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EVALUATING THE INCLUSION OF SUSTAINABLE ALTERNATIVES TO CONVENTIONAL FEEDING STRATEGIES IN POULTRY NUTRITION

A Thesis in

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by

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ABSTRACT

The overall objective of this thesis was to examine the inclusion of sustainable alternatives in poultry diets. In order to accomplish this objective two experiments were designed to further elucidate the use of various alternatives. The first experiment examined the impact of varying inclusion rates of mushroom stump waste (MSW) in broiler rations on day 1-21 performance and amino acid digestibility. The second experiment evaluated the addition of the direct-fed microbial (DFM) *Bacillus subtilis* C-3102 to tom turkey diets and its effect on performance and carcass composition.

Initially, an experiment was conducted involving MSW. Mushroom cultivation generates an unusable waste stream of mushroom stumps. In an effort to recycle these nutrients and incorporate mushroom waste into broiler rations a processed MSW product was created and utilized in this current study. The objectives of this study were to determine the nutrient profile of MSW (Experiment 1) and determine the optimal inclusion of MSW in broiler diets based on performance parameters (Experiment 2) and amino acid digestibility (Experiment 3). In Experiment 1, the nitrogen corrected true metabolizable energy (TME_n) of MSW (1,173 kcal/kg) and proximate analysis results were utilized to formulate two isocaloric and isonitrogenous diets containing 0 and 5% MSW. Four other treatments of varying MSW (1, 2, 3, and 4%) were then created via blending portions of the 0% and 5% MSW treatments together. In Experiment 2, the results from day 1-21 show that broilers provided 1% MSW improved live weight gain (LWG) per bird by 57.6 grams when compared to those consuming 5% MSW. Birds provided 0, 2, 3 or 4% MSW were intermediate for LWG per bird (P=0.024). Broilers provided 1% MSW improved average body weight (BW) when compared to broilers consuming 4% or 5% MSW. Birds fed 0, 2, or 3% MSW were intermediate for BW (P=0.026). Broilers provided 5% MSW increased feed conversion ratio (FCR) by 0.105 compared to birds consuming 1% MSW. Birds fed 0, 2, 3 or 4%

MSW were intermediate for FCR (P=0.0002). MSW inclusion did not affect feed intake per bird or mortality (P>0.05). In Experiment 3, results from apparent ileal amino acid digestibility show that MSW inclusion reduced digestibility for 16 of 19 reported amino acids (P<0.05). Amino acid digestibility coefficients demonstrate that MSW inclusion did not affect any of the 19 amino acids analyzed (P>0.05). These data indicate that MSW inclusions up to 3% of the diet do not affect broiler performance or amino acid digestibility coefficients.

Another experiment was conducted evaluating the efficacy of a direct-fed microbial (DFM) *Bacillus subtilis* C-3102 in turkey production. Due to antimicrobial use in food-producing animals becoming more scrutinized, DFM utilization is becoming more prevalent. Effects of various DFM on poultry performance have been studied; however, limited research has been conducted on the DFM *Bacillus subtilis* C-3102 in turkeys. Therefore, performance and processing responses of *Bacillus subtilis* C-3102 were investigated using 720 Nicholas Select tom turkeys. Control and DFM diets were formulated to be identical in nutrient composition and content aside from DFM inclusion. These diets were provided in a six-phase feeding program. Diets containing the DFM treatments were formulated to contain 500,000 cfu/g from d 1-35 (Stater 1 and Starer 2 phases) and 300,000 cfu/g of *Bacillus subtilis* C-3102 from d 36-133 (Grower 1, Grower 2, Finisher 1 and Finisher 2 phases). In total, each treatment was provided to 12 replicate pens of 30 turkeys. These pens were arranged in a randomized complete block design, with each block differing in pen location. Day 1-133 results indicate turkeys provided the DFM treatment exhibited a 0.08 improvement in FCR compared to birds provided the control treatment (P<0.0001). Turkey performance improved for the DFM treatment as *Lactobacillus* concentrations in fecal samples increased as well. This increase in *Lactobacillus* concentrations and subsequent improvement in performance occurred during the Finisher 1 and Finisher 2 phases, for birds provided the DFM treatment. Processing results from d 134 indicate turkeys

provided the control treatment improved pectoralis minor and total breast yields (P=0.023 and P=0.011, respectively) compared to turkeys consuming the DFM treatment. *Bacillus subtilis* C-3102 inclusion reduced overall feed cost by $0.20 per bird. These data indicate that *Bacillus subtilis* C-3102 inclusions improved tom turkey performance and decreased feed costs.

Key words: mushroom, TME_n, digestibility, direct-fed microbial, *Bacillus subtilis* C-3102, *Lactobacillus*, poultry production, performance, processing

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ABBREVIATIONS

AGP- Antibiotic growth promoters

AIAAD- Apparent ileal amino acid digestibility

BV- Biological value

BW- Body weight

C- Celsius

d- day

DFM- Direct-fed microbial

FCR- Feed conversion ratio

FI- Feed intake

g- gram

h- hour

LWG- Live weight gain

MSW- Mushroom stump waste

NRC- National Research Council

s- seconds

TME_n- True Metabolizable Energy corrected for nitrogen

TSAA- Total sulfur amino acids

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DEDICATION

Dedicated to my family, friends, and mentors for their constant support and guidance. They all molded me into who I am today, and I am thankful for being so blessed.

Chapter 1

Literature Review

POULTRY CONSUMPTION AND DEMAND

U.S. Poultry Consumption

According to the National Chicken Council (2020), per capita poultry consumption in the United States was 112.5 lb. in 2019. This rate of poultry consumption is an increase from previous years. In 2019, the average U.S. citizen consumed 13.8% more poultry than in 2009, 11.4% more than in 2014, and 2.2% more than in 2018 (National Chicken Council, 2020). Further breakdown of this mean consumption shows that of the 112.5 lb. consumed, 96.5 lb. were chicken and 16.0 lb. were turkey. Chicken meat consumption in 2019 increased 17.0% from 2009, 13.1% from 2014 and 2.7% from 2018 (National Chicken Council, 2020). The rate of turkey consumption in 2019 was 6.2% less than in 2009, 1.2% more than in 2014 and 1.2% less than in 2018 (National Chicken Council, 2020). Further breakdown of the total chicken meat consumed shows that of the 96.5 lb. consumed, 95.1 lb. was broiler meat. According to the National Chicken Council (2020), broiler consumption in 2019 increased 16.7% since 2009, 12.8% since 2014, and 2.7% since 2018. Per capita poultry consumption in the United States highlights the magnitude of U.S. poultry consumption while also revealing the steady increase in demand over the past decade. Although U.S. poultry consumption is increasing, U.S. poultry demands expand to global markets as well.

Global Markets and Exports

According to the U.S. Poultry and Egg Association (2020), the United States exported a total of 7.11 billion lb. of broiler meat in 2019. This is a 17.0% increase from 2009 and a 0.5% increase from 2018. However, this was a 5.4% decrease in exports since 2014. The largest consumer of U.S. poultry exports in 2019 was Mexico, who consumed nearly 780 million lb. of turkey and 3.01 billion lb. of chicken meat (U.S. Poultry and Egg Association, 2020). According to the United States Department of Agriculture (USDA, 2020) there is potential that poultry export values in 2019 and 2020 may be inflated due to the impact of African Swine Fever on the pork industry. African Swine Fever has caused devastating impacts around the world, decreasing pork production in 2019 and 2020. The year 2020 is the first time in recent years that global poultry production will exceed global swine production in pounds of meat produced (USDA, 2020). The USDA (2020) anticipates a 0-1% increase in U.S. poultry exports in 2021. This is virtually unchanged from years prior, in part due to recovering herds affected by African Swine Fever. The USDA (2020) anticipates that China will increase swine production approximately 9% in 2021 to 41.5 million tons. Although this is a substantial increase, China will still realize a 25% reduction in pork production compared to pre disease levels (USDA, 2020). Even with a rebounding swine market, these data expound the impact of the U.S. poultry industry on the world.

Economic Impact of the U.S. Poultry Industry

Poultry production in the United States incorporates billions of dollars into the economy. According to the U.S. Poultry and Egg Association (2020), overall broiler production in the U.S. totaled 9.18 billion birds in 2019, a 2% increase from 2018. Total broiler meat produced in 2019 was 58.3 billion pounds, up 3% from 2018 (U.S. Poultry and Egg Association, 2020). Although production of U.S. broilers increased, value of these broilers was only $28.3 billion in 2019, an 11% decrease from 2018. Total turkey meat produced in 2019 was 7.43 billion pounds, a 0.13% increase from 2018. Turkey

production in the U.S. totaled 229 million in 2019 and was down 4% from 2018 (U.S. Poultry and Egg Association, 2020). The demand and value associated with poultry related products elucidate the impact of the United States poultry industry locally and internationally. Increasing consumer demands have created a subsequent need to feed these animals efficiently and sustainably.

POULTRY NUTRITION AND ECONOMICS

Diet Cost and Formulation

The need to feed poultry affordably and efficiently is paramount. The feeding of production animals has been and will continue to be one of the more costly expenditures in raising livestock, regardless of species. Specifically, in poultry production, feed constitutes roughly 60-70% of the total production cost in an operation (Kidd et al., 2004; Corzo et al., 2005; Ravindran, 2012). This cost is reflective of the importance of nutrition and feed manufacturing to the poultry and animal industry. Individual components like cereal grains, synthetic amino acids, and vitamin/mineral premixes all vary in cost and nutrient content. In order to maximize animal performance, it is imperative to produce a balanced diet that provides an ideal ratio of carbohydrates, proteins, lipids, vitamins, and minerals with no deficiencies or excesses. In order to maximize profitability, a producer must balance feed costs with the nutritional requirements of the animals in production, as well as the varying income gained from live bird production. Formulation of diets on a least-cost basis has improved profitability tremendously over the last 70 years.

Least-cost Formulation

The idea of least cost formulation was first published by the University of Minnesota in the area of human nutrition (Stigler, 1945). Stigler set out to determine the minimum cost required to adequately

meet the nutritional demands of a man weighing 70 kilograms (1945). Stigler (1945) created an ingredient matrix of 77 commonly available foods and attempted to deduce the most cost-effective way of providing 9 nutrients. Munford (2005) explained that this was prior to the advent of many linear programming solutions and the personnel computer. Therefore, with a handheld calculator this was an overly complicated problem that Stigler was unable to solve prior to publishing.

The idea of studying linear relationships in terms of poultry production measurables dates back to 1928. Jull and Titus (1928) deduced live weight and feed consumption was expressible by the law of diminishing increment outlined by Spillman and Lang (1924). Although linear programming was an effective method at comparing production variables the potential of this software was not fully realized until the mid-1940's. Dantzing (1948) proposed the idea of "simplex method" for solutions to linear programming problems. This was revolutionary, as it was efficiently able to solve linear programming issues such as ones posed with Stigler's diet problem. The solution to Stigler's problem was critical for several industries including animal producers, as it has applications beyond human nutrition. The first instance where linear programming was used to formulate a least-cost livestock ration was in 1951, with dairy diets (Waugh, 1951).

Although the advances in linear programming were helpful, animal producers continued to find more problems that needed to be solved. For example, more specific dietary requirements were needed with lower and upper limits for certain nutrients (Newman, 1955). Newman also noted that formulating a least-cost diet is more difficult for animal producers, because in most cases integrators produce some of their own crops at a reduced cost. Another problem was the availability and variability of commonly used cereal grains. For example, the nutrient density of a grain like corn changes significantly depending on where it is grown and the conditions each crop is subjected too. Baidoo et al. (1991) found that the nitrogen corrected true metabolizable energy of corn, grown in different areas of the world, ranged from 3,681 to 3,962 (Kcal/kg). This could be due to kernel density, dry or wetness of the growing season as well as the type of maize produced (Baidoo et al., 1991). Nutrient variability can be managed in linear programming models by providing a margin of safety for each nutrient. This can be done by reducing the

mean nutrient value by 0.5 times the standard deviation of the nutrient (Saxena and Chandra, 2011). Margins of safety were an important finding; however, more accurate values and data are needed to maximize precision feeding efficiency (Ravindran, 2012). The solutions to the aforementioned issues were significant for decreasing feed-cost, however, animal producers are ultimately concerned with profitability rather than simply reduced production expenditures.

Animal producers are looking for the most profitable diet, not necessarily one that is the cheapest. Pesti et al. (1986) developed a quadratic program that revolved around the least-cost of broiler output in response to protein and energy input of the diet. Until this point, most diet formulation models contained a significant amount of nutritional knowledge, however, these economic models could be improved by including production response information. Pesti's quadratic model was compared to a linear programming model conventionally used in the poultry industry in 1986. Both models contained the same ingredients and were targeting the same nutrient density constraints, however, the quadratic model increased crude protein percentage by 1.8% and decreased energy by 43 (kcal/lb.). The quadratic model maximized growth at a cost of $0.71 per bird whereas the linear programming model cost $0.72-0.75 per bird. Even a $0.01 reduction in production cost per pound would save U.S. producers an estimated $120 million (Pesti et al., 1986). This (seemingly) marginal difference between two ration balancing software's, highlights the importance of constant improvement for the poultry industry. Pesti et al. (1986) did not change the nutrient requirements for the animal but rather, simply changed what question they wanted the software to answer. Least-cost formulation software's have and will continue to evolve to increase profitability for producers. Leeson (2008) found that although the nutrient requirements of birds have not changed dramatically over the last 50 years, diet specifications, feed programs and production goals have and will continue to evolve as market needs dictate. In order to maximize the potential savings of these software's it is imperative that producers understand the nutrient requirements and production goals for the animals they are currently feeding.

Nutrient Requirements and the Evolution of the Modern Bird

Leeson and Summers (2001) defined a nutrient requirement as "the minimum amount of the nutrient required to produce the best weight gain, feed efficiency, etc. and the lack of any signs of nutritional deficiency". Applegate and Angel (2014) said that nutrient requirements are commonly referred to as "minimum nutrient needs." Nutrient requirements are highly variable and are influenced by both bird-related and external factors (Ravindran, 2012). Bird-relates factors include variables such as genetics, sex, and stage of production, whereas external factors include environmental factors, biosecurity, stress, and general husbandry conditions (Ravindran, 2012).

The National Research Council (NRC) has historically been used by producers for the nutrient requirements of various species including poultry, beef, dairy and swine. However, for the poultry industry specifically, the latest publication of the NRC was released in 1994. The 26-year gap since the last publication has caused some issues for the poultry industry as commercial birds used in production today are drastically different then ones used in 1994. For example, Applegate and Angel (2014) found the national average body weight of 18-week-old tom turkeys was approximately 7.3 kg larger in 2014 (18.2 kg) compared to 1986 (10.9 kg). This improvement is due to a litany of reasons including nutrition and environmental management; however, the majority of the change has been attributed to genetic improvement. Havenstein et al. (2003) conducted an experiment comparing the effect of genetics, nutrition, and sex on broiler performance. These authors employed a 2 x 2 x 2 factorial arrangement comparing commercial broiler strains and diets used in 2001 and 1957, as well as their effect on performance of male or female broilers. Havenstein et al. (2003) concluded that approximately 85-90% of the improvements in performance of the modern broiler strain was due to genetic improvements, whereas only 10-15% of the improvement was due to advances in nutrition. These are significant findings as they expound the differences in nutritional requirements of birds today. Due to the outdated nutrient requirements of the NRC, and the difference in requirements between certain strains of broilers and turkeys, commercial breeding companies have begun to set their own recommendations. The

recommendations from these breeding companies match the nutritional requirements of these modern strains more closely than those set by the NRC in 1994 (Ravindran, 2012). The most notable changes in nutritional requirements of the modern bird, from the last publication of the NRC, are the amino acid requirements and the ratio of amino acids to energy (Gous, 2010; Applegate and Angel, 2014).

