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Improvement of the physical and nutritional quality of pelleted feed

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Improvement of the Physical and Nutritional Quality of Pelleted Feed

Curran Kenji Gehring

**Thesis submitted to the
Davis College of Agriculture, Forestry and Consumer Sciences
at West Virginia University
in partial fulfillment of the requirements for the degree of**

**Master of Science
in
Animal and Nutritional Sciences**

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Janet M. Tou, Ph.D.**

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Mixer-Added Fat, TMEn, TAAD, Exogenous Enzymes**

ABSTRACT

Improvement of Physical and Nutritional Feed Quality

Curran Kenji Gehring

Experiments were conducted to improve the physical and nutritional quality of pelleted feed. One experiment focused specifically on improvement of pellet quality using functional muscle proteins. A second experiment examined the effects of increasing mixer-added fat (MAF) on feed manufacture variables, broiler performance variables and carcass composition. In experiment 1, muscle proteins (myofibrillar and sarcoplasmic) were recovered using isoelectric/solubilization precipitation, and a functional paste (TPP) was formed by mixing recovered proteins with typical surimi additives. Trout protein paste was included in broiler diets at 0, 2.5 or 5%. A tap water treatment (3.9%) was included to match the moisture added by the 5% TPP treatment. Pellet manufacture variables, nitrogen-corrected true metabolizable energy (TMEn) and true amino acid digestibility were evaluated. Muscle protein inclusion improved pellet quality considerably, however 5% TPP decreased pellet mill throughput and increased relative electrical energy usage (REEU). Protein paste may be included in broiler diets below 2.5% to improve pellet quality without negative effects on pellet mill throughput and digestibility. Improving pellet quality is important because industry pellet quality is often low and our lab has demonstrated positive effects of feeding high quality pellets to broilers. Further experimentation revealed that nutrient availability may be reduced by adjusting feed manufacture strategies to improve pellet quality. Experiment 2 was conducted in order to determine if increasing mixer-added fat would improve broiler performance by maintaining nutrient availability in broiler diets pelleted at high temperature. . Increasing MAF reduced the electrical energy usage required to manufacture broiler feed. Feed intake and live weight gain were increased with enzyme addition. Enzyme addition, conditioning temperature and MAF interacted in their effects on feed conversion ratio. Overall, enzyme addition decreased feed conversion ratio, but the effect was greatest with 1% MAF and 82°C or 4% MAF and 85°C. Increasing MAF and conditioning temperature reduced abdominal fat pad yield. There was no difference in carcass, breast or leg yield due to any of the factors. It is likely that increased MAF improved exogenous enzyme retention and nutrient utilization by broilers. While striving to achieve high pellet quality, nutritionists should consider increasing mixer-added fat to increase profitability because of improved nutrient and exogenous enzyme retention.

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Key**CHAPTER ONE**

MAF = mixer-added fat

L:D = length (thickness) to diameter (hole diameter) ratio of the pellet die

PDI = pellet durability index

CP = crude protein

RS1 = resistant starch 1

RS2 = resistant starch 2

RS3 = resistant starch 3

CHAPTER TWO

TAAD = true amino acid digestibility

TPP = trout protein paste

TPI = trout protein isolate

HTTP = high trout protein paste (5.0% inclusion of TPP)

LTPP = low trout protein paste (2.5% inclusion of TPP)

MOIS = moisture (3.9% inclusion of tap water)

ANOVA = analysis of variance

LSD = Fisher's least significant difference

SEM = pooled standard error of the mean

PDI = pellet durability index

MPDI = modified pellet durability index

PREEU = relative electrical energy usage (pellet mill)

CREEU = relative electrical energy usage (conditioner)

BP = beef plasma protein

T-Gase = transglutaminase

α_2 M = α_2 -macroglobulin

CHAPTER THREE

MAF = mixer-added fat

ME = metabolizable energy

CP = crude protein

AA = amino acid

P = phosphorus

DDGS = distillers dried grains with solubles

DM = dry matter

LWG = live weight gain

FI = feed intake

FCR = feed conversion ratio

ANOVA = analysis of variance

HSD = Tukey's Honestly Significant Difference

SEM = pooled standard error of the mean

TEMP = conditioning temperature

NSP = non-starch polysaccharides

PDI = pellet durability index

MPDI = modified pellet durability index
ENZ = exogenous enzyme addition
PC = positive control

LITERATURE REVIEW

I. Pellet Quality

Although pelleting is an energy-intensive process, it is well understood that the ensuing physical and nutritional benefits more than justify the cost of production (Behnke, 1996). Consequently, more than 80 percent of all non-ruminant feed in the U.S. is pelleted (Behnke, 2001). Behnke (1994) attributed the benefits of pelleting to: 1) decreased feed wastage; 2) reduced selective feeding; 3) decreased ingredient segregation; 4) less time and energy expended for prehension; 5) destruction of pathogenic organisms; 6) thermal modification of starch and protein; 7) increased digestibility; and 8) improved palatability. Additionally, pelleting improves feed handling characteristics and may reduce feed formulation cost by allowing the use of alternative feed ingredients (Fairfield, 2003); likely due to densification of light, fibrous materials.

Several studies have revealed that pelleted diets improve broiler and swine performance over mash diets (Hussar and Robblee, 1962; Baird, 1973; Wondra *et al.*, 1995; Medel *et al.*, 2003). Performance and carcass composition may be further improved by increasing pellet quality. Pellet quality has been defined as the ability of a feed pellet to maintain post-manufacturing integrity throughout the mechanical agitations produced by conveying, shipping and handling by birds (Cramer *et al.*, 2003). In the broiler industry, due to sheer manufacture volume, pellet quality is often neglected in favor of least-cost diet formulation and high feed throughput. Because of production challenges associated with increased demand for larger broilers, low pellet quality may be exacerbated in older feed mills with capacities designed to accommodate production

of smaller birds (Dozier *et al.*, 2006). As a result, feed in the industry may contain an excessive amount of fines in relation to intact pellets. Scheideler (1991) reported that in some instances, 70 percent or more of feed pellets may be reduced to fines by the time they are consumed. Broilers provided high quality pellets spend less time eating and sit more than broilers fed mash or a mixture of low quality pellets and mash (Nir *et al.*, 1994). Additionally, when feed is pelleted, less time and energy are used for prehension (Jensen *et al.*, 1962; Behnke, 1994). When broilers consume mash or pelleted feed containing a high proportion of fines, fine particles spill from the sides of the beak and a reduced volume of feed is consumed per peck (Jensen *et al.*, 1962). Spilled feed represents squandered productive energy and increased feed wastage.

Feed form effects on broiler performance and carcass composition are well established. Feeding high quality pellets (94 pellet durability index, 5% fines) decreased broiler feed intake and feed conversion ratio compared to broilers fed slightly lower quality pellets (90 pellet durability index, 9% fines) (Cutlip *et al.*, 2008). Lilly *et al.* (2009) conducted a study to directly examine the effect of pellet quality on broiler performance, using the same diet formulation as in the previous study (3.09% soybean oil), and determined that increasing pellet quality from 60 to 90% pellets in the feed pan, improves carcass weight of broilers by nearly 2 fold. Previous experimentation by the same authors, used diets formulated at a reduced energy density (1.26% soybean oil), and no feed form effects on broiler performance were detected (Gehring *et al.*, 2009). Thus, it is likely that feed form effects may be masked by reduced nutrient quality in low fat formulations if significant nutrient loss occurs during pelleting.

Pellet quality is affected by several factors, namely diet formulation, ingredient particle size, mash moisture, mixer uniformity, steam conditioning parameters (temperature, pressure, retention time), die speed, die specifications, and cooling. Reimer (1992) deemed diet formulation the most influential factor (40%), followed by conditioning (20%) and particle size (20%) and to a lesser extent, die specification (15%) and drying/cooling (5%).

Although Reimer's theory has been repeated widely, there is little peer-reviewed evidence to support it. According to Thomas *et al.* (1997), with respect to modern conditioning systems, pellet quality may be influenced more by equipment than by diet formulation. Furthermore, it is known that die length to diameter ratio plays a substantial role in determining pellet quality (Buchanan *et al.*, 2008) and ambient temperature may greatly influence the amount of moisture that is added to mash in the conditioner. Gehring *et al.* (2009) reported that all factors are important and that interactions among factors may exist.

Factors that affect pellet quality by increasing feed temperature may also negatively affect broiler performance by reducing nutrient availability and exogenous enzyme retention. The pelleting process involves raising temperature and moisture by injecting steam into mash feed and further increasing feed temperature during pellet formation due to mechanical friction at the mash-die interface. Lubrication of the die ports by increasing the amount of fat added in the mixer (mixer-added fat; MAF) may reduce the extent of exogenous enzyme denaturation, loss of amino acids, and resistant starch formation during processing.

II. Diet Formulation

Diet formulation is one of the most apparent factors that influences pellet formation and quality. Because broiler diets are formulated to be least-cost, a wide variety of feedstuffs may be used to meet nutritional requirements. Depending on the ingredients used, pellet quality may vary widely. Additionally, the processing history and location of origin may influence the physicochemical properties of feedstuffs and their effect on the pelleting process (Thomas *et al.*, 1998). Ingredient selection should be determined with respect to price, bioavailability and the effect on physical and nutritional pellet quality.

Feed ingredients are comprised of primary constituents including starch, protein, sugar, non-starch polysaccharides, lipids, inorganic matter and water (Thomas *et al.*, 1998). Although corn is the major ingredient in broiler diets in the United States and starch gelatinization is often afforded primary importance, protein has been shown to affect pellet quality markedly (Winowiski, 1988; Thomas *et al.*, 1997; Briggs *et al.*, 1999). Briggs *et al.* (1999) increased crude protein from 16.3 to 21% and subsequently increased pellet durability from 75.8 to 88.8 %, respectively. This finding corroborates work by Winowiski (1988) who reported increased pellet durability when crude protein was increased by using wheat at the expense of corn. Recently, Buchanan *et al.* (2009) reported improved pellet quality with diets containing added moisture (2% and 4%) and added soybean meal (2%) compared to least-cost diet formulations.

Feed ingredient costs have increased substantially, especially in terms of corn and fat prices. Current industry practice is to reduce the amount of added fat in broiler diets to reduce diet cost and improve pellet quality (Gehring *et al.*, 2009). This reduction due

to least-cost diet formulation may be exacerbated by the use of exogenous enzymes that have substantial metabolizable energy matrix values. Formulation to a reduced metabolizable energy target may reduce the amount of added fat in broiler diets to 0.5 or 1% of the diet (Blair, 2009). Additionally, post-pellet spray application of supplemental fat is prevalent in the industry and consequently, very little fat may be added in the mixer. Skoch *et al.* (1983) reported reduced protein digestibility of steam-conditioned diets compared to mash diets in weanling pigs. The effect of pelleting on nutrient integrity is dependent on processing conditions and may significantly affect product quality even among pelleted feeds. Mounting evidence suggests that the nutritional quality of pelleted broiler diets may be compromised if the amount of fat added in the mixer is below a critical value (Gehring *et al.*, 2009).

Though increasing dietary calories from fat reduces pellet quality (Salmon, 1987; Cavalcanti and Behnke, 2005a; 2005b), industry pellet quality is sufficiently low such that performance responses associated with feed form effects are not likely to overshadow effects of nutrient availability. Fat added in the mixer may contribute to improved nutrient utilization and enzyme efficacy by coating feed particles (Eliasson *et al.*, 1981; Zimonja *et al.*, 2007) and lubricating the pellet die (Jones *et al.*, 1995; Cutlip *et al.*, 2008). Die lubrication may also provide further benefits associated with reduced electrical energy usage required to pellet broiler feed.

III. Steam Conditioning

Steam conditioning is a major part of the pelleting process. Moisture and temperature of mash feed are increased by steam injection into conditioner barrel, prior to extrusion through the pellet die. The addition of moist heat is essential for feed particle

agglomeration and formation of a durable pellet (Froetschner, 2005). Other benefits are apparent as well, such as improved throughput (Skoch *et al.*, 1981) and die lubrication (Skoch *et al.*, 1981; Skoch *et al.*, 1983) resulting in reduced electrical energy usage.

The ability to form high quality pellets by steam conditioning increases broiler performance compared to mash as previously described. Bayley *et al.* (1968) determined that steam conditioning at 70°C improved subsequent broiler performance compared to mash. Despite the benefits of steam conditioning, nutrient availability (Cutlip *et al.*, 2008) and broiler performance may be compromised above a specific temperature. The upper threshold for steam conditioning temperature appears to be approximately 80-95°C, as indicated by several broiler studies (Bayley *et al.*, 1968; Nissinen, 1994; Nir *et al.*, 1995; Silversides and Bedford, 1999). Estimation of the maximum steam conditioning temperature for a particular diet is not likely to be undertaken with further precision because of the multitude of factors that affect nutrient retention.

Further complication arises when comparing diets formulated with or without exogenous enzymes. Increasing steam conditioning temperature above 80°C improves broiler weight gain and feed conversion with exogenous xylanase addition but reduces broiler performance without xylanase, in wheat-based diets (Cowieson, *et al.*, 2005). Wheat- or barley-based diets may exhibit this effect because of the release of previously encapsulated non-starch polysaccharides (Cowieson *et al.*, 2005) that are a substrate for various carbohydrases.

The difficulty in estimating the effect of steam conditioning temperature on nutrient availability in broiler diets is potentially a serious problem in the industry. The amount of moisture that is added to mash feed depends on the diet composition as well as

ambient temperature (Cutlip *et al.*, 2008, Briggs *et al.*, 1999). Nutrient and enzyme retention may be particularly compromised in the winter months because more steam is required to reach conditioning temperature (Wyatt, 2009).

IV. Die Specifications

Pellets are formed by extrusion of conditioned mash through a pellet die. Within the die ports, frictional heat, compression force and shear significantly affect the structure and availability of feed polymers (Stevens, 1987; Briggs *et al.*, 1999). Pellet dies are typically characterized by their length (thickness) to diameter (hole diameter) ratio (L:D). An L:D ratio of 10 has been suggested as optimal for high grain poultry rations (CPM). Increasing the L:D ratio is associated with increased residence time in the die and subsequent improvement of pellet quality (Gehring *et al.*, 2009) by increasing the portion of dietary starch and protein that may participate in agglomeration reactions. However, increasing the L:D ratio may be detrimental to nutrient availability and subsequent broiler performance, especially if the conditioning temperature is within the 80-90°C range.

V. Mash Moisture

Mash moisture affects pellet manufacture in numerous ways by improving pelleting performance, electrical energy usage, pellet quality and feed stability (Skoch, 1981; Skoch, 1983; Cutlip *et al.*, 2008). Thus, feed ingredient and mash moisture should be monitored closely in order to predict feed production costs and benefits reliably. The magnitude of the aforementioned effects cannot be predicted accurately based upon moisture concentration *per se*, because water activity is arguably more important than total moisture with regard to pellet manufacture. The amount of moisture added to mash via steam conditioning is affected by numerous factors, including the moisture diffusivity

of the feed ingredients, steam quantity and quality, the degree of mixing during conditioning and conditioning chamber dimensions (residence time), as expressed by Briggs *et al.* (1999).

Pelleting parameters may also be affected by the location of moisture addition. Greer and Fairchild (1999) and Muirhead (1999) determined that control of cold mash moisture in the mixer could be utilized to maximize pellet durability and pelleting efficiency. Subsequently, Moritz *et al.* determined that moisture addition in the mixer (up to 5%) improves broiler feed efficiency (2001) and significantly reduces electrical energy usage (~17%), while maintaining or improving pellet quality and broiler performance, in diets formulated with decreased energy density (2002, 2003).

