ABSTRACT

CHUAH, XIN-YING SHARON. The Impact of Moisture on Physical and Chemical Variables in Pelleting Processing (Under the direction of Dr. Adam C. Fahrenholz).

In this experiment, diets containing varying moisture contents from mixer added water and conditioner added steam were pelleted. The impact of moisture on physical and chemical variables in pelleting processing were determined. The samples collected included unconditioned mash (UCM), conditioned mash (CM), and pellets (P). For physical variables, the parameters monitored included the moisture level in different feed forms, change in temperature between hot pellets and conditioned mash (ΔT), pellet durability index (PDI), and pellet mill energy consumption (PMEC). For chemical variables, the parameters monitored included starch gelatinization, glass transition temperature (Tg), and feed microstructure. ANOVA was adopted for statistical analysis and the means were separated using Tukey's HSD test. Regression model was utilized to study the correlation between variables. Overall, moisture addition through water and steam will significantly affect the physical and chemical variables. Water could not completely replace steam in terms of PDI and PMEC. However, additional 2% of mixer added water during the summer and when receiving dry or old crop corn may improve PDI and without affecting PMEC. In addition, moisture addition itself did not affect starch gelatinization. The study also shown that starch gelatinization did not have significant effect on PDI. Both water and steam addition will affect the structure integrity in pellets. Chemical variable like Tg could be an important factor in understanding the fundamental of pellet binding mechanism

The Impact of Moisture on Physical and Chemical Variables in Pelleting Processing

by
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DEDICATION

To MooMoo Family, thanks for being the greatest support system, always and forever.

BIOGRAPHY

Xin-Ying Sharon (X.S.) Chuah was born on June 21st, 1997, to Cindy Siew Hong Koh and Winson Kim Kwang Chuah in Penang, Malaysia. She grew up in Kampung Valdor, Penang and moved to Simpang Ampat, Penang. She graduated from Jit Sin High School in Bukit Mertajam, Penang in 2014 and attended Inti International College Penang (IICP) and transferred to NC State in 2017 to focus on academics. Sharon worked as an undergraduate research assistant at the NC State Poultry Science Nutritional Lab and Chicken Education Unit under the direction of Dr. Kimberly Livingston. She later worked as an intern and part time employee at NCSU Feed Mill Educational Unit, and graduated *Magna cum laude* with her Bachelor of Science in Poultry Science from NC State in May of 2019. With her passion in pelleting processing, Sharon continued to pursue a Master of Science in Poultry Science under the direction of Dr. Adam Fahrenholz. Upon graduation, Sharon will pursue a Ph.D. in Food Science in University of Florida under the director of Dr. Razieh Farzad.

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LITERATURE REVIEW

PELLETING

The pelleting of animal feeds has become common practice in the animal production industry due to improvements in feed handling and animal performance (Behnke, 2001). Enhanced animal performance may result from several attributes of pelleted feed including increased palatability, improved digestibility, decreased ingredient segregation and energy expenditure during prehension (Briggs *et al.*, 1999b; Behnke, 2001). Additionally, improved pellet durability has been shown to improve animal performance (Moran, 1989).

Pelleting is the process of pressing blended ingredients into a cylindrical form by compressing the individual particles of the blended ingredients together. The process involves passing the blended mash feed into the conditioning chamber. In the conditioner, heat and moisture are applied to facilitate the compaction of mash feed in the next step. After conditioning, the conditioned mash feed then flows to the pelleting chamber to be pressed through the die via pressure exerted by rollers to form pellets. Lastly, the pellets are dried and cooled in the cooler chamber to form a final feed for animal consumption. The pelleting process is not simply the sum of conditioning, pelleting, and cooling steps, but should be recognized as an integral system in which performance is dependent on interrelations between these three steps. Moisture, heat, and pressure play important roles in adhering particles together (Thomas & Van Der Poel, 1996).

Pelleting has become common process in the poultry industry due to both physical and nutritional benefits. Many studies have reported the positive effect of feeding pelleted feed to poultry such as increasing feed intake, growth performance and feed efficiency. From a physical perspective, pellets have shown to improve flowability and increase bulk density (Thomas &

Van Der Poel, 1996). In an automated feeding system, good flowability of feed is essential to convey feed to the broilers uniformly and efficiently (Pope, 2019). Greater tonnage of feed per a fixed volume can be transported and stored with increased bulk density. McKinney & Teeter (2004) had reported that feeding greater proportions of pellets compared to fines reduced selective feeding and energy expended for feeding. In the poultry industry, feed conversion ratio (FCR) is an indicator to measure poultry's performance by calculating the total feed consumed per unit body weight gain. Hence, better pellet quality may improve FCR due to its effective energy afforded from improved feed form (Lilly *et al.*, 2011).

Good physical feed quality is usually associated with enhanced animal performance. Thomas & Van Der Poel (1996) had categorized two physical quality parameters which were pellet hardness and pellet durability. Hardness is measured by determining the force needed to fragment the pellets. It can be a good indicator to predict the strength of the pellets to withstand statical pressure (i.e., weight) at the bottom of silo bins. On the other hand, Pellet Durability Index (PDI) is typically used as an indicator nowadays to determine pellet quality by measuring the ratio of pellets-to-fines in a fixed weight. It can be a good indicator to predict the strength of pellets during transportation and handling of feed. There are two methods that are widely recognized in the industry to determine PDI, which are the KSU tumble box method and the New Holmen Pellet Tester (NHPT) (ASAE, 1997). In the KSU tumble box method, pellets are sheared over one another and over the wall using mechanical agitation by rotating the tumble box for 10 minutes. The aggression can be modified by adding objects like hex nuts into the tumble box. The NHPT simulates a more rigorous treatment of pellets using pneumatic agitation by introducing a stream of air into the chamber for a period of 30, 60, 90, or 120s. Thomas & Van Der Poel (1996) reviewed that the tumbling can method measured abrasion while the NHPT method measured both fragmentation and abrasion. Fahrenholz (2012) later determined a strong linear relationship between both methods, indicating that they would predict differences in pellet durability in a very similar way.

PELLET BINDING

The mechanisms of pellet binding can be explained using the adhesive theories. Different theories on adhesion mechanisms were proposed and reviewed by several researchers. Wake (1978) and Allen (1987) categorized four mechanisms of adhesion as mechanical interlocking, diffusion theory, electronic theory, and adsorption. On the other hand, Behnke (2001) had described pellet binding mechanisms by reviewing the three theories on adhesive mechanisms proposed by Kinloch (1987), which were mechanical interlocking, diffusion and adsorption. Gardner (2017) later expanded the theories of adhesive into seven mechanisms, which were mechanical interlocking, electrostatic, adsorption (thermodynamics), diffusion, chemical (covalent) bonding, acid base, and weak boundary layers. However, there is an interplay between those parameters. These further highlighted the complication of adhesive mechanisms in pellet binding.

As an overview, the binding mechanisms can be divided into five primary mechanisms: i) “solid-solid” interactions (also known as solid bridges) between particles, ii) capillary forces by liquid necking (also known as interfacial forces and capillary pressures), iii) adhesive and cohesive forces between particles, iv) attraction forces between particles, and v) mechanical interlocking (interactions between particles due to folding and plying) (Thomas & Van Der Poel, 1996; Behnke, 2001; Kaliyan & Vance Morey, 2009; Gardner, 2017). Thomas & Van Der Poel (1996) had concluded that the binding in pellets is due to solubilization and subsequent crystallization of feed composition through liquid necking. Liquid necking held the feed particles in a three-phase system of water, air, and solid materials.

Solid-solid interactions maybe developed by diffusion of molecules from one particle to another at the points of contact when mechanical force was exerted by the friction and pressure in die (Kinloch, 1987; Thomas & Van Der Poel, 1996; Kaliyan & Vance Morey, 2009). The bonds are usually formed during the drying/cooling stage where recrystallization, solidification of materials, and hardening of binders will occur. In a porous agglomerate (i.e., pellets), feed particles are held by liquid necking. Rumpf (1958) determined that the surface tension of the binding liquid and the radius of the neighboring particle are the dependent factors for the force of the bonds in liquid necking. It indicated that smaller particle sizes may improve the binding force between particles. Besides, it also indicated that the binding agents (i.e., water) will influence pellet binding. In the presence of excess water, a two-phase system of water and particles was created as the moisture bridge between particles increased (Thomas & Van Der Poel, 1996). Hence, the porosity between feed particles was reduced and binding force was increased (Immergut & Mark, 1965; Levine & Slade, 2010).

Mechanical interlocking can be divided into groups: i) locking by friction, and ii) locking by dovetailing (Gardner, 2017). It takes place when adhesives penetrate/absorb into rough surfaces and become rigid by holding the materials together (Kinloch, 1987). A greater level of adhesion can be obtained when the contact areas have increased. This explained the reason why rougher surface had a better adhesion properties. In addition, adsorption plays an important role in mechanical interlocking. The adsorption adhesion energy is contributed by Van der Waals forces, which establishes across interface between all atoms and molecules when they are close together. In short, greater adsorption allows more penetration of adhesives into pores on adhered surfaces, thus improving the adhesion strength in mechanical interlocking system.

The diffusion theory is based on the concept that two materials which are soluble in one another will dissolve and form an interphase when in close contact (Voyutskii & Vakula, 1963). Diffusion takes place when materials are heated and allowed to diffuse across the interface between materials. For the diffusion mechanism of adhesion to occur, the temperature of the polymer must be above the glass transition temperature of the polymer (Kinloch, 1987). The attraction forces between particles can be categorized as ionic, covalent, hydrogen bonding dipole interactions, and Van der Waal forces (Behnke, 2001). Van der Waal forces may have greater effect in pellet integrity when the particles are small (Rumpf, 1958; Friedrich, 1977). In fact, the role of other binding mechanisms aforementioned like liquid necking could be more significant as the particle sizes in animal feed are generally larger (Thomas & Van Der Poel, 1996).

FACTORS AFFECTING PELLET BINDING

Feed formulation has been shown to be the most influential factor affecting pellet quality, followed by mash conditioning, particle size, pellet mill die specifications, and lastly pellet cooling (Behnke, 2001). Stark & Ferket (2011) and Fahrenholz (2012) later determined that pellet mill throughput (also known as production rate) had a pronounced influence on pellet quality. As technology advances, and more new ingredients are introduced over years, it is necessary to have a thorough understanding of factors affecting pellet quality.

A variety of raw materials are used during feed manufacturing to produce compound feed. The nutrients in feed can be classified as starch, protein, fat, sugar, non-starch polysaccharides (NSP), inorganic matter and water. Over the years, researchers have investigated the effect of ingredient compositions on the physical quality of feed (Skoch *et al.*, 1981; Wood, 1987; Stevens, 1987; Thomas *et al.*, 1998; Briggs *et al.*, 1999a; Zimonja & Svihus, 2009; Buchanan & Moritz, 2009; Fahrenholz, 2012; Abdollahi *et al.*, 2013; Pope *et al.*, 2018).

Proteins have four different levels of structure. The primary structure is composed of a linear chain amino acids. The next level of protein structure, secondary structure, refers to the folded structures in protein when the amino acids chains interact with each other to form alpha-helixes or beta-pleated sheets. The tertiary structures are the three-dimensional arrangements of a protein. Lastly, quaternary proteins comprised of multiple polypeptides. It was proven that starch and protein will impact pellets binding, as they are the major composition of broiler diets. During pelleting, the combination of heat, moisture, and pressure result in protein denaturation, which led to an increase in pellet durability (Wood, 1987; Thomas *et al.*, 1998). Denaturation is any change in the conformation of a protein but not the breaking of peptide bond (Cheftel, 1986). Since the denaturation reaction is not strong enough to disrupt the peptide bonds, the primary structure of protein remains the same. On the other hand, the secondary structure protein will lose its structure such as alpha-helixes or beta-pleated sheets and adopt a random coil configuration. The tertiary or quaternary protein structures are broken down into their primary structure by unfolding and refolding the molecular structures (Fellows, 2016). Wood (1987) concluded that protein had a greater influence on pellet quality than starch, which was later confirmed by Briggs *et al.* (1999a). Briggs *et al.* (1999a) observed an increase in pellet durability from 76% to 89% when the protein content increased from 16.3% to 21%. The strong gelling properties from denaturized proteins could contribute towards a more durable pellet by forming the long sticky fibrils that interconnect other feed particles (Abdollahi *et al.*, 2013)

STARCH

Starch is the most common carbohydrate source used in pelleted feed. Hence, it is important to understand the physiochemical properties of starch during pelleting. Starch consists of two main polysaccharides, amylose, and amylopectin. Amylose consists of linear chain in which 500-20000 D-glucose molecules are linked through α(1-4) glycosidic bonds while amylopectin consists of highly branched chains of up to 30 glucose molecules are linked through α(1-4) bonds through α(1-6) branch points (Camire, 1991; Takeda *et al.*, 1993; Parker & Ring, 2001; Fellows, 2016). The mass of amylopectin is 4 to 5 times greater than the mas of amylose and therefore is a larger molecule (Fellows, 2016). In most native starches, there are about 20-30% of amylose. For example, common corn starch and wheat have about 25% amylose, rice and potato have about 20% amylose, and tapioca starch has around 15-18% amylose. There are some starches that contain very little amylose (<1%) like waxy cornstarch or high amylose contents (>65%) such as high-amylose corn starch (Camire, 1991; Takeda *et al.*, 1993; Parker & Ring, 2001). Each starch has its own characteristic properties in cooking, gel formation and viscosity due to the different ratio of amylose and amylopectin within the starch granule (Singh *et al.*, 2007). Therefore, different starches have different uses. For examples, rice starch is used to produce baby food due to its opaque gel properties, potato starch is used to produce snacks, extruded cereal, or cake mix, while waxy maize is used to produce clear and cohesive pastes. The functional properties of starch can be improved after some modifications like improved gelatinization, improved solubility, increased or decreased in viscosity, freeze stability, improved gel strength and paste cohesiveness (Fennema, 1992).

STARCH FRACTIONS

Starch can be classified into three fractions according to its kinetics and degree of digestion in small intestine (Englyst *et al.*, 1992). The three fractions are rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS will be fully digested by amylase and absorbed in the mouth and upper intestine. SDS will be broken down by digestive enzymes in the stomach and absorbed by the small intestine at a slower rate. On the other hand, resistant starch (RS) will not be digested in small intestine but fermented mainly in the colon (Englyst *et al.*, 1992). In terms of structure, RDS is mainly amorphous; SDS is partially amorphous and crystalline, while RS is mainly crystalline. When the raw material is exposed to heat and moisture, the starch granule will be gelatinized. Gelatinized starch will be hydrolyzed by enzymes such as a-amylase, glucoamylase and sucrose-isoamylase. A higher percentage of starch gelatinization will result in a higher percentage of rapid digestible starch (RDS) in diets as more fractions of starch granules will be in the amorphous structure (Robin *et al.*, 2016).

Pelleting was shown to improve *in vivo* starch digestion rate (Carré *et al.*, 1987; Grosjean *et al.*, 1999). Pelleting affects starch digestion by particle size reduction and starch gelatinization. Weurding *et al.* (2003) observed an improvement in FCR in broilers fed diets containing greater levels of SDS compared to lesser levels of SDS. Vaugelade *et al.* (1994) stated that 20% of dietary energy is consumed to support digestive and absorptive processes. According to Fleming *et al.* (1997), glutamine and glucose are preferentially used to provide energy for the small intestine. When broilers are fed RDS diets, starch digestion takes place at the upper intestine. Thus, it does not provide the lower part of the intestine with glucose for its energy demand. More amino acids will be oxidized at the lower part of the small intestine for that purpose. On the contrary, with SDS diet, since not all starch is digested at the upper intestine, it provides the lower small intestine with glucose, hence glutamine is spared from being oxidized to provide energy for the enterocytes. This is especially important when the diet has a lower level of glutamine. A diet containing greater amounts of SDS enhances microbiome symbiosis in broilers, thus discouraging the colonization of entering pathogens such as *clostridium perfringens* in broilers. It is also important to formulate the diet with an ideal level of SDS, too much SDS may negatively impact the FCR due to poor digestion (Weurding *et al.*, 2001)

GELATINIZATION

Below temperature of starch gelatinization, native starch is insoluble in water due to the hydrogen bonds that exist between the helices and semi-crystalline structure of the starch granule. Individually these bonding forces are relatively weak, but together they are strong enough to prevent starch from dissolving in cold water. When starch is heated in excess water, gelatinization occurs, and it starts with the hydration and swelling of granules. The swelling of amorphous regions causes the loss of birefringence by breaking the crystalline regions. Upon continuous heating, granules will swell to a greater extent, the crystallites melt, resulting in increased molecular motion that eventually leads to complete separation of amylose and amylopectin. During preconditioning, the glassy preconditioned raw materials are plasticized with heat and moisture by the addition of water and steam prior to pelleting. Amylose plays an important role in the early stage of gelatinization (Parker & Ring, 2001; Ratnayake & Jackson, 2006). The gelatinization temperature (temperature of starch where it starts losing its birefringence) depends on the amount of available water. Water softens the amorphous regions and supplies crystalline regions with sufficient mobility to melt. If water is limited, more heat and or mechanical energy is needed to soften the amorphous regions and to disrupt the crystallinity. Hence, excess water helps lowering the glass transition temperature (Tg) and gelatinization temperature of starch (Rooney & Pflugfelder, 1986; Parker & Ring, 2001; Ratnayake & Jackson, 2006).

According to Lund (1984), the water to starch ratio of 0.3:1 is required for gelatinization, and a ratio of 1.5:1 would be required for complete gelatinization. Theoretically, higher mash moisture percentage will result in higher gelatinization percentage (Lund, 1984; Parker & Ring, 2001; Ratnayake & Jackson, 2006). However, water is a limiting factor for starch gelatinization during conventional pelleting conditions. Besides moisture levels, other factors like addition of salt, sucrose, or fiber will affect gelatinization as well as expansion. The addition of sugar will depress the enthalpy of gelatinization and increase the temperature of initiation (Jin *et al.*, 1994). Some chemicals accelerate gelatinization by disrupting the hydrogen bonds while some inhibit gelatinization by acting as dissolving agents (Bhattacharya & Hanna, 1987). Starch helps in structure forming and binding because gelatinized starch acts as gel and can provide better binding properties (Lund, 1984; Guy, 2001).

Previous findings had shown that only small extent of starch gelatinization, ranging from 5 – 30%, occurred during conventional pelleting due to the limited moisture content and moderate temperatures (Skoch *et al.*, 1981; Moritz *et al.*, 2005; Buchanan & Moritz, 2009; Zimonja & Svihus, 2009; Abdollahi *et al.*, 2010). Stevens (1987) reported that most gelatinization occurred on the outer portion (2mm thick) of pellets, indicating that mechanical shear across die had a greater impact on gelatinization during pelleting process. Several authors also observed that some degree of gelatinization occurred during conditioning, but the majority of gelatinization took place during pelleting (Heffner & Pfost, 1973; Zimonja & Svihus, 2009; Abdollahi *et al.*, 2010; Abdollahi *et al.*, 2011).

According to Moritz *et al.* (2003), the gelatinized starch content in pellets decreased from 18.4% to 10.6% and 6.1% when 25g/kg and 50g/kg of water were added. The final pellet moisture content of the control diet with no moisture addition was reported at 12.33%. In another study, Moritz *et al.* (2001a) also reported an addition of 50g/kg water resulted in an increase in gelatinized starch content from 15.8 to 29.6% when the dry matter of the maize-soy diet was greater than normal at 92.7%. It is noteworthy that further moisture addition above normal levels of moisture in a diet reduces starch gelatinization while moisture addition below normal levels of moisture in a diet increases starch gelatinization (Abdollahi *et al.*, 2013).

In addition, several studies have shown conflicting data on the relationship of starch gelatinization and pellet durability (Wood, 1987; Thomas *et al.*, 1998; Briggs *et al.*, 1999b; Moritz *et al.*, 2001a; Moritz *et al.*, 2002; Gilpin *et al.*, 2002; Zimonja & Svihus, 2009). Wood (1987) and Zimonja and Svihus (2009) observed the positive effect of pre-gelatinized starch on pellet quality. Gilpin *et al.* (2002) showed a negative correlation between starch gelatinization and pellet durability. Moritz *et al.* reported conflicting observations on the relationship of starch gelatinization and pellet durability in different studies when moisture increased (Moritz *et al.*, 2001b; Moritz *et al.*, 2002; Moritz *et al.*, 2003). On the other hand, Landon (2011) reported that the pellet durability stayed consistent when degree of gelatinization increased. Increased starch gelatinization of the total pellet may not improve pellet quality in corn-soybean based diets (Moritz *et al.*, 2003). In fact, the location and uniformity of the gelatinized starch may have a greater influence on pellet quality (Stevens, 1987; Behnke, 2001). For instance, pellet durability may improve in pellets with small amounts of evenly distributed gelatinization as opposed to high amounts of localized gelatinization.

GLASS TRANSITION

Glass transition temperature (Tg) is referred to as the physical change that reflects the increase in the atomic chains of the polymer backbone (Ferry, 1980; Levine & Slade, 2010). Factors like time, temperature, composition, molecular weight, and water activity will affect glass transition (Abiad *et al.*, 2009). Polymers will be glassy and immobile below Tg but rubbery, viscous, and flexible above Tg, but below the melting temperature (Tm). Hence, any alterations to the rigidity of the backbone of a polymer influences Tg. The level of plasticizers that exist in feed will change its glass transition temperature. Among the different types of plasticizer present in feed and food applications, water is one of the most ubiquitous plasticizers (Abiad *et al.*, 2009). Water exerts a plasticizing effect on many polymers and depresses the Tg (Zeleznak & Hoseney, 1987; Levine & Slade, 2010). Plasticizer changes the thermal and mechanical properties of polymers by reducing crystallization, lowering stiffness, and increasing chain flexibility. In addition to the role of moisture and its effect on Tg, temperature also plays a critical role in determining Tg. For example, stiffer chains require warmer temperatures to achieve the level of molecular motion necessary for the glass transition (Kaplan, 1976). Temperature also influences the rate of crystallization such that at a critical temperature, the crystalline phase of the polymers increases while the amorphous phase decreases. Hence, the change in crystallinity will lead to changes in Tg (Wang & Truong, 2017).

