ABSTRACT

HEINICHEN, BRENDAN O. Effects of Dietary Supplementation of Solid-State Fermentation Enzymes on the Growth Performance, Nutrient and Mineral Digestibility of Turkey Poults Fed Pelleted Corn-Soybean-Based Feed. (Under the direction of Dr. Peter Ferket).

Two experiments were conducted to study the effect of dietary supplementation with Allzyme Spectrum® (Alltech, Inc., Nicholasville, KY), an enzyme complex with both phytase and carbohydrase activities, in enhancing growth performance and nutrient digestibility for turkey diets of reduced nutrient density. The research was carried out with two projects designed to evaluate the impact of the enzyme complex on nutrient utilization—specifically phosphorus (P) and calcium (Ca) and apparent metabolizable energy (AMEn), growth performance, and carcass yield in turkey poults under both controlled research conditions and commercial production settings.

In the initial experiment, the effect of incremental dietary supplementation levels (100, 200, 300, and 400 grams per tonne of feed) of Allzyme Spectrum® in a pelleted corn-soybean meal diet were explored on growth performance indicators, nutrient digestibility, and tibia parameters in Nicholas Select turkey poults raised to 28 days of age. While no significant impacts were observed on growth performance, tibia-breaking force, and ash content, there were statistically significant effects observed in AMEn and ileal digestibility of Ca and P. These results suggest that the enzyme complex can effectively enhance nutrient digestibility including ME, Ca, and P in turkey diets by enhancing nutrient digestibility.

The subsequent study extended this investigation into a commercial research farm setting, evaluating a positive control diet against a nutrient-reduced negative control diet supplemented with two levels of the enzyme complex (150 and 300 grams per tonne of finished feed). The inclusion of this enzyme complex was shown to produce linear improvements in body weight

and yield percentage, thereby demonstrating its potential to counteract reductions in dietary nutrient density without adversely affecting performance. Statistically significant improvements in pre-chill carcass weights and yield percentages further highlighted the enzyme's effectiveness in a commercial turkey production environment.

Together, these studies suggest the potential of Allzyme Spectrum® as an effective feed additive for improving nutrient utilization and growth performance in turkeys. This presents a valuable strategy for reducing feed costs without compromising productivity. The findings advocate for the inclusion of this enzyme complex in turkey diets as a means to achieve both economic and nutritional efficiency, particularly in formulations with lowered nutrient specifications.

(Key words: turkeys, digestibility, performance, phytase, xylanase)

Effects of Dietary Supplementation of Solid-State Fermentation Enzymes on Growth Performance, Nutrient and Mineral Digestibility of Turkeys Fed Pelleted Corn-Soybean-Based Feed.

by
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DEDICATION

I dedicate this to my beautiful and supportive family. No matter how many times I faced the fear of failure, their love and support were steadfast, always by my side, providing the extra boost of confidence I needed. I couldn't have achieved any of this without the support of my wife, Samara, and the love from my wonderful children, Alicy and Henry. Eu amo vocês muito.

BIOGRAPHY

Born on March 18, 1987, in San Francisco, California, Brendan O'Rourke Heinichen was the son of Kathleen and Michael Heinichen. Raised by his devoted single mother, Brendan was deeply influenced by her strong work ethic and steadfast belief in the power of education. She instilled in him the idea that education is a priceless asset that opens doors and remains with a person for life.

After graduating high school, Brendan began a career in banking, where he demonstrated his ambition and determination by rapidly advancing through various positions. However, his career trajectory took an unexpected turn during his undergraduate studies at California Polytechnic State University. An elective course in Poultry Management captivated him, altering his professional path entirely. His newfound passion was significantly fostered and supported by Dr. Bob Spiller. This led him to complete all available courses in poultry science and poultry management, culminating in his receipt of the prestigious Max and Verda Foster Memorial Scholarship.

Upon completing his degree, Brendan transitioned into the poultry industry, starting in live production with broilers. His dedication and expertise led to a significant career advancement five years later, when he was appointed as the manager of the breeding department of a major poultry integrator. After a decade in live production, Brendan sought new challenges and shifted his focus to sales within a poultry nutrition company. This change reignited his passion for learning and marked the beginning of his journey in poultry nutrition.

Currently, Brendan is pursuing higher education to become a poultry nutritionist, driven by his lifelong commitment to learning and his deep-seated passion for the poultry industry.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

Literature review

1.1 Significance of the turkey industry

The turkey industry in the United States is a significant sector within the broader context of poultry production, contributing substantially to the agricultural economy, food supply, and nutritional status of the country. The industry has integrated various components, including breeding, nutrition, health management, processing, and marketing, each playing a critical role in the overall success and sustainability of turkey production.

From a nutritional standpoint, turkey meat is highly valued for its lean protein content, low fat levels, and variety of vitamins and minerals such as B vitamins, and selenium, making it an important component of a balanced diet (USDA, 2019). The industry's focus on nutrition has led to the development of specialized feeds and feeding strategies aimed at constantly improving growth rates, feed conversion ratios (FCR), health, and meat quality of turkeys, thereby enhancing economic efficiency and product appeal to consumers. The turkey industry relies heavily on advancements in genetics and breeding to produce birds that meet specific market demands for meat yield, quality, and efficiency of production (Nestor, 1976). Selective breeding programs have been successful in increasing the size and meat yield of turkeys, as well as improving their feed efficiency and resistance to diseases (USDA AgResearch Magazine, 2004).

The significance of the turkey industry is also evident in its contribution to employment and economic activity in rural areas where turkey production is often concentrated (National Turkey Federation, 2023). It provides a source of income for thousands of farmers, workers, and businesses involved in the supply chain from feed production to distribution and retail. Furthermore, the turkey industry plays a large role in the U.S. agricultural export market, with turkey meat being exported to various countries around the world. In 2023 alone over 400 million pounds of turkey meat was exported around the globe (*USDA ERS - Commodity Costs*

and Returns, 2023). This not only contributes to the U.S. trade balance but also showcases the quality and safety standards of U.S. poultry products on the international stage.

Environmental sustainability is another critical aspect of the turkey industry, with an ongoing drive for research and production practices aimed at reducing the environmental footprint of the turkey production (Alders & Tomley, 2022). This includes efforts to minimize water and energy usage, reduce greenhouse gas emissions, and implement sustainable manure management practices to protect soil and water quality.

The turkey industry in the United States is of significant importance due to its contributions to the agricultural economy, employment, nutrition of the population, and efforts towards sustainable production practices. The industry's reliance on scientific advancements in nutrition, genetics, health management, and environmental protection ensures its ongoing success and ability to meet the growing demand for turkey products both domestically and internationally.

1.2 Feedstuffs used in commercial turkey production and their antinutritional factors

In turkey production, corn, soybean meal, and wheat are primary feed ingredients due to their high nutritional profiles, essential for growth, maintenance, and reproduction (Bassi et al., 2021). Corn is selected for its starch-based energy content, while soybean meal, rich in essential amino acids such as lysine and methionine, serves as the primary protein source, addressing amino acid limitations in turkey diets (Attia et al., 2020). Wheat, although an energy source like corn, is often limited in use due to non-starch polysaccharides (NSPs) that can impair nutrient digestibility, yet it aids in pellet quality for high-fat starter diets (Winowiski et al., 2021).

Antinutritional factors (ANFs), such as phytic acid in corn and soybean meal and trypsin inhibitors in soybean meal, present challenges to nutrient absorption and digestion. Wheat's NSPs increase intestinal viscosity, further hindering nutrient uptake (Gorenz et al., 2022; Jha & Mishra, 2021; Kocher et al., 2003; Olukosi et al., 2020). Furthermore, phytic acid can also chelate trace minerals such as Cu, Mg and Fe leading to potential nutrient deficiencies (Frolich, 1990). Addressing these impacts involves strategies like thermal processing and the addition of enzymes (e.g., phytase, xylanase) to enhance nutrient bioavailability, thereby supporting turkey health and productivity (Cowieson et al., 2006; Kocher et al., 2003; Maqsood, 2013)

Feed formulations are carefully designed to meet turkeys' nutritional needs across various growth stages, balancing macronutrients and micronutrients, while minimizing the negative effects of ANFs, to foster optimal growth, health, and meat quality. This involves adjusting the composition based on nutritional content, cost, and ANF presence, with animal by-products, fats, oils, vitamins, and minerals playing significant roles (Ravindran et al., 2005; Romero et al., 2014).

Continuous evaluation of feedstuff composition is essential, utilizing techniques like near-infrared reflectance spectroscopy (NIR) for its efficiency and cost-effectiveness, ensuring that formulated diets meet actual nutritional requirements (Zaefarian et al., 2021). The primary roles of corn for energy, soybean meal for protein, and wheat for both energy and its higher protein content, albeit with NSPs, highlight the strategic formulation and management needed to counteract ANFs' effects on nutrient availability, underscoring the precision required in turkey feed formulation for efficient nutrition and sustainable production (Odetallah et al., 2002; Vargas et al., 2023).

1.3 Enzymes in General and Supplementation in Poultry Nutrition

Enzymes play a major role in the field of turkey nutrition, acting as biocatalysts that facilitate and accelerate biochemical reactions essential for the digestion, absorption, and utilization of nutrients (Robinson, 2015). Existing endogenously and exogenously (as additives), they are crucial for the efficient metabolism of feedstuffs, enabling turkeys to extract maximum nutritional value from their diets. The structure of enzymes is linked to their function, following the lock-and-key model where the enzyme (the key) precisely fits into the substrate (the lock) it acts upon. This specificity is due to the unique three-dimensional structure of the enzyme, which determines its ability to bind to a particular substrate and catalyze a specific reaction. This reaction specificity is fundamental to their role in metabolism, as each enzyme catalyzes only one type of biochemical reaction, thereby directing the metabolic pathways essential for life (Molnar & Gair, 2015).

In commercial turkey production, the application of enzymes has become increasingly common, with enzyme supplementation being integrated into feed formulations to enhance feed efficiency and nutrient availability (Cowieson et al., 2006; Maqsood, 2013). The use of enzymes in poultry diets is a direct response to the challenges posed by antinutritional factors and the inherent complexity of plant-based feedstuffs, which can limit the availability of certain nutrients (Odetallah et al., 2002). Enzyme supplementation in poultry nutrition, specifically for turkeys, addresses the need to improve the digestibility and absorption of feed ingredients. Enzymes such as phytase and xylanase are commonly added to turkey diets to break down phytic acid and non-starch polysaccharides, respectively (Gorenz et al., 2022). Phytic acid, which is the storage form of phosphorus in plants, is found in feedstuffs such as grains and oilseeds. Due to the presence of phosphate groups, it is a highly charged molecule which binds/chelates to important minerals in

the feed such as calcium, making them unavailable for absorption (Duodu & Apea-Bah, 2017; Martínez-Vallespín et al., 2022; Selle et al., 2009). Phytase catalyzes the hydrolysis of phytic acid, releasing bound phosphorus and making it available for uptake by the bird. This not only improves the nutritional value of the feed but also reduces the environmental impact of poultry production by decreasing phosphorus excretion (Cangussu et al., 2018).

Xylanase, on the other hand, targets the breakdown of xylans and arabinoxylans, a type of non-starch polysaccharide (hemicellulose) present in cell walls of cereals like wheat and barley. By breaking down xylans into smaller molecules such as xylooligosaccharides (XOS), xylanase reduces gut viscosity, enhancing the digestibility of the feed and the absorption of nutrients which results in improved feed conversion ratios, growth rates, and overall health of the turkey (González-Ortiz et al., 2017). This liberation of XOS can play a beneficial role in gut health by acting as a prebiotic, feeding the microbiome of the turkey which then creates short chain fatty acids, providing energy and nutrition to the bird.

However, the excessive use of xylanase should be a consideration, as full breakdown of the insoluble fiber can have a negative effect on performance (Singh & Kim, 2021). Once broken down into the XOS polymers, this provides a prebiotic effect on the fermentation of commensal microbiota (Singh & Kim, 2021) that produce short chain fatty acids, such as butyrate, propionate and acetate, which can be utilized by the liver and muscles as energy (Craig et al., 2020) and thus benefit growth performance. If further broken down into the monomer form, xylose is less efficiently utilized by the bird, diminishing satiety, increasing feed consumption, and reducing growth performance characteristics. According to a study conducted by (Huntley & Patience, 2018), monogastric animals have a limited ability to utilize xylose. This limitation can lead to a decrease in daily weight gain, food intake, and feed efficiency when the diet is high in

D-xylose. Despite the fact that most of the xylose is absorbed from the small intestine, a significant amount is excreted through urine. Only a small fraction is oxidized to CO2, indicating that xylose contributes minimally to the energy balance through oxidative pathways. Therefore, overconsumption of xylose can negatively impact the performance of these animals. Given the similarities in digestive systems among monogastric animals, it's plausible that the findings from Huntley and Patience's study on pigs could potentially be extrapolated to turkeys. However, further research would be necessary to confirm this hypothesis.

1.4 Methods of Enzyme Production

Traditional methods for enzyme production such as solid-state fermentation (SSF) and submerged liquid fermentation (SMF) are the most common methods of production due to their established efficiency and reliability (Maqsood, 2013). For its ease of production and ability to produce enzymes on a large scale, SMF remains to be the most widely used enzyme production method (Yoo et al., 2017). SSF is characterized by the cultivation of microorganisms on a moistened solid substrate with minimal liquid present, whereas SMF involves growth in a liquid nutrient medium. Despite the reliance on these established techniques, enzyme production is under continuous development, with research into novel methods aimed at enhancing the precision and efficiency of enzyme production for use in turkey nutrition.

Solid-state fermentation (SSF) is a technique where microorganisms, typically from a fungal or yeast source, are grown on a solid material substrate in the absence or near-absence of free water (Yoo et al., 2017). This method mimics the natural habitat of many fungi and yeast, which are commonly used in the production of feed enzymes. SSF is advantageous for producing high enzyme concentrations in a smaller and relatively simpler set up (Costa et al., 2018). The

enzymes produced through SSF are more stable and concentrated, making this method particularly suitable for producing feed enzymes that require minimal post-fermentation processing, yet require the capability to withstand the feed manufacturing process (Sankar et al., 2022).