Increasing mash moisture either through moisture addition in the mixer or steam conditioning is beneficial only if there is sufficient frictional force in the pellet die to extrude mash. When mash moisture exceeds approximately 18%, frictional force may be reduced to the point that mash cannot be effectively extruded through the die ports and mash feed accumulates between the rolls and die (Gilpin *et al.*, 2002). Electrical energy usage is significantly increased as the ability to push conditioned feed through the die is reduced (Gilpin *et al.*, 2002). Additionally, if the problem is not corrected immediately, the die may become plugged and “slip”, requiring an interruption of feed production and extensive time and labor to clear the machinery.

VI. Starch Gelatinization

Starch comprises the majority of commercial broiler diets and plays a role in feed particle agglomeration and pellet formation. Starches are homopolymers of α -D-glucose consisting of α -D-(1,4) and/or α -D-(1,6) linkages. The two main structural

polysaccharides in starch are amylose and amylopectin. Amylose comprises 18-33% of starch (Buleon *et al.*, 1998) and is essentially a linear polymer of glucose with few branch points. The majority of starch consists of amylopectin, a much larger molecule comprised of regular branch chains every 20-25 glucose residues (Sajilata *et al.*, 2006).

Higher order plants store energy in the form of starch that is packaged into distinct granules. Within the granule, adjacent amylopectin short chains form double helices that associate into clusters. The clusters pack into ordered arrays of alternating crystalline and amorphous lamellae (Smith *et al.*, 2001). Together the crystalline and amorphous lamellae create semi-crystalline regions that alternate with amorphous regions

Figure 1. Starch granule organization. Adapted from Smith *et al.*, 2001.

that contain amylose interspersed with amylopectin (Jane *et al.*, 1992). Amylopectin is much less organized in the amorphous regions because amylose disrupts the structural order of the amylopectin crystallites (Jenkins and Donald, 1995).

Cooking or other thermal processing with sufficient water may cause starch granules to gelatinize (Karim *et al.* 2000; Zaritzky, 2000). Gelatinization is characterized by water diffusion into the granule, granule swelling, loss of crystallinity, amylose

leaching and formation of a paste-like gel (Olkku and Rha, 1978; Hoover, 1995). The starch gel consists of gelatinized granules embedded within a continuous amylose network (Zaritzky, 2000) and may act as an adhesive to bind feed ingredients (Lund, 1984). The degree of starch gelatinization and subsequent particle binding are dependent on several factors, including granule size, amylose:amylopectin ratio (Madsen and Christensen, 1996), amylopectin branch chain length (Jane and Chen, 1992), diet composition (Bean and Osman, 1959; Ghiasi *et al.*, 1982), mash moisture (Moritz *et al.*, 2002, 2003), steam conditioning (Heffner and Pfoest, 1973; Skoch *et al.*, 1981), and die specifications.

Dietary starch gelatinization does not typically exceed 25-30% (Moritz *et al.*, 2003; Moritz *et al.*, 2005) due to the pelleting process. Moisture is a limiting factor for starch gelatinization in broiler diets. Moisture addition to the mixer was reported to decrease the peak temperature of starch gelatinization, as well as overall starch gelatinization (Moritz *et al.*, 2003). In a previous study, starch gelatinization was increased significantly by increasing mash moisture (Moritz *et al.*, 2001). Water acts as a plasticizer that decreases the glass transition temperature of starch (Zaritzky, 2000), as demonstrated by Moritz *et al.* (2003). However, water also acts as a lubricant (Moritz *et al.*, 2003) and reduces frictional and compression forces (Cavalcanti and Behnke, 2005a; 2005b) in the pellet die. Moritz *et al.* (2001) observed increased starch gelatinization when moisture was added to low moisture diets (~ 8%) possibly due to water being deficient for appreciable gelatinization at that level.

The extent that starch gelatinization affects pellet quality has been brought into question (Wood, 1987), although in practical broiler diets, gelatinization may play a

larger role in determining pellet quality than in the past. Wood (1987) pelleted diets with different types of starch (native and pregelatinized) and protein (native and predenatured). The pellet durability index (PDI) of pellets that contained 40% native starch and 60% native protein was 85. Replacing the starch fraction with pregelatinized starch only increased pellet durability by 11%. Starch gelatinization had a much greater affect when the protein fraction was predenatured, improving PDI from 19 to 70, with native vs. pregelatinized starch, respectively. Modern broiler diets are much more complex than those used in much of the research evaluating the relative effects of starch and protein on pellet quality. Therefore, the contribution of starch gelatinization on pellet quality remains equivocal, although it is likely that protein affects pellet quality to a greater extent.

VII. Protein Denaturation

Dietary protein has been regarded as the primary dietary factor that affects pellet formation and quality (Briggs *et al.*, 1999). This is despite protein comprising only 18-20% of most broiler diets compared to a 60-70% inclusion of starch. Moisture addition in the mixer decreased starch gelatinization but increased pellet durability index (Moritz, 2003), and modified pellet durability index increased with small increases (2 and 4%) of 48% CP soybean meal that were accompanied by reductions in total starch content (Buchanan and Moritz, 2009), indicating a greater contribution of dietary protein to feed ingredient agglomeration and subsequent pellet quality.

The major source of protein in United States broiler diets is solvent-extracted soybean meal. Approximately 90% of the protein fraction of soybeans is comprised of 7S (β -conglycinin) and 11 S (glycinin) storage globulins (Kinsella, 1979). Thermal

denaturation is a prerequisite for gel formation of globular proteins (Kinsella, 1976; Renkema *et al.*, 2002). At typical feed pH (~ 7.0), gelation coincides with glycinin denaturation (Renkema *et al.*, 2000). The amount of soy and animal protein denaturation that occurs during the pelleting process remains ambiguous. Aqueous dispersions of glycinin and soy protein isolate do not thermally denature below 85°C, as indicated by differential scanning calorimetry (Renkema *et al.*, 2000, Lakemond *et al.*, 2000). Broiler diets are low moisture systems (~10-15% moisture) and pelleting temperatures are typically below 85°C, so it is likely that much of the protein denaturation responsible for pellet formation is caused by frictional heat, as well as shear and compression forces in the pellet die.

The functionality of dietary proteins significantly affects pellet quality (Wood, 1987; Gehring *et al.*, 2009). Gehring *et al.* (2009) improved pellet quality with muscle proteins recovered from trout using a pH-shift process. Recovery of muscle proteins by solubilization at low or high pH and precipitation at their isoelectric point, yields partially-refolded proteins with increased gelation capacity compared to whole muscle (Gehring *et al.*, 2009). Pellet durability was increased by 33% compared to a corn-soybean meal-based control by addition of 5% trout protein paste.

VIII. Exogenous Enzymes

Broiler diets are commonly supplemented with exogenous enzymes in order to increase nutrient digestibility, decrease digesta viscosity, decrease diet cost and decrease nutrient excretion (Bedford, 2000, 2002). Although the benefits of exogenous enzyme addition are numerous and well-established, enzymes may be denatured during the pelleting process. Broiler diets are typically formulated below the intended nutrient

values, with the assumption that exogenous enzymes, such as phytase, protease, or carbohydrases, will improve nutrient utilization and make up the difference (Bedford, 2002). Thus, if a significant portion of the added enzyme preparation is denatured during pelleting, the diet will be nutrient deficient and may lead to poor broiler performance. Some research has focused on exogenous enzyme retention after pelleting (Inborr and Bedford, 1994; Spring *et al.*, 1996; Silversides and Bedford, 1999; Cowieson, *et al.*, 2005; Stark *et al.*, 2009), although the applicability of the data is questionable.

Intrinsic enzyme stability is determined by several factors such as amino acid sequence, three-dimensional structure and the sum of many non-covalent interactions such as hydrogen bonds, Van der Waals forces, hydrophobic interactions, and ion-pair networks (Yip *et al.*, 1995; Daniel *et al.*, 1996). Thermostable enzymes may be recovered from naturally-occurring thermophiles or thermostability may be conferred by targeted amino acid substitution or enzyme coating (Wyatt, 2009). Because there may be many different isoforms grouped under a single enzyme commission number, and commercial enzyme preparations differ greatly in terms of origin, production, and stability, it is not likely that retention data from one study can be applied to other enzymes, even of the same class. Care should be taken by feed mill managers to maintain pelleting temperatures below the published denaturation temperature for a particular enzyme. Additionally, lubrication of the pellet die by addition of fat in the mixer may improve enzyme retention and efficacy.

IX. Maillard Reaction

The Maillard reaction refers to a complex series of reactions occurring during food processing or storage, as well as *in vivo* in the tissues of birds, mammals, fish, and

reptiles (Panigrahy *et al.*, 1976; Cannon and Davison, 1978; Iqbal *et al.*, 1999; Fayle and Gerrard, 2002). Maillard reaction products are utilized to provide desirable flavors, aromas and colors to human foods (Ames, 1998; Martins *et al.*, 2001), although they are also associated with reduced nutritional quality (Martins *et al.*, 2001), especially with regard to production animal feed and thermally processed feed ingredients (Parsons *et al.*, 1992, Anderson-Haferman *et al.*, 1993; Zhang and Parsons, 1994). Various processing and storage variables influence the Maillard reaction (Ames, 1990), including diet formulation (nature and amount of reactants), cold mash moisture (water activity), steam conditioning (temperature and time) and die specification (retention time and pressure). Maillard reactions occur in three stages, described by Maureon (1981) as the initial Maillard reaction, the advanced Maillard reaction, and the final Maillard reaction.

The initial reaction involves the condensation of an amine and a carbonyl compound to form a Schiff base, and subsequent isomerization to form Amadori or Heyns products (Dills, 1993). The reaction typically occurs between a protein and a reducing sugar and primarily involves the ϵ -amino group of lysine (Dills, 1993), but may also involve the α -amino groups of terminal amino acids (Dills, 1993; Martin *et al.*, 2001) or crystalline amino acids used in broiler diets. The Schiff base cyclizes to form an N-substituted glycosylamine that undergoes subsequent Amadori or Heyns rearrangement (Dills, 1993).

The advanced Maillard reaction refers to the production of reactive intermediates and some of the initial flavor and aroma compounds (Maureon, 1981; Hodge, 1953). Amadori and Heyns products decompose to form a variety of highly reactive dicarbonyl degradation products that can further react with other reducing sugars or amino acids

(Hodge, 1953; Martins *et al.*, 2001). Further decomposition reactions yield heterocyclic compounds associated with flavor and aroma (Mauron, 1981; Hodge, 1953). The most apparent nutritional consequence of the Maillard reaction is the loss of sugars and amino acids (Dills, 1993). Glycosylamines are readily hydrolysable to yield the free amino acid and sugar but Amadori and Heyns products are not (Dills, 1993). Although the secondary Maillard reaction products do not yield available sugar or amino acids, many are absorbed as isopeptide moieties and are subsequently excreted in the urine (Valle-Riestra and Barnes, 1969; Ford and Shorrock, 1971; Hurrel and Carpenter, 1981), presenting problems with the interpretation of ileal and total tract digestibility assays (Wang and Parsons, 1998). Fernandez and Parsons (1996a) and Wang and Parsons (1998) expressed that most of the true digestible lysine determined using the precision-fed cecectomized rooster assay is available for protein synthesis and growth. In contrast, Batterham *et al.*, (1990; 1993), reported that ileal digestible Lys and Met in meat and bone meal were not totally bioavailable. According to Wang and Parsons (1998), most of the early Maillard reaction products would be determined to be digestible using the ileal digestibility assay but not in the precision fed rooster assay, because in the latter assay both urine and feces are collected.

Deoxysones and Strecker degradation products formed during the advanced Maillard reaction stage are precursors for high molecular weight brown polymers and copolymers known as Melanoidins (Ames, 1998; Kitts, 2007). Increased non-enzymatic browning has been associated with reduced nutritional quality of thermally-processed feed ingredients such as dried distillers grains with solubles (Fastinger *et al.*, 2006) and canola meal (Newkirk and Classen, 2002). Fastinger *et al.* (2006) reported that the

apparent and true amino acid digestibility of corn dried distillers grains with solubles decreased as the degree of lightness (L^*) and yellowness (b^*) reached values of 28-32 and 15-20, respectively.

Soluble non-starch polysaccharides reduce nutritional quality of poultry diets by increasing digesta viscosity (Antoniou *et al.*, 1981; Choct and Annison, 1990; Bedford, 2000) and reducing luminal nutrient and enzyme diffusion. It could be expected that melanoidins would increase digesta viscosity in a similar manner; however, the plasticization effect of water molecules released during the initial condensation reaction, apparently offsets the effect of the melanoidins, such that there is only a slight increase in viscosity (Manzocco and Maltini, 1999). Therefore, product darkening by melanoidins indicates decreased nutrient availability due to advanced Maillard reaction product formation rather than the final Maillard reaction products themselves.

X. Protein Cross-linking

Thermomechanical feed processing can also reduce amino acid digestibility by cross-linking reactions other than the Maillard reaction. Some of these reactions may hinder the interpretation of amino acid digestibility data because amino acids that are not released *in vivo* may be freed by acid hydrolysis (Evans and Butts, 1948; Moughan and Rutherford, 1996). One of the primary cross-linking reactions that may occur in soybean meal is the formation of an isopeptide linkage between the side-chain carboxyl groups of Asp or Glu and the ϵ -amino group of Lys (Block *et al.*, 1946). Such isopeptides are resistant to enzymic digestion but not to acid hydrolysis (Evans and Butts, 1948). Protein denaturation that occurs during thermomechanical processing may also be favorable for the formation of intra- and intermolecular cross-links involving other amino acids (Valle-

Riestra and Barnes, 1970). The resultant aggregated and cross-linked proteins are less accessible to digestive enzymes (Moughan and Rutherford, 2008) and thus, may reduce the utilization of amino acids that are not involved in the cross-links directly.

XI. Resistant Starch

A significant fraction (15-20%) of dietary starch may be recovered in the terminal ileum undigested (Noy and Sklan, 1995; Bedford, 2002). This fraction is termed resistant starch and consists of three general classes (Brown, 1996). Resistant starch 1 (RS1) is the portion of dietary starch that is unutilized by the bird because of encapsulation within plant material, such as cell wall components (Annison and Topping, 1994). The RS1 component may be highly available in terms of structure; however, the encapsulation retards the access of α -amylase to the substrate. Many cereal endosperm cells remain intact even after grinding and pelleting (Bedford, 2002) and thus, an appreciable amount of the RS1 starch may escape digestion.

Resistant starch 2 (RS2) refers to the portion of dietary starch that is undigested due to the size and structure of the native granule. Structural and chemical factors that may affect digestibility of starches include size (Franco *et al.*, 1992), protein encapsulation (Colonna and Mercier, 1985), crystalline structure (Brown, 1996) and amylose-lipid complexing (Holm *et al.*, 1983; Zimonja *et al.*, 2007). Starches that exhibit a B crystalline pattern are digested poorly and are found in potatoes, bananas and other tuber or high amylose starch sources (Annison and Topping, 1994; Brown, 1996). Conversely, cereal starches exhibit an A crystalline pattern and are more thoroughly digested than B pattern starches or legume starches, that exhibit a combination of A and B type structure (Annison and Topping, 1994). Native or ungelatinized starch is also

classified as RS2 (Englyst *et al.*, 1992) and is less accessible to pancreatic α -amylase than gelatinized starch.