It is important to understand how glass-transition influence the physical interactions of the polymer components in feed during the pelleting process (Thomas & van der Poel, 2020). In conventional pelleting conditions, feed ingredients have glass transition temperatures below the normal conditioning temperatures which ranged around 70-90° C. This will change the compacting behavior of feed materials and its physical quality as the feed materials are altered and transformed during conditioning. According to Thomas & van der Poel (2020), the impact of feed particles on pelleting behaviors are twofold. First, an increase in the fraction of the feed mash particles to go through the glass transition stage will lead to an easier deformation of particles. Second, the available moisture is bound to the surface of the particles at the start of the conditioning process. Hence, starch on the surface is more exposed to changes due to the presence of water during pelleting process.

EFFECT OF MOISTURE ON PELLET QUALITY

Improvement in pellet quality with a decrease in pellet mill energy consumption were observed when increasing moisture content of mash feed at the mixer (Fairchild & Greer, 1999). According to Skoch *et al.* (1981) steam conditioning improved pellet durability and production rates with a decrease in the number of fines. It has been proven that the practice of moisture addition through water or steam could improve PDI (Skoch *et al.*, 1981; Fairchild & Greer, 1999; Moritz *et al.*, 2002; Moritz *et al.*, 2003; Pope & Fahrenholz, 2020). Rumpf (1958) and Friedrich (1977) demonstrated that the binding of particles was caused by water absorption in particles and the hydrogen bonds between them. Buchanan and Moritz (2009) stated that moisture retention could increase starch gelatinization and improve pellet quality. As moisture increased, the feed particles were hydrated and softened after diffusion of water into these particles (Buchanan & Moritz, 2009; Thomas & van der Poel, 2020). During conditioning, both heat and water diffusion occurred at a different rate. According to Bouvier & Campanella (2014), water diffused at a rate of 100 times slower to hydrate particles compared to heat diffusion. This could result in an uneven moisture distribution across feed mash particles. The size and chemical composition of particles will affect the moisture gradient during conditioning, pelleting, and cooling (Hemmingsen *et al.*, 2008).

Conditioner added steam causes redistribution of water among feed particles, which increases the mobility of water between particles (Friedrich & Robohm, 1968). Water adsorbed on the surface of grain particles had more mobility and less hindrance compared to water bounded within capillaries in the grains. As a result, the water adsorbed on the surface was more available for chemical reaction compared to the water bounded within capillaries in the grains (Hemmingsen *et al.*, 2008a). Thus, it reduces the porosity between feed particles and increases the number of bonds, which then increases the strength in pellets.

However, die plugging or "roll slip" could happen when the conditioned mash moisture content is greater than 18% due to a loss of frictional force between the feed, rolls, and the die surface (Turner, 1995). During the summer, it is also challenging to pellet when increasing mash moisture through steam because less moisture is added while pelleting at the same common conditioning temperatures. Hence, it may be ideal to add water to rations to achieve the full binding potential in ingredients. In addition, Mohsenin & Zaske (1976) observed the effect of water on compaction behavior due to its influence on the stress relaxation in the feed pellet. When pelleting in excess water, greater residual stress induces a greater degree of expansion in feed, which results in lower bulk density of feed (Thomas *et al.*, 1997).

SUMMARY

Pelleting process is a complex integral system that depends on the interrelations between all the physical and chemical variables. Moisture addition through water and steam in the pelleting process have shown to improve the pellet quality. From the physical processing perspective, it is beneficial to understand how water addition through mixer and steam addition through conditioner could impact the moisture content in different feed forms, PDI and PMEC. On the other hand, it is important to understand how chemical variables influence the pelleting process as not much study and consistent data are available in this area. Cereal chemistry like starch gelatinization, protein denaturation, glass transition and crystallization could be the critical factors in understanding the fundamental of pellet binding mechanisms.

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CHAPTER 1

Impact of moisture on the physical and processing characteristics of pelleting using different moisture addition methods.

ABSTRACT

It has been proven that the practice of moisture addition through water or steam could improve pellet durability index (PDI). However, little work has been done on the effect of replacing conditioner added steam with mixer added water on pellet quality. In this experiment, diets containing varying moisture contents from mixer added water and conditioner added steam were pelleted. The impacts of moisture on the physical and processing characteristics of pelleting were evaluated. Poor pellet quality resulted when there was no moisture addition from mixer added water nor conditioner added steam ($p < .0001$). Moisture added to rations via mixer added water was not adequately removed from pellets as compared to moisture from conditioner added steam. It was determined that mixer added water and conditioner added steam impacted pellet durability index (PDI), ΔT, and pellet mill energy consumption (PMEC) ($p < .0001$). There was a negative correlation between moisture addition and ΔT ($p < .0001$). No interaction effects were observed between moisture level and moisture addition methods. Overall, increased moisture addition from either mixer added water or conditioner added steam resulted in improved PDI ($p < .0001$). Mixer added water could not completely replace conditioner added steam in terms of pellet quality. However, additional moisture from mixer added water during the summer and when receiving dry or old crop corn may be beneficial in terms of PDI without negatively affecting (PMEC).

Key words: Moisture, Water, Steam, Pelleting, PDI, PMEC

DESCRIPTION OF PROBLEM

The pelleting of animal feeds has become common practice in the animal production industry due to improvements in feed handling and animal performance (Behnke, 2001). Enhanced animal performance may result from several attributes of pelleted feed including increased palatability, improved digestibility, decreased ingredient segregation and energy expenditure during prehension (Briggs *et al.*, 1999b; Behnke, 2001). Pelleting can be a fairly expensive process, yet the expense is usually accounted for by improved animal performance (Behnke, 2012). Therefore, feed manufacturers are constantly looking for ways to improve pellet quality and reduce pelleting cost.

When the moisture content of mash feed at the mixer was increased, an improvement in pellet quality with a decrease in pellet mill energy consumption was observed (Fairchild & Greer, 1999). According to Skoch *et al.* (1981), steam conditioning improved pellet durability and production rates with a decrease in the amount of fines. Researchers have shown a positive correlation between moisture and pellet durability index (PDI) (Skoch *et al.*, 1981; Fairchild & Greer, 1999; Moritz *et al.*, 2002; Moritz *et al.*, 2003; Pope & Fahrenholz, 2020). During instances of low grain moisture, applying additional steam has been necessary to facilitate particle adhesion. However, conditioned mash moisture content greater than 18% has resulted in die plugging or "roll slip" due to a loss of frictional force between the feed, rolls and the die surface (Turner, 1995). It is also challenging to pellet when increasing mash moisture through steam during the summer because the higher ambient temperatures lead to less moisture addition while achieving the same common conditioning temperatures.

Hence, feed manufacturers started adding moisture via both mixer added water and conditioner added steam. However, not much work has been done on the effect of replacing conditioner added steam with mixer added water on pellet quality. The objective of this study was to evaluate the impact of moisture on the physical and processing characteristics of pelleting using different moisture addition methods. It was necessary to determine how much steam could be replaced by water without negatively impacting the pellet quality and pellet mill energy consumption. An in-depth review on the effect of moisture addition sources on processing characteristics of pelleting will be discussed.

MATERIALS AND METHODS

The experiment was conducted by pelleting twelve 340 kg batches of a broiler starter diet (Table I-1) to determine the effect of added moisture on pellet quality using different moisture addition methods. Treatments were arranged as a 2x3 factorials utilizing two levels of added moisture (2% vs 4%) and three moisture addition methods (mixer added water, conditioner added steam and both). Treatments consisted of a negative control and two positive controls with intermediate treatments in between (Table I-2). A mash diet not subjected to mixer added water nor conditioner added steam was dry pelleted to serve as a negative control (NC). 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).

There were three manufacturing runs per treatment which represented three replicates, and the run order was completely randomized. Each run consisted of two batches of 454kg, one batch of 680kg, and one batch of 907kg. Batches consisting of 454kg of feed were pelleted at one conditioning temperature, batches of 680kg of feed were pelleted at two conditioning temperatures and batches of 907kg were pelleted at three conditioning temperatures. Hence, each run consisted of 7 treatments. The pellet mill and conditioner were completely shut off between each run.

All feed was manufactured following the guidelines for Current Good Manufacturing Practice (CGPM). The research was conducted at the North Carolina State University Feed Mill Educational Unit utilizing a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) fitted with a 4.4 x 28.6 mm die and a conditioner feeder (Model C18LL4/F6 California Pellet Mill Co., Crawfordsville, IN). All corn was ground utilizing a hammermill (Model 1522, Roskamp Champion, Waterloo, IA.) equipped with a 2.4 mm screen on the impact side and a 3.2 mm screen on the release side.

Individual batches were sourced from a common basal to reduce variation in ingredient composition between batches. 2268 kg of common basal devoid of water was batched utilizing a twin shaft counterpoise ribbon mixer (Model TRDB126060, Hayes and Stolz, Fort Worth, TX) for 180 s of dry mixing time, followed by an additional 90 s of wet mixing time. The basal diet included phytase, xylanase, and fat. The individual batches were then blended utilizing the same equipment and methodology. For batches amended with water, the water was added after 180s of dry mixing, but before 90s of wet mixing. Upon mixer discharge, five composite mixer samples were collected in sealed sample bags, three of which were immediately frozen for subsequent moisture content analysis. The temperature of mixer mash feed was collected to determine the target conditioning temperature.

For batches amended with steam, conditioning temperature was increased by 25°F for every 1% of target conditioner added steam (Table I-5). The actual increase in conditioning temperature was calculated by subtracting the temperature of mixer mash feed from the conditioning temperature. The pellet mill die was warmed with 455kg of feed before proceeding with the experimental batches. All batches were conditioned for 30s and pelleted at a production rate of 771kg/hr. The average steam pressure was 255kPa.

After reaching the appropriate mash conditioning temperature, six hot conditioned mash and hot pellet samples were collected, sealed, and frozen immediately for moisture analysis over a five-minute period of pelleting. The moisture content was measured in accordance with AACC 44-19.01. The hot conditioned mash was collected as feed transitioned between the conditioner and the pellet mill die. At the same time, five pellet samples were collected and blended into composite samples. These pellets were then cooled in custom manufactured 30 x 30 cm trays, which were placed in a custom manufactured pellet cooler resembling a counterflow cooler. Cooled pellets were analyzed for moisture content and pellet durability index (PDI). Pellet durability was measured using a Holmen pellet durability tester (Model NHP100, Tekpro, Norfolk, UK). 100g of pellets screened using a #5 US sieve were placed into the pellet chamber for 30s. Pellets were removed directly from the pellet chamber and weighted to represent a proportion of the initial mass added to the testing chamber. Each sample was tested in triplicate and an average value was calculated.

For batch runs consisting of more than one conditioning temperature, the steam valve leading to the conditioning chamber was further opened to increase the conditioning temperature, and the sample collection process was repeated once reaching the appropriate conditioning temperature. During the pelleting process, parameters including the difference in temperature between hot pellets and conditioned mash (ΔT), and motor load and pellet mill energy consumption (PMEC) were monitored and collected. PMEC was calculated using the values of motor load (%), horsepower (hp), kilowatt per horsepower (kw/hp), and production throughput (ton/hr).

$$\text{PMEC} = \frac{Motor\ Load\ x\ Horsepower\ x\ Kilowatt}{Production\ Throughput}$$

Statistical Analysis

ANOVA from the Fit Model platform of JMP Pro 15.2 (Statistical Analysis, SAS Institute, Cary, NC) was employed and the means were separated using the LSMeans procedure of JMP. The means were considered statistically significant at $p \leq 0.5$. Significant differences were separated utilizing Tukey's HSD test. The relationship between dependent continuous factors generated during the pelleting process (PDI, ΔT, PMDC) and relative moisture (mixer added water, conditioner added steam, total moisture addition, feed form moisture) were regressed utilizing the Fit Y x X platform of JMP 15.2. The regression models were considered statistically significant at $p \leq 0.05$.

In addition, a 2x2 factorial randomized block design was analyzed for interaction effects utilizing the Fit Model platform (LS Means Procedure) of JMP 15.2 (Table 1-3). The 2x2 factorial designs consisted of two moisture addition methods (mixer added water and conditioner added steam) and two moisture addition levels (0% and 2%).

RESULTS AND DISCUSSION

Differences between target vs actual moisture contents were observed (Table I-4). For mixer added water, the actual water level reported was lower than targeted (Table 1-4). This may have been due to water evaporation occurring the intensive action inside the counterpoise mixer during mixing. Additionally, steam addition was observed for the treatment with 0% target steam moisture, possibly due to residual moisture in the conditioner. While the conditioning temperature was estimated to increase by 25°F for every 1% of target conditioner added steam, an interesting trend was observed in that the moisture retention from steam decreased when the mash feed was amended with more water. As the mixer mash moisture increased, the observed moisture addition due to steam decreased despite the increase in conditioning temperature being constant ($p < .0001$;

Table 1-5). When mixer mash was amended with 0% mixer added water, an increase in 51.52°F conditioning temperature resulted in 2.96% moisture addition. In contrast, when mixer mash was amended with 2% mixer added water, an increase in 50.10°F conditioning temperature resulted in 2.15% moisture addition ($p < .0001$; Table 1-5). An increase in 11.13°F conditioning temperature resulted in 0.59% moisture addition when the mixer mash was not subjected to mixer added water, as opposed to 0.34% moisture addition when mixer mash was amended with 2% mixer added water and 0.19% moisture addition when mixer mash was amended with 4% mixer added water. As mixer added water increased, the capability of hot conditioned mash to hold moisture from the steam decreased.

The moisture content of mixer mash increased in rations that were amended with 2% or 4% mixer added water when compared to rations that were not amended with water or 1% mixer added water ($p < .0001$; Table 1-6). Rations with 1%, 2%, and 4% conditioner added steam contained greater levels of moisture in hot conditioned mash and hot pellets when compared to rations with 0% conditioner added steam ($p < .0001$; Table 1-6). The moisture content of cool pellets increased in rations that were amended with 2% mixer added water and 2% conditioner added steam, and 4% mixer added water and 0% conditioner added steam when compared to rations that were amended with 0% mixer added water and 0% conditioner added steam ($p < .0001$; Table 1-6). In contrast, rations that were not subjected to mixer added water but 0%, 2% and 4% conditioner added steam contained statistically similar levels of moisture in cool pellets ($p < .0001$; Table 1-6). In addition, an interesting trend was observed that the die consistently reduced the amount of moisture in conditioned mash feed ($p < .0001$; Table 1-6). It might be due to the additional frictional heat generated in the die, which further removed moisture from the pellets as the hot air escaped. There was a positive correlation between cool pellet moisture and mixer added water ($p = .0113$; Figure 1-1) but not conditioner added steam. These results were in agreement with Pope and Fahrenholz (Pope & Fahrenholz, 2020), who observed moisture added to rations via mixer added water was not adequately removed from pellets, as opposed to moisture added via conditioner added steam. This may be because steam was more volatile and escaped from the pellet matrix, or because the heat introduced by the steam aided in drying during the cooling process. Another possibility could be that the rate of thermal diffusivity coefficient is 100 times more than water diffusivity. Hence, the addition of heat from steam may have caused the change in moisture content.

The hot conditioned mash and hot pellet temperatures of rations that were conditioned with 1%, 2%, and 4% steam were significantly greater than rations that were not conditioned with steam ($p < .0001$; Table 1-7). As the conditioned mash temperature increased, the difference in temperature between hot pellets and hot conditioned mash (ΔT) decreased, indicating that the moisture from steam reduced the friction generated during pelleting ($p < .0001$; Table 1-7). Similar findings were reported by several authors(Skoch *et al.*, 1981; Pope & Fahrenholz, 2020). Diets not subjected to steam, but rather to different levels of mixer added water did not show an effect on conditioned mash temperature nor hot pellet temperatures, but there was an effect on ΔT ($p < .0001$; Table 1-7). As mixer added water increased from 0% to 4%, ΔT decreased significantly despite no steam being added, indicating that the water acted as a lubricant and/or a plasticizer ($p < .0001$; Table 1-7). As moisture increased through the addition of either water, steam or a combination of both, the plasticization effect may have reduced the force needed to deform polymers during the pelleting process. Further discussion of this concept can be found in chapter 3.

A positive correlation between conditioning temperature, hot pellet temperature and PDI was observed (*p <.0001*, $R^2 = 0.75$; Figure I-2), (*p <.0001*, $R^2 = 0.57$; Figure I-2). The benefit of increasing mash conditioning temperature for better PDI has been reported widely (Skoch *et al.*, 1981)(Fahrenholz, 2012)(Thomas *et al.*, 1997). There was a strong negative correlation between ΔT and PDI (*p <.0001*, $R^2 = 0.86$; Figure I-2). This finding indicated that higher frictional force generated during pelleting at lower mash moisture did not improve PDI. It is noteworthy that the conditioning temperatures between treatments in this trial were different. In cases where conditioning temperatures is held constant, increased ΔT is often associated with increased PDI.

Increased moisture addition through conditioner added steam, mixer added water or a combination of both significantly improved PDI and reduced PMEC ($p < .0001$; Table 1-8) As conditioned mash moisture increased, PDI increased while PMEC decreased ($p < .0001$, $R^2 = 0.87$; Figure I-3, *p < .0001*, $R^2 = 0.74$; Figure I-4). The PDI was better in rations with 4% total added moisture as opposed to 2% total added moisture. At 2% total added moisture, moisture addition methods did not have a significant impact on PDI. At 4% total added moisture, the PDI of the treatment with 4% moisture from conditioner added steam was better compared to that of the treatment with 4% moisture from mixer added water or a combination of 2% moisture from mixer added water and 2% conditioner added steam. There was no significant difference in PDI between rations with 4% mixer added water and rations with 2% mixer added water and 2% conditioner added steam. However, PMEC was significantly lower in rations with 2% mixer added water and 2% conditioner added steam compared to 4% mixer added water. In addition, mash subjected to 4% steam resulted in the lowest PMEC while mash with 0% moisture addition had the highest PMEC. The PDI of the ration with 2% conditioner added steam and 2% mixer added water was significantly higher than the PDI of the ration with only 2% conditioner added steam. There was no significant difference between the PMEC of these diets. This appears to indicate that adding water to mash could improve PDI but not affect the PMEC. A significant correlation between PDI and PMEC was observed ($p < .0001$; Figure I-4). Moisture added through steam decreased PMEC while moisture added through mixer added water did not affect PMEC ($p < .0001$; Figure I-4). Moisture from mixer added water could not completely replace conditioner added steam. However, additional moisture from mixer added water during the summer when the pellet mill is not able to condition at high temperatures and when receiving dry or old crop corn maybe beneficial.

No significant interactions were observed between moisture addition methods (mixer added water and conditioner added steam) and moisture addition level (0% and 2%). For the main effect, a positive relationship between mixer mash moisture and PDI was observed ($p < .0001$; Figure I-5). There was no correlation between mixer mash moisture and PMEC ($p = .6382$; Figure I-6). In addition, as hot conditioned mash moisture increased, PDI increased while PMEC decreased ($p < .0001$; Figure I-5, $p < .0001$; Figure 1-6). The PDI of the ration with 2% conditioner added steam and 2% mixer added water was significantly better compared to the PDI of the ration with only 2% conditioner added steam ($p < .0001$; Table 1-9). There was no significant difference between PMEC of these two diets. This further indicated that adding water to mash could improve PDI but not affect the PMEC.

CONCLUSION AND APPLICATION

- Higher mash moisture content (4%) resulted in better PDI as opposed to lower mash moisture content (<2%).
- Moisture addition methods did not impact PDI when total moisture was lower (2%).

- With the same total moisture content (4%), 4% moisture from conditioner added steam resulted in better PDI compared to 4% moisture from mixer added water or a combination of 2% moisture from mixer added water and 2% conditioner added steam.
- Increased moisture from steam decreased pellet mill energy consumption while increased moisture from mixer added water did not affect pellet mill energy consumption.
- Moisture from mixer added water could not completely replace conditioner added steam.
- However, additional moisture from mixer added water during the summer and when receiving dry or old crop corn may improve pellet quality without affecting PMEC.

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TABLES AND FIGURES

Table I-1. Ingredient composition of broiler starter diets containing phytase and xylanase.

Ingredients	(%)
Corn	58.62
SBM (48%)	34.77
Poultry by-product Meal	2.26
Poultry fat	2.00
Limestone	0.84
Salt	0.50
DL-Methionine[a]	0.24
Choline chloride (60%)	0.20
Trace mineral premix[1]	0.20
Dicalcium phosphate (18.5% P)	0.14
L-Lysine[b]	0.08
Vitamin premix[2,c]	0.05
Selenium premix[3] (0.06%)	0.05
Phytase[4]	0.02
L-Threonine[d]	0.02
Xylanase[5]	0.01
Calculated nutrients	
Protein	22.95
Calcium	0.85
Available phosphorous	0.43
Total lysine	1.29
ME, kcal/g	3.00

[1] Trace mineral premix provided the following per kg of diet: Mn, 120 mg; Zn, 120 mg; Fe, 80 mg; Cu, 10 mg; I, 2.5 mg; Co, 1 mg.
[2] Vitamin premix provided the following per kg of diet: vitamin A, 6600 IU; vitamin D3, 1980 IU; vitamin E, 33 IU; vitamin B12, 0.02 mg; biotin, 0.13 mg, menadione, 2 mg; thiamine, 2 mg; riboflavin, 6.6 mg; pantothenic acid, 11 mg; vitamin B6, 4 mg; niacin, 55 mg; folic acid, 1.1 mg.
[3] Selenium premix provided Se at 0.3 mg/kg of feed.
[4] Quantum Blue 5G was added to provide 1000 FTU/kg of feed.
[5] Econase XT was added to provide 16,000 BXU/kg of feed.
[a,b,c,d] Dl-Methionine, L-Lysine, Vitamin premix, and L-Threonine were donated by Evonik, Ajinomoto, DSM, and Ajinomoto.

