8-2017

EFFECT OF PROTEASE SUPPLEMENTATION IN BROILER FEED ON GROWTH PERFORMANCE, CARCASS YIELD AND TOTAL NITROGEN RETENTION IN FECAL MATTER AND LITTER

Jawad Al-juboori
Stephen F Austin State University, j.s1997@yahoo.com

EFFECT OF PROTEASE SUPPLEMENTATION IN BROILER FEED ON GROWTH PERFORMANCE, CARCASS YIELD AND TOTAL NITROGEN RETENTION IN FECAL MATTER AND LITTER

EFFECT OF PROTEASE SUPPLEMENTATION IN BROILER FEED ON GROWTH PERFORMANCE, CARCASS YIELD AND TOTAL NITROGEN RETENTION IN FECAL MATTER AND LITTER

By

JAWAD K. AL-JUBOORI, Bachelor of Animal Science

Presented to the Faculty of the Graduate School of

Stephen F. Austin State University

In Partial Fulfillment

Of the Requirements

For the Degree of

Master of Science

STEPHEN F. AUSTIN STATE UNIVERSITY
August 2017

EFFECT OF PROTEASE SUPPLEMENTATION IN BROILER FEED ON GROWTH PERFORMANCE, CARCASS YIELD AND TOTAL NITROGEN RETENTION FECAL MATTER AND LITTER

By

JAWAD K. AL-JUBOORI, Bachelor of Animal Science

APPROVED:

____

Joey Bray, Ph.D., Thesis Director

____

John Mehaffey, Ph.D., Committee Member

____

Christopher Comer, Ph.D., Committee Member

____

Wayne Weatherford, Ms., Committee Member

____

Richard Berry, D.M.A

Dean of the Graduate School

ABSTRACT

The objectives of this study were to determine the effects of protease supplementation on commercial broiler performance, carcass yield, and nitrogen retention in fecal matter and litter. Total of 4,800 female (Ross 708) birds split into 96 floor pens, and randomly assigned to one of four treatment groups. Birds were placed within 96, 5'x10' floor pens in a randomized-block design at the SFASU Poultry Research Center. Birds were randomly divided among the pens at a stocking density of 1.00 ft^2/bird (50 birds/pen*24 pens/treatment=1200 birds/treatment), and reared for 49 days on used pine shavings. The target average weight for the birds was 6.25lbs. Dietary treatments consisted of: treatment #1 positive control (PC) Pilgrim's Standard Diet (Basal diet), treatment # 2 negative control (NC) Pilgrim's Diet with Protease Matrix removed (only the amino acids' credit – no energy credit), treatment # 3 (PC+ Protease) Pilgrim's Diet (Basal diet) + Protease "on top", and treatment # 4 (NC+ Protease) Pilgrim's Diet with Protease Matrix removed + Protease "on place". groups were analyzed for bird performance, carcass yield, and Nitrogen retention in fecal matter and litter. A yield study was completed at the end of the study to determine meat yield for all retail cuts. Results indicated that the protease addition on top of protein matrix in treatment 3 had significant effect on live body weight at day 49, and had no significant effect on feed conversion ratio (FCR) & adjusted feed conversion ratio (AFCR). Also, the protease had no significant effect on carcass yield. However, the inclusion of protease on low protein diet (NC+ Protease, Tx4) lowered the nitrogen retention in fecal matter.

ACKNOWLEDGEMENTS

The author would like to take this opportunity to express his sincere appreciation and thanks to God and all those who helped him in making this research project a success especially my uncle Ali for the guidance and support. A very special thank you and gratification goes to his loving wife, Salwa. Without her divine efforts to encourage him to pursue his dreams, none of this would be possible. He would like to thank Dr. Joey Bray for the guidance, support, patience, and encouragement he has given throughout this process and during the author's Master's degree. He also, want to show his utmost appreciation to his committee members for dedicating time and effort during the process. He would also like to thank his friends and family for supporting and encouraging him throughout his Master's degree.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

Introduction

For any broiler producer, the main goal is higher production with a lower cost and environmental impact. Working on a complicated production equation to increase the variables in one side like the bird's weight and decrease the variables in the other side like feed cost is not an easy concept.

Protein is the second major nutrient and the most expensive in the broiler diet, and all other poultry industries. The protein sources in modern broiler diets are mostly derived from corn and soybean meal along with other sources like animal by-products (Buttin et.al, 2016). Soybean products are the most common source of protein in broiler diets and have rapidly increased in price since 2000 (Buttin et.al, 2016). Despite this, a valuable amount (18-20 %) of protein passes through the gastrointestinal tract without being completely digested and absorbed (Angel et.al, 2011, Applegate et.al, 2008). The environmental impact from nitrogen and phosphorus that comes from undigested proteins and other excreted substances in the poultry manure (Gerber et.al, 2015) has led to the idea of using supplemental exogenous enzymes like proteases in poultry diets to improve protein digestibility and reduce the amount of protein wasted, production cost, and environmental impact (Buttin et.al, 2016)

Protease enzymes have several benefits including decreasing undigested proteins in the diet, increasing amino acid availability, reducing protein needs in the diet, maintaining weight gain and feed efficiency, reducing proteolytic fermentation, and decreasing biogenic amines and bacterial toxins (Buttin et.al, 2016). Therefore, protease enzymes are of interest for many poultry companies and nutrition supplementation companies for use as an important supplement digestive enzyme in broiler diets and other poultry diets.

In our study, we were focusing on the evaluation of the effects of protease supplementation on broiler performance by measuring growth performance parameters and carcass yield over 49 days. We also measured the growth rate at different growth stages to quantify the birds' performance under inclusion of protease in their diet. The protease supplementation was added on top or in place of the protease matrix in commercial broiler diets.

Statement of Problem

On the averages about (34-46 lbs./ton) nitrogen, and (60 lbs./ton) phosphorus are extracted in solid poultry litter (Spiehs, 2005). This valuable amount of protein and non-protein nitrogen that are extracted in broiler manure have a value of (18-20%) of the protein cost in the diet indicate the amount of dollars wasted that need to be decreased to reduce the production cost and environment impact (Applegate et.al, 2008). This study was to determine if it is beneficial to include protease in broiler diets to improve growth performance, carcass yield, and nitrogen retention in fecal matter and litter.

Objectives

The objectives of this study were:

- To evaluate the effects of protease inclusion on growth performance parameters such as average body weight, feed conversion ratio, and adjusted feed conversion ratio.
- To evaluate the carcass yield, and the weights of front-half carcass, weight Without Giblets (WOG), hind-half carcass, breast, tenders, wings, drums, thighs, frame, back, abdominal fat pad, and skin with protease inclusion in broiler diets.
- To evaluate the potential of using protease in the broiler diet to reduce the nitrogen footprint in fecal matter and litter from broiler production.

CHAPTER II

Literature Review

Enzyme supplementation in poultry diets is nutritionally, economically, and environmentally justified (Kamel et. al, 2015). Enzymes are used to increase the energy value of feed ingredients and enhance the utilization of protein, fats, carbohydrates, and phosphorus from plant materials, leading to a lower excretion rate of undigested nutrients into the environment and, hence, reduced environmental pollution. This is the most important function for most feed supplement enzymes, especially proteases, as digestion of nitrogenous compounds in feed materials is essential for reducing nitrogen (N) excretion – a major pollutant worldwide (Kamel et. al, 2015).

The use of exogenous enzymes in diets of domestic animals is not a new concept and has been extensively studied and reported. However, studies have shown that response to exogenous enzymes ranges from adverse to beneficial (Campbell and Bedford, 1992, Smits and Annison, 1996, Madrid et. al, 2010, and Oxenboll et. al, 2011,). Some research has pointed out that protein is less digestible (80-85%) compared to starch (90%) in corn-soy diets (Kamel et.al, 2015). Also, certain amounts of protein pass through the gastrointestinal tract without being completely digested. Thus, the nitrogen content in the undigested protein is going into the environment, and this protein is wasted rather than used for production. As a result, using enzyme products such as proteases is very important to maximizing protein utilization and minimizing protein waste (Kamel et. al, 2015).

Proteins

Proteins are complex compounds made up of amino acids subunits which are comprised of carbon, hydrogen, oxygen, nitrogen, and sometimes sulfur. A protein molecule consists of one or more chains of amino acids. Proteins are essential components of all body cells (such as enzymes, hormones, and antibodies) that are necessary for certain body functions. They are essential in the animal's diet for growth, tissue repair, and reproduction and can be derived from many feedstuffs such as meat and fish meals, cereal grains, and legume byproducts such as soybean meal (Bailey et.al, 2016*)*.

After a bird consumes protein, the digestive tract breaks down the protein into amino acids by extracting protein degradation oxygenated enzymes such as protease, pepsin, and trypsin. The amino acids are then absorbed by the blood and transported to cells that convert the individual amino acids into the specific proteins required by the animal. Proteins are used in the construction of body tissues such as muscles, nerves, cartilage, skin, feathers, and beak, and so on. Egg white is also high in protein. Proteins have major roles in poultry production because They are essential for growth, body maintenance, production, and reproduction (Dale, 2009). Furthermore, some research has shown that the rate and efficiency of growth is reduced, and carcass composition is inferior when the crude protein (CP) level is reduced by more than 3%, even when all nutrient requirements are met (Bregendahl et al., 2002).

Amino Acids

Amino acids are typically divided into two categories, essential and nonessential. Essential amino acids such as arginine, glycine, histidine, leucine, isoleucine, lysine, methionine, cystine, phenylalanine, threonine, tryptophan, and valine are those that cannot be made in the body to meet the needs of the animal. The nonessential amino acids are those that the body can generate if certain materials are available. There are 22 amino acids commonly found in feed ingredients. About ten of them are essential and must be supplied in the feed. Poultry diets typically contain a variety of feedstuffs because no single ingredient can supply all the necessary amino acids at the correct levels (Dale, 2009).

Essential amino acids must be supplied by the diet, and some non-essential amino acids that are in sufficient amount should be supplied to avoid the conversion of essential amino acids into non-essential amino acid. Furthermore, amino acid requirements depend on the needs of the animal, and the excess amino acids from the bird's needs will be used as a source of energy instead for body protein synthesis. This breakdown of amino acids will also result in higher nitrogenous excretions in the fecal matter (Applegate et.al, 2008).

The best way to reduce nitrogen in poultry manure is to lower the amount of CP that is fed to the broiler by supplementing diets with amino acids. Reducing the nonessential amino acid amount, combined with adding more essential amino acids in the diet, can increase the efficacy of total N retention by the bird (Applegate et.al, 2008). Formulation based on bird amino acid requirements not on CP requirement can minimize N excretion because it simply reduces total N intake (Ferguson et al., 1998). Furthermore, broiler litter N was reduced more than 16% when dietary CP was reduced by 2%, while maintaining similar levels of dietary amino acids (Applegate et al. 2008). However, Reducing CP content of broiler diets by less than two percentage units resulted in decreased litter N content but no significant differences in NH_3 concentration in the house (Ferguson et al., 1998). Additionally, total N losses in the houses averaged 18% to 20% of total N input (Applegate et al., 2008).

Angel et al. (2006) examined the possibility of reducing dietary N intake in broilers to 42 days of age. Feed conversion was similar between groups after 5 flocks, but live body weight was 77 g lower in the lowest protein group. However, breast yield (%) was not affected by diet in the third or fourth flocks. Consumption of N was 8.3% lower resulting in a 20% reduction in N excretion. Pope et al. (2004) also studied the advantages of increasing the number of phases during the broiler growth cycle. By changing diets every two days to better meet the bird’s amino acids needs from 21 to 63 days of age, performance and carcass yield didn’t change, but N excretion was reduced by 7 - 13%.

Amino acids which are essential cannot be synthesized by the bird. These essential amino acids must be fed to supply the building blocks needed in the synthesis of body proteins to support growth. Dozier et al, (2008) recently summarized the amino acid requirements of broilers in weekly durations based that is shown in table below (Table 1).