Amino Acid Requirements

Research of varying amino acid concentrations affecting growth rate and feed efficiency, in farm animals, has been well documented. This research dates to the early 1940's when the idea of dispensable and indispensable amnio acids were still being discovered. Almquist (1942) conducted a review and deduced the essential nature of arginine, histidine, tryptophan, lysine, methionine, and isoleucine. Almquist and Grau (1944) conducted several experiments limiting concentrations of specific amino acids and concluded that leucine, phenylalanine, threonine, and valine were dietarily essential for chickens. These authors also found that glycine, tyrosine, and glutamic acid were semi-dispensable (Almquist and Grau, 1944). In total, poultry diets require the addition of ten dietarily essential amino acids (Ravindran, 2012).

Although supplementation of these essential amino acids is required for optimal growth and livelihood, at what rate should producers supplement these amino acids? Hankins and Titus (1939) deduced that based on the chemical composition of the empty body of poultry, it is possible to estimate the amino acid requirement for a desired growth rate. Based on these ideas, Munks et al. (1945) proposed that the amino acid concentrations of tissues produced could be used as a measure of amino acid requirements. These authors concluded that amino acid requirements for growth were different from those for egg production (Munks et al., 1945). In a review, Almquist (1952) deduced the amino acid concentration required for adequate performance in young and laying chickens, as well as young turkeys. This author concluded that with supplementation of various synthetic amino acids, young chickens and turkeys only required 20% and 24% crude protein, respectively, for optimal growth and performance

(1952). Interestingly, prior to the advent of purified synthetic amino acids, typical poultry diets consisted of approximately 71% soybean meal, 14% corn and contained roughly 35.6% crude protein simply to meet indispensable amino acid requirements (Pesti, 2009). Considering, of all dietary constitutes, essential amino acids and energy are the most expensive, this was a critical discovery for poultry producers (Pesti, 2009; Ravindran, 2012). It is noteworthy that although only ten amino acids are dietarily essential, non-essential amino acids are needed for optimal growth and muscle deposition (Pesti, 2009). For example, Wang and Fuller (1989) found that the most efficient ratio of essential to non-essential amino acids was 45:55 in swine. Considering the cost of protein in poultry diets, a more precise ratio of specific, essential amino acids is required for maximum efficiency and sustainability.

The Concept of Ideal Protein Ratio

The concept of ideal protein ratio and the application to livestock was first used in swine models in the mid to late-1980's. These swine models were an extension of a rat model used in the 1960's. Bender (1965) calculated a biological value (BV) of varying amino acid densities based on net protein utilization divided by digestibility. For reference, the BV associated with a dried egg product that is highly digestible was found to be 97. This author found that supplementation of lysine, threonine, methionine, and valine resulted in the highest BV (93) for the rat models (1965). Based on these ideas Wang and Fuller (1989) created a general linear model in swine to determine the amino acid requirement by deduction. This was done in an effort to calculate a dietary amino acid pattern where each amino acid would be equally limiting to maximize efficiency. Considering the similarities between swine and poultry digestive systems and diet composition these ideas were further elucidated in broiler diets.

Interestingly, traditional corn-soy diets have shown that the order of limiting amino acids in broiler diets is methionine (1), lysine (2), and threonine (3; Kidd et al., 2013). Conversely, the ideal protein ratio uses lysine as the reference amino acid. This is largely because lysine is an economically feasible dietary supplement, lysine is only used for protein accretion and maintenance in the body, and it is the second

limiting amino acid in traditional broiler diets as mentioned previously (Baker, 1996). Baker and Han (1994) deduced the ideal ratio for broilers up to three weeks post hatching. These authors deduced the ideal ratios of digestible amino acids as: lysine, 100%; methionine + cystine (TSAA), 72%; threonine, 67%; valine, 77%; arginine, 105%; histidine, 32%; isoleucine, 67%; tryptophan, 16%; leucine, 109%; phenylalanine+ tyrosine, 105%; glycine (or serine), 65%; and proline, 44% (1994). These ratios provided a more precise estimate to the amino acid requirements of broilers than those set in the 1994 NRC. Considering the cost of protein in broiler diets, this aforementioned precision is critical for reduction in feed cost and production expenditures as a whole. This ratio has evolved slightly over the years based on more empirical data, as well as changes in genetics, production stage and sex of the broilers used in production (Baker et al., 2002). Increasing the feeding precision of amino acids has resulted in more profitable and sustainable diets, however, energy is another costly dietary constituent for poultry diets as well.

Metabolizable Energy

It is well documented that broilers and layers alike consume feed to meet energy requirements (NRC, 1994; Leeson, 2008; Ravindran, 2012); provided that there is a lack of extraneous environmental challenges, lack of physical limitations of the bird and that the diet contains adequate ratios of vitamins and minerals (Kamran et al., 2008). This is critical in understanding the optimal nutrient densities of poultry diets and their effect on bird performance. Increasing metabolizable energy values has shown the ability to reduce feed intake and muscle deposition while subsequently increasing abdominal fat pad yield (Garcia Neto et al., 2000; Karman et al., 2008). Leeson et al. (1996) deduced that increasing metabolizable energy within the diet decreased feed intake but not live body weight. Considering producers income is based on the quality and amount of salable muscle/meat these results should be considered and manipulated when formulating rations, in order to maximize profitability. Metabolizable energy requirements have not substantially changed over the last 50 years, however, the ratio of dietary

amino acids to metabolizable energy has increased due to the “fast growing” nature of the modern bird (Leeson, 2008). This is due to several reasons including increasing protein maintenance requirements of the modern bird and the constant pressure to increase breast tissue deposition by poultry producers (Garcia Neto et al., 2000; Leeson, 2008). Improved efficiency and nutritional precision have resulted in more advantageous production results, profitability and sustainability for producers. Although diet formulation is vital for adequate performance and efficiency, feed manufacturing is another critical component that affects diet composition and subsequent performance responses. Proper feed manufacturing ensures that diet formulations and nutrient requirements produced in theory are provided to birds in reality.

FEED MANUFACTURING

Processing Techniques

In order to meet the nutritional requirements of poultry individual feed ingredients such as cereal grains, soybean meal, fats, and vitamin/mineral premixes must be thoroughly mixed or processed in a way that avoids nutrient variation and segregation. This mixing must be done in a way that is effective in producing minor variation during processing, handling, and feeding but does not decrease the bioavailability of the nutrients present in the ration. There are many different processes that can be used to maximize the nutrient content within rations such as hammer and rolling mills, which are used to reduce particle sizes of cereal grains (Amerah et al., 2007). Reduction in particle size increases the surface area of the grain and subsequently improves digestibility while decreasing nutrient segregation (Behnke, 1996). Soybean meal is often formed through solvent extraction or extrusion of dehulled soybeans, this soybean meal undergoes steam heating and in turn denatures anti-nutritional factors such as trypsin inhibitors found in raw soybeans (Anderson-Hafermann et al., 1992). The heating of soybean meal can be a time sensitive procedure as underheating will result in a low-quality meal high in antinutritive factors

(Ao, 2011). Conversely overheating will result in the reduced digestibility of several amino acids such as lysine and arginine (Hayward et al., 1936). These processing techniques are designed to increase the bioavailability of nutrients but are costly due to the equipment and energy needed to form these final products (Behnke, 1996; Ao, 2011). In terms of the poultry industry there is one process that has revolutionized the way feed is manufactured and that is pelleting. Pelleting has been in the past, is now, and will continue to be a significant feed manufacturing process in the poultry industry.

Pellet Quality and Bird Performance

Pellet mills were first commercialized in the United States in the early 1930's (Behnke et al., 1994). For much of the next ninety years, research has compared mash and pelleted diets in an effort to understand differences and similarities between the two. Patton et al. (1937) reported that chicks provided pelleted rations consumed 5.6% less feed than chicks fed mash diets. These authors reported that chicks receiving pelleted diets were 6.6% higher in body weights than mash fed chicks, which resulted in a 10.5% improvement in feed: gain ratio, for the birds consuming pelleted feed (1937). Heywang and Morgan (1944) reported that the average body weights of 12 and 22-week-old cockerels, receiving pelleted diets, were significantly larger than that of the cockerels receiving a mash diet in five of six experimental treatments. These authors found improved feed efficiency in cockerels fed pelleted diets compared to cockerels fed mash diets (1944). Conversely, Heywang and Morgan (1944) found cockerels receiving pelleted feeds had a slightly larger feed intake than cockerels receiving a mash-based diet.

It is generally accepted that pelleting increases economic returns and sustainability in the poultry industry due to increases in growth rate and feed efficiency (Abdollahi et al., 2011). Improvements in performance responses of birds consuming pelleted diets compared to mash diets is well documented (Calet, 1965; Behnke, 1994; Behnke 1996). These improvements could be due to several reasons such as reductions in prehension energy expenditure, feed wastage, nutrient segregation and deleterious organisms present in the feed (Behnke 1994; Jones et al., 1995; Jensen, 2000). Jensen et al. (1962) found

that birds consuming mash diets spent 9.6% more time eating per day than those consuming pelleted feed. Eley and Hoffmann (1949) reported that drinkers in pens where birds consumed pellets, recovered on average only 0.39 grams of filtrates in the water per day, compared to 2.47, 3.12, and 3.41 grams where coarse, medium, and finely ground, mash diets were fed, respectively. This feed wastage can decrease feed efficiency, farm cleanliness and profitability. Cox et al. (1986) found that varying steam conditioning temperatures reduced *Salmonella* incidence by 50% and *Enterobacteria* plate counts in pelleted broiler starter diets compared to unconditioned mash diets.

Factors such as pellet quality and pellet to fine ratio can cause significant changes in bird performance as well. Proudfoot and Sefton (1978) found that broilers consuming mash diets or higher level of fines in the finisher phase had lower body weights and higher feed conversion ratios. These authors found a linear trend that as percentage of fines increases monetary return decreases 0.04 cents per bird per percentage increase in fines (1978). For example, a diet containing an 80:20 pellet to fine ratio would result in a 0.8 cent decrease in return per bird compared to a diet containing 100:0 pellet to fine ratio. Proudfoot and Hulan (1982) found similar results in turkeys. These authors found that increasing pellet to fine ratio in both grower and finisher phases led to increased body weights and improved feed conversion ratios. Lilly et al. (2011) found that increases in pellet: fine ratio resulted in a subsequent improvement in feed conversion ratio, carcass weight and breast weight. Cutlip et al. (2008) found that increases in pellet quality due to increasing conditioning temperatures resulted in improved body weight and feed conversion ratio for broilers. The culmination of research shows the importance of pelleting and overall pellet quality on bird performance and efficiency. Two ways in which pellet quality is enhanced is through increases in steam conditioning temperatures and inclusions of pellet binders.

Steam Conditioning Temperature and Pellet Binders

It is noteworthy that feed mill throughput demands often decrease feed quality while increased pellet quality/durability often comes at the expense of nutritional digestibility (Abdollahi et al., 2011).

These authors found that although increasing steam conditioning temperatures of pelleted feed significantly increased pellet durability; increasing steam conditioning temperatures resulted in reduced feed efficiency (2011). Loar II et al. (2014) found that increasing steam conditioning temperatures during pelleting resulted in improved pellet quality as well as a reduction in methionine digestibility. Skoch et al. (1981) found, for pelleted diets, production rates can be increased with increasing steam conditioning temperatures. As previously mentioned, increasing steam conditioning temperatures decreases the presence of deleterious organisms while also increasing pellet durability (Cox et al., 1986). The review of past literature highlights the complexity and the balancing required when pelleting rations. Qualities such as throughput demands, pellet durability, pellet to fine ratio, steam conditioning temperatures and presence of pathogens should be considered prior to feeding or manufacturing.

Much research has been conducted reviewing major physiochemical factors required to enhance pellet formation, quality, and durability. Thomas et al. (1998) reported that protein can act as a binding agent when heat, pressure and water are added. These authors hypothesized that this heat partially denatures the proteins, and the subsequent cooling causes the proteins to reassociate, establishing new bonds and increasing pellet quality (1998). These findings have been supported by an accumulation of more research. Buchanan and Moritz (2009) who reported a 5% inclusion of soy-protein isolate or cellulose improves pellet quality and durability. Gehring et al. (2009) who found that a 5% inclusion of a trout protein paste (CP = 85.77%) improved pellet quality and durability. Boney et al. (2017) and Evans et al. (2015) who reported *Spirulina* algae (CP = 76.00%) inclusions of 1, 5, 10, 16 and 21% elicit an improvement in pellet quality and durability. Similar to protein content, reducing particle sizes of cereal grains prior to pelleting have also been reported to enhance pellet quality. Wondra et al. (1995) found that decreasing the particle size of cereal grains led to an increase in pellet durability and overall pellet quality. Gehring et al. (2009) expressed the need for nutritive pellet binders in order to increase pellet durability while also increasing nutrient density within poultry diets. Given the results from previous studies, increasing the use of nutritional pellet binders is critical in improving pellet durability and subsequent bird performance.

SUBTHERAPEUTIC ADMINISTRATION OF ANTIBIOTICS IN POULTRY DIETS

Use of Antibiotics in Poultry Diets

In the mid 1940's and early 1950's an abundance of research was conducted showing the benefits of various antibiotics on growth performance measurables of animals (Moore et al., 1946; Stokstad and Jukes, 1950; Biely and March, 1951). These authors associated antibiotic inclusions in poultry diets with improvements in performance responses such as growth rate, feed efficiency and mortality. These conclusions were found rather serendipitously, as scientists could not deduce a direct mode of action or mechanism that would elicit an increase in bird performance parameters. However, Laxminarayan et al. (2015) deduced two potential mechanisms in which antibiotics improve performance responses. Enhancing growth rate and feed efficiency as well as decreasing the need for hygienic-management practices during transportation and in animal houses. Potential modes of action of antibiotic growth promoters (AGP) include reductions in total microbial populations within the gut, allowing for more nutrients to be absorbed by the animal; as well as reductions in opportunistic pathogens and morbidity (Dibner and Richards, 2005). Although little was known about the ramifications of AGP, subtherapeutic inclusions of antibiotics in animal diets were cost effective. This, coupled with the impoverished state of the United States following World War II, put pressure on legislatures to support farmers during these difficult times. Therefore, in 1951 the United States Food and Drug Administration approved the use of antimicrobials as feed additives without veterinary prescription (Jones and Ricke, 2003). Due to increased profitability and unknown ramifications poultry producers continued to supplement antimicrobials for growth promotant purposes. In 1981, it was estimated that animal producers saved approximately $3.5 billion a year in production costs from the practice of antibiotic feed supplementation (Antibiotics in Animal Feeds, 1981). In 2015, Laxminarayan et al. (2015) estimated that complete removal of AGP would result in a 1.3% to 3% reduction in global meat production. This would equate to a loss of approximately $13.5 to $44.1 billion in revenue.

Prevalence of Antibiotic Resistance

Although, there were some reports of the incidence of antibiotic resistance (Luria and Delbruck, 1943; Demerec, 1945; Demerec 1948; Starr and Reynolds 1951), the connection between feeding antibiotics to animals and its potential association with human health was not yet widely known. Smith (1968) reviewed the discovery of multiple drug resistant strains of *Escherichia* coli, *Staphylococcus Aureus*, and *Salmonella* in various animals and its relation to direct feeding of antimicrobials. According to Dibner and Richards (2005) recommendations to ban the subtherapeutic use of antibiotics were not brought to government attention until 1969, when the Swann report was presented to British Parliament. Further research by White et al. (2001) showed a high incidence of antibiotic resistant *Salmonella* serotypes in the ground meat of chicken, beef, turkey, and pork fed antimicrobials. Barton (2000) highlighted the difficulty of treating antibiotic resistant strains of bacteria in human medicine, as well as the incidence of antibiotic resistant pathogens in livestock production and advised for reduction in the use of AGP in animal diets. Considering these data many countries have adopted protocols limiting the use of AGP in animal feeds. Sweden and Denmark eliminated AGP use in food producing animals in 1986 and 1998-1999, respectively. In 2006, the European Union banned the use of all antibiotic growth promoters in animal diets. The United States followed in 2015 in part due to lawsuits filed against the FDA by the Natural Resources Defense Council. It is worth noting that eliminating the use of AGP has not eliminated the administration of antibiotics to production animals. In countries such as Sweden and Denmark overall antibiotic use has decreased dramatically; however, therapeutic administration of antibiotics has increased in the immediate years following removal of AGP. For instance, Sweden saw a 21% increase in the use of therapeutic antimicrobial two years after banning AGP (McEwen et al., 2017). Denmark nearly doubled the amount of therapeutic antibiotics used in food producing animals two years following the banning of AGP (Grave et al., 2006). These data further expound the effect of AGP on reducing clinical disease in food producing animals and show the impact of eliminating AGP use.

