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The Effect of Feed Manufacture Techniques on Pathogen Reduction and Feed Nutrient Value

Timothy Paul Boltz

West Virginia University, tpb0015@mix.wvu.edu

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The Effect of Feed Manufacture Techniques on Pathogen Reduction and Feed Nutrient Value

Timothy P. Boltz

Thesis submitted
to the Davis College of Agriculture, Natural Resources, and Design
at West Virginia University

in partial fulfillment of the requirements for the degree of

Master of Science
In
Nutrition and Food Science

Joseph S. Moritz, Ph.D., Chair
Jacek Jaczynski, Ph.D.
Cangliang Shen, Ph.D.

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ABSTRACT

The Effect of Feed Manufacture Techniques on Pathogen Reduction and Feed Nutrient Value

Timothy P. Boltz

Experiments were conducted to evaluate how differing feed manufacture techniques, such as varying conditioning times and new equipment, can affect pathogen mitigation, feed manufacture metrics, amino acid digestibility, and enzyme recovery in feed. Feed hygienics are of increasing importance in providing safe feed to animals, and ultimately safe food for consumers. Salmonella has been identified as a major microbial hazard in animal feed that has been linked to animal and human illness. In chapter 2, the effects of standard pelleting and more thermally aggressive techniques were utilized to determine pathogen mitigation potential. Use of antibiotics has decreased in recent years due to policies and practices of poultry production, increasing opportunities for potential pathogens in feed to affect poultry and poultry products. More thermally aggressive pelleting decreased pellet mill motor load ($P=0.02$), increased hot pellet temperature ($P=0.02$), and tended to increase pellet durability ($P=0.07$). *E. faecium* ATCC 8459, as a *Salmonella* surrogate colonies were reduced with standard pelleting relative to inoculated mash and reduced further with more thermally aggressive pelleting ($P<0.05$). Standard pelleting and more thermally aggressive pelleting resulted in a 3 and 4-log reduction in *E. faecium* ATCC 8459 colonies respectively, relative to inoculated mash. In chapter 3, varying steam conditioning temperature and conditioning time were utilized to examine the how these interacted to affect pellet mill motor load, pellet quality, digestible amino acid concentration, and enzyme recovery. Corn and soybean meal based diets that included DDGS and meat and bone meal were conditioned at either 76°C, 82°C, or 88°C with conditioning times of either 30 or 60 seconds. Conditioning temperature and time interacted to affect pellet mill motor load ($P<0.05$). Motor load decreased for 30 second conditioning compared to 60 second conditioning when diets were subjected to 76 and 82°C, but motor load was similar among conditioning times at 88°C. This interaction was likely due to the result of lower feed volume in the conditioner as per 30 second conditioning obtaining greater moisture condensation and lubrication at lower conditioning temperatures. Pellet quality increased with increased conditioning temperature ($P<0.05$), likely associated with greater starch gelatinization and protein gelation. Lysozyme activity decreased when conditioned at 88°C ($P<0.05$), but was not affected by retention time. Conditioning temperature and time interacted to affect digestible amino acid concentrations of methionine, lysine, threonine, alanine, aspartic acid, glutamic acid, isoleucine, leucine, proline, and valine ($P<0.05$). Increased conditioning temperatures at 30 seconds increased digestible amino acid concentrations. Similarly, diets conditioned at 60 seconds increased digestible amino acid concentration between 76 and 82°C. However, the digestible amino acid concentration decreased when diets were conditioned at 60 seconds and 88°C. Perhaps negative effects on digestibility of trypsin inhibitor complexes and ingredient aleurone layers were decreased with increasing temperatures; however, the 60 second 88°C treatment may have been too thermally aggressive and rendered the proteins indigestible.

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ABBREVIATION KEY

Chapter 1

1. Dried Distiller's Grains with Solubles – DDGS
2. Mixer added fat – MAF
3. Hazard Analysis and Risk-Based Preventive Controls – HARPC
4. Veterinary Feed Directive – VFD
5. Species – Spp.
6. American Type Culture Collection – ATCC
7. Agriculture Research Service Culture Collection – NRRL

Chapter 2

1. Food Safety Modernization Act – FSMA
2. Horse Power – HP
3. Metric Ton – MT
4. Programmable Logic Control – PLC
5. New Holman Pellet Tester – NHPT
6. Brain-heart infusion broth – BHI
7. Tryptic soy broth – TSB
8. Phosphate Buffered Saline – PBS
9. Colony Forming Unit – CFU
10. Generalized Linear Model – GLM
11. Statistical Analysis System – SAS

Chapter 3

1. Dried Distiller's Grains with Solubles – DDGS
2. Trypsin Inhibitor Unit – TIU
3. Horse Power – HP
4. Metric Ton – MT
5. Programmable Logic Control – PLC
6. New Holman Pellet Tester – NHPT
7. Single Comb White Leghorn – SCWL
8. Amino Acid – AA
9. Generalized Linear Model – GLM
10. Statistical Analysis System – SAS
11. Lysine – Lys
12. Methionine – Met
13. Threonine – Thr

CHAPTER ONE

LITERATURE REVIEW

I. Feed Manufacture

Feed manufacture is a crucial step in the broiler production system. In 2016, it was estimated on a global scale that one billion tons of feed were produced, with around 45% going towards poultry [1]. Feed costs are associated with 60-65% of total production costs, along with a large initial capital investment [2-3]. Pelleting feed was introduced to Europe around 1920, and then came to the United States around 1930 [4]. Pelleting decreases energy expenditure of the animal, spillage, ingredient segregation, selective feeding, and pathogens that may be associated with ingredients [5-6]. All of these factors come together to produce a feed that permits animals to have increased daily gain and decreased feed conversion relative to mash diets [3,7]. Since the late 20th century, animals have been selectively bred for production traits, resulting in improved breeds with genetically superior animals [5]. Feed manufacturers now have the responsibility to provide feed for these animals that allows for these genetic improvements to be realized.

II. Grinding, Mixing, and Pelleting

Before pellets can be produced, ingredients must first be ground and mixed together. Grinding results in particle reduction, which is needed to allow the feed to have improved binding ability with other ingredients in the diet to form a pellet [7]. Grinding is usually done for cereals such as corn before being mixed. The smaller particles also create more surface area for digestion to occur within the gastrointestinal tract of the animal. This especially helps poultry, as these animals have short digestive tracts and digestibility of feed must be high [8]. Particle reduction can be accomplished using machinery such as hammer and roller mills. Mixing is critical and essential to ensure that all the feed ingredients are thoroughly mixed and are ready for the pelleting process [5]. After all ingredients are ground and mixed, the mixture can then be

pelleted. Pellets are formed by agglomerating smaller feed particles with the help of machinal pressure, moisture, and heat from steam to heat the larger particles [8].

Pellets are easier to store and handle than a mash feed, which is beneficial to an operation that needs to feed tens of thousands of animals on a daily basis. Pellets flow more efficiency through conveying equipment and have superior discharging behavior from silos than mash feed [9-11]. Pellets also have greater bulk density than mashed feed, which allows for more pelleted feed to be hauled in a load which can potentially reduce costs on trucking [7]. Quality of pellets can be measured on the hardness and durability of the pellet, which these measures can be used to evaluate the effects of diet formulation, conditioning, expander treatment, pellet binders, and die selection [12].

III. Steam Conditioning

Steam conditioning temperature is a manipulable thermo-mechanical processing variable that influences the quality of feed that will be produced, with common temperatures of 76°C to 93°C commonly being used [2]. Utilizing higher temperatures have been shown to result in high quality pellets, which is desired in the poultry industry, as well as possibly decreasing pathogen population in the feed [2,13]. Alternatively, these higher temperatures can negatively affect the nutrients of feed which has been shown when utilizing 70°C has shown to increase broiler performance, whereas at 90°C performance was observed to be decreased [14]. The observed decrease could be associated with lowered nutrient availability in the feed due to the high temperature not allowing the birds to get all required nutrients to obtain their maximum genetic potential.

Heat is commonly applied to feed as it can aid in destruction of pathogens and anti-nutritive factors, such as trypsin inhibitors that can be found in soybean meal [2]. This makes steam

conditioning the most important step in the feed manufacture process to reduce potential pathogens associated with the feed [15]. Though the effectiveness of this step can be reduced if there is equipment malfunctions, uneven heating or incomplete mixing [16].

Temperatures used to condition the feed can have an impact on the amino acid and vitamin availability as well as enzyme activity of the final feed. Conditioning temperatures of 85°C and 96°C have been shown to significantly reduce digestibility of methionine, isoleucine, and proline when compared to diets conditioned at 74°C [17]. Boney and Moritz later found that at 91°C leucine, valine, isoleucine, alanine, and aspartic acid digestibility decreased when compared to diets conditioned at 74°C and 82°C [18]. Vitamins are also susceptible to damage during steam conditioning as well, where Cutlip and colleagues found a 6.7% loss in vitamin A at 93°C [2]. Feed-borne and exogenous enzymes can also lose activity due to certain temperatures denaturing the enzyme, which could cause deficiencies in the birds that are provided this feed [19-20]. Feed producers must consider that at too low of temperatures pathogen mitigation might not be as much as desired but, these temperatures could maintain nutrient availability of the feed that will ultimately get to the bird. Steam conditioning temperature not only can affect the nutrient availability and pathogen load of feed, but also has an important role in producing quality pellets.