Table I-2. Treatments design.

Treatments	Mixer Added Water	Conditioner Added Steam	Total Moisture Addition
	Moisture, %		
Negative Control	0	0	0
Treatment 1	0	2	2
Treatment 2	1	1	2
Treatment 3	2	0	2
Treatment 4	2	2	4
Positive Control	0	4	4
Positive Control 2	4	0	4

Table 1-3. 2x2 factorial designs for interaction effect.

		Conditioner Added Steam, %	
		0	2
Mixer Added Water, %	0	0,0 (NC)	0,2 (Trt 1)
	2	2,0 (Trt 3)	2,2 (Trt 4)

Table I-4. Target vs actual moisture addition through water and steam.

Treatments	Water Addition[1]		Steam Addition [2]		Total Moisture Addition[3]	
	Moisture, %[1,2,3]					
	Target	Actual	Target	Actual	Target	Actual
Negative Control	0	0	0	0.59	0	0.59
Treatment 1	0	0	2	2.96	2	2.96
Treatment 2	1	0.59	1	0.99	2	1.58
Treatment 3	2	1.51	0	0.34	2	1.85
Treatment 4	2	1.51	2	2.15	4	3.66
Positive Control	0	0	4	4.08	4	4.08
Positive Control 2	4	3.13	0	0.19	4	3.32

[1,2,3] Moisture content was measured in accordance with AACC 44-19.01.
[1] Water was added through mixer and the amount of water added was calculated by weight percentage.
[2] Steam was added through conditioner and the amount of steam added was calculated by weight percentage.
[3] Total moisture addition was calculated by adding the amount of water and steam.

Table I-5. Target vs actual increase in conditioning temperature and conditioner added steam.

Treatments and Target Moisture[1]		Increase in conditioning temperature		Steam Addition	
		°F		%	
		Target[2]	Actual[3]	Target[4]	Actual[5]
0% Water, 0% Steam	Negative Control	0	11.13[D]	0	0.59[DE]
0% Water, 2% Steam	Treatment 1	50	51.52[B]	2	2.96[B]
1% Water, 1% Steam	Treatment 2	25	24.93[C]	1	0.99[D]
2% Water, 0% Steam	Treatment 3	0	11.57[D]	0	0.34[E]
2% Water, 2% Steam	Treatment 4	50	50.10[B]	2	2.15[C]
0% Water, 4% Steam	Positive Control	100	101.80[A]	4	4.08[A]
4% Water, 0% Steam	Positive Control 2	0	16.82[CD]	0	0.19[E]
	SEM[6]	-	2.25	-	0.13
	P-value	-	*<.0001*	-	*<.0001*

[1]The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2,4] Conditioning temperature was increased by 25°F for every 1% of target conditioner added steam.

[3] The actual increase in conditioning temperature was calculated by subtracting ambient temperature from the conditioning temperature.

[5]Moisture content was measured in accordance with AACC 44-19.01. Steam was added through conditioner and the amount of steam added was calculated by weight percentage.

[6] SEM= Standard error of the mean for moisture effect (n=3).

Table I-6. Total moisture content of each feed form between treatments.

Treatments and Target Moisture[1]		Mixer Mash[2]	Hot Conditioned Mash[3]	Hot Pellet[4]	Cooled Pellet[5]
		Moisture, %[6]			
0% Water, 0% Steam	Negative Control	12.73 C	13.32 D	13.05 D	11.74 B
0% Water, 2% Steam	Treatment 1	12.73 C	15.69 B	15.04 ABC	12.77 AB
1% Water, 1% Steam	Treatment 2	13.32 C	14.32 C	14.16 CD	12.26 AB
2% Water, 0% Steam	Treatment 3	14.24 B	14.58 C	14.28 BCD	12.4 AB
2% Water, 2% Steam	Treatment 4	14.24 B	16.40 AB	15.84 A	13.44 A
0% Water, 4% Steam	Positive Control	12.73 C	16.81 A	16.39 A	13.06 AB
4% Water, 0% Steam	Positive Control 2	15.86 A	16.05 AB	15.69 AB	13.64 A
	SEM[7]	0.16	0.19	0.30	0.35
	P-value	*<.0001*			

[A,B,C,D] Means within a column with different superscripts differ significantly (P < 0.0001).

[1] The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2] Mixer Mash was collected as the mash was coming out of the mixer and was froze immediately for further laboratory analysis.

[3] Hot conditioned Mash was collected as the mash was coming out of the conditioner and was froze immediately for further laboratory analysis.

[4] Hot Pellets were collected as the pellets were coming out of the pellet mill and was froze immediately for further laboratory analysis.

[5] Cooled Pellets were collected after the hot pellets were completely cooled down and stored in a cool environment.

[6] Moisture content was measured in accordance with AACC 44-19.01.

[7] SEM= Standard error of the mean for moisture effect (n=3).

Table I-7. Treatment effects on hot conditioned mash temperature, hot pellet temperature and difference in temperature between hot pellets and hot conditioned mash (ΔT).

Treatments and Target Moisture[1]		Hot Conditioned Mash Temperature[2]	Hot Pellet Temperature[3]	ΔT
			°F	
0% Water, 0% Steam	Negative Control	75.2 ^{D}	130.02 ^{D}	54.82 ^{A}
0% Water, 2% Steam	Treatment 1	115.59 ^{B}	150.41 ^{B}	34.82 ^{D}
1% Water, 1% Steam	Treatment 2	89.5 ^{C}	136.58 ^{CD}	47.08 ^{BC}
2% Water, 0% Steam	Treatment 3	76.83 ^{D}	128.84 ^{D}	52.01 ^{AB}
2% Water, 2% Steam	Treatment 4	115.36 ^{B}	145.35 ^{BC}	30.00 ^{D}
0% Water, 4% Steam	Positive Control	165.87 ^{A}	172.82 ^{A}	6.96 ^{E}
4% Water, 0% Steam	Positive Control 2	82.25 ^{CD}	125.88 ^{D}	43.62 ^{C}
	SEM[4]	2.52	2.39	1.36
	P-value		*<.0001*	

^{A,B,C,D} Means within a column with different superscripts differ significantly ($P < 0.0001$).

[1] The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2] Hot conditioned mash temperature was determined based on target moisture %, where every 25°F is equivalent to 1% steam. The hot conditioned mash temperature was collected from Repete automation system.

[3] Hot pellet temperature was collected by taking temperature of hot pellets in an insulated Thermos.

[4] SEM= Standard error of the mean for moisture effect (n=3).

Table I-8. Treatments effects on pellet durability index (PDI) as determined by the Holmen method for 30 seconds of testing and pellet mill energy consumption (PMEC).

Treatments and Target Moisture[1]		PDI[2]	PMEC
		%	kWh/ton
0% Water, 0% Steam	Negative Control	5.37 ^{D}	18.44 ^{A}
0% Water, 2% Steam	Treatment 1	22.67 ^{C}	12.55 ^{C}
1% Water, 1% Steam	Treatment 2	17.54 ^{C}	17.13 ^{AB}
2% Water, 0% Steam	Treatment 3	19.74 ^{C}	17.55 ^{AB}
2% Water, 2% Steam	Treatment 4	35.93 ^{B}	11.97 ^{C}
0% Water, 4% Steam	Positive Control	57.86 ^{A}	9.39 ^{D}
4% Water, 0% Steam	Positive Control 2	27.91 ^{B}	15.53 ^{B}
	SEM[3]	2.17	0.51
	P-value	<.0001	<.0001

^{A,B,C,D} Means within a column with different superscripts differ significantly (P < 0.0001).

[1] The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2] Pellet durability index (PDI) was measured in accordance with ASAE S269.4.

[3] SEM= Standard error of the mean for moisture effect (n=3).

Table I-9. Main effects on pellet durability index (PDI) as determined by the Holmen method for 30 seconds of testing and pellet mill energy consumption (PMEC).

Treatments and Target Moisture[1]		PDI[2]	PMEC
		%	kWh/ton
0% Water, 0% Steam	Negative Control	5.37 ^{C}	18.44 ^{A}
0% Water, 2% Steam	Treatment 1	22.67 ^{B}	12.55 ^{B}
2% Water, 0% Steam	Treatment 3	19.74 ^{B}	17.55 ^{A}
2% Water, 2% Steam	Treatment 4	35.93 ^{A}	11.97 ^{B}
	SEM[3]	2.02	0.48
	P-value	<.0001	<.0001

^{A,B,C,D} Means within a column with different superscripts differ significantly ($P < 0.0001$).

[1] The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4).

[2] Pellet durability index (PDI) was measured in accordance with ASAE S269.4.

[3] SEM= Standard error of the mean for moisture effect (n=3).

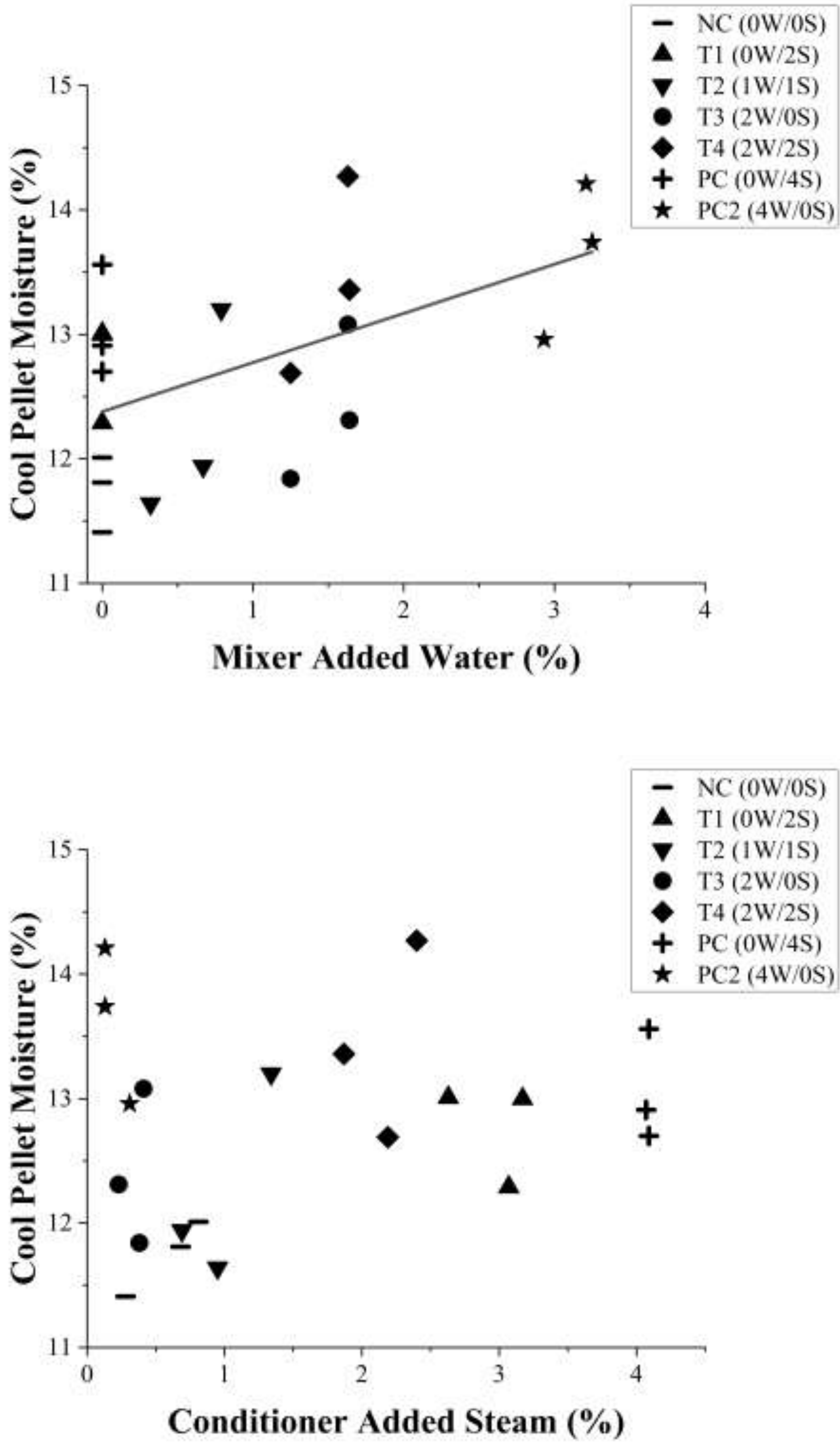


Figure I-1. Cool pellet moisture vs a) mixer added water (p = .0113, R^2=0.29) and b) conditioner added steam (p = .29, R^2=0.06).

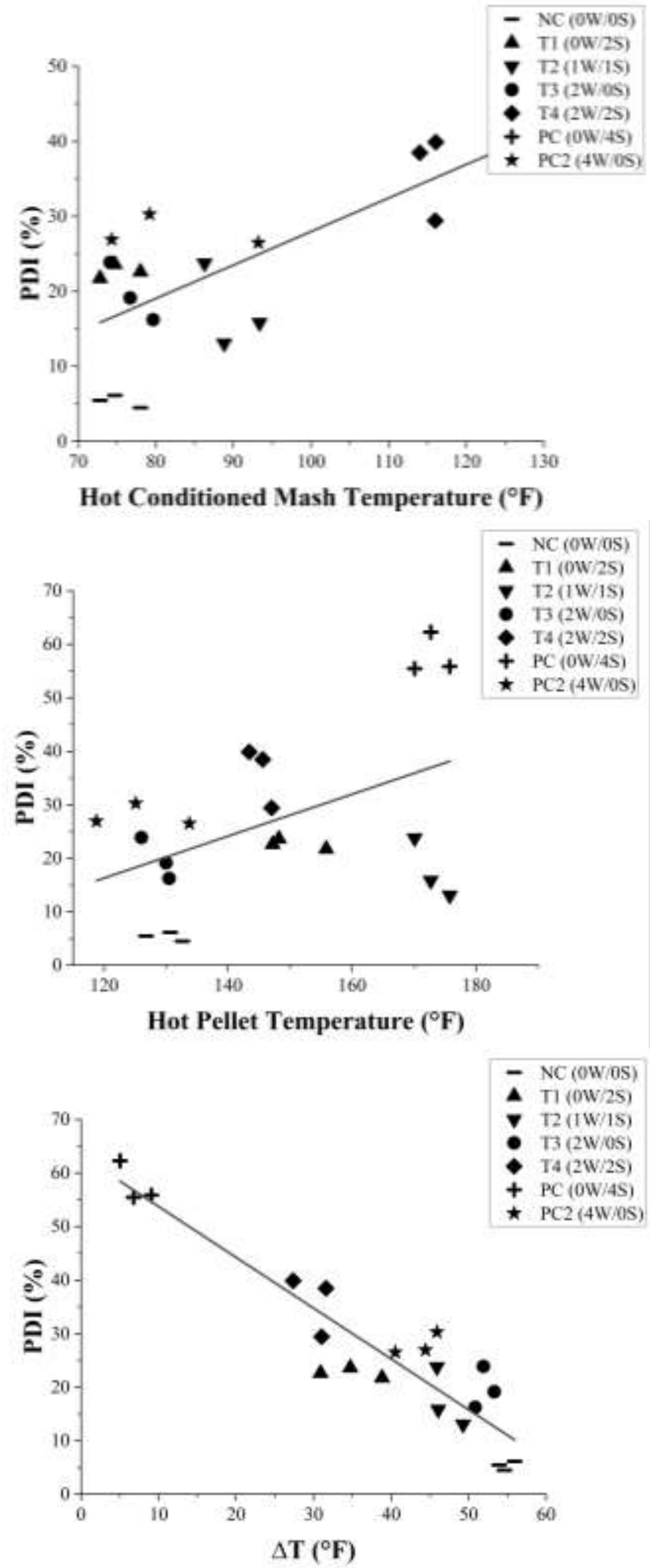
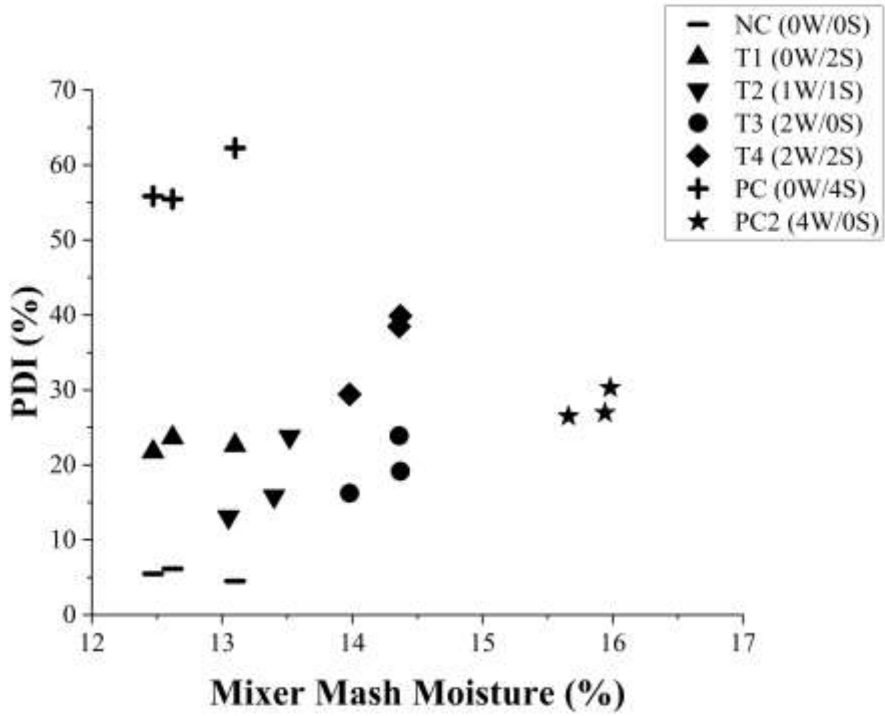


Figure I-2. Pellet durability index (PDI) vs a) hot conditioned mash temperature (p <.0001, R^2=0.75) b) hot pellet temperature (p <.0001, R^2=0.57) and c) change in temperature between hot pellet and hot conditioned mash (ΔT) (p <.0001, R^2=0.86).



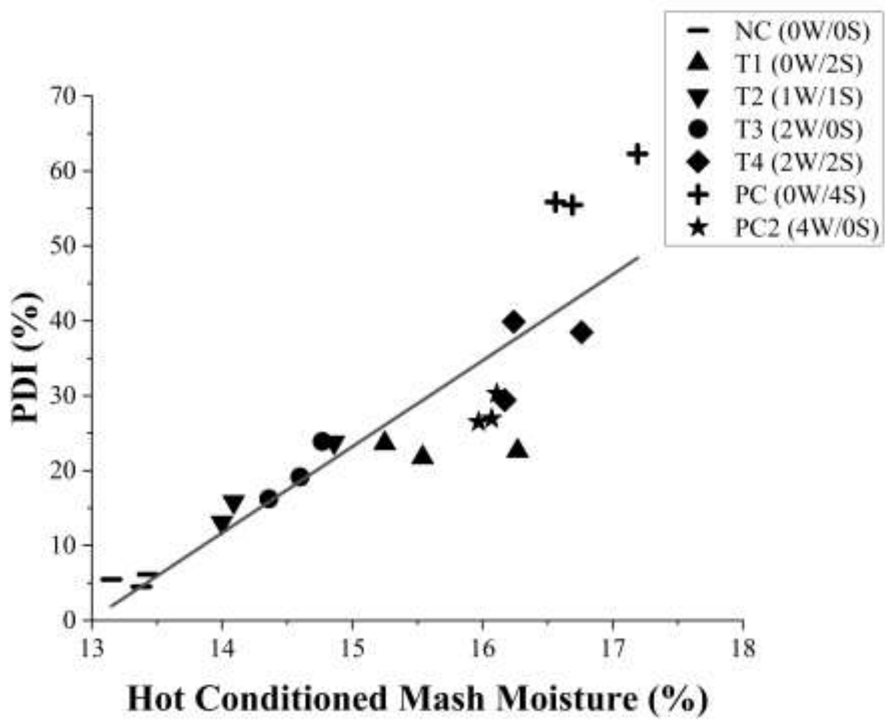


Figure I-3. Pellet durability index (PDI) vs a) mixer mash moisture (p = 0.87, R^2=0.001) and b) hot conditioned mash moisture (p <.0001, R^2=0.78).