Submerged liquid fermentation (SMF) involves growing microorganisms, typically from a bacterial derived source, in a liquid nutrient medium under highly controlled conditions. This method is easier to maintain proper conditions as the water temperature stability is easier to control than that of the surrounding air and chamber of an SSF production set up. This liquid SMF method is widely used for enzyme production due to its ease of scaling up and the ability to precisely control the fermentation environment (pH, temperature, oxygen levels) (Robinson, 2015). SMF can facilitate the production of a wide range of enzymes however, it typically requires more extensive downstream processing to concentrate and purify the enzymes compared to SSF which are ready to be used once fermentation is complete (Yoo et al., 2017).

In comparative studies of enzymes derived from SSF and SMF, there have been observed distinct superiority of SSF in terms of hydrolytic performance (Karimi et al., 2021; Prévot et al., 2013) versus that of enzymes derived from SMF. This suggests an enhanced efficiency of SSF in the production of enzymes that exhibit higher activity and effectiveness in the degradation and hydrolysis of biomass (Sankar et al., 2022). The study conducted by Sankar et al. (2022) identifies specific proteins and catalytic efficiencies that are differentially expressed in SSF, thereby contributing to its increased hydrolytic capability. Notably, even when the enzyme concentration is held constant, the enzymes produced from SSF tend to outperform those derived from SMF, releasing significantly more free sugars (Alhomodi et al., 2022). This differential in performance is attributed to the variation in enzyme stability and rate of reaction between SSF

and SMF, with enzymes derived from SSF demonstrating a higher hydrolytic capacity (Saritha et al., 2016). This suggests that the method of fermentation and production significantly influences the quality and effectiveness of the enzymes produced (Sankar et al., 2022). This implies that SSF is a potentially more potent option, necessitating a lower concentration of enzymes to perform the same work as that achieved by enzymes produced by SMF (Karimi et al., 2021).

Novel techniques in enzyme production are being explored to overcome the limitations of traditional methods and to enhance the efficiency and specificity of enzyme synthesis. These include genetic engineering and recombinant DNA technology, which allow for the modification of microorganisms to overproduce desired enzymes or to express enzymes with altered properties, such as improved stability or altered pH optimum, which can be beneficial in the harsh environments of feed manufacturing (Mousavi et al., 2022). Immobilized enzyme technology is another innovative approach, where enzymes are attached to a solid support, enhancing their stability and allowing for their reuse, which can significantly reduce production costs (Cherry & Fidantsef, 2003). Additionally, advancements in bioreactor design and process optimization can potentially be used to improve the yield and efficiency of enzyme production processes (Nikita et al., 2023).

1.5 The use of Allzyme Spectrum®, a phytase and xylanase complex, in poultry diets

According to Alltech, Inc. (Nicholasville, KY), Allzyme Spectrum® enhances the efficiency of poultry feeds, leading to significant cost savings. The enzyme complex drives energy and phosphorus release, promoting optimal digestion and nutrient absorption. The use of Allzyme Spectrum® contributes to more sustainable poultry production, by incorporating it into a feed program, the amounts of certain feed ingredients formulated into poultry diets can be reduced (*Allzyme Spectrum*®, 2024). The nutrient credit values provided by the manufacturer,

are 90 calories per kilogram of feed, plus 0.15% phosphorus and 0.15% calcium. Prior research trials on broilers and layers have demonstrated the benefits of including Allzyme Spectrum® in nutrient-reduced diets. The current commercial use of the product is not publicly known, as this is a private company, but the availability of the product appears to be global.

Previous research with this product includes research performed at the University of Kentucky with broilers and also with laying hens. In a trial performed on Cobb 500 broilers, it was found that chickens fed a low nutrient diet which incorporated 200 grams/tonne of Allzyme Spectrum®, had a similar weight gain and a feed to gain ratio than that of a control diet (Ao et al., 2021). In a trial performed on Hy-Line brown laying hens, with a similarly nutrient reduced diet, it was found that the body weights, feed intake and hen day eggs were not different than that of the control diet (Ao et al., 2022). These trials suggest that both broilers and layers which are fed low nutrient diet, as described above, will not suffer performance losses, and therefore create more ROI for the producer.

1.5 Hypothesis and Objectives

The rising costs of feed production promote innovative strategies which enhance the efficiency of turkey diets (Okonkwo et al., 2022). Given the substantial portion of cost attributed to feed in commercial turkey operations, which is more than 65% of production costs (Moss et al., 2021), enzyme supplementation, particularly with complexes like Allzyme Spectrum®, presents a promising avenue to improve nutrient digestibility and overall growth performance while mitigating dietary costs. This thesis aims to investigate the impact of Allzyme Spectrum® on growth performance, nutrient digestibility, and bone quality in turkeys, hypothesizing that such supplementation can compensate for reduced nutrient diets, thus maintaining or enhancing growth and health outcomes.

Chapter 2 Objectives and Hypothesis:

The objective of this chapter is to assess the effects of Allzyme Spectrum® on the growth performance, nutrient digestibility, and bone quality of turkey poults in a battery cage. This study posits that the dual action of phytase and carbohydrase enzymes within Allzyme Spectrum® will enhance the utilization of nutrients from reduced nutrient diets, leading to improved growth metrics and bone development compared to turkeys fed conventional diets. It hypothesizes that Allzyme Spectrum® supplementation will demonstrate a marked improvement in the efficiency of nutrient use, reflecting positively on both the growth performance and skeletal health of the turkeys. It also aims at determining the proper dose amount of the product in order to deliver the best return for investment.

Chapter 3: Objectives and Hypothesis

This chapter aims to evaluate the efficacy of Allzyme Spectrum® in a commercial turkey production setting, focusing on growth performance, carcass yield, and bone quality when dietary reductions in calcium, phosphorus, and energy are offset by enzyme supplementation. The hypothesis here is that the inclusion of Allzyme Spectrum® at strategic dosages will not only counterbalance the nutrient reductions but also lead to incremental improvements in growth performance and carcass quality. This could aid in reducing costs to the producer by using lower nutrient dense diets, yet receiving the same performance as their typical diet.

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CHAPTER 2

Effects of dietary supplementation of Allzyme Spectrum® on the growth performance, nutrient digestibility, and bone quality of turkey poults.

ABSTRACT

HEINICHEN, BRENDAN O. Effects of dietary supplementation of solid-state fermentation enzymes on the growth performance, nutrient and mineral digestibility of turkeys fed pelleted corn-soybean-based feed. (Under the direction of Dr. Peter Ferket, Dr. Jesse Grimes, Dr. Ramon Malheiros and Dr. Tuoying Ao).

Dietary inclusion of phytase and carbohydrase enzymes is common practice to reduce feed cost and improve growth performance of turkeys. Allzyme® Spectrum (Alltech, Inc., Nicholasville, KY), produced through solid state fermentation, is an enzyme complex with both phytase and carbohydrase activities. Dietary supplementation of this enzyme in a corn-soybean meal diet with low nutrient density was hypothesized to increase growth performance and nutrient (P and Ca) digestibility and AMEn of turkey poults. Nicholas Select turkey poults were randomly assigned to 48 battery cages (7 poults/cage; 8 cages/treatment) and raised to 28 d. Six pelleted dietary treatments included a positive control (PC) corn-soy diet that met breeder recommendations for nutrients; a negative control (NC) corn-soy diet with reduced nutrient content relative to PC (88 kcal/kg less ME and 0.15% less Ca and 0.15% less avP, 0.03% less dLys and 0.02% less dTSAA); and NC diets supplemented with 100, 200, 300, and 400 mg enzyme/kg feed, respectively. Celite was included in the diet as internal marker for the determination of nutrient digestibility. Body weight and feed intake were determined weekly. At the end of trial, ileal digesta and excreta pooled by cage were collected for the determination of nutrient digestibility and two birds per cage were sampled for tibias to measure bone ash and breaking strength. Data were subjected to ANOVA and PROC GLM regression analysis (SAS, Inc. Cary, NC). There were no significant treatment effects ($P>.05$) on growth performance indicators (BW, FCR, and mortality rate), tibia breaking strength, and tibia ash content. However, significant difference ($P<0.05$) in nutrient retention and energy digestibility was observed between the PC and NC treatment. As dietary supplemental levels of the enzyme

product increased, AMEn increased linearly (AMEn = 0.3779x+3013.51, *P<0.0001*); ileal digestible Ca increased linearly (dCa = 0.00025557x+0.569, *P < 0.005*), and ileal digestible P increased quadratically (dP = $0.0000006263x^2$+0.0000393291x+0.4445001408, *P=.0238*). Based on the results of this study, dietary supplementation of Allzyme® Spectrum could increase AMEn and digestibility of Ca, and P in pelleted turkey diets

(Key words: turkeys, digestibility, performance, bone quality, enzyme, phytase, xylanase)

Introduction

Optimizing nutrient utilization is pivotal for the economic sustainability of poultry production. Phosphorus (P) is a critical element for poultry, essential for skeletal development and various metabolic processes (Bassi et al., 2021). However, in predominantly plant-based poultry diets, phosphorus, is bound within phytate, significantly compromising its bioavailability (Coon et al., 2007). Phytate phosphorus is not metabolically useful to poultry as they lack endogenous enzymes capable of hydrolyzing the bonds responsible for the release the inorganic phosphorus molecules (Ravindran, 1995). This limitation necessitates the use of enzyme supplements, particularly phytase, to effectively release phosphorus from phytate, enhancing its absorption and optimizing nutrient utilization. While phytase has been extensively documented for its efficacy in enhancing P availability in broiler and layer diets (Cangussu et al., 2018), the specific responses of turkeys to enzyme supplementation, remains less explored. This is particularly noteworthy given the distinct physiological and nutritional requirements of turkeys compared to other poultry species in which a physiological response to enzyme supplementation should be evaluated rather than assuming research data from one poultry species will hold consistent to use in turkey nutrition (González-Ortiz et al., 2020).

Recent advancements in enzyme technology have led to the development of multifunctional enzyme complexes, including combinations of phytase with other enzymes such as xylanase. These complexes are positioned to offer synergistic benefits (Cowieson & Bedford, 2009) in nutrient digestion and absorption, enhancing both phosphorus and energy bioavailability (Attia et al., 2020; Cherry & Fidantsef, 2003; Costa et al., 2018). Such combinations may not only improve P and energy bioavailability but also streamline feed formulation processes, reducing logistical challenges in feed manufacturing, by including multiple technologies in one

product, freeing up micro-bin space or room in the premix. Despite these prospects, the specific response of turkeys to these combined enzyme formulations, is still underexplored. This is underscored by studies indicating species-specific responses to dietary enzyme supplementation, suggesting that findings in broilers may not directly translate to turkeys (Olukosi et al., 2020) specifically regarding the response to phosphorus utilization (Rodehutscord & Dieckmann, 2005).

This study was designed to evaluate and validate a specific enzyme complex, produced via solid-state fermentation (SSF), for its impact on growth performance and nutrient digestibility in turkey diets. This complex combines phytase and carbohydrase activities, aiming to enhance the digestibility of corn-soy and wheat-based diets, common in turkey nutrition. The primary objective was to validate the specific uplift values assignable to the phytase and carbohydrase enzyme complex in turkey diets, focusing on digestibility improvements and the potential for growth performance improvements. We hypothesized that supplementation with this enzyme complex, would result in improvements in nutrient digestibility and energy utilization. Through this approach, the study will not only validate the beneficial impacts of the enzyme on nutrient availability but also support the optimization of feed formulas, contributing to improved growth performance and overall health in turkeys.

MATERIALS AND METHODS

Design of the study

Experimental procedures were in accordance with the North Carolina State University's Animal Care and Use (IACUC) guidelines. A total of 400 day-old Nicholas Select turkey female poults were provided by a commercial hatchery (Select Genetics, Goldsboro NC), individually weighed, to ensure that they were within 3-gram body weight ranges of each other. Then 48 groups of 7 poults (total 336 poults) were selected, wing tagged, and placed in the replicate cages. About 50 extra poults were maintained in separate cages and provided basal starter diet and water for ad libitum consumption so that they can be used to replace mortalities or cull birds that occurred during the first 4 days of the study. All mortality was weighted and documented on the pen sheets and used in the adjusted feed conversion calculations.

The poults were reared in a climate-controlled house using a central heat source to maintain the ambient room temperature, and then individual cage heaters to ensure even temperature maintenance throughout the replications. The poults were divided into Alternative Design (Siloam Springs, AR, USA) turkey brooder cages in a completely randomized block design, separated by six different treatment feed. In total there were 48 pens, 4 blocks total, 12 pens per block, and 7 poults per pen. In accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 4th edition (Tucker et al., 2020) the space requirements for the turkeys met 90 square inches per bird at their projected 4wk body weight. Each cage provided 672 square inches of floor space, which would allow for the housing of up to 7 birds per cage. Poults in each of the 48 groups described above were individually identified using wing bands and weighed before being placed into their respective replicate treatment cage. Feed and water were provided *ad libitum*. Lights were provided at 24 hours per day for the first

three days, then reduced to 14 hours of light per day until termination when the poults were 28 days of age. Each week the residual feed was weighed to determine the weekly feed consumption and the birds were weighed as a group, other than the first week where each bird was weighed individually to assure a normal distribution in each cage that represented the whole population of the poults received. On day 25, the fecal trays were cleaned and lined with paper to start the daily fecal collection for three consecutive days total, until experiment termination. On day 27, feed was taken away in order to fast the birds for about 8 hours, and then feed was provided on the day of ileal collection, day 28, so each replicate cage had access to feed for at least five hours of consumption to ensure sufficient time of digestion and ileal digesta content. Two of the poults were randomly selected per cage, and their left tibial bones were removed and stored for laboratory evaluation. Bones were kept wrapped separately per cage.

Treatment diets, formulas and nutrient levels

Three Basal Diets were manufactured at the North Carolina State University Feed Mill, based on the below specifications (Table 2.1): positive control corn-soy diet; negative control diet; and the summit enzyme diet (NC+400g/tonne spectrum). All three of these basal mash diets contained 1% celite as an acid insoluble ash (AIA) indigestible marker. Basal diet 2 and 3 were blended to produce 6 experimental diets as follows: 1) 100% PC; 2) 100% NC; 3) 75% NC + 25% NC-400 = NC-100; 4) 50% NC + 50% NC-400 = NC-200; 5) 25% NC + 75% NC-400 = NC-300; and 6) 100% NC-400. A double ribbon mixer was used to mix the basal diets. Due to the volume of the treatment diets, a smaller single ribbon mixer was used to blend the treatment diets for 300 seconds to ensure complete and total homogeneity. All experimental mash diets were conditioned and then pellet crumbled. The retention time for the conditioner was 30

seconds at 81 to 82 degrees Celsius. The six manufactured treatment diets consisted of a Positive Control that met or exceeded the breeder nutrient recommendations for turkeys, a Negative Control (NC) that contained 88 kcal ME/kg, 0.15% less non-phytate phosphorus, and 0.15% less Ca than the positive control, and 4 diets of increasing enzyme supplementation to the NC diet: NC +100 grams of enzyme per tonne, NC +200 grams of enzyme per tonne, NC +300 grams of enzyme per tonne, and NC +400 grams of enzyme per tonne. The reason for using a summit diet for the treatments, as opposed to mixing each treatment diet independently, was to ensure that the treatment diets had the exact same nutrient profile, only differing in the inclusion rate of the enzyme. A mash sample from each treatment diet was collected before conditioning, and subsequently, samples of each diet were collected post-pelleting for enzyme recovery analysis. The assay methods used for analysis were "WIMA142 Phytase in Animal Feed", and the Megazyme endo-xylanase assay procedure (XylX6 method) (Bailey et al., 1992).