IV. Pellet Quality

Pellet quality is an important factor to consider when wanting to maximize animal performance, but is often considered poor in the commercial US industry [3]. It has been shown that one of the simplest way to improve pellet quality is to use higher steam conditioning temperatures, but this could have a negative impact on the final nutrient composition of the feed [2,13,17]. An increase of feed conversion by 2.4 percentage points resulted when broilers were fed a combination of 75% pellet and 25% fines when compared to being fed 25% pellet and 75% fines

[21]. Modest improvements in pellet quality from 50% crumble/pellet to 70% may improve broiler performance and decreased feed conversion ratio by 3 points [22]. Commercial diets are commonly formulated on a least cost basis, which frequently include inexpensive by-products like meat and bone meal, which can negatively impact pellet quality and can potentially introduce pathogens to the feed [3,23]. Use of varying inclusions of non-by-product ingredients and using different sized pellet dies are other ways to improve pellet quality without having to use as extreme of condition temperatures.

Addition of certain ingredients can improve pellet quality, while certain inclusions of others can be detrimental to pellet quality. Dried distillers' grains with solubles (DDGS) and oat hulls are two examples of by-products that when added at certain amounts decrease pellet quality [3,24]. Inclusion of 2% and 4% oat hulls in a diet negatively affected the pellet quality when compared to no addition [3]. Where additions of 15% and 30% DDGS in grower-phase yielded decreased pellet quality and increased amount of total fines and lower amount of intact pellets [25]. Inclusion of DDGS at 8% in grower diets and 16% in finisher diets have also shown to decrease pellet quality [26]. Wamsley and colleagues alternately demonstrated increasing DDGS in the diet tended to not affect pellet quality until the finisher phase, which contained 10% or 20% DDGS [27]. Diet formulation should be taken into consideration if pellet quality is desired to be improved, as addition of certain alternative ingredients have been shown to improve the quality of pellets produced.

Addition of protein and fibrous material pre-pelleting should be done [6]. Buchanan and Moritz found that adding fiber in the form of cellulose and protein in the form of soy protein isolate at 5% improved pellet quality [3]. Buchanan and Moritz also found that pellet durability index and modified pellet durability index can be improved through small inclusions of protein

(around 2%) in the form of soybean meal and moisture (3-4%) in the form of tap water [3].

Moritz also found that moisture can be added at the mixer to improve pellet quality [28].

The use of *Spirulina* algae has been shown to be a promising pellet binder due to the large amount of protein (76%) associated with the algae. Using a corn-soy diet, it was demonstrated that going from 0% to 10% algae in the diet improved pellet quality by 31-percentage points when the feed was conditioned at 74°C [18]. When the same diets were pelleted at 91°C the difference was only 5-percentage points [18]. When pellet quality was tested using a new Holmen pellet tester, a 51-percentage point difference was seen for the 74°C, while a 7-percentage point difference was seen for the 91°C [18]. Buchanan and colleagues found that research based diets that were pelleted utilizing a thick die and slow production rate resulted in higher pellet durability index and modified pellet durability index scores when compared to diets that were formulated on a least cost basis and pelleted using a thin die at a high production rate [23]. The research based diets were formulated to contain 3.87% more crude protein than the least cost formulated diets with additional moisture added [23]. These additions could explain why the research diet had improved pellet quality when compared to the commercial least cost diet. Addition of fat and oils, either pre-or post-pelleting can also have an impact on the quality of the final pellets.

Fat addition pre-and post-pelleting has been shown to both improve and decrease pellet quality, depending on the inclusion percentage. Low mixer added fat (MAF) (1%) can be added pre-pelleting to a diet, and the remaining fat added post pelleting to improve pellet quality [29]. Increased amounts of MAF can prevent reduction of nutrient availability [30]. The fat could reduce the friction between the pellet die and mash feed, preserving some nutrients that may be loss to the frictional heat that is generated [30]. Addition of fat under 5.6% and protein content around 20% showed to not impact pellet quality negatively [21]. Addition of too much fat at the mixer can be

detrimental though, where Wamsley and Moritz found inclusion of 3% mixer added fat with a thicker die (44.9 mm), negatively impacted pellet quality [29]. Inclusion of 7.5% or greater oil in the diet has shown to have a negative impact on the pellet quality as well [21]. It should also be noted that fat and oil addition can possibly introduce pathogens to feed if added post pelleting as these ingredients do not undergo any pathogen reduction treatments [16]. *Salmonella* contamination of feed is a major concern for the feed industry and requires a great deal of management to control.

V. *Salmonella* in Feed

Salmonella spp. are major microbial hazards in animal feed and have been linked between infected feed and illness in both animals and humans [31]. *Salmonella spp.* are gram negative, facultative anaerobic, nonspore forming, rod shaped bacteria that have the potential to cause illness and death in humans and livestock [32]. Poultry can consume feed that is contaminated with *Salmonella* and not exhibit any clinical signs of the disease. Later these birds can contaminate processing facilities when being eviscerated, which can transfer *Salmonella* to the carcasses [33]. *Salmonella* is native to the gastrointestinal tract of livestock and is commonly found in nature in a variety of places, such as poultry feed and feces [34-37]. Most of these places in nature contain low moisture, so supplemental moisture is needed for reproduction, like in feed and the feed mill environment [31]. The water activity reported by Greco et al. for feed is 0.537, which is lower than the optimal water activity for pathogenic bacteria to thrive in [38].

Numerous serotypes of *Salmonella spp.* have been isolated from feed mills, with *Salmonella typhimurium* and *Salmonella enteritidis* being sampled frequently [39]. *Salmonella enterica* being the leading cause of death and hospitalizations in the United States and *Salmonella enterica* serovar Kentucky being the leading serovar isolated from poultry and

poultry products [40-41]. In Great Britain a study was conducted to test the different serovars sampled from various feed ingredients from 1987 to 2006. Mbandaka was sampled most frequently for soybean, Montevideo for meal and bone meal, and Schwarzemgrund for corn [42]. Government regulations have been put into place to aid the production of safe feed and ultimately safe food for consumers.

VI. Food and Feed Safety

It has been estimated that every year in the United States approximately 48 million people get sick, 128,000 are hospitalized, and 3,000 die from food borne illness [43]. This means that 1 in 6 American will become ill annually from a food borne illness [43]. On January 4, 2011, the Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) was signed into effect by President Obama, which was passed by Congress to help address public food safety challenges. This new piece of regulation shifted the focus from reacting to food borne outbreaks to protection and prevention of outbreaks. This new legislation introduced mandated a comprehensive, science-based preventive controls in facilities. Facilities must conduct Hazard Analysis and Risk-Based Preventive Controls (HARPC) and establish science-based preventive control measures that reduce risk of contamination, whereas before programs like this were voluntary.

Another way that the FDA is trying to keep the food supply safe with is the Veterinary Feed Directive (VFD), which was signed into effect January 1, 2017 [44]. These are stricter federal rules that regulate how medically important medications, such as tetracycline and penicillin, are administered to animals in feed (require a VFD) and water (require a prescription). With proper administration of antibiotics to reduce the opportunity for resistance to develop and keep a supply of antibiotics when there are situations of true need to keep both humans and

animals healthy. To aid in producing safe food control measures have to be in place to control possible *Salmonella* contamination.

VII. *Salmonella* Control

Salmonella spp. are ubiquitous in nature and have superb survivability [35]. Because of this ability multiple tools for control should be utilized and control efforts should be a regular part of the feed manufacture process, which can be referred to as a multiple hurdle approach [45]. Three broad categories of *Salmonella* control principles for feed are recognized: 1. Efforts to prevent contamination, 2. Effort to reduce microbial multiplication, and 3. Plans to kill the pathogen [46]. Contaminated feed is the main vector of introduction, so it is prudent to assume all incoming feed is contaminated and should be handled as such [47]. The more handling that the feed is subjected to, the higher the chance of contamination to uncontaminated feed is, so handling should be kept to only when required [48].

Multiple practices can be implemented to help prevent contamination from occurring. Purchasing feed from suppliers that test ingredients is advised since this reduces the chance of bringing *Salmonella* into the production system [49]. Another area of significance to control is dust at receiving and during grinding to reduce the possibly of aerosol spread of *Salmonella*. Dust from raw material receiving is the largest source of dust emissions in the mill environment, but can also be produced by size reduction equipment like hammer mills and roll mills if not maintained properly [50-51]. Dust and caked material should not be at any location in the mill as these provide suitable substrate for *Salmonella* colonization [46]. Venting to the outside that is separate from the intake should be done as this may remove contaminated dust from the feed mill environment [46]. Filters installed on intakes can help to prevent any recontamination due to contaminated dust being up taken [46].

Preventing cross contamination between area of raw materials and finished pellets can be accomplished by implementing boot covers or other methods of disinfecting footwear between these areas and restricting the flow of equipment [31]. Reducing buildup of fats and oil in the feed mill decreases possible locations for *Salmonella* to survive and multiply, as these tend to protect *Salmonella* from outside stressors [52-54]. Controlling pests, such as rodents and wild birds, is another way to reduce the chance of having *Salmonella* contamination occur in the mill [52,55]. Jones and Ricke suggested that intake pits be cleaned with a neutral feed like corn containing organic acid as this can help to prevent any cross contamination that could happen in the feed mill [56]. Transport vehicles have been identified as another vector for this species of bacteria [57-58]. It is recommended to have trucks solely deliver raw ingredients and after each load be thoroughly cleaned to reduce any contamination to the next load [31]. Since this is not always practical, it is recommended to have drivers disclose the contents of the last three loads prior to delivery of raw ingredients [48].