Table 1. Dietary amino acid (% of diet) requirements for high-yielding broilers (Dozier et al., 2008).

Amino Acid	Age, day						
	7	14	21	28	35	42	56
Total sulfur amino acids	0.94	0.90	0.85	0.81	0.77	0.74	0.70
Methionine	0.62	0.55	0.50	0.48	0.46	0.47	0.50
Lysine	1.36	1.26	1.19	1.12	1.06	1.01	0.97
Threonine	0.84	0.81	0.77	0.74	0.71	0.69	0.67
Isoleucine	0.91	0.86	0.82	0.78	0.75	0.72	0.70
Valine	1.03	0.98	0.94	0.90	0.87	0.84	0.82
Arginine	1.47	1.37	1.28	1.21	1.14	1.09	1.04

According to Applegate et al., (2008) the long-term reductions in CP formulation with adoption of the digestible amino acid should reduce feed cost and N retention in the broiler manure. However, inconsistent methodologies make it difficult to switch to using digestible amino acid values, especially for non-traditional feed ingredients.

Proteins Digestion

The digestion of protein is driven mainly by endogenous protease in the case of monogastric animals there are two stages of the digestion process (Bedford et al., 2014). The gastric stage is the first stage, which is a low pH environment. During the gastric stage pepsin breaks certain chemical bonds in proteins, producing smaller molecules called peptides and beginning protein digestion. The second stage is the small intestinal stage, a neutral phase where trypsin, chymotrypsin, elastase, and several other exo-proteases are present to complete the process of protein digestion (Bedford et al., 2014). The pancreas synthesizes trypsin and chymotrypsin, and these enzymes are released into the small intestine through the pancreatic duct. When partially digested food moves from the stomach into the intestine, trypsin, and chymotrypsin complete protein digestion, producing simple amino acids that are absorbed into the blood (Rogers, 2015).

The secreted proteases are very effective in degrading dietary proteins and, as a result, are potentially dangerous as they could digest the animal's gastrointestinal (GI) tract and the cells in which they are produced (Bedford et al., 2014). However, this problem is avoided since the enzymes are secreted in an inactive form and only activated by pH or enzymes within the lumen. In addition, the gastrointestinal (GI) tract is protected by a layer of mucus which is relatively inert to proteolytic destruction. Generally, this system works well but protein digestion may be compromised, and certain amounts of protein pass through the gastrointestinal tract without being completely digested. Thus, the nitrogen content in the undigested protein is going into the environment. Several factors influence protein digestion rate including (Kamel et al., 2015): protease inhibitors within feed ingredients, damage to intestinal structure and absorptive surface area, rapid transit time through the gastrointestinal tract, and insufficient secretion of endogenous proteases.

The latter includes impediments like viscous non-starch polysaccharides (NSPs) which reduce the transformation rate of all digestive enzymes, including proteases, thus resulting in insufficient proteases being secreted to complete digestion (Bedford et al., 2014). Young and sick animals may also be limited in their ability to produce or secrete digestive enzymes. In many cases the animal is faced with one or more of the above situations. Under such circumstances, supplementation of the diet with enzymes which treat one or more of the factors limiting digestion enhances more complete protein digestion and more efficient growth (Kamel et al., 2015).

Recent work has shown significant improvements in protein digestibility when proteases are used, but the improvement in performance is not always clear (Angel et al., 2011). However, in the work of Liu et al. (2013) the effectiveness of protease was correlated to protein level in the diet. Also, the efficacy of a protease may be dependent upon the ingredients used in the ration (Kocher et al., 2003). The benefit of a protease may also depend on the presence of other enzymes, for example the benefit is lost or limited when the protease is tested with a xylanase and/or phytase (Kalmendal, 2012). However, in the work of Yan et al. (2012) it was clear that the benefit of the protease was higher in the starter diet compared with the finisher diet, which suggested that the young animal may be more responsive to protease. An interaction between protein and protease was observed in which digestibility of CP and energy were greater when protease was added to high-protein diets as compared with the low-protein diets. Another interaction between energy and protease was associated with a greater increase in energy digestibility when protease was added to high-energy diets, as compared with the low-energy diets (Freitas et al., 2011).

Kamel et al. (2015) showed that protease addition has a significant effect on increasing the level of CP digestibility. The results were compatible with Freitas et al., (2011) who pointed out an improvement of 1.8% in crude protein digestibility when the protease was added to the high-protein diets, while an improvement of only 1% was in the low protein diets. In addition, Angel et al. (2011) reported an improvement of crude protein and amino acid digestibility in diets supplemented with graded levels of protease fed to 22-day old broiler chickens. Moreover, Fru-Nji et al., (2011) concluded that exogenous protease enzymes enhanced protein and energy digestibility. Gitoee et al., (2015) pointed out the effects of multi-enzyme (ME) including protease dietary treatments on feed intake (FI), body weight (BW) and feed conversion ratio (FCR) at 10, 24 and 49 days of age. Results showed that the ME main effects and their interaction had no significant effect on FI of broilers at 10 days and 24 days. Although, no effect of the enzyme or its interaction could be detected in 49 days, the ME significantly affected the FI of birds in the finisher diet (49 days). On the other hand, other research showed that there was no effect for protease alone or in combination with other enzymes on BW and FCR (Kocher et al., 2003). Marsman et al. (1997) found no beneficial effects of protease inclusion in a maize-soybean diet on broiler performance. Some other research showed that the source of the protease is important in the effectiveness of the enzyme in the improvement in broiler performance by including a specific protease P2 (isolated from *Aspergillus* strains) in a SBM diet.

However, broiler performance did not improve when another specific protease P1 (isolated from *Bacillus* strains) was added (Ghazi et al., 1997a).

Protease Inhibitors

Protease inhibitors are small protein molecules that can interfere with the action of the proteolytic enzymes involved in breaking down protein into amino acid components. Inhibitors have been isolated from many legumes, including soybeans, and they can be destroyed by heat, which is why whole soybeans must be roasted before they can be included in poultry diets (Jacob, 2015). For maximum conversion of the proteins of soybeans and other legumes into products with good nutritional quality, the conditions of heat treatment must inactivate the antinutritional substances as well as transform the raw protein into a more bird-available digested form (Rackis et al., 2014). Protease inhibitors are limiting factors for protein digestibility and growth performance (Jacob, 2015).

Anti-nutritional Factors

The addition of enzymes in broiler diets can help to improve the utilization of dietary energy and amino acids and eliminate the effects of anti-nutritional factors resulting in improved performance of chickens (Gitoee et al., 2015). Anti-nutritional factors are substances that when present in animal feed or water reduce the availability of one or more nutrients. Anti-nutritional factors include substances such as protease inhibitors, phytate, beta-glucans, gossypol, and lectins (Jacob, 2015). Phytate is the principal storage form of phosphorus in many plant tissues. Also, phytate’s main function is to block the absorption of not only phosphorus but also other minerals, particularly calcium, magnesium, iron, and zinc, and negatively affect the absorption of lipids and proteins (Jacob, 2015). Beta-glucans bind with water in the intestines, resulting in the formation of gels that increase the viscosity of the intestinal contents. However, there is a negative correlation between intestinal viscosity and nutrient availability because the increase in viscosity associated with increased gel formation affects digestion and absorption of nutrients (Jacob, 2015). Gossypol is a toxic compound found in the cotton plant. Although it can exist throughout the plant (in the hulls, leaves, and stems), it is concentrated in the cottonseed. Two forms of gossypol exist: free and bound. The free form is the toxic form. Bound gossypol binds to proteins, making it nontoxic but decreasing protein digestion (Jacob, 2015). Lectins are proteins that have the unique property of binding carbohydrate-containing molecules which cause the agglutination of red blood cells. In the digestive tract, agglutination causes the atrophy of the microvilli, decreases the viability of the epithelial cells, and increases the weight of the small intestine caused by hyperplasia of crypt cells. Moist heat treatment will destroy much of the lectin in grain legumes (Jacob, 2015).

Protease

Proteases are a class of enzymes that are responsible for the breakdown of protein into its basic building blocks. The digestive tract produces several types of enzymes, but the three main proteases are pepsin, trypsin, and chymotrypsin. Special cells called gastric chief cell, peptic cell, or gastric zymogenic cell in the stomach produce an inactive enzyme, pepsinogen, which changes into pepsin when it contacts the acidic environment in the stomach (Mótyán et al., 2013).

Proteolytic enzymes hydrolyze peptide bonds and are also referred to as peptidases, proteases, or proteinases (Mótyán et al., 2013). The physiological function of proteases is necessary for all living organisms, and proteolytic enzymes can be classified based on their origin: microbial (bacterial, fungal, and viral), plant, animal and human (Mótyán et al., 2013). Proteolytic enzymes belong to the hydrolase class of enzymes, and are grouped into the subclass of the peptide hydrolases or peptidases. Depending on the site of enzyme action the proteases can also be subdivided into exopeptidases or endopeptidases. Endopeptidases cleave peptide bonds within and distant from the ends of a polypeptide chain. Exopeptidases catalyze the hydrolysis of the peptide bonds near the *N*- or *C*-terminal ends of the substrate. Aminopeptidases can liberate single amino acids, dipeptides (dipeptidyl peptidases) or tripeptides (tripeptidyl peptidases) from the N-terminal end of their substrates. Single amino acids can be released from dipeptide substrates by dipeptidases or from polypeptides by carboxypeptidases, while peptidyl dipeptidases liberate dipeptides from the C-terminal end of a polypeptide chain (Figure 1) (Mótyánet al., 2013).

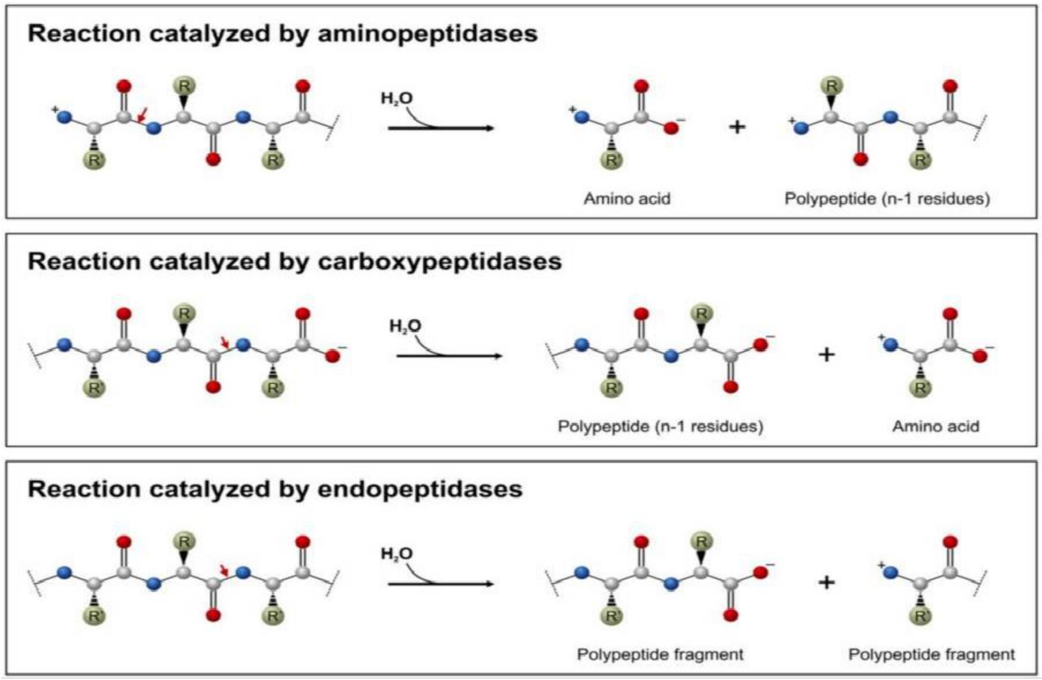


Figure 1: Action of aminopeptidases and carboxypeptidases removing the terminal amino acid residues as well as endopeptidases on a polypeptide substrate (having n residues). Red arrows show the peptide bonds to be cleaved (Mótyánet al., 2013).