Procedures and methods for the performance, digestibility assay, and bone quality assay

Growth performance was measured on a weekly basis by removing each poult from its battery cage, weighing it individually in grams using an A&D Company balance, models HV-15KGL and HV-10KGL (A&D Company LTD., Tokyo, JP), and recording it into excel to calculate mean body weight, standard deviation (SD) and coefficient of variance (CV) per cage. Any poult whose body weight that was outside of 2 SD of the mean on weigh day was removed and euthanized. Average weekly weight gain per bird was calculated by subtracting the previous week's mean body weight from the current week's mean body weight plus the weight of any mortality observed during that observation period. The weekly starting feeder weight (i.e. the weight of the residual feed determined on the weigh day of the last observation period) plus any

additional feed additions were summed and then the residual feed weight at the end of the observation period was subtracted in order to determine the weekly feed consumption (FC) per cage. The weekly feed consumed was then divided by the body weight gain (BWG) for each replicate cage of poults to calculate the weekly feed conversion ratio (FCR = FC/BWG).

Dietary metabolizable energy was determined as described by (Vogtmann et al., 1975) and ileal phosphorus and calcium digestibility was determined as described by Liu et al. (Liu et al., 2013). A composite sample of each dietary treatment feed was collected during the manufacturing process. Each composite feed sample was then put through a riffle separator to separate down to a 500-gram composite sample, which was then stored in a moisture impermeable freezer bag and stored at -20 C until further analysis.

On day 24 of the trial, new clean paper was added to the excreta collection trays beneath each cage. On days 25 through 27, 200 grams of excreta was collected from each of the 3 days from each cage and stored at -20 C until further processing and analysis. To process each excreta sample, they were placed on an aluminum tray and refrigerated overnight to thaw, weighed, and then dried in a forced-air oven at 65 C for 72 hours, and weighed again to determine dry matter content (Kocher et al., 2003). Equal amounts of each of these dried daily fecal samples were pooled per cage, ground to a fine and uniform powder, and stored at -20 C for further analysis.

On day 28, poults were euthanized by cervical dislocation, sequentially in time by block such that birds had at least 5 to 6 hours of access to feed so as to have sufficient amount of ileal digesta for collection. The ileal contents were collected by gently squeezing the contents into a 50ml plastic snap top container (Kheravii et al., 2017). The ileum is defined as the portion of the digestive tract from the Meckel's diverticulum to approximately 5cm proximal to the ileocecal junction. In order to have a sufficient of ileal digesta to analyze, the ileal digesta from each cage

group of 6-7 poults was pooled. Extracted digesta was immediately frozen to stop any degradation or microbial alterations of the material. The ileal digesta samples were then freeze-dried (Harvest Right, Home Pro Freeze Dryer, Salt Lake City UT) to determine dry matter content, and then the dried digesta samples were ground to a fine and uniform powder, then stored at -20 C until further analysis (Ahiwe et al., 2020). From the birds which were euthanized, two poults were randomly selected for tibial evaluation. The left tibia was removed from each of the two poults, labeled, and stored at -20 C until further analysis.

To ascertain the nutrient digestibility among the dietary treatments, celite was included at 1% of the diet as an indigestible marker, and assayed as acid-insoluble ash (AIA) in the feed, ileal digesta, and excreta on a dry matter basis as described by (Vogtmann et al., 1975). This approach enables the determination of ratio of the marker's concentration between the feed and the excreta or digesta, thereby determining the relative amount of nutrients absorbed or metabolized. In this method, two grams of each sample were placed into individual beakers and boiled with 20 mL of 4N HCl for 7 minutes. The contents were then filtered through ashless filters, and the beakers were rinsed with 20 mL of distilled water (dH2O) to ensure all residual contents were transferred onto the ashless filter papers. The filters were further rinsed with 50 mL of dH2O to eliminate any acid residue. After all of the dH2O was drained away, the ashless filter papers were placed in pre-weighed dried and labeled crucibles. These sample containing crucibles were then incinerated in a muffle furnace at 600 degrees Celsius for 10 hours, cooled in a desiccator, and then dried crucibles with the residual ash were weighed, and the AIA percentage concentration in the original samples was calculated as follows:

{AIA = (crucible weight after ashing – empty crucible weight)/ starting sample weight}

This calculation yields the percentage of celite and other acid insoluble fractions in the sample, based on the weight difference before and after ashing. The resulting digestibility factor is represented by the AIA ratio Ti/To (total ingested/total out). Digestibility quantifies the proportion of a consumed dietary nutrient that is not present after digestion and absorption. This is determined by comparing the total nutrient intake against the nutrient quantity remaining in the excreta or digesta, using the AIA as a reference. The AIA analysis is conducted on a dry matter basis to ensure consistent comparisons, irrespective of the samples' moisture content. Once the AIA data was obtained, phosphorus and calcium digestibility were evaluated by three separate methods. First, ileal digestibility was calculated as a percent digestible phosphorus and calcium (Liu et al., 2013). Second, digestible ilea phosphorus and calcium were calculated as an absolute value based on the formulated values (Hill & Anderson, 1958). Thirdly, calcium and phosphorus retention was calculated, as a percentage, based on the values found in the excreta, which accounts for cecal absorption and microbial fermentation (Plumstead et al., 2008). Nutrient digestibility was calculated as follows:

AMEn= (GEfeed-(GEexcreta X (Ti/To)))-((8.22 X (N_{diet} – ($N_{excreta}$ X (Ti/To)))

Digestible P (%) = Feed P% - (Ilea digesta P% X (Ti/To))

Digestible Ca (%) = Feed Ca% - (Ilea digesta Ca% X (Ti/To))

APU (%) = 100 − [(Ti/To) × (Po/Pi) × 100]

ACaU (%) = 100 − [(Ti/To) × (Po/Pi) × 100]

Whereas:

APU = Apparent Phosphorus utilization

AcaU = Apparent Calcium utilization

AMEn= Apparent metabolizable energy corrected for nitrogen

GE= Gross energy

Ti/To= acid indigestible ash factor

N= Nitrogen content

P= phosphorus

Ca= Calcium

Pi = the Phosphorus concentration of dietary intake

Po= the Phosphorus concentration of output (in pre-cecal digesta)

Nitrogen content in the feed and excreta was measured by a third-party lab (Carolina Analytical Services, LLC, Bear Creek, NC) using the method as described in AOAC 990.03. Gross energy of the feed and excreta was determined by bomb calorimetry (IKA LABORTECHNIK C5000, Wilmington, NC), which was calibrated using benzoic acid pellets. AMEn was then determined from the GE readings using the nitrogen results and the equations described above.

The dried and ground ilea digesta was weighed into one-gram samples using a Santorius CP 124s balance (Santorius, Wood Dale IL) to prepare for analysis of phosphorus and calcium content. The one gram of sample was placed into a flask with 10mL of 6N HCL, covered with a concave glass cover to avoid splatters, and boiled on a hot plate for 7 minutes. The slurry was then poured into an ashless filter which was placed above a volumetric flask and rinsed with 50mL of distilled water. Once drained, distilled water was then added to the contents in the Erlenmeyer flask to equal 200mL of final solution. This final solution was then mixed, poured into 50mL plastic centrifuge tubes and analyzed for phosphorus and calcium concentration

(Environmental and Agricultural Testing Service Laboratory, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC).

Bone (tibia) deformation and force to breaking was evaluated using a twin column texture analyzer (Model TA HDPlus C, Stable Micro Systems, London, GB) as described in (Toscano et al., 2013). The bones were measured with a digital caliper to evaluate for thickness (mm). Bones were then placed within the texture analyzer. The analyzers central probe was then lowered to the bone until fracture was achieved, resulting in a reading of bending moment strength (g/mm^2) The amount of force was then logged.

Tibia ash was determined as described by (Kolakshyapati et al., 2019). The tibias were wrapped in cheesecloth, then left to soak in an ether solution (90% ethyl ether, 10% methanol) for 72 hours to extract any fat in the bone samples. They were then removed from the ether solution and left to dry under a vent hood for 24 hours. Dried tibias were then removed from the cheese cloth, crushed to fit inside of the crucible, weighed, then incinerated in a muffle furnace at 600 degrees Celsius for 12 hours. The remaining ash was then weighed to determine the ash content of the bones and expressed as a percentage of the original bone.

Data Management and Statistical Analysis

A cage of 7 birds was considered an experimental unit, with 8 replicates cages per dietary treatment. All data were subjected to ANOVA to determine dietary treatment effects and PROC GLM regression analysis of dietary enzyme levels in the NC diets (SAS, Inc. Cary, NC). There were 6 treatments involved in each model (A, B, C, D, E, F), representing the positive control (PC) group, negative control (NC) group, then the enzyme treatment levels of 100g, 200g, 300g,

400g, respectively. ANOVA was used to determine statistical difference among the 6 dietary treatments. When evaluating the data, all sample data must be considered normal. With a small sample size per treatment, the need for normality evaluation becomes more critical, as tests such as ANOVA and regression analysis, assume the data are normally distributed around the mean. The data were evaluated for normality following the empirical rule and any data above or below two standard deviations of the mean was rejected. The test for significance was determined by a setting the p-value at a threshold of 0.05. For any model that returned results showing significant differences ($P<0.05$), further analysis of differences between the independent variables was determined by running a Tukey's LSD (least significant difference) post-hoc analysis. To determine the most appropriate post-hoc analysis for the study, Levene's test was first conducted to assess homogeneity of variance across the treatment groups. The test confirmed homogeneity, validating the use of analyses that assume equal variances. Consequently, Tukey's Honestly Significant Difference (HSD) test was utilized for post-hoc analysis. This approach was chosen because it allowed comparison across all treatment groups with each other. In contrast, a post-hoc test like Dunnett's would have limited our comparisons to each treatment group against only the control group (PC), which was not the primary focus of the analysis.

Block effect was also evaluated, as all treatments were equally spread among four separate blocks. Each of the 4 experimental blocks consisted of 12 experimental units (cages). The purpose of blocking was to ensure that if there was any effect from the blocks (i.e. location within the housing), then that effect would be equally applied to all treatments. A block effect was identified in three of the dependent variables, AMEn, body weight (BW) and feed conversion ratio (FCR).

RESULTS

The nutrient profiles were met according to the trial design (Table 2.1) but the analyzed protein values for the three diets (NC, PC and summit) were lower than the calculated amount. This was due to the feeds being formulated with constraints set on amino acids, not on crude protein. Phosphorus and calcium levels for all diets were analyzed at higher levels for all three diets than what was calculated for.

Enzyme Recovery Analysis

The mash samples exhibited phytase recovery levels above theoretical expectations at all enzyme inclusion rates. Conversely, the pelleted feed samples demonstrated phytase levels below the guaranteed values, indicating potential loss during heat treatment. The xylanase analysis for both mash and pelleted feeds tested significantly higher than the guarantee. Even at the lowest inclusion rate of 100g/tonne, the xylanase levels from the mash and pellet tested substantially higher (pelleted=0.1083 XylX6/g, mash=0.115 XylX6/g) than the guaranteed levels (0.004 XylX6/g) (Table 2.6).

Growth Performance Evaluation in Response Dietary Treatments

The effects of dietary treatments on growth performance metrics, including body weights (BW) and feed conversion ratios (FCR), are presented in Table 2.5. During the experimental period, no significant treatment effects were observed on BW among the different dietary groups. The average BW recorded was 0.972 kg (Table 2.2), which did not meet the Aviagen benchmark of 1.16 kg at four weeks of age (Aviagen Turkeys, 2023). The statistical analysis

reported a P-value of 0.9043 (Table 2.2) for BW differences among treatments, indicating no significant impact from the dietary conditions applied. This suggests that variations in BW among the treatment groups were likely due to random factors rather than the dietary treatments themselves.

Similarly, FCR showed no significant differences between the dietary treatments, with a recorded P-value of 0.6275 (Table 2.2). This indicates a lack of statistical significance in how the different diets affected feed efficiency among the groups. The R-square value reported for FCR was 0.0768, suggesting that the treatment variations accounted for approximately 7.68% of the variance in feed conversion efficiency. Given the high P-value and the low explanatory power of the model, it can be inferred that the dietary treatments did not significantly affect FCR during the study period.

Impact of Dietary Treatments on AMEn

The effects of dietary treatments on Apparent Metabolizable Energy corrected for nitrogen (AMEn), showed significant differences across treatments (Table 2.2). The AMEn values ranged from 3026 kcal/kg in the negative control group to 3,168 kcal/kg in the NC+0.04% group, indicating a distinct response to enzyme supplementation levels. The statistical analysis yielded a highly significant p-value (< 0.0001) and an F-value of 18.48, revealing the statistical significance of these variations in AMEn among the dietary treatments. The PC group, which is considered the baseline threshold to meet or exceed, resulted in an AMEn of 3,112kcal/kg. The treatment groups at higher levels (0.03% and 0.04%) resulted in 3129kcal/kg and 3168kcal/kg, respectively. These findings indicate that the inclusion of the enzyme at higher concentrations

(0.03% and 0.04%) can match or exceed the energy utilization efficiency of the positive control diet.

Impact of Dietary Treatment on Nutrient Digestibility

The effects of dietary treatments on nutrient digestibility are presented in Table 2.4. In the regression analysis, both calcium (Ca) and phosphorus (P) digestibility showed a positive response to increasing enzyme levels, with Ca digestibility increasing linearly and P digestibility increasing quadratically (Figures 2.2 and 2.3), with a general trend for Ca digestibility to increase linearly and P digestibility to increase quadratically as the enzyme level increases. Although the calculated slopes for Ca and P digestibility were similar across the three methods of calculation, indicating consistent uplift values, the low R-square value for Ca digestibility suggests caution in the interpretation and application of these results.