VIII. *Salmonella* Reduction

Following control, there are practices that can be utilized to reduce *Salmonella* multiplication. The simplest way to reduce *Salmonella* is to control excess moisture in the mill environment [31]. Biofilms are a layer of microorganisms that created a colony that is difficult to eradicate and can form in niches that are not easily accessible [59]. Biofilms are common areas of cross-contamination where *Salmonella* can live and multiply. The lack of accessibility into the cooler for cleaning permits *Salmonella* to multiply and can cross-contaminate any feed that comes into contact with the biofilm. All feed facilities are unique in design, so the workers should be compelled to find any potential growth niches [59].

A common place that *Salmonella* can multiply rapidly in is the pellet cooling system. Hot pellets coming from the pellet mill release water vapor onto the cold surfaces in the cooler that condensate and trap dust in a moist area [61]. Contamination of new pellets entering the cooler can occur due to the colonization of *Salmonella* in the cooler [61]. Heating of the equipment to temperatures above the dew point of the air is the most effective way to prevent environments for contamination in the cooler [61]. Reduction of *Salmonella* is the final step to eliminate any *Salmonella* cells that survived the control and reduction practices.

IX. *Salmonella* Reduction

Feed producers have options on how to aid in the mitigation of *Salmonella* in feeds that they are producing. These options can be thermal processing associated with pelleting, applying a chemical to the feed, or a combination of both which is the most effective way to mitigate *Salmonella* [31]. There are three factors associated with microbial lethality which are: 1. Temperature 2. Time of exposure at a certain temperature, and 3. Moisture [62]. It was speculated that at temperatures of approximately 71°C cells of pathogens start to be destroyed and can result in about a 10^3 cfu/100 g reduction, with 79°C to 85°C the target temperatures for pathogen reduction [63-64]. Later it was demonstrated that pelleting at 80°C showed to reduce most *Salmonella* and other coliforms, while pelleting over 90°C was not a lethal temperature for spore forming bacteria [64-68]. Boney et al. found that mitigation potential was apparent with short term steam conditioning for 30 seconds was applied to a mash that was inoculated with an appropriate *Salmonella* surrogate at 71°C, and that potential increased with increased temperature to 81°C and 88°C [69]. Other pieces of equipment like expanders, have been shown to reduce the pathogen load in feed around 10^5 to 10^6 cfu/g utilizing temperatures between 115°C and 125°C, pressures up to 1,200 psi and exposure times of 10 to 20 seconds [70]. This piece of equipment is not

commonly used for poultry feed, but could be utilized more if hygiene of the feed is desired to be improved, but could impact the nutrient availability due to the high temperatures utilized. Temperature is not the only factor that should be considered when looking at mitigation as the moisture in the feed can aid in mitigation potential.

Increasing moisture in feed from 5% to 15% showed to reduce the exposure time required to eliminate *Salmonella* in the temperature range of 71°C-82°C [71]. To achieve high levels of mitigation, temperatures from 85°C to 93°C are needed for 90 seconds up to 4 minutes with moisture around 15% [71-72]. The extra moisture in the feed could aid in the thermal breaking of peptide bonds in the microorganism that will ultimately lead to the death of cell. These extended exposure times may have a positive impact on mitigation of pathogens like *Salmonella*, but once again could be detrimental to the nutrient availability of the feed. Temperature, moisture, and exposure time are not the only ways to mitigate pathogen load, but organic acids can also aid in the mitigation of pathogens if applied in the right concentrations and time of manufacture.

Inclusion of chemicals such as organic acids like, formic and propionic acid, to feed can aid in the destruction, inhibit growth, and prevent recontamination of microorganisms during and after the pelleting process [73]. Though, extended retention times of feed in the feed mill are needed for organic acids to achieve maximum efficiency. It has been demonstrated that mixtures of organic acids applied at 3% to meat meals eliminated *Salmonella* over a 3-day period [74]. Use of 0.25% propionic acid added to feed has been shown to reduce colony forming units to less than 10 within 72 hours of application, with higher concentrations needed to reduce colony counts within 2 hours of application [75]. This may not be feasible for meat by-products and poultry feed, as large volumes are needed daily and so the by-product and feed may not get the needed exposure time to achieve maximum reduction. Use of chemicals can add another risk to feed mill personal,

and so personal should be properly trained and protected from overexposure to chemicals [76]. Using organic acids could be a way to aid in mitigating pathogens in feed like *Salmonella* if the initial concentrations are low. This could be another method to aid in controlling pathogens in feed without having to use high conditioning temperatures that could result in negative effects on the final feed. Mitigation research can be conducted using nonpathogenic microorganisms, which is ideal in the research and commercial feed mill environments.

X. Surrogates

Enterococcus faecium (*E. faecium*) is a common surrogate used in research looking at reduction of *Salmonella*, with two common lab strains being ATCC 8459 and NRRL B-2354 [77-78]. These strains of *E. faecium* are commonly used as they both lack the majority of virulence factors known for this species and are sensitive to medically relevant antibiotics [78]. Using a minimum temperature of 73°C has demonstrated to be able to produce a 5-log reduction in ATCC 8459 counts, while temperatures above 80°C resulted in no detectable levels of the organism to be counted [79]. Studies in the feed mill environment using short-term steam conditioning of 10 seconds demonstrated a 3-log reduction in a surrogate *E. faecium*, while long-term steam conditioning of 60 seconds resulted in a 4-log reduction in *E. faecium* [69]. Another surrogate that could be considered is *Pediococcus acidilactici* (*P. acidilactici*), which has been used in low-moisture pet food testing, which is comparable to poultry feeds as these are low moisture. Both *E. faecium* strains and *P. acidilactici* are ideal for thermal validation studies, *P. acidilactici* may be the superior option for studies under 90°C [77]. What could make *P. acidilactici* superior is that this organism has a lower heat resistance than *E. faecium*, which could make for *P. acidilactici* to be easier to work with when testing between temperatures of 60°C and 90°C [77]. These surrogates have an important role in mitigation research as they can be used in the feed mill

environment without the worry of contaminating feed. These traits are what allows surrogates to be used to investigate how steam conditioning can impact the pathogen load of feed in the feed mill environment.

XI. Conclusions/Recommendations

Feed manufacture and feed hygienics have major roles in the poultry production systems. Multiple factors go into producing hygienic and quality feed, and all factors must be considered and monitored. The most important factor to consider during feed manufacture is steam conditioning. If done properly, this step has the greatest impact on mitigation of pathogens. Use of higher steam conditioning temperatures also can improve bird performance by producing quality pellets and can reduce microbial load in feed, but could negatively impact nutrient availability of the feed. Obtaining ingredients from trusted suppliers that can verify there is no detectable pathogen is another way to aid in prevention of introducing pathogens to the feed and feed mill environment. Inclusion of pellet binders or other ingredients that act in a similar manner to pellet binders can be utilized to improve pellet quality when pelleted at lower conditioning temperatures, but could increase the risk of pathogens in the feed if not sourced from a reputable supplier. Time of addition of fat to the diet can have impacts on the final feed and thus must be considered during the feed manufacture process. New technology and feed ingredients will continue to come out and so feed producers must keep up to date on this information. A delicate balancing act must be done to achieve proper nutrition for the birds while also producing a hygienic feed.

XII. Future Research

Future studies could use different conditioning temperatures and retention times and how these factors impact nutrient availability of the feed and pellet quality. Past research would suggest

that pellet quality might be improved, but nutrient availability could be affected. Conducting more testing in commercial feed mills with surrogate organisms to see how these hold up in a commercial environment with larger batch sizes and equipment could also be beneficial to the knowledge base of feed microbiology. New technology and antimicrobials can also be considered to aid in pathogen reduction while still possibly maintaining the nutrient availability of the feed. New feed mill equipment, like hygenisers which subject feed to heat for an extended period, on pathogen reduction in feed and nutrient availability of feed. Additionally, antimicrobials could be tested with hygiensers to see if this combination improves feed hygiene and how it affects nutrient availability. Pellet binders, like *Spirulina* algae, could also be used in conjunction with hygiensers to see if the extra protein associated with the algae can negate some of the negative effects that could come from prolonged exposure to high temperatures.

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CHAPTER TWO

The Effect of Standard Pelleting and More Thermally Aggressive Pelleting utilizing a Hygieniser on Feed Manufacture and Reduction of *Enterococcus faecium*, a *Salmonella* Surrogate

T. P. Boltz¹, J. W. Boney², C. Shen¹, J. Jaczynski¹ and J. S. Moritz¹

¹ Division of Animal and Nutritional Science, West Virginia University, Morgantown, West Virginia, 26506

Phone: 304-293-1911
Fax: 304-293-2232

² Department of Animal Science, Pennsylvania State University, University Park, Pennsylvania, 16802

Phone: 814-865-1362
Fax: 814-863-6042

SUMMARY

Feed hygienics are of ever increasing importance in providing safe feed to animals, and ultimately safe food for consumers. *Salmonella* has been identified as a major microbial hazard in animal feed that has been linked to illness in both animals and humans. Use of antibiotics has decreased in recent years due to policies and practices of poultry production, increasing opportunities for potential pathogens in feed to affect poultry and poultry products. New feed equipment technology provides an option to combat feed pathogens. For example, hygienisers have been suggested to decrease *Salmonella* associated with mash feeds due to the ability to maintain conditioned feed temperature for an extended time. *Enterococcus faecium* (*E. faecium*) is a non-pathogenic surrogate used to study reduction of *Salmonella*. The objective of the current study was to compare feed manufacture and *Salmonella* surrogate (*E. faecium* ATCC 8459) reduction differences between standard pelleting and more thermally aggressive pelleting utilizing a hygieniser. More thermally aggressive pelleting decreased pellet mill motor load ($P=0.02$), increased hot pellet temperature ($P=0.02$), and tended to increase pellet durability ($P=0.07$). *E. faecium* ATCC 8459 colonies decreased with standard pelleting relative to inoculated mash and were reduced further with more thermally aggressive pelleting ($P<0.05$). Standard pelleting and more thermally aggressive pelleting resulted in a 3 and 4-log reduction in *E. faecium* ATCC 8459 respectively, relative to inoculated mash. More thermally aggressive pelleting utilizing a hygieniser may improve manufacture efficiency, pellet quality, and *Salmonella* reduction.