There has been a great deal of research about using protease in broiler diets. Some of research indicates that most the broilers that have been tested by adding protease in their diet have shown improvement in feed efficiency especially in birds fed low protein diets (Buttin et al., 2016). However, many researchers have reported improvement of crude protein digestibility by the addition of protease enzyme (Kamel et al., 2015). Furthermore, other researchers have concluded that exogenous serine protease enzyme supplementation enhanced protein and energy digestibility (Gitoee et al., 2015).

POULTRYGROW 250™ (Protease)

The protease product that we used in this trail is called POULTRYGROW 250™**.** It is a mixture of fermentation extracts primarily providing proteolytic enzyme activity from yeasts. POULTRYGROW 250™ main functions are to improve gain and feed conversion, and it allows a reduction of crude protein and amino acid content in the feed.

Nitrogen Environmental Impact

The poultry industry has made adjustments to meet the increasing demand for meat and egg supplies. Over the past three decades, the poultry sector has been growing at more than 5 percent annually, and its part in world meat production increased from 15 percent three decades ago to 30 percent in 2006 (FAO, 2006). This growth has been accompanied by intensifying and concentrative of poultry operations. The pressure to lower production costs and increase supply led to more efficient operations, by growing to larger, more specialized, and more integrated facilities, and through improvements in the use of animal genetics, optimized nutrition, and new production technologies. Animals reared in intensive production systems consume a considerable amount of protein and other nitrogen-containing substances in their diets. The conversion of dietary nitrogen to animal products is relatively inefficient, with 50 to 80 percent of the nitrogen is excreted (Gerber et al., 2015). Nitrogen is excreted in both organic and inorganic compounds. Nitrogen emissions from manure take four main forms: ammonia (NH_3^+), dinitrogen (N_2), nitrous oxide (N_2O) and nitrate (NO_3^-; Gerber et al., 2015). The excretion of nitrogen originating from intensive livestock and poultry operation is a serious environment concern. In addition to polluting the air and water, nitrogen in poultry fecal matter or litter is converted to volatile ammonia through microbial fermentation and can affect the health of birds and farm workers (Hassan et al., 2011).

Nitrogen pollution has been identified as a risk to the quality of soil and water. These risks relate to high levels of nitrates, which can be leached to the groundwater table or to surface water causing eutrophication. In its nitrate form, nitrogen can easily be leached below the rooting zone and into groundwater. Poultry manure contributes to the structural nutrient overload in these areas. Moreover, the manure may be applied to crops or fish ponds in excess or in addition to chemical fertilizers or fish feed, resulting in an over-supply of nutrients. Such saturated systems will release a huge amount of nutrients into the environment. Excessive levels of nitrogen in the environment lead to negative effects (De

Vries et al., 2003). Enhanced levels of nitrogen in the environment may have several adverse effects, including decreased plant species diversity in the ecosystems, eutrophication of surface waters, pollution of groundwater due to nitrate leaching, and global warming due to nitrous, nitrogen oxide, and ammonia (N_2O, NO_x, and NH_3) emissions (Gerber et al., 2015).

Atmospheric ammonia (NH_3) is increasingly being recognized as a major air pollutant because of its role in regional and global-scale negative effects when deposited into ecosystems. Ammonia is a soluble and reactive gas (Sutton and Fowler, 1995). This means that it dissolves, for example in water, and that it will react with other compounds to form ammonia-containing compounds. The concentrations of ammonia in the air are greatest in areas where there is intensive livestock farming. Agricultural land receiving large inputs of nitrogen from manures normally acts as a source of ammonia. There is little deposition of ammonia gas to intensively managed farmland, which is largely a net source of ammonia (Sutton and Fowler, 1995). Ammonia in the atmosphere can be absorbed by land, water, and vegetation (known as dry deposition). It also can be removed from the atmosphere by rain or snow (wet deposition). Impacts of ammonia deposition include; soil and water acidification, eutrophication caused by nitrogen enrichment with consequent species loss, vegetation damage, and increases in emissions of the greenhouse gases such as nitrous oxide (Gerber et al., 2015).

Nitrogen excretion from farm animals is part of an unfriendly environmental footprint. So, the new idea for using protease enzymes may not only be to improve feed efficiency and utilization by the animal to decrease production cost, but also to reduce the total content of nitrogen being excreted in the manure (Kamel et. al, 2015). This indicates that when aiming to improve the environmental performance of broilers, the use of a protease in feed is one of the more promising nutritional strategies, either used alone or combined with other dietary alterations or changes in poultry production (Smith, 2015). Hassan et al., (2011) found that the addition of protease in broiler diet decreased the N excretion by 8.33, 7.60, and 7.97% in starting, growing, and finishing periods, respectively. Moreover, the combination of xylanase, amylase, protease and phytase is effective in improving the digestibility of DM, N, lipid, amino acids, energy, Ca, and P of maize/soybean meal-based diets for broiler chickens (Cowieson et al., 2006). Also, Ghazi et al., (2010b) have found that the protease increased apparent nitrogen (N) digestibility and apparent N retention across the whole digestive tract in broilers. On the other hand, nitrogen was lower for chicks fed low-protein diets; however, no significant effect of protease enzyme supplementation was observed (Yamazaki et al., 2002).

One of the aims of our study was to examine the effect of the protease in the broiler diet on nitrogen excretion in the manure of the broiler at age 1, 12, 32, and 48 days across four treatments.

CHAPTER III

Materials and Methods

Experimental Animals

This study began on February 24, 2017, when 4,800 one day-old, female Ross 708 commercial broiler chicks supplied by Pilgrim's Corporation (Nacogdoches, Tx) were placed at the Stephen F. Austin State University Poultry Research Center. The birds were randomly assigned to one of four treatment groups with a total of 1,200 birds /treatment group. Birds were randomly placed into 96, 50 ft^2 pens at a stocking density of 1.00 ft^2/bird (50 birds/pen). Each pen was then assigned to one of four treatment groups in a randomized complete block design within 24 blocks, and four pens for each block (Figure 2). A randomized block design was used to minimize any effect due to environmental variation dependent on position within the test facility. The birds were reared on used bedding for a total of 49 days. Two hanging tube feeders and a nipple drinker were placed in each pen.

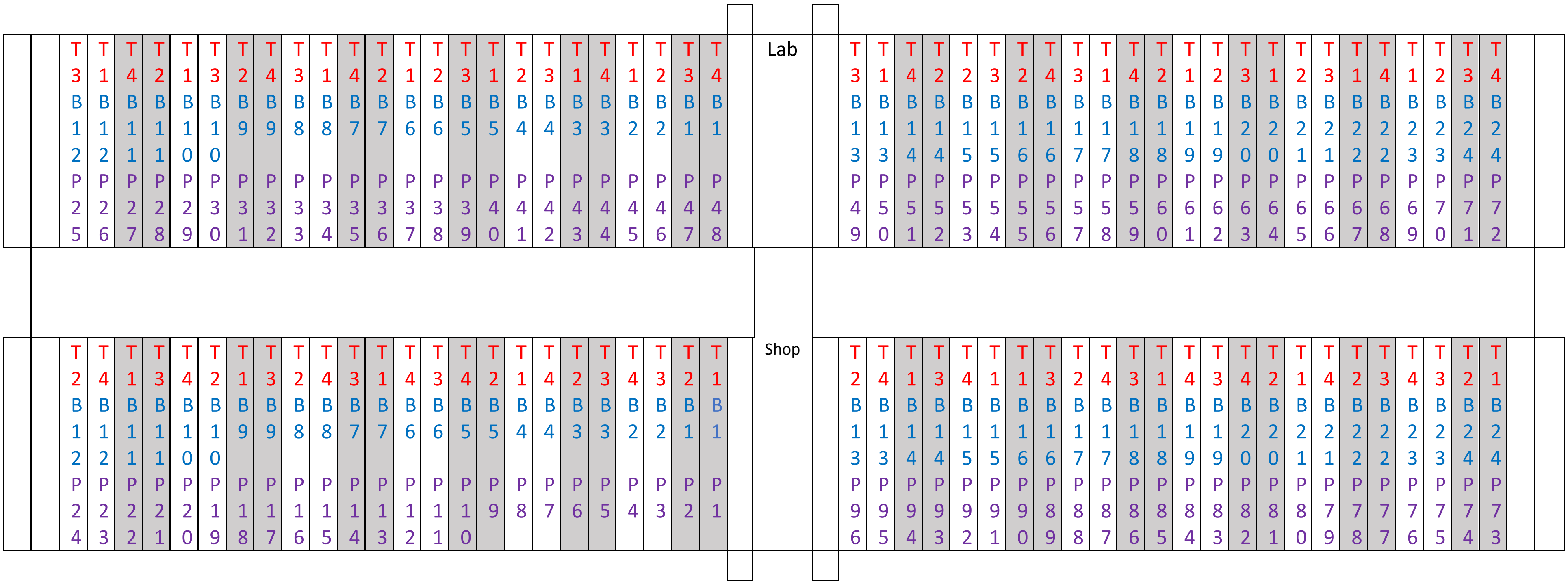


Figure 2: Blocks and Treatments Design (T= Treatment, B= Block, P= Pen)

Experimental Treatments and Groups

This study had a total of four different treatment groups (Table 2). Each treatment group consisted of 1,200 birds and had 24 replicates per treatment where pen is the experimental unit. For each of the below groups, feed changes mimicked Pilgrim's standard feeding regimen as follows: Starter diet – 1 lb. complete feed/bird (~d1-13), Grower diet – 4 lbs. complete feed/bird (~d14-32), Finisher (Withdrawal) diet - ~7 lbs. complete feed/bird (~d33-49). Pilgrim's supplied all basal diets. Diets were back formulated prior to arrival at the SFASU Research Feed Mill. Diets were then formulated per the treatment specifications, mixed, crumbled and/or pelletized, weighed and recorded.

Table 2. Dietary treatment groups PC, NC, PC +Protease, and NC +Protease

Treatment #		Diet		
		Starter	Grower	Finisher
1	Positive Control (PC)	Pilgrim's Diet (Basal diet)		
2	Negative Control (NC)	Pilgrim's Diet with Protease Matrix removed (only the amino acids' credit – no energy credit)		
3	PC + Protease	Pilgrim's Diet (Basal diet) + Protease "on top"		
4	NC + Protease	Pilgrim's Diet with Protease Matrix removed + Protease "on top"		

* Protein or protease matrix= all protein and amino acids credit in the diet

Performance Parameters

All birds in each pen were counted and weighed collectively on days 13, 32 & 49. These days represent approximate times for feed change (day 13 – End of starter phase, day 32 – End of grower phase, and day 49 – End of finisher phase.). A five shelf (Doran® XL8000) scale used to weigh all the pen's content of birds as shown in (Figure 2). The scale was attached to the pen's door (Figure 3) where the scale shelves' doors were facing the inside of the pen. Two of our weighing team were inside the pen to load 15 birds into each layer. No more than 50 birds per pen were weighed. The birds' total weight and number were recorded for each pen individually. However, before weighing the birds, the tube feeders, and any feed in the feed bags from the last feed phase were placed on top of the scale and weighed. The feed measurements were used to calculate the intake. Pens total live weight were used to determine average body weight per treatment group. All feeds were weighed and recorded prior to delivery in each pen with the feed remaining in each pen on assigned weigh days were used to calculate total feed intake, feed conversion ratio, and adjusted feed conversion ratio. Mortality was checked daily, and all mortality was collected, weighed, and recorded. Probable cause of death was noted.