The final class of resistant starch is RS3 or retrograded starch. Retrogradation occurs following thermal processing and is characterized by reassociation amylose molecules and stabilization of the structure by hydrogen bonding (Haralampu, 2000). When starch granules gelatinize, amylose is leached from the granules in the form of random coil polymers (Roos, 1995; Haralampu, 2000). Cooling initiates the formation of double helices and subsequent reassociation and hydrogen bonding to form indigestible crystalline (Wu and Sarko, 1978) complexes with proteins and cell wall structures (Bedford, 2002).

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**Improvement of pellet quality with proteins
recovered from whole fish using isoelectric solubilization-precipitation**

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SUMMARY

Muscle proteins are known to form thermally induced gel networks and may bind particles such as feed constituents. Dietary incorporation of functional proteins may improve pellet quality. Muscle proteins were recovered from rainbow trout by using isoelectric solubilization-precipitation. The objectives of this study were 1) to determine the proximate composition and amino acid content, AMEn, TMEn and true amino acid digestibility of trout protein isolate and paste (TPP), and 2) to determine the effects of TPP on feed manufacturing, pellet quality and true amino acid digestibility of broiler diets formulated to include TPP. Four treatments, consisting of 1) a control, 2) 5% TPP (HTPP), 3) 2.5% TPP (LTPP), and 4) 3.9% added moisture (MOIS) were manufactured at the West Virginia University pilot feed mill using a Latin square design. Five percent TPP resulted in the highest pellet quality, followed by LTPP, MOIS and the control. The improvement in pellet quality could be attributed to gelation of the recovered proteins. Addition of TPP reduced pellet mill production rate. Relative electrical energy usage decreased with MOIS but increased with HTPP relative to the control and LTPP treatments. Negative effects of increased viscosity likely associated with TPP, were absent with LTPP. Digestibility of Asp, Glu, Pro, Ile and Leu were lower for HTPP than the control. However, Ala digestibility was decreased in LTPP compared to with the control. Therefore, fish muscle proteins incorporated at less than 5% of the diet may be beneficial to the broiler industry as a pellet binder and nutritive feed additive.

DESCRIPTION OF PROBLEM

Pelleted diets improve broiler feed conversion and decrease overall broiler production costs compared to mash diets. However, feed in the broiler industry often contains mostly fines, instead of whole pellets. It is apparent that production cost benefits are more likely to be realized with high-quality pellets. Feed costs represent the majority of the total cost of broiler production [1]; consequently, substantial economic losses may be incurred by feeding diets with low pellet quality. Factors that may reduce pellet quality include a fast production rate, ingredients that inhibit pellet binding, and attrition stresses associated with transportation and handling [2]. Several strategies have been explored to improve the physical quality of pelleted feed, including moisture addition at the mixer [3-5], ingredient selection [6, 7], and the use of specific pellet binders, as reviewed by Thomas et al. [7]. Although various commercial pellet binders have demonstrated efficacy, increased pellet quality may be somewhat offset by nutrient dilution. Currently, very few nutritive pellet binders are available to the poultry industry.

Proteins affect pellet formation by binding feed particulates [7]. However, binding capability is inherently dependent upon protein functionality. To possess optimum functionality, proteins must be in a native or undenatured state. Wood reported that pellets had a harder texture in feeds containing raw soybean meal than in feeds using denatured soybean meal [8]. The ability of native, functional proteins to immobilize water and particulates within a stable gel network [9] may be applied to improve the physical quality of pelleted feeds.

A novel method for recovery of muscle proteins, known as isoelectric solubilization-precipitation, has been developed as an alternative to conventional surimi

processing [10-13], resulting in increased yield. Additionally, functionality may be improved, compared with surimi, after frozen storage or with addition of functional additives, or both [14]. Isoelectric solubilization-precipitation results in a 3-phase fractionation of fish raw materials (Figure 1): 1) a top phase (neutral lipids); 2) a middle phase (a soluble protein solution from which proteins are subsequently precipitated at their isoelectric point to yield a protein isolate); and 3) a bottom phase (bone, skin, stromal proteins, and membrane lipids). To curtail production expenses, functional muscle proteins may be recovered from fish processing by-products, low-value pelagic species or alternative underutilized species such as Antarctic krill [15]. Isoelectric solubilization-precipitation may be used to make novel and cost-effective fish-based feed additives for the poultry industry.

Muscle proteins recovered using isoelectric solubilization-precipitation retain functionality and their gel-forming ability is well documented [14-16]. Gelation of recovered muscle proteins may lead to improved pellet durability and resilience to post-pellet stresses. Pellet quality must be taken into account when developing strategies to optimize broiler performance. Thus, functional feed additives that lead to pellets better suited to withstand the rigors of transportation and handling will be beneficial to the poultry industry. The potential to support dietary amino acid requirements may also be advantageous. The objectives of this study were 1) to determine the proximate and amino acid compositions, AMEn, TMEn and true amino acid digestibility (**TAAD**) of proteins recovered from whole eviscerated fish using isoelectric solubilization-precipitation, and 2) to determine the effects of trout protein paste (**TPP**) on feed manufacturing, pellet quality, and the TAAD of broiler diets formulated to include TPP.

MATERIALS AND METHODS

Recovery of Trout Muscle Protein

Rainbow trout (*Onchorynchus mykiss*) were obtained from a West Virginia University aquaculture research facility. Trout were eviscerated, minced [17], and immediately vacuum packaged [18], blast frozen, and stored at -20°C. Trout protein isolate (**TPI**) was obtained using isoelectric solubilization-precipitation (Figure 1) [19]. Cryoprotectants [20], at a concentration of 8.3%, were added to the TPI before vacuum packaging and freezing at -20°C. After isolation of a sufficient volume of TPI to proceed with the experiment, 10.43 kg of TPI (with cryoprotectants) was thawed prior to each day of feed manufacture. Proteins in thawed TPI were extracted with 2.5% NaCl and mixed with protease-inhibiting and gel-enhancing additives [21]. The pH and moisture content were adjusted to 7.5 and 78%, respectively, to yield TPP. Care was taken throughout the process to maintain a temperature of 1 to 4°C to minimize autoproteolysis.

Before diet formulation, TPI was analyzed for amino acid content [22]. Concentrations of individual amino acids were calculated and corrected for moisture and content of additives. The AMEn of TPP was determined by precision feeding intact Single Comb White Leghorn roosters [23]. Proximate compositions of TPI and TPP also were determined [24].

Diet Formulation and Feed Manufacture

Experimental broiler grower diets were formulated to meet or exceed Cobb-Vantress specifications [25]. Diets were formulated to be isocaloric and similar in concentrations of amino acids and added fat. Sand was included in order to facilitate a high soybean oil inclusion and retain a similar nutrient profile. Accordingly, CP was

allowed to fluctuate to meet the aforementioned criteria. The concentration of soybean oil in each diet was formulated to be approximately equal, because pellet quality is known to decrease with increasing fat inclusion [26, 27]. Dietary treatments consisted of 1) the control; 2) 5% TPP (high TPP, **HTPP**); 3) 2.5% TPP (low TPP, **LTPP**); and 4) 3.9% added moisture (**MOIS**; Table 1). The addition of 3.9% moisture at the mixer is equivalent to the moisture added by a 5% inclusion of TPP. Moritz et al. [3-5] have demonstrated a substantial increase in pellet durability with addition of moisture at the mixer. Thus, it was imperative that a moisture treatment be incorporated to differentiate moisture and protein effects. Mash for all treatments was mixed for 20 min and batched before commencing with the experiment. Allotments of mash for each treatment were set aside in four batches to equal 300 lb (136 kg) after TPP or moisture addition. On each day of manufacture, TPP and moisture were added to their respective treatments. Diets were manufactured at the West Virginia University pilot feed mill [28] over four d, using a Latin square design. Trout protein paste was added to approximately 60 lb (27 kg) of mash in a horizontal ribbon mixer [28] and mixed for 5 min prior to addition to the LTPP or HTPP treatment. Moisture was added at the mixer in the form of a fine mist [29]. Mash containing TPP or added moisture was mixed for 20 min, and control mash was mixed for 5 min. After mixing, mash was conveyed to the conditioner-pellet mill. Mash was conditioned [30] to a steady-state temperature of 82°C (180°F). Steam pressure at the guage was 262 kPa (38 psig). Feed temperature was monitored with a digital thermometer inserted directly into the stream of conditioned mash and was controlled by manual adjustment of steam flow rate into the conditioner. Feeder rate of mash into the conditioner was constant among all treatments. Pellets were formed with a California

Pellet Mill [28] and cooled in a horizontal belt cooler. The time from pellet formation until exiting the cooler was approximately 2 min. Relative electrical energy usage of the pellet mill and conditioner were determined [31], as well as pellet mill production rate, bulk density, and percentage of fines.

One representative bag from each manufacturing run was reserved for determination of pellet durability. Pellet durability index (**PDI**) and modified pellet durability index (**MPDI**) [32] were determined on the day of manufacture.

TAAD and TME_n

True amino acid digestibility of pelleted treatments, TPI, TPP, and TME_n for TPI and TPP were determined by precision feeding cecectomized [33] Single Comb White Leghorn roosters (Hy-Line W-36). Digestibility assays were performed according to a modification of the procedure described by McNab and Blair [23]. Roosters were placed in individual raised wire cages equipped with cup drinkers, and feed was withheld for 24 h to evacuate their gastrointestinal tracts. Seven treatments were assigned to roosters in a randomized complete block design: 1) cornstarch, 2) TPI, 3) TPP, 4) control pellets, 5) LTPP pellets, 6) HTPP pellets, and 7) MOIS pellets. Four replications were conducted for each treatment. Cornstarch was used as a nitrogen-free control to account for endogenous losses. Wet samples (TPI and TPP) were lyophilized and ground to facilitate precision feeding. Aliquots of 25 g each were precision-fed using a 0.5-in (1.27-cm) tube inserted into the crop. Excreta were collected on plastic trays placed directly beneath each bird during a 24-h fasting period. Samples were weighed, air-dried and ground [34] prior to analysis. All treatments were evaluated for TAAD, and TPI and TPP samples were evaluated for TME_n. Excreta samples for TAAD determination were analyzed for

amino acid content [22], and excreta samples for TME_n determination were analyzed for gross energy [35] and N content [24]. TME_n was calculated according to the method of Parsons et al. [36], and TAAD was calculated according to the method of Sibbald [37]. Roosters used in the assays were handled in accordance with West Virginia University Animal Care and Use Committee guidelines.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS [38] to determine the effect of TPP inclusion on manufacturing variables and TAAD. Multiple comparisons of means were made using Fisher's LSD test. Values were considered significant at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Trout Protein Nutritional Value

Proximate composition and ME values for TPI and TPP are presented in Table 2. The TPI (% of DM) contained greater than 90% protein, as indicated by amino acid analysis (Table 3) [22]. Concentrations of CP, lipids, and ash in rainbow trout filet (% of DM) have been reported as 74.4-81.4, 12.9-13.4 and 5.1-5.6%, respectively [39, 40]. Comparatively, TPI contained 85.8, 5.1, and 3.1%, the latter two values becoming negligible on a wet (as-fed) basis. Isoelectric solubilization-precipitation likely resulted in removal of nonmuscle protein constituents and thus concentration the protein. Similar results were obtained by Chen et al. [45] using trout processing by-products.

Trout protein paste contained 2.9% fat (% of DM; Table 2) compared to 12.9-13.4% for trout filet. Hence, there would be little concern for reduction of sensory quality (i.e., off-flavors, odors, or both) of poultry meat caused by inclusion of fish-

derived muscle protein paste. Although some researchers have speculated that membrane lipids are sedimented with the bottom phase [15], it is possible that membrane lipids (phospholipids) are retained with the recovered proteins. Fish phospholipids are generally highly unsaturated and therefore readily oxidizable; however, at such a small inclusion of the total diet, their presence may not be a negative factor. Inclusion of fish meal is usually limited in commercial broiler diets because of product taint associated with highly oxidizable polyunsaturated fatty acids and trimethylamine [42], as well as the possibility of gizzard erosion or ulceration caused by gizzerosine formed through thermal processing [43]. The presence of prooxidants, such as heme proteins, also may contribute to product quality issues. Dietary fish meal has been associated with off-flavors and odors in broiler meat [44, 45]. Therefore, the isoelectric solubilization-precipitation product may be of interest to nutritionists, because muscle protein isolates retain only a small percentage of lipids and heme proteins, and the aforementioned concerns may be alleviated.

Nitrogen-corrected AME and TME (% as is; Table 2) of TPI and TPP are lower than that of most common feed additives [46]. The addition of functional additives to create TPP decreased the ME of TPI by approximately 350 kcal/kg, presumably because of decreased fat (5.1 vs 2.9%) and increased ash (.1 vs. 15.4%).

Acidic amino acids and lysine were found in the highest concentrations in recovered trout protein (Table 3). Trout protein paste had a similar amino acid composition to NRC [46] values for yellow dent corn. The concentration of each amino acid, with the exception of Cys, Leu, Phe, and Ser, was higher in TPP than in corn.

Concentrations of the first limiting amino acids in corn- and soybean-based diets, Met, Lys and Thr, were higher in TPP compared to corn by 11, 223 and 41%, respectively.

Manufacturing Variables

Several manufacturing variables were influenced by addition of TPP (Table 4). Pellet mill production rate was decreased with TPP addition at either 2.5 or 5% compared with the control ($P \leq 0.05$). Unexpectedly, production rate was decreased with moisture addition. This reduction was possibly due to pellet mill operator apprehensions of slipping or plugging the die, as the pellet mill may become plugged with excessive moisture in the mash [47]. Relative electrical energy usage by the pellet mill was decreased by MOIS but increased by HTPP (Table 4) compared with the control ($P \leq 0.05$). Past research has shown that moisture addition and reduction of electrical energy usage are correlated [5]. Relative electrical energy usage was not affected by LTPP. Five percent TPP likely affected production rate and relative electrical energy usage by increasing mash viscosity. Although higher viscosity may have negatively affected some manufacturing variables, higher viscosity may increase friction (i.e., frictional heat, at the mash-die interface), which may contribute to increased starch gelatinization and protein gelation, resulting in increased pellet durability.

Bulk density decreased with TPP or moisture addition compared with the control ($P \leq 0.05$; Table 4). This reduction of bulk density may be related to pellet quality. Although there were no treatment effects on production of fines ($P = 0.2682$), total fines were numerically reduced with either concentration of TPP or moisture addition. Moritz et al. [5] reported reduced feed bulk density as graded levels of moisture were added to corn- and soybean-based diets. They attributed this phenomenon to increased air space

with a greater percentage of intact pellets in relation to fines.

Pellet and Mash Moisture

The concentration of moisture in feed samples numerically followed the expected patterns for mash and hot pellets. Cool pellets demonstrated expected patterns of moisture concentration ($P \leq 0.05$; Table 5). Cool pellet moisture was 13.4, 14.5, 16.0, and 15.7% for the control, LTPP, HTPP, and MOIS treatments, respectively. As formulated, there was no difference between HTPP and MOIS ($P \leq 0.05$).

TAAD of Trout Proteins and Corn- and Soybean-Based Diets

The TAAD of pelleted diets and recovered trout protein (TPI and TPP) are presented in Table 6. Digestibility of TPI was greater than 95% for the majority of amino acids assayed. Digestibility of the majority of TPI amino acids decreased ($P \leq 0.05$) after addition of salt and functional additives (TPP). The cause of this phenomenon is unknown; however, the component responsible may be related to protein-based functional additives, specifically beef plasma protein (**BPP**) or transglutaminase (**TGase**).