Key words: Hygieniser, *Salmonella*, Bacterial Reduction, Pelleting, Feed

DESCRIPTON OF PROBLEM

Feed safety has been of ever increasing concern since the 2011 signing of the Food and Drug Administration's Food Safety Modernization Act (FSMA) into law. The Centers for Disease Control and Prevention estimate that every year in the United States approximately 48 million people get sick, 128,000 are hospitalized, and 3,000 die from a food borne illness [1]. *Salmonella* is a major microbial hazard in animal feeds, and is difficult to eliminate from feed ingredients [2]. Poultry can become infected with *Salmonella* from feed manufactured with contaminated ingredients, and maintained in poultry products. Feed microbiology is a new field of science that may help control pathogens in animal feed.

Industry standards for manufacturing pelleted feeds do not exist, and commercial and integrated feed mills have varied standards of production practices. Antibiotic use in live bird production is on the decline due to policies, practices and marketing; therefore, new methods to control possible pathogenic bacteria in feed such as *Salmonella*, should be investigated. Use of new feed technology available to feed producers may be implemented to reduce the microbial load in feed, which could ultimately aid in producing safe poultry products for consumers.

Use of higher conditioning temperature, antimicrobial inclusion such as organic acids, and other pieces of equipment like expanders have been used to reduce feed *Salmonella* [2]. A new piece of feed equipment technology that could further aid in pathogen reduction in poultry feed is a hygieniser. High temperatures have been shown to have destructive effects on *Salmonella* and other pathogens that may be present in food [3]. Feed can be retained in the barrel of the hygieniser and maintained at a desired conditioning temperature through jacketed heat, which may aid in the destruction of pathogens. Extended retention times have been shown in past literature to reduce contamination of feed. A retention time of 60 seconds at conditioning temperatures of 71°C, 82°C,

and 88°C has demonstrated a reduction of *Enterococcus faecium* ATCC 8459 in feed up to 4-log, while short term conditioning for 10 seconds at the same temperatures demonstrated a 3-log reduction in the same organism [4]. *Enterococcus faecium* ATCC 8459 has been used in past research as an appropriate surrogate for mitigation in feed and poultry products as well as being inactivated at higher temperatures [4-6]. Surrogate organisms do not carry the risks associated with the pathogen of interest and are safer to use when investigating mitigation techniques.

Thermal processing in the form of steam conditioning is a variable that can be manipulated by feed producers. Steam conditioning temperature serves as the critical control point in feed manufacture that reduces the microbial load of the feed [7]. In addition to mitigation potential, pellet quality can be improved with increased conditioning temperature [8-10]. Conversely, steam conditioning can have detrimental effects on nutrient digestibility and retention of mixer added enzymes [8-11]. Therefore, understanding thermal processing variables that mitigate pathogens, improve pellet quality, and avoid nutrient digestibility detriment are critical to optimizing poultry production.

The objective of the current study was to compare differences between standard pelleting and more thermally aggressive pelleting utilizing a hygieniser on feed manufacture efficiency, pellet quality and the viability of *Enterococcus faecium* ATCC 8459, an appropriate non-pathogenic surrogate for *Salmonella*.

MATERIALS AND METHODS

Experiential Design

Two pelleting techniques, standard pelleting with a goal conditioning temperature of 70°C for 15 seconds without hygieniser use and more thermally aggressive pelleting with a goal conditioning

temperature of 80°C for 30 second and hygieniser use for 45 seconds, were applied to three replicated 226.8 kg batches of broiler feed inoculated with *Enterococcus faecium* ATCC 8459 over a three-day feed manufacture period in a randomized complete block design.

Diet Formulation, Batching, and Feed Manufacture

A basal diet was formulated using AgriStat data [12] as a nutrient reference for growing broiler chickens. A master batch of 1,360.8 kg was created and then split into three replications of 453.6 kg. This was then split again into two 226.8 kg batches for the two dietary treatments to be manufactured each day. From each 226.8 kg batch, 21.5 kg of feed was collected to be inoculated with *Enterococcus faecium* inoculum broth. All feed was manufactured at the West Virginia University pilot feed mill located in Morgantown, West Virginia. A California Pellet mill conditioner, hygieniser and 40 HP California Pellet Mill [13] were used for conditioning and pelleting, where pellets were extruded through a 4.7 x 38 mm pellet die. Production rate was constant at 1.2 MT/hr among all replications of manufacture. One allotment of basal diet, 204 kg, was conveyed to a surge bin above the conditioner, and used to obtain and maintain desired conditioning temperature of 70°C for standard pelleting and 80°C for more thermally aggressive pelleting. After the desired temperature had been achieved and the feed probe sensor in the back of the surge bin above the pellet mill was exposed, 22.8 kg of inoculated feed was added into the surge bin to be pelleted. This was done when the probe was exposed to ensure the right samples would be collected and to prevent the system from shutting down and losing the desired conditioning temperature.

Standard pelleting runs had the hygieniser turned off so no additional jacketed heat was applied to the feed as there is no way to bypass the hygieniser in this feed manufacture system. More thermally aggressive pelleting had the hygieniser turned on, so the mash feed was exposed

to additional heat for another 45 seconds after initial conditioning for 30 seconds. Conditioner, hygieniser temperature and pellet mill motor amperage were recorded at this time using a Beta Raven programmable logic control (PLC) system [14]. Two minutes after the inoculated feed was added into the surge bin, pellet samples were collected directly post extrusion from the pellet die. This technique ensured that pellet samples were from the inoculated feed. Pellet samples were placed on cheesecloth on top of a large agriculture fan that pulled ambient air across the pellets for a standardized time of 12 minutes to allow the pellets to cool and dry. This follows methodologies from Reese et al. [15], that limits potential nutrient segregation effects of post pellet auguring. In addition, a portion of pelleted sample was assayed for hot pellet temperature using an insulated container to catch pellets, then immediately closing the lid, and inserting a thermocouple thermometer [16] with an 80PK-24 temperature probe.

After the 12-minute cooling and drying period, a 500 g portion of the pellet sample was placed in a Whirl-Pak bag [17] and flash frozen in liquid nitrogen to maintain bacterial integrity. Following flash freezing, samples were stored at -80°C until *Enterococcus faecium* analysis. Additional pellets sampled from the cooler deck were assayed for pellet quality.

Pellet Quality Analysis

Approximately 24 hours post-pelleting, pellet quality was determined utilizing a New Holman pellet tester (NHPT) [18]. A pelleted sample from each treatment was sifted using a No. 6 W.S. Tyler testing sieve [19]. One hundred grams samples of sifted pellets were placed in the NHPT perforated chamber, where forced air was applied for 30 seconds and the remaining pellet samples were weighed and recorded as a percentage to determine pellet durability. Pellet quality analyses were conducted in triplicate for each treatment and results reflect average pellet durability.

Non-pathogenic Surrogate Microorganism

Enterococcus faecium ATCC 8459 (American Type Culture Collection, Manassas, Virginia), herein referred to as *E. faecium* was selected to be the non-pathogenic surrogate microorganism for this experiment based on use in previous work [4-6,20]. Brain heart infusion (BHI) broth was used to prepare *E. faecium* culture [3, 21]. BHI broth was prepared by dissolving BHI powder in deionized water followed by sterilization in an autoclave at 121°C for 15 minutes. A freeze-dried *E. faecium* pellet was rehydrated in sterile BHI broth. A five percent inoculum of rehydrated *E. faecium* was then aseptically transferred to tryptic soy broth (TSB) [22] with five percent defibrinated sheep blood [23] and incubated for 24 hours at 26°C using a rotary incubator shaker [24] at 100 revolutions per minute. This provided the initial culture of *E. faecium*. A stock culture of *E. faecium* was prepared by combining equal volume of glycerol and the initial culture. The stock culture was and stored at -80°C.

Prior to feed inoculation, the stock culture was thawed under running tap water. A five percent inoculum of thawed stock culture was aseptically transferred to 500 mL of sterile TSB supplemented with five percent defibrinated sheep blood followed by incubation for 24 hours at 26°C using a rotary incubator shaker. This process yielded the *E. faecium* culture that was used to inoculate feed.

Feed Inoculation and Incubation

Feed inoculation and bacterial incubation followed Boney et al. [3], where TSB powder was dissolved in deionized water and sterilized by autoclaving at 121°C for 15 minutes. The sterile TSB was cooled to approximately 47°C before defibrinated sheep blood was added at a five percent inclusion and then gently mixed. A sterilized inoculation loop was inserted into the of *E.*

faecium stock culture and then aseptically transferred to the TSB to initiate bacterial propagation. Inoculum was placed in an incubator shaker for 24 hours at 26°C at 100 revolutions per minute.