Figure 3: Five shelf (Doran® XL8000) scale

Figure 4: five shelves (Doran® XL8000) scale attached to floor pen

Yield Study

At the completion of the study, 4 randomly- selected birds per pen, for a total of 384 birds, were individually weighed, recorded, and wing tagged. A numbered wing tag was placed in the wing web of each bird for further individual identification throughout the yield process. Birds from each treatment group remained together and were placed in individual isolation pens until time for processing. The birds were provided feed and water until 10 hours prior to processing, when the feed was removed for gut passage. The process steps are shown in (Figures 5 & 6). Birds were first placed in the Killing cones, where the birds were stunned in the Pulsed DC Poultry Stunner from (Executrol Systems) stunning unit (Figure 5). Next birds were bled by using a knife to sever the carotid artery and jugular vein, and allowing approximately 2 minutes bleed time. The third step was placing the birds in the scalder in 140^{o} F water to prepare them to be defeathered. Birds were transferred from the scalder into the plucker and defeathered until most of the feathers were removed. Finally, the feet were manually removed, and then the carcasses were hooked to the shackle line to manually remove the head and neck. The intestines and internal organs were eviscerated manually. The whole carcass was cut into the standard poultry cuts and placed in one basket. Standard cuts were weighed using two computer capturing scales. The basket was placed on the first scale to record the whole carcass weight, and then as each part was removed from the basket weights were captured. The software subtracted each part weight from the whole carcass weight and saved that part weighed until all the carcass parts were recorded separately. The front half part went to the deboning table to be cut for breast, tenders, wings, frame, skin, and all those parts went to the second scale to be weighed as we done with hind half. The following weights were recorded: weight without giblets (WOG), front-half carcass, hind-half carcass, breast, tenders, wings, drums, thighs, frame, back, abdominal fat pad and skin. The remaining broilers in the houses were taken to the Pilgrims' processing plant and slaughtered for commercial distribution. The yield study was to determine if protease addition in broiler diet had any effect on whole carcass, and retail cuts weight.

Figure 5: Step (1) in the processing procedure. The Pulsed DC Poultry Stunner from Executrol Systems

Figure 6: The Steps of the processing procedure from Step (2) to Step (6)

Nitrogen study

A. Preparation of sample

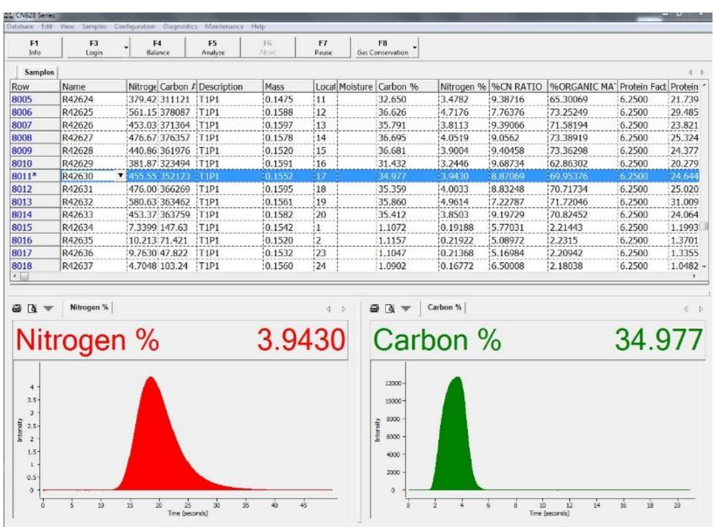
Fecal matter samples and litter samples including used bedding materials consisting of wood shaving and fecal matter from previous trials were taken with 12 replicates for each treatment at four intervals during the study on days 1, 12, 32, and 49. Days 12 and 32 represented a day before the transition of the starter feed phase to grower feed phase, and switching from grower feed phase to finisher feed phase respectively. Samples were taken at the end of each feeding phase plus the first day of the trial. We picked those sample dates to investigate the effect of each diet during the feeding phases. The samples were air dried at room temperature (approximately 20 C^{o}) until dry. All samples were ground to a particle size less than 2mm.

B. Nitrogen Analysis

Samples were analyzed using a Leco CN628 instrument for total Carbon/Nitrogen content by combustion (Figures 7a &7b). Instrument was set for operating parameters (oven temperature, oxygen flow, helium flow, calibration values, etc.) according to the method of application (LECO CN628 Manual). The furnace of the instrument was allowed to reach the operating temperature (950^{o} C), and then allowed to stabilize. The fecal matter, chicken litter, and feed were then weighed to 150-175 mg into a tared combustion foil cup and transferred into a loading carousel on top of the instrument. The samples were analyzed to compare the proportion of nitrogen on the first day with the remaining samples, as well as the nitrogen proportion in the (PC)control diet with diet number 3, and (NC) control diet with diet number 4. Also, the proportion of nitrogen in feed compared to the chicken litter and fecal matter to calculate the amount of nitrogen utilized in the body and the amount of nitrogen excreted outside the body.

Figure 7a: The LECO CN628 Carbon/ Nitrogen Analyzer

Figure 7b: Carbon and Nitrogen Detected graphs by spectral and thermal detector



Statistical Analyses

Data collected from the study were analyzed using the Statistical Analysis System (SAS 9.2). The data were interpreted using one-way analysis of variance (ANOVA). Differences were accepted as significant at $p<0.05$. Dependent variables of performance and yield data were analyzed according to the independent variables of treatment and block in separate ANOVA tables. The significant differences were identified using Duncan's Multiple Range Test, and paired t Test (LSD) when overall ANOVA was significant.

CHAPTER IV

Results and Discussion

At the completion of the study, all data collected during the study was evaluated. The following is a compilation of the results determined from this research trial. As stated previously, treatment 1 was used as a positive control (Pilgrim's Standard Basal diet) in starter, grower, and finisher feed phases as shown in appendixes (A, E, and I) respectively. Treatment 2 was used as a negative control (Pilgrim's Diet with Protease Matrix removed only the amino acids' credit – no energy credit) in starter, grower, and finisher feed phases as shown in appendixes (B, F, and J) respectively. Treatment 3 was positive control + protease as shown in appendixes (D, G, and K) respectively. Treatment 4 was negative control + protease as shown in appendixes (C, H, and L) respectively.

PERFORMANCE PARAMETERS

Average Body Weight and Feed Conversion parameters

Average body weight was measured on multiple occasions throughout the study. Days 1, 13, 33, and 49 were chosen as they were the intervals that the broilers switched diets. Birds were weighed on Day 1 to compare the trial pens in order to minimize differences between treatment groups. At day 13, the chickens had finished their consumption of starter diets and were switched to a grower diet. At day 33, they switched from grower diets to finisher diets. At day 49, all feed was removed as the birds were prepared for processing.

There was no difference at day 1 among treatments as shown in (Table3). At day 13 and 33, no significant differences were seen in average body weight between the four treatments (Tables 4 and 5). By day 49, there was significant difference seen in body weight (Table 5). Specifically, treatment # 3 showed higher mean body weight (6.48 lb.) when compared to the other treatments (Table 7).

Table 3: ANOVA Table for Average Body Weight Day 1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.00502674	0.00021855	5.47	<.0001*
Treatment	3	0.00005828	0.00001943	0.49	0.6931
Model	26	0.00508502	0.00019558	4.89	<.0001*
Error	69	0.00275797	0.00003997		
Total	95	0.00784299			

*Significant at the (0.05) level of probability.

Table 4: ANOVA Table for Average Body Weight Day 13

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.08277100	0.00359874	2.17	0.0073*
Treatment	3	0.00532892	0.00177631	1.07	0.3677
Model	26	0.08809992	0.00338846	2.04	0.0099*
Error	69	0.11456858	0.00166041		
Total	95	0.20266850			

*Significant at the (0.05) level of probability.

Table 5: ANOVA Table for Average Body Weight Day 33

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.27938862	0.01214733	0.86	0.6526
Treatment	3	0.10152313	0.03384104	2.38	0.0767
Model	26	0.38091175	0.01465045	1.03	0.442
Error	69	0.97963687	0.01419764		
Total	95	1.36054862			

*Significant at the (0.05) level of probability.

Table 6: ANOVA Table for Average Body Weight Day 49

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	23	0.57308691	0.02491682	1.04	0.4370
Treatment	3	0.34078645	0.11359548	4.72	0.0047*
Model	26	0.91387335	0.03514898	1.46	0.1080
Error	69	1.66061630	0.02406690		
Total	95	2.57448966			

*Significant at the (0.05) level of probability.

Table 7: Duncan's Multiple Range Test for Average Body Weight Day 49

Duncan Grouping	Mean	N	Treatment
A	6.48	24	3
B	6.36	24	1
B			
B	6.34	24	4
B			
B	6.34	24	2

*Means with the same letter are not significantly different.
*Alpha 0.05
*Error Degrees of Freedom 69
*Error Mean Square 0.02406

Table 8: Average body weight for day s1, 13, 33, and 49 & Feed Conversion Ratio and Adjusted Feed Conversion Ratio for day 49

	Average Body Weight Lbs.					
Treatment	Day 1	Day 13	Day 33	Day 49	FCR	AFCR
TX 1 (PC)	0.08	0.79	3.22	6.36	1.84	1.65
TX 2 (NC)	0.08	0.77	3.30	6.34	1.85	1.67
TX 3 (PC + Protease)	0.08	0.78	3.23	6.48*	1.85	1.64
TX 4 (NC + Protease)	0.08	0.78	3.28	6.34	1.85	1.67

*Significant at the 0.05 level of probability

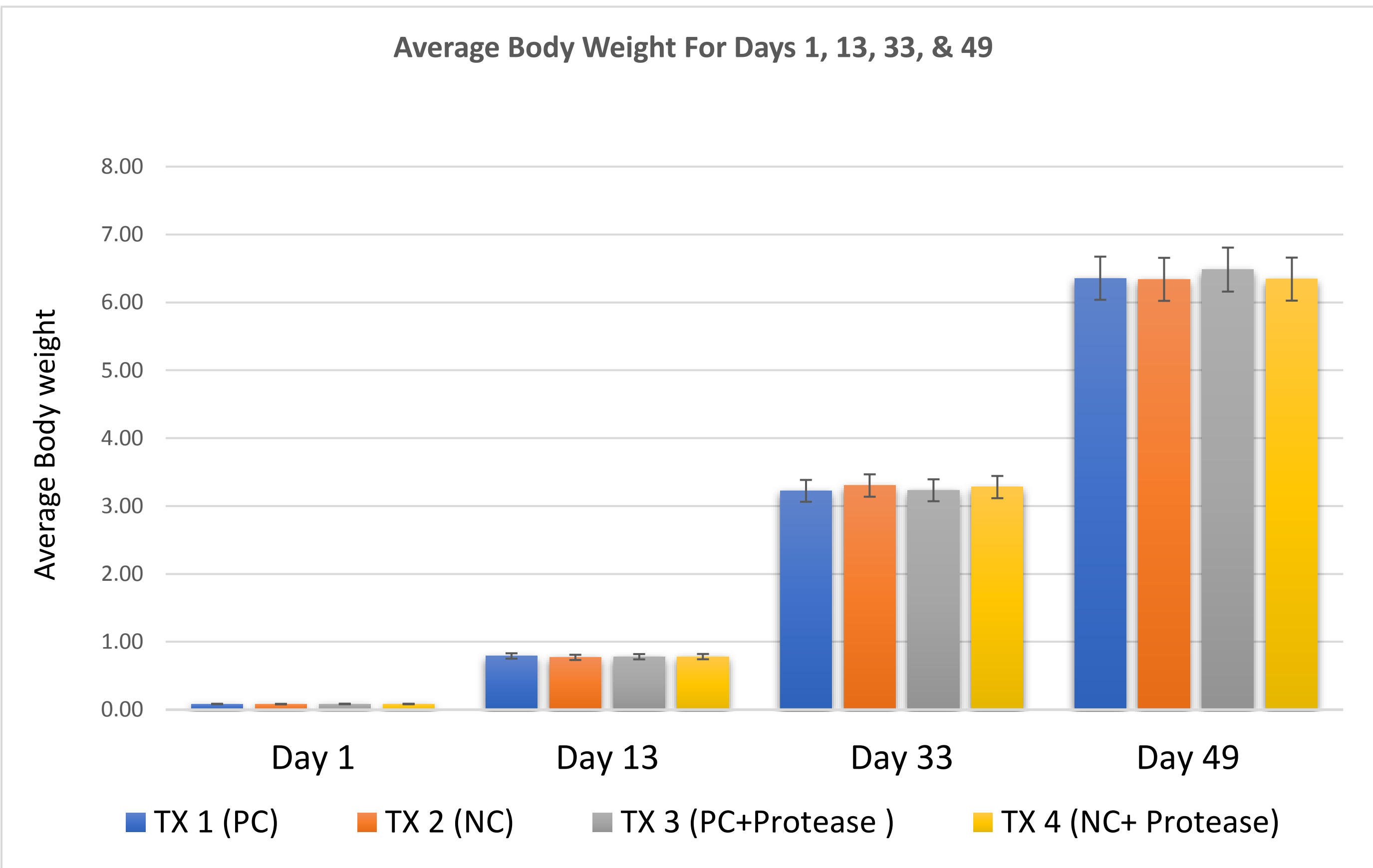


Figure 8: Average Body Weight by Treatment for Days 1, 13, 33, and 49

Feed Conversion Ratio (FCR) & Adjusted Feed Conversion Ratio (AFCR)

Feed Conversion Ratio= Total Feed Consumed/Pen Total Body weight

Adjusted Feed Conversion Ratio:

(Actual Average Body Weight - 6)/7 = X1

Actual Feed Conversion ratio - X1) = X2

(X2 * 1450 average kcal of all diets) / 1,500 standard kcal = Adjusted Feed Conversion for Body Weight.