Spray-dried BPP is known as an effective protease inhibitor and therefore as a gelation-enhancing additive for use in surimi products [48]. However, the use of BPP in the surimi seafood industry has been abandoned, largely because of fears of transmission of bovine spongiform encephalopathy [49]. Of the more than 60 different proteins [50] found in BPP, protease inhibition of BPP is attributed to kininogens and α_2 -macroglobulin ($\alpha_2\mathbf{M}$) [51]. Kininogens are specific inhibitors of cysteine proteases [52], such as lysosomal cathepsins, responsible for weakening surimi gels [53]. α_2 -Macroglobulin is a nonspecific protease inhibitor able to inhibit all 4 classes of proteases

[54]. Protease inhibition by α_2 M is facilitated by irreversible binding of substrate, resulting in steric hindrance to large macromolecules [54]. It is possible that a quantity of α_2 M would be complexed to endogenous proteases in trout muscle; thus the concentration of functional α_2 M available to inhibit gastric and pancreatic enzymes would be less than that added to TPI. Additionally, under certain conditions, BPP has been shown to improve performance of broilers [55] and pigs [56]. Ravindran and Morel [57] demonstrated higher ileal amino acid digestibility of spray dried BPP compared with soybean meal in broiler chickens, indicating that digestibility may not be diminished by inclusion of bovine plasma in broiler diets. Further research is required in order to determine if BPP is, in fact, responsible for decreased amino acid digestibility of TPP as compared to TPI.

The TGase (protein-glutamine γ -glutamyltransferase, E.C. 2.3.2.13), is a widely disseminated enzyme [58] commonly used in the food industry to catalyze the formation of ϵ -(γ -glutamyl) lysine covalent cross-links [59], that are responsible for an increased “suwari” effect, or gel setting [60]. Digestibility of ϵ -(γ -glutamyl)lysine bonds has been disputed. Tang et al. [61] showed that in vitro digestibility of casein by pepsin or trypsin was decreased significantly following microbial TGase treatment [65]. In contrast, high bioavailability of the isopeptide moiety was reported in rats [62], chicks [62], and pigs [63].

With respect to the extremely low concentration of BPP and TGase in the LTPP and HTPP treatments, there may have been no effect on amino acid digestibility of the pelleted feed. Diets containing TPP, compared with the control, did not affect digestibility of Cys, Lys, Met, Thr or Val ($P > 0.05$) regardless of the inclusion level. At

a concentration of 2.5%, TPP decreased digestibility only for Ala ($P > 0.05$). Digestibility of Asp, Glu, Ile, Pro and Leu were lower for HTPP than the control ($P < 0.05$); however, TAAD of HTPP was not different ($P > 0.05$) from that of LTTP or MOIS for all amino acids, with the exception of Ile. Reduced digestibility associated with protein paste may be a function of feed viscosity. Increased digesta viscosity has been associated with decreased performance and nutrient availability in chickens [64, 65]. Large inclusions of TPP may decrease the availability of nutrients to digestive enzymes and reduce amino acid availability in a dose-dependent manner. However, this effect may be offset by increased pellet quality. To minimize undesirable effects on pellet mill production rate and electrical energy usage, as well as prevent decreased amino acid digestibility, it is the recommendation of the authors that TPP inclusion not exceed 5%.

TPP on Pellet Quality

Broiler diets in the United States are comprised primarily of corn; thus, starch gelatinization is typically the major physicochemical factor that affects pellet formation and integrity. As expected, feed industry representatives may be concerned predominantly with starch gelatinization [47]; however, protein also has been shown to affect pellet quality [47, 66] and protein gelation may be used to enhance the quality of pelleted feeds.

Muscle proteins added to broiler diets improved pellet quality ($P \leq 0.05$; Table 4). Pellet durability was increased by 33% compared with the control with a 5% inclusion of TPP. High TPP increased the PDI by 15% when compared with an equal addition of moisture (MOIS treatment). There was no difference in PDI between LTTP and MOIS; however, LTTP pellets had a greater MPDI ($P \leq 0.05$), despite having only half the

volume of moisture added to the cold mash. Inclusion of sand in the control was nearly 2-fold greater than in HTPP to maintain similar nutrient profiles within treatments. Sand inclusion may affect pellet quality; however, because LTPP had a higher pellet quality compared with MOIS, despite having more than 2 orders of magnitude more sand and without added-moisture, it is the opinion of the authors that sand inclusion did not confound the effect of TPP on pellet quality.

Increased PDI and MPDI of the TPP treatments (HTPP and both LTPP and HTPP, respectively) compared with the control and MOIS treatments indicate that moisture addition per se, was not responsible for these results. Pellet quality is defined by Cramer et al. [67] as the ability of a pellet to withstand mechanical agitations produced through conveying, transportation, and handling by birds. Thus, quality is not determined merely by post-manufacturing durability, but more notably by the integrity of pellets at the feeder. According to a survey of broiler pellet quality, pellet integrity is exhausted rapidly between the feed mill and the farm, resulting in fewer than 30% whole pellets presented to birds [68]. Incorporation of recovered trout proteins into broiler grower diets had a protective effect on pellet integrity (Figure 2). In a commercial setting, it is likely that an increased number of whole pellets would survive transportation and handling. Despite a higher PDI, MOIS pellets had a reduction of durability (PDI minus MPDI) equal to the control. Conversely, LTPP and HTPP pellets had a reduced loss of integrity in an inclusion-dependent fashion. The immobilization of large volumes of water and other ingredients by muscle protein gels has been well established. Chen and Jaczynski [69] demonstrated formation of a strong gel with trout muscle proteins, isolated under similar conditions, using isoelectric solubilization-precipitation.

Therefore, the data support the theory that gelation of functional recovered proteins improved pellet quality.

Inclusion of functional muscle proteins in broiler diets will likely result in a greater percentage of intact pellets in the feeder and may improve broiler performance. However, reduced digestibility observed for some amino acids is of concern and future research should elucidate the causative factor(s) affecting amino acid availability of feeds containing recovered muscle proteins. A feeding trial should be conducted to determine the effects of pelleted diets containing muscle protein paste on broiler performance.

The suitability of using isoelectric solubilization-precipitation to produce muscle protein pellet binders for production animal agriculture also must be ascertained. Laboratory batch processing would be impractical for commercial recovery of muscle proteins; however, Jaczynski [70] developed a method for continuous protein recovery using an industrial bioreactor system. Thus, recovery of muscle proteins for use in animal feeds may ultimately be economically viable, especially if used as a functional ingredient. Another concern is the amount of moisture present in the material. Muscle protein pastes are approximately 78% moisture and thus may present problems associated with transportation and handling. It may be beneficial to remove moisture (i.e., lyophilize recovered muscle protein liquid or isolate and add water at the mixer) to reduce shipping costs and ease handling. Currently, the best application for this technology would be in the US Southeast, where broiler production and aquaculture are concentrated [77]. The close proximity of catfish-processing facilities to feed mills in the region may lead to reduced transportation and handling costs.

CONCLUSIONS AND APPLICATIONS

1. Muscle proteins recovered using isoelectric solubilization-precipitation improved pellet quality.
2. Five percent, but not 2.5%, trout protein paste increased pellet mill relative electrical energy usage and decreased pellet mill production rate.
3. Digestibility of some amino acids decreased in corn- and soybean-based diets containing trout protein paste.
4. Further research should 1) attempt to elucidate the ideal concentration of protein paste to maximize pellet quality improvement while retaining the nutritional quality of the diet; 2) determine the causative factors affecting amino acid digestibility in pelleted diets containing muscle protein paste; 3) determine the effects of pelleted feeds containing muscle protein paste on broiler performance; and 4) identify means to reduce production and handling costs.

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19. A processing flowchart for recovery of muscle protein from whole trout is shown in Figure 1. Cold (1 °C) distilled and deionized water (ddH₂O) was added to partially frozen, ground whole trout at a 1:6 ratio (trout:water, wt/volume). Because of endogenous protease activity, the temperature during the entire processing to recover trout muscle protein was carefully controlled at 1-4 °C. The homogenization-mixing was continued with a laboratory homogenizer (PowerGen 700, Fisher Scientific, Fairlawn, NJ) set at speed three. The pH of the homogenate was adjusted to 11.0 ± 0.05 with 10 *N* NaOH in order to solubilize trout proteins. The pH meter (model AB15, Fisher Scientific, Fairlawn, NJ) was calibrated frequently to ensure accurate readings during experiments. Once the desired pH was obtained, the solubilization reaction was allowed to proceed for 10 min, followed by centrifugation (Sorvall RC-5B Refrigerated Superspeed, Kendro Laboratory Products, Newton, CT) at 10000 x *g* for 10 min. The supernatant was collected, and the pH was adjusted to 5.50 ± 0.05 by 10 *N* HCl in order to isoelectrically precipitate trout proteins. Once the desired pH was obtained, the precipitation reaction was allowed to proceed for 10 min. The precipitated protein was centrifuged at 10000 x *g* for 10 min to remove free water.

20. Recovered trout proteins were mixed with a cryoprotectant mixture of 4% crystalline dihydrate trehalose (Cargill Inc., Wayzata, MN), 4% D-sorbitol (Sigma Aldrich Inc., St. Louis, MO) and 0.3% phosphate (PP; Kena FP-28, Innophos, Cranbury, NJ) (Cryoprotectant:TPI, wt/wt) in a benchtop mixer (Sunbeam Mixmaster model 2350, Sunbeam Products Inc., Boca Raton, FL).

21. Muscle proteins were extracted with 2.5% NaCl (wt/wt), followed by addition of functional additives, consisting of 4% potato starch (Penbind 1000 modified potato starch, Penford Food Ingredients Corp., Centennial, CO), 1% spray-dried beef plasma protein (Proliant Inc., Ankeny, IA), 1% microbial transglutaminase (Avtiva RM, Ajinomoto USA Inc., Teaneck, NJ) and 0.3% PP. Functional additives were incorporated as a percentage of the final weight.

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29. Moisture was added at a rate of 22 L/hr (determined by adding a known volume of water to the sprayer and timing the evacuation) using a 2 gal (7.57-L) compression sprayer.

30. A 4.25 ft length, 1.02 ft diameter short term California Pellet Mill conditioner (3 steam inlet ports), 429 rpm shaft speed; 21 picks; 10-s feed retention time.

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Table 1. Dietary Treatment Formulations and Composition.

Item	Dietary Treatment, ¹ %			
	Control	LTPP	HTPP	MOIS
Ingredient				
Corn	55.13	53.93	52.81	57.97
Soybean Meal	32.89	31.07	29.13	28.97
Soybean Oil	4.74	4.74	4.74	4.74
Sand	3.12	3.54	3.90	0.01
Tap Water	—	—	—	3.91
TPP	—	2.50	5.00	—
Dicalcium Phosphate	1.72	1.74	1.77	1.75
Limestone	1.21	1.21	1.21	1.23
Salt	0.41	0.41	0.41	0.41
DL-Methionine	0.28	0.31	0.38	0.33
NB-3000 ²	0.25	0.25	0.25	0.25
L-Lysine hydrochloride ³	0.11	0.17	0.23	0.26
Coban 60 ⁴	0.08	0.08	0.08	0.08
BMD 50 ⁵	0.05	0.05	0.05	0.05
L-Threonine	0.01	0.01	0.04	0.01
Calculated Values				
Crude Protein, %	21.00	21.00	21.00	19.50
Ether Extract, %	7.12	7.06	7.00	7.19
AME _n , kcal/kg	3172	3107	3041	3172
Lysine, %	1.25	1.25	1.25	1.25
Methionine, %	0.61	0.62	0.68	0.63
TSAA, %	0.96	0.96	1.00	0.96
Threonine, %	0.83	0.80	0.80	0.80
Tryptophan, %	0.28	0.26	0.25	0.25
Calcium, %	0.88	0.88	0.88	0.88
P, Available, %	0.42	0.42	0.42	0.42
Sodium, %	0.17	0.17	0.17	0.17
Analyzed Values ⁵				
Crude Protein, %	19.24	18.28	19.40	16.82
Ether Extract, %	5.18	5.27	5.30	5.22
Ash, %	9.49	9.57	10.84	6.19

¹CON = control; LTPP = low trout protein paste (2.5%); HTPP = high trout protein paste (5%); MOIS moisture

²Supplied per kg of diet: manganese, 0.02%; zinc 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69mg; choline, 386mg; riboflavin, 6.61mg; biotin, 0.03mg; vitamin B 6, 1.38mg; niacin, 27.56mg; pantothenic acid, 6.61mg; thiamine, 2.20mg; manadione, 0.83mg; vitamin B12, 0.01mg; vitamin E, 16.53 IU; vitamin D₃, 2133 ICU; vitamin A, 7716 IU.

³Ajinomoto Heartland LLC (Chicago, IL).

⁴Active drug ingredient Monensin Sodium 60gpb (90g/ton inclusion).- Elanco Animal Health, Indianapolis, IN. As an aid in the prevention of coccidiosis caused by Eimeria necarix, Eimeria tenella, Eimeria acervulina, Eimeria brunette, Eimeria mivati, and Eimeria maxima

⁵Bacitracin Methylene Disalicylate 50g/lb (50g/ton inclusion)- Alpharma, Fort Lee, NJ. For increased rate of weight gain and improved feed efficiency.

⁶Analysis performed in triplicate on cool pellets manufactured on day 3.

Table 2. Proximate composition and ME of trout protein isolate and trout protein paste (mean $\pm$ SD).

Component (%)	Trout Protein Isolate (mean $\pm$ SD)	Trout Protein Paste (mean $\pm$ SD)
Moisture	73.27 $\pm$ 0.02	78 $\pm$ 1
Dry Matter ¹	26.73 $\pm$ 0.02	22 $\pm$ 1
Ash ¹	3.12 $\pm$ 0.01	15.40 $\pm$ 0.08
Crude Protein ¹	85.77 $\pm$ 0.18	36.78 $\pm$ 0.43
Amino Acids ^{1,2}	90.37 $\pm$ 1.15	40.33 $\pm$ NA ³
Ether Extract ¹	5.11 $\pm$ 0.02	2.89 $\pm$ 0.38
(kcal/kg)		
AME _n ⁴	1,117 $\pm$ 115	794 $\pm$ 53
TME _n ⁴	1,232 $\pm$ 114	902 $\pm$ 52

¹Calculated on a DM basis.

²The value for trout protein isolate was determined by HPLC [21]. The value for trout protein paste is estimated based on a conversion factor of 6.96, which explains the difference between CP and HPLC data.

³NA = not applicable.

⁴Calculated on a wet (as-is) basis.

Table 3. Amino acid profile of trout protein isolate and trout protein paste (mean $\pm$ SD)¹.