It is important to note that none of the feed was sterilized before inoculation. A 21.5 kg feed allotment was placed in a Triumph paddle mixer, where five percent (wt/wt) (1.3 kg) inclusion of inoculum was applied to the feed and allowed to mix for five minutes. This provided a 22.8 kg treatment that would be added back to an untreated 204 kg of basal diet to achieve the total 226.8 kg experimental unit for each treatment. After mixing, mash samples (~500 gram) were collected in Whirl-Pak bags and submerged in liquid nitrogen to maintain bacterial integrity and stored (-80°C) until analysis. The remaining inoculated feed was transferred to the West Virginia University pilot feed mill to be pelleted.

Enumeration of *Enterococcus faecium* in Mash and Pelleted Feed

The following procedures were conducted to enumerate *E. faecium*, which follow Boney et al. [3]. Pelleted feed samples were removed from storage at -80°C and pulverized using a sterile mortar and pestle. A two-gram sample of either unconditioned mash or pulverized pellet was weighed and aseptically placed in a sterile, 50 mL conical tube [25]. Phosphate buffered saline (1xPBS) [26] was added to the conical tube containing either mash or pulverized pellets to create a 50-mL solution. The solution was agitated by hand to suspend *E. faecium* in the 1xPBS solution. Following agitation, serial dilutions were prepared by placing nine mL of 1xPBS in five sterilized, 20 mL glass tubes. A quantity of one mL of the feed and PBS solution was transferred to a serial dilution tube, covered with parafilm, and inverted multiple times, creating a 1:10 dilution. Subsequent serial dilutions were carried out (1:10–1:1,000,000). Contents from each dilution tube were passed through a 0.45µm gridded membrane filter [27] using vacuum filtration. Post vacuum filtration, each filter was placed on an m-Enterococcus agar plate [28], inverted, and placed in an

incubator for 48 hours at 35°C. Following incubation, typical pink/purple *E. faecium* colonies were counted and recorded. Colony counts were derived from two g feed samples, so counts were divided by two and reported as colony forming units per gram of feed ($\log_{10}$ CFU/g). Enumeration was performed in triplicate for each pelleting technique and then averaged. These methods were performed on each of the three replicates of feed samples. Relative reduction percentage was calculated using the following equation:

$$100 - ((\text{Pellet CFU/g}) / (\text{Mash CFU/g})).$$

Statistical Analysis

Feed manufacture pelleting technique was replicated three times over a three-day period with a single 226.8 kg allotment of feed serving at the experimental unit. Treatments were randomly ordered per day to achieve a randomized complete block design. Data were analyzed using the GLM procedure of Statistical Analysis System [29] and alpha was designated to be $P \leq 0.05$. Mash and pelleted samples were utilized in the analysis for bacteria colony count. Significant effects were further explored via post hoc protected Fisher's least significant difference test for bacteria colony count data.

RESULTS AND DISCUSSION

The basal diet is shown in Table 1. Feed manufacture and bacteria colony count results are depicted in Table 2. The more thermally aggressive pelleting technique increased conditioner and hygieniser temperature relative to standard pelleting ($P < 0.05$) as designed. More thermally aggressive pelleting also decreased motor load and increased hot pellet temperature ($P < 0.05$). Pellet temperature effect was associated with treatment design. The decreased pellet mill motor load can be attributed to increased moisture at the pellet die due to greater steam volume to obtain the higher conditioning temperature providing more lubrication. These results agree with past

literature that demonstrated a reduction of motor load with elevated conditioning temperatures [8, 30-31]. Pellet durability tended to increase from standard pelleting to more thermally aggressive pelleting ($P=0.07$). Table 2 shows an increase of 21 percentage points for more thermally aggressive pelleting. The additional moisture and heat retention time could aid in starch gelatinization and protein gelation, as demonstrated in past literature [10, 32-33]. This particular magnitude of pellet durability improvement is important due to past literature describing broiler performance improvement when fed pellets that varied in similar percentage points [8, 34-35].

Reduction of *E. faecium* was significant between standard and more thermally aggressive pelleting. The untreated mash started with 88,389 colonies and after standard and more thermally aggressive pelleting, it was reduced to 2,822 and 320 colonies respectively. From a microbiology perspective, this is a 3- and 4-log reduction for standard pelleting and more thermally aggressive pelleting. These results agree with general claims suggesting that pelleting aids in the destruction of pathogens in feed [36]. Generally, a 5-log reduction of a target pathogen is considered sufficient for pasteurization, which is defined as “partial sterilization of a substance at a temperature and for a period of exposure that destroys objectionable organisms without major chemical alteration of the substance” [37]. The current study did not achieve pasteurization, but future research could investigate increased temperature and time to achieve pasteurization.

Addition of an antimicrobial, such as a formaldehyde amalgam, and use of a hygieniser could result in greater mitigation of bacteria in feed. Boney et al. [4], utilized mixer-added antimicrobial inclusion and varying temperatures of steam conditioning and found that inclusion of an antimicrobial decreased *E. faecium* colony counts when applied to mash feed that was inoculated and mitigation potential increased when mash was conditioned at higher conditioning temperatures with antimicrobial addition. Jones et al. saw that application of formaldehyde to feed

that was preconditioned at 82°C for 60 seconds, and then subjected to extended heat in a hygieniser for 4.5 minutes had minimal impact on amino acid digestibility [38]. The effect of hygieniser use on feed nutrients using additional feed manufacture variables should be addressed with additional studies.

Essential nutrients can be damaged at high conditioning temperatures, which could be utilized to combat pathogens associated with feed. Cutlip et al. found a 6.7% loss of vitamin A when feed was conditioned and pelleted at 93°C [8]. Leucine, valine, isoleucine, alanine, and aspartic acid digestibility have been shown to decrease in diets conditioned at 91°C relative to 74 and 82°C [10]. Loar et al. also found that methionine, isoleucine, and proline had significantly reduced digestibility when conditioned at 85 and 96°C relative to 74°C [9]. Use of more thermally stable enzymes should be considered as certain enzymes can be compromised at high conditioning temperatures and retention times [11,39-41]. Though conditioning temperatures and retention times utilized for this study were not as extreme relative to some commercial practices, more research is needed determine optimal temperatures and retention times for pathogen reduction while still considering nutrient availability and pellet quality.

Results from this study suggest that use of a hygieniser can not only reduce possible pathogenic organisms in feed, but also improve feed manufacture metrics and pellet quality.

CONCLUSION AND APPLICATIONS

1. More thermally aggressive pelleting, i.e. 80°C conditioning for 30 seconds and 45 seconds retention in a hygieniser, increased pellet durability and showed more *Salmonella* reduction potential compared to standard pelleting.
2. Pellet mill motor load was decreased with more thermally aggressive pelleting.
3. Within the parameters of this study, standard pelleting demonstrated a 3-log reduction in an inoculated surrogate organism while more thermally aggressive pelleting demonstrated a 4-log reduction.

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TABLES AND FIGURES

Table 1. Dietary composition and calculated nutrients of the basal diet used for inoculation.

Ingredient	Inclusion (%)
Corn	57.52
Soybean Meal (48%)	30.82
Wheat Middlings	5.00
Soybean Oil	2.18
Dicalcium Phosphate	1.68
Limestone	1.31
DL-Methionine	0.36
Salt	0.35
L-Lysine HCL	0.27
Vitamin/Mineral Premix ¹	0.25
Sodium Bicarbonate	0.15
Threonine	0.12
Calculated Nutrients	
ME (kcal/kg)	3,030.5
Crude Protein (%)	20.71
Digestible Lysine (%)	1.18
Digestible Met+Cys (%)	0.89
Digestible Threonine (%)	0.77
Calcium (%)	0.96
Available Phosphorus (%)	0.45
Sodium (%)	0.20

¹ Supplied per kilogram of diet: 0.02% manganese; 0.02% zinc; 0.01% iron; 0.0025% copper; 0.0003% iodine; 0.00003% selenium; 0.69 mg of folic acid; 386 mg of choline; 6.61 mg of riboflavin; 0.03 mg of biotin; 1.38 mg of vitamin B6; 27.56 mg of niacin; 6.61 mg of pantothenic acid; 2.20 mg of thiamine; 0.83 mg of menadione; 0.01 mg of vitamin B12; 16.53 IU of vitamin E; 2,133 IU of vitamin D3; and 7,716 of vitamin A.

Table 2. Pelleting Technique and Hygieniser Use on Feed Manufacturing Metrics and *E. faecium* 8459 mitigation.

Run Type	Goal Conditioning Temperature (°C)	Conditioning Time (sec)	Hygieniser (On/Off)	Recorded Conditioning Temperature (°C)	Recorded Hygieniser Temperature (°C)	Motor Load ¹ (%)	Hot Pellet Temperature ² (°C)	New Holman Pellet Tester Survivability ³ (%)	<i>E. faecium</i> Mitigation ⁴ (Colonies)	Relative Reduction ⁵ (%)
Mash	-	-	-	-	-	-	-	-	88389.0 ^a	-
Standard	70	15	Off	70.0	55.4	51.0	70.8	68.7	2822.3 ^b	99.97
More Thermally Aggressive	80	30	On	80.2	82.8	44.0	79.7	89.7	320.0 ^c	99.99
Anova P-value	-	-	-	0.0003	0.0036	0.0198	0.0209	0.0692	<.0001	.0529
SEM ⁶	-	-	-	0.132	1.16	0.707	0.928	3.54	327.4	0.005
Fisher's LSD ⁷	-	-	-	-	-	-	-	-	1285.6	-

¹ A 100% motor load was based on FLA (full load amps) that was 47 amps based on the pellet mill motor name plate.