There were no significant differences (p >0.05) among the treatments for feed conversion ratio (FCR) and adjusted feed conversion ratio (AFCR) (Tables 8, 9). However, AFCR values are slightly different from each other between treatments (Figure 9). Table 8 shows that treatment 3 has the lowest AFCR. AFCR adjusts the feed efficiency of the birds for an equal body weight of 6 lbs. Since treatment 3 had the highest average body weight that shows the lowest feed conversion when all treatments are adjusted to the same body weight.

Table 9: ANOVA Table for Feed Conversion Ratio Day 49

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	23	0.05040583	0.00219156	0.92	0.5688
Treatment	3	0.00309967	0.00103322	0.44	0.7283
Model	26	0.05350550	0.00205790	0.87	0.6483
Error	69	0.16371583	0.00237269		
Total	95	0.21722133			

*Significant at the (0.05) level of probability.

Table 10: ANOVA Table for Adjusted Feed Conversion Ratio Day 49

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	23	0.05816896	0.00252909	0.84	0.6701
Treatment	3	0.00908088	0.00302696	1.01	0.3951
Model	26	0.06724983	0.00258653	0.86	0.6572
Error	69	0.20744113	0.00300639		
Total	95	0.27469096			

*Significant at the (0.05) level of probability.

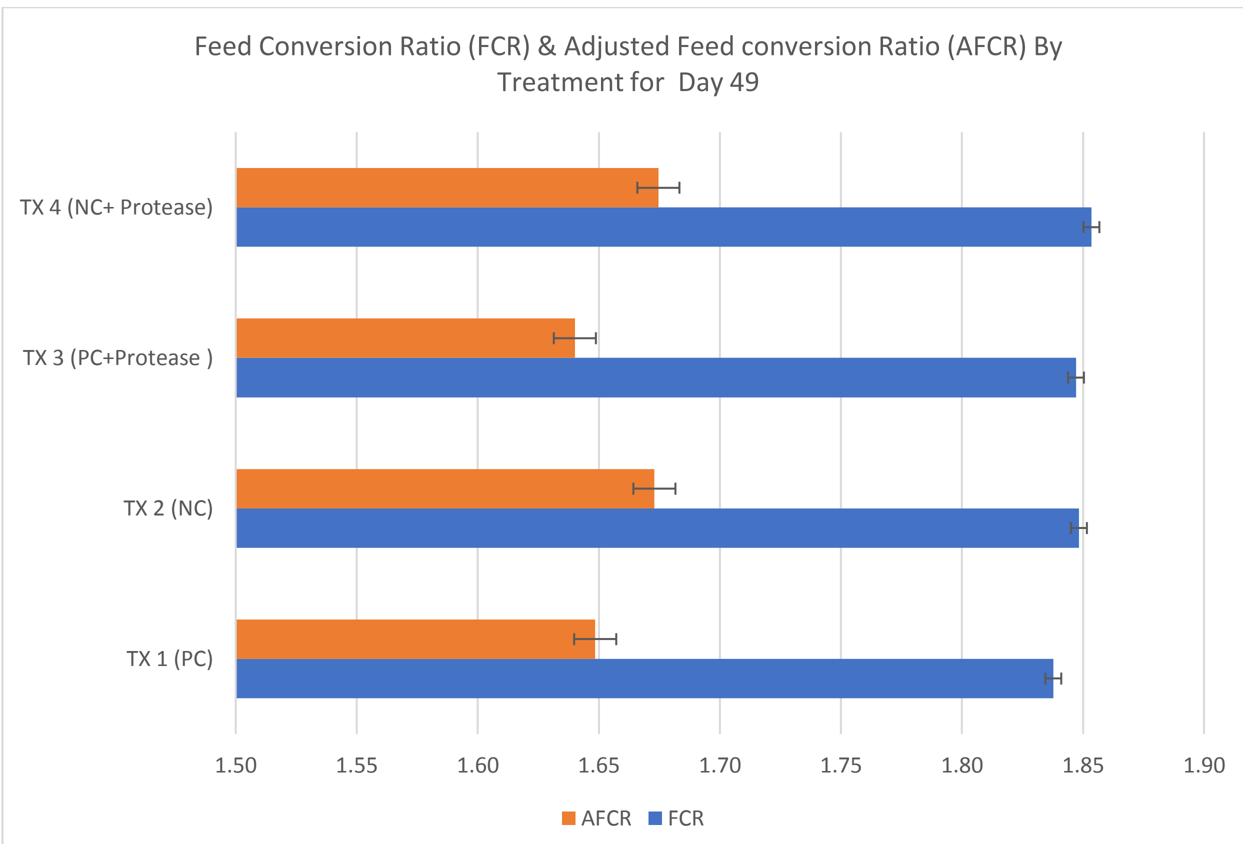


Figure 9: Feed Conversion Ratio (FCR) and Adjusted Feed Conversion Ratio (AFCR) by Treatment

Yield Study

No significant difference was observed for average live weights of the sample birds processed among all treatments (Table 11). Treatment 3 had the highest body weight among the treatments similar to the average body weight per pen at the day 49. This shows there was no selection bias within selecting sample birds. Furthermore, no significant differences were seen in the retail cuts (WOG, fat Pad, front half, hind half, frame, wings, tenders, drums, thighs, back, skin, and brest) among treatments (p >0.05) as shown in (Tables 12-25). Treatment 3 had the highest breast weight, while treatment 4 had the lowest breast weight (Table 11). Treatment 3, PC + Protease, was consistently higher in average live weight, fat pad, front half, hind half, frame, breast, and skin compared to other treatments (Table 11).

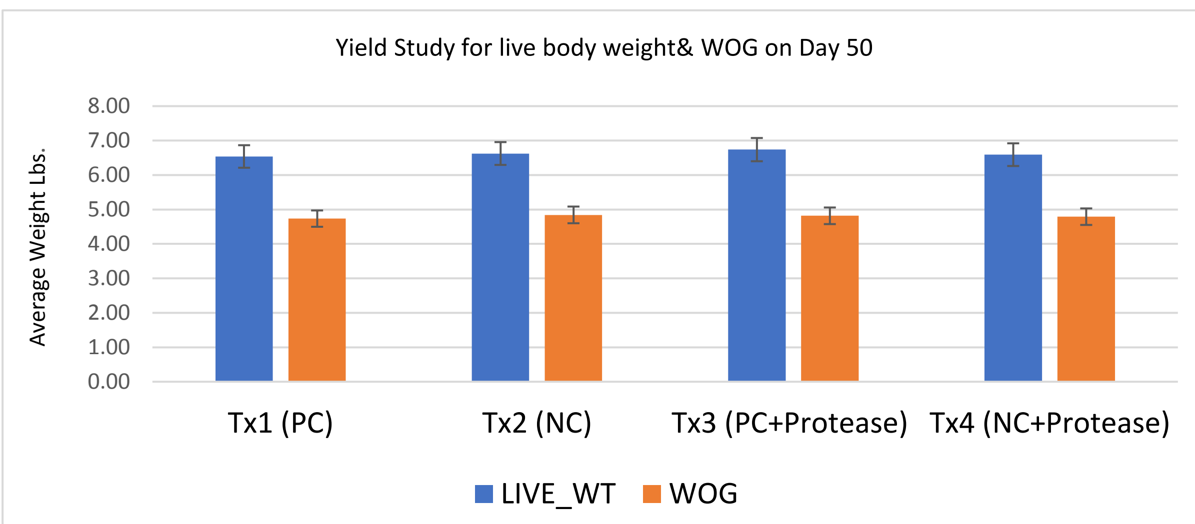


Figure 10: Yield Study for Live Weight & Weight without Giblet (WOG) on Day 50

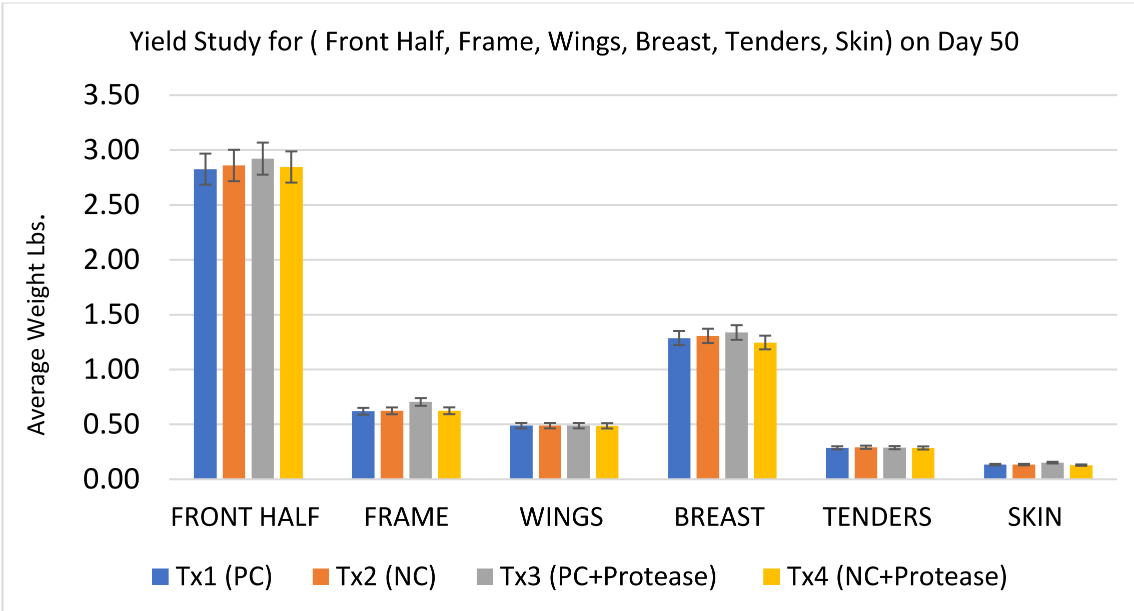


Figure 11: Yield Study for (Front Half, Frame, Wings, Breast, Tenders, Skin) on Day 50