Amino Acid	Trout Protein Isolate, %		Trout Protein Paste, %	
	DM Basis	As-Is Basis (73.27% Moisture)	DM Basis	As-Is Basis (78% Moisture)
Alanine	5.2 $\pm$ 0.1	1.4 $\pm$ 0.0	2.2 $\pm$ 0.0	0.5 $\pm$ 0.0
Arginine ²	5.8 $\pm$ 0.1	1.5 $\pm$ 0.0	2.4 $\pm$ 0.0	0.5 $\pm$ 0.0
Aspartic Acid	9.4 $\pm$ 0.1	2.5 $\pm$ 0.0	4.0 $\pm$ 0.0	0.9 $\pm$ 0.0
Cysteine	1.0 $\pm$ 0.0	0.3 $\pm$ 0.0	0.6 $\pm$ 0.0	0.1 $\pm$ 0.0
Glutamic Acid	13.5 $\pm$ 0.2	3.6 $\pm$ 0.0	6.0 $\pm$ 0.0	1.3 $\pm$ 0.1
Glycine	3.9 $\pm$ 0.1	1.0 $\pm$ 0.0	1.6 $\pm$ 0.0	0.4 $\pm$ 0.0
Histidine ²	2.6 $\pm$ 0.1	0.7 $\pm$ 0.0	1.1 $\pm$ 0.0	0.2 $\pm$ 0.0
Hydroxylysine	0.01 $\pm$ 0.01	—	—	—
Hydroxyproline	—	—	—	—
Isoleucine ²	4.6 $\pm$ 0.1	1.2 $\pm$ 0.0	2.0 $\pm$ 0.0	0.4 $\pm$ 0.0
Lanthionine	0.02 $\pm$ 0.02	0.01 $\pm$ 0.00	0.05 $\pm$ 0.01	0.01 $\pm$ 0.00
Leucine ²	8.4 $\pm$ 0.1	2.2 $\pm$ 0.0	3.5 $\pm$ 0.0	0.8 $\pm$ 0.0
Lysine ²	8.9 $\pm$ 0.1	2.4 $\pm$ 0.0	3.8 $\pm$ 0.0	0.8 $\pm$ 0.0
Methionine ²	3.1 $\pm$ 0.0	0.8 $\pm$ 0.0	0.9 $\pm$ 0.0	0.2 $\pm$ 0.0
Ornithine	0.12 $\pm$ 0.00	0.03 $\pm$ 0.00	0.01 $\pm$ 0.01	—
Phenylalanine ²	4.0 $\pm$ 0.1	1.1 $\pm$ 0.0	1.8 $\pm$ 0.0	0.4 $\pm$ 0.0
Proline	2.7 $\pm$ 0.0	0.7 $\pm$ 0.0	1.5 $\pm$ 0.0	0.3 $\pm$ 0.0
Serine	3.0 $\pm$ 0.1	0.8 $\pm$ 0.0	1.6 $\pm$ 0.0	0.3 $\pm$ 0.0
Taurine	0.14 $\pm$ 0.00	0.04 $\pm$ 0.0	0.09 $\pm$ 0.01	0.02 $\pm$ 0.00
Threonine ²	4.1 $\pm$ 0.1	1.1 $\pm$ 0.0	1.8 $\pm$ 0.0	0.4 $\pm$ 0.0
Tryptophan ²	1.1 $\pm$ 0.0	0.3 $\pm$ 0.0	0.7 $\pm$ 0.0	0.1 $\pm$ 0.0
Tyrosine	3.5 $\pm$ 0.1	0.9 $\pm$ 0.0	1.5 $\pm$ 0.0	0.3 $\pm$ 0.0
Valine ²	5.4 $\pm$ 0.1	1.4 $\pm$ 0.0	2.4 $\pm$ 0.0	0.5 $\pm$ 0.0
TEAA ³	48.0	12.8	20.2	4.5
TAA ⁴	90.1	24.1	39.0	8.6
TEAA:TAA	53.2	53.2	51.9	51.9

¹Values are rounded for clarity. Mean and standard deviation for hydroxylated amino acids, ornithine and taurine are presented to the hundredths place because they comprise only a small fraction of total amino acids yet are detectable in some samples.

²Essential amino acids.

³Total essential amino acids.

⁴Total amino acids (hydroxylysine, hydroxyproline, lanthionine, ornithine and taurine are omitted because they are not included in the 20 most common amino acids).

Figure 1. Flowchart for recovery of muscle proteins from whole trout using isoelectric solubilization/precipitation.

Table 4. Effect of trout protein paste on manufacturing variables.

Item	P-Rate, ¹ metric tons/h	REEU – P ² (kW·h/metric ton)	REEU – C, ³ kW·h/metric ton	Bulk Density, lb/ft ³ (kg/m ³)	Total fines ⁴ ,%	PDI, ⁵ %	MPDI, ⁶ %
Treatment ⁷							
CON	0.95 ^a	5.51 ^b	0.35 ^b	40.38 ^a (646.83)	36.09	65.49 ^c	37.02 ^d
LTPP	0.91 ^b	4.92 ^{bc}	0.34 ^b	39.78 ^b (637.21)	28.59	78.37 ^b	54.46 ^b
HTPP	0.89 ^b	6.45 ^a	0.41 ^a	39.15 ^c (627.12)	26.45	87.19 ^a	70.94 ^a
MOIS	0.91 ^b	4.48 ^c	0.35 ^b	39.20 ^c (627.92)	26.17	75.58 ^b	47.15 ^c
P-value	0.0413	0.0090	0.0303	0.0018	0.2682	<0.0001	<0.0001
LSD ⁸	0.035	0.922	0.049	0.4571	—	3.126	4.453
SEM ⁹	0.010	0.267	0.014	0.1321	3.571	0.9033	1.287

^{a-c}Means within a column with no common superscript differ significantly ($P \leq 0.05$).

¹Pellet mill production rate; kW·h = kilowatt hour.

²Relative electrical energy usage (pellet mill).

³Relative electrical energy usage (conditioner).

⁴Total fines were calculated as [(% of bagged fines x kg of bagged feed) + (% of cooler fines x kg of cooler feed)]/kg of feed produced. Bagged fines were determined with feed from the same bag that was used for pellet durability index and modified pellet durability index determinations. Cooler feed is designated as feed collected below the cooler. Fines were determined by weighing 500 g of feed, sifting through a no. 6 American Society for Testing and Materials screen, weighing the sifted pellets, and determining the percentage of fines.

⁵Pellet durability index.

⁶Modified pellet durability index (utilizing five 13-mm hex nuts for added pressure on pellets).

⁷LTPP = low trout protein paste (2.5%); HTPP = high trout protein paste (5%); MOIS = moisture

⁸Fisher's LSD.

⁹Pooled SEM.

Figure 2. Protective effect of TPP against pellet degradation. ^{a-c}Bars with no common letter differ significantly ($P \leq 0.05$). CON = control; LTPP = low trout protein paste (2.5%); HTPP = high trout protein paste (5%); MOIS = moisture.

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Table 5. Pellet and mash moisture

Item	Mash	Hot Pellet ¹	Cool Pellet ²	Day-3 Pellet ³
Treatment ⁴				
Control	10.68 ^b	14.93	13.44 ^c	12.25 ^b
LTTP	12.58 ^a	16.89	14.48 ^b	13.25 ^b
HTTP	14.22 ^a	18.41	15.96 ^a	14.56 ^a
MOIS	13.59 ^a	18.89	15.68 ^a	14.53 ^a
<i>P</i> - value	0.0082	0.0595	<0.0001	0.0100
LSD ⁵	1.643	—	0.4238	1.231
SEM ⁶	0.4747	0.8545	0.1225	0.3556

^{a-c}Means within a column with no common superscript differ significantly ($P \leq 0.05$).

¹Samples taken as pellets exited the pellet mill.

²Samples taken as pellets exited the cooler.

³Cool pellets analyzed for moisture after 3 d of ambient temperature storage.

⁴LTTP = low trout protein paste (2.5%); HTTP = high trout protein paste (5%); MOIS = moisture

⁵Fisher's LSD value.

⁶Pooled SEM.

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Table 6. True amino acid digestibility of pelleted diets and isoelectrically solubilized-precipitated trout proteins¹

Item	Amino acid, %											Average ²
	Ala	Asp	Cys	Glu	Ile	Leu	Lys	Met	Pro	Thr	Val	
Treatment ³												
Control	83.66 ^{bc}	89.30 ^c	74.71 ^{bc}	92.41 ^b	88.41 ^c	90.69 ^{bc}	93.28 ^{bc}	93.55 ^{bc}	90.86 ^{ab}	83.27 ^{bc}	86.46 ^c	87.64
LTPP	77.17 ^d	86.57 ^{cd}	65.13 ^c	90.11 ^{bc}	84.69 ^{cd}	88.13 ^{cd}	90.13 ^c	92.17 ^c	88.25 ^{bc}	82.27 ^c	83.89 ^c	88.13
HTPP	80.35 ^{cd}	84.95 ^d	65.94 ^c	89.29 ^c	83.47 ^d	87.20 ^d	89.96 ^c	92.09 ^c	84.98 ^c	80.28 ^c	82.51 ^c	87.20
MOIS	80.71 ^{cd}	88.46 ^{cd}	73.13 ^{bc}	91.79 ^{bc}	87.73 ^c	90.25 ^{cd}	91.42 ^c	93.13 ^{bc}	91.10 ^{ab}	82.48 ^c	86.51 ^c	90.25
TPI	96.30 ^a	97.86 ^a	89.75 ^a	97.87 ^a	97.86 ^a	98.13 ^a	97.18 ^a	98.40 ^a	96.08 ^a	96.74 ^a	97.54 ^a	98.13
TPP	87.99 ^b	93.44 ^b	78.86 ^b	93.13 ^b	92.94 ^b	93.99 ^b	96.25 ^{ab}	94.81 ^b	88.75 ^{bc}	90.45 ^{ab}	91.79 ^b	93.99
<i>P</i> -value	0.0001	<0.0001	0.0011	0.0005	<0.0001	<0.0001	0.0035	0.0002	0.0156	0.0021	<0.0001	—
LSD ⁴	6.252	3.736	10.10	3.113	4.109	3.350	3.868	2.201	5.539	7.517	4.784	—
SEM ⁵	2.074	1.239	3.352	1.033	1.363	1.111	1.283	0.7303	1.838	2.494	1.587	—

^{a-d}Means within a column with no common superscript differ significantly ($P \leq 0.05$).

¹Determined with cecectomized SCWL roosters.

²Mean digestibility of amino acids assayed within a row.

³LTPP = low trout protein paste (2.5%); HTPP = high trout protein paste (5%); MOIS = moisture; TPI = trout protein isolate; TPP = trout protein paste. TPP contained cryoprotectants (4% sorbitol, 4% trehalose, 0.3% polyphosphate, wt/wt), 2.5% NaCl and functional additives (3% potato starch, 1% beef plasma protein, 1% transglutaminase, 0.3% polyphosphate, wt/wt).

⁴Fisher's LSD value.

⁵Pooled SEM.

**Increasing Mixer-Added Fat Reduces the Energy Required for
Pelleting and Improves Exogenous Enzyme Efficacy for Broilers**

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Running Title: **Fat Addition and Broiler Performance**

Primary Audience: feed manufacturers, nutritionists, researchers

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SUMMARY

The price of fat and diet formulation with exogenous enzymes have reduced the amount of fat added to broiler diets. Although diets may be cheaper to produce, there is potential for nutrient and exogenous enzyme destruction with low inclusions of fat (0.5-1%) because of frictional heat and shear forces produced in the pellet die. Additionally, much of the added fat may be applied using post-pellet spray application and the amount of fat added in the mixer may become negligible. Thermal inactivation of exogenous enzymes and occurrence of unfavorable reactions (e.g., Maillard reaction, protein cross-linking, etc.) may be abated by increased levels of fat included at the mixer prior to pelleting, i.e., mixer-added fat (MAF). This study evaluated the effects of MAF (1, 2.5 or 4%) with or without exogenous enzyme addition (carbohydrase, protease and phytase), and at different conditioning temperatures (82 or 85°C), on feed manufacture and finisher phase broiler performance variables, and processing yields. Increasing MAF reduced the electrical energy usage required to manufacture broiler feed ($P < 0.0001$). Feed intake and live weight gain were increased with enzyme addition ($P < 0.0001$). Enzyme addition, conditioning temperature and MAF interacted in their effects on feed conversion ratio ($P = 0.0093$). Overall, enzyme addition decreased feed conversion ratio, but the effect was greatest with 1% MAF and 82°C or 4% MAF and 85°C. Increasing MAF and conditioning temperature reduced abdominal fat pad yield ($P = 0.0259$ and 0.0240 , respectively). There was no difference in carcass, breast or leg yield due to any of the factors ($P > 0.05$). It is likely that increased MAF improved exogenous enzyme retention and nutrient utilization by broilers. Subject to current fat prices, increasing MAF may reduce the total cost of broiler production, particularly when reduction of electrical energy usage is considered.

DESCRIPTION OF PROBLEM

Factors that enhance pellet quality by increasing feed temperature may also be deleterious to broiler performance by reducing nutrient availability and exogenous enzyme retention. The pelleting process involves raising temperature and moisture by injecting steam into mash feed and further increasing feed temperature during pellet formation due to mechanical friction at the mash-die interface. Exogenous enzymes may denature with sufficiently high feed temperature and moisture, and amino acids may become unavailable because of Maillard reaction or cross-linking.

Least-cost diet formulation does not account for interaction among feed ingredients or the effects of pellet manufacture on nutrient quality and subsequent broiler performance. The assumption that feed ingredient nutrient content is invariably static and additive is a fallacy [1] that may limit the performance of broilers fed least-cost diets. Current industry practice is to reduce the amount of added fat in broiler diets to reduce diet cost and improve pellet quality [2]. Very little fat may be added in the mixer because of the prevalence of exogenous enzymes that spare ME, and post-pellet fat application. The nutritional quality of pelleted broiler diets may be compromised if the amount of fat added in the mixer is below a critical level [2].

Fat added in the mixer may contribute to improved nutrient utilization and enzyme efficacy by coating feed particles [3, 4] and lubricating the pellet die [5, 6]. Die lubrication may also reduce the electrical energy usage required to pellet broiler feed [7]. The effects of fat added in the mixer on feed manufacture, broiler performance, and carcass composition were assessed by conducting feed manufacture and broiler grower and finisher phase performance studies using practical diets conditioned at different temperatures.

MATERIALS AND METHODS

Diet Formulations and Pellet Manufacture

Experiments were conducted to evaluate the effects of mixer-added fat (**MAF**) on finisher feed manufacture variables and broiler performance during 11-22 d grower and 22-38 d finisher production cycles. Broiler grower and finisher diets that contained 1 or 4% MAF were formulated using 2008 AgriStats, Inc. averages [8] with decreased ME, CP, AA and available P commensurate with producer provided enzyme nutrient matrix values (Tables 1 and 2). Diets that contained 2.5% MAF, were created by blending the 1 and 4% MAF diets. Grower and finisher positive control diets were formulated to have a higher plane of nutrition equal to the enzyme nutrient matrix values. The concentration of Trp was allowed to fluctuate to maintain equal or nearly equal inclusions of dried bakery product, corn distillers dried grains with solubles (**DDGS**), wheat middlings and corn gluten meal in diets differing in MAF. All diets were formulated to be isocaloric and isonitrogenous. Calculated nutrient density was held constant by the addition of washed play sand. All diets were formulated with practical ingredients found in commercial formulations.

Feed was manufactured at the West Virginia University pilot feed mill [9]. The experiments consisted of a 2 x 3 x 2 factorial arrangement of treatments with the addition of a positive control diet. Factors were enzyme inclusion (with or without), MAF (1, 2.5 or 4%) and conditioning temperature (82 or 85°C). Factor levels were constant between grower and finisher phases, except grower feed was conditioned at a high temperature of 93, rather than 85°C. The levels of MAF were chosen because previous research from the authors' lab indicates that nutrient availability may be compromised at industry levels (approximately 1%) but not at higher

levels (>3%) [2]. Enzymes were included to provide the following minimum activities: 300 U xylanase, 400 U α -amylase, and 4000 U subtilisin protease/kg of feed from a commercial enzyme cocktail [10] and 1,000 FTU phytase/kg of feed from a commercial *E. coli* phytase [11]. Diets were top-dressed with either 0.05% enzyme-cocktail and 0.0125% phytase, or an equal amount of sand.