The analysis of variance revealed significant differences in all of the treatment groups (Table 2.4). While the ileal digestibility of Ca was initially significant ($P=0.032$), this significance was not maintained in post-hoc comparisons, indicating no specific treatment effect over the controls. Conversely, ileal digestibility for P was highly significant ($P<0.0001$), with post-hoc analysis identifying treatments NC+0.03% and NC+0.04% as having notably improved digestibility over the positive (PC) and negative controls (NC). Retention data mirrored these findings, with P retention being highly significant ($P<0.0001$). The Ca retention model hinted at a potential treatment effect with a borderline significance ($P=0.052$), indicating a trend could be of interest for further investigation. Absolute ileal digestible values for Ca and P both resulted high levels of significance ($P=0.0002$ and $P<0.0001$). Absolute digestible P was significantly

lower than the PC in the NC+0.01% treatment group, but then this reversed and surpassed significantly the PC group at the NC+0.04% treatment level.

Impact of Dietary Treatment on Bone Quality

In examining the effects of dietary treatments on bone quality, which is an indication of nutrient bioavailability, tibia force and tibia ash percentage were used as indicators. The results presented in Table 2.3 reveal numeric variations across treatment groups in terms of tibia strength, with the highest mean value observed in the group receiving NC+0.03% (10.765 g), suggesting a potential optimal level of enzyme dose. The observed differences in tibia strength among the treatment groups were not statistically significant (P=0.5851), implying that the variations could be due to random chance rather than a systematic effect of the treatments. Regarding tibia ash percentage, which is a measure of mineralization, the mean values ranged narrowly between 63.50% and 64.51%. The positive control yielded a tibia ash percentage of 63.86%, while the negative control was slightly lower at 63.56%. Treatment groups showed a minimal variation in tibia ash percentage, with the highest value recorded for the NC+0.03% group (64.51%) and the lowest for the NC+0.01% group (63.50%). Similar to the results for tibia strength, the differences in tibia ash percentage among treatment groups were not statistically significant (P=0.8637), indicating that the dietary enzyme supplementation did not have a distinguishable impact on the mineral deposition of the tibia within the scope of this trial.

Discussion

The primary objectives of this study were to assess the impact of dietary enzyme supplementation on growth performance and nutrient digestibility in turkeys subjected to a nutrient-deficient diet, to identify the optimal level of enzyme supplementation and to determine appropriate nutrient uplift values for turkeys under these conditions.

It is noteworthy that the mash samples revealed enzyme recovery levels surpassing theoretical expectations across all enzyme inclusion rates, indicating reasonable stability for phytase and xylanase in these samples (Table 2.6). Conversely, the findings for pelleted feed samples, where phytase levels fell below the guaranteed values, suggest a potential loss during the heat treatment process, suggesting less stability for these enzymes during pelleting. Similarly, the xylanase analysis for both mash and pelleted feeds showed levels significantly exceeding the guaranteed standards. Even at the lowest inclusion rate of 100g/tonne, xylanase levels from both the mash (0.155 XylX6/g) and pellet (0.1083XylX6/g) samples were substantially higher than the guaranteed level (0.004 XylX6/g). Furthermore, the similarity in enzyme recovery between the 300g/tonne and 400g/tonne treatments raises questions. This could suggest that either the 300g/tonne sample was inadvertently analyzed again as the 400g/tonne level, or that the laboratory assay procedure was not adequately standardized for higher dose levels. It is common in enzyme assays for activities near the top of the assay's credibility range to reach a plateau at higher dosages, indicating a limit to the detectable increase in activity. Given that the observed enzyme recovery showed decreased resilience at higher conditioning temperatures, these findings underline the importance of further research into the enzyme's thermostability.

Our findings revealed no significant effects of the treatments on the growth performance parameters, a result that is consistent with previous research conducted in battery cage settings (Feijo et al., 2023). This lack of significant impact may be attributed to the combination of optimal housing conditions and the experimental diets not being markedly deficient in nutrients, as outlined in the National Research Council's nutrient guideline (Nutrient Requirements of Poultry, 1994). Despite the variations in dietary enzyme treatments, there were no significant differences in feed conversion ratio (FCR) between the treatment groups. These findings indicate that the dietary formulations satisfied the basic nutritional requirements, and the enzyme treatments did not confer additional growth benefits compared to the control diets as also seen by Kocher et al. (Kocher et al., 2003). Kocher et al. (2003) also noted that a lack of performance response could be attributed to the short duration of a trial, rather than the lack of enzyme efficacy.

In shorter-term trials, it is proposed that the Apparent Metabolizable Energy (AME) assay offers a more effective method for evaluating enzyme performance compared to traditional growth performance response approaches (Kocher et al., 2003). In the context of this study where we set out to assign uplift values to the enzyme, the apparent metabolizable energy adjusted for nitrogen (AMEn) assay showed significant findings amongst treatment groups. Contrasting with the results on growth performance, this study identified significant variances in AMEn values among the different treatments, particularly when comparing enzyme-supplemented diets to the negative control (NC), showing a linear dose response. The enhancement in energy utilization efficiency with increased enzyme concentrations aligns with findings from prior studies, which have documented the advantages of enzyme supplementation in poultry feed (Amerah et al., 2017; Bernardes et al., 2022; Pirgozliev et al., 2007). When

evaluating AMEn against the baseline positive control (PC) group, the findings show that the lower levels of enzyme inclusion (0.01% and 0.02%), couldn't offset the energy reductions of the NC diet, necessitating a higher level of enzyme inclusion. These results imply that the inclusion of higher levels of enzymes in diets deficient in energy could equate to or surpass the energy yields of standard control diets. This has the potential to counterbalance the financial implications of utilizing higher-quality feed inputs (Shirley & Edwards, 2003).

In the context of nutrient utilization, calcium (Ca) and phosphorus (P), both showed positive response with increasing enzyme level consistent with prior studies (Bassi et al., 2021; Shirley & Edwards, 2003). The methodology used in this study incorporated calculations for ileal digestibility, digestible ileal P and Ca, and retention rates, following established protocols (Hill & Anderson, 1958; Liu et al., 2013; Plumstead et al., 2008). Our findings showed, with all three methods of calculations, Ca digestibility increasing linearly and P digestibility increasing quadratically, in response to higher enzyme levels. This trend is consistent with the hypothesis that enzyme supplementation can enhance nutrient utilization, an assertion supported by previous research (Amerah et al., 2017; Bernardes et al., 2022). Post-hoc tests from the analysis of variance identified significant differences across treatment groups in both Ca and P digestibility, with specific treatments (0.03% and 0.04%) showing marked improvement over control groups. The enzyme showed to have had the greatest influence on the response to phosphorus, in relation to the baseline of the PC diet, as also seen by other researchers (Kozlowski et al., 2010; Shirley & Edwards, 2003). This contrasts to the response of calcium, which was less consistent in meeting or exceeding the PC levels. This makes sense, since phytase works directly on releasing phosphorus by degrading phytic acid and as a secondary benefit, bound minerals such as calcium are liberated. Work done by Zhang et al. showed similar responses to calcium where the

digestibility levels remained near or below the basal diet levels (Zhang et al., 2000). Each method of calculation has its own practical application. Retention, is not something that a nutritionist would use to formulate from, it is more of a gauge of environmental impact and how much of the mineral is being excreted into the manure. The absolute digestible value is a measurement relative to the actual nutrient values formulated for this particular study, which has merit in the determination of uplifts relative to this study. The digestibility calculated as a percentage, can be applied to all formulations, as it assesses the impact of the enzyme regardless of the formulation.

Tibia bone ash and breaking strength were evaluated as a measure of relative bioavailability of Ca and P, which is a common method of determining mineral utilization (Shastak et al., 2012; Yan et al., 2005). Despite observable numerical variations across treatment groups- most notably, the NC+0.03% group exhibited the highest mean tibia force (10.765 kg) and ash (64.51%), statistical analysis did not indicate significant differences in either parameter ($P=0.5851$ for force and $P=0.8637$ for ash), consistent with observations from (Srikanthithasan et al., 2020). These findings concur with prior investigations that observed a numerical increase of bone ash in relation to increasing levels of enzyme supplementation, albeit without reaching statistical significance (Bassi et al., 2021). Bassi (2021) explained that the absence, of significant difference in bone ash could be attributable to the immature gastrointestinal tract of young turkeys. In the initial weeks post-hatch, the GIT is characterized by gradual development, impacting the efficacy of nutrient absorption in the intestine of young poults (Applegate et al., 2005; Sell et al., 1991). With both force and ash increasing numerically in relation to the NC group, and surpassing the PC group at treatment level NC+0.02% and above, it suggests that the bioavailability of Ca and P is increased in turkeys fed enzyme-supplemented diets.

Conclusion

This study contributes to the understanding of the role of enzyme supplementation in turkey nutrition. While no significant effects were observed on growth performance and mineral bioavailability measured by bone strength and ash, the significant improvements in nutrient digestibility and apparent metabolizable energy adjusted for nitrogen (AMEn) are noteworthy. The effects on digestibility and AMEn were the aim of this research in order to be able to assign appropriate nutrient uplift values, and these digestibility results validated the hypothesis of this study that the enzyme complex can compensate for a diet reduced in energy, calcium (Ca) and phosphorus (P), fed to turkeys. These findings suggest a potential avenue for improving feed efficiency and nutrient utilization in turkey diets, which is increasingly relevant in the context of sustainable animal production. Based on the results of this study, dietary supplementation of Allzyme® Spectrum could compensate for reduced levels of metabolizable energy (ME), Ca, and P in turkey diets by increasing nutrient digestibility and AMEn. In the context of this individual study, the dose to get the maximum energy, calcium and phosphorus digestibility response is apparently 300g per tonne, and the recommended nutrient values (88 kcal/kg less ME and 0.15% less Ca and 0.15% less avP, 0.03% less dLys and 0.02% less dTSAA) are valid. Future research should focus on long-term studies encompassing different rearing conditions, such as commercial settings, to validate these findings.

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TABLES AND FIGURES

Table 2.1 Basal Diet Composition

Ingredient	Positive Control	Negative Control	Summit
	---------- Percentage of Diet Composition ----------		
Corn	45.54	47.91	47.91
Soybean Meal (48% CP)	38.55	39.13	39.13
Poultry Meal	5.00	5.00	5.00
Poultry Fat	4.54	2.46	2.46
Mono-Dical Phosphate (21% P)	2.13	1.36	1.36
Limestone	1.53	1.57	1.57
Celite	1.00	1.00	1.00
L-Lysine	0.48	0.41	0.41
Sodium bicarbonate	0.24	0.25	0.25
DL-Methionine	0.23	0.20	0.20
Mineral Premix[1]	0.20	0.20	0.20
Choline Choride 60%	0.20	0.20	0.20
Vitamin Premix[2]	0.15	0.15	0.15
Salt, Plain (NaCl)	0.13	0.13	0.13
Selenium Premix	0.05	0.05	0.05
L-Threonine	0.02	0.00	0.00
Spectrum	0.00	0.00	0.04
Total	100.00	100.00	100.00
Protein, Crude (calculated)	25.78	25.59	25.59
Protein, Crude (analyze)	21.89	24.73	24.73
Av Calcium, (calculated)	1.35	1.2	1.2
Av Calcium, (analyzed)	1.56	1.37	1.37
Av Phosphorus, (calculated)	0.65	0.5	0.5
Av Phosphorus, (analyzed)	0.89	0.78	0.78
dLys, Total	1.6	1.57	1.57
dTSAA, Total	0.9	0.88	0.88
ME, kcal/kg	3,000	2,912	2,912

[1]Mineral premix containing guaranteed minimum values of 15.75% calcium, 6.0% manganese, 6.00% zinc, 4.00% iron, 2.68% magnesium, 5000 ppm copper, 1250 ppm iodine, and 500 ppm cobalt, respectively.
[2] Vitamin premix containing guaranteed minimum values of 13,227,513 IU/kg vitamin A, 3,968,254 IU/kg vitamin D3, 66,138 IU/kg vitamin E, 40 mg/kg vitamin B12, 254 mg/kg Biotin, 3,968 mg/kg menadione, 3,968 mg/kg thiamin, 13,228 mg/kg, riboflavin, 22,046 mg/kg d-pantothenic acid, 7,937 mg/kg vitamin B6, 110,229 mg/kg niacin, and 3,205 mg/kg folic acid, respectively

Table 2.2 Effects of dietary treatments on the performance of turkey poults

Treatment	BW (kg)	FCR	AMEn
Positive control	1.002	1.40	3112[ab]
Negative control	0.964	1.44	3026[a]
NC + 0.01% Alzyme® Spectrum	0.968	1.42	3035[a]
NC + 0.02% Alzyme® Spectrum	0.963	1.39	3087[ab]
NC + 0.03% Alzyme® Spectrum	0.977	1.39	3129[ab]
NC + 0.04% Alzyme® Spectrum	0.962	1.40	3168[b]
mean	0.972	1.41	3093
P-value	0.9043	0.6275	<0.0001
F Value	0.31	0.70	18.48
SEM(42)[1]	0.0112	0.0097	17.042

[1]SEM is calculated by taking RMSE and dividing it by square root of n.
a,b,c,d Means covered by the same superscript are not significantly different.
AMEn is the apparent metabolizable energy corrected for zero nitrogen retention
FCR is the feed conversion ratio

Table 2.3 Effects of dietary treatments bone strength and bone ash

Treatment	Tibia break strength (g)	Tibia ash (%)
Positive control	10.211	63.86
Negative control	9.577	63.56
NC + 0.01% Allzyme® Spectrum	10.234	63.50
NC + 0.02% Allzyme® Spectrum	10.085	64.31
NC + 0.03% Allzyme® Spectrum	10.765	64.51
NC + 0.04% Allzyme® Spectrum	10.085	63.67
P-value	0.5851	0.8637
SEM(42)[1]	0.1804	0.0028
[1]SEM is calculated by taking RMSE and dividing it by square root of n.		