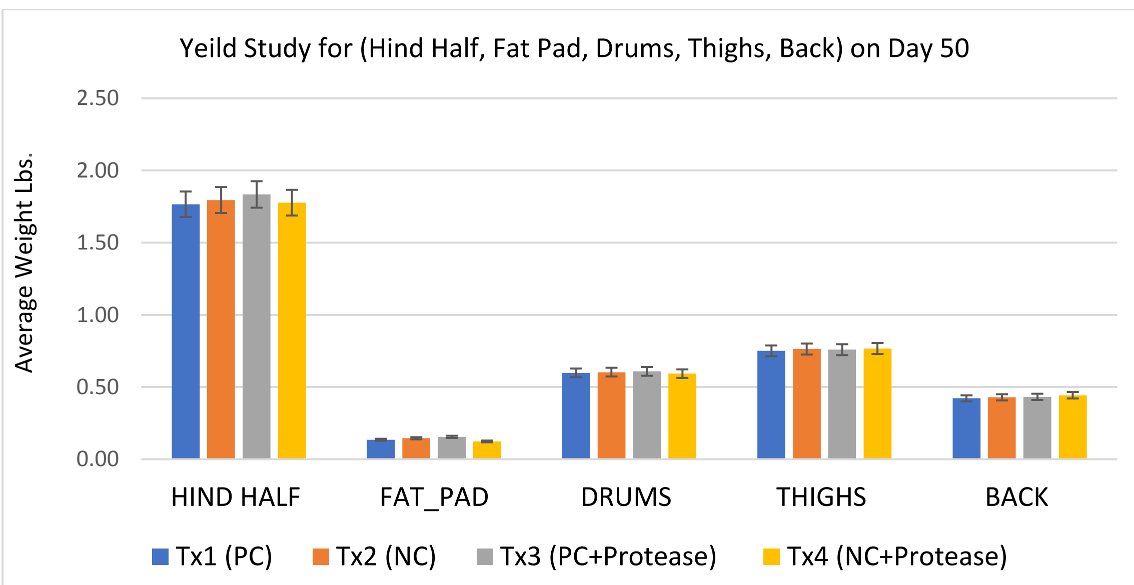


Figure 12: Yield Study for (Hind Half, Fat Pad, Drums, Thighs, Back) on Day 50

Table 11: Yield Data Result by Treatments on Day 50

Retail Cuts	Treatments			
	Tx1 (PC)	Tx2 (NC)	Tx3 (PC+Protease)	Tx4 (NC+Protease)
LIVE WEIGHT	6.54	6.62	6.74	6.59
WOG	4.73	4.84	4.82	4.79
FAT PAD	0.13	0.15	0.15	0.12
FRONT HALF	2.83	2.86	2.92	2.85
HIND HALF	1.77	1.80	1.83	1.78
FRAME	0.62	0.62	0.70	0.62
WINGS	0.49	0.49	0.49	0.49
BREAST	1.29	1.31	1.34	1.25
TENDERS	0.29	0.29	0.29	0.29
SKIN	0.13	0.13	0.15	0.13
DRUMS	0.60	0.60	0.61	0.59
THIGHS	0.75	0.76	0.76	0.77
BACK	0.42	0.43	0.43	0.44

*Significant at the (0.05) level of probability

Table 12: ANOVA Table for live body weight

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	9.25053448	0.40219715	1.13	0.3049
Treatment	3	1.95673510	0.65224503	1.84	0.1396
Model	26	11.2058998	0.4309961	1.22	0.2178
Error	342	121.2130221	0.3544240		
Total	368	132.4189220			

*Significant at the (0.05) level of probability.

Table 13: ANOVA Table for WOG

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	4.79644851	0.20854124	0.92	0.5691
Treatment	3	0.45983230	0.15327743	0.68	0.5663
Model	26	5.25815555	0.20223675	0.89	0.6177
Error	340	76.91269744	0.22621382		
Total	366	82.17085300			

*Significant at the (0.05) level of probability.

Table 14: ANOVA Table for Thighs

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	23	0.23867402	0.01037713	1.10	0.3429
Treatment	3	0.00433762	0.00144587	0.15	0.9276
Model	26	0.24305078	0.00934811	0.99	0.4799
Error	340	3.20805494	0.00943546		
Total	366	3.45110572			

*Significant at the (0.05) level of probability.

Table 15: ANOVA Table for Back

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.06943377	0.00301886	0.75	0.7973
Treatment	3	0.01659733	0.00553244	1.37	0.2529
Model	26	0.08590851	0.00330417	0.82	0.7264
Error	340	1.37667732	0.00404905		
Total	366	1.46258583			

*Significant at the (0.05) level of probability.

Table 16: ANOVA Table for Fat-Pad

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.26259905	0.01141735	0.98	0.4936
Treatment	3	0.04666839	0.01555613	1.33	0.2637
Model	26	0.31158408	0.01198400	1.03	0.4313
Error	338	3.94638030	0.01167568		
Total	364	4.25796438			

*Significant at the (0.05) level of probability.

Table 17: ANOVA Table for Front Half

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	2.99347971	0.13015129	1.73	0.0215
Treatment	3	0.34022479	0.11340826	1.50	0.2133
Model	26	3.32312634	0.12781255	1.70	0.0199
Error	340	25.63748735	0.07540437		
Total	366	28.96061369			

*Significant at the (0.05) level of probability.

Table 18: ANOVA Table for Hind Half

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.69634970	0.03027607	0.68	0.8630
Treatment	3	0.13086036	0.04362012	0.98	0.4007
Model	26	0.83107875	0.03196457	0.72	0.8417
Error	340	15.08088637	0.04435555		
Total	366	15.91196512			

*Significant at the (0.05) level of probability.

Table 19: ANOVA Table for Drums

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.12984388	0.00564539	0.97	0.5057
Treatment	3	0.00397550	0.00132517	0.23	0.8773
Model	26	0.13358002	0.00513769	0.88	0.6354
Error	340	1.98127938	0.00582729		
Total	366	2.11485940			

*Significant at the (0.05) level of probability.

Table 20: ANOVA Table for Frame

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	23	2.37483295	0.10325361	1.01	0.4552
Treatment	3	0.37010928	0.12336976	1.20	0.3084
Model	26	2.72130249	0.10466548	1.02	0.4385
Error	333	34.13353617	0.10250311		
Total	359	36.85483866			

*Significant at the (0.05) level of probability.

Table 21: ANOVA Table for Wings

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.95064809	0.04133253	0.87	0.6456
Treatment	3	0.19656177	0.06552059	1.37	0.2511
Model	26	1.14475537	0.04402905	0.92	0.5774
Error	339	16.18716157	0.04774974		
Total	365	17.33191694			

*Significant at the (0.05) level of probability.

Table 22: ANOVA Table for Tenders

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.04621170	0.00200920	1.22	0.2201
Treatment	3	0.00151478	0.00050493	0.31	0.8198
Model	26	0.04805518	0.00184828	1.13	0.3076
Error	336	0.55118665	0.00164044		
Total	362	0.59924182			

*Significant at the (0.05) level of probability.

Table 23: ANOVA Table for Skin

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.20240186	0.00880008	1.69	0.0262
Treatment	3	0.02349270	0.00783090	1.50	0.2136
Model	26	0.22617996	0.00869923	1.67	0.0231
Error	331	1.72446396	0.00520986		
Total	357	1.95064392			

*Significant at the (0.05) level of probability.

Table 24: ANOVA Table for Breast

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	23	0.63867998	0.02776869	1.05	0.3969
Treatment	3	0.20155448	0.06718483	2.55	0.0557
Model	26	0.84373712	0.03245143	1.23	0.2046
Error	338	8.90741098	0.02635329		
Total	364	9.75114810			

*Significant at the (0.05) level of probability.

Table 25: Duncan's Multiple Range Test for Breast

Duncan Grouping	Mean	N	Treatment
A	1.33733	91	3
A			
B A	1.32100	90	2
B A			
B A	1.30086	93	1
B			
B	1.27374	91	4

*Means with the same letter are not significantly different
*Alpha 0.05
*Error Degrees of Freedom 338
*Error Mean Square 0.026353

Nitrogen Retention in Fecal Matter & Litter.

Fecal matter samples and litter samples were taken with 12 replicates for each treatment at four intervals during the study on days 1, 12, 32, and 49. Days 12 and 32 represented a day before the transition of the starter feed phase to grower feed phase, and switching from grower feed phase to finisher feed phase respectively. Samples were taken at the end of each feeding phase plus the first day of the trial. No significant difference in nitrogen retention was observed in chicken litter samples at days 1, 12, 32, and 48 among all treatments ($p > 0.05$), (Tables 26 to 29, Figure 13). Day 1 litter samples were used as starting baseline since the litter had birds previously grown on it. Nitrogen dropped constantly through days 12, 32, and 48.

Table 26: ANOVA Table for Chicken Litter Nitrogen Retention on Day 1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	17	3.90941021	0.22996531	0.89	0.5942
Treatment	3	0.53622687	0.17874229	0.69	0.5668
Model	20	4.44988521	0.22249426	0.86	0.6341
Error	27	7.00730646	0.25952987		
Total	47	11.45719167			

*Significant at the (0.05) level of probability.

Table 27: ANOVA Table for Chicken Litter Nitrogen Retention on Day 12

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	17	0.29980744	0.01763573	0.94	0.5381
Treatment	3	0.15204911	0.05068304	2.71	0.0645
Model	20	0.48576369	0.02428818	1.30	0.2588
Error	27	0.50423422	0.01867534		
Total	47	0.98999792			

*Significant at the (0.05) level of probability.

Table 28: ANOVA Table for Chicken Litter Nitrogen Retention on Day 32

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	17	0.56350887	0.03314758	0.90	0.5784
Treatment	3	0.16327554	0.05442518	1.48	0.2418
Model	20	0.73669845	0.03683492	1.00	0.4892
Error	27	0.99193280	0.03673825		
Total	47	1.72863125			

*Significant at the (0.05) level of probability.

Table 29: ANOVA Table for Chicken Litter Nitrogen Retention on Day 48

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	17	1.06287815	0.06252224	1.73	0.0989
Treatment	3	0.04590315	0.01530105	0.42	0.7380
Model	20	1.09462815	0.05473141	1.51	0.1562
Error	27	0.97643852	0.03616439		
Total	47	2.07106667			

*Significant at the (0.05) level of probability.