² Hot pellet temperature was determined on pellets directly following extrusion from the die. Pellets were collected into an insulated container and temperature was measured using a thermocouple thermometer and an 80PK-24 temperature probe.

³ Measurements of PDI were obtained using the New Holmen Pellet Tester, where 100 g pelleted samples are subjected to air flow within a perforated chamber for 30 s.

⁴ *E. faecium* mitigation = Determined using serial dilutions, vacuum filtration, bacteria specific m-ENT media, and incubation. Pink colonies were counted following 35°C incubation for 48 hours.

⁵ Relative Reduction = 100 - (Pellet Colony Count/Mash Colony Count)

⁶ SEM = Pooled Standard error of the mean.

⁷ Fisher's least significant difference value

**The Effect of Varying Steam Conditioning Temperature and Time on Pellet Manufacture
Variables, Digestible Amino Acid Concentration, and Feed Enzyme Recovery**

T. P. Boltz ¹, N. E. Ward ², V. E. Ayres¹, A. E. Lamp¹ and J. S. Moritz ¹

¹ Division of Animal and Nutritional Science, West Virginia University, Morgantown, West
Virginia, 26506

Phone: 304-293-1911

Fax: 304-293-2232

² DSM Nutritional Products Inc., Parsippany, NJ 07054

Phone: +1-973-257-8500

SUMMARY

This study hypothesized that increased steam conditioning temperature and conditioning time would improve feed manufacture metrics while decreasing the digestible amino acid concentration and recovery of a lysozyme feed additive. The objectives of the study were to assess how varying steam conditioning temperatures and conditioning times interacted to affect pellet mill motor load, pellet quality, digestible amino acid concentration, and enzyme recovery. Corn and soybean meal based diets that included DDGS and meat and bone meal were conditioned at either 76°C, 82°C, or 88°C with conditioning times of either 30 or 60 seconds. Treatments were arranged in a 3 (steam conditioning temperature) x 2 (conditioning time) factorial in a randomized complete block design. Digestible amino acid concentration was estimated using cececotomized roosters in a Latin Square with crossover design. Motor load decreased for 30 second conditioning compared to 60 second conditioning when diets were subjected to 76 and 82°C, but the time effect was lost at 88°C. This interaction was likely the result of lower feed volume in the conditioner as per 30 second conditioning obtaining greater moisture condensation and lubrication at lower conditioning temperatures. Lysozyme activity decreased when conditioned at 88°C ($P=0.0001$), but was not affected by retention time. Conditioning temperature and time interacted to affect digestible amino acid concentrations of methionine, lysine, threonine, alanine, aspartic acid, glutamic acid, isoleucine, leucine, proline, and valine ($P<0.05$). Increased conditioning temperatures at 30 seconds increased digestible amino acid concentrations. Similarly, diets conditioned at 60 seconds increased digestible amino acid concentration between 76 and 82°C.

Key words: Conditioning, Pelleting, Amino Acid, Digestibility, Enzyme

DESCRIPTION OF PROBLEM

Commercial broiler production relies on pelleting as it has been shown to decrease feed wastage, ingredient segregation, prehensile energy expenditure, selective feeding, and reduce pathogen mitigation [1]. Due to increased regulation on feed safety and the desire to reduce pathogens as well as a desire for improved pellet quality, higher conditioning temperatures and longer conditioning times are being utilized in pelleted feed manufacture. The use of more thermally aggressive conditioning techniques may be a concern for nutrient availability. Use of high temperatures over 85°C have been shown to significantly reduce digestibility of amino acids such as methionine and valine [2-3]. Vitamins are also susceptible to heat as demonstrated that pelleting at 93°C decreased vitamin A by 6.7% [4]. Along with amino acids being negatively impacted by higher temperatures, exogenous enzymes can also become denatured and negatively affect bird performance [5-6].

Positive benefits of increased conditioning temperature could be improved pellet quality, which in turn could improve bird performance. It has been well documented that having an improved pellet-to-fine ratio allows broilers to have improved performance and decreased feed conversion ratios [7-9]. More specially, modest improvements in pellet quality from 50% crumble/pellet to 70% may improve broiler performance and decreased feed conversion ratio by 3 points [7]. Increased steam conditioning temperature results in a higher amount of moisture being added to the mash feed which can improve pellet quality [10]. Increased conditioning time (60 sec vs 30 sec) has also been shown to increase pellet durability [11].

Use of cecectomized roosters is a common and reliable method for determining nutrient digestibility. Cecectomized roosters are better models for estimating amino acid digestibility than conventional roosters as removal of the ceca reduces microbial activity in the hind-gut [12]. In addition, cecectomized roosters are commonly used for determination of amino acid digestibility

due to the relatively simple surgical procedure, easy maintenance, and no need for digesta markers [13].

More pressure from markets worldwide have seen a decrease in antibiotic use in animal feeds. One alternative that has been investigated are lysozymes. Lysozyme are naturally occurring enzymes that are found in tears, saliva, and milk and are also used in feed. Lysozymes cleave glycosidic linkages in the peptidoglycan components of bacterial cell walls which lead to loss of cellular membrane integrity and ultimately cell death [14]. Feeding this enzyme to swine has shown decreased *Campylobacter* prevalence in the digestive tract and increased growth rates, while in poultry reduced numbers of *E. coli* were reported when compared to birds being fed a diet with an antibiotic [14-16].

The objective of the current study was to assess how varying steam conditioning temperatures and conditioning times interacted to affect feed manufacture metrics, amino acid digestibility using single comb white leghorn cecectomized roosters, and enzyme recovery.

MATERIALS AND METHODS

Diet Formulation and Batching

A corn and soybean basal diet with DDGS and meat and bone meal was formulated to meet industry specifications for nutritional needs of growing broiler chickens. Soybean meal sent to a commercial laboratory to be analyzed for trypsin inhibitor which was reported to be 3.64 TIU/mg [17]. One master batch of basal diet, 3,266.1 kg, was manufactured and separated into three replications of 1,088.7 kg. From each replication, six allotments of 362.9 kg were used for the varying conditioning temperatures (76°C, 82°C, 88°C) and conditioning time (30 and 60 seconds), creating a total of six experimental diets. A lysozyme product was added to all diets to determine the thermostability of the enzyme.

Feed Manufacture

All feed was manufactured at the West Virginia University pilot feed mill located in Morgantown, West Virginia. A California Pellet mill conditioner, hygieniser and 40 HP pellet mill [18] was used for pelleting and pellets were extruded through a 4.7 x 38mm pellet die. Production rate was constant at 1.2 MT/hr among all replications of manufacture. For this experiment the hygieniser was not on and so no heated retention of feed occurred. Feed still passed through the hygieniser as there is no way to bypass it within this feed manufacture system. Conditioner, hygieniser temperature, and pellet mill motor amperage were recorded using a Beta Raven programmable logic control (PLC) [19] system after goal temperature had been achieved for each treatment. Pellet samples were taken directly after pellet die extrusion and placed on a cheesecloth on top of a large agriculture fan that pulled ambient air across the pellets for a standardized time of 12 minutes to allow the pellets to cool and dry. This follows methodologies from Reese et al. [20], that limits potential nutrient segregation effects of post pellet auguring. In addition, a portion of the pelleted samples was assayed for hot pellet temperature using an insulated container to catch pellets and then immediately closing the lid, inserting a thermocouple [21] with an 80PK-24 temperature probe.

Pellet Quality Analysis

24 hours post-pelleting, pellet quality was determined utilizing a New Holman pellet tester (NHPT) [22]. All pelleted samples from each treatment were sifted using a NO. 6 W.S. Tyler testing sieve [23]. One hundred gram samples of sifted pellets were placed in the NHPT perforated chamber, where forced air was applied for 30 seconds and the remaining pellet samples were weighed and recorded as a percentage. Pellet quality analyses were conducted in triplicate for each treatment and results reflect average pellet durability.

True Amino Acid Digestibility (TAAD)

Dietary treatments were precision fed to six, 18-month-old cecectomized Single Comb White Leghorn (SCWL) roosters for determination of TAAD. This was done using a Latin Square with crossover design where each rooster was fed all six of the experimental diets over a 14-week period in a way that no two roosters were fed the same diet during the same week. A wash out period of one week was utilized between precision feedings to ensure birds did not have residual diet. Corn starch pellets were precision fed to each rooster during the last week to determine endogenous amino acid losses. Precision feeding followed modified methodologies of Sibbald [24], where SCWL roosters were fasted for 24 hours and then precision fed 30 grams of sample directly into the crop. Excreta was collected for 48 hours post feeding. Amino acid analysis on feed and excreta were conducted by a commercial laboratory [25]. The following equations was used to calculate the true amino acid digestibility and then amino acid concentration:

$$\text{TAAD (\%)} = \frac{(\text{Grams Fed} * \% \text{AA}_{\text{Feed}}) - (\text{Grams of Excreta} * \% \text{AA}_{\text{Excreta}}) - (\text{Grams of Excreta}_{\text{endogenous}} * \% \text{AA}_{\text{Endogenous}})}{(\text{Grams Fed} * \% \text{AA}_{\text{Feed}})} * 100$$

$$\text{AA Concentration} = (\text{TAAD}/100) * (\text{Grams of AA in diet})$$

Statistical Analysis

Variables were analyzed in a 3 (conditioning temperature) x 2 (conditioning time) factorial arrangement in a randomized complete block design. The experimental unit consisted of one 362.9 kg batch of feed or one SCWL rooster. A multiple comparison was performed to compare main effect means. Digestible amino acid concentration was estimated using cececotomized roosters in a Latin Square with crossover design. The conditioning temperature and conditioning time interactions were further determined using Fisher's least significant difference test. Data was analyzed using the GLM procedure of SAS [26] and alpha was designed to be $P \leq 0.05$.