Direct-Fed Microbials

The removal of AGP from animal diets, although good for human medicine, have been detrimental to the poultry industry around the world increasing production costs an estimated $0.03 per bird (Maria Cardinal et al., 2019). Poultry producers across the world are trying to replicate the positive effects of feeding antimicrobials without the negative consequences. One potential alternative may be the dietary supplementation of direct-fed microbials (DFM). Direct-fed microbials are commonly referred to as probiotics. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). The dietary administration of DFM to poultry and other livestock have shown improvements in production parameters such as weight gain and feed conversion ratio (Chiang and Hsieh, 1995; Zani et al., 1998; Fritts et al., 2000; Willis and Reid, 2008; Smialek et al., 2018). Although the direct mechanism in which DFM evoke beneficial changes to bird health and performance is unknown, it is theorized that the supplementation of commensal intestinal microbiota inhibits the proliferation of pathogens (Patterson and Burkholder, 2003). Fuller (1991) and Rolfe (2000) found that dietary inclusions of DFM decrease the prevalence of infection and enteric disease. Maruta et al. (1996) reported that dietary inclusions of *Bacillus subtills* C-3102 to infected chickens, decreased the prevalence of pathogens like *Campylobacter*, *Salmonella*, and *Enterobacteriaceae* while increasing *Lactobacillus* populations in the excreta. Similarly, Fritts et al. (2000) reported that birds consuming *Bacillus subtills* C-3102 significantly reduced the incidence of *Salmonella* found on prechilled carcasses compared to that of the control.

Although the direct mechanism of various DFM is unknown, there are numerous modes of actions in which DFM may protect the host from intestinal disorders. These modes of action include, but are not limited to, competitive exclusion, blocking of adhesion sites, pathogen inhibition, and stimulation of immunity (Rolfe, 2000; Revolledo et al., 2006; Mountzouris et al., 2009). Competitive exclusion is "a process by which an organism is prevented from colonizing a given environment due to the presence of other organisms that are better able to establish and maintain themselves in that environment" (Revolledo

et al., 2006). This process is difficult to elucidate due to the complexity of the gastrointestinal microflora, however, previous research has seen beneficial effects similar to the aforementioned definition (Maruta et al., 1996; Fritts et al., 2000; Mountzouris et al., 2009). Conway et al. (1987) and Goldin et al. (1992) found that different strains of DFM inhibited pathogenic adhesion to the intestinal epithelium. In a review, Rolfe (2000) discussed that various strains of DFM produce inhibitory substances such as lactic acid, hydrogen peroxide and bacteriocins. Direct fed microbials may exhibit one or several of these modes of action as these are not mutually exclusive mechanisms (Patterson and Burkholder, 2003).

Although there are many benefits of DFM supplementation such as improved production parameters and bird health, these beneficial responses are not seen in every application. Torres-Rodriguez et al. (2007) reported the effects of the dietary supplementation of DFM (FloraMax-B11) to 60 commercial turkey houses. Interestingly, these authors found that performance measurables were only significantly improved from the farms historically ranked in the bottom 75% by the integrator (2007). Considering many DFM are chosen specifically to increase *Lactobacillus* and *Bifidobacterium* populations within the gastrointestinal tract, it is noteworthy these microorganisms are particularly vulnerable to stress (Patterson and Burkholder, 2003). Therefore, it would suffice that increases in production stressors such as inadequate nutrition, biosecurity, environmental challenges, etc. may improve the efficacy of dietary DFM supplementation.

CONCLUSIONS AND APPLICATIONS

This literature review was designed to highlight the magnitude of the U.S. poultry industry while showing the importance of constant improvement in several areas of the industry, most notably nutrition. The sheer number of birds produced per year requires immense amounts of goods and services. To sustainably meet consumer demands, new products must be created and advances in technology must continue to evolve. The continuous change in production goals and bird genetics require the use of precise and current nutrition requirements. Understanding feed manufacturing research is paramount in meeting

these production goals as well. Balancing the effects of varying steam conditioning temperatures, pathogen loads, and nutrient digestibility's is critical for optimum growth and efficiency. The removal of antibiotic growth promoters from animal diets has increased production costs significantly for producers. Therefore, the production of alternatives such are direct-fed microbials is vital to meet performance goals and increase profitability for producers.

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Chapter 2

Effects of mushroom stump waste (MSW) inclusions to broiler diets on amino acid digestibility and performance during the first 21 days

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SUMMARY

Mushroom cultivation in the United States produced 408,183 tons of edible product in the 2019-2020 crop. Mushroom production subsequently generates a waste stream of inutile mushroom stumps. This waste stream was dried and ground to create a mushroom stump waste (MSW) product in the current study. The objectives of this study were to determine the nutrient profile of MSW and the optimal inclusion of MSW in broiler diets based on performance measurables and amino acid digestibility. The TME_n of MSW (1,173 kcal/kg) and proximate analysis results were used to formulate broiler diets containing 0, 1, 2, 3, 4 and 5% MSW. These diets were provided to straight-run broiler from d 1-21 of age. Day 21 results indicate birds consuming 1% MSW improved LWG by 57.6 g per bird compared to birds fed 5% MSW. Birds fed 0, 2, 3 or 4% MSW were intermediate for LWG (P=0.024). Broilers provided 1% MSW improved average body weight (BW) compared to those fed 4% or 5% MSW. Birds consuming 0, 2, or 3% were intermediate for BW (P=0.026). Birds fed 1% MSW improved FCR by 0.105 compared to those fed 5% MSW. Broilers consuming 0, 2, 3 or 4% MSW were intermediate for FCR (P=0.0002). Neither mortality nor feed intake were affected by MSW inclusion (P>0.05). Apparent ileal amino acid digestibility results demonstrated that MSW inclusion reduced digestibility for 16 of 19 reported amino acids (P<0.05). Amino acid digestibility coefficients were not affected by MSW inclusion (P>0.05). These data support MSW inclusion in broiler diets up to 3% without detriment to performance or amino acid digestibility coefficients.

DESCRIPTION OF PROBLEM

Mushrooms are a source of many essential nutrients as they are rich in rich in carbohydrates and proteins. Mushrooms are known to contain therapeutic bioactive compounds that have antimicrobial and

antioxidant activities (Atila et al., 2017). The United States produced roughly 398,000 tons of *Agaricus* mushrooms in the 2019-2020 crop (USDA-NASS, 2020). Pennsylvania is the foremost producer of mushrooms in the U.S., accounting for 66% of all *Agaricus* mushrooms produced in the U.S. from 2019-2020. While harvesting, the mushroom head is used for human consumption, the stump, including the stipe, volva, hypha, and mycelium is disposed of as an undesired waste product that is composted after harvest. On average, the stump waste is nearly 29% of the total mushroom weight. Therefore, roughly 115,420 tons of mushroom stumps are composted yearly. Mushroom stump waste may be a viable feedstuff generated from material previously deemed as waste. Camay (2016) found that small inclusions of a mushroom waste powder did not affect the body weight of broilers through a 42 d production period. Similarly, Mahfuz et al. (2019) found that inclusions of mushroom stem waste, up to 2% of the diet, did not affect average daily gain, average daily feed intake or feed conversion ratios of broilers. Considering these data, the objectives were to determine the nutrient profile of MSW (Experiment 1), determine the optimal inclusion of MSW in broiler diets based on performance measurables (Experiment 2), and measure amino acid digestibility at various MSW inclusions **(**Experiment 3). Considering results from past research, authors hypothesized that varying MSW inclusions would have no effect on broiler performance through the first 21 days of age.

MATERIALS AND METHODS

Mushrooms were plucked from the soil and the edible cap removed. The remaining stipe, volva, hypha, and mycelium made up the “mushroom stump”. Mushroom stumps were sourced from a commercial mushroom farm in southwest Pennsylvania, in coordination with the American Mushroom Institute (American Mushroom Institute, Avondale, PA), and transported to Penn State University. Mushroom stumps were shoveled into burlap bags and then placed on racks in a small grain drier. These racks allowed the grain drier to serve as a forced draft oven set to 66℃. The burlap sacks were turned daily and were removed after 120 h of drying. The dried product was placed on a #5 sieve and

mechanically shaken to remove dried peat moss that had adhered to the mycelium. After sieving, the dried mushroom stumps were passed through a hammermill that reduced particle size to 463 µm and yielded the final MSW product. All live animal procedures used in the experiments herein were approved by the Pennsylvania State University Animal Care and Use Committee (IACUC PROTO201900873).

Experiment 1

Treatments of 100% corn, 100% MSW and a non-fed control were precision-fed to 12, 20-week-old intact Single Comb White Leghorn (SCWL) roosters. The non-fed control accounted for endogenous losses of energy and nitrogen. The 12 (n=4 birds per treatment) roosters were fasted for 24 h, precision fed, and individually placed in battery cages with excreta collection trays placed under each pen. The roosters were arranged in a randomized complete block design and excreta were collected 24 h post-feeding and then weighed. These methodologies are modified from that of Sibbald (1976). Physical properties of the MSW, such as its propensity to absorb water, created precision feeding challenges. Therefore, the amount of MSW precision fed into the crop ranged between 13.1 and 18.0 g. Following excreta collection, the excreta were dried in a convection oven at 65°C for 72 h. Once dried, the excreta were sent to a commercial laboratory (Eurofins Nutrition Analysis Center, Des Moines IA) for crude protein and gross energy analyses. True metabolizable energy corrected for nitrogen (TME_n) calculations followed those of Parsons et al., (1982):

$$TME_n = (FEf - (EEf + 8.22\ Nf) + (EEU + 8.22\ Nu)) / FC$$

FEf = gross energy of the total feed consumed
EEf and EEu = energy in the excreta collected from the fed birds and fasted birds, respectively
Nf and Nu = grams of nitrogen retained by the fed birds and fasted birds, respectively
FC = grams of dry feed consumed

Experiment 2

Dietary Treatments

A control diet containing 0% MSW was formulated to satisfy the nutrient requirements of Cobb x Cobb straight-run broilers from d 1-21 (Cobb-Vantress Inc. 2018a). Nitrogen corrected true metabolizable energy values from Experiment 1 and proximate analysis were used to create an ingredient matrix for the MSW product (Table 2.1), to be used in the formulation of the 5% MSW diet. Diets were formulated to be isocaloric and isonitrogenous (Table 2.2). The 0% MSW and 5% MSW diets were mixed at the Penn State University Poultry Education and Research Center in a Scott Surge Hopper horizontal mixer (Scott Equipment Co., New Prague, MN) for three minutes dry and three minutes post-soybean oil addition. Additional treatments (1, 2, 3, 4% MSW) were created by blending portions of the 0% MSW and 5% MSW diets in a Rapids Marion Mixer (Rapids Machinery Co., Marion, Iowa) for three minutes. To ensure that each treatment was subjected to the same mixing conditions, the 0% MSW and 5% MSW treatments were also subjected to the same three minutes of mixing. Titanium dioxide was included as an indigestible marker in each treatment diet at 0.20% from d 18-21 of Experiment 2. Titanium dioxide concentrations were necessary for calculation of apparent ileal amino acid digestibility (AIAAD) and amino acid digestibility coefficients in Experiment 3.

Broiler Management

A total of 480 Cobb x Cobb straight-run broilers were vaccinated for Marek's disease and purchased from a commercial hatchery on day of hatch (Longenecker's Hatchery, Elizabethtown, PA). Ten birds were randomly selected, weighed and placed in one of 48 cages (Alternative Design Manufacturing and Supply Inc., Siloam Springs, AR). Feed and water were provided ad libitum. The six dietary treatments and eight replicate cages per treatment totaled 48 cages arranged in a randomized complete block design. The experimental unit was one cage of 10 broilers. Lighting and temperature regimen followed recommendations from Cobb-Vantress (Cobb-Vantress Inc. 2018b). Mortalities were replaced through d 3, after which mortalities were weighed and recorded. On d 21, lights were turned on 4 h prior to bird handling to ensure full gastrointestinal tracts for digesta collection. Upon handling, body weights and remaining feed weights were recorded.

Experiment 3

Selected treatments from Experiment 2 were used to determine the AIAAD and amino acid digestibility coefficients. Following weighing, birds provided 0, 1, 3, or 5% MSW were euthanized via cervical dislocation. Distal ilea, defined as the distal half of the small intestine from the Meckel's diverticulum to approximately 1 cm proximal to the ileocecal junction, were extracted and their contents flushed into a cup using a 20 mL syringe and distilled water. Digesta from approximately five birds per cage were pooled, frozen, and stored at -4°C until they were processed. This followed similar methodologies to that of Ravindran et al. (1999) and Evans et al. (2015). Digesta contents were lyophilized at -40°C for 65 h, and then both digesta and feed samples were sent to a commercial laboratory (The University of Missouri-Columbia Agricultural Experiment Station and Chemical Laboratories, Columbia, MO) for titanium concentration determination and complete amino acid profile analysis. These data were used to calculate AIAAD and amino acid digestibility coefficients following equations outlined by Evans et al. (2015) and Ravindran et al. (1999):

AIAAD (%) =

$$[((AAdiet/Tidiet) - (AAdigesta/Tidigesta))/(AAdiet/Tidiet)]\times AAdiet$$

Amino Acid Digestibility Coefficient (%) =

$$[((AAdiet/Tidiet) - (AAdigesta/Tidigesta))/ (AAdiet/Tidiet)]\times 100$$

Statistical Analysis

Experiment 2 was arranged in a randomized complete block design and subjected to analysis of variance (ANOVA) using the GLM procedure of SAS version 9.4 (SAS Institute, 2020). Significant differences were determined based on alpha ≤ 0.05. Additionally, a post hoc Fisher’s least significant

difference (LSD) test was used to further differentiate significant treatment means. Letter superscripts were used to denote differences among treatment means.

Select treatments from Experiment 2 were used in Experiment 3. The experimental unit in Experiment 3 was a pooled digesta sample from one cage of broilers. These data were subjected to ANOVA using the GLM procedure of SAS version 9.4 (SAS Institute, 2020) and alpha was designated as ≤ 0.05. A Fisher's LSD test was used to further explore significant multiple comparison analyses. Letter superscripts were used to denote differences among treatment means.

RESULTS AND DISCUSSION

Experiment 1

The 100% MSW treatment resulted in a TME_n value of 1,173 kcal/kg (Table 2.1). The corn treatment TME_n value was 3,754 kcal/kg, which is higher than previously published corn TME_n values. Evans et al., (2015) reported a TME_n value of 3,335 (kcal/kg) for corn. However, authors hypothesized age of the roosters used in the TME_n assay may have affected these results. Sibbald et al., (1960) found the age of roosters used for the metabolizable energy assay can cause small differences in metabolizable energy values. Experiment 1 utilized 20-week-old SCWL roosters, conversely, Evans et al., (2015) utilized 30-week-old SCWL roosters. It is worth noting that multiple factors affect the nutrient density of corn such as kernel density and environmental factors during the growing season. This is highlighted by Baidoo et al., (1991) who reported TME_n of corn grown in different areas of the world ranged from 3,681 to 3,962 (kcal/kg). Proximate analysis results show that MSW has a crude protein of 24.9%, crude fiber content of 22.8% and is 15.5% ash (Table 2.1). Given the TME_n and proximate analysis results, diets in Experiment 2 were formulated to be isocaloric and isonitrogenous across all treatments (Table 2.2). Particle size analyses were conducted to see how varying MSW inclusions affected feed form in the various mash diets (Table 2.3).