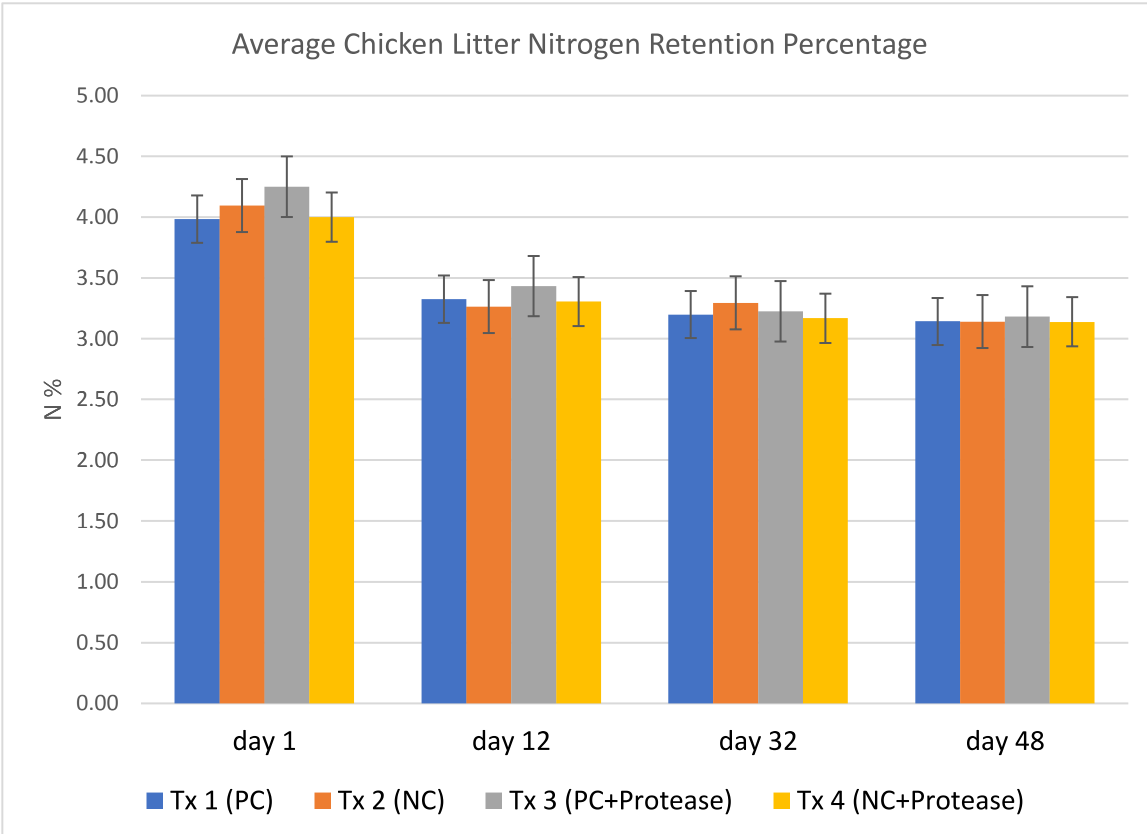


Figure 13: Average Chicken Litter Nitrogen Retention Percentage

Fecal matter samples were analyzed for N content, and there was no significant difference observed in days 1 and 32 among all treatments (Tables 30 & 33). However, there was a significant difference observed among treatments in N content for fecal matter on day 12 (Table 31). Treatments 1 with a 3.51 % N had the lowest nitrogen retention, and treatment 3 with 3.82 % N had the highest nitrogen retention. On day 48 there was also a significant difference observed among treatments as shown in Table 33. Treatment 4 NC + Protease had the lowest nitrogen retention which coincides with Yamazaki et al (2002) finding (Figure 14). Average feed matter nitrogen retention can be seen across treatments in Table 36.

Table 30: ANOVA Table for Fecal Matter Nitrogen Retention Day1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	17	7.75834189	0.45637305	1.39	0.2141
Treatment	3	2.18900855	0.72966952	2.23	0.1076
Model	20	9.94339189	0.49716959	1.52	0.1539
Error	27	8.83417478	0.32719166		
Total	47	18.77756667			

*Significant at the (0.05) level of probability.

Table 31: ANOVA Table for Fecal Matter Nitrogen Retention Day12

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	17	1.14132227	0.06713660	1.62	0.1272
Treatment	3	0.63550560	0.21183520	5.12	0.0062*
Model	20	1.59109519	0.07955476	1.92	0.0569
Error	27	1.11775273	0.04139825		
Total	47	2.70884792			

*Significant at the (0.05) level of probability.

Table 32: t Tests (LSD) for Fecal Matter Nitrogen Retention Day 12

t Grouping	Mean	N	Treatment
A	3.82333	12	3
A			
A	3.75750	12	2
A			
B A	3.72917	12	4
B			
B	3.56083	12	1

*Means with the same letter are not significantly different.
*Alpha 0.05
*Error Degrees of Freedom 27
*Error Mean Square 0.041398

Table 33 ANOVA Table for Fecal Matter Nitrogen Retention Day32

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	17	1.38812946	0.08165467	0.76	0.7157
Treatment	3	0.38846280	0.12948760	1.21	0.3250
Model	20	1.67082946	0.08354147	0.78	0.7130
Error	27	2.88947054	0.10701743		
Total	47	4.56030000			

*Significant at the (0.05) level of probability.

Table 34: ANOVA Table for Fecal Matter Nitrogen Retention Day48

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	17	2.72709295	0.16041723	3.05	0.0047
Treatment	3	0.47046795	0.15682265	2.98	0.0489*
Model	20	2.97749920	0.14887496	2.83	0.0063
Error	27	1.42019872	0.05259995		
Total	47	4.39769792			

*Significant at the (0.05) level of probability.

Table 35: t Tests (LSD) for Fecal Matter Nitrogen Retention Day 48

t Grouping	Mean	N	Treatment
A	3.82083	12	1
A			
B A	3.77833	12	3
B A			
B A	3.74833	12	2
B			
B	3.62667	12	4

*Means with the same letter are not significantly different.
*Alpha 0.05
*Error Degrees of Freedom 27
*Error Mean Square 0.0526

Table 36: Average Feed Matter % N

Feed % N	Tx1	Tx2	Tx3	Tx4
Starter	3.77	2.96	3.80	3.84
Grower	2.90	3.24	3.37	3.15
Finisher	2.98	2.72	2.81	2.96

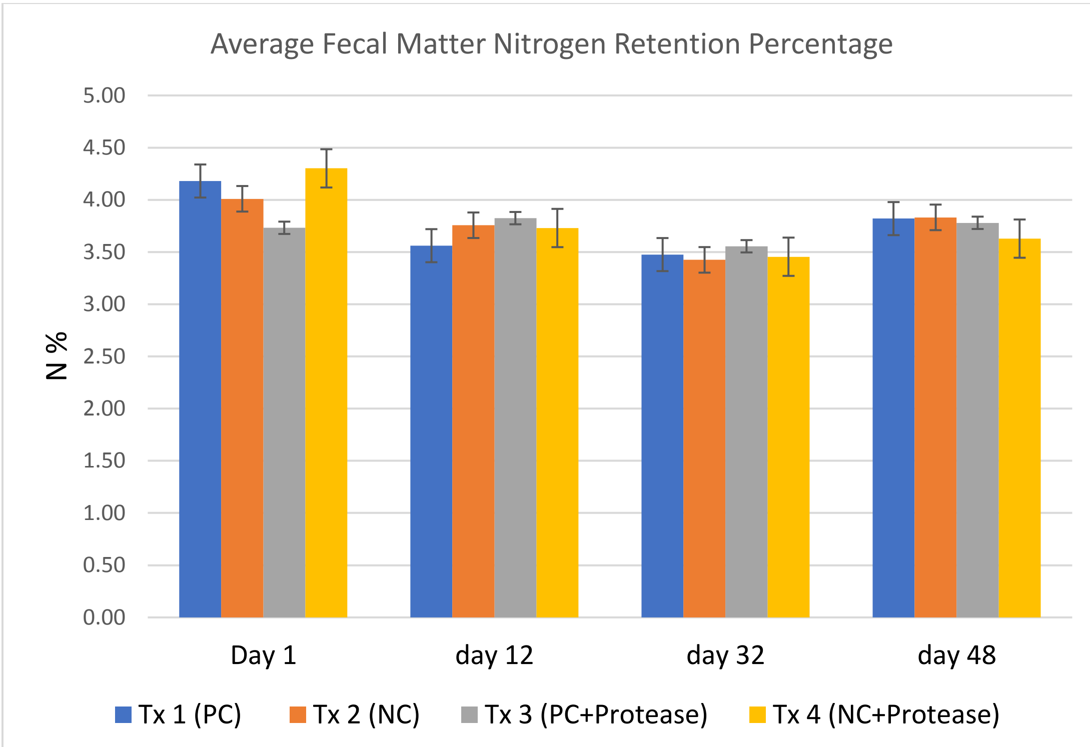


Figure 14: Average Fecal Matter Nitrogen Retention Percentage

CONCLUSION

The results from this research demonstrates that the addition of protease on top of a diet with a complete protein matrix (treatment 3) significantly increased average body weight over a 49 days rearing period. The addition of protease on the negative control (NC) was not beneficial, treatment #2 (NC) had the lowest body weight. As result, the only difference in the average body weight among treatments was in treatment 3 on day 49, suggesting a positive influence of protease on the top of the protein matrix had the highest effect on growth performance.

. If we subtract treatment 1 mean body weight from treatment 3:

6.48 lb. – 6.36 lb. = 0.12 lb.

the difference is (0.12 lb.). This represents the improvement seen from protease inclusion in broiler diets within a complete protein matrix. This amount of performance improvement can be considering significant to the commercial poultry industry. If we multiply the difference of the average body weight by the number of birds in a whole flock as seen below:

0.12 lb. of body weight increase * 20,000 birds/flock = 2,400 lb. of additional live body weight

However, if we multiply the difference by the Pilgrim's total production in east Texas which is (4,000,000 birds/week)

0.12 lb. * 4,000,000 birds = 480,000 lbs./week

Furthermore, with 72% average carcass dressing percentage the additional 0.12 lbs. of body weight can be a tremendous increase in meat yield across the industry. This result coincides with numerous researchers' findings (Buttin et al., (2016), Liu et al., (2013), Kamel et.al, (2015)). The inclusion of protease in this study had no significant effect on FCR & AFCR among treatments and feed phases. However, with FCR & AFCR relatively similar among treatments, the increase in body weight comes with no adverse effects to feed efficiency. For all yield data, the protease inclusion had no significant effect on any of the retail parts weights.

No significant difference was observed in chicken litter nitrogen retention at days 1, 12, 32, and 48 among all treatments. Also, for fecal matter, there was no significant difference observed in days 1 and 32 among all treatments. Fecal matter N retention at day 12 showed a significant difference among treatments. Treatment 1 is significantly lower than treatments 2&3, but not significantly lower than treatment 4. Treatment 1 that had lowest nitrogen retention maybe because the digestive system of the birds was not effectively responsive to the effect of the enzyme. On the other hand, on day 48 treatment 4 is significantly lower than treatment 1, but not significantly different from treatments 2&3, which is indicates that the addition of protease in place of protein matrix (low protein diet) had a significant effect to reduce the nitrogen retention in fecal matter which coincides with Yamazaki et al (2002). As a result, we can say that the addition of protease on top of broiler standard diet has no effect on reducing nitrogen excretion in the fecal matter.

In conclusion, the result from the study showed that addition of protease in top of a diet with a complete protein matrix significantly increased the body weight. However, the protease inclusion had no significant effect on FCR, AFCR, retail cuts, litter N retention, and fecal matter N retention.

BIBLIOGRAPHY

Angel, R., W. Powers, S. Zamzow, and T. Applegate. (2006). "*Dietary modifications to reduce nitrogen consumption and excretion in broilers*". Poult. Sci. 85(Suppl. 1):25.

Angel, C.R., Saylor, w., Vieira, S.L., and Ward, N. (2011). "*Effects of a monocomponent. protease on performance and protein utilization in 7- to 22-day-old broiler chickens*". Metabolism and Nutrition. Department of Animal and Avian Sciences, University of Maryland.

Applegate, T., Angel, R. (2008). "*Protein and Amino Acid Requirements for Poultry".* NRCS, USDA.

Buttin, P., Yan, F., Dinbner, J., Knight, C.D., Vazquez-Anon, M., Odetallah, N., Carter, S. (2016). "*Effect of dietary protein and protease supplementation on performance and gut health of broiler chicks*" NOVUS.

Barekatain, M. R., Antipatis, C., Walkden-Brown S.W., Iji, P. A., and Choct, M. (2013). "*Evaluation of high dietary inclusion of distillers dried grains with solubles andsupplementation of protease and xylanase in the diets of broiler chickens under necrotic enteritis challenge*." School of Environmental and Rural Science, University of New England, Armidale, NSW, Australia. Poultry Science 92 :1579–1594.

Bailey, C., Cantor, A. (2016). *"POULTRY NUTRITION".* Poultry Science Manual, 6th Edition. 13 pages. Texas A&M Poultry Science Department.

Bedford, M., and Walk C., (2014). "*Enzymes and their effect on amino acid nutrition".* AB Vista Feed Ingredients, Marlborough, Wilts UK.

Bregendahl, K., Sell L., and Zimmerman R., (2002). "*Effect of low protein diets on performance and body composition of broiler chicks*". Poult. Sci. 81:1156 –1167.