RESULTS AND DISCUSSION

The basal diet formulation can be found in Table 1. Feed manufacture and pellet quality main effect and interaction data are presented in Table 2. Conditioning temperature and conditioning time interacted in their effect on motor load ($P=0.0168$) and hot pellet temperature ($P=0.0477$). This interaction was likely the result of lower feed volume in the conditioner as per 30 second conditioning obtaining greater moisture condensation and lubrication at lower conditioning temperatures. Conditioning time had a greater influence on motor load at low conditioning temperatures, where a longer retention time increased motor load. Increasing conditioning temperature decreased motor load and conditioning time had no effect at high conditioning temperatures. This effect is most likely due to gelatinization of ingredients, which lead to a more viscous diet. The increased volume of feed to steam ratio provided more moisture to the diet, which added extra lubrication at the die which resulted in decreased motor load [3, 27-29]. Boney and Moritz found that as conditioning temperature increased, so did hot pellet temperature [3]. As conditioning temperature and time was increased so did hygieniser temperature, which could have contributed to the interaction for hot pellet temperature. The varying feed volume to steam ratio could affected the hot pellet temperatures as the shorter retention time had a lower feed volume allowing the steam to penetrate further into the mash feed which raised the overall temperature of the feed.

Conditioning temperature affected all metric measured except for motor load and hot pellet temperature variables measured. Conditioning temperature ($P=0.0001$) and hygieniser temperature ($P=0.0011$) increased with increasing steam conditioning temperature, which is by design. New Holman Test results improved with increasing temperature ($P=0.0005$). Past literature has demonstrated that increased conditioning temperature improves pellet quality, which was observed

in this study with a 24.8 percentage point difference between 77°C and 88°C treatments [3-4, 27, 29]. The additional moisture added during the heating process could aid in starch gelatinization and improved protein denaturation, which would result in improved pellet quality [30-31].

Conditioning time affected hygieniser temperature ($P=0.0435$). The hygieniser was turned off for this experiment, but conditioned feed still had to pass through it before being pelleted. The feed volume to steam ratio for the different times is what caused a difference in the temperature the hygieniser was displaying on the PLC. The lower feed volume for the 30 second treatment allowed heat to penetrate the mash feed and concentrate in the center of the mash. This allowed for the hygieniser to be heated more when feed passed through it when compared to the 60 second treatments as the heat could not penetrate the mash feed as deep.

Conditioning temperature and conditioning time interacted their effect on all measured amino acids, other than cysteine and glycine (Table 3). Interactions for the 3 most limiting essential amino acids (Lys, Met, and Thr) are shown in Figures 1-3. These interactions are most likely due to the extended conditioning time and increased conditioning temperature having positive effects on anti-nutritional factors that were in the diet. Trypsin inhibitor was high in this diet at 3.64 TIU/mg, which is above the industry standards of <0.2 to 3.5 [32]. Past research has shown that diets containing 1.46 TIU/mg can have negative effects on the performance of non-ruminant animals [33]. In the current study conditioning at 30 seconds for 77, 82, and 88°C and 60 seconds at 77 and 82°C demonstrated to increase digestibility when compared to 88°C for 60 seconds. Bergeron et al. speculated that thermal processing can affect the cell wall of feed ingredients by breaking down the aleurone layer, which can lead to increased opportunities for nutrient utilization [34].

Proteins in the diet could have undergone gelation, which is the aggregation of denatured molecules with a certain degree of order, resulting in the formation of a continuous network [35]. Before gelation can occur, proteins must first be denatured and then heat and moisture are added to initiate the gelation process [36]. Proteins in the feed could have been denatured and gelled to point that was still available to the birds when conditioned at 76, 82, and 88°C for 30 seconds and 72 and 82°C for 60 seconds, but not when conditioned at 88°C for 60 seconds. The addition moisture and heat required for the 88°C treatment may have led to a greater proportion of gelation of proteins in the diet, which resulted in less amino acids being available to the bird.

Lysozyme retention decreased with conditioning temperature ($P=0.0001$), but was not affected by conditioning time. Similar recovery was observed for the 77°C and 82°C treatments, but a significant drop in recovery was observed for the 88°C treatment. These results suggest that conditioning time does not affect the thermostability of an enzyme, but the temperature at which the feed is processed does. Past literature has shown that increased conditioning temperatures denature enzymes, which is in agreement with results from this study [37].

CONCLUSION AND APPLICATIONS

1. Increasing conditioning temperature and time decreased pellet mill motor load.
2. Increasing conditioning time does not affect exogenous enzyme recovery. However conditioning temperature of 88°C decreased recovery.
3. Conditioning broiler diets at 88°C for 60 seconds demonstrated a decrease in digestible amino acid concentration when the same diets were conditioning at 77, 82 and 88°C for 30 second and 76 and 82°C for 60 seconds.

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TABLES AND FIGURES

Table 1. Dietary composition and calculated nutrients of the basal diet.

Ingredients	% in Diet
Corn	52.31
Soybean Meal (46%) ¹	35.13
Soybean Oil	3.40
DDGS	3.00
Meat and Bone Meal	2.61
Limestone	1.38
DiCalcium Phosphate	0.65
Methionine	0.48
Threonine	0.29
Vitamin/Mineral Premix ²	0.25
Salt	0.25
Lysine	0.14
Sodium Bicarbonate	0.10
Calculated Nutrients	
ME (kcal/kg)	3,000
Crude Protein (%)	23.00
Calcium (%)	1.01
Available Phosphorus (%)	0.36
Sodium (%)	0.14
Lysine (%)	1.20
Methionine (%)	0.79
Methionine + Cysteine (%)	1.08
Threonine (%)	1.01
Tryptophan (%)	0.23

¹ Trypsin inhibitor was analyzed and determined to be 3.64 TIU/mg, where TIU is Trypsin Inhibitor Units.

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

Table 2. The effects of conditioning temperature and conditioning time on feed manufacture variables and pellet quality.

Conditioning Temperature (°C)	Conditioning Time (sec)	NHPT ¹ (%)	Conditioner Temperature ² (°C)	Hygieniser Temperature ³ (°C)	Motor Load ⁴ (%)	Hot Pellet Temperature ⁵ (°C)	Muramidase Activity ⁶ (LSU(F)/kg)
77	30	47.3	77.0	66.7	41.7 ^b	77.5 ^c	24,900.8
82	30	61.3	81.9	70.7	39.7 ^c	81.0 ^b	25,260.0
88	30	68.7	85.6	76.3	39.3 ^c	86.2 ^a	19,216.3
77	60	44.0	76.7	62.8	44.7 ^a	75.4 ^d	25,938.0
82	60	59.0	82.0	67.8	41.3 ^b	80.6 ^b	23,925.3
88	60	72.3	87.6	72.8	39.7 ^c	86.8 ^a	16,739.1
Treatment <i>P</i> -Value	---	0.0037	<0.0001	0.0051	<0.0001	<0.0001	<0.0001
Treatment SEM ⁷	---	4.14	0.649	1.83	0.375	0.490	1,269.8
Conditioning Temperature							
77	---	45.7 ^c	76.9 ^c	64.7 ^c	43.2 ^a	76.5 ^c	25,419.4 ^a
82	---	60.2 ^b	81.9 ^b	69.3 ^b	40.5 ^b	80.8 ^b	24,592.7 ^a
88	---	70.5 ^a	86.6 ^a	74.5 ^a	39.5 ^c	86.5 ^a	17,977.7 ^b
Conditioning Temperature SEM	---	2.93	0.459	1.30	0.265	0.347	943.7
Conditioning Time							
---	30	59.1	81.5	71.2 ^a	40.2 ^b	81.6	23,287.1
---	60	58.4	82.1	67.8 ^b	41.9 ^a	80.9	22,447.2
Conditioning Time SEM	---	2.39	0.374	1.06	0.217	0.283	802.0
Probability Values							
Conditioning Temperature	---	0.0005	<0.0001	0.0011	<0.0001	<0.0001	<0.0001
Conditioning Time	---	0.8476	0.2708	0.0435	0.0003	0.1433	0.3611
Conditioning Temperature by Conditioning Time	---	0.6691	0.2015	0.9683	0.0168	0.0477	0.3984

¹ Durability was measured using the New Holmen Pellet Tester where 100 gram pelleted samples are subjected to air flow within a perforated chamber for 30 seconds.

² Conditioning temperature was measured as the reading from the conditioner temperature probe at the time of sample collection.

³ The hygieniser was not turned on during this experiment; however, feed must run through the hygieniser for 45 seconds post conditioning and prior to pellet die extrusion based on the WVU feed manufacture system.

⁴ A 100% motor load was based on FLA (full load amps) that was 47 amps based on the pellet mill motor name plate.

⁵ Hot pellet temperature was determined on pellets directly following extrusion from the die. Pellets were collected into an insulated container and temperature was measured using a thermocouple thermometer and an 80PK-24 temperature probe.

⁶ Mash mean activity = 31,437.4 LSU(F)/kg, where LSU(F) is the amount of enzyme that increases the fluorescence of 12.5 µg/ml fluorescein-labelled peptidoglycan per minute at pH 6.0 and 30 °C by a value that corresponds to the fluorescence of approximately 0.06 nmol fluorescein isothiocyanate isomer.