Experiment 2

Data for d 1-21 live bird performance is shown in Table 2.4. Neither feed intake nor mortality were affected by MSW inclusion (P>0.05). Birds consuming 1% MSW increased LWG per bird by 57.6 grams per bird compared to those fed 5% MSW. Birds provided 0, 2, 3 and 4% MSW were intermediate for LWG per bird (P=0.024). Birds consuming 1% MSW had higher average body weight (BW) compared to broilers provided 4% and 5% MSW. On average, broilers consuming 1% MSW were 56 grams heavier per bird than those fed 5% MSW. Treatments containing 0, 2 and 3% MSW were intermediate for BW (P=0.026). Broilers consuming 1% MSW improved mortality corrected feed conversion ratio (FCR) by 0.105 compared to those fed 5% MSW. Broilers fed 0, 2, 3, and 4% MSW were intermediate in FCR (P=0.0002).

Feed intake results from Experiment 2 are supported by Mahfutz et al. (2019) who reported no differences in feed intake when *Flammulina velutipes* mushroom stem waste was included up to 2% of the diet. However, these authors reported that MSW inclusions did not affect FCR or LWG. Camay (2016) reported no differences in BW, LWG or FCR when *Pleurotus ostreatus* mushroom waste powder was included up to 2.5% of the diet. Results from the current study support these findings as birds consuming diets up to 3% MSW did not affect performance parameters compared to those provided the control (0% MSW). Conversely, birds fed 5% MSW resulted in reduced performance measurables when compared to the control. Conflicting reports may be associated with the strains of both the mushrooms and broilers used in the current experiment, as well as the rate of mushroom waste included in the diet.

The crude fiber fraction of MSW (Table 2.1) may have increased digesta viscosity. This increase could have prevented endogenous enzymes from accessing substrates and may have contributed to low body weights and high feed conversion ratio when birds were provided 5% MSW. Almirall et al. (1995) replaced corn with either high or low-viscosity barley and reported a 19.3% reduction in the body weights of broilers fed the high-viscosity barley diets compared to those consuming the traditional corn-soy diets.

Therefore, future mushroom stump waste research should consider the effects of MSW on viscosity and subsequent performance.

Experiment 3

Birds consuming 0, 1, 3, and 5% MSW were utilized in Experiment 3 to determine apparent ileal amino acid digestibility and amino acid digestibility coefficients were calculated. Apparent ileal amino acid digestibility of essential amino acids is shown on Table 2.5. Broilers provided 1, 3, and 5% MSW reduced the AIAAD of leucine, histidine, phenylalanine, and valine compared to birds provided the control ($P<0.05$). Birds fed 1% MSW decreased the AIAAD of lysine by 10.04% compared to broilers consuming the control. Birds provided 3% and 5% MSW were intermediate for lysine digestibility ($P<0.0001$). Birds provided 1% MSW reduced the AIAAD of methionine by 29.3% compared to broilers fed the control. Birds consuming 3% and 5% MSW were intermediate for methionine digestibility ($P<0.0001$). Birds provided 1% and 3% MSW had lower AIAAD for threonine when compared to birds consuming the control and 5% MSW ($P<0.0001$). Broilers fed 1% and 5% MSW diets reduced the AIAAD of tryptophan compared to birds consuming the control and 3% MSW ($P<0.0001$). Birds provided 5% MSW reduced the AIAAD of arginine by 14.9% when compared to birds consuming the control. Broilers fed 3% MSW had similar arginine digestibility to those provided the 5% MSW treatment. Broilers consuming 1% MSW were intermediate for arginine digestibility ($P<0.0001$). Isoleucine digestibility followed arginine digestibility patterns ($P<0.0001$; Table 2.5).

The AIAAD results of non-essential amino acids are located in Table 2.6. Birds provided 1, 3 and 5% MSW had lower AIAAD for alanine, aspartic acid, cysteine, glycine, serine, and tyrosine compared to birds consuming the control ($P<0.05$). Birds provided 5% MSW decreased the AIAAD of glutamic acid compared to broilers fed the control. Birds consuming 1% and 3% MSW were intermediate for glutamic acid digestibility ($P<0.0001$). Proline digestibility followed glutamic acid digestibility patterns ($P<0.0001$). Taurine digestibility was unaffected by MSW inclusion ($P=0.812$; Table 2.6).

Using the total amount of amino acid digested in comparison to total amount of amino acid provided, digestibility coefficients were calculated. Amino acid digestibility coefficients were unaffected by MSW inclusion ($P>0.05$; Table 2.7 and Table 2.8). However, trending differences were apparent for cysteine and methionine digestibility coefficients ($P<0.10$). Feeding 1, 3 and 5% MSW reduced cysteine and methionine digestibility compared to birds consuming the control ($P=0.055$; $P=0.066$).

Considering the novelty of MSW, limited research has been conducted on the amino acid digestibility of this product. However, amino acid digestibility coefficient results from Experiment 3 are supported by Abro et al., (2016) who found that overall crude protein digestibility of *Pleurotus Ostrearus* mushroom inclusions up to 1% of the diet did not affect crude protein digestibility. Conversely, inclusions of 1.5% *Pleurotus Ostrearus* mushrooms improved crude protein digestibility when compared to the other treatments (2016). Conflicting digestibility results may be due to utilizing the entire mushroom, including the cap, rather than just the stem. Buwjoom et al., (2004) found that the cap of shiitake mushrooms yielded higher crude protein values than that of the stem. Considering these data, true amino acid digestibility analyses are needed to complete the MSW ingredient matrix and further elucidate the impact of MSW inclusions on broiler diets.

Performance results from Experiments 2 indicate that MSW could be used as a viable feed additive in broiler diets at inclusions up of the 3% of the diet. The decrease in performance attributed to inclusion rates greater than 3% MSW could be due to the high crude fiber and ash fraction of the MSW product (Table 2.1). These highly indigestible components could cause an increase in viscosity of digesta as previously mentioned. As seen with previous research (Mahfutz et al., 2019; Camay, 2016) the strain of the mushroom and broilers used in these studies can affect performance results as well. The AIAAD results indicated that increasing MSW inclusions reduced digestibility for 16 of the 19 analyzed amino acids. However, these differences did not affect the amino acid digestibility coefficients. Given these results, a full-term production trial, true amino acid digestibility values and viscosity results are necessary

to further elucidate the efficacy of this MSW product. A method for commercially and consistently producing this MSW product is also required to conduct further research on the benefits of MSW inclusions in broiler diets.

CONCLUSIONS AND APPLICATIONS

1. Day 1-21 performance results indicate that supplementation of 1% MSW improved feed efficiency, LWG per bird and average body weight when compared to broilers consuming 5%MSW.
2. Mushroom stump waste inclusion reduced AIAAD for 16 of the 19 analyzed amino acids.
3. Amino acid digestibility coefficients were not affected by MSW inclusions.
4. Mushroom stump waste inclusions in broiler diets up to 3% exhibited no detriment to performance or amino acid digestibility coefficients when compared to birds provided the control.

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Table 2.1: Mushroom stump waste nutrient profile used for diet formulation.

Nutrient	Value
TME_n[1] (kcal/kg)	1,173
Gross energy (kcal/kg)	3,836
Moisture (%)	6.93
Crude protein (%)	24.88
Crude fat (%)	1.65
Crude fiber (%)	22.77
Ash (%)	15.51
Calcium (%)	2.69
Total phosphorus (%)	0.632
Phytic acid (%)	0.14
Non-Phytate phosphorus[2] (%)	0.592
Average particle size (µm)	463

[1]True Metabolizable energy was calculated following modified methodologies from Sibbald (1976) and Parson et al., (1982).

[2]Non-phytate Phosphorus = Total Phosphorus - (Phytic Acid * 0.282). (Angel et al., 2002).

Table 2.2: Diet formulation and nutrient composition used to feed Cobb x Cobb straight-run broilers from d 1-21.

Ingredients (%)	**0% MSW**	**1% MSW**	**2% MSW**	**3% MSW**	**4% MSW**	**5% MSW**
Corn	57.64	57.42	57.20	56.98	56.76	56.55
Soybean meal (48%)	35.36	34.84	34.33	33.81	33.30	32.79
Mushroom stump waste	0.00	1.00	2.00	3.00	4.00	5.00
Mono calcium phosphate	1.72	1.69	1.67	1.65	1.63	1.61
Limestone	1.73	1.67	1.61	1.55	1.49	1.44
Soybean oil	2.44	2.22	2.01	1.80	1.59	1.38
Salt	0.46	0.45	0.45	0.44	0.44	0.44
DL-Methionine	0.24	0.24	0.25	0.25	0.26	0.27
Vitamin/Mineral premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine HCl	0.08	0.09	0.10	0.12	0.13	0.15
L-Threonine	0.07	0.08	0.09	0.10	0.11	0.12
Calculated Nutrients						
Metabolizable energy (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000
Crude protein (%)	21.25	21.25	21.25	21.25	21.25	21.25
Calcium (%)	1.05	1.05	1.05	1.05	1.05	1.05
Available phosphorus (%)	0.48	0.48	0.48	0.48	0.48	0.48
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18
Moisture (%)	10.50	10.48	10.46	10.44	10.42	10.40
Digestible lysine (%)	1.13	1.13	1.13	1.13	1.13	1.13
Digestible methionine (%)	0.53	0.53	0.53	0.53	0.53	0.54
Digestible TSAA (%)	0.81	0.81	0.81	0.81	0.81	0.81
Digestible threonine (%)	0.76	0.76	0.76	0.76	0.76	0.76
Analyzed Nutrients						
Gross energy (kcal/kg)	3,880	3,858	3,858	3,836	3,836	3,813
Crude protein (%)	21.56	20.75	22.31	20.94	21.00	21.50
Crude fat (%)	4.53	4.41	4.09	4.05	3.78	3.58
Crude fiber (%)	2.10	2.00	2.30	2.60	2.70	3.30
Ash[2] (%)	5.88	6.24	6.06	6.17	5.88	5.98
Moisture[2] (%)	13.38	13.01	12.89	13.01	12.93	13.02

[1]The vitamin and mineral premix contained the following (per lb of diet): menadione, 150 mg; B_{12}, 2,000 MCG; B_6, 250 mg; Vit. A, 1,400 KU; Vit. E, 3,000 IU; folic acid, 125 mg; choline, 70,000 mg; pantothenic acid, 1,200 mg; riboflavin, 1,200 mg; niacin, 5,000 mg; manganese, 40,000 ppm; zinc, 40,000 ppm; iron, 20,000 ppm; copper, 4,500 ppm; iodine, 600.0001 ppm; selenium, 60 ppm.

[2]Moisture (%) and Ash (%) analysis were completed in triplicate and reported as an average.

Table 2.3: Descriptive particle size[1] data of dietary treatments and mushroom stump waste.

MSW Inclusion	Rep 1 (µm)	Rep 2 (µm)	Mean (µm)
0%	680.00	676.84	678.42
1%	664.82	642.26	653.54
2%	587.71	618.38	603.04
3%	553.98	548.89	551.43
4%	602.28	603.18	602.73
5%	586.19	587.33	586.76
Mushroom Stump Waste	486.93	439.75	463.34

[1]Particle size was determined using a Ro-Tap tester, Model RX-29 (W.S. Tyler. Mentor, Ohio).

Table 2.4: Effects of performance results for day 1 to 21 Cobb x Cobb straight-run broilers fed diets containing 0, 1, 2, 3, 4, and 5% mushroom stump waste (Experiment 2).

MSW Inclusion	**FI/cage[1] (kg)**	**FI/bird[2] (kg/bird)**	**LWG/cage[3] (kg)**	**LWG/bird[4] (kg/bird)**	**BW[5] (kg)**	**Mortality (%)**	**FCR[6] (kg:kg)**
0%	9.490	1.073	7.254	0.783^{ab}	0.831^{ab}	7.5	1.355^{bc}
1%	9.848	1.078	7.340	0.809^{a}	0.854^{a}	8.75	1.334^{c}
2%	10.135	1.068	7.435	0.783^{ab}	0.831^{ab}	5.0	1.357^{bc}
3%	10.262	1.080	7.416	0.781^{abc}	0.829^{ab}	5.0	1.379^{b}
4%	9.844	1.046	7.077	0.752^{bc}	0.801^{b}	6.25	1.388^{b}
5%	9.793	1.091	6.724	0.746^{c}	0.798^{b}	10.0	1.439^{a}
P-Value	0.897	0.468	0.471	0.024	0.026	0.834	0.0002
LSD	0.950	0.044	0.803	0.035	0.0357	9.078	0.04
SEM[7]	0.331	0.015	0.279	0.0125	0.012	3.162	0.014

[1]Feed intake per pen.
[2]Feed intake per bird.
[3]Live weight gain per pen.
[4]Live weight gain per bird.
[5]Average body weight.
[6]Mortality corrected feed conversion ratio (FCR= FI/ (LWG+ mortality weight)).
[7]Pooled Standard error of the mean.

Table 2.5: The apparent ileal amino acid digestibility[1] of essential amino acids when Cobb x Cobb straight-run broilers were fed 0, 1, 3 and 5% mushroom stump waste.

MSW Inclusion	Arginine (%)	Histidine (%)	Isoleucine (%)	Leucine (%)	Lysine (%)	Methionine (%)	Phenylalanine (%)	Threonine (%)	Tryptophan (%)	Valine (%)
0%	1.385^{a}	0.501^{a}	0.814^{a}	1.513^{a}	1.185^{a}	0.628^{a}	0.940^{a}	0.713^{a}	0.194^{a}	0.850^{a}
1%	1.211^{b}	0.448^{b}	0.753^{b}	1.394^{b}	1.066^{c}	0.444^{d}	0.851^{b}	0.634^{b}	0.180^{b}	0.798^{b}
3%	1.188^{bc}	0.435^{b}	0.737^{bc}	1.369^{b}	1.083^{bc}	0.461^{c}	0.837^{b}	0.635^{b}	0.199^{a}	0.778^{b}
5%	1.178^{c}	0.430^{b}	0.723^{c}	1.366^{b}	1.104^{b}	0.489^{b}	0.830^{b}	0.696^{a}	0.174^{b}	0.799^{b}
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001
LSD	0.025	0.013	0.027	0.048	0.028	0.007	0.027	0.032	0.007	0.033
SEM[2]	0.0085	0.0047	0.0093	0.0164	0.0096	0.0024	0.0094	0.0110	0.0025	0.0113

[1]AIAAD =[((AAdiet/Tidiet) − (AAdigesta/Tidigesta))/(AAdiet/Tidiet)] ×AAdiet. Percent digestible amino acid refers to the percentage of digestible amnio acid within the total diet.
[2]Pooled Standard error of the mean.
[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 2.6: The apparent ileal amino acid digestibility[1] of non-essential amino acids when Cobb x Cobb straight-run broilers were fed diets containing 0, 1, 3 and 5% mushroom stump waste.

MSW Inclusion	Alanine (%)	Aspartic Acid (%)	Cysteine (%)	Glutamic Acid (%)	Glycine (%)	Proline (%)	Serine (%)	Tyrosine (%)	Taurine (%)
0%	0.880[a]	1.928[a]	0.286[a]	3.499[a]	0.742[a]	0.997[a]	0.802[a]	0.650[a]	0.071
1%	0.810[b]	1.687[b]	0.222[b]	3.111[b]	0.670[b]	0.925[b]	0.696[b]	0.585[b]	0.077
3%	0.806[b]	1.679[b]	0.216[b]	3.097[bc]	0.659[b]	0.906[bc]	0.695[b]	0.583[b]	0.071
5%	0.813[b]	1.670[b]	0.221[b]	3.038[c]	0.649[b]	0.872[c]	0.709[b]	0.573[b]	0.067
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.812
LSD	0.029	0.065	0.017	0.071	0.027	0.033	0.028	0.019	0.022
SEM[2]	0.0100	0.0222	0.0058	0.0241	0.0094	0.0114	0.0096	0.0064	0.0075

[1]AIAAD =[((AAdiet/Tidiet) − (AAdigesta/Tidigesta))/(AAdiet/Tidiet)] ×AAdiet. Percent digestible amino acid refers to the percentage of digestible amnio acid within the total diet.
[2]Pooled Standard error of the mean.
[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 2.7: Essential amino acid digestibility coefficients[1] when Cobb x Cobb straight run broilers were fed diets containing 0, 1, 3 and 5% mushroom stump waste.