Table 2.4 Effects of dietary treatments on the calcium and phosphorus digestibility of turkey poults

Treatment	Ca Ileal digestibility (%)	P Ileal digestibility (%)	Ca Excreta retention	P Excreta retention	Ileal digestible Ca	Ileal digestible P
Positive control	0.428[a]	0.5012[b]	0.532[a]	0.418[c]	0.7585[a]	0.5065[bc]
Negative control	0.367[a]	0.5102[b]	0.473[a]	0.524[a]	0.5725[b]	0.4520[d]
NC + 0.01%	0.366 [a]	0.4962[b]	0.469[a]	0.472[b]	0.5705[b]	0.4396[d]
NC + 0.02%	0.414[a]	0.5395[b]	0.509[a]	0.497[ab]	0.6457[ab]	0.4779[cd]
NC + 0.03%	0.421[a]	0.5951[a]	0.494[a]	0.520[ab]	0.6565[ab]	0.5272[ab]
NC + 0.04%	0.421[a]	0.6244[a]	0.517[a]	0.534[a]	0.6573[ab]	0.5531[a]
P-value	0.032	<0.0001	0.052	<0.0001	0.0002	<0.0001
SEM(42)[1]	0.0077	0.0082	0.007	0.0074	0.0141	0.007

[1]SEM= Standard Error of the mean for n=42
a,b,c,d means covered by the same superscript are not significantly different

Table 2.5 Growth Performance Data

Treatment	BW (kg) Wk1	BW (kg) Wk2	BW (kg) Wk3	BW (kg) Wk4	FCR Wk1	FCR Wk2	FCR Wk3	FCR Wk4	Cumm FCR
A (PC)	0.162	0.356	0.626	1.002	1.100	1.216	1.444	1.548	1.399
B (NC)	0.157	0.350	0.612	0.964	1.085	1.252	1.472	1.611	1.437
C (NC+0.01%)	0.161	0.349	0.609	0.968	1.075	1.261	1.439	1.511	1.389
D (NC+0.02%)	0.161	0.357	0.613	0.963	1.050	1.239	1.459	1.531	1.394
E (NC+0.03%)	0.159	0.349	0.611	0.968	1.062	1.298	1.497	1.515	1.388
F (NC+0.04%)	0.159	0.344	0.603	0.962	1.070	1.308	1.534	1.565	1.447
P-value	0.968	0.866	0.937	0.909	0.873	0.054	0.601	0.642	0.5705
SEM(42)[1]	0.0038	0.0081	0.0152	0.0277	0.0292	0.023	0.042	0.0435	0.0272
[1]SEM= Standard Error of the mean for n=42									

Table 2.6 Enzyme recovery data

Experiment 1			Lab A			
Enzyme Level	Phytase Guarantee (SPU/g)	Xylanase Guarantee (XylX6/g)	Pelleted Feed Recovery		Mash Feed Recovery	
			Phytase	Xylanase	Phytase	Xylanase
100g/tonne	0.1	0.004	0.07	0.1083	0.16	0.115
200g/tonne	0.2	0.008	0.16	0.2194	0.25	0.2582
300g/tonne	0.3	0.011	0.26	0.2274	0.45	0.6325
400g/tonne	0.4	0.015	0.26	0.3929	0.7	0.6484

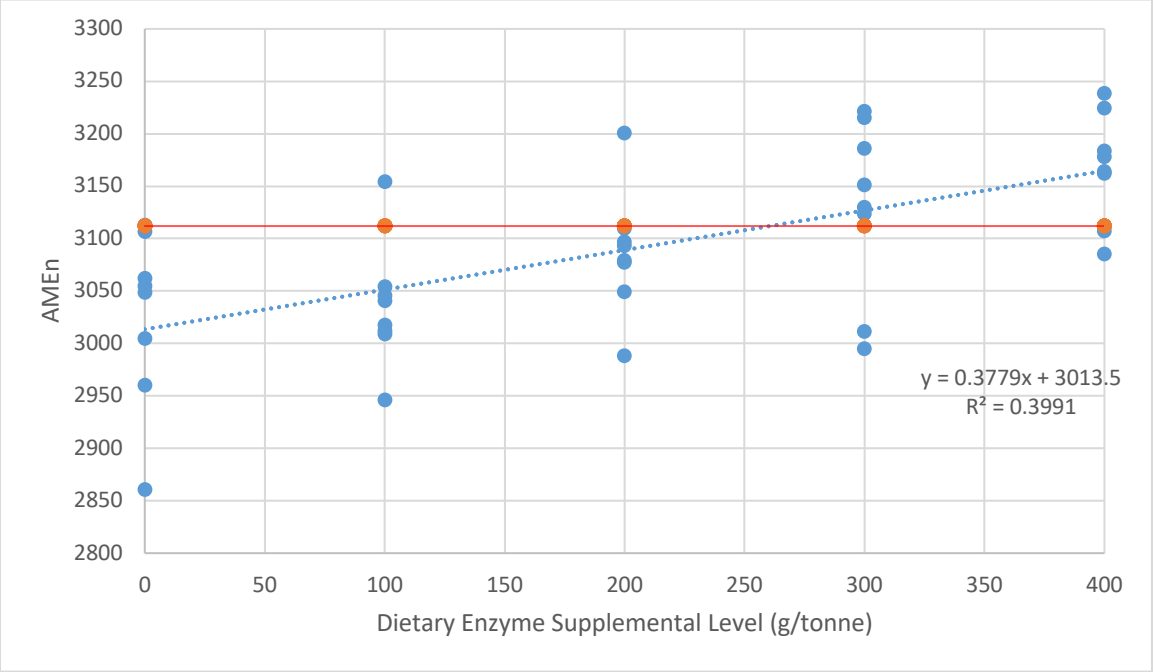

Figure 2.1 Effects of dietary treatments on apparent metabolizable energy corrected for nitrogen **(**AMEn)

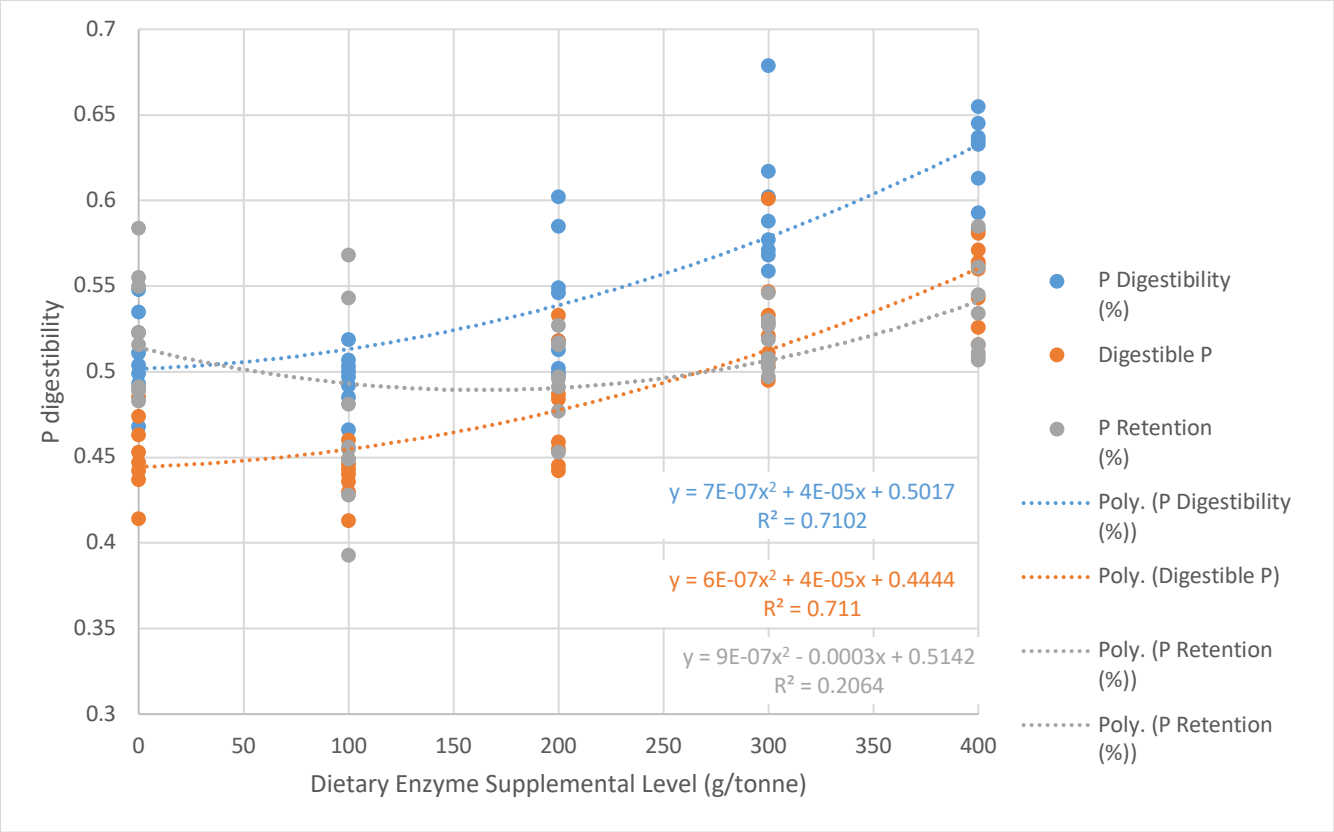

Figure 2.2 Effect of dietary treatments on P digestibility and retention

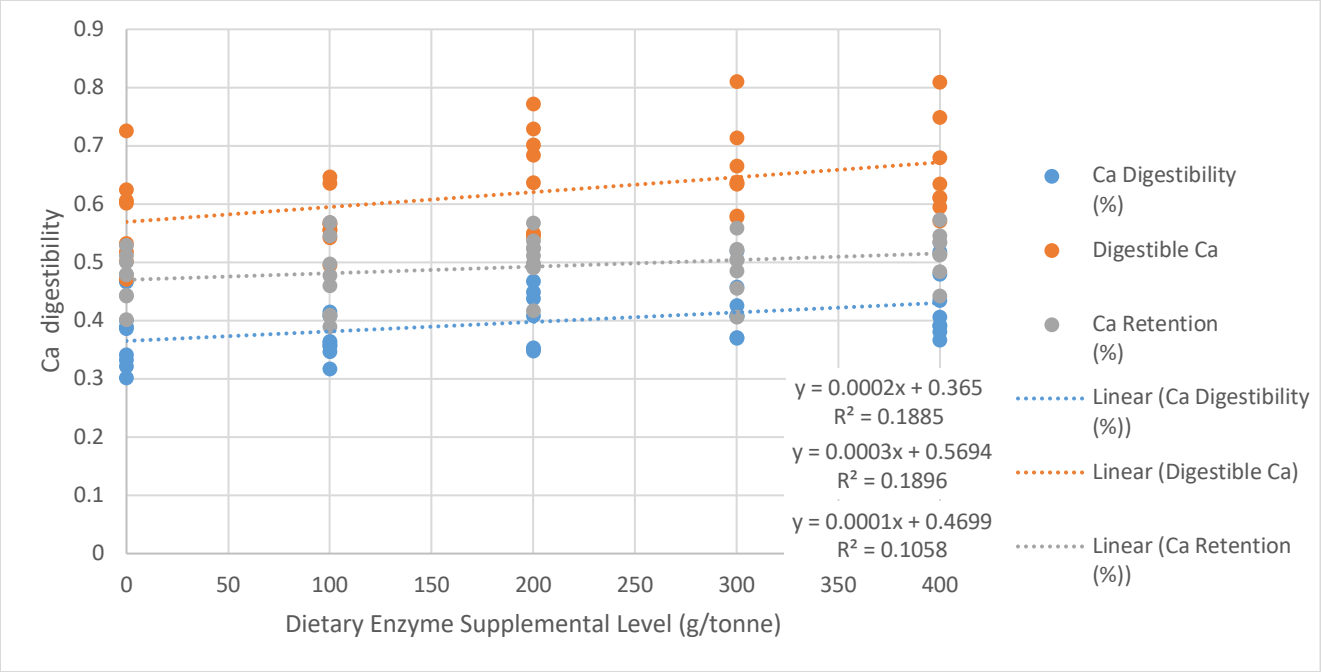

Figure 2.3 Effect of dietary treatments on Ca digestibility and retention

Chapter 3:

Effects of dietary supplementation of Allzyme Spectrum® on the growth performance, carcass yield, and bone quality of turkeys in commercial condition

ABSTRACT

The integration of solid-state fermentation enzyme complexes such as Allzyme Spectrum® (Alltech, Inc., Nicholasville, KY) into poultry diets has been shown to potentially offset reductions in nutrient density, without adversely affecting performance. This study tests the hypothesis that dietary reductions in calcium (Ca), phosphorus (P), and energy can be compensated by the addition of Allzyme Spectrum® in turkey diets under commercial management conditions. Ninety-three Nicholas Select turkey male poults were allocated to each of 32 pens in a complete randomized design, resulting in a total of 2976 poults across all pens, and eight replicates for each of 4 treatment groups: 1) a positive control (PC) diet formulated to meet current Aviagen Turkey recommendations; 2) a negative control (NC) diet with reduced levels of Ca, P, and energy (88 kcal/kg less ME and 0.15% less Ca and 0.15% less avP, 0.03% less dLys and 0.02% less dTSAA) as compared to PC; 3) NC diet supplemented with 150 g Allzyme Spectrum®/tonne; and 4) NC diet supplemented with 300 g Allzyme Spectrum®/tonne. The poults were raised to 6 weeks of age in a brooder barn under industry-standard conditions for heat, water, and feed delivery, and then transferred to a grow-out barn until marketing at 20 weeks of age. Growth performance, including body weight (determined by automatic hanging scales) and feed intake, was monitored weekly. Final performance data were calculated, including feed conversion ratio (FCR), average daily gain, and feed intake. In comparison to the PC and NC, the enzyme supplemented treatment groups had significantly ($P<0.05$) heavier body weights through to 17 weeks of age without effect on FCR, but thereafter insufficient number of automatic scale weights compromised detection of treatment effects. However, statistical analysis on eviscerated body weight and breast meat yield was conducted on a random sample of

35 birds per treatment post-processing. Statistically significant differences were observed in pre-chill carcass weights ($p<0.0001$) and yield percentages ($p=0.0018$) between the PC and enzyme-supplemented groups. Incremental increases in enzyme supplementation resulted in linear improvements in body weight ($y=39.71+.0056x$, $P<0.005$) and carcass breast meat yield percentage ($y=0.34 + 0.000015x$, $P<0.005$)). Final on-farm body weights determined by truck scale weight were 21.49 kgs/tom for PC, 21.78 kgs for NC, 22.28 kgs for the 150g/tonne enzyme treatment, and 21.82 kgs for the 300g/tonne enzyme treatment. Dietary inclusion of Allzyme Spectrum® can enhance growth performance, more than compensating for reduced nutrient density in turkey diets.