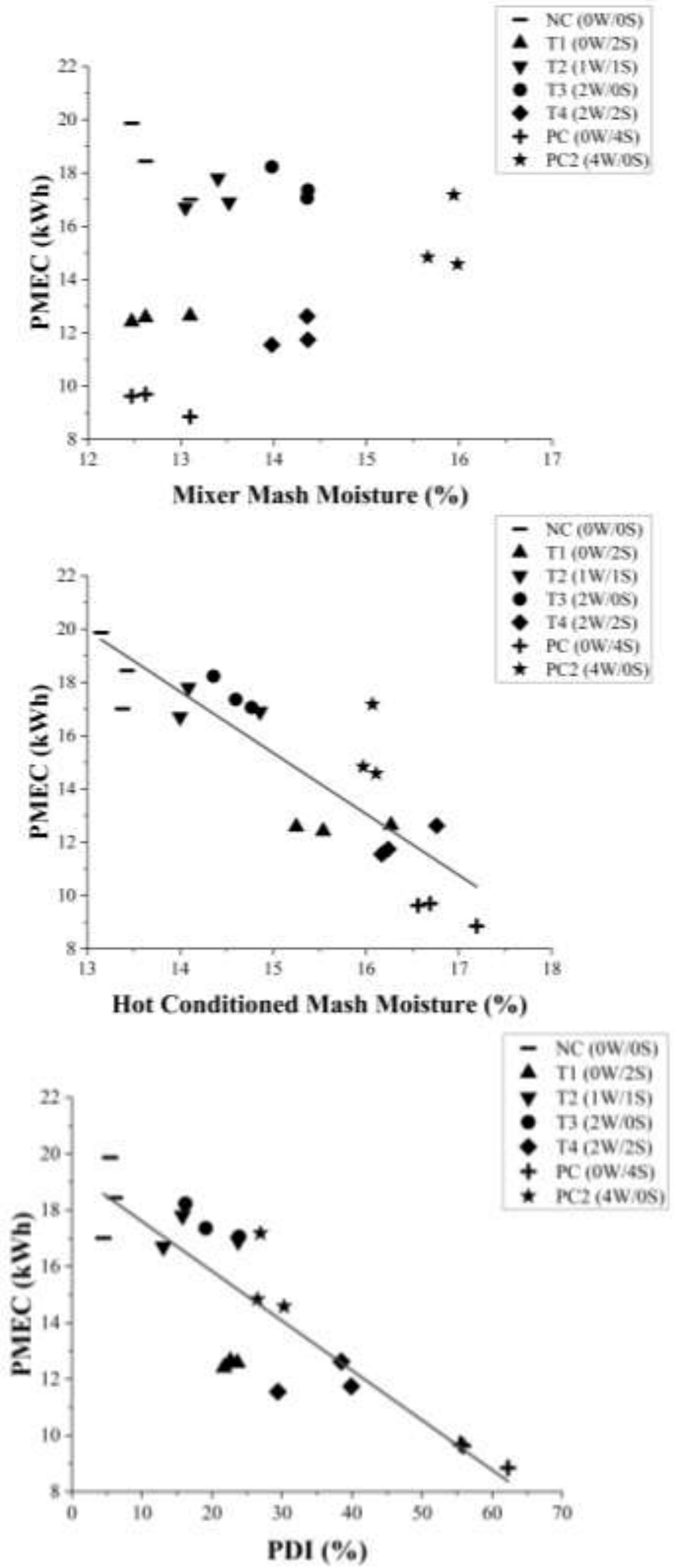
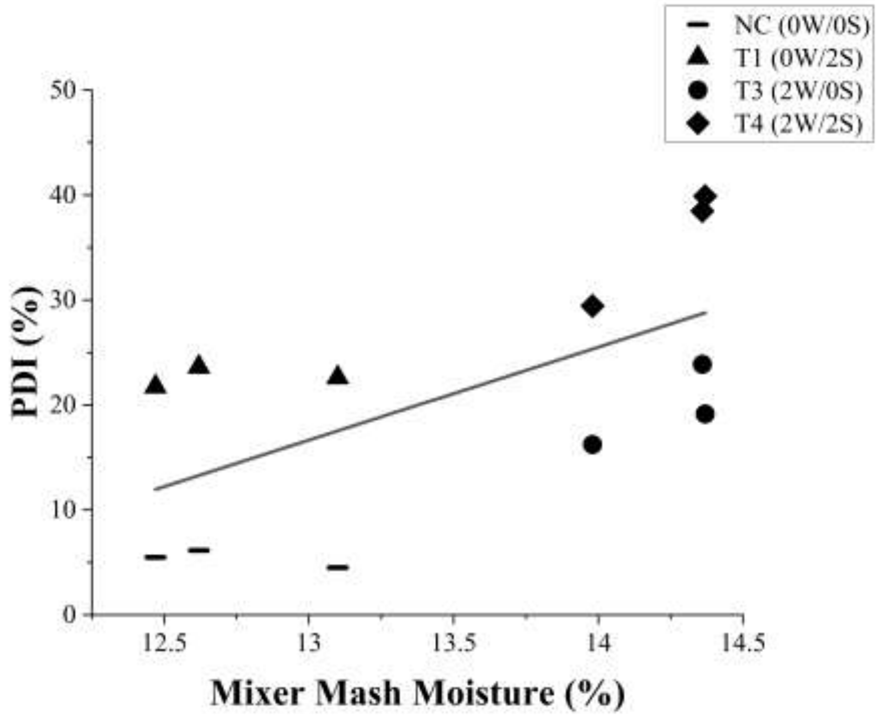


Figure I-4. Pellet mill energy consumption (PMEC) vs a) mixer mash moisture (p 0.46, R^2=0.03) b) hot conditioned mash moisture (p <.0001, R^2=0.74) and c) pellet durability index (PDI) (p <.0001, R^2=0.74).



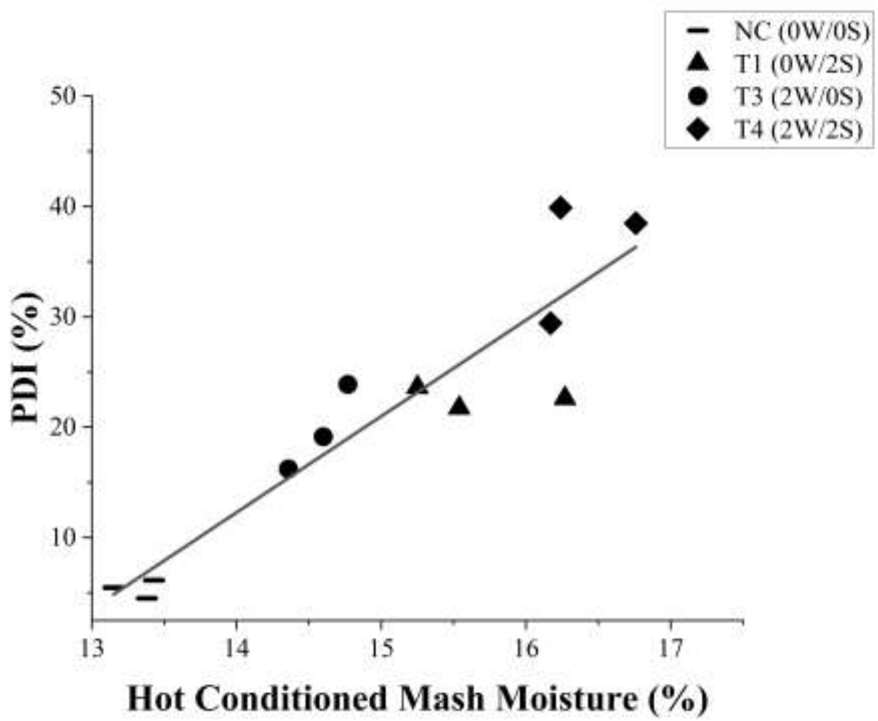


Figure I-5. Main effect of pellet durability index (PDI) vs a) mixer mash moisture (p = .0308, R^2=0.39) and b) hot conditioned mash moisture (p <.0001, R^2=0.86).

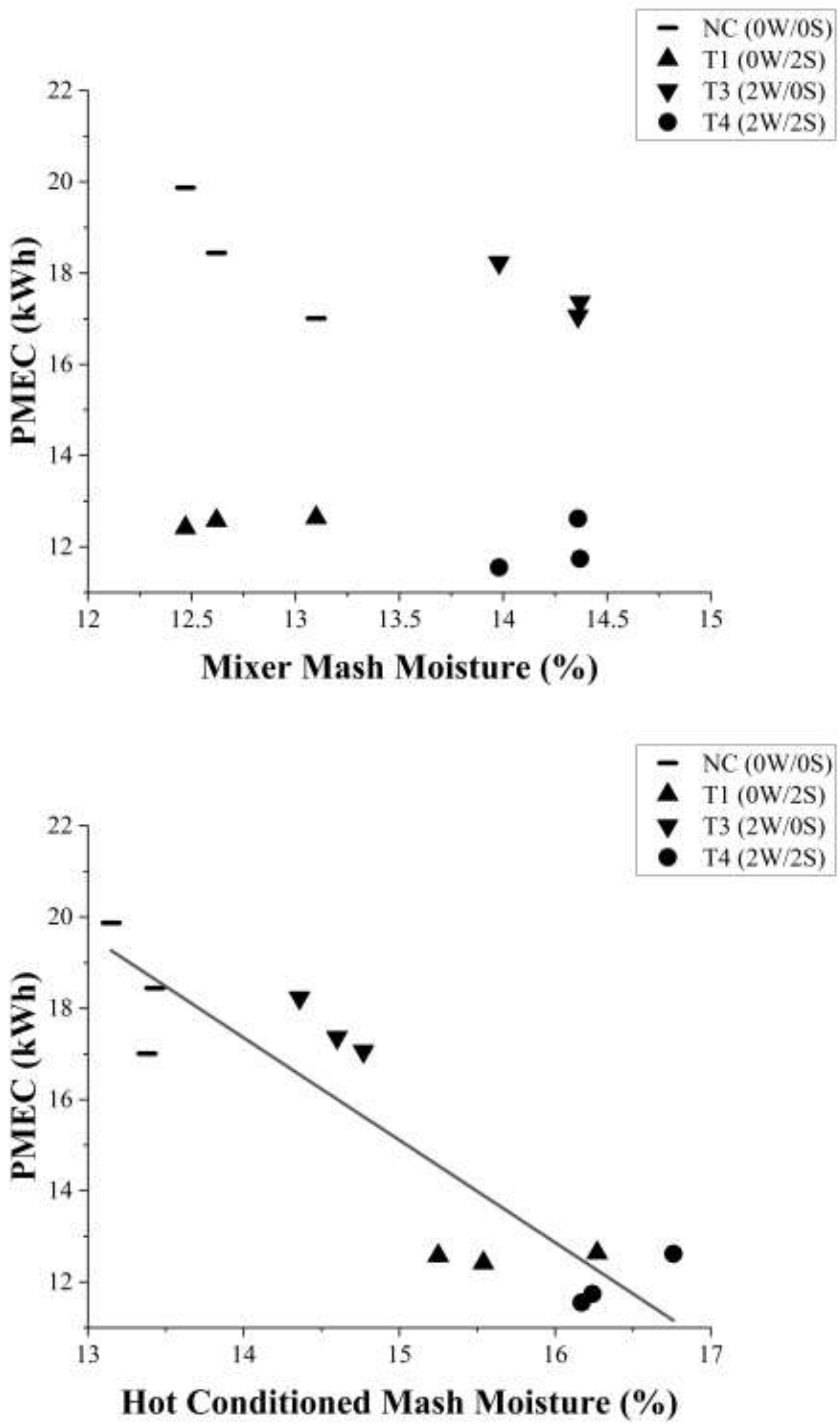


Figure I-6. Main effect of pellet mill energy consumption (PMEC) vs a) mixer mash moisture (p = .6382, R^2=0.02) and b) hot conditioned mash moisture (p <.0001, R^2=0.81).

CHAPTER 2

Moisture retention across particle sizes in conditioned mash feed.

ABSTRACT

Conditioning has been determined to be an important factor in influencing pellet quality. However, not much work has been done on evaluating the moisture retention properties across particle sizes in mash feed. Diets containing varying moisture contents from mixer added water and conditioner added steam were pelleted. The impacts of moisture levels on moisture retention across particle sizes using different moisture addition methods were assessed. This study also evaluated the amount of steam retention in conditioned mash feed at different base moistures. The capability of hot conditioned mash to hold moisture from the steam decreased as mixer mash moisture increased ($p < .0001$; Table II-4). The moisture retention across mash particles of rations amended with 2% and 4% mixer added water was significantly different compared to rations amended with 0% and 1% mixer added water ($p < .05$; Table II-7; Table II-8). When additional moisture level was ≥ 2%, the moisture distribution was uneven in feed mash particles. Increasing moisture addition by 4% through conditioner added steam, mixer added water or a combination of both significantly influenced the moisture retention across different particle sizes in hot conditioned mash ($p < .0001$; Table II-8), indicating that the feed particle size had an effect on the moisture gradient during conditioning.

Key words: Conditioning, Water, Steam, Moisture retention, Particle sizes

DESCRIPTION OF PROBLEM

The pelleting process involves conditioning, pelleting, and cooling. Conditioning has been determined to be the second most important factor in influencing pellet quality (Behnke, 1996). During conditioning, steam and feed particles are mixed in the conditioning chamber. Conditioning involves adding moisture and heat, via steam, to the particles to facilitate the compaction of the mash during the pelleting process (Thomas *et al.*, 1997). Moisture added through conditioner steam lubricates the surface of feed particles, which reduces friction as the particles move through the pellet die. The gluing effect of water through “liquid necking” may increase when the particle sizes are reduced, and the surface area of particles increase (Thomas & Van Der Poel, 1996). At the pelleting stage, the blended ingredients are pressed into cylindrical pellets by adhering the individual particles of the blended ingredients with one another. After pelleting, excess water and heat are removed from the pellets during the process of cooling (Thomas & Van Der Poel, 1996).

Researchers have shown that the practice of moisture addition through water or steam could improve PDI (Skoch *et al.*, 1981; Fairchild & Greer, 1999; Moritz *et al.*, 2002; Moritz *et al.*, 2003; Pope & Fahrenholz, 2020). The binding of particles was caused by water absorption in particles and the hydrogen bonds between them (Rumpf, 1958; Friedrich, 1977). After the water was diffused into the feed particles, the feed particles were hydrated and softened (Buchanan & Moritz, 2009; Thomas & van der Poel, 2020). During conditioning, both heat and water diffused at a different rate. According to Bouvier & Campanella (2014), water diffused at a rate of 100 times slower to hydrate particles compared to heat diffusion. As a result, wet steam will have an uneven moisture distribution across feed mash particles compared to saturated steam.

Hemmingsen *et al.* (2008b) stated that the particle sizes and chemical composition of particles will affect the moisture gradient during conditioning, pelleting, and cooling.

However, feed manufacturers do not necessarily control the particle size of every ingredient. Particle size distribution of ingredients like corn and wheat may be controlled by grinding, while protein meals like soybean meal and byproduct meals are pre-ground by the supplier. This results in an unequal particle size distribution across mash feed. Larger particle sizes may require a longer time to be fully hydrated as opposed to smaller particle sizes. The capability of different ingredients to absorb moisture can be affected by the differences in chemical composition and physical conditions on the surface of the particles (Hemmingsen *et al.*, 2008b; Bouvier & Campanella, 2014).

Yet not much work has been done on evaluating the moisture retention properties across particle sizes in mash feed, nor on the impact of moisture levels on moisture retention across particle sizes using different moisture addition methods. Mixer added water and conditioner added steam were the two sources of moisture addition methods considered. This study also evaluated the amount of steam retention in conditioned mash feed at different base moistures.

MATERIALS AND METHODS

The experiment was conducted by pelleting twelve 340 kg batches of a broiler starter diet (Table II-1) to determine the effect of added moisture on pellet quality using different moisture addition methods. Treatments were arranged as a 2x3 factorials utilizing two levels of added moisture (2% vs 4%) and three moisture addition methods (mixer added water, conditioner added steam and both). Treatments consisted of a negative control and two positive controls with intermediate treatments in between (Table II-2). A mash diet not subjected to mixer added water nor conditioner added steam was dry pelleted to serve as a negative control (NC). 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).

There were three manufacturing runs per treatment which represented three replicates, and the run order was completely randomized. Each run consisted of two batches of 454kg, one batch of 680kg, and one batch of 907kg. Batches consisting of 454kg of feed were pelleted at one conditioning temperature, batches of 680kg of feed were pelleted at two conditioning temperatures and batches of 907kg were pelleted at three conditioning temperatures. The pellet mill and conditioner were completely shut off between each run.

All feed was manufactured following the guidelines for Current Good Manufacturing Practice (CGPM). The research was conducted at the North Carolina State University Feed Mill Educational Unit utilizing a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) fitted with a 4.4 x 28.6 mm die and a conditioner feeder (Model C18LL4/F6 California Pellet Mill Co., Crawfordsville, IN). All corn was ground utilizing a hammermill (Model 1522, Roskamp Champion, Waterloo, IA.) equipped with a 2.4 mm screen on the impact side and a 3.2 mm screen on the release side.

Individual batches were sourced from a common basal to reduce variation in ingredient composition between batches. 2268 kg of common basal devoid of water was batched utilizing a twin shaft counterpoise ribbon mixer (Model TRDB126060, Hayes and Stolz, Fort Worth, TX) for 180 s of dry mixing time, followed by an additional 90 s of wet mixing time. The basal diet included phytase, xylanase, and fat. The individual batches were then blended utilizing the same equipment and methodology. For batches amended with water, the water was added after 180s of dry mixing, but before 90s of wet mixing. Upon mixer discharge, five composite mixer samples were collected in sealed sample bags, three of which were immediately frozen for subsequent moisture content analysis. The temperature of mixer mash feed was collected to determine the target conditioning temperature.

For batches amended with steam, conditioning temperature was increased by 25°F for every 1% of target conditioner added steam (Table II-4). The actual increase in conditioning temperature was calculated by subtracting the temperature of mixer mash feed from the conditioning temperature. The pellet mill die was warmed with 455kg of feed before proceeding with the experimental batches. All batches were conditioned for 30s and pelleted at a production rate of 771kg/hr. The average steam pressure was 255kPa.

After reaching the appropriate mash conditioning temperature, six hot conditioned mash and hot pellet samples were collected, sealed, and frozen immediately for moisture analysis over a five-minute period of pelleting. The moisture content was measured in accordance with AACC 44-19.01. The hot conditioned mash was collected as feed transitioned between the conditioner and the pellet mill die. For batch runs consisting of more than one conditioning temperature, the steam valve leading to the conditioning chamber was further opened to increase the conditioning temperature, and the sample collection process was repeated once reaching the appropriate conditioning temperature.

The particle size distribution was determined by the American Society of Agricultural and Biological Engineers (ASAE) method S319.4 with 0.5g of silicon dioxide dispersion agent and sieve agitators. A 100g sample was sifted through the 14-sieve stack with US sieve numbers 4, 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270 and a pan on a Ro-Tap shaker for 10 minutes. The weight of particles retained on each screen size was then recorded to determine the geometric mean diameter by mass (d_{gw}) and the geometric standard deviation of particle diameter by mass (S_{gw}).

The particle sizes distribution of corn, soybean meal, and hot conditioned mash feed of all treatments were analyzed (Table II-5, Table II-6).

To measure moisture retention across particles in mixer mash feed and hot conditioned mash feed, the mash feed was separated into four fractions according to the particle sizes. Sieves were set up by placing one ball and one carnucle in US sieve number 20, one ball and two carnucles in sieve number 40, and no ball and carnucle in sieve number 16 or the pan. A 50g frozen sample was sifted through this 3-sieve plus pan stack by shaking the stack side to side for 90 seconds. The samples on each sieve represent the fraction of particle sizes >1180 microns, 1180 - 850 microns, 850 – 425 microns, and < 425 microns (Supplemental Figure II-i). The moisture of the feed samples from each fraction were then analyzed using AACC 44-19.01.

STATISTICAL ANALYSIS

ANOVA from the Fit Model platform of JMP Pro 15.2 (Statistical Analysis, SAS Institute, Cary, NC) was employed and the means were separated using the LSMeans procedure of JMP. The means were considered statistically significant at $p \leq 0.05$. Significant differences were separated utilizing Tukey's HSD test.

RESULTS AND DISCUSSION

Differences between target vs actual moisture contents were observed (Table II-3). For mixer added water, the actual water level reported was lower than targeted (Table II-3). This may have been due to water evaporation occurring the intensive action inside the counterpoise mixer during mixing. Additionally, steam addition was observed for the treatment with 0% target steam moisture, possibly due to residual moisture in the conditioner. While the conditioning temperature was estimated to increase by 25°F for every 1% of target conditioner added steam, an interesting trend was observed in that the moisture retention from steam decreased when the mash feed was amended with more water. As the mixer mash moisture increased, the observed moisture addition due to steam decreased despite the increase in conditioning temperature being constant ($p < .0001$; Table II-4). When mixer mash was amended with 0% mixer added water, an increase in 51.52°F conditioning temperature resulted in 2.96% moisture addition. In contrast, when mixer mash was amended with 2% mixer added water, an increase in 50.10°F conditioning temperature resulted in 2.15% moisture addition ($p < .0001$; Table II-4). An increase in 11.13°F conditioning temperature resulted in 0.59% moisture addition when the mixer mash was not subjected to mixer added water, as opposed to 0.34% moisture addition when mixer mash was amended with 2% mixer added water and 0.19% moisture addition when mixer mash was amended with 4% mixer added water. As mixer added water increased, the capability of hot conditioned mash to hold moisture from the steam decreased.

The d_{gw} of corn was 368microns, while the d_{gw} of soybean meal was 1109 microns (Table II-5). There was no difference in particle sizes of hot conditioned mash across treatments ($p = .13$; Table II-6),indicating that the particle size of hot conditioned mash was equally distributed across treatments.

The moisture retention across mixer mash particles behaved differently in rations amended with 2% and 4% mixer added water in comparison to rations amended with 0% and 1% (Table II-7). When the diet was amended with 2% mixer added water, feed particles smaller than 425 microns had significantly lower moisture content at 12.15% as opposed to feed particles of 425 - 850 microns, 850 -1180 microns and >1180 microns with moisture contents of 13.35%, 14.12% and 14.21%, respectively ($p = .0011$; Table II-7). The effect was more apparent when the diet was amended with 4% mixer added water. Feed particles of 425 microns had the lowest moisture content at 13.04%, followed by feed particles of 425 – 850 microns with 14.64% moisture, while feed particles of 850-1180 microns and >1180 microns had the highest moisture contents of 16.16% and 16.54%, respectively (*$p < .0001$*; Table II-7). For diets amended with 0% or 1% mixer added water, there were no significant differences in moisture contents across particle sizes.