Finisher feed was manufactured using 168 kg batches in a randomized complete block design. The blocking factor was day of manufacture and pelleting was conducted over four replicate days. Mixing was accomplished using a single-screw vertical mixer [9]. Each 168 kg batch represented the experimental unit to be steam conditioned and pelleted. Pellets were formed in a California Pellet Mill [9], fitted with a 30.48 cm (12 in), 4.76 mm (3/16 in) x 38.1 mm (1.5 in) die. Feed screw rate was adjusted to achieve an approximate production rate of 1.37 metric tons/hr. Speed of the conditioner [9] was constant (429 rpm) and mash feed was conditioned to a constant temperature of 82 or 85°C. Finisher feed was not able to be pelleted at 93°C, because the pellet die began to slip above 85°C. Prior to the start of each days pelleting, the die was warmed up with approximately 90 kg of feed. The first 45 kg of each batch used to reach conditioning temperature was discarded so that all feed used to assess broiler performance was manufactured at either 82 or 85°C.

Grower diets were mixed in a horizontal ribbon mixer [9] in 54.43 kg batches and combined to create 163.29 kg batches of feed to be pelleted. Each 163.29 kg batch was remixed for 5 min in the vertical screw mixer and conditioned and pelleted in an identical manner as finisher feed, with the exception of being steam conditioned at 93°C. However, due to a lower feed volume need pelleting was not replicated.

All pellets were reground to mash form using a roller mill to eliminate feed form effects on

broiler performance. Lilly *et al.* [12] demonstrated that pellet quality affects live weight gain and carcass weight of broilers when nutritional quality is maintained. The current study was designed to specifically evaluate the effects of MAF on exogenous enzyme efficacy at different conditioning temperatures without confounding from differences in pellet quality.

Variables Measured

Relative electrical energy usage of the pellet mill (**PREEU**) and conditioner (**CREEU**) [13], pellet mill motor load, and production rate were determined. Samples of mash, conditioned mash and cool pellets (pellets sampled immediately after exiting the cooler) were obtained for moisture analysis. Proximate analysis was conducted on cool pellet samples. Cool pellets were subsequently analyzed for crude protein, crude fat, and ash (% of DM) [14].

Separation of Sand and MAF Effects on Feed Manufacture

Sand inclusion increased with MAF inclusion because the diets were formulated to be isocaloric. To separate the effects of sand and MAF on pellet manufacture variables, a supplemental feed manufacture experiment was conducted utilizing a completely randomized design with a 2 x 2 factorial arrangement of treatments and 4 replications. Treatment combinations consisting of the low and high levels of sand and MAF used in the previous feed manufacture and performance studies (1.5 or 6% sand and 1 or 4% MAF), were manufactured during 1 d using a conditioning temperature of 85°C, and a die and feed screw rate identical to the previous feed manufacture experiments. Treatments were 1) 1% MAF and 1.5% sand, 2) 1% MAF and 6% sand, 3) 4% MAF and 1.5% sand, and 4) 4% MAF and 6% sand. Two 2,100 lb (953 kg) batches of a basal corn-soybean meal-based diet (treatment 1) [15] were mixed and allocated evenly into 4 sub-batches. A portion of the basal diet was removed from 3 sub-batches and replaced with the appropriate amounts of MAF and washed, play sand to create treatments 2-

4. In addition to PREEU and CREEU, bulk density, percentage of fines, and standard (PDI) and modified (**MPDI**) pellet durability [16] were determined.

Broiler Management

Feather-sexed Cobb 500 broiler chicks were obtained from a commercial hatchery. Chicks were placed in alternating, gender specific, 1.68 m² floor pens containing 22 chicks each. The research facility was a solid-sided negative pressure house with 3 mesh curtain-separated rooms. Temperature was regulated using thermostatically controlled forced air heaters in each room. Each pen had one hanging feeder, one drinker line with 3 nipples, and built-up litter top-dressed with clean pine shavings. During the initial 5 d, feed pans were added to each pen to ensure that chicks had sufficient access to feed. Brooding temperature was maintained initially at 33°C and decreased by ~3°C each week to achieve a final set point of 21°C. Broilers' thermal neutral zone was assessed by visual inspection and temperature was adjusted accordingly. The lighting regimen consisted of continuous light from d 1-3, 23L:1D from d 4-7, 20L:4D from d 8-24, and 23L:1D for the remainder of the study. Broilers were provided an industry-style corn-soybean meal-based starter diet in mash form for the initial 10 d post-hatch that met or exceeded 2008 AgriStats averages [8]. The starter diets were formulated to be as similar as possible to the 2.5% MAF diets of either the grower or finisher phase formulations. Feed and water were provided *ad libitum*.

On d 11, broilers were weighed individually by gender and placed in groups of 10 that did not differ significantly in weight. A total of 1,638 broilers (819 cockerels and 819 pullets) were used for the experiment. The experimental unit was a pen and each pen consisted of 9 male and 9 female broilers. Prior research conducted using the same facility has indicated blocking by location was ineffective with 12 or more treatments, because of blocks spanning rooms.

Therefore, a completely randomized design was implemented.

Performance and Carcass Measurements

On d 22 and 38, broilers were weighed and feed consumption was calculated. Mortalities were collected and recorded daily. Performance variables calculated included live weight gain (**LWG**), feed intake (**FI**), FCR (adjusted for mortality), and mortality. On d 38, one male and one female bird from each pen, whose weights were within ± 100 g of the mean weight of their respective genders, were selected for processing. Approximately 11% of each experimental unit was processed and used to determine carcass variables. On d 39, selected birds were placed in crates and transported to the West Virginia University pilot processing facility. Birds were stunned electrically, exsanguinated by cutting the jugular vein, scalded, plucked and eviscerated manually. Hot carcass, abdominal fat pad and bone-in leg quarter weights were recorded. The front half of each carcass was chilled in ice overnight and breasts were deboned manually and weighed. All carcass traits were calculated as weight relative to live weight. This was done to center the data so that the weights of carcass components were not confounded by live weight.

Statistical Analysis

Finisher phase and sand x MAF feed manufacture data was analyzed using three- or two-way factor effects ANOVA models with interaction terms, respectively. For broiler performance and carcass measurements, data was analyzed by analysis of covariance, with room as a covariate. Significance was determined by $\alpha \leq 0.05$. Multiple comparisons of means were made using Tukey's honestly significant difference test. The statistical analyses were conducted using the R statistical software package [17].

Animal Care and Use

All animals and facilities were maintained in compliance with guidelines established by the

West Virginia University Animal Care and Use Committee.

RESULTS AND DISCUSSION

Feed Manufacture

Increasing the level of MAF or conditioning temperature (**TEMP**) had a marked effect on the electrical energy required to pellet broiler feed (Table 3). Increasing MAF caused a concurrent decrease in PREEU ($P<0.0001$). On average, increasing MAF from 1 to 4% of the diet reduced PREEU by 2.97 kW·h/metric ton. Contrary to the effect of MAF, increasing TEMP from 82 to 85°C increased PREEU ($P=0.0512$). Mixer-added fat also affected CREEU and pellet mill motor load. Increasing MAF increased CREEU ($P=0.0181$) but decreased motor load ($P<0.0001$). Because of the relatively minor effect of CREEU on total energy usage compared to PREEU, overall, increasing MAF from 1 to 4%, decreased total electrical energy usage (PREEU minus CREEU) by 42%. Added fat lubricates the pellet die [5, 6, 18], decreasing the coefficient of friction between the interior of the die ports and conditioned mash. Lubrication of the mash-die interface reduces the amount of energy necessary to overcome the opposing frictional force and extrude conditioned feed. In addition to decreasing relative electrical energy usage and loss of heat-labile nutrients, die lubrication by MAF may reduce wear on the pellet die and motor [18]. Pellet mill motor load ($P<0.0001$) was reduced by approximately 12% by increasing MAF from 1 to 4%. It is unclear why relative electrical energy usage increased when feed was conditioned at 85 compared to 82°C. Several authors have reported reduced pellet mill electrical energy usage for steam conditioned and pelleted diets compared to dry pelleted diets [19, 20]. Skoch *et al.* [20] proposed that because steam acts a lubricant, mechanical friction is reduced. Cowieson *et al.* [21] reported increased diet viscosity when wheat-based diets were conditioned at temperatures above 80°C. They attributed the occurrence to release of previously

encapsulated non-starch polysaccharides (**NSP**). In the current study, the finisher diet formulations had nearly 7% wheat middlings and DDGS, although it is the difference in free NSP following conditioning at 82 compared to 85°C was not assessed in the current study. The effect of mixer-added fat on conditioner relative electrical energy usage may be related to the ability of fat to coat feed particles [3, 4] and reduce their interaction with heat and moisture. Thus, slightly reduced plasticization and mobility of conditioned feed ingredients may be responsible for marginally increasing the energy required for the conditioner picks to move through the feed mass.

Mash and cool pellet moisture (Table 3) were only slightly affected by treatments (range of < 1 percentage point). Increasing MAF decreased mash and cool pellet moisture ($P \leq 0.05$); however, the effect was small enough that no differences were detected by the mildly conservative HSD test. Moisture added by steam conditioning increased mash moisture by approximately 4 percentage points. No differences were detected ($P > 0.05$) due to any of the treatments.

Sand is often used as an inert filler or diluent in nutrition studies. However, little is known about the effects of sand on pellet manufacture variables, especially electrical energy usage and pellet quality. Sand has a coarse, grainy texture, similar to feed phosphates, and may affect pellet manufacture variables by die scouring. Die scouring may be dependent on particle size and density. For example, tricalcium phosphate has been reported to improve feed production rate [22] and reduce pellet mill electrical energy usage [23] compared to other feed phosphates in corn-soybean meal-based diets. In the current study, diets were made to be isocaloric and have similar ingredient compositions by adding washed play sand. The effects of sand and MAF were confounded, and therefore, a second feed manufacture experiment was

conducted to separate their effects (Table 4). Increasing sand or MAF increased production rate by 1 and 2%, respectively ($P < 0.05$). The effect of sand on production rate is likely due to the die scouring effect attributed to some feed phosphate sources [22]. The level of MAF significantly affected pellet mill motor load ($P < 0.0001$). From 1 to 4% MAF, motor load decreased by more than 6% ($P \leq 0.05$). Sand inclusion numerically increased pellet mill motor load ($P = 0.0878$). Increased electrical energy usage indicates greater frictional force at the mash-die interface. Conversion of mechanical energy to heat in the die affects pellet formation by increasing starch gelatinization and protein denaturation in the conditioned feed and this may have offset the physical effect of sand on pellet quality. Sand inclusion did not affect the production of fines ($P > 0.05$). The level of MAF significantly increased all pellet manufacture variables associated with fines production, including the amount of feed that fell through the cooler, cooler fines, bagged fines, and total fines ($P \leq 0.05$).

Sand and MAF inclusion affected PDI, MPDI, and bulk density of pelleted feed ($P \leq 0.05$). Increasing MAF significantly decreased PDI and MPDI, by 23 and 42 percentage points, respectively. The disparity between the PDI and MPDI at both levels of MAF indicate that increasing MAF reduces pellet elasticity, likely by coating feed particles and disrupting starch and protein reactions that are requisite for agglomeration. The difference between PDI and MPDI with 1% MAF and 1.5% sand was 6.25 percentage points and with 4% MAF and 1.5% sand was 24 percentage points. Other ingredients, such as water, impart a shift from control treatments in PDI and MPDI, without changing the difference [24], indicating a change in hardness but not elasticity. Pellets formed with greater amounts of fat would be less able to withstand transportation and handling stress than those formed by current industry practices. However, despite reduced pellet quality, increasing MAF up to 4% in corn-soybean meal-based

diets may not affect FCR or carcass yield, based solely on feed form, of broilers [12]. Lilly *et al.* [12] reported no difference in carcass weight or FCR of broilers fed diets with 30 or 60% pellets. Significant improvement of carcass weight was only achieved by increasing the percentage of pellets in the feed pan to 90%. Based on past surveys, this level of pellet quality is not typical of the broiler industry [25, 26].

Increasing sand up to 6% of the diet only slightly decreased PDI and MPDI ($P \leq 0.05$). Feed particles are primarily agglomerated by starch gelatinization and protein denaturation-aggregation. Feed constituents that disrupt the interaction of feed polymers reduce pellet quality, e.g., pellets often break along the outside of a corn particle. Sand would likely disrupt pellet formation in the same manner, although the effect was small, even at 6% of the diet. The effect may also be related to increased production rate and thus, reduced retention time in the pellet die. Bulk density increased as the level of sand or MAF was increased ($P \leq 0.05$). Sand likely improved bulk density because of its density, rather than by a change in feed form. In contrast, increasing MAF likely increased bulk density because of the greater percentage of fines associated with 4 versus 1% MAF. Moritz *et al.* [7] attributed decreased bulk density with greater pellet durability to the air space between high quality pellets. In the current study, the difference in bagged fines between 1 and 4% MAF treatments (7.99 compared to 26.08%, respectively) likely affected bulk density, because fines were able to fill in gaps between whole pellets.

Grower Phase Broiler Performance

Broiler performance variables are presented in Tables 5 and 6. Initial pen weight at 11 d of age did not differ significantly among treatments ($P > 0.05$). Factorial analysis (without the positive control) yielded a MAF x TEMP interaction ($P = 0.0248$). The interaction occurred

because on average, 2.5% MAF had a 10 g lower initial pen weight at 82°C, and a 15 g higher initial pen weight at 93°C, compared to 1 and 4% MAF. The difference equals less than 1g per bird and is probably why there was no difference detected on a treatment basis (Table 5.).

Exogenous enzyme addition (**ENZ**) and MAF interacted in their effects on feed intake ($P = 0.0299$). The addition of exogenous enzymes increased feed consumption of broilers fed 1 or 2.5% MAF diets compared to the same diets without ENZ. In contrast, broilers fed 4% MAF diets consumed less feed with ENZ compared to without. When the data was combined with the PC, there were no differences between individual treatments ($P > 0.05$). The interaction may have occurred because of an increase in transit time of ingesta. Mateos *et al.* [27] reported a linear increase in transit time when graded levels of yellow grease were included in diets fed to White Leghorn hens. Increased transit time may improve the digestibility of all diet components by providing digesta increased exposure to endogenous and exogenous enzymes.

There was an ENZ x MAF interaction on 11-22 d live weight gain (**LWG**) ($P = 0.0538$). On average, ENZ improved LWG by 200 g per bird; however, LWG of broilers increased when the level of MAF was increased in diets without ENZ but there was no effect of MAF on LWG in diets with ENZ. Past research conducted during a similar growth phase (8-21d), demonstrated that addition of α -amylase, xylanase and protease did not affect LWG [28]. However, Café *et al.* [29] demonstrated improved 16 and 35d LWG, using the same strain of broiler as the current study, when the same enzyme cocktail was fed. It is likely that phytase also improved LWG, although the individual effects of the different enzymatic activities on broiler performance variables cannot be elucidated in the current study. The PC diet elicited greater LWG than diets without ENZ formulated to a lower plane of nutrition ($P = 0.0399$). The addition of exogenous enzymes brought LWG back to the level of the PC ($P > 0.05$). Thus, nutrient matrix values for

the enzymes were appropriate for the grower phase. Steam conditioning feed at 93°C reduced live weight gain by 10 g per bird on average compared to conditioning at 82°C. Moreover, conditioning at 93°C decreased LWG compared to the PC ($P = 0.0508$). Pelleting improves broiler performance compared to mash [30, 31] primarily by reducing time and energy for prehension, improving palatability and thermal modification of starch and protein [32]. The beneficial effects of pelleting on starch and protein availability have been attributed to destruction of cell walls, destruction of heat-labile anti-nutrients and starch gelatinization [33]. Although the benefits of pelleting are numerous, over-processing may reduce broiler performance. Results of the current study are in agreement with prior research [21], demonstrating reduced LWG when feed is conditioned above approximately 80°C. Reduction of LWG may have occurred due to reduced protein solubility [34, 35], Maillard reaction product formation [33, 35] and/or release of previously encapsulated non-starch polysaccharides [21].