MSW Inclusion	Arginine (%)	Histidine (%)	Isoleucine (%)	Leucine (%)	Lysine (%)	Methionine (%)	Phenylalanine (%)	Threonine (%)	Tryptophan (%)	Valine (%)
0%	90.0	84.9	82.2	83.2	85.9	93.9	84.0	76.7	81.2	79.5
1%	89.7	84.6	82.8	83.5	86.0	92.7	84.3	76.4	82.0	79.8
3%	89.4	83.8	82.0	83.0	86.0	92.4	83.7	76.6	83.0	78.6
5%	88.0	82.1	80.3	81.8	84.9	92.3	82.2	76.6	79.4	76.9
P-value	0.108	0.115	0.356	0.598	0.682	0.066	0.375	0.998	0.162	0.241
LSD	1.725	2.448	2.872	2.747	2.111	1.282	2.558	3.587	3.232	3.174
SEM[2]	0.586	0.832	0.976	0.934	0.718	0.435	0.869	1.061	1.098	1.079

[1]Amino Acid Digestibility Coefficient = (AAdiet/Tidiet − AAdigesta/Tidigesta)/(AAdiet/Tidiet)×100. These values are reported as a percentage out of 100.
[2]Pooled Standard error of the mean.
[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 2.8: Non-essential amino acid digestibility coefficients[1] when Cobb x Cobb straight-run broilers were fed diets containing 0, 1, 3 and 5% mushroom stump waste.

MSW Inclusion	Alanine (%)	Aspartic Acid (%)	Cysteine (%)	Glutamic Acid (%)	Glycine (%)	Proline (%)	Serine (%)	Tyrosine (%)	Taurine (%)
0%	82.3	81.0	71.7	88.1	78.1	81.1	81.0	83.4	44.5
1%	82.7	80.6	67.4	88.1	77.9	81.2	81.0	83.6	48.7
3%	82.2	80.7	67.7	88.0	77.6	80.9	80.9	83.4	44.9
5%	81.4	79.2	65.0	86.6	75.5	78.6	79.7	82.0	42.2
P-value	0.810	0.543	0.055	0.257	0.278	0.224	0.739	0.539	0.812
LSD	2.864	2.853	4.685	1.861	3.045	2.834	2.979	2.161	13.940
SEM[2]	0.974	0.970	1.593	0.632	1.035	0.963	1.012	0.859	4.739

[1]Amino Acid Digestibility Coefficient (%) = (AAdiet/Tidiet − AAdigesta/Tidigesta)/(AAdiet/Tidiet)×100. These values are reported as a percentage out of 100.
[2]Pooled Standard error of the mean.
[a-b] Values within comparisons with different superscripts differ (P<0.05).

Chapter 3

Effects of dietary direct-fed microbial inclusion on production and processing parameters of Nicholas Select tom turkeys

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SUMMARY

The dietary inclusion of direct-fed microbials (DFM) is becoming more common as antimicrobial use in food-producing animals becomes more scrutinized. Effects of various DFM on poultry performance has been studied; however, limited research has been conducted on the DFM *Bacillus subtilis* C-3102 in turkeys. Therefore, performance and processing responses to *Bacillus subtilis* C-3102 were investigated using 720 Nicholas Select tom turkeys. Diets were formulated to be nutritionally identical aside from DFM inclusion and were provided in a six-phase feeding program. Diets containing the DFM treatments were formulated to contain 500,000 cfu/g from d 1-35 and 300,000 cfu/g of the DFM from d 36-133. Each treatment was provided to 12 replicate pens of 30 turkeys arranged in a randomized complete block design. Results from d 1-133 show turkeys provided the DFM treatment exhibited a 0.08 improvement in FCR compared to those provided the control treatment ($P<0.0001$). Fecal microbial analysis from d 112 d 132 showed *Lactobacillus* concentrations were unaffected by DFM inclusion ($P=0.164$ and $P=0.886$, respectively). When compared to the prior four samplings, *Lactobacillus* counts increased for birds provided the DFM treatment for d 112 and d 132. This microbial population change aligned with improved performance. Day 134 processing results indicated birds consuming the control treatment increased pectoralis minor and total breast yields ($P=0.023$ and $P=0.011$, respectively) compared to turkeys consuming the DFM treatment. DFM supplementation reduced overall feed cost by $0.20 per bird. These data indicate that *Bacillus subtilis* C-3102 inclusions improved tom turkey performance and decreased feed costs.

DESCRIPTION OF PROBLEM

Poultry producers have utilized the subtherapeutic supplementation of various antibiotics for improved performance responses since the mid 1940's (Moore et al., 1946; Stokstad and Jukes, 1950; Biely and March, 1951). Although, some reports of antibiotic resistance existed (Luria et al., 1943; Demerec, 1945, 1948; Starr and Reynolds, 1951), the penurious state of the United States following World War II pressured legislatures to support farmers during these challenging times. Furthermore, the Food and Drug Administration of the United States approved the use of antibiotics as feed additives without veterinarian prescription in 1951 (Jones and Ricke, 2003). Although profitable for animal producers, research has demonstrated the ramifications of subtherapeutic supplementation of antibiotics. Smith (1968) examined the relationship between antimicrobial consumption and the discovery of multiple drug resistant strains of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* in various animals. Barton (2000) advised for the reduction of antibiotic use in animal diets due to the incidence of antibiotic resistant pathogens in livestock production. Due to the substantial amount of evidence from past literature and the treacherous effects on human health, several countries have limited the use of antimicrobials in animal diets. In 2006, the European Union banned the use of all antibiotic growth promoters in animal diets. The United States eliminated the use of antibiotic growth promoters in 2015 following a lawsuit filed against the FDA by the Natural Resources Defense Council. These changes, have been detrimental to the poultry industry by increasing production costs approximately $0.03 per bird (Maria Cardinal et al., 2019). Therefore, poultry producers are trying to replicate the positive effects of feeding antimicrobials, including growth promotion and reduction in subclinical disease challenge, without the negative consequences.

One potential solution to these issues is the supplementation of direct-fed microbials (DFM). This area of research has been reviewed extensively in poultry literature and has found some favorable results (Chiang and Hsieh, 1995; Patterson and Burkholder, 2003; Willis and Reid, 2008; Smialek et al., 2018). Many different strains of *Lactobacillus* and *Bacillus subtills* have been studied as DFM and shown

positive results under conditions of microbial challenge or stress (Torres-Rodriguez et al., 2007; Vicente et al., 2007; Mohammadigheisar et al., 2019). Vicente et al. (2007) reported that turkey poults consuming a *Lactobacillus* DFM and lactose prebiotic exhibited improvements in BW and FCR, when challenged with *Salmonella* Enteritidis, in comparison to birds provided the control diet. In contrast, these authors reported unchallenged turkey poults, consuming DFM, exhibited no differences in BW or FCR when compared to the control (2007). Although there is an abundance of research on DFM, little work has been conducted specifically on *Bacillus subtilis* C-3102 (Calsporin®) in turkeys. *Bacillus subtilis* C-3102 is a spore forming DFM that is thought to elicit commensal effects to the host. Fritts et al., (2000) reported improved BW and FCR in the grower period over two separate trials for broilers fed *Bacillus subtilis* C-3102 when compared to those consuming the control. The mode of action for *Bacillus subtilis* C-3102 is still unknown; however, past research has reported that competitive exclusion may be one mechanism of action. Maruta et al. (1996) reported that diets supplemented with *Bacillus subtilis* C-3102 were effective at excluding pathogens like *Campylobacter, Salmonella,* and *Enterobacteriaceae* while increasing the microbial population of *Lactobacillus* in the excreta. These authors also reported that *Bacillus subtilis* C-3102 supplementation proved effective in protecting chickens from *C. perfringens* (1996). Fritts et al. (2000) also reported that birds fed *Bacillus subtilis* C-3102 substantially reduced the incidence of *Salmonella* found on prechilled carcasses compared to broilers provided the control diet. Considering past research and the lack of knowledge on the effects of *Bacillus subtilis* C-3102 on turkey performance, this study was conducted to explore the impacts of this specific DFM. Therefore, the objective of this study was to determine the effects of dietary *Bacillus subtilis* C-3102 on production, microbial populations, and processing measurables of tom turkeys.

MATERIALS AND METHODS

Feed Manufacture

All diets were manufactured at a commercial feed mill (Kalmbach Feed Inc., Upper Sandusky, OH) and transported to the Pennsylvania State University. Diets were formulated to be identical in nutrient composition per phase, for six different feeding phases (Table 3.1), differing only in DFM inclusion. The control diets contained no added DFM while the experimental diets contained varying inclusions of DFM across the six feeding phases. The experimental diets for both Starter 1 (d 1-21) and Starter 2 (d 22-35) phases were formulated to contain 500,000 cfu/g (0.5lbs/ton), whereas the Grower 1 (d 36-56), Grower 2 (d 57-84), Finisher 1 (d 85-112) and Finisher 2 (d 113-133) phases were formulated to contain 300,000 cfu/g (0.33lbs/ton) of *Bacillus subtilis* C-3102. Standard operating procedures such as sequencing and flushing between batches were used to avoid contamination amongst treatments. To ensure adequate DFM levels were met, diets were manufactured prior to each phase change so samples could be collected and sent to a laboratory (Calpis American, Peachtree City, GA) where *Bacillus subtills* spores were enumerated. These results ensured that the activity level of the DFM were adequate, for each treatment, prior to feeding (Table 3.2). Starter 1 and Starter 2 diets were fed as crumbles while diets from the grower and finisher phases were provided as pellets. Descriptive feed quality data are presented on Table 3.3.

19-week Production Period

An experiment was designed to study the effect of dietary DFM inclusion (*Bacillus subtilis* C-3102) on tom turkey performance and processing measurables across a 19-week grow out. This project was planned to occur over the summer months, May 2020 to September 2020, to include the effects of high ambient temperatures on bird performance and subsequent DFM efficacy (Table 3.4). Two dietary

treatments were arranged in a randomized complete block design with 12 replicate pens per treatment. A total of 720 Nicolas Select tom turkeys were purchased from a commercial hatchery (Select Genetics LLC, Harrisonburg, VA) on day of hatch. Poults were randomly distributed across 24 floor pens (8.36 m^2/pen), each containing 30 poults (0.278 m^2/bird). All live animal procedures used in the experiments herein were approved by the Pennsylvania State University Animal Care and Use Committee (PROTO201901221). The experimental unit for this study was one pen of 30 turkeys. Feed and water were provided ad libitum while lighting and temperature regimen followed commercial recommendations (Aviagen, 2015a).

Diets were formulated to meet or exceed nutrition requirements of Nicholas Select tom turkeys (Aviagen, 2015b) except the digestible amino acid concentration. Digestible lysine was provided at 90% of breed recommendations and all other amino acids were provided at recommended ratios to digestible lysine. Prior to poult placement, shavings from a previous flock were removed. However, the barn was neither cleaned nor disinfected. Fresh shavings were added to all pens prior to poult placement. The combination of high ambient temperatures, reduced amino acid plane, and potential challenge vectors from previous flocks were used to create an environment suitable for potential performance uplift from DFM inclusions.

Fecal and Litter Samples

Due to the spore forming capabilities of this specific DFM, birds will excrete these spores onto the litter. To ensure there was no transfer of spores between pens, disposable boot covers were required when moving between treatment pens. Daily sweeping of the barn and isles minimized potential for spore adhesion to dust particles and controlled pen contamination. Litter, dust, and other foreign materials gathered during sweeping were disposed of daily. Litter and fecal samples were collected on the day prior to changing feeding phases (d 20, 34, 55, 83, 111, and 132). Composite litter samples were gathered from

nine defined areas per pen. Composite fecal samples were collected from eight individual droppings per pen. These samples were placed in insulated shipping containers with ice packs and were sent overnight to a private laboratory (Calpis American, Peachtree City, GA) where microbial populations were enumerated. Due to routine sampling, pens were top dressed with fresh pine shavings once throughout the study, on d 85.

Turkey Slaughter and Processing Procedures

All processing procedures were performed at the Pennsylvania State University Poultry Education and Research Center pilot processing facility. On d 133, birds were weighed individually, and random birds were systematically selected for processing measurements. Here, the 10^{th}, 15^{th}, and 20^{th} weighed birds per pen were wing tagged and transported to the processing facility. Turkeys were hung by their feet in steel shackles, stunned with electrical current (Midwest Processing Systems, Eden Prairie, MN), and dispatched via exsanguination. Following exsanguination, birds were scalded in a SuperScald Rotary Scalder (Brower Equipment, Houghton, IA) at a temperature of 60°C for 30 s. Following scalding, feathers were removed with an Ashley Sure Pick (Ashley Machine, Greensburg, IN) and carcasses were manually eviscerated. The ceca and sections of the ileum were tied shut, placed on ice, and transported overnight to a private laboratory (Calpis American, Peachtree City, GA) where microbial populations were determined. Following evisceration hot carcass weights were recorded and carcasses were placed in an ice bath overnight. Chilled carcass weights were recorded prior to deboning for yield determinations.

Statistical Analysis

Data from the turkey performance, fecal and litter microbial analysis, and processing experiments were arranged in randomized complete block designs and subjected to analysis of variance (ANOVA) using the GLM procedure of SAS version 9.4 (SAS Institute, 2020). Significant differences were

determined based on $\alpha \leq 0.05$. Additionally, post hoc Fisher's least significant difference (LSD) tests were used to differentiate significant treatment means.

RESULTS AND DISCUSSION

D 1-133 Overall Performance, Fecal, Litter, Ileal and Cecal Results

Performance results for the entire study period (d 1-133) are shown in Table 3.5. Overall, birds consuming the DFM treatment improved FCR by 0.08 when compared to those consuming the control ($P<0.0001$). Birds fed the DFM treatment tended to improve average body weight (BW); these turkeys were 400-g heavier than those provided the control treatment ($P=0.0999$). Overall mortality, feed intake (FI), live weight gain (LWG) and coefficient of variation were not affected by DFM inclusion ($P>0.05$; Table 3.5).

The benefits of DFM inclusions are variable. Efficacy may be dependent on DFM strain, bird strain, DFM dose, age, and stressors encountered during the production trial. There have been several applications in which DFM inclusions have resulted in positive effects on performance (Torres-Rodriguez et al., 2007; Russel and Grimes, 2009; Mohammadigheisar et al., 2019). Russel and Grimes (2009) reported that tom turkeys consuming DFM (*Lactobacillus acidophilus*) improved body weights at 8, 10, and 12 weeks of age. These authors reported improved FCR for each period throughout the 20-week production period (2009). Conversely, past literature has reported several instances where DFM inclusions have not affected various performance parameters (Francis et al., 1978; Potter et al., 1979; Owings, 1992; Wolfenden et al., 2011). Wolfenden et al. (2011) conducted two separate experiments evaluating three isolates of *Bacillus subtilis* and reported turkey poults provided the various DFM exhibited no improvements in BW or body weight gain when compared birds consuming the negative control. These authors reported birds provided a fourth isolate (*Bacillus subtilis* isolate PHL-NP122) exhibited improvements in BW similar to birds consuming the positive control during the first experiment

but not the second (2011). Conflicting results between experiment one and two were attributed to a lack of environmental stressors in experiment two (Wolfenden et al., 2011).