Key words: turkey, solid-state enzyme complex, growth performance, carcass yield

Introduction

In the realm of commercial poultry nutrition, the optimization of nutrient utilization, particularly phosphorus (P) and Calcium (Ca), is of importance due to the significant cost contribution in the diet formulation, and role in skeletal development and overall growth performance. The utilization of these nutrients is often hindered by their natural form in plant-based ingredients like corn and soybean meal, where P is predominantly found as phytate, a compound that monogastric animals poorly digest due to the lack of intrinsic phytase. Consequently, this unavailability can lead to the excessive inclusion of inorganic P sources in diets, escalating feed costs and environmental P load through manure (Lu et al., 2017). The reduction of energy inputs is also of large importance from a financial perspective, especially in growing heavy turkey toms, due to the high energy density of their diets (Hurwitz et al., 1980). Fat being a major source of energy, can have major impacts not only on growth performance, but to the cost of the feeding program (Kagan et al., 1982), so increases, or in this case reductions, need to be considered carefully.

To address this challenge, exogenous phytase and carbohydrase enzyme products have been introduced into poultry diets, demonstrating efficacy in enhancing the digestibility of phytate-bound P, Ca, and other nutrients, thus reducing the need for inorganic mineral supplementation and decreasing the amount of energy required in formulation (Amerah et al., 2017; Gorenz et al., 2022). Despite the wealth of research in broiler chickens, there is a lack of data on the impact of such enzymes on turkey nutrition, particularly concerning growth performance when dietary energy is also reduced alongside Ca and P.

The present study is designed to fill this knowledge gap by investigating the dietary

inclusion of Allzyme Spectrum®, an enzyme complex with both phytase and carbohydrase activities, in a pelleted corn-soybean meal diet for Nicholas Select heavy turkey toms. With the goal of reducing feed cost without compromising growth, diets were formulated with reductions in energy, Ca, and P, theorizing that the enzyme's action could liberate bound nutrients, thus maintaining or enhancing performance and nutrient digestibility (Bassi et al., 2021; Fritts & Waldroup, 2006).

Furthermore, this study was designed to be conducted within a commercial research farm environment, a setting that mirrors the decision-making context of a typical live production manager. The significance of this setting lies in its relevance to the decision-making process for feed additives and other program changes. This process is typically multi-pronged, with the initial phase involving an examination of research data on the product or practice in question, a review of previous trial flock performance data, and an evaluation of the compatibility of the product or practice with the farm's capabilities and practices. The objective was to ascertain whether the product or practice would be beneficial to the company. Subsequent to this evaluation, the new product usually undergoes a field trial within the company's facilities. The present research is intended to obviate this costly and time-consuming step. By conducting the study in a commercial setting, with all commercial practices, densities, and programs implemented, we aim to expedite and streamline the production management team's decision-making process regarding product implementation.

A four-treatment experimental design at a commercial facility was employed, using a complete randomized design setup to compare a current nutrition program, meeting genetic company standards (PC) (Table 3.1), negative control with reduced nutrient content (NC) (Table 3.2), and negative control diets supplemented with two levels of Allzyme Spectrum® (150g/tonne

and 300g/tonne). This approach aimed to evaluate the effects of Allzyme Spectrum® on heavy turkey tom growth performance and the potential to offset nutrient reductions in a practical, commercial setting.

Materials and Methods

All animal care and humane treatment standards were designed to exceed commercial industry standards, in compliance with the ethical guidelines for animal treatment in research. The study utilized Nicholas Select turkey poults, with a total of 2976 birds allocated into 32 pens at a commercial facility, ensuring eight replicates per treatment. The pens were configured to provide an initial density of 0.12 square meters per poult, aligning with industry standards for turkey rearing. The birds were accommodated in a facility outfitted with thermostat controlled forced air heating, ad libitum fresh drinking water, mechanical feeding equipment, and side wall fan ventilation.

Pelleted diets were produced at a commercial feed mill, using a corn and soybean meal-based diet. Each batch underwent a mixing process for 120 seconds using a double ribbon mixer, succeeded by conditioning at temperatures between 57 to 63 degrees Celsius to prepare for pelleting. The Allzyme Spectrum® enzyme was weighed using a digital scale and incorporated into the mixer prior to pelleting. Pellets were fabricated to a 4.4 mm diameter, ensuring homogeneity across all treatment groups. Starter diets were crumbled to provide easier consumption by the poults.

Enzyme activity for each diet was assessed using specific assay methods. The assays were “WIMA142 Phytase in Animal Feed” and the Megazyme endo-xyalanase assay procedure (XylX6 method) (Bailey et al., 1992). To ensure the reliability and reproducibility of our

findings, two separate laboratories, designated as Laboratory (Lab) A and Laboratory (Lab) B, were utilized to assess the enzyme recovery in the feed. This dual-laboratory approach allowed for cross-verification of the assay results, thereby strengthening the validity of our analysis.

The diets (Tables 3.1 and 3.2) were formulated as a positive control (PC), a diet adhering to current genetic company nutrient standards without enzyme supplementation, a negative control (NC), with diminished levels of Ca, P, and energy, without enzyme supplementation, the negative control diet supplemented with 150 grams/tonne of Allzyme Spectrum® (NC+150g), and finally the negative control diet supplemented with 300 g/tonne of Allzyme Spectrum® (NC+300g). The negative control diet was formulated with a reduced nutrient content relative to the PC diet, specifically 88 kcal/kg less metabolizable energy, 0.15% less Ca, 0.15% less available P, 0.03% less digestible lysine, and 0.02% less digestible total sulfur amino acids. Each treatment group had seven formulas through the duration of the trial, including starter, grower 1, grower 2, finisher 1, finisher 2, finisher 3, and finisher 3 WD (Tables 3.1-3.2).

Feed and water were supplied to the pens via an automated system, and consumption was recorded to accurately calculate feed intake. Water was provided *ad libitum* through nipple and cup drinkers in each pen. Feeders were weighed back on each weigh day to ensure accurate computation of feed conversion ratios.

The turkeys were weighed on a weekly basis to monitor growth performance, with all mortalities documented, weighed, and assigned a cause of death. During the brooding stage from placement to week six, all birds were weighed by hand. At six weeks of age, the poults were relocated to a finisher building, with a finishing pen size of 22’x15’, which provided a density of approximately 4 square feet per bird. All treatment groups remained the same as in the brood facility, with the same pen identifying numbers. At this point forward, all body weights were

recorded using a Rotem RSC-2SE automated platform scale (Munters, Amesburry, MA. USA). Performance data for feed conversion ratio (FCR), average daily gain, and live weight were recorded until the conclusion of the study.

Upon flock termination, despite the loss of individual pen data due to logistical limitations, the treatment groups remained distinct for processing. At the processing plant, a sample of 35 birds per treatment was chosen by selecting every tenth bird from the processing line for further analysis to ascertain breast meat yield. Each carcass was individually weighed and tagged for identification and preserved for statistical analysis. The breast lobe and tender were then removed by hand and weighed together to get individual white meat weight yield per bird.

Statistical Analysis Data were subjected to ANOVA and PROC GLM regression analysis (SAS, Inc., Cary, NC). The statistical model incorporated the effects of dietary treatments on growth performance indicators measured from the processing plant facility, including pre-chill carcass weight, and breast meat yield. Breast meat yield was transformed to arcsines to normalize the data distribution (Attia et al., 2020). Differences were deemed significant at $P<0.05$. FCR was calculated using the on-farm feed scale consumption records, and live weight was taken as an average bird weight from the live haul trailer scales.

Additionally, a mixed-effects linear regression model was utilized to examine further the week-to-week effect of enzyme dose treatments on weekly body weights, FCR and weekly feed consumption across the 20 weeks grow period, utilizing the R software environment (R Core Team, 2021) with the lme4 package. Heteroskedasticity was observed in the weekly body weights, so a natural logarithm transformation was applied to normalize the variance. This analysis allowed for the assessment of both fixed effects (treatment, week, and their interaction)

and random effects (individual pens) to account for the repeated measures within pens across the study period. Significant contrasts between treatment groups were identified using the post-hoc Tukey's HSD test to adjust for multiple comparisons.

RESULTS AND DISCUSSION

The primary focus of the study was to assess the effect of Allzyme Spectrum® supplementation on the growth performance of heavy turkey toms fed a nutrient-reduced diet in a commercial setting. The nutrient uplifts were derived from the findings of chapter 2 in this thesis. As such, the data presented is from reports which are representative of a closed flock report used by a commercial integrator in the industry (Table 3.6). The data suggested that the NC+150g enzyme treatment group exhibited enhanced overall growth performance as compared to the positive control (PC) group, despite the diet's reduced levels of Ca, P, and energy. Specifically, the NC+150g group demonstrated a 1.67% increase in white meat yield relative to the PC group. This increase could potentially translate into a significant financial advantage considering the current market value of breast meat to producers, which is approximately $2.60/lb at the time of this study (*Weekly National Turkey Report*, 2024).

The feeds manufactured during this experiment were conditioned at 60 degrees Celsius, so it was important to evaluate the response and recovery of the enzyme. Two laboratories, Lab A and B, analyzed the feeds, revealing significant variation in enzyme activity results (Table 3.4). Notably, the enzyme activity levels determined by Lab B were five times higher in phytase activity and seven to ten times higher in xylanase activity compared to Lab A. This is considerable disparity highlights that despite following identical assay procedures and methods,

enzyme activity measurements can vary significantly between laboratories, corroborating the findings of previous research (Bailey et al., 1992). To delve deeper into the methodological aspects, it's pertinent to acknowledge the assay methods, while robust, may be subject to variability due to factors such as reagent sensitivity and dilution coeficients, instrument calibration, operator technique or perhaps even the possible variation of particular sample of feed which was collected and sent to each laboratory. This variability emphasizes the necessity for stringent standardization and calibration across laboratories to ensure consistency in enzyme activity measurements. The stability and recovery of phytase and xylanase under these conditions open discussions on the biochemical resilience of these enzymes to processing conditions. The heat sensitivity of enzymes, coupled with the processing environment, can significantly influence enzyme activity (Pope et al., 2020). These findings prompt a closer examination of the enzyme properties and the investigation of the range of temperatures which the enzyme will remain viable.

The weights of the birds were calculated based on two methods and therefore are reported herein as such. The on-farm data is reported based on the weights recorded by the automated platform scales as described in the materials and methods section, whereas the flock close-out report uses the bird weights recorded from the actual truck weights at the processing facility. The liveweight, according to the truck scale weights before processing, of the NC+150g group was right on target according to the genetic company recommendations, whereas all of the other groups were behind by at least 0.5 kg, representative in the flock close-out data report (Table 3.6). The FCR of all groups, calculated from the liveweight of the birds according to the truck weights at the processing facility, (PC: 2.17, NC 2.19, NC+150g: 2.19, NC+300g 2.27) was significantly better (Table 3.6) than the genetic company’s target of 2.43 (Aviagen Turkeys,

2023). Accounting for body weight and livability, the NC+150g treatment group yielded nearly 8% more pounds of meat to the processing plant than did the control group (Table 3.6).

The General Linear Model (GLM) procedure was used to detect significant differences across all four groups: Positive Control (PC), Negative Control (NC), and the two enzyme-supplemented groups (NC+150g and NC+300g). The objective was to evaluate the impact of enzyme supplementation on pre-chiller carcass weights and processing plant breast meat yield percentages in comparison to both the PC and NC diets. In addition to the GLM procedure, we leveraged R's mixed-effects linear regression analysis to delve deeper into the enzyme supplementation's week-by-week impact on body weights, FCR, and feed consumption. This assessment provided an overview of the treatment effects. An R-square value of 15.96% for pre-chiller carcass weights and 10.61% for breast meat yield (Tables 3.4 and 3.5) indicates that the model accounts for a modest proportion of the variance in these outcomes. The F-values were 8.42 for carcass weights and 5.26 for breast meat yield, with corresponding p-values of <0.0001 and 0.0018, respectively (Table 3.3). These statistics confirm the model's significance at the 0.05 level for both measured parameters. To further delineate the influence of each treatment, a post hoc Tukey's HSD test was applied. The results from the Tukey's HSD test (Tables 3.6 and 3.7) revealed that there was significant improvement in both carcass weights and breast meat yield relative to the PC group for the NC+150g and NC+300g enzyme supplementation group, at the predetermined significance level of 0.05. These findings suggest the potential of the enzyme supplementation to positively effect turkey carcass weight and breast meat yield.

To analyze the on-farm performance data, including FCR, body weight, and weekly feed consumption over the 20-week growth period (Tables 3.8-3.10), PROC MIXED was utilized. This method was chosen due to its ability to handle the correlated structure of the data arising

from multiple observations over time for each turkey, accommodating varying growth rates and feed efficiency across different treatment groups and time points. This data was calculated based on the recordings from the automated hanging platform scales and the automatic feed delivery system.

For body weight growth analysis (BW), conversion of weight data to a logarithmic scale significantly enhanced model accuracy and variance homogeneity, effectively addressing initial heteroscedasticity. Analysis revealed that treatment, time, and their interaction significantly influenced growth rates ($p < .0001$), illustrating substantial variations in growth trajectories dependent on treatment type and duration (Table 3.8). Comparisons between treatment groups demonstrated statistically significant differences in growth outcomes. Particularly between the NC+150g group and control groups (NC and PC), as shown by the larger least square means estimates, indicating higher overall weight gain by the NC+150g group. In the further analysis for difference week-by-week (Table 3.8) it was seen from weeks 2 to 7, that treatments with NC+150g and NC+300g doses significantly enhanced weight gain compared to both the negative control (NC) and the positive control (PC), showing the treatments' effectiveness. In the initial weeks (2 and 3), the NC+150g treatment outperformed the NC+300g, NC, and PC groups, indicating its efficacy in promoting early weight gain. Throughout weeks 4 to 14, both the NC+150g and NC+300g treatments continued to show significant benefits over NC and PC. This pattern revealed the consistent effectiveness of enzyme dose treatments in supporting weight gain. By week 15 the significant difference effect by week between treatment groups disappeared.