During the 11-22 d grower phase there were no differences ($P > 0.05$) in FCR or mortality. Feed conversion ratio followed similar trends as live weight gain (Table 4.) although the differences were not detectable with 95% confidence. Failure to detect differences in FCR may be related to the degree of feed consumption in the grower phase. The strain of broilers used in the current study typically only consume between 23 and 33% of their total feed intake from hatch to 21 d, when marketed at 35 to 42 d of age [37]. Thus, differences in performance due to dietary treatment are more likely to be realized during the finisher phase when the majority of feed is consumed.

Finisher Phase Broiler Performance

Initial body weights reflected differences in live weight gain during the grower phase (Table 5). Exogenous enzyme addition and higher conditioning temperature increased and

decreased initial (22 d) body weight, respectively. Use of 22 d body weight as a covariate was not significant for any of the variables tested. Feed intake and live weight gain were not affected by TEMP or MAF ($P > 0.05$). However, ENZ increased feed intake by 7.7% (179 g per bird) and live weight gain by 10.1% (120 g per bird) compared to feeding diets without exogenous enzymes ($P \leq 0.05$). Feeding the PC diet yielded the lowest LWG ($P \leq 0.05$). This likely occurred because there was not a great enough difference between the PC diet and experimental diets without ENZ, to yield improved broiler performance due to the PC.

Mixer-added fat, TEMP and ENZ interacted in their effects on 22-38 d FCR ($P = 0.0293$). On average, ENZ decreased FCR by 3 points (Table 4; $P \leq 0.05$) but the magnitude of the FCR response to ENZ depended on the level of TEMP and MAF. Exogenous enzyme addition to diets with 4% MAF, conditioned at 85°C, reduced FCR by 9 points compared to the same diet without ENZ. Diets with ENZ at 2.5 or 1% MAF, conditioned at 82°C, yielded reduced FCR of 6 and 4 points, respectively, compared to their non-ENZ counterparts. The primary substrate for carbohydrases is the portion of the digesta that becomes the ileal undigested fraction. Exogenous enzymes typically act upon a fixed percentage of the undigested fraction and therefore, their response is dependent on diet composition and digestibility [38]. A diet with lower basal digestibility will generally be improved more by ENZ than a diet that is more inherently digestible. Some of the reduction in nutritional quality that is attributed to overprocessing is not likely to be overcome by ENZ, specifically loss of amino acids due to cross-linking or Maillard reaction. The ileal undigested fraction may become larger, but rather than increasing the pool of substrate, the increase would likely be associated with the dietary enzyme-resistant fraction (typically 80-85% of the ileal undigested fraction). Thus, increased MAF in diets processed at high conditioning temperatures maximized enzyme substrate, that in part, improved enzyme

efficacy.

Increasing the inclusion of MAF to 3% or more may prevent reduced nutrient availability [2]. Cutlip et al [6] observed a 20 point reduction in FCR when broilers were fed diets conditioned at 93 versus 82°C with 3.09% MAF. The increase in temperature corresponded to increased pellet durability. Subsequent studies [2, 12] were designed to evaluate feed form effects on broiler performance. When MAF was included at 1.26% there were no differences in FCR even with a range in PDI from 71-96%. However, in a following study, when 3.09% MAF diets were used, there were significant feed form benefits on live weight gain, carcass weight and breast weight. In the current study, feed manufactured with 4% MAF and conditioned at 85°C was most responsive to ENZ. Prevention of exogenous enzyme denaturation and reduction of nutrient availability because of die lubrication may have also increased enzyme activity and subsequent release of previously unavailable nutrients.

One or 4% MAF reduced FCR compared to the PC ($P \leq 0.05$); however, individual factor level (marginal) means are confounded by ENZ, as indicated by the significant three-way interaction ($P = 0.0293$) on FCR and the overall large response to ENZ in the present study. The difference between the grower and finisher phase data may be related to conditioning temperature and the enzyme nutrient matrix values. A high conditioning temperature of 93°C was reached during grower feed manufacture and this may have exacerbated the lower nutrient density of the experimental treatments. Finisher feed could not be conditioned at 93°C because the pellet die began to slip near 85°C. Thus, the high conditioning temperature used for finisher feed was the highest temperature that could be reached for all diet formulations. In the finisher phase, experimental treatments yield LWG and FCR equal to or better than the PC. Only an available P matrix value was used for the exogenous phytase (1%) and amino acid values for the

enzyme cocktail were marginal ($\sim 0.01\%$). The data indicates that nutrient matrix values used for diet formulation may have been too conservative. It is likely that reduction of the ileal undigested fraction and thus, improved energy and protein utilization with the phytase addition, elicited performance above the positive control.

The addition of exogenous enzymes decreased mortality by nearly a full percentage point (0.92). The effect of exogenous enzymes on broiler mortality is equivocal. Some researchers have reported reduced mortality with enzyme supplementation [29, 39] and others have reported no effect on mortality [40, 41, 42]. In the current study late period mortality due to leg problems, began to increase marginally in non-ENZ birds. However, in general, the response to enzyme addition on mortality may be related to dietary or environmental stressors, such that exogenous enzymes only reduce mortality when basal mortality is sufficiently high.

39 d Broiler Processing Yields

Carcass and leg yields were not affected by any of the treatments (Table 6; $P > 0.05$). The author's feel that there was a strong trend ($P = 0.0683$) for a MAF x ENZ interaction on breast yield. The trend for an interaction occurred because, on average, ENZ to 1 and 2.5% MAF diets increased breast yield and ENZ to 4% MAF diets decreased breast yield compared to 1, 2.5 and 4% MAF diets without exogenous enzymes, respectively. Phytase addition to mash diets marginal in available P has been reported to improve carcass yield of broilers at 42 d of age [43]. It is likely that the trend for an interaction in the current study was associated with differences in enzyme or substrate concentrations due to pelleting. Although the conditioning temperature in the current study was not particularly high, other factors, such as mash moisture may have contributed to enzyme denaturation. It is estimated that steam conditioning causes a 1% increase in moisture for every 20-30°F increase in mash temperature [44]. In the current study, feed was

manufactured in January when ambient temperature was below freezing. Exogenous enzymes may be denatured to a greater extent in the winter months compared to summer because of increased moisture added during steam conditioning [45]. The increase in moisture in the current study may have promoted enzyme denaturation and provided opportunity for MAF to improve retention of the thermostable phytase.

The abdominal fat pad is the most apparent adipose depot in the broiler chick. The amount of adipose tissue deposition in the abdominal fat pad is correlated ($R^2 > 0.5$) to body and carcass fat [46]. In the current study, increasing MAF from 1 to 4% decreased abdominal fat pad yield ($P \leq 0.05$). Steam conditioning at 85°C decreased abdominal fat pad yield compared to conditioning at 82°C ($P \leq 0.05$). The main effect occurred because the data was pooled across levels of ENZ and MAF and the lowest abdominal fat pad yields occurred when broilers were fed diets that were steam conditioned at 85°C with 4% MAF. This data along with the previous FCR data indicate that broilers fed diets conditioned at 85°C with 4% MAF and ENZ, converted nutrients into saleable carcass more efficiently than all other broilers. Increasing MAF likely prevented the loss of amino acids during pelleting and improved protein utilization by promoting phytase, xylanase, and protease retention. Diets were formulated to have equal protein:calorie ratios, and a reduction of the protein:calorie ratio caused by reduced protein digestibility may increase whole body adipose tissue deposition [47, 48]. These results indicate that mixer-added fat lubricates the mash-die interface and at a minimum inclusion, improves exogenous enzyme efficacy and subsequent broiler performance by maximizing substrate and enzyme concentrations, and limiting the loss of amino acids that may occur during pelleting.

CONCLUSIONS AND APPLICATIONS

1. Increasing mixer-added fat improves exogenous enzyme efficacy and subsequent broiler performance by three possible mechanisms; 1) maximizing substrate and 2) exogenous enzyme concentrations, and 3) reducing the loss of amino acids associated with feed manufacture at high conditioning temperatures or with high mash moisture.
2. Increasing mixer-added fat reduces the electrical energy required for pelleting.
3. Broilers fed diets that were conditioned at 85°C with exogenous enzymes and 4% mixer-added fat demonstrated the most efficient conversion of nutrients to saleable carcass.
4. Sand included below 6% of the diet has little effect on pellet manufacture variables, and thus, can be used as an inert filler in both broiler performance and feed manufacture research.

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Table 1. Grower diet formulations and compositions.

Item	Positive Control	1% MAF	2.5% MAF	4% MAF
Ingredients, %				
Corn	46.81	50.66	45.22	39.78
Soybean Meal (44%)	16.52	10.94	15.97	21.0
Dried Bakery Product	9.0	9.0	9.0	9.0
Extruded Soybean Meal	11.05	13.5	11.05	8.59
Corn Gluten Meal (60%)	1.09	2.0	1.71	1.41
Wheat Middlings	1.84	1.98	1.98	1.98
Corn DDGS	1.98	4.0	4.12	4.24
Sand	4.32	1.5	3.75	6.0
Poultry By-product Meal	0.71	1.01	0.71	0.40
Hydrolyzed Feather Meal	0.48	0.69	0.48	0.27
Porcine Meat and Bone Meal	0.60	0.87	0.60	0.34
Limestone	1.07	1.36	1.38	1.39
Animal/Vegetable Fat Blend	2.5	1.0	2.5	4.0
Trical Phosphate	1.09	0.48	0.57	0.66
NB3000 ¹	0.25	0.25	0.25	0.25
DL-Methionine	0.24	0.23	0.25	0.26
L-Lysine HCl	0.17	0.23	0.18	0.13
Salt	0.18	0.17	0.18	0.19
Coban-90 ²	0.05	0.05	0.05	0.05
BMD-60 ³	0.04	0.04	0.04	0.04
L-Threonine	0.02	0.03	0.03	0.02
Calculated Analysis				
ME (kcal/lb)	1415	1380.6	1380.6	1380.6
CP (%)	21.0	20.5	20.5	20.5
TSAA (%)	0.91	0.90	0.90	0.90
Lys (%)	1.19	1.18	1.18	1.18
Thr (%)	0.80	0.79	0.79	0.79
Trp (%)	0.24	0.22	0.23	0.24
Ca (%)	0.85	0.85	0.85	0.85
P _{av} (%)	0.40	0.30	0.30	0.30
Na (%)	0.22	0.22	0.22	0.22
Analyzed Value				
Crude Protein $\pm$ SD, % of DM	22.25 $\pm$ 0.05	20.79 $\pm$ 0.52	21.11 $\pm$ 0.51	21.84 $\pm$ 0.79

Crude Fat $\pm$ SD, % of DM	10.29 $\pm$ 0.37	8.42 $\pm$ 0.22	10.04 $\pm$ 0.57	10.75 $\pm$ 0.58
Ash $\pm$ SD, % of DM	7.37 $\pm$ 0.58	6.63 $\pm$ 0.50	8.34 $\pm$ 2.05	10.66 $\pm$ 0.67

¹Supplied per kg of diet: manganese, 0.02%; zinc 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69mg; choline, 386mg; riboflavin, 6.61mg; biotin, 0.03mg; vitamin B 6, 1.38mg; niacin, 27.56mg; pantothenic acid, 6.61mg; thiamine, 2.20mg; manadione, 0.83mg; vitamin B12, 0.01mg; vitamin E, 16.53 IU; vitamin D₃, 2133 ICU; vitamin A, 7716 IU.

²Active drug ingredient Monensin Sodium 90g/lb (120g/ton inclusion).- Elanco Animal Health, Indianapolis, IN. As an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*

³Bacitracin Methylene Disalicylate 60g/lb (60g/ton inclusion)- Alpharma, Fort Lee, NJ. For increased rate of weight gain and improved feed efficiency.

Table 2. Finisher diet formulations and compositions.

Item	Positive Control	1% MAF	2.5% MAF	4% MAF
Ingredients, %				
Corn	53.60	57.36	51.77	46.17
Soybean Meal (44%)	19.44	16.93	19.89	22.85
Dried Bakery Product	9.0	9.0	9.0	9.0
Corn Gluten Meal (60%)	1.51	2.37	1.94	1.5
Corn DDGS	2.10	3.0	3.0	3.0
Wheat Middlings	3.81	3.81	3.81	3.81
Washed, Play Sand	2.87	1.5	3.75	6.0
Poultry By-product Meal	0.98	0.98	0.69	0.39
Hydrolyzed Feather Meal	0.67	0.68	0.47	0.27
Porcine Meat and Bone Meal	0.84	0.84	0.59	0.34
Limestone	1.05	1.37	1.39	1.4
Animal/Vegetable Fat Blend	2.5	1.0	2.5	4.0
Deflourinated Phosphate	0.75	0.21	0.3	0.39
NB3000	0.25	0.25	0.25	0.25
DL-Methionine	0.18	0.18	0.20	0.21
L-Lysine HCl	0.17	0.23	0.17	0.11
Salt	0.17	0.17	0.18	0.19
Coban-60	0.05	0.05	0.05	0.05
BMD	0.04	0.04	0.04	0.04
L-Threonine	0.03	0.04	0.04	0.03
Calculated Analysis				
ME, kcal/lb	1441	1406.6	1406.6	1406.6
CP, %	18.69	18.19	18.19	18.19
TSAA, %	0.80	0.79	0.79	0.79
Lys, %	1.02	1.01	1.01	1.01
Thr, %	0.71	0.70	0.70	0.70
Trp, %	0.20	0.19	0.20	0.21
Ca, %	0.79	0.79	0.79	0.79
Available Phosphorus, %	0.35	0.25	0.25	0.25
Na, %	0.22	0.22	0.22	0.22
Analyzed Value				
Crude Protein $\pm$ SD, % of DM	20.41 $\pm$ 0.08	19.74 $\pm$ 0.48	19.49 $\pm$ 0.57	20.09 $\pm$ 0.76
Crude Fat $\pm$ SD, % of DM	7.89 $\pm$ 0.77	6.99 $\pm$ 0.77	8.70 $\pm$ 0.77	8.50 $\pm$ 0.37
Ash $\pm$ SD, % of DM	6.31 $\pm$ 0.08	6.42 $\pm$ 0.23	8.50 $\pm$ 0.37	10.30 $\pm$ 0.54

¹Supplied per kg of diet: manganese, 0.02%; zinc 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69mg; choline, 386mg; riboflavin, 6.61mg; biotin, 0.03mg; vitamin B 6, 1.38mg; niacin, 27.56mg; pantothenic acid, 6.61mg; thiamine, 2.20mg; manadione, 0.83mg; vitamin B12, 0.01mg; vitamin E, 16.53 IU; vitamin D₃, 2133 ICU; vitamin A, 7716 IU.