Overall performance results are concurrent with previous literature that showcased how environmental stress, similar to stress in commercial environments, is suitable for studying DFM efficacy (Torres-Rodriguez et al., 2007; Vicente et al., 2007). Torres-Rodriguez et al. (2007) evaluated the effects of DFM (FloraMax-B11) inclusions across 60 commercial turkey flocks. These authors reported that DFM inclusion only improved body weights for the flocks on farms that historically ranked in the bottom 75% of production efficiency (2007). Past research elucidates the variability of DFM inclusions in research settings as the positive effects of DFM may be diminished when birds are achieving genetic potential. The birds in this study did not meet genetic potential and the average body weights for both the control and DFM treatment were below breed performance objectives (Aviagen, 2015c; Figure 3.1). This was expected and birds consuming the control treatment were 14.5% below breed expectations, while turkeys provided the DFM treatment were 12.9% below breed expectations. Day 1-133 performance results indicate DFM inclusions, to tom turkeys in a commercial environment can elicit improved performance response.

The microbial analysis results of fecal samples collected on d 132 are displayed in Table 3.5. Birds fed the DFM treatment increased concentrations for total *Bacillus*, *Bacillus subtills* C-3102 and *Staphylococcus* when compared to those provided the control treatment. Birds consuming the DFM treatment show a 1.03 log increase in total *Bacillus* cfu/g compared to those fed the control treatment ($P<0.0001$). *Bacillus subtilis* C-3102 concentrations show that birds provided the DFM treatment exhibited a 1.80 log increase compared to those fed the control treatment ($P<0.0001$). These results were expected and ensured that the randomized complete block model utilized in this study did not lead to contamination between treatments. *Staphylococcus* concentration shows that birds provided the DFM treatment exhibited a 0.55 log increase when compared to those fed the control treatment ($P<0.0022$). Total anaerobe concentrations show that birds provided the control treatment exhibited a 0.16 log increase

when compared to those fed the DFM treatment (P<0.0334). Differences were not apparent for the concentration of *Enterobacteriaceae*, *C. perfringens*, *Enterococcus* or *Lactobacillus* (P>0.05; Table 3.5).

Microbial analysis results of litter samples collected on d 132 are shown in Table 3.5. Similar to fecal results, litter microbial analysis results indicate that turkeys consuming the DFM treatment increased concentrations for total *Bacillus* (P<0.0001) and *Bacillus subtills* C-3102 (P<0.0001) when compared to those fed the control treatment. However, birds provided the control treatment had significantly greater Staphylococcus and *Enterococcus* concentrations. Interestingly, birds provided the control treatment showed a 0.40 log increase in *Staphylococcus* cfu/g compared to turkeys consuming the DFM treatment (P=0.004), this was inconsistent with results from fecal microbial analysis. *Enterococcus* concentrations show that turkeys consuming the control treatment exhibited a 0.28 log increase compared to those provided the DFM treatment (P=0.014). DFM inclusion did not affect the concentration of *Enterobacteriaceae* or *C. perfringens* (P>0.05; Table 3.5).

Microbial analysis of ileal digesta results on d 133 are shown in Table 3.6. Similar to fecal and litter results, ileal microbial analysis data indicate that turkeys consuming the DFM treatment increased concentrations for total *Bacillus* (P<0.0001) and *Bacillus subtills* C-3102 (P<0.0001) when compared to those fed the control treatment. This was expected due to the dietary inclusion of *Bacillus subtilis* C-3102. This provides further evidence that DFM spores did not contaminate control pens throughout the study. *Enterobacteriaceae*, *C. perfringens*, *Lactobacillus*, total anaerobes, *Staphylococcus*, and *Enterococcus* counts were not different (P>0.05; Table 3.6).

Microbial analysis results of cecal digesta on d 133 are displayed in Table 3.6. Similar to fecal, litter and ileal results, cecal microbial analysis data indicate that turkeys consuming the DFM treatment increased concentrations for total *Bacillus* (P<0.0001) and *Bacillus subtills* C-3102 (P<0.0001) when compared to those fed the control treatment. As previously stated, these are expected results due to dietary inclusions of *Bacillus subtilis* C-3102 from the DFM diet. However, birds fed the DFM treatment displayed a 0.33 log increase in *Enterococcus* counts when compared to those consuming the control treatment. These results were inconsistent with fecal and litter analyses; however, this is potential

reasoning for improved performance for the DFM treatment. *Enterococcus* is a lactic acid producing bacteria that has shown beneficial effects on performance and health of poultry (Dhama et al., 2011). Trending differences were shown for *C. perfringens* cfu/g as the DFM treatment tended to increase these counts when compared to birds consuming the control. DFM inclusion did not affect the concentration of *Enterobacteriaceae*, *Lactobacillus*, total anaerobes, or *staphylococcus* (P>0.05; Table 3.6).

Overall, microbial populations in the fecal and litter samples fluctuated between treatments throughout the study. Interestingly, bird performance improved for the DFM fed birds as *Lactobacillus* counts in fecal samples increased. This increase in *Lactobacillus* concentrations and subsequent improvement in performance occurred during the Finisher 1 (Table 3.7) and Finisher 2 phases (Table 3.5; Table 3.8). The aforementioned microbial population fluctuations and lack of performance response suggests that *Lactobacillus* concentration is a critical parameter in initiating a performance response. Mountzouris et al. (2007) supports these findings as they reported broilers consuming two DFM treatments improved BW compared to those provided a diet containing antibiotics. These authors also reported that cecal digesta samples for birds consuming the antibiotic treatment resulted in a 0.9 and 0.8 log reduction in *Lactobacillus* concentrations when compared to birds provided the DFM treatments (2007). Considering these data, increasing *Lactobacillus* concentrations in the lower gastrointestinal tract is imperative for enhanced performance responses in tom turkeys.

D 134 Processing Results

Processing results on d 134 are displayed in Table 3.9. DFM inclusion did not affect live body weight (P=0.283) or hot carcass yield (P=0.821). However, birds fed the control treatment tended to improve chilled carcass yield when compared to turkeys provided the DFM treatment (P=0.054). Turkeys consuming the control treatment had higher pectoralis minor yield by 0.3% (P=0.023) when compared to birds fed the DFM treatment. Birds consuming the control treatment increased total breast yield by 1.2% (P=0.011) compared to birds provided the DFM treatment. Turkeys provided the control diet tended to

improve pectoralis major yield (P=0.056) when compared to birds consuming the DFM treatment. Birds provided the DFM treatment tended to increase thigh yield when compared to turkeys consuming the control treatment (P=0.096). Drum and wing yields were not affected by DFM inclusion (P>0.05).

This data is not concurrent with past research reported by Blair et al. (2004). These authors reported that dietary inclusions of *Bacillus subtilis* C-3102 to tom turkeys had no effect on pectoralis minor, pectoralis major or total breast yield (2004). In the current study, storage conditions may have affected carcass yield and subsequent breast yields. Due to limited cooler space, carcasses were chilled in an ice bath at but in sperate locations. Variations in chilling temperature may have affected water gain percentage as seen in past research. Babji et al. (1982) found that chilling carcasses at 37.8ºC increased water gain percentage by 0.33% when compared to carcasses stored at 21.1ºC. It is noteworthy that past research has found the varying inclusions of DFM can increase the production of volatile fatty acids (VFA). Mohammadigheisar et al. (2019) found that tom turkeys consuming *Bacillus subtilis* (DSM29784) significantly increased VFA production when compared to turkeys provided the control. This could have impacted the d 134 processing results as increased VFA production may have partitioned energy to fat pad deposition rather than muscle accretion. Fat pad yield was not assessed in the current experiment. Day 134 processing data indicate that DFM inclusion resulted in a reduction in protein accretion and subsequent processing yields; however, due to the small sample size (36 birds per treatment) additional yield studies should be conducted to further elucidate the impact of DFM inclusion on processing measurables.

Overall data indicates that DFM inclusion resulted in beneficial performance and microbial population responses. To further examine the effect of DFM inclusions on performance and microbial populations, phase specific data is included below. These data illustrate the impact of *Bacillus subtilis* C-3102 inclusions throughout the 19-week production period.

D 1-21 Performance, Fecal and Litter Results

Starter 1 (d 1-21) live bird performance results are shown in Table 3.10. During the Starter 1 period birds consuming the control treatment demonstrated higher FI, LWG, BW, and FCR when compared to turkeys consuming the DFM treatment. On a per bird basis, birds provided the control treatment increased FI by 70-g (P<0.0001), LWG by 40-g (P<0.0001) and BW by 30-g (P=0.0002) when compared to turkeys provided the DFM treatment. Considering the Starter 1 period contained a higher dosage of DFM compared to the grower and finisher periods, it may have caused detriment to performance. Bielke et al. (2003) reported that the lowest concentration of dietary DFM exhibited the greatest protection against *Salmonella enteritidis*. Mohammadigheisar et al., 2019 reported that the lowest inclusions of *Bacillus subtilis* (DSM29784) resulted in improved body weights and feed efficiency when compared to the control and higher inclusions of DFM. These authors also reported that high DFM inclusions reduced body weights and feed efficiency when compared to the control or lower inclusions of DFM (2019). Interestingly, birds fed the DFM treatment demonstrated a 0.06 improvement in FCR when compared to those fed the control treatment (P<0.0001). Mortality was not affected by DFM inclusion (P=0.674; Table 3.10).

The microbial analysis results of fecal samples collected on d 20 are displayed in Table 3.10. Turkeys consuming the DFM treatment had greater total *Bacillus* (P<0.0001) and *Bacillus subtills* C-3102 (P<0.0001) when compared to those fed the control treatment. These results were expected due to dietary inclusion of *Bacillus subtilis* C-3102 in the DFM treatment. However, *Lactobacillus* concentration increased for birds consuming the control treatment when compared to those provided the DFM treatment (P=0.009). This was unexpected as Maruta et al. (1996) reported that dietary inclusions of *Bacillus subtilis* C-3102 increased the amount of *Lactobacillus* present in fecal samples of broilers. Past research has shown that increasing *Lactobacillus* concentration has produced beneficial results in both turkeys and broilers (Jin et al., 1998; Vicente et al., 2007). Therefore, increasing *Lactobacillus* concentration of the fecal samples from the control treatment may have contributed to improved body weights and LWG

exhibited by turkeys provided the control diet for this period. Differences were not apparent for *Enterobacteriaceae*, *C. perfringens*, total anaerobes, *Staphylococcus*, or *Enterococcus* cfu/g (P>0.05; Table 3.10). The microbial analysis results of litter samples collected on d 20 are displayed in Table 3.10. DFM inclusion had no effect on litter microbial analysis results for this period (P>0.05).

D 22-35 Performance, Fecal and Litter Results

Performance measurables for the Starter 2 period (d 22-35) are displayed in Table 3.11. Birds consuming the control treatment had greater FI, LWG, BW, percent mortality, and feed efficiency compared to those fed the DFM treatment. Birds consuming the control treatment increased FI by 210-g per bird (P<0.0001), improved LWG by 170-g per bird (P<0.0001), BW by 210-g per bird (P<0.0001) and decreased FCR by 0.04 when compared to those fed the DFM treatment. As previously mentioned, high DFM inclusion may have enacted detrimental effects for the Starter 1 and Starter 2 periods. Although the greater FCR for the birds consuming the DFM treatment was unexpected as generally smaller birds as well as birds consuming DFM are more feed efficient (Russel and Grimes, 2009). However, Mohammadigheisar et al. (2019) reported that high DFM concentrations reduced feed efficiency when compared to the control and lower inclusions of DFM. Interestingly, birds consuming the control treatment had a greater incidence of d 22-35 mortality by 1.40% when compared to those consuming the DFM treatment (P=0.017; Table 3.11).

Microbial analysis results of fecal samples collected on d 34 are displayed in Table 3.11. Turkeys consuming the DFM treatment increased the concentration of total *Bacillus* (P<0.0001) and *Bacillus subtilis* C-3102 (P<0.0001) when compared to those provided the control treatment. However, birds fed the control treatment tended to have a greater concentration of *Lactobacillus* when compared to the DFM treatment (P=0.076). As previously mentioned, this trending difference may have affected performance

between treatments. No differences were shown for total anaerobes, *Enterobacteriaceae, C. perfringens, Staphylococcus,* or *Enterococcus* (P>0.05; Table 3.11).

Microbial analysis results of litter samples collected on d 34 are shown in Table 3.11. These results indicate that birds consuming the control treatment increased *Staphylococcus* cfu/g compared to turkeys provided the DFM treatment (P=0.007). DFM inclusion did not affect the concentration of *Enterobacteriaceae*, total *Bacillus, Bacillus subtilis* C-3102, *C. perfringens*, or *Enterococcus* (P>0.05; Table 3.11). The analysis for total *Bacillus* and *Bacillus subtilis* C-3102 were unexcepted however, these results show that the DFM treatment was numerically higher for these microbes. Considering these microorganisms naturally inhabit the gut microflora of turkeys and spore formation could result in cross contamination among pens this may explain why significant differences between treatments was not apparent for this period.

D 36-56 Performance, Fecal and Litter Results

Grower 1 (d 36-56) performance results are displayed in Table 3.12. Birds consuming the control treatment increased FI, LWG, BW and FCR compared to those fed the DFM treatment. Birds fed the control treatment increased FI by 360-g per bird (P=0.0003), LWG by 190-g per bird (P=0.0006), BW by 400-g per bird (P<0.0001) and FCR by 0.03 (P=0.016) when compared to turkeys provided the DFM treatment. Mortality was not affected by DFM inclusion (P=0.923; Table 3.12).

The microbial analysis results of fecal samples collected on d 55 are shown in Table 3.12. Turkeys provided the DFM treatment increased the concentrations of total *Bacillus* (P<0.0001) and *Bacillus subtills* C-3102 (P<0.0001) when compared to those fed the control treatment. However, *Lactobacillus* concentration increased for birds consuming the control treatment compared to those provide the DFM treatment (P=0.006). Differences were not apparent for *Enterobacteriaceae, C. perfringens,* total anaerobes, *Staphylococcus,* or *Enterococcus* (P>0.05; Table 3.12).

The microbial analysis results of litter samples collected on d 55 are displayed in Table 3.12. These results signify that birds fed the DFM treatment increased total *Bacillus* (P<0.0001) and *Bacillus subtills* C-3102 (P<0.0001) concentrations when compared to those provided the control treatment. These results are consistent with fecal microbial analysis results for d 55. DFM inclusion did not affect the concentration of *Enterobacteriaceae*, *C. perfringens*, *Staphylococcus* or *Enterococcus* (P>0.05; Table 3.12).

D 57-84 Performance, Fecal and Litter Results

Live-bird performance results for the Grower 2 period (d 57-84) are shown in Table 3.13. Similar to results from the Grower 1 period, d 57-84 results show birds consuming the control treatment increased FI, LWG, BW and FCR compared to turkeys provided the DFM treatment. Turkeys fed the control treatment increased FI by 660-g per bird (P<0.0001), LWG by 250-g per bird (P=0.014), BW by 610-g per bird (P<0.0001) and FCR by 0.07 (P<0.0001) when compared to those consuming the DFM treatment. Direct-fed microbial inclusion did not affect mortality for this period (P=0.398; Table 3.13).

Microbial analysis results of fecal samples collected on d 83 are displayed in Table 3.13. Birds fed the DFM treatment increased concentrations for total *Bacillus* (P<0.0001) and *Bacillus subtills* C-3102 (P<0.0001) when compared to those provided the control treatment. However, birds provided the control treatment increased *Lactobacillus* (P=0.015) and total anaerobe (P=0.035) concentrations when compared to birds consuming the DFM treatment. Microbial analysis differences were not apparent for *Enterococcus*, *Enterobacteriaceae*, *C. perfringens*, or *Staphylococcus* (P>0.05; Table 3.13).

Microbial analysis results of litter samples collected on d 83 are shown in Table 3.13. These results indicate that turkeys consuming the DFM treatment increased the concentrations of total *Bacillus* (P<0.0001) and *Bacillus subtills* C-3102 (P<0.0001) when compared to those fed the control treatment. Birds provided the control treatment increased the concentration of *Staphylococcus* cfu/g compared to

turkeys provided the DFM treatment (P=0.036). Interestingly, the *Staphylococcus* population is ubiquitous in a poultry production environment but increases susceptibility to dermatitis or infection as many species of *Staphylococcus* are opportunistic pathogens (Szafraniec et al., 2020). Therefore, this level of *Staphylococcus* in the litter may bw detrimental to performance if these numbers persist in the following periods. DFM inclusion did not affect the concentration of *Enterobacteriaceae, C. perfringens,* or *Enterococcus* (P>0.05; Table 3.13).