The differences in the body weights observed from the on-farm data between the treatment groups are reported in Table 3.8. As previously noted, these weights were recorded

from the automated hanging platform scales. It was observed that the number of weights recorded significantly declined from the time of placement to the termination of the research (Figure 3.2). In the beginning of the grow out period, over 1600 weights were recorded per day, per pen. From a pen of 93 birds, it is reasonable to assume that this number of weights recorded, reflects a very accurate and representative evaluation of the average bird weight. As the birds increased in age and weight, there was a sharp decline in the number of weights recorded per day, per pen. By the end of the study, around 200 or less weights were recorded per day, per pen. Between weeks 19 and 20, there was an unexpected change in average bird weights (Table 3.8), in which the NC group suddenly appeared to weigh more than all of the other treatment groups. This was unexpected because the NC+150g and the NC+300g treatment groups were heavier than the NC group for the entire rearing period aside from the final 20th week weight recording. Upon evaluation of the number of weights recorded, it cannot be assumed that this is representative of an accurate estimation of the average bird body weight. This agrees with research which shows that as birds get heavier, they don't utilize the scales as often (Chedad et al., 2003). Upon weighing every bird at processing, the weights re-normalized along the trend that was observed throughout the flock. The use of these automated platform scales is a practical tool for a commercial producer to utilize, but the accuracy of the readings should be taken with caution.

FCR data, indicated significant variations in feed efficiency across treatments and over time ($p < .0001$), with distinct differences observed in the efficiency of feed conversion among treatment groups (Table 3.8). The week-by-week statistical analysis revealed where those differences occurred. The results were adjusted using the Tukey method for multiple comparisons (Table 3.8). Week 6 and Week 18 stand out as showing statistically significant

differences between some treatments. At Week 6, the contrast between NC+150g and NC+300g treatments (p-value = 0.0174) and between NC and NC+300g treatments (p-value = 0.0081) are significant, suggesting notable differences in FCR between these treatments during this week. At Week 18, several contrasts show significant differences, especially between NC+300g and PC treatments (p-value = 0.0001), indicating a strong effect of these treatments on FCR at this time point. Most other weeks and comparisons do not reach statistical significance (p-value < 0.05), suggesting that the differences in FCR between the treatments during those weeks might not be statistically significant. Ultimately, the NC+300g treatment group showed less efficiency compared to NC and PC groups (p<.0001).

Analysis of the weekly feed consumption data, revealed significant treatment and time effects on feed intake levels. Notable differences were observed between the NC+150g/NC+300g groups and NC/PC groups, with the former consuming more feed (Table 3.7). The data suggest that while turkeys in treatments NC+150g and NC+300g consumed more feed, this did not necessarily translate to proportionately higher weight gain compared to NC and PC, considering the FCR findings.

The analysis of weekly feed consumption by week (Table 3.7) revealed at what age the differences in feed consumption across the treatments occurred. In week 6, treatment NC+300g significantly consumed more than the NC group. In week 11, increased feed consumption was observed with both NC+150g and NC+300g treatments compared to NC and PC. Weeks 12 through 19 consistently showed that treatments NC+150g and NC+300g had significantly higher feed consumption compared to PC. The efficiency of feed conversion (lower FCR in NC and PC) suggests that these treatments achieved similar or better growth outcomes with less feed, ultimately making the decision for the producer come down to the value of meat versus the cost

of feed.

One of the most surprising findings of this study was that the NC+300g inclusion level group did not perform as well as the NC+150g group. This could suggest an optimal enzyme inclusion level, and for anything beyond that, performance may decline. This finding aligns with studies showing that increased enzyme levels do not necessarily improve feed efficiency (Rao et al., 2021) but contrasts with others demonstrating a linear response to varying levels of xylanase or enzyme combinations (Amerah et al., 2017; Boontiam et al., 2022; Gorenz et al., 2022). The natural assumption would be that more is better, but the findings of this study prove that assumption to be incorrect.

The observed variations in performance across the NC, NC+150g, and NC+300g groups suggest a complex interaction between enzyme levels, gut microbiota, energy utilization, and nutrient absorption. Xylanase, a key component of the enzyme complex used, is known to affect the gut microbiome's composition. Studies such as those by Craig et al. and Wu et al. (Craig et al., 2020; Wu et al., 2017), have documented this impact, although the specific effects of high levels of enzyme supplementation on microbial symbiosis and gut health in turkeys require further exploration. Research in chickens, for instance, has indicated that xylanase can modulate the bird’s microbial ecology beneficially (Van Hoeck et al., 2021). In contrast, studies in swine have observed that xylanase supplementation can decrease gut bacterial population variety without necessarily affecting growth performance (Dong et al., 2018; Luise et al., 2020).

This decrease in bacterial diversity could be attributed to the enzyme's action on more fibrous feedstuffs, leading to their increased solubilization and digestion. This process leaves less fiber available for microbial fermentation in the gut, which typically acts as a prebiotic for beneficial gut bacteria. Dietary fiber is known to play a role in gastrointestinal health and

function in poultry, including nutrient digestion, absorption, and gut microbiota diversity (Singh & Kim, 2021). Thus, the excessive degradation of insoluble fibers might not always be advantageous, as insoluble fibers maintain gut health by acting as prebiotics and supporting proper gut mucosa, motility, and function (Jha & Mishra, 2021). While xylanase supplementation is generally beneficial for improving the digestibility of NSPs in poultry diets, excessive amounts could lead to over-solubilization of insoluble fibers, potentially resulting in poorer nutrient and energy utilization, as seen in this present study and as also noted in Jorgensen et al. (JøRgensen et al., 1996).

The observed performance decline with increasing enzyme inclusion levels might be explained by the diet's primary composition of corn and soybean meal, which are inherently lower in fiber than other cereal grains like wheat. This composition suggests that the impact of xylanase might be more significant in such diets, leading to a substantial reduction in fiber content available for maintaining gut health. It suggests the importance of considering the source, type, and quantity of dietary fiber in poultry diets in order to ensure the proper class of enzyme supplementation that would be most beneficial.

The study encountered a few logistical challenges, typical in commercial settings, which could have influenced the outcomes. For instance, a feed tank malfunction for the NC+300g group and formulation errors in the early diet stages demonstrate the potential discrepancies between formulated and delivered diets. At week two the boot of the feed bill broke for the bin holding the NC+300g level feed. This created a scenario where new feed had to be ordered and delivered. It took approximately one week for the new feed to arrive and the bin to be repaired, so the birds may have consumed feed that was exposed to outside elements, although care was taken to ensure that no contamination occurred. The treatment diets for starter and grower 1 were

formulated incorrectly to include 150 grams per short ton and 300 grams per short ton of Allzyme Spectrum® instead of the proper amounts of 136 grams per short ton and 272 grams per short ton, and were corrected for the remaining diets of the treatment. Although this small discrepancy is below the typical accuracy ability of a micro bin scale and represents approximately 13% of their lifetime feed consumption, these incidents emphasize the importance of stringent quality control measures in feed production and delivery processes.

CONCLUSIONS

The study demonstrates the potential of enhancing growth performance and nutrient utilization in heavy turkey toms with the use of this enzyme complex, particularly at the 150g treatment level. The increase in white meat yield and livability, combined with reduced feed costs, presents a strong case for incorporating this enzyme into turkey diets. The reduction in feed cost, ranging from $17.69 to $39.68 per treated tonne, due to nutrient reductions inclusive of enzyme costs, proves the economic viability of this particular enzyme supplemented into turkey diets. These feed costs were relative to Iowa commodity prices recorded by Feedstuffs on September 2023 (*Commodity Prices*, 2023). Moreover, the decreased reliance on inorganic mineral sources aligns with environmental sustainability goals by potentially reducing the environmental phosphorus (P) load, a concern highlighted by (Lu et al., 2017). Future research should aim to explore the impact of varying enzyme levels on gut microbiota and overall health. Additionally, understanding the implications of formulation and manufacturing discrepancies in commercial feed production will enhance the reliability of feed trials.

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TABLES AND FIGURES

Table 3.1 Positive Control Diets

Ingredients		Starter	Grower 1	Grower 2	Finisher 1	Finisher 2	Finisher 3	Finisher 3 WD
		---------- Percent of Dietary Composition ----------						
Corn		38.34	51.47	56.94	60.54	66.10	71.39	74.06
Soybean Meal (46% CP)		32.39	30.09	24.16	19.84	14.63	9.77	7.89
Wheat Midds		10.00	0.00	0.00	0.00	0.00	0.00	0.00
Meat and Bone Meal		10.00	10.00	10.00	10.00	10.00	9.77	9.03
Soybean Oil		5.31	4.84	5.63	7.02	7.05	7.25	7.18
Calcium Carbonate		0.68	0.38	0.11	0.17	0.11	0.01	0.06
L-Lysine		0.64	0.56	0.66	0.58	0.53	0.48	0.51
Mono-Cal Phosphate (21% P)		12.38	0.62	0.83	0.58	0.23	0.17	0.00
DL-Methionine		0.41	0.38	0.36	0.33	0.29	0.26	0.26
Salt		0.28	0.28	0.31	0.31	0.31	0.31	0.32
Mineral Premix[1]		0.25	0.20	0.20	0.20	0.20	0.20	0.20
Choline Chloride 60%		0.17	0.15	0.15	0.14	0.14	0.11	0.09
L-Threonine		0.14	0.15	0.13	0.10	0.03	0.03	0.05
L-Arginine		0.11	0.07	0.14	0.03	0.05	0.07	0.09
Vitamin Premix[2]		0.11	0.10	0.10	0.09	0.08	0.08	0.07
Selenium Premix		0.02	0.02	0.02	0.02	0.02	0.02	0.02
Tryptophan		0.01	0.00	0.03	0.01	0.02	0.02	0.02
Allzyme Spectrum®		0.00	0.00	0.00	0.00	0.00	0.00	0.00
Calculated Analysis								
Nutrient Name	Units							
Crude Protein	Pct	26.25	24.25	22.15	20.07	18.00	16.00	15.00
Met. Energy Poultry	kcal/kg	3053	3197	3306	3428	3483	3549	3571
Crude Fat	Pct	8.28	7.84	8.70	10.12	10.25	10.51	10.42
Crude Fiber	Pct	2.77	1.98	1.84	1.73	1.62	1.51	1.48
Calcium	Pct	1.35	1.21	1.05	1.00	0.95	0.85	0.80
Phosphorus - Avail	Pct	0.66	0.66	0.60	0.52	0.50	0.45	0.42
Phosphorus - Total	Pct	0.95	0.92	0.85	0.76	0.73	0.67	0.64
TSAA	Pct	1.13	1.14	1.06	0.99	0.89	0.82	0.79
AVAILABLE LYSINE	Pct	1.55	1.42	1.35	0.19	0.19	0.19	0.19
AVAILABLE TSAA	Pct	1.01	0.95	0.88	1.20	1.05	0.91	0.87
AVAILABLE THREONINE	Pct	0.89	0.85	0.76	0.82	0.73	0.67	0.65
AVAILABLE TRYPTOPHAN	Pct	0.25	0.22	0.22	0.67	0.54	0.48	0.47
AVAILABLE ARGININE	Pct	1.68	1.50	1.41	0.18	0.16	0.13	0.12
AVAILABLE METHIONINE	Pct	0.73	0.69	0.64	1.18	1.06	0.93	0.88
Sodium	Pct	0.18	0.18	0.19	0.59	0.53	0.48	0.47

[1]Mineral premix containing guaranteed minimum values of 15.75% calcium, 6.0% manganese, 6.00% zinc, 4.00% iron, 2.68% magnesium, 5000 ppm copper, 1250 ppm iodine, and 500 ppm cobalt, respectively.
[2] Vitamin premix containing guaranteed minimum values of 12,494 IU/kg vitamin A, 5,247 IU/kg vitamin D3, 100,352 IU/kg vitamin E-50%, 31.06 mg/kg vitamin B12, 251 mg/kg Biotin, 4,498 mg/kg vitamin K, 4,000 mg/kg Thiamine (B1), 13,995 mg/kg Riboflavin (B2), 5,998 mg/kg Pyridoxine (B6), 12,700 mg/kg Pantothenic Acid (B5), 84,960 mg/kg Niacin (B3), 4,000 mg/kg Folic Acid (B9), respectively

Table 3.2 Negative Control Diets

Ingredients		Starter	Grower 1	Grower 2	Finisher 1	Finisher 2	Finisher 3	Finisher 3 WD
		---------- Percent of Dietary Composition ----------						
Corn		42.19	55.93	60.53	61.88	67.03	71.24	73.93
Soybean Meal (46% CP)		32.05	28.90	24.17	22.91	18.27	14.86	12.98
Wheat Midds		10.00	0.00	0.00	0.00	0.00	0.00	0.00
Meat and Bone Meal		9.43	10.00	9.20	7.15	6.77	5.56	4.83
Soybean Oil		3.03	2.38	3.42	5.48	5.63	6.14	6.08
Calcium Carbonate		0.67	0.29	0.13	0.46	0.45	0.48	0.53
L-Lysine		0.61	0.55	0.63	0.52	0.46	0.39	0.42
Mono-Cal Phosphate (21% P)		0	0.00	0.11	0.00	0.00	0.00	0.00
DL-Methionine		0.39	0.36	0.34	0.31	0.26	0.23	0.23
Salt		0.29	0.28	0.32	0.35	0.35	0.37	0.38
Mineral Premix		0.25	0.20	0.20	0.20	0.20	0.20	0.20
Choline Chloride 60%		0.17	0.15	0.15	0.13	0.13	0.10	0.08
L-Threonine		0.15	0.15	0.14	0.09	0.02	0.02	0.03
L-Arginine		0.13	0.09	0.16	0.03	0.05	0.06	0.09
Vitamin Premix		0.11	0.10	0.10	0.09	0.08	0.08	0.07
Selenium Premix		0.02	0.02	0.02	0.02	0.02	0.02	0.02
Tryptophan		0.01	0.01	0.03	0.00	0.00	0.00	0.00
Allzyme Spectrum®		0.00	0.00	0.00	0.00	0.00	0.00	0.00
Calculated Analysis								
Nutrient Name	Units							
Crude Protein	Pct	26.05	24.05	21.95	19.873	17.8	15.8	14.8
Met. Energy Poultry	kcal/kg	2965	3108	3219	3340	3395	3461	3483
Crude Fat	Pct	6.092	5.542	6.555	8.351	8.549	8.994	8.9
Crude Fiber	Pct	2.805	1.998	1.883	1.841	1.741	1.668	1.63
Calcium	Pct	1.203	1.06	0.9	0.85	0.8	0.7	0.65
Phorphorus - Availab	Pct	0.51	0.51	0.45	0.37	0.35	0.3	0.27
Phorphorus - Total	Pct	0.802	0.774	0.702	0.611	0.582	0.522	0.49
TSAA	Pct	1.11	1.117	1.035	0.966	0.87	0.8	0.77
AVAILABLE LYSINE	Pct	1.519	1.394	1.319	0.19	0.19	0.19	0.19
AVAILABLE TSAA	Pct	0.989	0.931	0.86	1.17	1.024	0.877	0.84
AVAILABLE THREONINE	Pct	0.891	0.849	0.763	0.799	0.712	0.651	0.63
AVAILABLE TRYPTOPHAN	Pct	0.249	0.221	0.22	0.674	0.54	0.481	0.47
AVAILABLE ARGININE	Pct	1.684	1.504	1.409	0.175	0.155	0.134	0.12
AVAILABLE METHIONINE	Pct	0.703	0.666	0.616	1.18	1.057	0.933	0.88
Sodium	Pct	0.18	0.18	0.19	0.567	0.498	0.454	0.44

[1]Mineral premix containing guaranteed minimum values of 15.75% calcium, 6.0% manganese, 6.00% zinc, 4.00% iron, 2.68% magnesium, 5000 ppm copper, 1250 ppm iodine, and 500 ppm cobalt, respectively.