A similar trend was observed for the moisture retention across different particle sizes in hot conditioned mash. The moisture retention in hot conditioned mash particles of rations amended with 2% and 4% conditioner added steam behaved differently compared to rations amended with 0% and 1% conditioner added steam (Table II-8). For diets amended with either 2% mixer added water or conditioner added steam, the feed particles smaller than 425 microns had a lower moisture level compared to feed particles of 425 - 850 microns, 850 -1180 microns and >1180 microns. Increasing the moisture addition to 4% through conditioner added steam, mixer added water or a combination of both significantly influenced the moisture retention across different particle sizes in hot conditioned mash ($p < .0001$; Table II-8). Feed particles smaller than 425 microns had the lowest moisture level, followed by feed particles of 425 - 850 microns, 850 - 1180 microns, and feed particles with >1180 micron had the highest moisture level ($p < .0001$; Table II-8). Overall, when additional moisture level was ≥ 2%, the moisture distribution was uneven in feed mash particles, indicating that the feed particle sizes had an effect on the moisture gradient during conditioning. At additional moisture levels ≥ 2%, coarser particles had higher moisture retention capacity. According to Hemmingsen *et al.*, the differences in water content across particles sizes could be explained by water mobility in grains (Hemmingsen *et al.*, 2008b). Water adsorbed on the surface of grain particles had more mobility and was less hindered compared to water bound within capillaries in the grains. As a result, the water adsorbed on the surface was more available for chemical reactions. The gluing effect of water through "liquid necking" may also increase when the particle size is reduced (Thomas & Van Der Poel, 1996). As finer particles have greater surface area-to-mass ratio, more surface bound water is available to migrate to the surroundings (Al-Muhtaseb *et al.*, 2002). This explained why it is easier for finer particles to lose moisture. Another reason could be due to the capacity limit of water bound within capillaries for smaller particle sizes. Finer particle sizes have smaller volume, thus the moisture retention capacity may be limited when moisture increased (≥2%) as opposed to coarser particle size. In addition, soybean meal used in this study had bigger particle sizes at 1109 Dgw as opposed to corn at 368 Dgw. Since the diet consisted of 58.6% of corn and 36.8% of SBM, corn might represent most of the finer particle sizes sample while SBM might represent most of the larger particle sizes sample. An uneven distribution of feed ingredients might have interfered the results. Besides, during the mixing process, fat was added in the basal diet before moisture was added to avoid variations between treatments. Finer particle sizes might have been coated with more fat due to the larger surface area. Hence, this may also explain why less moisture was retained in finer particle sizes due to the hydrophobic property from fat.

CONCLUSION AND APPLICATION

- This study showed that as mixer added water increased, the capability of hot conditioned mash to hold moisture from the steam decreased. Finer particle sizes had lower moisture content as opposed to bigger particle sizes.
- However, more studies need to be conducted to further confirm the theory.

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TABLES AND FIGURES

Table II-1. Ingredient composition of broiler starter diets containing phytase and xylanase.

Ingredients	(%)
Corn	58.62
SBM (48%)	34.77
Poultry by-product Meal	2.26
Poultry fat	2.00
Limestone	0.84
Salt	0.50
DL-Methionine[a]	0.24
Choline chloride (60%)	0.20
Trace mineral premix[1]	0.20
Dicalcium phosphate (18.5% P)	0.14
L-Lysine[b]	0.08
Vitamin premix[2,c]	0.05
Selenium premix[3] (0.06%)	0.05
Phytase[4]	0.02
L-Threonine[d]	0.02
Xylanase[5]	0.01
Calculated nutrients	
Protein	22.95
Calcium	0.85
Available phosphorous	0.43
Total lysine	1.29
ME, kcal/g	3.00

[1] Trace mineral premix provided the following per kg of diet: Mn, 120 mg; Zn, 120 mg; Fe, 80 mg; Cu, 10 mg; I, 2.5 mg; Co, 1 mg.
[2] Vitamin premix provided the following per kg of diet: vitamin A, 6600 IU; vitamin D3, 1980 IU; vitamin E, 33 IU; vitamin B12, 0.02 mg; biotin, 0.13 mg, menadione, 2 mg; thiamine, 2 mg; riboflavin, 6.6 mg; pantothenic acid, 11 mg; vitamin B6, 4 mg; niacin, 55 mg; folic acid, 1.1 mg.
[3] Selenium premix provided Se at 0.3 mg/kg of feed.
[4] Quantum Blue 5G was added to provide 1000 FTU/kg of feed.
[5] Econase XT was added to provide 16,000 BXU/kg of feed.
[a,b,c,d] Dl-Methionine, L-Lysine, Vitamin premix, and L-Threonine were donated by Evonik, Ajinomoto, DSM, and Ajinomoto.

Table II-2. Treatments design.

Treatments	Mixer Added Water	Conditioner Added Steam	Total Moisture Addition
	Moisture, %		
Negative Control	0	0	0
Treatment 1	0	2	2
Treatment 2	1	1	2
Treatment 3	2	0	2
Treatment 4	2	2	4
Positive Control	0	4	4
Positive Control 2	4	0	4

Table II-3. Target vs actual moisture addition through water and steam.

Treatments	Water Addition[1]		Steam Addition [2]		Total Moisture Addition[3]	
	Moisture, %[1,2,3]					
	Target	Actual	Target	Actual	Target	Actual
Negative Control	0	0	0	0.59	0	0.59
Treatment 1	0	0	2	2.96	2	2.96
Treatment 2	1	0.59	1	0.99	2	1.58
Treatment 3	2	1.51	0	0.34	2	1.85
Treatment 4	2	1.51	2	2.15	4	3.66
Positive Control	0	0	4	4.08	4	4.08
Positive Control 2	4	3.13	0	0.19	4	3.32

[1,2,3] Moisture content was measured in accordance with AACC 44-19.01.
[1] Water was added through mixer and the amount of water added was calculated by weight percentage.
[2] Steam was added through conditioner and the amount of steam added was calculated by weight percentage.
[3] Total moisture addition was calculated by adding the amount of water and steam.

Table II-4. Target vs actual increase in conditioning temperature and conditioner added steam.

Treatments and Target Moisture[1]		Increase in conditioning temperature		Steam Addition	
		°F		%	
		Target[2]	Actual[3]	Target[4]	Actual[5]
0% Water, 0% Steam	Negative Control	0	11.13[D]	0	0.59[DE]
0% Water, 2% Steam	Treatment 1	50	51.52[B]	2	2.96[B]
1% Water, 1% Steam	Treatment 2	25	24.93[C]	1	0.99[D]
2% Water, 0% Steam	Treatment 3	0	11.57[D]	0	0.34[E]
2% Water, 2% Steam	Treatment 4	50	50.10[B]	2	2.15[C]
0% Water, 4% Steam	Positive Control	100	101.80[A]	4	4.08[A]
4% Water, 0% Steam	Positive Control 2	0	16.82[CD]	0	0.19[E]
	SEM[6]	-	2.25	-	0.13
	P-value	-	*<.0001*	-	*<.0001*

[1]The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).
[2,4] Conditioning temperature was increased by 25°F for every 1% of target conditioner added steam.
[3] The actual increase in conditioning temperature was calculated by subtracting ambient temperature from the conditioning temperature.
[5]Moisture content was measured in accordance with AACC 44-19.01. Steam was added through conditioner and the amount of steam added was calculated by weight percentage.
[6] SEM= Standard error of the mean for moisture effect (n=3).

Table II-5. Particle size of corn and soybean meal were determined by Ro-tap® sieve shaker using 14 sieves (#4, #6, #8, #12, #16, #20, #30, #40, #50, #70, #100, #140, #200 and #270).

	Particle Size[1]	Standard Deviation
	Dgw	Sgw
Corn	368	3.72
Soybean Meal	1109	2

[1]Particle Size was measured in accordance with ASAE/ANSI S319.4 (n=3).

Table II-6. Particle size of hot conditioned mash was determined by Ro-tap® sieve shaker using 14 sieves (#4, #6, #8, #12, #16, #20, #30, #40, #50, #70, #100, #140, #200 and #270).

Treatments and Target Moisture[1]		Particle Size[2]	Standard Deviation
		Dgw	Sgw
0% Water, 0% Steam	Negative Control	532.33 [A]	2.87
0% Water, 2% Steam	Treatment 1	546.67 [A]	2.68
1% Water, 1% Steam	Treatment 2	592.67 [A]	2.69
2% Water, 0% Steam	Treatment 3	576.67 [A]	2.75
2% Water, 2% Steam	Treatment 4	573.33 [A]	2.70
0% Water, 4% Steam	Positive Control	587.00 [A]	2.85
4% Water, 0% Steam	Positive Control 2	587.67 [A]	2.72
	Mean of Y	570.90	
	SEM[3]	16.08	
	R-square[4]	0.46	
	P-value	*0.13*	

[A,B] Means within a column with different superscripts differ significantly ($P < 0.0001$).
[1]The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).
[2]Particle Size was measured in accordance with ASAE/ANSI S319.4.
[3]SEM= Standard error of the mean for moisture effect (n=3).
[4]R-square= Coefficient of determination

Table II-7. Moisture content of mixer mash in different particle sizes.

Treatments and Target Moisture[1]		>1180 Micron	1180-850 Micron	850-425 Micron	<425 Micron	SEM	*P-value*
		Moisture %[2]					
0% Water, 0% Steam	Negative Control	12.32^{A}	12.53^{A}	12.28^{A}	11.27^{A}	0.35	*.1607*
0% Water, 2% Steam	Treatment 1	12.32^{A}	12.53^{A}	12.28^{A}	11.27^{A}	0.35	*.1607*
1% Water, 1% Steam	Treatment 2	13.71^{A}	13.75^{A}	13.16^{A}	11.98^{A}	0.41	*.0782*
2% Water, 0% Steam	Treatment 3	14.21^{A}	14.12^{A}	13.35^{A}	12.15^{B}	0.23	*.0011*
2% Water, 2% Steam	Treatment 4	14.21^{A}	14.12^{A}	13.35^{A}	12.15^{B}	0.23	*.0011*
0% Water, 4% Steam	Positive Control	12.32^{A}	12.53^{A}	12.28^{A}	11.27^{A}	0.35	*.1607*
4% Water, 0% Steam	Positive Control 2	16.54^{A}	16.16^{A}	14.64^{B}	13.04^{C}	0.19	<*.0001*

^{A,B} Means within a row with different superscripts differ significantly ($P < 0.0001$).

[1]The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2] Moisture content was measured in accordance with AACC 44-19.01.

[3] SEM= Standard error of the mean for moisture effect (n=3).

Table II-8. Moisture content of hot conditioned mash in different particle sizes.

Treatments and Target Moisture[1]		>1180 Micron	1180-850 Micron	850-425 Micron	<425 Micron	SEM	*P-value*
		Moisture %[2]					
0% Water, 0% Steam	Negative Control	12.76[A]	12.67[A]	12.80[A]	12.28[A]	0.20	*.3197*
0% Water, 2% Steam	Treatment 1	16.12[A]	15.51[A]	15.11[A]	13.45[B]	0.31	*.0016*
1% Water, 1% Steam	Treatment 2	14.66[A]	14.20[A]	14.01[A]	12.85[A]	0.53	*.1774*
2% Water, 0% Steam	Treatment 3	14.88[A]	14.56[A]	14.21[A]	12.85[B]	0.18	*.0002*
2% Water, 2% Steam	Treatment 4	17.27[A]	16.48[B]	15.68[C]	14.13[D]	0.14	<*.0001*
0% Water, 4% Steam	Positive Control	18.60[A]	16.98[B]	15.79[C]	14.19[D]	0.10	<*.0001*
4% Water, 0% Steam	Positive Control 2	16.75[A]	16.17[B]	15.53[C]	13.61[D]	0.11	<*.0001*

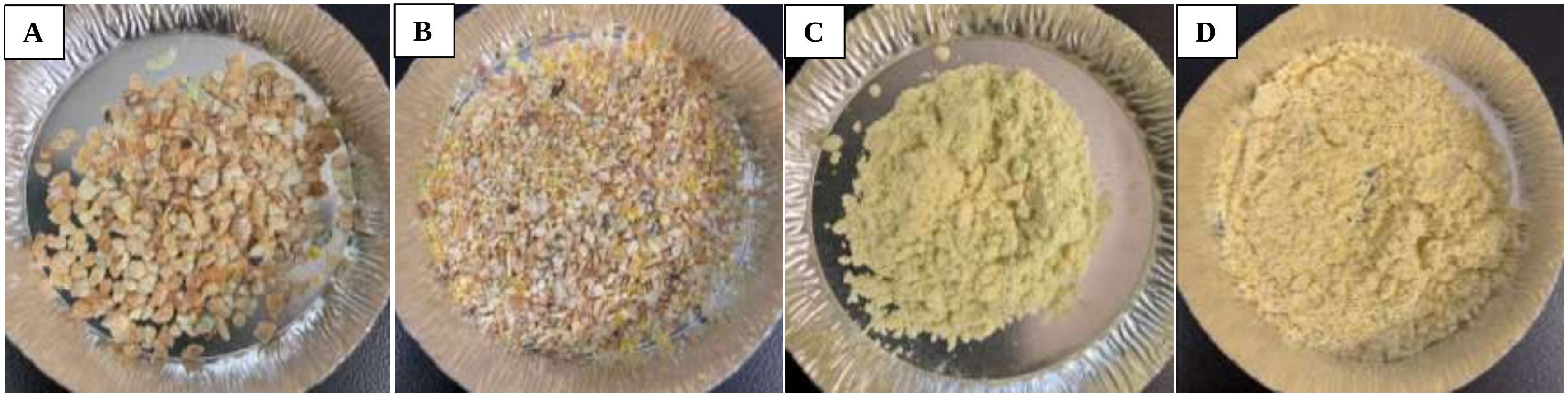
[A,B] Means within a row with different superscripts differ significantly ($P < 0.0001$).

[1]The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2] Moisture content was measured in accordance with AACC 44-19.01.

[3] SEM= Standard error of the mean for moisture effect (n=3).

Supplemental Figures



Supplemental Figure II-i. Samples represented the fraction of particle sizes A) >1180 microns, B) 1180 - 850 microns, C) 850 – 425 microns, and D) < 425 microns.

CHAPTER 3

The impact of moisture on the chemical and physiochemical properties of pellets.

ABSTRACT

Feed formulation has been shown to be the most influential factor affecting pellet quality. However, there was no consistent data on the relationship of moisture, starch gelatinization and pellet quality. In addition, the nature of glass-transition was discussed as it could be one of the most important parameters that affects the physical interactions of the polymer components in feed during the pelleting process. Yet, little work has done to investigate the role of glass transition in feed processing. In this experiment, diets containing varying moisture contents from mixer added water and conditioner added steam were pelleted. This study had evaluated: 1) The impact of moisture addition methods on starch gelatinization and glass transition temperatures (Tg). 2) Their effects on pellet quality in terms of durability and other processing parameters like change in temperature between hot pellet and hot conditioned mash (ΔT) and pellet mill energy consumption (PMEC). To further study the morphology of feed particles in pellets, Scanning Electron Microscopy (SEM) imaging was applied to generate optical observations to provide qualitative understanding of the microstructure of pellets. In short, starch gelatinization might have a negligible effect on pellet durability index (PDI). However, future work characterizing the interaction effect of heat and moisture addition on the degree of starch gelatinization and PDI are needed to confirm the theory. Tg could be an important factor in understanding the fundamental of pellet binding mechanism

Key words: Moisture, Water, Steam, Pelleting, PDI, Glass transition, Starch gelatinization

DESCRIPTION OF PROBLEM

Feed formulation has been shown to be the most influential factor affecting pellet quality, followed by mash conditioning, particle size, pellet mill die and throughput, and lastly pellet cooling (Behnke, 2001). Feed manufacturers constantly face a challenge that the nutritionists are striving for the maximum nutrition for animals at least cost when formulating feed, but feed manufacturers are constantly looking for ways to improve pellet quality. A variety of raw materials are used during feed manufacturing to produce compound feed. The feed ingredients contain nutrients that can be classified as starch, protein, fat, sugar, non-starch polysaccharides (NSP), inorganic matter and water. Over the years, researchers have grown interest on the effect of ingredients compositions on the physical quality of feed (Skoch *et al.*, 1981; Wood, 1987; Stevens, 1987; Thomas *et al.*, 1998; Briggs *et al.*, 1999a; Zimonja & Svihus, 2009; Buchanan & Moritz, 2009; Fahrenholz, 2012; Abdollahi *et al.*, 2013; Pope *et al.*, 2018). However, there was no consistent data on the relationship of moisture, starch gelatinization and pellet quality.

Due to the limited moisture content and moderate temperatures in conventional pelleting, it was shown that only a small extent of starch gelatinization, ranging from 5 – 30% occurred in the pelleting process (Skoch *et al.*, 1981; Moritz *et al.*, 2005; Buchanan & Moritz, 2009; Zimonja & Svihus, 2009; Abdollahi *et al.*, 2010). Several authors also observed that the majority of gelatinization took place during pelleting (Heffner & Pfost, 1973; Zimonja & Svihus, 2009; Abdollahi *et al.*, 2010; Abdollahi *et al.*, 2011). Water is a limiting factor to starch gelatinization during conventional pelleting as only about 30g/kg moisture is added to feed (Thomas *et al.*, 1998). Theoretically, a higher mash moisture percentage would result in higher gelatinization percentage (Lund, 1984; Parker & Ring, 2001; Ratnayake & Jackson, 2006). However, conflicting data was reported on the effect of moisture levels on starch gelatinization.

In addition, conflicting data on the relationship of starch gelatinization and pellet durability was reported (Wood, 1987; Thomas *et al.*, 1998; Briggs *et al.*, 1999b; Moritz *et al.*, 2001a; Moritz *et al.*, 2002; Gilpin *et al.*, 2002; Zimonja & Svihus, 2009). There was no consistent data on the effect of starch gelatinization on pellet durability. In fact, the location and uniformity of the gelatinized starch may have a greater influence on pellet quality compared to total starch gelatinization (Stevens, 1987; Behnke, 2001).

On the other hand, moisture content has been shown to be negatively correlated to glass transition temperature (Tg). Tg is referred to as the physical change that reflects the segmental motion polymer backbone (Ferry, 1980; Levine & Slade, 2010). Water is one of the most ubiquitous plasticizers that depresses the Tg of other polymers (Zeleznak & Hoseney, 1987; Abiad *et al.*, 2009; Levine & Slade, 2010) Hence, it is important to understand the nature of glass-transition as it is one of the most important parameters that affects the physical interactions of the polymer components in feed during the pelleting process (Thomas & van der Poel, 2020).

In summary, Tg could be an important factor in improving the understanding of the fundamentals of the pellet binding mechanism. Yet, little work has been done on the effect of Tg on pellet quality. The objective of this study was to evaluate: 1. Both the effect of water (moisture) and steam (moisture and heat) on starch gelatinization and Tg. 2. The relationship of starch gelatinization and Tg on the chemical and physiochemical properties of pellets. To further study the morphology of feed particles in pellets, Scanning Electron Microscopy (SEM) imaging was applied to generate optical observations to provide qualitative understanding of the microstructure of pellets. An in-depth review on the effect of moisture addition methods on chemical and physiochemical properties of pellets will be discussed.

MATERIALS AND METHODS

The experiment was conducted by pelleting twelve 340 kg batches of a broiler starter diet (Table III-1) to determine the effect of added moisture on pellet quality using different moisture addition methods. Treatments were arranged as a 2x3 factorials utilizing two levels of added moisture (2% vs 4%) and three moisture addition methods (mixer added water, conditioner added steam and both). Treatments consisted of a negative control and two positive controls with intermediate treatments in between (Table III-2). A mash diet not subjected to mixer added water nor conditioner added steam was dry pelleted to serve as a negative control (NC). 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).

There were three manufacturing runs per treatment which represented three replicates, and the run order was completely randomized. Each run consisted of two batches of 454kg, one batch of 680kg, and one batch of 907kg. Batches consisting of 454kg of feed were pelleted at one conditioning temperature, batches of 680kg of feed were pelleted at two conditioning temperatures and batches of 907kg were pelleted at three conditioning temperatures. The pellet mill and conditioner were completely shut off between each run.

All feed was manufactured following the guidelines for Current Good Manufacturing Practice (CGPM). The research was conducted at the North Carolina State University Feed Mill Educational Unit utilizing a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) fitted with a 4.4 x 28.6 mm die and a conditioner feeder (Model C18LL4/F6 California Pellet Mill Co., Crawfordsville, IN). All corn was ground utilizing a hammermill

(Model 1522, Roskamp Champion, Waterloo, IA.) equipped with a 2.4 mm screen on the impact side and a 3.2 mm screen on the release side.

Individual batches were sourced from a common basal to reduce variation in ingredient composition between batches. 2268 kg of common basal devoid of water was batched utilizing a twin shaft counterpoise ribbon mixer (Model TRDB126060, Hayes and Stolz, Fort Worth, TX) for 180 s of dry mixing time, followed by an additional 90 s of wet mixing time. The basal diet included phytase, xylanase, and fat. The individual batches were then blended utilizing the same equipment and methodology. For batches amended with water, the water was added after 180s of dry mixing, but before 90s of wet mixing. Upon mixer discharge, five composite mixer samples were collected in sealed sample bags, three of which were immediately frozen for subsequent moisture content analysis. The rest of the samples were used to determine the degree of gelatinization and glass transition temperature (Tg). The temperature of mixer mash feed was collected to determine the target conditioning temperature.

For batches amended with steam, conditioning temperature was increased by 25°F for every 1% of target conditioner added steam (Table III-3). The actual increase in conditioning temperature was calculated by subtracting the temperature of mixer mash feed from the conditioning temperature. The pellet mill die was warmed with 455kg of feed before proceeding with the experimental batches. All batches were conditioned for 30s and pelleted at a production rate of 771kg/hr. The average steam pressure was 255kPa.