D 85-112 Performance, Fecal and Litter Results

Finisher 1 (d 85-112) performance results are shown in Table 3.7. Interestingly, DFM inclusion only affected FCR during the Finisher 1 period. Birds consuming the DFM treatment improved FCR by 0.21 compared to birds fed the control treatment (P<0.0001). Mortality, FI, LWG, and BW were not affected by DFM inclusion (P>0.05). This is the first instance throughout the study in which the DFM treatment have displayed similar FI, BW and LWG performance compared to that of the control treatment (Table 3.7).

The microbial analysis results of fecal samples collected on d 111 are displayed in Table 3.7. It is noteworthy that these samples were transported the same way as all other samples, however, shipping of these samples were delayed one day and may have affected results. Total *Bacillus* (P<0.0001) and *Bacillus subtills* C-3102 (P<0.0001) concentrations increased for turkeys consuming the DFM treatment when compared to those provided the control treatment. *Enterococcus* concentrations increased for birds provided the control treatment when compared to those fed the DFM treatment (P<0.0001). Differences were not apparent for *Enterobacteriaceae, Staphylococcus C. perfringens, Lactobacillus,* or total anaerobes (P>0.05; Table 3.7). It is worth noting that that this is the first time throughout the study that *Lactobacillus* fecal counts have been similar between treatments. This increase and the subsequent

improvement in performance results may have contributed to the performance improvements experienced by the birds provided the DFM treatment during this period.

The microbial analysis results of litter samples collected on d 111 are shown in Table 3.7. As previously mentioned with fecal sampling results, sample transportation was consistent with other samples, but shipping was delayed for d 111 litter samples. It is noteworthy, that fresh pine shavings were added on d 85 and may have affected these results as well. Results indicate that turkeys consuming the control treatment increased the concentration of total *Bacillus* ($P<0.0001$), *Enterococcus* ($P=0.002$) and *Enterobacteriaceae* ($P=0.030$) when compared to those fed the DFM treatment. The total *Bacillus* concentration results were unexpected and inconsistent with the results from d 111 fecal microbial analysis; however, the addition of fresh shavings coupled with the ability of *Bacillus subtilis* C-3102 to adhere to dust particles may be reasoning for these results. Birds consuming the DFM treatment increased the concentration of *Staphylococcus* when compared to turkeys provided the control treatment ($P=0.021$). DFM inclusion did not affect the concentration of *Bacillus subtills* C-3102 or *C. perfringens* ($P>0.05$; Table 3.7).

D 113-133 Performance Results

Performance results for the Finisher 2 period (d 113-133) are displayed in Table 3.8. Birds provided the DFM treatment increased FI by 490-g per bird ($P=0.025$), and decreased FCR by 0.14 ($P=0.040$) when compared to those fed the control treatment. Birds tended to have higher LWG ($P=0.075$) and BW ($P=0.0999$) when provided the DFM treatment. Mortality was not affected by DFM inclusion during this period ($P=0.224$; Table 3.8). These results are noteworthy as the DFM treatment displayed reduced growth and feed intake during the initial four periods of this study. However, by the end of the 19-week production period, birds consuming the DFM treatment tended to be 400-g heavier than those provided the control.

Economic Analysis

Dietary treatments for this production trial were manufactured at a commercial feed mill (Kalmbach Feed Inc., Upper Sandusky, OH). Therefore, an economic analysis was performed. Feed costs were calculated based on FI in each of the six phases (Table 3.14). For the Starter 1 and Starter 2 phases, feeding the DFM treatment reduced feed cost per bird by $0.04 and $0.10, respectively. For the Grower 1 and Grower 2 phases, birds provided the DFM treatment reduced feed cost per bird by $0.18 and $0.33. During the Finisher 1 and Finisher 2 phases, feeding the control treatment reduced feed cost per bird by $0.20 and $0.26, respectively. Overall, feeding the DFM treatment reduced feed cost by $0.20 per bird.

Overall, dietary inclusions of *Bacillus subtilis* C-3102 to tom turkeys throughout a 19-week production period yielded favorable results to performance. Birds consuming the DFM treatment tended to increase average body weight by 400-g when compared to those provided the control treatment. DFM inclusion resulted in a 0.08 improvement in FCR when compared to birds consuming the control treatment. Although birds provided the control treatment exhibited greater pectoralis minor and total breast yields than those consuming the DFM treatment, a larger sample size is needed to further assess the impact of DFM inclusion on processing responses. However, even if DFM inclusion results in reduced proetin accretion these deleterious effects may be offset by decreased feed costs. Birds consuming the DFM treatment resulted in a $0.20 reduction in feed cost per bird when compared to turkeys provided the control.

CONCLUSIONS AND APPLICATIONS

1. Day 1-133 performance results indicate that supplementation of *Bacillus subtilis* C-3102 improved feed conversion ratio by 0.08.
2. Dietary inclusions of *Bacillus subtilis* C-3102 did not affect FI, LWG or mortality; however, average body weight tended to be higher by 400-g for the DFM fed turkeys.
3. DFM supplementation reduced overall feed cost per bird by $0.20.

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Appendix A

Figures and Tables for Chapter 3

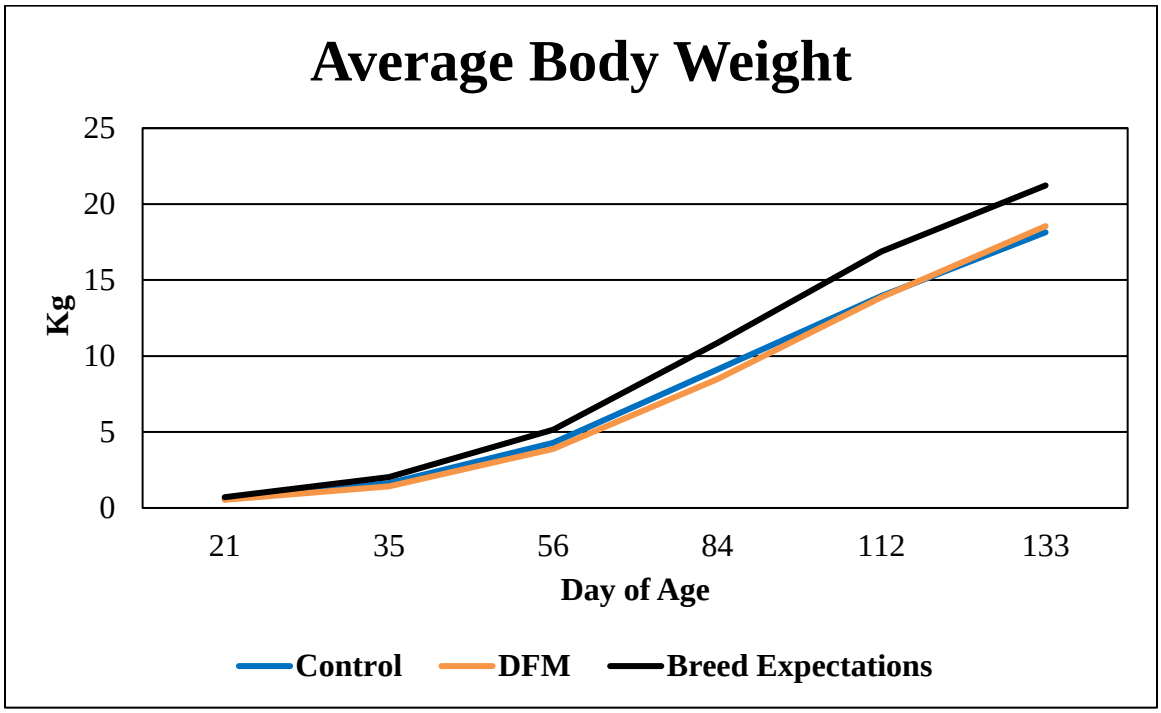

Figure 3.1: Average body weights of tom turkeys fed control and DFM treatments compared to Nicolas Select performance objectives.

Table 3.1: Diet formulation and diet composition used to feed Nicholas Select tom turkeys from D 1-133

Diet Formulations						
Ingredients (%)	Starter 1	Starter 2	Grower 1	Grower 2	Finisher 1	Finisher 2
Corn	42.13	45.59	51.14	57.57	62.80	66.36
Soybean meal	48.42	44.92	39.50	34.01	27.78	23.18
Corn oil	4.58	5.02	5.21	4.63	5.98	7.50
Limestone	0.94	0.79	0.77	0.73	0.72	0.68
Defluorinated phosphate	2.13	2.13	2.07	1.82	1.54	1.04
Monocalcium phosphate	0.35	0.12	0.05	0.00	0.00	0.00
Salt	0.16	0.16	0.17	0.17	0.18	0.18
Sodium bicarbonate	0.00	0.00	0.00	0.04	0.07	0.15
Choline chloride, 70%	0.10	0.10	0.10	0.10	0.10	0.10
L-Lysine HCl	0.20	0.20	0.17	0.16	0.14	0.13
DL-Methionine	0.37	0.36	0.30	0.25	0.20	0.21
L-Threonine	0.06	0.06	0.05	0.05	0.04	0.02
Vit/Min premix[1]	0.50	0.49	0.42	0.41	0.40	0.39
Calculated Nutrients						
Nutrient	Starter 1	Starter 2	Grower 1	Grower 2	Finisher 1	Finisher 2
Metabolizable energy (kcal/kg)	3,042	3,108	3,174	3,207	3,340	3,472
Crude protein (%)	26.21	24.78	22.51	20.31	17.64	15.71
Crude fat (%)	6.61	7.13	7.45	7.04	8.49	10.08
Crude fiber (%)	2.69	2.61	2.48	2.37	2.20	2.08
Dig. lysine (%)	1.56	1.46	1.30	1.16	0.98	0.85
Dig. methionine (%)	0.70	0.67	0.59	0.52	0.44	0.42
Dig. TSAA (%)	1.01	0.96	0.86	0.77	0.66	0.63
Dig. threonine (%)	0.92	0.87	0.79	0.71	0.61	0.53
Dig. tryptophan (%)	0.32	0.30	0.27	0.24	0.20	0.18
Dig. isoleucine (%)	1.01	0.95	0.86	0.77	0.66	0.57
Dig. arginine (%)	1.57	1.48	1.33	1.18	1.00	0.88
Dig. valine (%)	1.10	1.04	0.95	0.86	0.74	0.66
Calcium (%)	1.53	1.42	1.35	1.22	1.10	0.90
Available Phosphorus (%)	0.76	0.71	0.67	0.61	0.55	0.45
Sodium (%)	0.17	0.17	0.17	0.17	0.17	0.17
Chloride (%)	0.19	0.19	0.19	0.19	0.19	0.19
Potassium (%)	1.23	1.16	1.06	0.95	0.83	0.74

[1]Contains: K-Trace Mineral PMX 220; Avalia Zn 120 (Zinc); Selenium, 0.06%; K-Vitamin PMX 211; K-Vitamin E, 20,000; 25-OH-D3 add Pack, 100 mg/lb (Hy-D); Econase XT25 (16,000 BXU/KG - 30 kcal/lb); Quantum Blue 5G (500FTU/kg - 0.15%AvP).

Table 3.2: Analyzed nutrients of feed provided to Nicholas Select tom turkeys from D 1-133

Analyzed Nutrients												
Diet Phase	Starter 1		Starter 2		Grower 1		Grower 2		Finisher 1		Finisher 2	
Treatment	Control	DFM	Control	DFM	Control	DFM	Control	DFM	Control	DFM	Control	DFM
Bacillus subtilis C-3102 (cfu/g)	$1.58x10^4$	$2.15x10^5$	$1.16x10^5$	$5.67x10^5$	$6.21x10^3$	$1.92x10^5$	$4.24x10^2$	$1.43x10^5$	$2.54x10^3$	$2.3x10^5$	$8.2x10^2$	$1.82x10^5$
Gross Energy (kcal/kg)	4,012	4,166	4,078	4,122	4,144	4,166	4,122	4,166	4,144	4,210	4,276	4,254
Crude Protein (%)	25.88	25.75	27.0	23.88	23.44	20.56	20.75	22.81	17.94	17.88	16.25	16.31
Crude Fat (%)	5.90	7.16	6.72	7.10	7.62	6.58	6.55	7.29	8.30	8.58	9.31	9.22
Crude Fiber (%)	2.60	2.40	2.50	2.30	2.50	2.30	2.50	2.30	2.30	2.20	2.10	2.00
Ash[1] (%)	7.55	6.37	6.97	6.52	6.86	6.59	6.17	5.31	5.75	5.50	5.01	4.41
Moisture[1] (%)	12.01	11.54	12.59	12.09	10.95	11.03	11.25	9.41	11.01	10.45	10.26	10.63

[1] Moisture (%) and Ash (%) analyses were completed in triplicate and reported as an average.

Table 3.3: Descriptive feed[1] quality data of dietary treatments

Diet Phase	Treatment	Particle Size Analysis[2] (µm)	PDI[3] (%)	MPDI[4] (%)	NHPT[5] (%)
Starter 1	Control	1,283	-	-	-
	DFM	1,203	-	-	-
Starter 2	Control	1,300	-	-	-
	DFM	1,139	-	-	-
Grower 1	Control	-	97.7	97.0	97.2
	DFM	-	97.8	96.2	96.5
Grower 2	Control	-	98.1	97.4	97.2
	DFM	-	97.8	96.7	96.3
Finisher 1	Control	-	95.0	90.5	93.8
	DFM	-	93.3	89.1	92.1
Finisher 2	Control	-	96.6	90.6	93.0
	DFM	-	93.3	86.0	91.4

[1]Feed was manufactured at Kalmbach Feed Inc. in Upper Sandusky, OH.
[2]Particle size was determined in duplicate and reported as an average. Values were determined using a Ro-Tap tester, Model RX-29.
[3]PDI= pellet durability index; percentage was determined by inserting 500-g samples of sifted pellets into a P: Fost tumbler (Gamet Manufacturing Inc., Saint Paul, MN). Samples tumbled for 10 min at 50 rpm. After tumbling, the sample was sifted and weighed.
[4]MPDI = modified pellet durability index; percentage was determined similar to PDI but was modified by adding 5 hexagonal nuts to the 500-g samples prior to tumbling.
[5]NHPT= New Holmen Pellet Tester; percent pellet survivability was determined by placing 100-g samples of sifted pellets into the New Holmen Pellet Tester (NHPT100; TekPro Ltd., North Walsham, Norfolk, UK). The pellets were subjected to air flow for 30 sec within the test chamber. The surviving pellets were then removed and weighed

Table 3.4: Ambient temperature[1] ranges for each phase of production

Diet Phase	Date	Avg. Temperature[2] (°C)	Low Temperature[3] (°C)	High Temperature[4] (°C)
Starter 1	May 19 – June 8	20.1	7.2	31.6
Starter 2	June 9 – June 22	22.7	13.3	32.2
Grower 1	June 23 – July 13	26.6	20.0	34.4
Grower 2	July 14 – August 10	27.2	20.0	34.4
Finisher 1	August 11- September 7	24.7	16.6	33.8
Finisher 2	September 8- September 29	21.5	8.3	28.3

[1]Daily temperatures were recorded by The Pennsylvania State Climatologist.
[2]Average of daily temperatures for the period.
[3]Lowest temperature during the period.
[4]Highest temperature during the period.