⁷ SEM: Pooled Standard error of the mean.

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

Table 3. The effects of conditioning temperature and conditioning time on Digestible Amino Acid Concentration.

Conditioning Temperature (°C)	Conditioning Time (sec)	Alanine (%)	Aspartic Acid (%)	Cysteine (%)	Glutamic Acid (%)	Glycine (%)	Isoleucine (%)	Leucine (%)	Lysine (%)	Methionine (%)	Proline (%)	Threonine (%)	Valine (%)
77	30	1.02 ^b	2.31 ^c	0.29	3.64 ^d	0.64	0.92 ^{cd}	1.76 ^d	1.31 ^c	0.73 ^d	1.46 ^{bc}	0.99 ^c	1.01 ^c
82	30	1.08 ^a	2.47 ^a	0.31	3.80 ^b	0.69	0.98 ^a	1.83 ^b	1.40 ^a	0.74 ^c	1.49 ^a	1.04 ^b	1.09 ^a
88	30	1.09 ^a	2.46 ^a	0.31	3.90 ^a	0.69	0.99 ^a	1.87 ^a	1.40 ^a	0.80 ^b	1.49 ^a	1.07 ^a	1.08 ^a
77	60	1.03 ^b	2.35 ^b	0.30	3.74 ^c	0.62	0.93 ^c	1.81 ^{bc}	1.32 ^c	0.74 ^c	1.48 ^{ab}	1.02 ^c	1.02 ^c
82	60	1.04 ^b	2.39 ^b	0.30	3.74 ^c	0.65	0.95 ^b	1.79 ^c	1.36 ^b	0.81 ^a	1.45 ^c	1.05 ^b	1.05 ^b
88	60	1.03 ^b	2.31 ^c	0.29	3.64 ^d	0.61	0.91 ^d	1.75 ^d	1.32 ^c	0.72 ^e	1.44 ^c	1.01 ^c	1.01 ^c
Treatment P-Value	---	<0.0001	<0.0001	0.0617	<0.0001	0.7753	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment SEM ¹	---	0.008	0.012	0.005	0.015	0.053	0.004	0.008	0.008	0.002	0.007	0.006	0.006
Conditioning Temperature													
77	---	1.03 ^b	2.33 ^c	0.30	3.69 ^b	0.63	0.92 ^c	1.78 ^b	1.32 ^b	0.73 ^c	1.47	1.00 ^b	1.02 ^c
82	---	1.06 ^a	2.43 ^a	0.31	3.77 ^a	0.67	0.97 ^a	1.81 ^a	1.38 ^a	0.77 ^a	1.47	1.05 ^a	1.07 ^a
88	---	1.06 ^a	2.39 ^b	0.30	3.77 ^a	0.65	0.95 ^b	1.81 ^a	1.36 ^a	0.76 ^b	1.47	1.04 ^a	1.04 ^b
Conditioning Temperature SEM	---	0.005	0.008	0.004	0.011	0.037	0.003	0.005	0.006	0.001	0.005	0.005	0.005
Conditioning Time													
---	30	1.06 ^a	2.41 ^a	0.30	3.78 ^a	0.68	0.96 ^a	1.82 ^a	1.37 ^a	0.75	1.48 ^a	1.03	1.06 ^a
---	60	1.03 ^b	2.35 ^b	0.30	3.71 ^b	0.62	0.93 ^b	1.78 ^b	1.33 ^b	0.75	1.46 ^b	1.03	1.03 ^b
Conditioning Time SEM	---	0.004	0.007	0.003	0.009	0.031	0.002	0.004	0.005	0.001	0.004	0.004	0.004
Probability Values													
Conditioning Temperature	---	0.0003	<0.0001	0.0747	<0.0001	0.7479	<0.0001	0.0010	<0.0001	<0.0001	0.8432	<0.0001	<0.0001
Conditioning Time	---	<0.0001	<0.0001	0.1500	<0.0001	0.2322	<0.0001	<0.0001	<0.0001	0.4425	0.0003	0.1261	<0.0001
Conditioning Temperature by Conditioning Time	---	0.0007	<0.0001	0.1398	<0.0001	0.8204	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001

^{a-c} Means within a column not sharing a common superscript differ significantly (P<0.05).¹ SEM: Pooled Standard Error of the Mean.

Figure 1. Interaction of Digestible Lysine Concentration.

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

Figure 2. Interaction of Digestible Methionine Concentration.

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

Figure 3. Interaction of Digestible Threonine Concentration.

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

CIRRICULUM VITAE

Timothy P. Boltz

28 Drake Road, Morgantown, WV 26501

tpb0015@mix.wvu.edu

(307) 286-6989

EDUCATION

Degree Obtained: Associate of Science Degree, Agriculture; May 2015

University Attended: Laramie Country Community College

Undergraduate GPA: 3.913

Degree Obtained: Bachelor of Science, Animal Science; May 2017

University Attended: Colorado State University

Undergraduate GPA: 3.967

Expected Degree: Master of Science, Animal and Food Science

Expected Graduation Date: May 2019

University Attended: West Virginia University

Current GPA: 3.875

EDUCATION HONORS/AWARDS

Scholarships/Grants Received

- Presidential Scholarship: 2013-2015
- Janie Smith Memorial 4-H Scholarship: 2013

Awards and Honors

- Graduation with Honors (Summa Cum Laude): Spring 2017
 - College of Agricultural Sciences Dean's List: Fall 2015-Spring 2017
 - Obtained Eagle Scout Rank from Troop 221: 2013
-

PUBLICATIONS

First Author Publications

T. P. Boltz, J. W. Boney, C. Shen, J. Jaczynski and J. S. Moritz. The Effect of Standard Pelleting and More Thermally Aggressive Pelleting utilizing a Hygieniser on Feed Manufacture and Reduction of *Enterococcus faecium*, a *Salmonella* Surrogate. (In Preparation)

T. P. Boltz, N. E. Ward, V. E. Ayres, A. E. Lamp and J. S. Moritz. The Effect of Varying Steam Conditioning Temperature and Time on Pellet Manufacture Variables, Digestible Amino Acid Concentration, and Feed Enzyme Recovery. (In Preparation)

Abstracts

T. P. Boltz, J.W. Boney, and J.S. Moritz. 2018. Feed manufacture and *Salmonella* surrogate mitigation differences between standard pelleting and more thermally aggressive pelleting utilizing a hygieniser. Poult. Sci. (Accepted Abstract (161)).

T. P. Boltz, N. E. Ward, V. E. Ayres, A. E. Lamp and J. S. Moritz. The Effect of Varying Steam Conditioning Temperature and Time on Pellet Manufacture Variables, Digestible Amino Acid Concentration, and Feed Enzyme Recovery. (In Preparation)

K. Harding, A. E. Lamp, **T. P. Boltz**, V. Ayres, and J. S. Moritz. The Effect of Pelleting Diets Composed of Large Particle Corn using Standard Conditioning and More Thermally Aggressive Conditioning Utilizing a Hygieniser. (In Preparation)

RESEARCH EXPERIENCE

National Meeting Paper Presentations

- 2018 Poultry Science Association Annual Meeting (San Antonio, TX) (Graduate Student)
 - “Feed manufacture and *Salmonella* surrogate mitigation differences between standard pelleting and more thermally aggressive pelleting utilizing a hygieniser.”

Graduate Teaching Assistant

- Teaching Assistant for Companion Animal Science Class: Fall 2018
 - Assisted Dr. Joseph Moritz instructing classes, graded assignments, and lectured on reptile and amphibian management/nutrition.

Graduate Research Assistant

- WVU Pilot Feed Mill Manager: Fall 2017-Present
- Led multiple pelleting trials with United Animal Health to test thermostability of enzyme premixes: May, August, December 2018
- Led a contract study with Huvepharma to test a novel pellet binder: May 2018

- Led a pelleting trial with DSM Nutritional Products, Inc. utilizing a phytase and murimadase product added to diets to determine thermostability: April 2018
- Lead a pelleting trial to determine *Salmonella* mitigation potential of new hygieniser: March 2018
- Attended the Poultry Science Association's Annual Meeting: 2018
 - San Antonio, TX (2018)
- Assisted with a traveling backyard poultry demonstration in various locations throughout the state of West Virginia: Fall 2017-Present.
- Assisted with numerous West Virginia University Animal Science Farm tours: Fall 2017-present
- Assisted with the Poultry Festival: 2018
 - Moorefield, WV
 - Assisted with conducting an annual poultry judging competition
- Assisted with the West Virginia State FFA Poultry CDE Competition: 2017, 2018
 - Helped organized the competitions
- Davis College Welcome Back BBQ: 2017, 2018
 - Assisted Dr. Joseph Moritz preparing and delivering food

SKILLS

- Feed mill management
- Ingredient sourcing and ordering
- Efficient in Microsoft Word, Microsoft Excel, and Microsoft PowerPoint
- California Pellet Mill operation
- Poultry processing
- Cecectomy surgery
- Precision-feeding
- Tibia extraction
- Ileum extraction
- Experience using SAS software
- Pellet Quality Analysis (Pfast Tumbling Box and New Holman Pellet Tester)
- Dry matter analysis
- Kjeldahl analysis
- Freezer dryer operation
- Salmonella surrogate inoculum preparation
- Sample preparation for analysis of Salmonella surrogate in poultry feed
- Vacuum filtration
- Agar plate preparation