²Active drug ingredient Monensin Sodium 90g/lb (120g/ton inclusion).- Elanco Animal Health, Indianapolis, IN. As an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*

³Bacitracin Methylene Disalicylate 60g/lb (60g/ton inclusion)- Alpharma,- Fort Lee, NJ. For increased rate of weight gain and improved feed efficiency.

Table 3. Feed Manufacture Variables.¹

Item	Pro Rate, Metric Ton/hr	PREEU, kW·h/ Metric Ton	CREEU, kW·h/ Metric Ton	Motor Load, A	Mash Moisture, %	Conditioned Mash Moisture, %	Cool Pellet Moisture ² , %
Treatments ³							
NE, low, 1%	0.80	6.72 ^{abc}	0.15	16.70 ^{abc}	10.42	14.61	10.57
NE, high, 1%	0.81	8.59 ^a	0.13	18.15 ^a	10.11	15.00	10.39
NE, low, 2.5%	0.79	4.61 ^{bc}	0.16	15.18 ^{bc}	9.92	15.20	10.29
NE, high, 2.5%	0.80	6.14 ^{abc}	0.15	15.68 ^{bc}	9.95	14.66	10.45
NE, low, 4%	0.80	3.99 ^c	0.12	14.75 ^c	9.46	14.30	9.96
NE, high, 4%	0.81	5.04 ^{bc}	0.15	14.98 ^{bc}	9.31	14.07	9.53
E, low, 1%	0.81	6.34 ^{abc}	0.15	16.08 ^{abc}	10.27	14.60	10.64
E, high, 1%	0.78	7.56 ^{ab}	0.14	17.00 ^{ab}	10.34	15.02	10.33
E, low, 2.5%	0.81	4.74 ^{bc}	0.16	15.20 ^{bc}	9.87	13.95	10.26
E, high, 2.5%	0.79	6.30 ^{abc}	0.16	15.68 ^{bc}	10.01	14.22	9.86
E, low, 4%	0.78	4.44 ^{bc}	0.16	15.30 ^{bc}	9.62	14.27	10.30
E, high, 4%	0.78	3.86 ^c	0.16	14.75 ^c	9.92	14.22	9.52
Positive Control ⁴	0.79	5.06 ^{bc}	0.16	15.55 ^{bc}	10.22	14.56	10.65
Tukey's HSD ⁵	—	3.47	—	2.12	—	—	—
SEM ⁶	0.013	0.691	0.004	0.423	0.228	0.440	0.270
Marginal Means							
No Enzyme	0.80	5.85	0.14	15.90	9.86	14.63	10.20
Enzyme	0.79	5.54	0.16	15.65	10.01	14.36	10.15
82°C	0.80	5.14	0.15	15.53	9.93	14.49	10.34
85°C	0.80	6.25	0.15	15.96	9.94	14.50	10.01
1% MAF	0.80	7.30	0.15	16.98	10.29	14.79	10.48
2.5% MAF	0.79	5.45	0.16	15.31	9.94	14.51	10.21
4% MAF	0.79	4.33	0.16	14.94	9.58	14.21	9.83
Probability (> F) ⁷							
Treatment	0.7361	0.0003	0.3046	0.0015	0.0377	0.7303	0.0532
Enzyme	0.6730	0.9026	0.3419	0.9809	0.2911	0.2828	0.7542
Temperature	0.8151	0.0512	0.5660	0.3077	0.9255	0.9893	0.0428
Fat inclusion	0.9536	<0.0001	0.0181	<0.0001	0.0006	0.2418	0.0058
E X T	0.4089	0.7819	0.5660	0.9285	0.2482	0.4353	0.2597
E X F	0.6370	0.2735	0.4741	0.5409	0.4510	0.4028	0.4574
T X F	0.1961	0.4419	0.7790	0.3959	0.7781	0.9307	0.4220
E X T X F	0.2037	0.3047	0.2034	0.5159	0.8585	0.8479	0.8498

¹Pro Rate = production rate; PREEU = relative electrical energy usage (pellet mill); CREEU = relative electrical energy usage (conditioner).

² Pellets sampled immediately after exiting the cooler.

³NE = No Enzyme; E = Enzyme; low = 82°C; high = 85°C; 1%, 2.5% or 4% Mixer-added Fat (MAF).

⁴²NE, low, 2.5% formulated without enzyme matrix values

⁵Honestly significant difference.

⁶Pooled standard error of the mean (n=4).

⁷E = Enzyme, T = Conditioning Temperature, F = MAF.

Table 4. Effects of Washed Play Sand and Mixer-Added Fat on Pellet Manufacture Variables.¹

Item		Pro Rate, Metric Ton/hr	PREEU, kW·h/ Metric Ton	CREEU, kW·h/ Metric Ton	Motor Load, A	Total Fines, %	PDI, %	MPDI, %	BD, kg/m ³
Treatments									
Fat, %	Sand, %								
1	1.5	0.95 ^b	7.79 ^a	0.69	20.36 ^a	12.42 ^b	90.20 ^a	83.95 ^a	637.45 ^c
1	6	0.96 ^{ab}	7.78 ^a	0.76	20.45 ^a	11.75 ^b	89.04 ^a	82.02 ^a	648.27 ^{ab}
4	1.5	0.96 ^{ab}	6.36 ^b	0.76	18.95 ^b	29.89 ^a	68.47 ^b	44.47 ^b	645.22 ^b
4	6	0.98 ^a	6.34 ^b	0.75	19.18 ^b	31.78 ^a	64.75 ^c	37.97 ^c	654.11 ^a
Tukey's HSD ⁵		0.023	1.009	—	0.353	4.082	3.100	6.298	6.886
SEM ⁶		0.006	0.240	0.021	0.084	0.972	0.738	1.500	1.64
Probability (> F)									
Fat		0.0072	<0.0001	0.1925	<0.0001	<0.0001	<0.0001	<0.0001	0.0013
Sand		0.0188	0.9372	0.1957	0.0878	0.5451	0.0062	0.0159	<0.0001
Fat x Sand		0.6393	0.9835	0.0658	0.4295	0.2127	0.1084	0.1547	0.5688
Marginal Means									
1% Fat		0.95	7.79	0.73	20.41	12.08	89.62	82.98	642.86
4% Fat		0.97	6.35	0.75	19.06	30.83	66.61	41.22	649.67
1.5% Sand		0.96	7.08	0.73	19.66	21.16	79.34	64.21	641.34
6% Sand		0.97	7.06	0.75	19.81	21.76	76.89	59.99	651.19

¹Fat = animal/vegetable blended fat; Sand = washed play sand. Factorial design consisting of low and high levels of fat and sand used in grower-finisher feed manufacture.

Means without a common superscript differ significantly ($P < 0.05$).

²MT = tonne; kWh = kilo watt hour; Pro Rate = production rate; PREEU = relative electrical energy usage (pellet mill); CREEU = relative electrical energy usage (conditioner); Motor Load = pellet mill motor load.

³Cooler Fines = Percent of feed that fell through the cooler during production; Cooler Fines Fines = Percentage of Fines that passed through a #6 U.S. standard sieve; Bagged Fines were determined from the fourth bag of feed from each batch and are the percentage of the bagged feed that passed through a #6 U.S. standard sieve.

⁴PDI = pellet durability index; MPDI = modified pellet durability index. Bulk density (BD) was determined from the fourth bag of feed from each batch.

⁵Honestly significant difference.

⁶Pooled standard error of the mean (n=4).

Table 5. Broiler performance variables.

Item	Initial Body Weight (kg per pen)		Feed Intake (kg per pen)		LWG (kg per bird)		FCR (kg:kg)		Mortality (%)	
	11-22 d	22-38 d	11-22 d	22-38d	11-22 d	22-38 d	11-22 d	22-38 d	11-22 d	22-38 d
Broiler Age	11-22 d	22-38 d	11-22 d	22-38d	11-22 d	22-38 d	11-22 d	22-38 d	11-22 d	22-38 d
Treatments ¹										
NE, low, 1%	3.18	12.94 ^{ab}	14.87	41.34 ^{abcde}	0.54 ^{abc}	1.22 ^{abc}	1.52	1.92a ^b	0.00	2.38
NE, high, 1%	3.15	12.55 ^b	14.59	44.16 ^{abc}	0.52 ^c	1.33 ^a	1.55	1.89 ^b	0.00	0.00
NE, low, 2.5%	3.17	13.06 ^{ab}	15.29	43.46 ^{abcde}	0.55 ^{abc}	1.17 ^c	1.54	1.98 ^a	0.00	0.00
NE, high, 2.5%	3.18	12.66 ^{ab}	15.04	44.91 ^a	0.53 ^{bc}	1.30 ^a	1.59	1.94 ^{ab}	0.00	0.79
NE, low, 4%	3.18	13.05 ^{ab}	15.21	40.44 ^{de}	0.55 ^{ab}	1.18 ^c	1.54	1.92a ^b	0.79	2.38
NE, high, 4%	3.18	13.00 ^{ab}	15.29	44.61 ^{ab}	0.55 ^{abc}	1.30 ^a	1.54	1.95a ^b	0.79	0.79
E, low, 1%	3.17	13.02 ^{ab}	15.22	40.14 ^c	0.56 ^a	1.20 ^{bc}	1.52	1.88 ^b	1.59	3.17
E, high, 1%	3.17	13.09 ^{ab}	15.28	44.31 ^{ab}	0.55 ^{ab}	1.32 ^a	1.54	1.86 ^b	0.00	0.79
E, low, 2.5%	3.16	13.20 ^a	15.57	41.17 ^{bcde}	0.56 ^a	1.17 ^c	1.55	1.88 ^b	0.00	1.59
E, high, 2.5%	3.18	13.06 ^{ab}	15.17	44.25 ^{ab}	0.55 ^{abc}	1.29 ^{ab}	1.54	1.91a ^b	0.00	1.59
E, low, 4%	3.18	13.12 ^{ab}	15.28	40.54 ^{cde}	0.56 ^a	1.18 ^c	1.54	1.93 ^{ab}	0.79	0.79
E, high, 4%	3.16	12.97 ^{ab}	15.00	43.80 ^{abcd}	0.55 ^{abc}	1.32 ^a	1.51	1.86 ^b	0.79	0.00
Positive Control ²	3.17	13.07 ^{ab}	15.30	41.19 ^{bcde}	0.55 ^{ab}	1.17 ^c	1.54	1.96 ^{ab}	0.79	0.00
Tukey's HSD ³	—	0.6055	—	3.646	0.028	0.104	—	0.09	—	—
SEM ⁴	0.009	0.126	0.187	0.753	0.132	0.021	0.017	0.019	0.055	1.05
Marginal Means										
No Enzyme	3.17	12.88	15.04	41.18	0.54	1.19	1.55	1.93	0.34	1.85
Enzyme	3.17	13.08	15.25	44.34	0.55	1.31	1.53	1.90	0.53	0.66
82°C	3.17	13.06	15.24	43.15	0.55	1.25	1.54	1.92	0.57	1.06
High Temp	3.17	12.89	15.06	42.37	0.54	1.25	1.55	1.90	0.26	1.46
1% MAF	3.17	12.90	14.99	42.49	0.54	1.27	1.54	1.89	0.40	1.59
2.5% MAF	3.17	12.99	15.26	43.45	0.55	1.23	1.55	1.93	0.16	1.19
4% MAF	3.18	13.04	15.20	42.35	0.55	1.25	1.53	1.91	0.79	0.99
Probability (> F) ⁵										
Treatment	0.2801	0.0263	0.2362	<0.0001	0.0003	<0.0001	0.2693	0.0037	0.8131	0.3646
Enzyme	0.6092	0.0081	0.8734	<0.0001	0.0003	<0.0001	0.1132	0.0018	0.4776	0.0295
Temperature	0.2567	0.0172	0.4748	0.2538	0.0019	0.3872	0.2516	0.0800	0.4776	0.4175
Fat inclusion	0.5928	0.3562	0.1090	0.3451	0.0785	0.1602	0.1075	0.0099	0.2237	0.7604
E x T	0.8305	0.1040	0.3487	0.8101	0.1729	0.5502	0.1175	0.5568	0.3733	0.9812
E x F	0.6109	0.2105	0.0299	0.6203	0.0538	0.6742	0.5852	0.7776	0.7202	0.4073
T x F	0.0248	0.5957	0.7529	0.9725	0.3896	0.8573	0.4721	0.9968	0.6154	0.2283
E x T x F	0.2718	0.2324	0.3577	0.4750	0.3377	0.8361	0.4598	0.0093	0.6484	0.8660

¹NE = No Enzyme; E = Enzyme; low = 82°C; high = 85°C; 1%, 2.5% or 4% Mixer-added Fat (MAF).

²NE, low, 2.5% formulated without enzyme matrix values.

³Honestly Significant Difference.

⁴Pooled standard error of the mean (n = 7).

⁵E = Enzyme, T = Conditioning Temperature, F = MAF.

Table 6. 38 d Broiler processing variables.

Item	Body Weight, kg	Carcass Yield, %	Breast Yield, %	Leg Yield, %	Fat Pad Yield, %
Treatments ¹					
NE, low, 1%	2.03	67.25	20.13	24.63	1.57 ^{ab}
NE, high, 1%	2.01	65.78	20.34	23.57	1.52 ^{ab}
NE, low, 2.5%	2.01	67.53	20.13	24.02	1.58 ^{ab}
NE, high, 2.5%	1.99	66.49	20.31	22.93	1.55 ^{ab}
NE, low, 4%	2.03	66.72	20.64	24.05	1.64 ^{ab}
NE, high, 4%	2.02	67.49	21.05	24.38	1.26 ^b
E, low, 1%	2.15	68.16	20.65	23.78	1.79 ^a
E, high, 1%	2.18	67.62	20.87	24.03	1.67 ^{ab}
E, low, 2.5%	2.13	68.16	20.44	24.68	1.60 ^{ab}
E, high, 2.5%	2.08	66.82	20.30	23.79	1.52 ^{ab}
E, low, 4%	2.13	67.54	20.22	23.90	1.49 ^{ab}
E, high, 4%	2.14	68.45	20.46	24.75	1.39 ^{ab}
Positive Control ²	2.04	67.16	20.96	22.93	1.47 ^{ab}
Tukey's HSD ³					
SEM ⁴	—	0.625	0.350	0.457	0.097
Marginal Means					
No Enzyme	2.02	67.07	20.43	23.93	1.52
Enzyme	2.13	67.62	20.49	24.15	1.58
82°C	2.08	67.39	20.37	24.18	1.61
High Temp	2.07	67.30	20.56	23.91	1.48
1% MAF	2.09	66.94	20.50	24.01	1.64
2.5% MAF	2.05	67.25	20.29	23.85	1.56
4% MAF	2.08	67.84	20.59	24.27	1.45
Probability (> F) ⁵					

Treatment	—	0.3961	0.4059	0.1000	0.0536
Enzyme	—	0.0425	0.4797	0.4005	0.3209
Temperature	—	0.4304	0.1843	0.3176	0.0260
Fat inclusion	—	0.3993	0.3882	0.4382	0.0282
E x T	—	0.5256	0.8855	0.6000	0.6550
E x F	—	0.5096	0.0683	0.8130	0.2456
T x F	—	0.1872	0.8564	0.4531	0.3422
E x T x F	—	0.1786	0.9304	0.0791	0.3952

¹NE = No Enzyme; E = Enzyme; low = 82°C; high = 85°C; 1%, 2.5% or 4% Mixer-added Fat (MAF).

²NE, low, 2.5% formulated without enzyme matrix values.

³Honestly Significant Difference.

⁴Pooled standard error of the mean (n = 7).

⁵E = Enzyme, T = Conditioning Temperature, F = MAF.