Table 3.5: Effects of DFM inclusion to d 1-133 Nicholas Select turkey performance and microbial populations of feces and litter

Day 1-133 Performance Results						
Treatment	**Feed Intake (kg/bird)**	**Live Weight Gain (kg/bird)**	**Body weight (kg)**	**Mortality (%)**	**Feed Conversion Ratio[1]**	**CV[2] (%)**
Control	39.57	18.09	18.15	10.56	2.12[a]	7.81
DFM	39.91	18.49	18.55	14.17	2.04[b]	8.39
P-Value	0.664	0.101	0.0999	0.121	<0.0001	0.389
LSD	1.647	0.496	0.497	4.732	0.0256	1.432
SEM[3]	0.529	0.159	0.159	1.520	0.008	0.460

Day 132 Fecal Microbial Analysis								
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Lactobacillus* (Log_{10} CFU/g)	Total anaerobes (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	7.05	4.04[b]	3.23[b]	5.88	9.11	9.66[a]	7.45[b]	6.70
DFM	7.16	5.07[a]	5.03[a]	6.09	9.10	9.50[b]	8.00[a]	7.00
P-Value	0.651	<0.0001	<0.0001	0.385	0.886	0.033	0.002	0.365
LSD	0.4991	0.0787	0.1923	0.528	0.1738	0.1512	0.3029	0.6822
SEM[2]	0.1603	0.0252	0.0617	0.1696	0.0558	0.0485	0.0973	0.2191

Day 132 Litter Microbial Analysis						
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	6.91	4.21[b]	3.98[b]	5.91	9.38[a]	7.22[a]
DFM	6.59	5.19[a]	5.15[a]	5.77[b]	8.98[b]	6.94[b]
P-Value	0.201	<0.0001	<0.0001	0.365	0.004	0.014
LSD	0.5193	0.1897	0.1807	0.3426	0.2418	0.2147
SEM[2]	0.1668	0.0609	0.0580	0.1100	0.0776	0.0689

[1]Mortality corrected feed conversion ratio (FCR = FI/ (LWG + Weight of Mortality)).
[2]Average body weight coefficient of ariation= (Standard Deviation/Mean) *100.
[3]Pooled Standard error of the mean.
[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 3.6: Effects of DFM inclusion to d 133 Nicholas Select turkey microbial populations of ileal and cecal digesta

Day 133 Ileal Contents Microbial Analysis Results								
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Lactobacillus* (Log_{10} CFU/g)	Total anaerobes (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	6.34	3.67[b]	2.93[b]	3.85	7.49	8.47	5.62	5.36
DFM	6.04	4.82[a]	4.75[a]	4.01	7.83	8.57	4.46	5.57
P-Value	0.493	<0.0001	<0.0001	0.799	0.202	0.506	0.402	0.536
LSD	0.9143	0.2763	0.4951	1.3746	0.5457	0.3067	1.0954	0.717
SEM[1]	0.2937	0.0887	0.1590	0.4416	0.1753	0.0985	0.3519	0.2303
Day 133 Cecal Contents Microbial Analysis Results								
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Lactobacillus* (Log_{10} CFU/g)	Total anaerobes (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	7.42	3.97[b]	2.64[b]	5.79	8.98	9.63	5.86	6.14[b]
DFM	7.31	5.06[a]	4.99[a]	6.48	9.07	9.77	6.18	6.47[a]
P-Value	0.584	<0.0001	<0.0001	0.072	0.278	0.160	0.198	0.007
LSD	0.4408	0.2086	0.595	0.7624	0.1677	0.1987	0.5144	0.2165
SEM[1]	0.1416	0.0670	0.1911	0.2449	0.0538	0.0638	0.1652	0.0695

[1]Pooled Standard error of the mean.

[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 3.7: Effects of DFM inclusion to d 85-112 Nicholas Select turkey performance and microbial populations of feces and litter

Day 85-112 Performance Results					
Treatment	Feed Intake (kg/bird)	Live Weight Gain (kg/bird)	Body weight (kg)	Mortality (%)	Feed Conversion Ratio[1]
Control	12.00	4.54	13.95	3.02	2.49[a]
DFM	12.37	4.87	13.88	5.63	2.28[b]
P-Value	0.184	0.107	0.732	0.165	<0.0001
LSD	0.580	0.416	0.410	3.862	0.071
SEM[2]	0.186	0.133	0.131	1.240	0.023

Day 111 Fecal Microbial Analysis								
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Lactobacillus* (Log_{10} CFU/g)	Total anaerobes (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	7.89	4.02[b]	3.11[b]	4.75	9.12	9.59	7.09	7.96[a]
DFM	7.69	5.12[a]	5.11[a]	5.11	9.10	9.58	7.31	7.58[b]
P-Value	0.200	<0.0001	<0.0001	0.200	0.866	0.955	0.096	<0.0001
LSD	0.3223	0.052	0.1411	0.5957	0.1642	0.1644	0.2729	0.1405
SEM[2]	0.1035	0.0167	0.0453	0.1913	0.0527	0.0528	0.0876	0.0451

Day 111 Litter Microbial Analysis						
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	6.56[a]	5.46[a]	5.08	4.88	8.79[b]	7.16[a]
DFM	5.95[b]	5.20[b]	5.13	4.93	9.02[a]	6.54[b]
P-Value	0.030	<0.0001	0.182	0.845	0.021	0.002
LSD	0.5369	0.0552	0.0721	0.4974	0.1867	0.3266
SEM[2]	0.1724	0.0177	0.0231	0.1598	0.0599	0.1049

[1]Mortality corrected feed conversion ratio (FCR= FI/ (LWG+ mortality weight)).
[2]Pooled Standard error of the mean.
[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 3.8: Effects of DFM inclusion to d 113-133 Nicholas Select turkey performance

Day 113-133 Performance Results					
Treatment	Feed Intake (kg/bird)	Live Weight Gain (kg/bird)	Body weight (kg)	Mortality (%)	Feed Conversion Ratio[1]
Control	11.07[b]	3.98	18.15	2.86	2.66[a]
DFM	11.56[a]	4.25	18.55	1.51	2.52[b]
P-Value	0.025	0.075	0.0999	0.224	0.040
LSD	0.413	0.302	0.497	2.302	0.125
SEM[2]	0.132	0.097	0.159	0.739	0.040

[1]Mortality corrected feed conversion ratio (FCR= FI/ (LWG+ mortality weight)).
[2]Pooled Standard error of the mean.
[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 3.9: Effects of different levels of *Bacillus subtilis* C-3102 on day 134 Nicholas Select tom turkey processing[1] measurables results

Treatment	Live Weight (kg)	Hot Carcass Yield[2] (%)	Chilled Carcass Yield (%)	Pec. Major Yield[4] (%)	Pec. Minor Yield[4] (%)	Breast Yield[2,4] (%)	Drum Yield[4] (%)	Thigh Yield[4] (%)	Wing Yield[4] (%)
Control	18.712	82.8	86.3	24.2	5.6[a]	29.8[a]	12.8	13.4	9.5
DFM	18.308	82.9	85.7	23.3	5.3[b]	28.6[b]	12.8	14.0	9.4
P-Value	0.283	0.821	0.054	0.056	0.023	0.011	0.911	0.096	0.751
LSD	0.746	0.434	0.546	0.895	0.250	0.884	0.437	0.680	0.390
SEM[3]	0.263	0.153	0.193	0.316	0.088	0.312	0.154	0.240	0.137

[1]Measurables were calculated with 36 birds per treatment.
[2]Breast weight and yield include both pectoralis major and minor.
[3]Pooled Standard error of the mean.
[4]Pec. Major, Pec. Minor, breast, drum, thigh and wing yields were calculated as a percentage of chilled carcass weight.
[a-b] Values within comparisons with different superscripts differ ($P<0.05$).

Table 3.10: Effects of DFM inclusion to d 1-21 Nicholas Select turkey performance and microbial populations of feces and litter

Day 1-21 Performance Results					
Treatment	Feed Intake (kg/bird)	Live Weight Gain (kg/bird)	Body weight (kg)	Mortality (%)	Feed Conversion Ratio[1]
Control	0.72[a]	0.52[a]	0.57[a]	0.55	1.40[a]
DFM	0.65[b]	0.48[b]	0.54[b]	0.83	1.34[b]
P-Value	<0.0001	<0.0001	0.0002	0.674	<0.0001
LSD	0.021	0.011	0.011	1.415	0.015
SEM[2]	0.006	0.003	0.003	0.454	0.004

Day 20 Fecal Microbial Analysis								
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Lactobacillus* (Log_{10} CFU/g)	Total anaerobes (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	7.02	4.27[b]	0.32[b]	2.22	8.11[a]	9.12	5.23	6.76
DFM	6.70	5.27[a]	5.24[a]	2.53	7.49[b]	9.10	5.23	6.55
P-Value	0.113	<0.0001	<0.0001	0.600	0.009	0.921	0.989	0.495
LSD	0.404	0.123	0.723	1.244	0.431	0.296	0.515	0.641
SEM[2]	0.1299	0.0397	0.2324	0.3996	0.1386	0.0951	0.1654	0.2060

Day 20 Litter Microbial Analysis				
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	7.37	3.38	6.46	7.09
DFM	7.12	3.24	6.41	6.93
P-Value	0.308	0.704	0.791	0.474
LSD	0.5081	0.8084	0.4762	0.4788
SEM[2]	0.1632	0.2597	0.1530	0.1538

[1]Mortality corrected feed conversion ratio (FCR= FI/ (LWG+ mortality weight)).

[2]Pooled Standard error of the mean.

[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 3.11: Effects of DFM inclusion to d 22-35 Nicholas Select turkey performance and microbial populations of feces and litter

Day 22-35 Performance Results					
Treatment	Feed Intake (kg/bird)	Live Weight Gain (kg/bird)	Body weight (kg)	Mortality (%)	Feed Conversion Ratio[1]
Control	1.45[a]	1.05[a]	1.63[a]	1.68[a]	1.37[b]
DFM	1.24[b]	0.88[b]	1.42[b]	0.28[b]	1.41[a]
P-Value	<0.0001	<0.0001	<0.0001	0.017	<0.0001
LSD	0.028	0.019	0.028	1.098	0.012
SEM[2]	0.009	0.006	0.008	0.352	0.003

Day 34 Fecal Microbial Analysis								
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Lactobacillus* (Log_{10} CFU/g)	Total anaerobes (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	6.61	4.65[b]	4.62[b]	1.42	8.27	9.36	5.77	6.04
DFM	6.89	5.41[a]	5.38[a]	2.91	7.95	9.35	5.51	6.26
P-Value	0.096	<0.0001	<0.0001	0.051	0.076	0.844	0.397	0.313
LSD	0.3363	0.0309	0.0328	1.4963	0.3593	0.0787	0.6661	0.4684
SEM[2]	0.1080	0.0099	0.0105	0.4807	0.1154	0.0252	0.2139	0.1504

Day 34 Litter Microbial Analysis						
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	7.66	4.30	4.27	3.66	8.14[a]	7.24
DFM	7.41	4.62	4.56	3.48	7.43[b]	7.07
P-Value	0.392	0.102	0.127	0.248	0.007	0.462
LSD	0.6372	0.3675	0.3949	0.3272	0.4682	0.5138
SEM[2]	0.2046	0.1180	0.1268	0.1051	0.1504	0.1650

[1]Mortality corrected feed conversion ratio (FCR= FI/ (LWG+ mortality weight)).
[2]Pooled Standard error of the mean.
[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 3.12: Effects of DFM inclusion to d 36-56 Nicholas Select turkey performance and microbial populations of feces and litter

Day 36-56 Performance Results					
Treatment	Feed Intake (kg/bird)	Live Weight Gain (kg/bird)	Body weight (kg)	Mortality (%)	Feed Conversion Ratio[1]
Control	4.13[a]	2.60[a]	4.29[a]	3.73	1.55[a]
DFM	3.77[b]	2.41[b]	3.89[b]	3.85	1.52[b]
P-Value	0.0003	0.0006	<0.0001	0.923	0.015
LSD	0.152	0.087	0.119	2.605	0.021
SEM[2]	0.049	0.027	0.038	0.837	0.006

Day 55 Fecal Microbial Analysis								
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Lactobacillus* (Log_{10} CFU/g)	Total anaerobes (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	6.72	4.08[b]	3.38[b]	2.47	8.22[a]	9.04	7.65	7.20
DFM	6.70	5.14[a]	5.12[a]	3.05	7.75[b]	9.08	7.68	6.92
P-Value	0.913	<0.0001	<0.0001	0.344	0.006	0.623	0.765	0.102
LSD	0.4573	0.0832	0.1396	1.2796	0.2986	0.148	0.2495	0.352
SEM[2]	0.1469	0.0267	0.0448	0.4110	0.0959	0.0475	0.0801	0.1130

Day 55 Litter Microbial Analysis						
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	7.64	4.11[b]	3.83[b]	3.87	9.25	7.87
DFM	7.79	4.96[a]	4.82[a]	3.68	9.34	7.85
P-Value	0.443	<0.0001	<0.0001	0.477	0.324	0.899
LSD	0.4287	0.1327	0.1569	0.5713	0.1842	0.3929
SEM[2]	0.1377	0.0426	0.0504	0.1835	0.0591	0.1262

[1]Mortality corrected feed conversion ratio (FCR= FI/ (LWG+ mortality weight)).

[2]Pooled Standard error of the mean.

[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 3.13: Effects of DFM inclusion to d 57-84 Nicholas Select turkey performance and microbial populations of feces and litter

Day 57-84 Performance Results					
Treatment	Feed Intake (kg/bird)	Live Weight Gain (kg/bird)	Body weight (kg)	Mortality (%)	Feed Conversion Ratio[1]
Control	9.09[a]	4.78[a]	9.10[a]	0.83	1.89[a]
DFM	8.43[b]	4.53[b]	8.49[b]	1.75	1.82[b]
P-Value	<0.0001	0.014	<0.0001	0.397	<0.0001
LSD	0.255	0.183	0.223	2.305	0.019
SEM[2]	0.081	0.058	0.071	0.740	0.006

Day 83 Fecal Microbial Analysis								
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Lactobacillus* (Log_{10} CFU/g)	Total anaerobes (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	6.39	4.06[b]	2.61[b]	4.35	9.02[a]	9.45[a]	7.94	6.37
DFM	6.39	5.14[a]	5.01[a]	4.30	8.59[b]	9.21[b]	8.04	6.71
P-Value	0.991	<0.0001	<0.0001	0.889	0.015	0.035	0.581	0.089
LSD	0.5922	0.0851	0.5774	0.7424	0.3304	0.2149	0.3806	0.4022
SEM[2]	0.1902	0.0273	0.1854	0.2385	0.1061	0.0690	0.1222	0.1291

Day 83 Litter Microbial Analysis						
Treatment	*Enterobacteriaceae* (Log_{10} CFU/g)	Total *Bacillus* (Log_{10} CFU/g)	*Bacillus subtilis* C-3102 (Log_{10} CFU/g)	*C. perfringens* (Log_{10} CFU/g)	*Staphylococcus* (Log_{10} CFU/g)	*Enterococcus* (Log_{10} CFU/g)
Control	6.04	4.18[b]	3.83[b]	4.70	9.64[a]	7.03
DFM	5.73	5.07[a]	4.97[a]	4.38	9.54[b]	6.97
P-Value	0.387	<0.0001	<0.0001	0.088	0.036	0.626
LSD	0.7647	0.0921	0.1219	0.3725	0.087	0.2676
SEM[2]	0.2456	0.0295	0.0391	0.1196	0.0279	0.0859

[1]Mortality corrected feed conversion ratio (FCR= FI/ (LWG+ mortality weight)).
[2]Pooled Standard error of the mean.
[a-b] Values within comparisons with different superscripts differ (P<0.05).

Table 3.14: Feed[1]cost per bird[2]associated with the control and DFM treatments

Treatment	Starter 1	Starter 2	Grower 1	Grower 2	Finisher 1	Finisher 2	Overall[3]
Control	0.39	0.77	2.13	4.75	5.86	5.33	19.26
DFM	0.35	0.67	1.95	4.42	6.06	5.59	19.06
Difference	$0.04	$0.10	$0.18	$0.33	$0.20	$0.26	$0.20

[1]Feed was manufactured at Kalmbach Feed Inc. in Upper Sandusky, Ohio.
[2]Feed cost per bird is based on feed intake in each phase and the cost of the feed in that given phase. The diet cost used in this calculation did not include the cost of bagging.
[3]Overall denotes the cost required to feed each respective bird from D 1-133. The cost difference to feed birds per phase is provided.