[2] Vitamin premix containing guaranteed minimum values of 12,494 IU/kg vitamin A, 5,247 IU/kg vitamin D3, 100,352 IU/kg vitamin E-50%, 31.06 mg/kg vitamin B12, 251 mg/kg Biotin, 4,498 mg/kg vitamin K, 4,000 mg/kg Thiamine (B1), 13,995 mg/kg Riboflavin (B2), 5,998 mg/kg Pyridoxine (B6), 12,700 mg/kg Pantothenic Acid (B5), 84,960 mg/kg Niacin (B3), 4,000 mg/kg Folic Acid (B9), respectively

Table 3.3 Treatment effects on yield and pre-chill body weight

	Yield (%)	Pre-Chill BW (lbs)
PC	33.13%[b]	38.28[b]
NC	33.90%[b]	39.86[b]
NC + 150g	34.80%[a]	40.25[a]
NC + 300g	34.35%[a]	41.52[a]
P-Value	0.0018	<.0001
F Value	5.26	8.42
SEM(133)[1]	0.00153	0.604

[1]SEM is calculated by MSE divided by the square root of n.
Yield is calculated by divided the amount of white meat by the carcass weight
a,b,c,d means covered by the same superscript are not significantly different

Table 3.4 Enzyme recovery data

			Lab A		Lab B	
Enzyme Level	Phytase Guarantee (SPU/g)	Xylanase Guarantee (XylX6/g)	Pelleted Feed Recovery		Pelleted Feed Recovery	
			Phytase	Xylanase	Phytase	Xylanase
150g/tonne	0.17	0.0055	0.2	0.173	0.9	2.9
300g/tonne	0.3	0.011	0.3	0.432	1.2	3.19

Table 3.5 Effects on FCR

	Age (Wk)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Treatment																				
PC		0.99	1.09	1.15	1.18	1.27^{b}	1.28^{b}	1.37	1.41	1.47	1.49	1.55^{b}	1.63	1.71	1.79	1.85	1.92^{b}	1.97^{c}	2.00^{c}	2.14^{b}
NC		0.97	1.06	1.13	1.19	1.23^{b}	1.23^{b}	1.33	1.41	1.47	1.50	1.58^{b}	1.65	1.72	1.79	1.87	1.96^{b}	2.00^{bc}	2.06^{b}	2.16^{b}
NC+150g		0.97	1.04	1.11	1.18	1.23^{b}	1.28^{b}	1.31	1.40	1.47	1.51	1.61^{a}	1.68	1.74	1.85	1.91	1.98^{a}	2.04^{ab}	2.10^{ab}	2.23^{a}
NC+300g		0.94	1.05	1.14	1.21	1.31^{a}	1.32^{a}	1.37	1.42	1.50	1.55	1.62^{a}	1.67	1.74	1.82	1.92	1.99^{a}	2.08^{a}	2.12^{a}	2.25^{a}
P-value		0.004	0.22	0.37	0.25	<.0001	0.002	0.055	0.85	0.49	0.16	0.03	0.66	0.46	0.16	0.29	0.03	0.001	0.003	0.009
SEM(32)[1]		.006	.009	.008	.006	.007	.009	.008	.009	.009	.010	.014	.008	.007	.011	.011	.010	.011	.013	.014

a,b,c,d means covered by the same superscript are not significantly different
[1]SEM is calculated by MSE divided by the square root of n.

Table 3.6 Flock close-out data

	PC	NC	NC+150g	NC+300g
Age (days)	140	140	140	140
Yield (%)	33.13%	33.90%	34.80%	34.35%
Yield difference from PC	0.00%	0.78%	1.67%	1.22%
Pre-chill carcass weights (lbs)	38.28	39.86	40.25	41.52
Live bird weights (lbs)	47.38	48.01	49.13	48.1
Livability (%)	86.29%	86.56%	89.79%	87.63%
Live pounds to the plant	30420	30920	32820	31360
FCR	2.174	2.186	2.188	2.270
ADG	0.338	0.343	0.351	0.344
Feed cost per pound of gain	0.516	0.490	0.492	0.512

Table 3.7 Effects on Weekly Feed Consumption

	Age (Wk)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Treatment																				
PC		68	62	80	141	181[b]	205	308	354	393	474[b]	456[b]	587	650[b]	697[a]	763[b]	729[b]	748	598[b]	773
NC		65	64	82	144	165[b]	204	307	355	391	464[b]	504[a]	542	664[a]	677[b]	832[a]	754[a]	771	673[a]	792
NC+150g		71	67	90	159	189[b]	222	336	377	428	528[a]	540[a]	589	703[a]	738[a]	836[a]	795[a]	798	692[a]	818
NC+300g		62	63	97	169	228[a]	237	331	387	416	526[a]	535[a]	591	699[a]	743[a]	819[a]	782[a]	763	656[a]	795
P-value		<.001	.31	<.001	<.001	<.001	<.001	0.02	0.006	<.001	<.001	0.042	0.304	0.043	0.073	0.087	0.078	0.32	0.014	0.196
SEM(32)[1]		.736	.99	1.73	2.48	4.53	3.08	4.28	4.3	3.88	7.06	12.0	10.5	8.15	10.7	11.6	9.84	9.66	11.4	7.46

[1]SEM= Standard Error of the mean for n=48
a,b,c,d means covered by the same superscript are not significantly different
P-value derived from ANOVA of individual weeks
Post-hoc contrasts derived from mixed model repeated measure identify treatment effect over time

Table 3.8 Effects on Body Weights

	Age (Wk)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Treatment																				
PC		0.74[b]	1.31[b]	2.01[b]	3.27[b]	4.66[b]	6.40[c]	8.69[b]	11.29[b]	13.91[b]	17.31[b]	20.18[b]	23.39[b]	26.73[b]	30.50[b]	34.66	38.41	42.33	45.53	47.63
NC		0.73[b]	1.33[b]	2.04[b]	3.26[b]	4.69[b]	6.52[c]	8.86[b]	11.30[b]	13.95[b]	17.31[b]	20.15[b]	23.14[b]	26.90[b]	30.51[b]	34.85	38.09	42.44	45.67	48.49
NC+150g		0.79[a]	1.45[a]	2.26[a]	3.60[a]	5.13[a]	6.87[b]	9.70[a]	12.17[a]	14.86[a]	18.48[a]	21.18[a]	24.56[a]	28.41[a]	31.60[a]	35.76	39.44	43.24	46.10	48.07
NC+300g		0.72[b]	1.31[b]	2.14[a]	3.55[a]	5.22[a]	7.21[a]	9.75[a]	12.43[a]	14.96[a]	18.32[a]	21.29[a]	24.74[a]	28.35[a]	31.86[a]	35.68	39.44	42.33	45.75	48.14
P-value		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.0013	0.003	<.001	0.008	0.096	0.029	0.593	0.899	0.649
SEM(32)[1]		.006	.012	.02	.032	.048	.064	.097	.11	.11	.14	.15	.202	.19	.191	.195	.21	.27	.27	.232

[1]SEM= Standard Error of the mean for n=48
a,b,c,d means covered by the same superscript are not significantly different
P-value derived from ANOVA of individual weeks
Post-hoc contrasts derived from mixed model repeated measure identify treatment effect over time

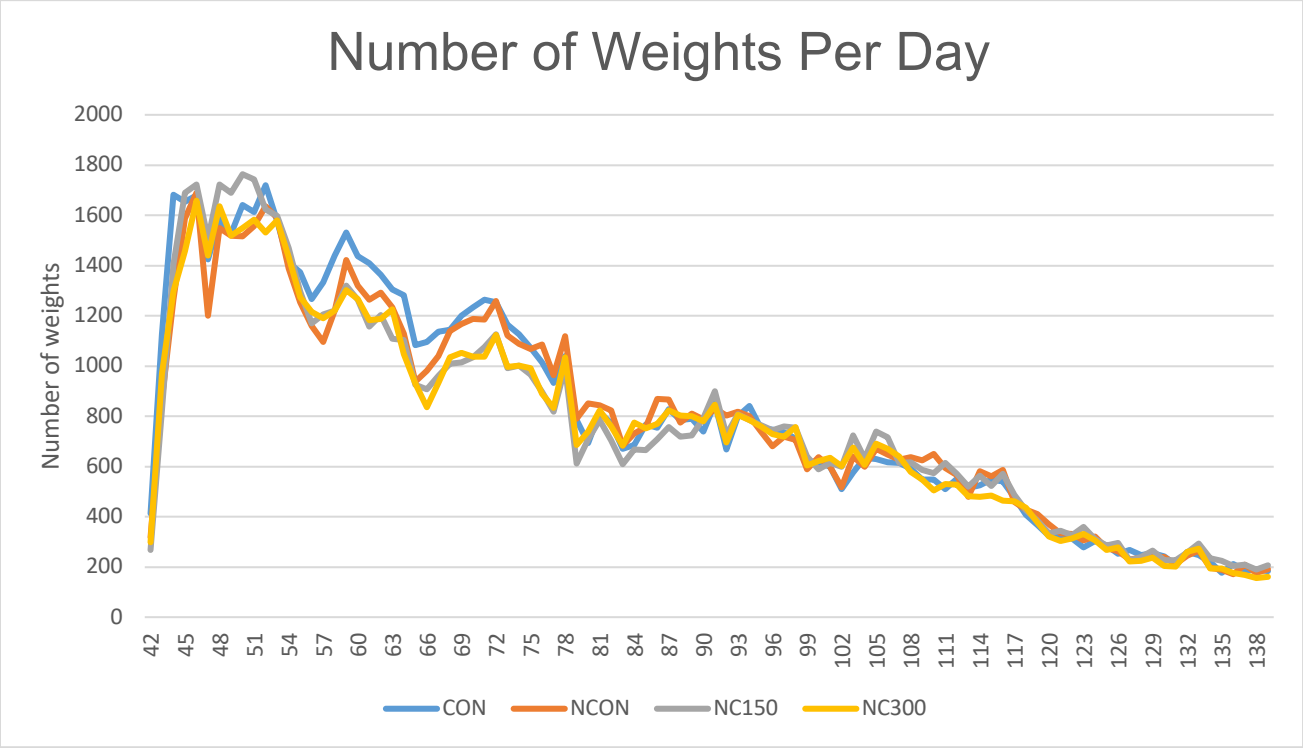

Figure 3.2 Number of weights recorded per day during the grow out stage

CHAPTER 4

FINAL CONCLUSIONS

This two-part study demonstrates the role of dietary enzyme supplementation, specifically Allzyme Spectrum®, in overcoming the impacts of reduced nutrient density in turkey diets, showing the potential for enhanced growth performance and nutrient utilization without compromising economic efficiencies. The core of this investigation revolved around the hypothesis that strategic enzyme integration could counterbalance dietary reductions in critical nutrients—calcium (Ca), phosphorus (P), and energy—thereby sustaining or even improving growth performance in turkeys, within both controlled and commercial settings.

The initial segment of our research underlined the enzyme complex's capacity to significantly improve apparent metabolizable energy (AMEn) and the ileal digestibility of Ca and P in turkey poults. These enhancements were notable against diets of diminished nutrient density, suggesting that the enzymatic complex can effectively unlock the bioavailability of these expensive nutrients. This finding met our goal of sustaining performance under low nutrient conditions, given the constant challenge to reduce feed costs while ensuring nutritional adequacy. Thus, providing an opportunity in turkey nutrition towards more sustainable and cost-effective practices.

Expanding upon these insights, the subsequent commercial setting evaluation affirmed the enzyme complex's efficacy in improving growth metrics, including body weight and yield percentages, amidst nutrient reductions. Notably, the 150g enzyme treatment level emerged as an apparent optimal dose level, improving growth performance and carcass yield, thereby offering a tangible financial leverage in the context of current market dynamics. This optimal dose level

suggests that enzyme efficacy is subject to diminishing returns beyond a certain threshold, a revelation that challenges the conventional 'more is better' approach to enzyme supplementation.

Furthermore, the study's outcomes raised questions about the relation of enzyme supplementation and gut microbiota dynamics, hinting at the potential for high enzyme levels to disrupt microbial equilibrium. This observation merits further investigation into the microbiome's role in nutrient absorption and overall health while supplementing, particularly with xylanase enzymes, in turkey rations.

The logistical challenges encountered during the study, including feed tank malfunctions and formulation discrepancies, underscore the difficulties inherent in translating controlled research findings to commercial production realities. These experiences highlight the need for rigorous quality control measures in feed production and delivery processes to ensure the product trial will be evaluated properly.

In conclusion, the primary aim of this study was to validate the inclusion levels and nutrient uplift values provided by the enzyme when fed to turkeys. Our findings indicate that the tested uplifts, derived from existing broiler matrix values, which are 88 kcal/kg of energy, 0.15% calcium, and 0.15% phosphorus, are valid and also suitable for turkeys when fed at 200 grams per tonne. These results support the efficacy of Allzyme Spectrum® in turkey diets, demonstrating its potential to improve nutrient utilization and growth performance. This aligns with the broader objectives of enhancing economic efficiency and environmental sustainability in turkey production. Further research is warranted to deepen our understanding of the interactions between enzymes and microbiota, and to refine enzyme inclusion levels for different diets, emphasizing the overarching importance of enzyme supplementation within poultry nutrition strategies.