After reaching the appropriate mash conditioning temperature, six hot conditioned mash and hot pellet samples were collected, sealed, and frozen immediately for moisture analysis over a five-minute period of pelleting. The moisture content was measured in accordance with AACC 44-19.01. The hot conditioned mash was collected as feed transitioned between the conditioner and the pellet mill die. At the same time, three mash samples and five pellet samples were collected and blended into composite samples. The mash samples were collected in sieves #100 and cooled on top of fans that were placed horizontally on the floor. These pellets were then cooled in custom manufactured 30 x 30 cm trays, which were placed in a custom manufactured pellet cooler resembling a counterflow cooler. Cooled conditioned mash was analyzed for the degree of gelatinization and glass transition temperature (Tg); Cooled pellets were analyzed for moisture content and pellet durability index (PDI), degree of gelatinization, glass transition temperature (Tg) and Scanning Electron Microscopy (SEM) imaging. PDI was measured using a Holmen pellet durability tester (Model NHP100, Tekpro, Norfolk, UK). 100g of pellets screened using a #5 US sieve were placed into the pellet chamber for 30s. Pellets were removed directly from the pellet chamber and weighted to represent a proportion of the initial mass added to the testing chamber. Each sample was tested in triplicate and an average value was calculated.

For batch runs consisting of more than one conditioning temperature, the steam valve leading to the conditioning chamber was further opened to increase the conditioning temperature, and the sample collection process was repeated once reaching the appropriate conditioning temperature. During the pelleting process, parameters including the difference in temperature between hot pellets and conditioned mash (ΔT), and motor load and pellet mill energy consumption (PMEC) were monitored and collected. PMEC was calculated using the values of motor load (%), horsepower (hp), kilowatt per horsepower (kw/hp), and production throughput (ton/hr).

$$\text{PMEC} = \frac{Motor\ Load\ x\ Horsepower\ x\ Kilowatt}{Production\ Throughput}$$

Two methods were utilized to determine starch gelatinization. Enzymatic method developed by Kemin Agrifood Asia (Singapore) was used to determine starch gelatinization. However, the enzymatic method developed by Kemin Agrifood Asia (Singapore) was proprietary. In addition, differential scanning calorimetry (DSC) (Model Q2000, TA Instruments, New Castle, DE) was used to determine starch gelatinization and glass transition temperatures (Tg). Distilled water was added to the sample in the pan to obtain a solid to water ratio of 1:2. The pans were hermetically sealed, and the samples were equilibrated overnight. An empty sealed pan was used as a reference for all other sample sets. When running the DSC, the samples were equilibrated at 10°C. The pans were heated from 10°C to 140°C at the rate of 10°C/min and cooled down from 140°C to 10°C at the rate of 25°C/min. The end of cooling cycle was marked with nitrogen gas flow rate of 50mL/min. The samples were again rescanned with heating from 10°C to 140°C at the rate of 10°C as the final phase of the test. The data was then analyzed using the TA Universal Analysis software (TA Instruments, New Castle, DE). Tg values could be obtained by placing the marker on the glass transition (Tg) peak in the software. The enthalpy values of both unprocessed feed sample (i.e. mixer mash) and processed feed sample (i.e. conditioned mash/ pellets) were obtained from the software by analyzing the area under the crystallization temperature (Tc) peak. Degree of gelatinization was calculated using the formula:

$$Degree\ of\ Gelatinization\ (\%) = \frac{(\Delta\ H_{native} -\ \Delta\ H_{Sample})}{\Delta\ H_{native}}\ x\ 100\%$$

For SEM analysis, cooled pellets from each replicate of the same treatment were mixed to represent a uniform sample group. A few pellets from each treatment were randomly chosen and and then flash frozen in liquid nitrogen for 10 seconds. To observe the microstructure of the outer layer and cross-sectional of pellets, the pellets were cut both vertically and horizontally in the middle (Supplemental Figure III-i). The flat surface was then placed on a prepared tub with carbon tape. Next, carbon sputter coatings coated the pellets for SEM imaging (Hitachi TM4000II). An accelerating voltage of 5kV and a detector setting of mixed secondary electrons

(SE) and backscattering electrons (BSE) imaged the pellets with the SEM. The magnification of SEM imaging was set at 500x.

STATISTICAL ANALYSIS

ANOVA from the Fit Model platform of JMP Pro 15.2 (Statistical Analysis, SAS Institute, Cary, NC) was employed and the means were separated using the LSMeans procedure of JMP. The means were considered statistically significant at $p \leq 0.5$. Significant differences were separated utilizing Tukey's HSD test. The Fit Y x X platform of JMP 15.2 was utilized to generate regression models. The regression models were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

In this study, Differential Scanning Calorimetry (DSC) was first used to characterize starch gelatinization in different feed forms. However, the data was not adopted due to the inconsistent peaks in the DSC curves (Supplemental Figure III-ii). The enthalpy values were unreadable as the peaks in DSC were either indiscernible or corresponding to a pan explosion. These were probably due to an uneven distribution in feed particles and complications in feed components. The cool pellets were crushed to fine instead of ground to avoid any potential heat treatment on the samples. However, the fine samples were not sieved through a finer screen before being characterized by DSC. According to Landon (2011), an indiscernible peak was observed when the feed sample was ground through a 0.5mm screen but a distinct peak was observed when the feed sample was ground though a 0.2mm screen. Due to limited access to DSC testing, an enzymatic method developed by Kemin Agrifood Asia (Singapore) was used to determine the degree of starch gelatinization in this study.

The degree of gelatinization in mixer mash feed and conditioned mash feed ranged between 4.89% -7.71% as opposed to 16.97% -19.32% in pelleted feed ($p \leq .0003$; Table III-7). The degree of gelatinization was significantly higher in pelleted feed compared to conditioned mash feed or mixer mash feed across treatments ($p \leq .0003$; Table III-7). There were no differences in the degree of gelatinization between conditioned mash feed nor mixer mash feed across treatments (Table III-7). These data indicated that mechanical sheer across the pellet mill die had a greater impact on gelatinization during the pelleting process when compared to the impact of the conditioner. This result was in agreement with Stevens (1987), who reported that most gelatinization occurred on the outer portion (2mm thick) of pellets.

There were no significant differences between treatments for gelatinized starch in any feed form ($p > .05$; Table III-8). Hot conditioned mash moisture was neither correlated with conditioned mash gelatinization nor pellet gelatinization ($p > .05$; Figure III-1). These data showed that moisture addition through either steam, water, or a combination of both did not significantly affect starch gelatinization in any feed form in this experiment. However, conflicting findings were reported. Moritz *et al.* (2003) observed that the gelatinized starch content in pellets decreased from 18.4% to 10.6% and 6.1% when 25g/kg and 50g/kg of water were added. The final pellet moisture content of the control diet with no moisture addition was reported at 12.33%. In another study, Moritz *et al.* (2001a) also reported that an addition of 50g/kg water resulted in an increase in gelatinized starch content from 15.8 to 29.6% when the dry matter of the maize-soy diet was very high at 92.7% (8.13% final pellet moisture). In this study, the cool pellet moisture content of diet with no water addition was 11.74%, which was in the zone between 8.13% and 12.33% reported in Moritz's studies. This highlighted the effect of dry matter of diet and moisture addition on starch gelatinization. It is noteworthy that further moisture addition above normal levels of moisture in a diet reduces starch gelatinization while moisture addition below normal levels of moisture in a diet increases starch gelatinization (Abdollahi *et al.*, 2013). Conditioning process could be another variable that causes the differences in observations. The conditioning temperature was set at 180°F in both studies conducted by Moritz *et al.* as opposed to this study in which the conditioning temperatures were set at a range of 75°F to 165°F. Higher conditioning temperatures resulted in an increase in the degree of gelatinization in conditioned mash but not pellet ($p = .01$; Figure III-2). A similar finding was reported by Abdollahi *et al.* (2011), in which the highest gelatinized starch content was found in mash diets when the conditioning temperature was increased from 68°F to 194 °F. In contrast, gelatinized starch content in pellets was higher when the diet was conditioned at 68°F compared to 140°F and 167°F. This study further accentuated the impact of die friction on starch gelatinization. Moisture addition did not impact the degree of gelatinization in conditioned mash nor pellets. This indicated that moisture addition itself was not sufficient to gelatinize starch. Future studies should focus on the interaction effect of heat and moisture addition on the degree of starch gelatinization. In addition, pellet durability index (PDI) was neither correlated to conditioned mash gelatinization ($p = .26$; Figure III-3) nor pellet gelatinization ($p = .86$; Figure III-3). These data indicated that starch gelatinization might have a negligible effect on pellet durability

In this experiment, the Tg of conditioned mash ranged between 71.27 °C to 75.44 °C with moisture ranging between 13.32% to 16.81% (Table III-4, Table III-9). According to Zhong & Sun (2005), the Tg of gelatinized cornstarch at 11.9% water content was 98.3 ± 1.8°C while the Tg of gelatinized cornstarch at 18.5% water content was 47.5 ± 4.6°C. Nithya *et al.* (2015), reported a substantial decrease in Tg as moisture content of cereal-pulse blend increased from 9% to 27%. The Tg of conditioned mash feed between treatments was significantly different (p =

.0068; Table III-9). The Tg of rations amended with 2% water and 2% steam was lower compared to rations with no moisture addition, 1% water and 1% steam, 2% water and 0% steam, and 4% water and 0% steam (p = *.0068*; Table III-9). Besides, Tg of conditioned mash subjected to 2% water and 2% steam was not significantly different from conditioned mash subjected to 0% water and 2% steam or 0% water and 4% steam (p = *.0068*; Table III-9). Furthermore, Tg of conditioned mash feed was negatively correlated to conditioned mash moisture (p = *.013*; Figure III-5). A similar finding was also reported by Zeleznak and Hoseney (1987). Their data indicated that the plasticizing effect of water depressed the temperature at which the feed material became mobile as moisture increased. There was no correlation between Tg and hot conditioned mash temperature (p = *.06*; Figure III-5). However, it might be due to the limited range in conditioning temperature. Future work investigating the relationship of Tg and a wider range of conditioning temperatures is needed to confirm the theory. Overall, Tg decreased when moisture addition increased. Despite the levels of moisture addition, steam might have a greater influence on Tg in conditioned mash feed compared to just moisture alone.

The relationship of Tg on other processing characteristics during pelleting was also evaluated in this experiment. As Tg of polymers increased, the change in temperature between hot pellet and hot conditioned mash (ΔT) increased (p = *.044*; Figure III-6). When the heat energy was applied, the molecular motion was initiated and the stiffer molecules had sufficient energy to slide past one another (Kaplan, 1976). When the glass transition temperature (Tg) of feed increased, the molecules required more energy to alter the rigidity backbones of polymer. In the absence of moisture and heat, the polymers will be glassy and immobile, thus more frictional force was generated during pelleting. Thus, the change in temperature between hot pellet and hot conditioned mash will increase. On the contrary, as moisture increased through water, steam or a combination of both, the plasticization and thermal effect reduced the force needed to deform the polymers during pelleting process. This also further explained the positive relationship between Tg and pellet mill energy consumption (PMEC) (*p = .0055;* Figure III-6). As Tg increased, more energy was needed during pelleting as the movement of polymer chains was restricted.

To understand the fundamental pellet binding mechanisms, it is crucial to evaluate the relationship between glass transition and pellet quality. When the temperature of a polymer is above its glass transition temperature, the polymers become rubbery and flexible. This allows the polymers to diffuse across the interface between materials and adhere to one another (Kinloch, 1987). In this study, Tg was negatively correlated with Pellet Durability Index (PDI) (*p = .035*; Figure III-7). These data further highlighted the impact of glass transition on the pelleting behavior of feed materials. As discussed, an increase in moisture through water and steam could lower the glass transition temperature (Tg) in the feed material. This causes a higher volume fraction of the feed mash particles to go through the glass transition stage. Thus, it improves the binding properties in particles (Thomas & van der Poel, 2020).

To further study the morphology of feed particles in pellets, two sets of Scanning Electron Microscopy (SEM) images were taken. Figure III-8 exhibited SEM images of pellets subjected to 0% water, 2% water, and 4% water with no steam addition. On the other hand, Figure III-9 exhibited SEM images of pellets subjected to 0% steam, 2% steam, and 4% steam with no water addition. The SEM images on the top layer were the outer portion of pellets while the SEM images on the bottom layer were the cross section of the pellets. These images served as optical observations to provide qualitative understanding of the feed's microstructure. However, quantitative image analysis was not performed such as measuring grain size, porosity etc.

Therefore, the images do not completely support the discussed theory in the following paragraphs. Future work on image analysis is needed to confirm the theory.

From the observation on SEM images, as mixer added water increased, the binding of particles on the outer layer of pellet decreased. Yet, the binding of particles on the cross section of pellet increased (Figure III-8). It indicated that at 0% moisture addition, the particles in feed were mainly bound by the solid-solid interaction force and the pellet structure was held by the mechanical force exerted by the friction and pressure in die (Kinloch, 1987; Thomas & Van Der Poel, 1996). According to Thomas & Van Der Poel (1996), water, air, and solid materials from the feed particles are held together by liquid necking. As mixer added water increased, the excess water increased the moisture bridge between particles, creating a two-phase system of water and particles. Phenomenally, this reduces the porosity between feed particles and increases the binding force (Immergut & Mark, 1965; Levine & Slade, 2010). On the other hand, excess water reduced the binding between particles on the outer layer of pellet (Figure III-8). Mohsenin & Zaske (1976) observed the effect of water on compaction behavior due to its influence on the stress relaxation in the feed pellet. When pelleting in excess water, higher residual stress induces a higher degree of expansion in feed (Thomas *et al.*, 1997). This probably explained the observation of particles segregation on the outer layer of pellets.

As conditioner added steam increased, the binding of particles on both the outer layer and cross section increased. This might be due to the increase volume fraction of rubbery stage in polymer as the Tg was lower and the conditioning temperature was higher than the Tg of feed. The feed particles subjected to higher steam showed more rubbery behavior, which allowed better adhesion between particles in the pellet feed as the pellet was pushed through the die. In addition, conditioner added steam increases the mobility of water between particles as water adsorbed on the surface of grain particles had more mobility and less hinderance compared to water bound within capillaries in the grains (Friedrich & Robohm, 1968). Hence, water adsorbed on the surface was more available for chemical reaction. Thus, it reduces the porosity between feed particles and increases the number of bonds, which then increases the strength in pellets. In short, both water and steam addition will affect the structural integrity of pellets by altering the Tg of polymers. However, the additional heat from steam may have a greater influence on the physiochemical properties in feed compared to moisture alone which further improved the pellet quality.

CONCLUSION AND APPLICATION

- The degree of gelatinization increased significantly when comparing pellets to conditioned mash. This indicated that mechanical sheer across the pellet mill die had a larger impact on gelatinization during the pelleting process when compared to the conditioner.
- Moisture addition did not impact the degree of gelatinization in conditioned mash nor pellets. This indicated that moisture addition itself was not enough to gelatinize starch. Future studies could focus on the interaction effect of heat and moisture addition on degree of gelatinization and PDI.
- Gelatinization was not correlated with PDI, indicating that the effect of starch gelatinization on PDI could be negligible.
- Moisture levels and Tg were inversely correlated. It further highlighted the plasticizing effect of water on Tg. An increase in moisture through water and steam could lower the set point of glass transition temperature (Tg) in the feed material. This causes a higher volume fraction of the feed mash particles to go through the glass transition stage, in which the

polymers in feed are rubbery and flexible. Thus, it induces better binding capability in pellets and reduces the PMEC and ΔT.

- Both water and steam addition will affect the structural integrity in pellets by altering the set point of Tg. However, the additional heat from steam may have a greater influence on the physiochemical properties in feed which further improved the pellet quality.

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TABLES AND FIGURES

Table III-1. Ingredient composition of broiler starter diets containing phytase and xylanase.

Ingredients	(%)
Corn	58.62
SBM (48%)	34.77
Poultry by-product Meal	2.26
Poultry fat	2.00
Limestone	0.84
Salt	0.50
DL-Methionine[a]	0.24
Choline chloride (60%)	0.20
Trace mineral premix[1]	0.20
Dicalcium phosphate (18.5% P)	0.14
L-Lysine[b]	0.08
Vitamin premix[2,c]	0.05
Selenium premix[3] (0.06%)	0.05
Phytase[4]	0.02
L-Threonine[d]	0.02
Xylanase[5]	0.01
Calculated nutrients	
Protein	22.95
Calcium	0.85
Available phosphorous	0.43
Total lysine	1.29
ME, kcal/g	3.00

[1] Trace mineral premix provided the following per kg of diet: Mn, 120 mg; Zn, 120 mg; Fe, 80 mg; Cu, 10 mg; I, 2.5 mg; Co, 1 mg.
[2] Vitamin premix provided the following per kg of diet: vitamin A, 6600 IU; vitamin D3, 1980 IU; vitamin E, 33 IU; vitamin B12, 0.02 mg; biotin, 0.13 mg, menadione, 2 mg; thiamine, 2 mg; riboflavin, 6.6 mg; pantothenic acid, 11 mg; vitamin B6, 4 mg; niacin, 55 mg; folic acid, 1.1 mg.
[3] Selenium premix provided Se at 0.3 mg/kg of feed.
[4] Quantum Blue 5G was added to provide 1000 FTU/kg of feed.
[5] Econase XT was added to provide 16,000 BXU/kg of feed.
[a,b,c,d] Dl-Methionine, L-Lysine, Vitamin premix, and L-Threonine were donated by Evonik, Ajinomoto, DSM, and Ajinomoto.

Table III-2. Treatments design.

Treatments	Mixer Added Water	Conditioner Added Steam	Total Moisture Addition
	Moisture, %		
Negative Control	0	0	0
Treatment 1	0	2	2
Treatment 2	1	1	2
Treatment 3	2	0	2
Treatment 4	2	2	4
Positive Control	0	4	4
Positive Control 2	4	0	4

Table III-3. Target vs actual moisture addition through water and steam.

Treatments	Water Addition[1]		Steam Addition [2]		Total Moisture Addition[3]	
	Moisture, %[1,2,3]					
	Target	Actual	Target	Actual	Target	Actual
Negative Control	0	0	0	0.59	0	0.59
Treatment 1	0	0	2	2.96	2	2.96
Treatment 2	1	0.59	1	0.99	2	1.58
Treatment 3	2	1.51	0	0.34	2	1.85
Treatment 4	2	1.51	2	2.15	4	3.66
Positive Control	0	0	4	4.08	4	4.08
Positive Control 2	4	3.13	0	0.19	4	3.32

[1,2,3] Moisture content was measured in accordance with AACC 44-19.01.
[1] Water was added through mixer and the amount of water added was calculated by weight percentage.
[2] Steam was added through conditioner and the amount of steam added was calculated by weight percentage.
[3] Total moisture addition was calculated by adding the amount of water and steam.

Table III-4. Total moisture content of each feed form between treatments.

Treatments and Target Moisture[1]		Mixer Mash[2]	Hot Conditioned Mash[3]	Hot Pellet[4]	Cooled Pellet[5]
		Moisture, %[6]			
0% Water, 0% Steam	Negative Control	12.73 [C]	13.32 [D]	13.05 [D]	11.74 [B]
0% Water, 2% Steam	Treatment 1	12.73 [C]	15.69 [B]	15.04 [ABC]	12.77 [AB]
1% Water, 1% Steam	Treatment 2	13.32 [C]	14.32 [C]	14.16 [CD]	12.26 [AB]
2% Water, 0% Steam	Treatment 3	14.24 [B]	14.58 [C]	14.28 [BCD]	12.4 [AB]
2% Water, 2% Steam	Treatment 4	14.24 [B]	16.40 [AB]	15.84 [A]	13.44 [A]
0% Water, 4% Steam	Positive Control	12.73 [C]	16.81 [A]	16.39 [A]	13.06 [AB]
4% Water, 0% Steam	Positive Control 2	15.86 [A]	16.05 [AB]	15.69 [AB]	13.64 [A]
	SEM[7]	0.16	0.19	0.30	0.35
	P-value		*<.0001*		

[A,B] Means within a column with different superscripts differ significantly ($P < 0.0001$).

[1] The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2] Mixer Mash was collected as the mash was coming out from the mixer and freeze immediately for further laboratory analysis.

[3] Hot conditioned Mash was collected as the mash was coming out from the conditioner and froze immediately for further laboratory analysis.

[4] Hot Pellet was collected as the pellet was coming out from the pellet mill and froze immediately for further laboratory analysis.

[5] Cooled Pellet was collected after the hot pellet was completely cooled down and stored in a cool environment.

[6] Moisture content was measured in accordance with AACC 44-19.01.

[7] SEM= Standard error of the mean for moisture effect (n=3).

Table III-5. Treatments effects on hot conditioned mash temperature, hot pellet temperature and difference in temperature between hot pellets and hot conditioned mash (ΔT).

Treatments and Target Moisture[1]		Hot Conditioned Mash Temperature[2]	Hot Pellet Temperature[3]	ΔT
			°F	
0% Water, 0% Steam	Negative Control	75.2 D	130.02 D	54.82 A
0% Water, 2% Steam	Treatment 1	115.59 B	150.41 B	34.82 D
1% Water, 1% Steam	Treatment 2	89.5 C	136.58 CD	47.08 BC
2% Water, 0% Steam	Treatment 3	76.83 D	128.84 D	52.01 AB
2% Water, 2% Steam	Treatment 4	115.36 B	145.35 BC	30.00 D
0% Water, 4% Steam	Positive Control	165.87 A	172.82 A	6.96 E
4% Water, 0% Steam	Positive Control 2	82.25 CD	125.88 D	43.62 C
	SEM[4]	2.52	2.39	1.36
	P-value		*<.0001*	

[A,B] Means within a column with different superscripts differ significantly ($P < 0.0001$).

[1] The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2] Hot conditioned mash temperature was determined based on target moisture %, where every 25°F is equivalent to 1% steam. The hot conditioned mash temperature was collected from Repete automation system.

[3] Hot pellet temperature was collected by taking temperature of hot pellets in an insulated Thermos.

[4] SEM= Standard error of the mean for moisture effect (n=3).

Table III-6. Treatments effects on pellet durability index (PDI) as determined by the Holmen method for 30 seconds of testing and pellet mill energy consumption (PMEC).

Treatments and Target Moisture[1]		PDI[2]	PMEC
		%	kWh/ton
0% Water, 0% Steam	Negative Control	5.37 [D]	18.44 [A]
0% Water, 2% Steam	Treatment 1	22.67 [C]	12.55 [C]
1% Water, 1% Steam	Treatment 2	17.54 [C]	17.13 [AB]
2% Water, 0% Steam	Treatment 3	19.74 [C]	17.55 [AB]
2% Water, 2% Steam	Treatment 4	35.93 [B]	11.97 [C]
0% Water, 4% Steam	Positive Control	57.86 [A]	9.39 [D]
4% Water, 0% Steam	Positive Control 2	27.91 [B]	15.53 [B]
	SEM[3]	2.17	0.51
	P-value	<.0001	<.0001

[A,B] Means within a column with different superscripts differ significantly ($P < 0.0001$).

[1] The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2] Pellet durability index (PDI) was measured in accordance with ASAE S269.4.

[3] SEM= Standard error of the mean for moisture effect (n=3).

Table III-7. Total starch gelatinization % of each feed form within treatment.

Treatments and Target Moisture[1]		Mixer Mash[2]	Cool Conditioned Mash[3]	Cool Pellet[4]	SEM[5]	*P-value*
		Starch Gelatinization[6] %				
0% Water, 0% Steam	Negative Control	6.79 ^{B}	6.17 ^{B}	17.05 ^{A}	0.67	<*.0001*
0% Water, 2% Steam	Treatment 1	6.79 ^{B}	6.52 ^{B}	19.08 ^{A}	0.93	*.0001*
1% Water, 1% Steam	Treatment 2	6.30 ^{B}	6.10 ^{B}	16.33 ^{A}	0.63	<*.0001*
2% Water, 0% Steam	Treatment 3	6.57 ^{B}	5.77 ^{B}	16.86 ^{A}	0.95	*.0003*
2% Water, 2% Steam	Treatment 4	6.57 ^{B}	5.86 ^{B}	17.05 ^{A}	0.81	*.0001*
0% Water, 4% Steam	Positive Control	6.79 ^{B}	7.71 ^{B}	16.97 ^{A}	0.72	*.0001*
4% Water, 0% Steam	Positive Control 2	4.97 ^{B}	4.89 ^{B}	19.32 ^{A}	0.72	<*.0001*

^{A,B} Means within a row with different superscripts differ significantly ($P < 0.0001$).

[1] The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2] Mixer Mash was collected as the mash was coming out from the mixer and stored in a cool environment.

[3] Cool Conditioned Mash was collected after the hot conditioned mash was completely cooled down and stored in a cool environment.

[4] Cool Pellet was collected after the hot pellet was completely cooled down and stored in a cool environment.

[5] SEM= Standard error of the mean for moisture effect (n=3).

[6] Starch gelatinization testing method was proprietary to Kemin Agrifood Asia (Singapore).

Table III-8. Total starch gelatinization % of each feed form between treatments.

Treatments and Target Moisture[1]		Mixer Mash[2]	Cool Conditioned Mash[3]	Cool Pellet[4]
		Starch Gelatinization[5] %		
0% Water, 0% Steam	Negative Control	6.79 [A]	6.17 [A]	17.05 [A]
0% Water, 2% Steam	Treatment 1	6.79 [A]	6.52 [A]	19.08 [A]
1% Water, 1% Steam	Treatment 2	6.30 [A]	6.10 [A]	16.33 [A]
2% Water, 0% Steam	Treatment 3	6.57 [A]	5.77 [A]	16.86 [A]
2% Water, 2% Steam	Treatment 4	6.57 [A]	5.86 [A]	17.05 [A]
0% Water, 4% Steam	Positive Control	6.79 [A]	7.71 [A]	16.97 [A]
4% Water, 0% Steam	Positive Control 2	4.97 [A]	4.89 [A]	19.32 [A]
	SEM[6]	0.91	0.71	0.69
	P-value	*0.79*	*0.26*	*0.054*

[A,B] Means within a column with different superscripts differ significantly ($P < 0.0001$).
[1] The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).
[2] Mixer Mash was collected as the mash was coming out from the mixer and stored in a cool environment.
[3] Cool Conditioned Mash was collected after the hot conditioned mash was completely cooled down and stored in a cool environment.
[4] Cool Pellet was collected after the hot pellet was completely cooled down and stored in a cool environment.
[5] Starch gelatinization testing method was proprietary to Kemin Agrifood Asia (Singapore)
[6] SEM= Standard error of the mean for moisture effect (n=3).

Table III-9. Glass transition temperature (Tg) between treatments.

Treatments and Target Moisture[1]		Tg[2]
		℃
0% Water, 0% Steam	Negative Control	74.50 [A]
0% Water, 2% Steam	Treatment 1	73.61[AB]
1% Water, 1% Steam	Treatment 2	75.44 [A]
2% Water, 0% Steam	Treatment 3	74.40 [A]
2% Water, 2% Steam	Treatment 4	71.27 [B]
0% Water, 4% Steam	Positive Control	73.36 [AB]
4% Water, 0% Steam	Positive Control 2	74.69 [A]
	SEM[3]	0.61
	P-value	0.0068

[A,B] Means within a column with different superscripts differ significantly ($P < 0.0001$).

[1] The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0.59% water and 0.99% steam (treatment 2), 1.51% water and 0.34% steam (treatment 3), 1.51% water and 2.15% steam (treatment 4), 0% water and 4.08% steam (positive control), 3.13% water and 0.19% steam (positive control 2).

[2] Tg, glass transition temperature was obtained from differential scanning calorimetry (DSC) analysis.

[3] SEM= Standard error of the mean for moisture effect (n=3).

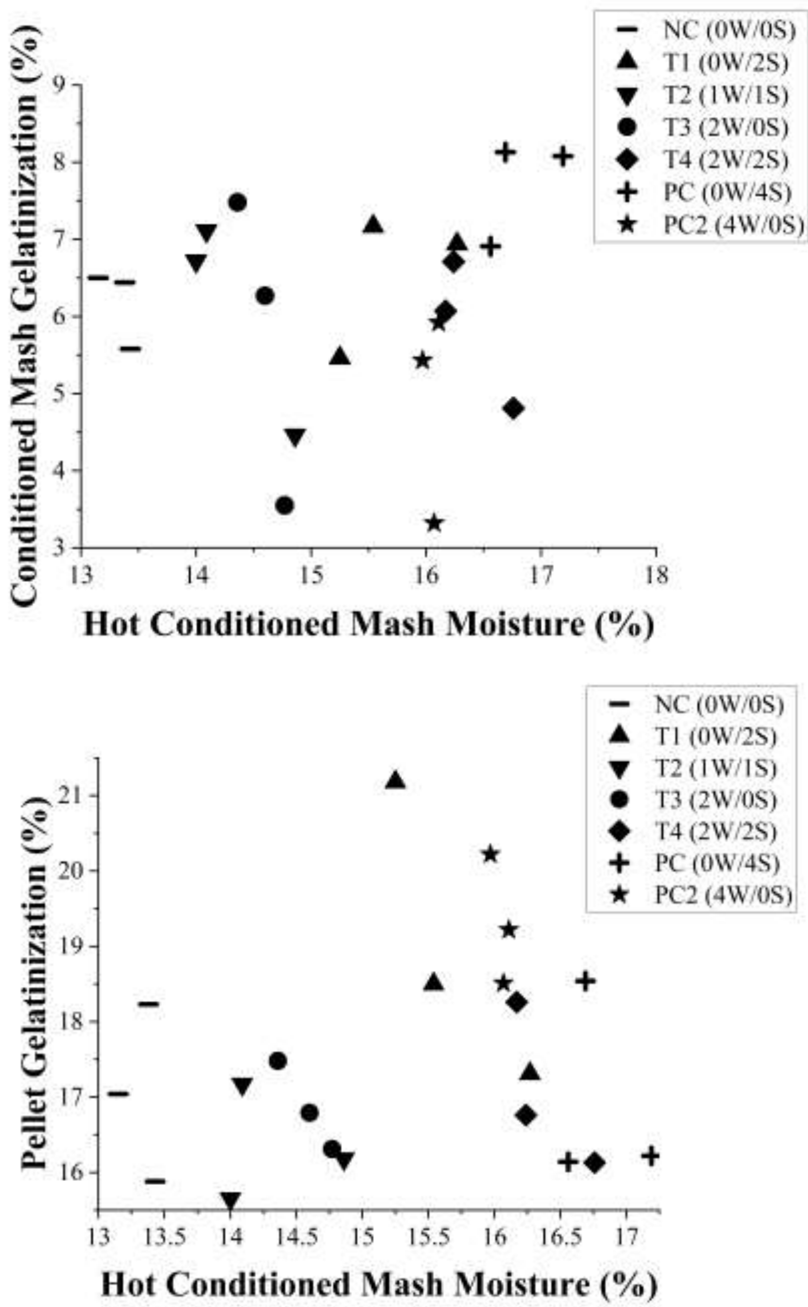


Figure III-1. Hot conditioned mash moisture vs a) conditioned mash gelatinization (p = .72, R^2=0.007) and b) pellet gelatinization (p = .46, R^2=0.03).

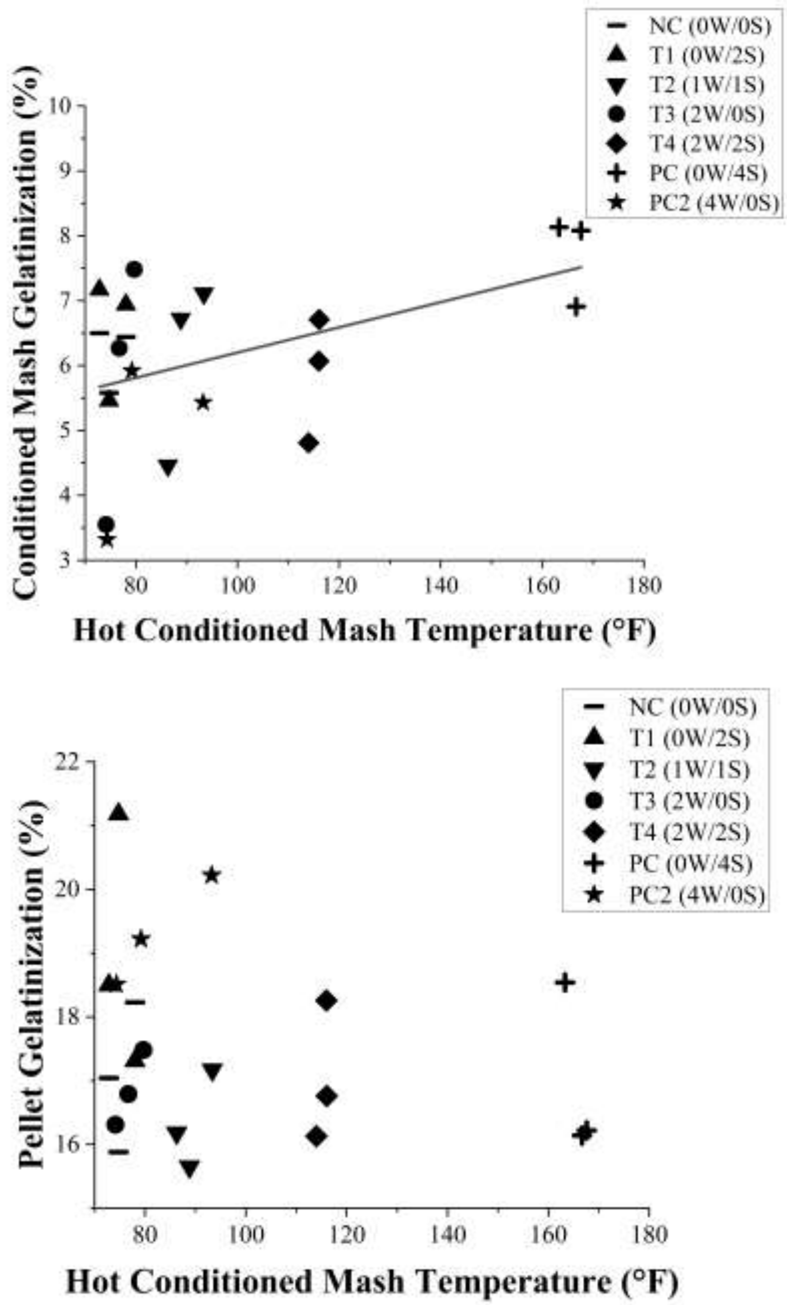


Figure III-2. Hot conditioned mash temperature vs a) conditioned mash gelatinization (p = *.01*, R^2=0.3) and b) pellet gelatinization (p = *.92*, R^2=0.0005).

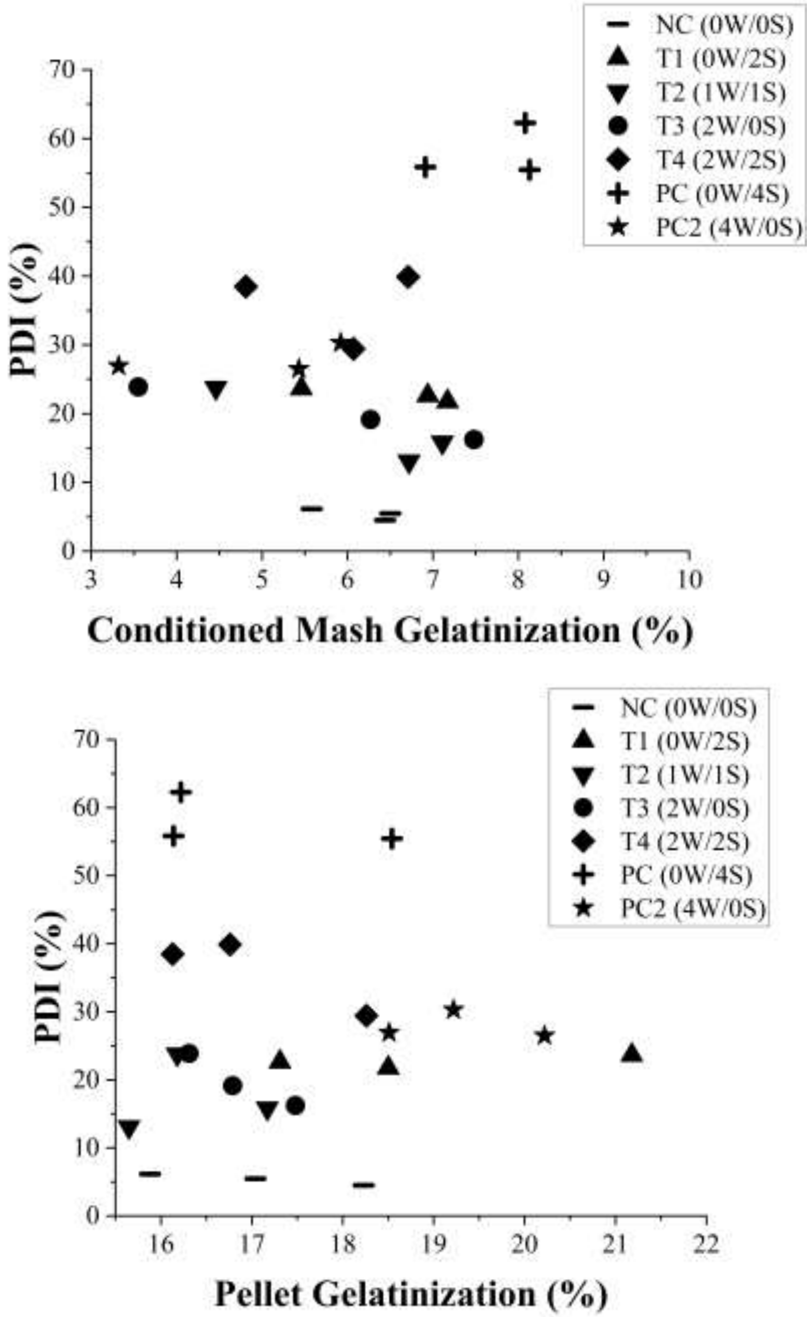


Figure III-3. Pellet durability index (PDI) vs a) conditioned mash gelatinization (p=.*26*, R^2=0.07) and b) pellet gelatinization (p= .*86*, R^2=0.002).

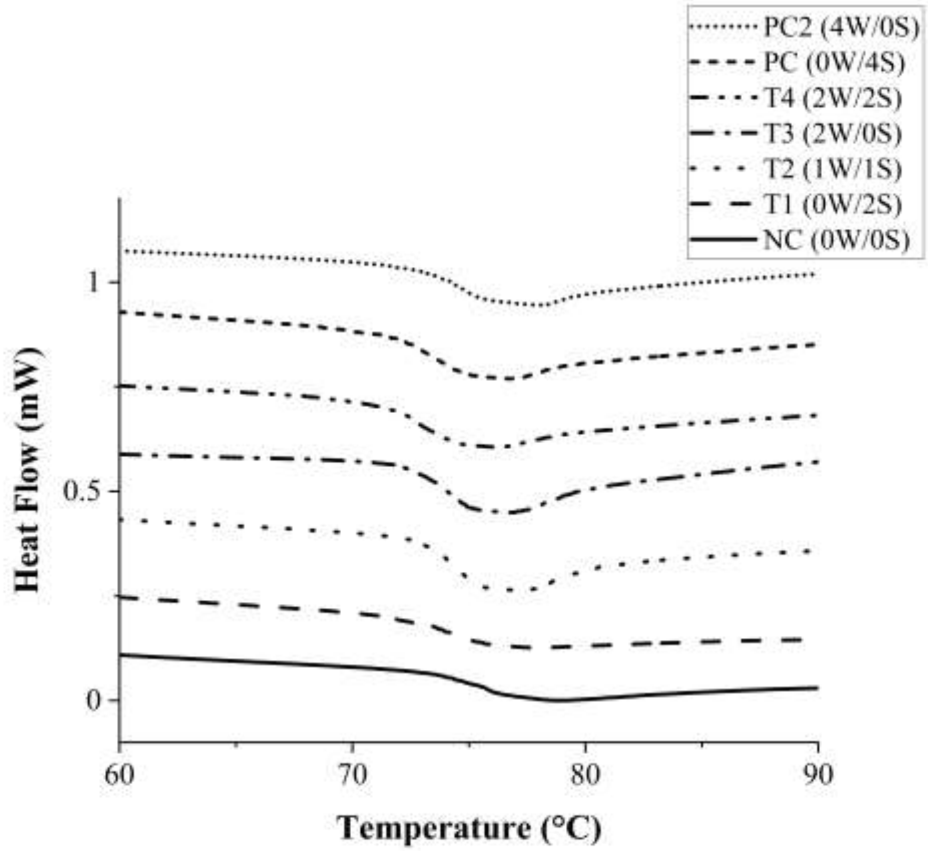


Figure III-4. Glass transition temperatures (Tg) of each treatments.

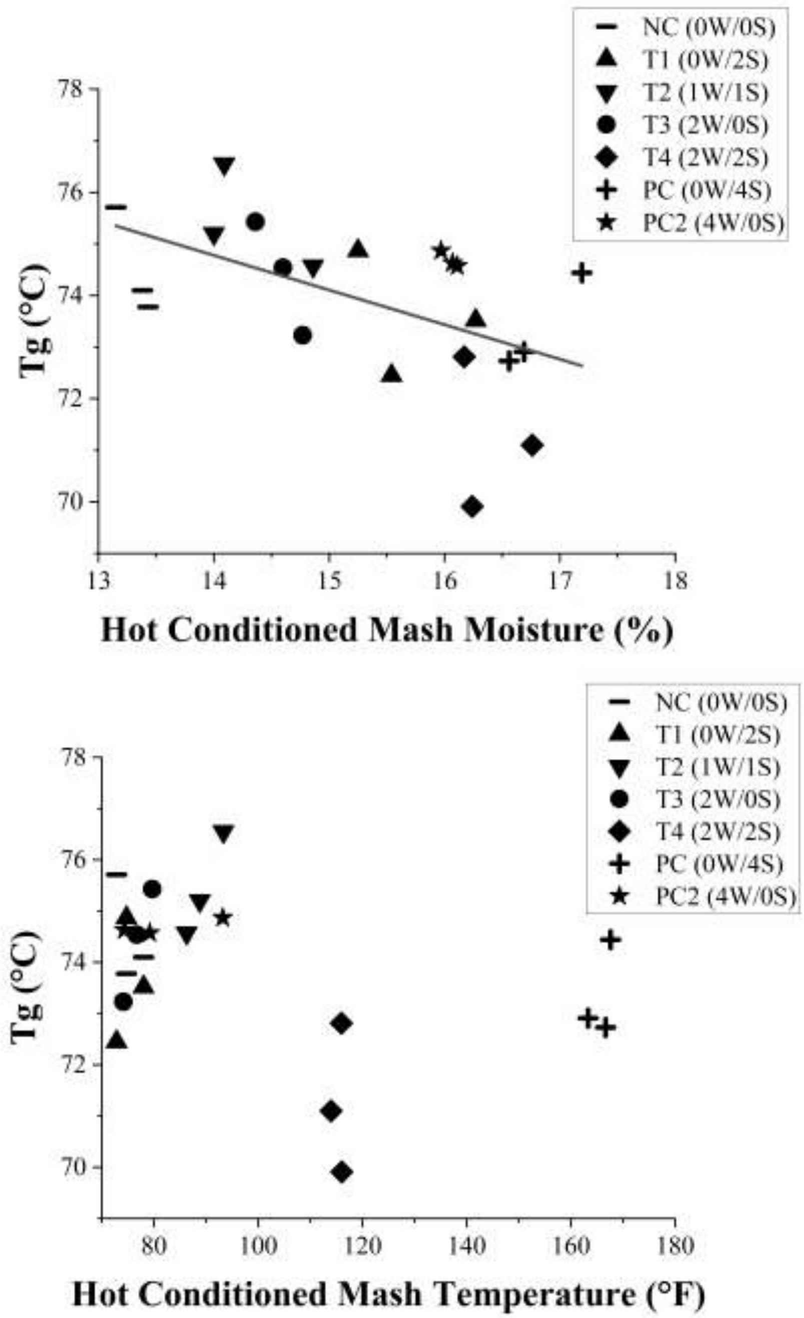


Figure III-5. Glass transition temperature (Tg) vs a) hot conditioned mash moisture (*p*= *.013*, R^2=0.28) and b) hot conditioned mash temperature (*p*= *.06*, R^2=0.17).

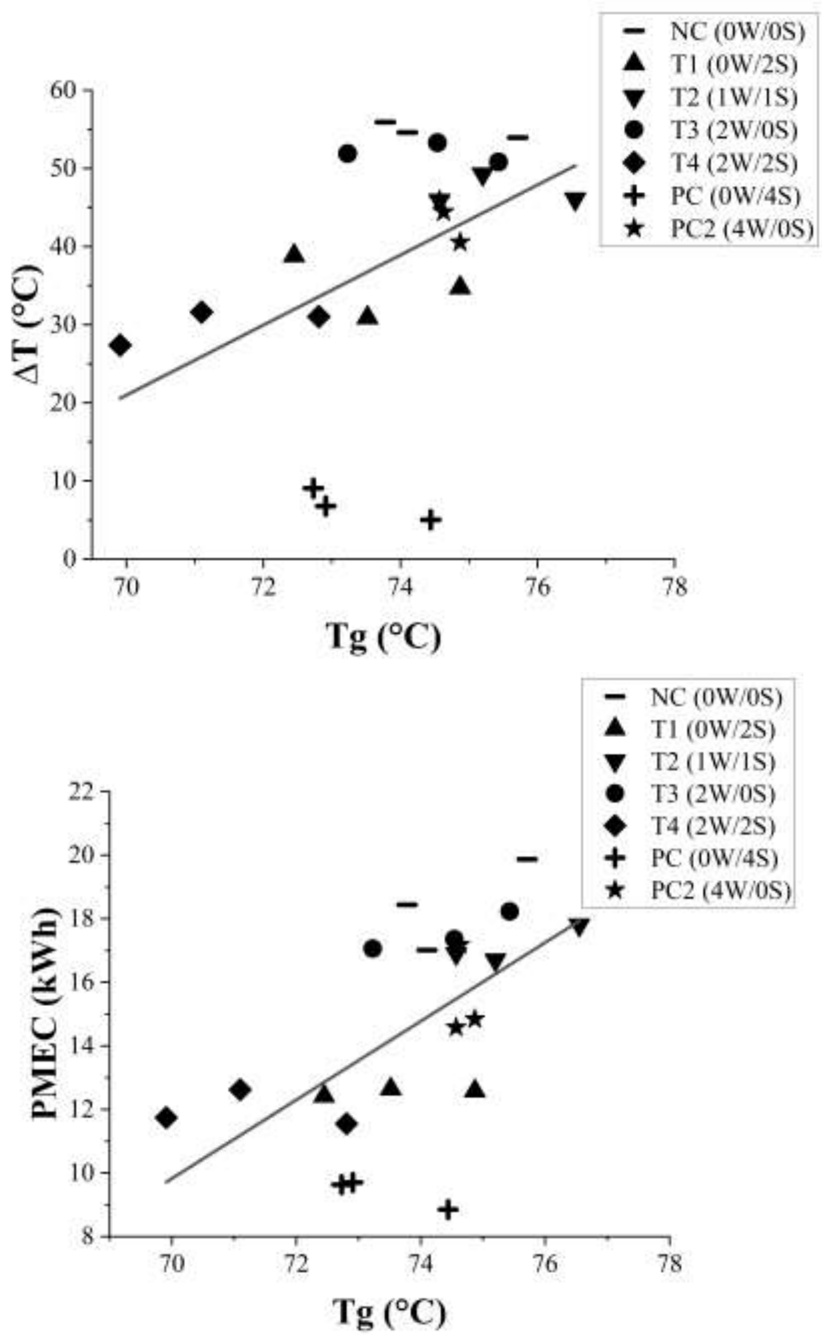


Figure III-6. Glass transition temperature (Tg) vs a) change in temperature between hot pellet and hot conditioned mash (ΔT) (p = *.044*, R^2=0.20) and b) pellet mill energy consumption (PMEC) (p = *.0055*, R^2=0.34).

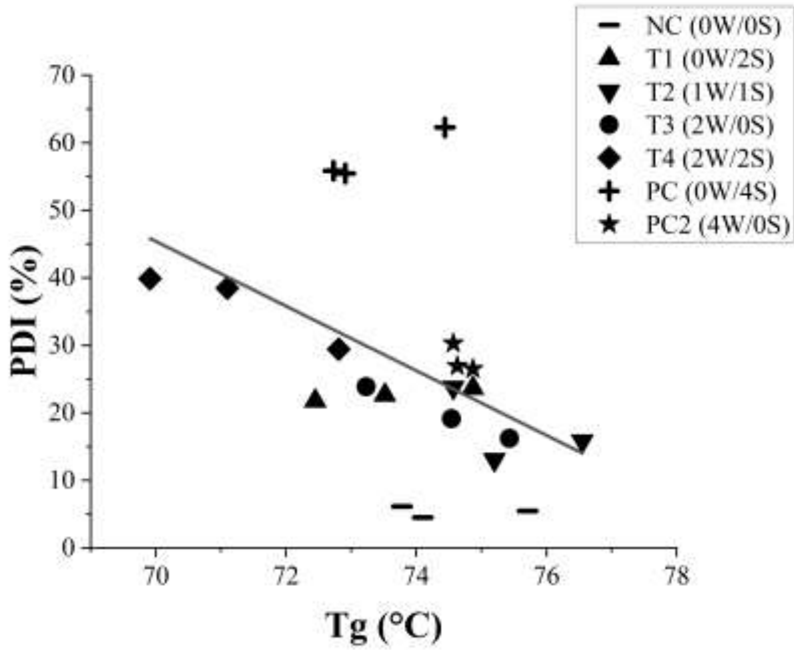


Figure III-7. Glass transition temperature (Tg) vs pellet durability index (PDI) (p = .*035*, R^2=0.21).

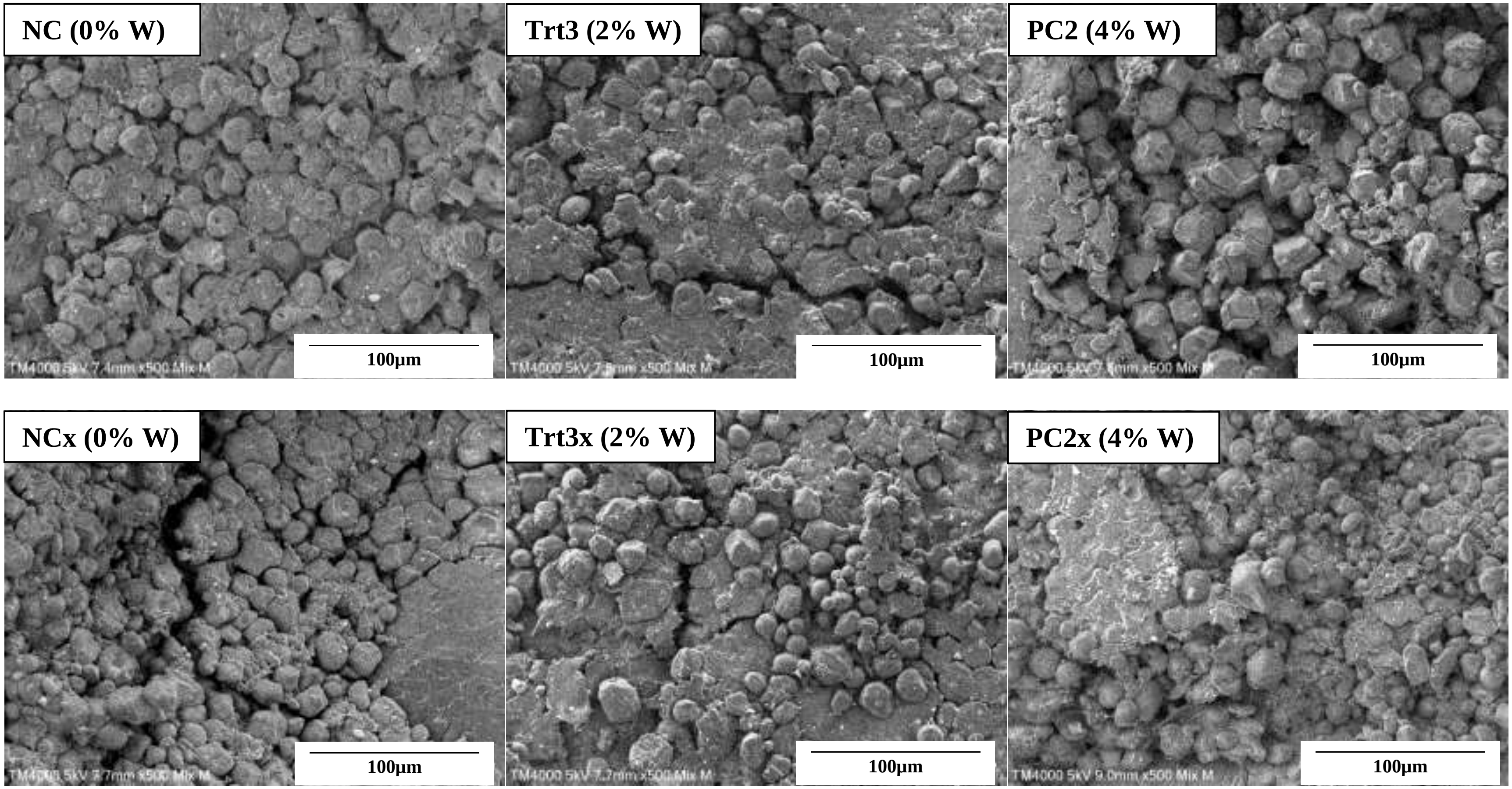


Figure III-8. Scanning electron microscopy (SEM) images of pellets: NC, Trt 3, and PC2.
Top row represented the **outer layer** of pellets; **Bottom** row represented the **cross-section** of pellets.
The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 1.51% water and 0.34% steam (treatment 3), 3.13% water and 0.19% steam (positive control 2).
The actual Tg of the treatments were as follows:74.50 ℃ (negative control), 74.40℃ (treatment 3), 74.69℃ (positive control 2).
The conditioning temperature of the treatments were as follows: 75.2℃ (negative control), 76.83℃ (treatment 3), 82.25℃ (positive control 2).
The PDI of the treatments were as follows: 5.37% (negative control), 19.74% (treatment 3), 27.91% (positive control 2).

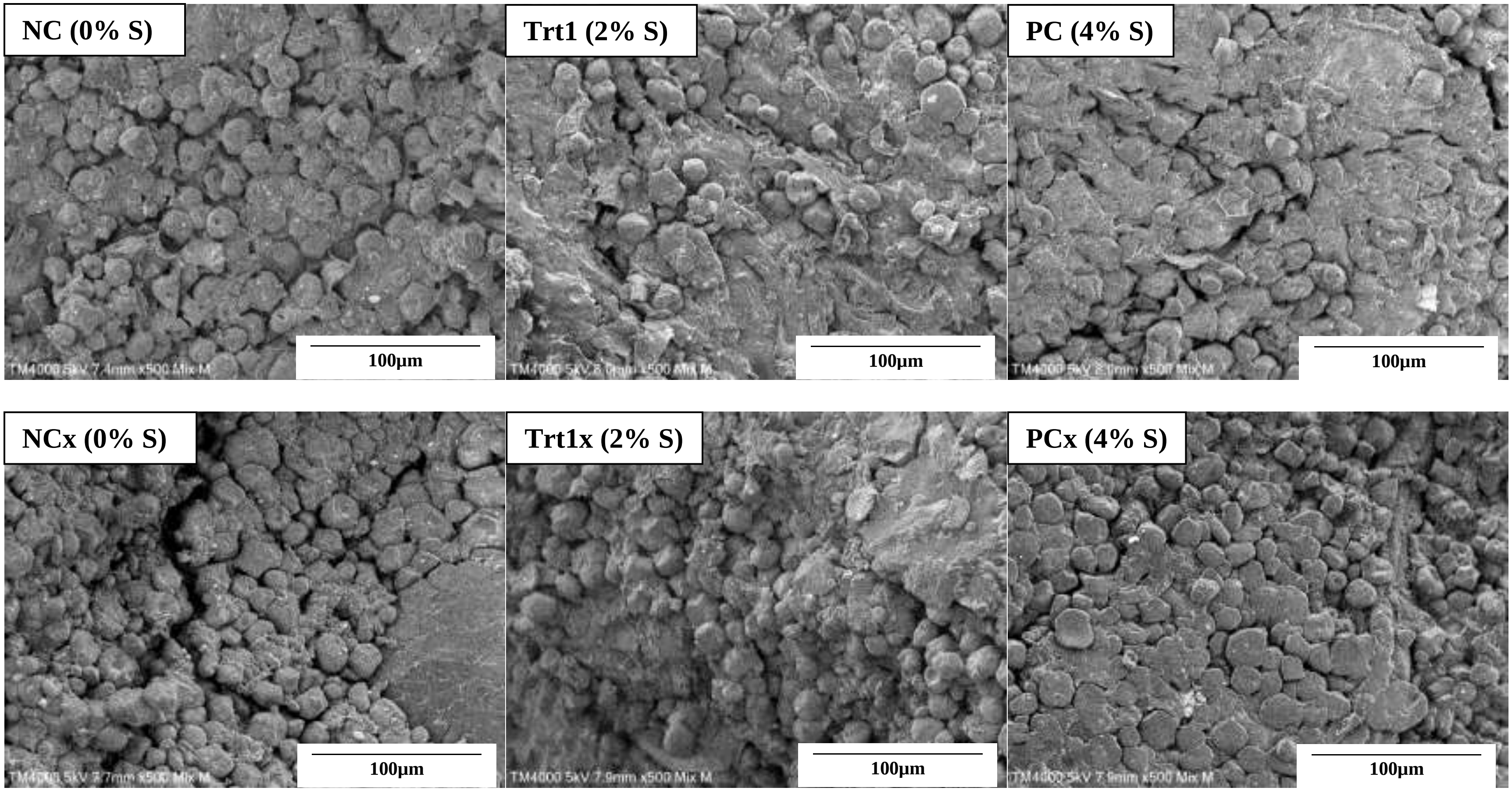
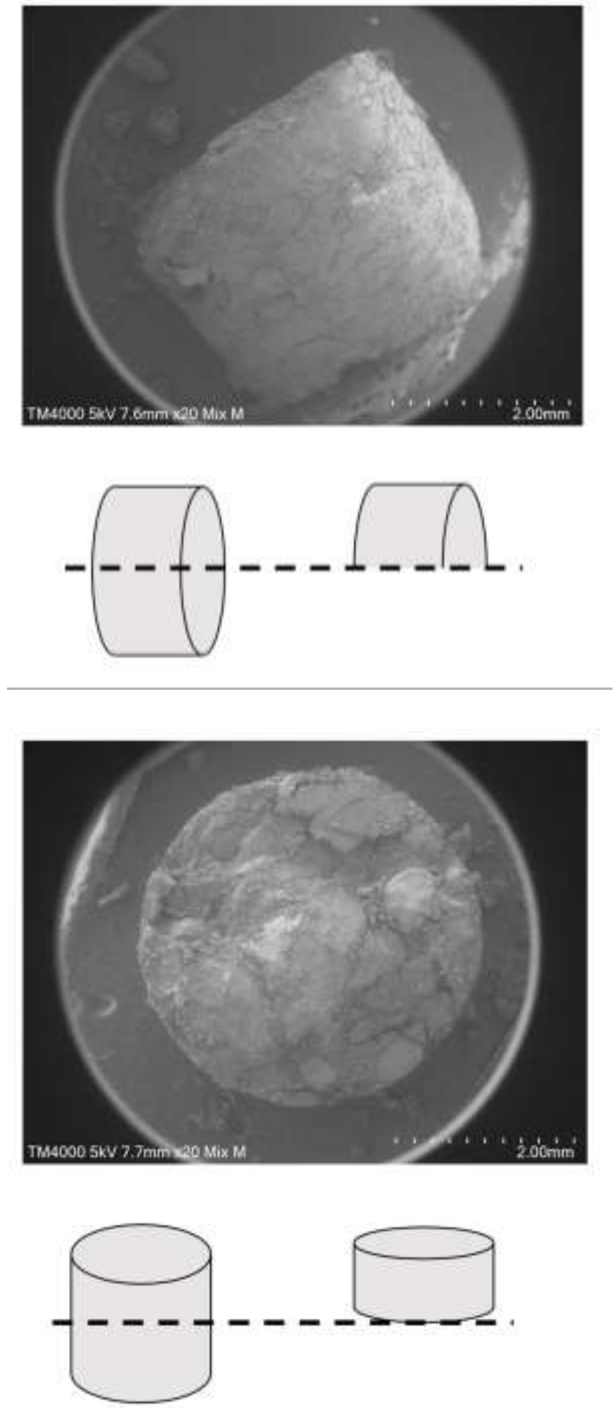
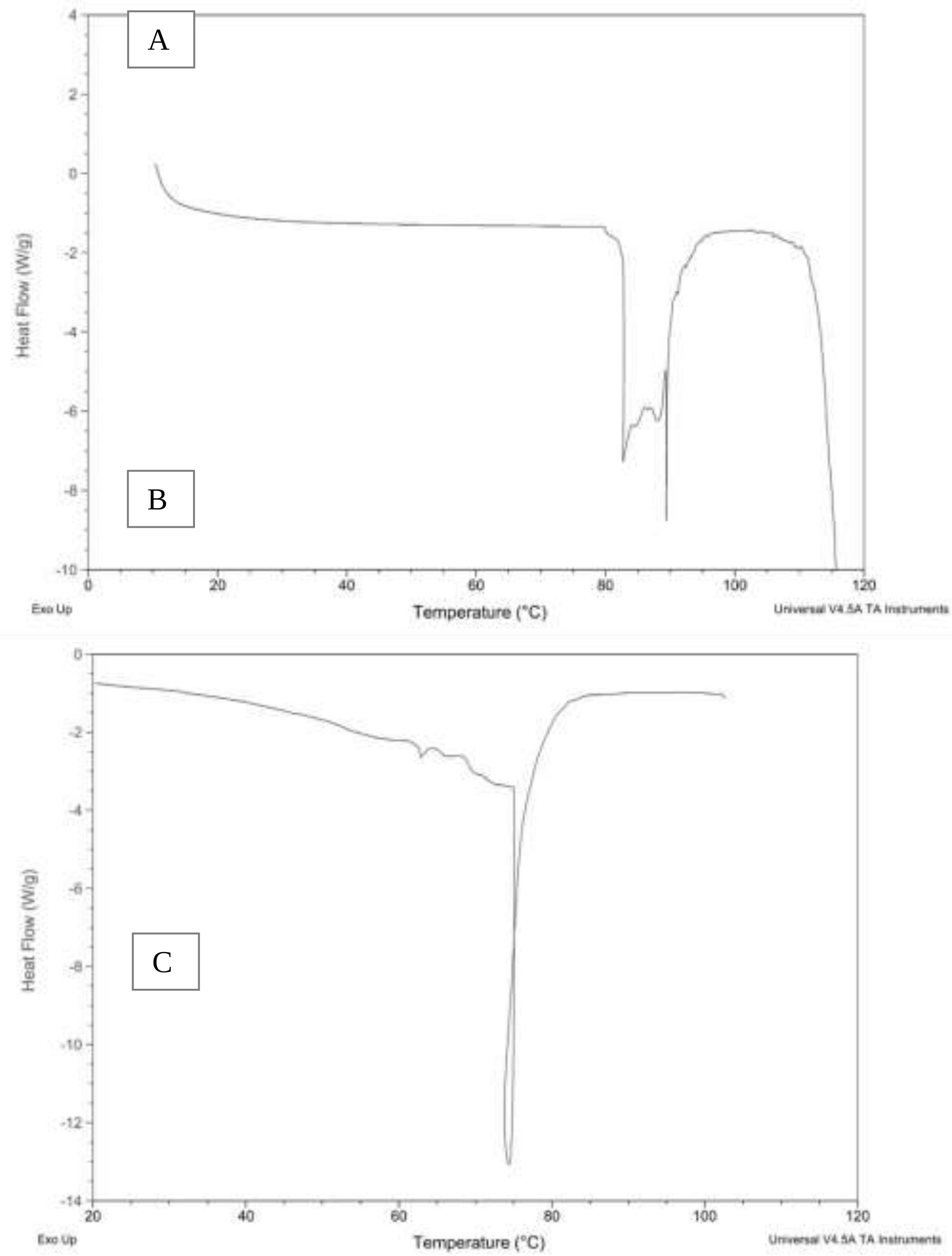


Figure III-9. Scanning electron microscopy (SEM) images of pellets: NC, Trt 1, and PC.
Top row represented the **outer layer** of pellets; **Bottom** row represented the **cross-section** of pellets.
The actual moisture contents of the treatments were as follows: 0% water and 0.59% steam (negative control), 0% water and 2.96% steam (treatment 1), 0% water and 4.08% steam (positive control).
The actual Tg of the treatments were as follows:74.50 ℃ (negative control), 73.61℃ (treatment 1), 73.16℃ (positive control).
The conditioning temperature of the treatments were as follows: 75.2℃ (negative control), 115.59℃ (treatment 1), 165.87℃ (positive control).
The PDI of the treatments were as follows: 5.37% (negative control), 22.67% (treatment 1), 57.86% (positive control).

Supplemental Figures



Supplemental Figure III-i. Scanning electron microscopy (SEM) images of pellets: a) the outer layer of pellets vs b) the cross-section of pellets.



Supplemental Figure III-ii. Starch gelatinization of broiler feed as determined by differential scanning calorimetry. Inconsistent peaks were observed: a) indiscernible b) corresponded to a sample pan explosion.