Cowieson, A. J., Singhi, D. N., and Adeola, O. (2006). "*Prediction of ingredient quality and the effect of a combination of xylanase, amylase, protease and phytase in the diets of broiler chicks. 2. Energy and nutrient utilization".* British Poultry Science Volume 47, Number 4, pp. 490—500. Campbell G.L., Bedford M.R. (1991). "*Enzyme applications for monogastic feeds*". Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Dale, Nick (2009). "*A Few Basic Points about Protein*". The Poultry Site. De Vries, W., Kros, J., Oenema, O. & de Klein, J. **(**2003). "*Uncertainties in the fate of nitrogen II: a quantitative assessment of the uncertainties in major nitrogen fluxes in the Netherlands*". Nutrient Cycling in Agroecosystems, 66(1): 71–102.

Dozier, W.A., III, M.T. Kidd, and A. Corzo. (2008). "*Amino acid responses of broilers*". J. Appl. Poult. Res. 17:157-167.

Ferguson, N.S., R.S. Gates, J.L. Taraba, A.H. Cantor, A.J. Pescatore, M.L. Straw, M.J.Ford, and D.J. Burnham, (1998). "*The effect of dietary protein and phosphorus on ammonia concentration and litter composition in broilers*". Poultry Sci. 77:1085-1093.

FAO. (2006). "*World agriculture: towards 2030/2050 interim report"*. Rome.

Freitas, D. M., Vieira, S.L., Angel, C.R., Favero, A., Maiorka, A., (2011). "*Performance and nutrient utilization of broilers fed diets Supplemented with a novel mono component protease*". J. Appl. Poult. Res. 20, 347–352.

Fru-Nji, F., Kluenter, A.M., Fischer, M., Pontoppidan, K., (2011). "*A feed serine protease improves broiler Performance and energy digestibility*". The Journal of Poultry Science, vol. 48, No. 10, 239–246.

Group, E. (2013). "*The Health Benefits of Protease*". Global Health Center.

Gitoee, A., Janmohammadi, H., Taghizadeg, A., Rafat, S.A. (2015). "*Effects of multi-enzyme on performance and carcass characteristics of broiler chickens fed corn-soybean meal basal diets with different metabolizable energy levels*". Journal of Applied Animal Research, 2015 Vol. 43, No. 3, 295–302.

Gerber, P., Opio, C., Steinfeld, H. (2015). "*Poultry production and the environment – a Review". Poultry in the 21st Century*. Animal Production and Health Division, Food and Agriculture Organization of the United Nations.

Ghazi, S., Rooke, T.A., Galbraith, H. & Morgan, A. (1997a). "*The potential for improving soya-bean meal in diets for chickens: treatment with different proteolytic enzymes*". British Poultry Science, **37:** 554–555.

Ghazi, S., Rooke, J.A., Galbraith H. & Bedford, M.R. (2010b). "*The potential for the improvement of the nutritive value of soya-bean meal by different proteases in broiler chicks and broiler cockerels".* British Poultry Science Journal. Pp. 70-77.

Hassan, A., Abd-Elsamee, O., El-Sherbiny, E., Samy, A., and Mohamed, A. (2011). "*Effect of Protein Level and Avizyme Supplementation on Performance, Carcass Characteristic, and Nitrogen Excretion of Broiler Chicks*" American-Eurasian J. Agric.& Environ. Sci., 10(4):551-560,2011

Jacob, J. (2015). "*Antinutritional Factors in Feed Ingredients*". University of Kentucky. eXtension. extension.org.

Kamel, N.F., Naela, Ragaa M., El-Banna, R.A., Mohamed, F.F. (2015). "*Effects of a Monocomponent Protease on Protease on Performance Parameters and Protein Digestibility in Broiler Chickens.*" Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University, Egypt. Agriculture and Agricultural Science Procedia 6 (2015) 216 – 225. www.sciencedirect.com. Elsevier B.V.

Kocher, A., Choct, M., Ross, G., Broz, J., Chung, T., (2003). "*Effects of enzyme combinations on apparent metabolizable energy of corn-soybean meal-based diets in broilers*". *School of Rural Science and Agriculture, University of New England, Armidale, NSW 2351, Australia In: pp. 275-283.

Kalmendal, R., (2012). "*Effects of a xylanase and protease, individually or in combination, and an ionophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet*". Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, S-75323 Uppsala, Sweden.

Liu, S., Selle, P., Court, S., Cowieson, A., (2013). "*Protease supplementation of sorghum based diets enhances amino acid digestibility coefficients in four small intestinal sites and accelerates their rates of digestion*". Animal Feed Science and Technology. In.

LECO CN628 Manual. Version 1.3x, Part Number 200-747 (2014). LECO Corporation. www.leco.com.

Mótyán J., Tóth F., and Tőzsér J., (2013). "*Research Applications of Proteolytic Enzymes in Molecular Biology".* US National Library of Medicine National Institutes of Health. Biomolecules Journal 2013 Dec; 3(4): 923-942.

Madrid, J., Catalá-Gregori, P., García, V., Hernández, F. (2010). *"Effect of a Multienzyme complex in wheat-soybean meal diet on digestibility of broiler chickens under different rearing conditions."* Italian Journal of Animal Science 2010; volume 9: e.

Marsman, G.J.P., Gruppen, H., Vander P., Kwakkel B., Verstegen P., Voragon J. (1997). "*The effect of thermal processing and enzyme treatments of soybean meal on growth performance, ileal nutrient digestibility, and chyme characteristics in broiler chicks*". *Poultry Science*, **76:** 864–872.

Pope, T., L.N. Loupe, P.B. Pillai, and J.L. Emmert. (2004). "*Growth performance and nitrogen excretion of broilers using a phase feeding approach from twenty-one to sixty-three days of age*". Poult. Sci. 83:676-682.

Smith, A., (2015). "*Protease reduces environmental impact of broiler production".* Feed Enzymes EMEA Animal Nutrition & Health DSM. Poultry world.

Spiehs, M. (2005). “*Nutritional and Feeding Strategies to Minimize Nutrient Losses in Livestock Manure*”. The College of Agriculture, Food and Environmental Science, University of Minnesota.

Oxenboll, K.M., Pontoppidan, K., and Fru-Nji, F. (2011). “*Use of a Protease in Poultry Feed Offers Promising Environmental Benefits”.* International Journal of Poultry Science 10 (11): 842-848.

Rogers, k. (2015). “*Proteolytic enzyme*”. Encyclopædia Britannica.

Rackis, J., Gumbmann, R. (2014). *“Protease Inhibitors: Physiological Properties and Nutritional Significance”* Northern Regional Research Center Agricultural Research Science and Education Administration. USDA.

Smits. C., Annison G. (1996). “*Non-starch plant polysaccharides in broiler nutrition – towards a physiologically valid approach to their determination*”. World's Poultry Science Journal.

Sutton, M. & Fowler, D. (1995). “*Atmospheric deposition of nitrogen compounds to heathlands*”. *Aarhus Geoscience,* 4, 61–71.

SAS User’s Guide. 2007. Version 9.2 ed. SAS Inst. Inc., Cary, NC.

Yan, F., Garribay, L., Arce, J., Lopez-Coello, C., Camacho, D., Disbennet, P., Vazquez-Anon, M., Manangi, M., Odetallah, N.H., Carter, S., (2012). “*Effects of protease supplementation on broiler performance and in vitro protein digestibility*”. Australian Poultry Science Symposium, 134-137.

Yamazaki, M. Murakami, H., and Nakashima, K. (2002). “*Effect of Enzyme Supplementation on Performance and Nitrogen Excretion of Broiler Chick Fed Low-Protein Diet Based on Corn and Soybean Meal*”. Bulletin Nat. Ins. Lives Grassland Sci. 2:9-13.

APPENDIXES

APPENDIX A

Diet # 1: Pilgrim's Broiler Starter Positive Control (PC).

Date Printed: 05/17/17
Date Optimized: 02/16/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 4
Page: 1

Nacogdoches
Broiler Starter Postive Control

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Pct	-- Range -- Low	High	-- Restriction -- Min Pct	Max Pct	Rcost
101	CORN, Fine	1018.61	50.931		0.1402			
111	SOYBEAN MEAL	796.03	39.802	0.0879				
143	Soy Oil	71.58	3.579	0.1157		0.5000		
153	DISTILLER'S GR	40.00	2.000			2.0000	2.0000	
28	LIMESTONE FINE	23.43	1.171		1.0742			
299	NEXPHOS MONO-D	18.86	0.943		10.9765	0.2500		
41	ALIMET	8.21	0.411		7.9681			
36	SALT PLAIN	5.23	0.262		1.4862			
13	LIQ LYSINE50%	5.03	0.251		1.9294			
919	Adisodium	3.00	0.150			0.1500		
45	L-THREONINE	2.45	0.122		5.9177			
78	BIOAVAIL TRACE	1.50	0.075			0.0750		
63	CHOLINE LIQ.	1.32	0.066		51.1009			
166	Opti Bac S/L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500		
177	NICARB 25%	0.900	0.045			0.0450	0.0450	
60	BROILER VITAMI	0.500	0.025			0.0250		
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
775	Hemicell Dry	0.400	0.020			0.0200	0.0200	
72	LIQ ETHOXYQUIN	0.250	0.012			0.0125		
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT	0.99	0.9995	1.01
2	PROTEIN		23.28	
3	FAT		5.52	
4	FIBER		2.54	
5	MOISTURE		12.54	
6	ASH		4.96	
7	CALCIUM	0.90	0.9060	0.93
8	TOTAL PHOS.		0.5969	
9	AVAIL. PHOS.	0.45	0.4499	
10	SALT		0.3086	
11	CHLORIDE	0.21	0.2100	0.28
12	SODIUM	0.19	0.2204	0.22
13	POTASSIUM	0.65	0.9262	
14	MAGNESIUM		0.1767	
15	MANGANESE		94.80	
18	COPPER		156.70	
21	SULFUR		0.2898	
22	LINOLEIC ACID		1.78	
23	XANT. ACTIVITY		5.11	
36	CHOLINE	825.00	838.31	
37	ME; POULTRY	1375.00	1381.47	
53	AVAIL. ARGININ		1.44	
54	AVAIL. LYSINE	1.25	1.26	
55	AVAIL. METHION		0.6402	
56	AVAIL. METH+CY		0.9496	
57	AVAIL. TRYPTOP		0.2502	
58	AVAIL. ISOLEUC		0.9044	
59	AVAIL.VALINE		0.9678	
61	AVAIL. CYSTINE		0.6688	
62	AVAIL. THREONI		0.8873	
69	ANALYZED CALCI		0.7496	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		95.87	
81	Potassium mEq/		236.88	
82	Chloride mEq/K		59.23	
83	DEB mEq/Kg	160.00	273.51	
84	DEB+S		108.60	
100	Nacogdoches		90.73	

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
12	SODIUM	PCT	0.02 PCT
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name		Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed		0.0150	0.0150	146.1
293	Hostazym X WSP	Suppressed		0.0100	0.0100	82.20
288	Poultry Grow 2	Suppressed		0.0125	0.0125	102.9
46	BIOLYS	Suppressed				1.98

APPENDIX B

Diet # 2: Pilgrim's Broiler Starter Negative Control (NC)

Date Printed: 05/17/17
Date Optimized: 02/16/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 9
Page: 1

Nacogdoches
Broiler Starter Negative Control

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Unrounded Pct	Range Low	Range High	Restriction Min Pct	Restriction Max Pct	Restriction Rcost
101	CORN, Fine	1054.11	52.705		0.1402			
111	SOYBEAN MEAL	765.52	38.276	0.0879				
143	Soy Oil	66.06	3.303	0.1157		0.5000		
153	DISTILLER'S GR	40.00	2.000			2.0000	2.0000	
28	LIMESTONE FINE	23.56	1.178		1.0742			
299	NEXPHOS MONO-D	19.07	0.953		10.9765	0.2500		
41	ALIMET	7.94	0.397		7.9681			
36	SALT PLAIN	5.22	0.261		1.4862			
13	LIQ LYSINE50%	5.21	0.261		1.9294			
919	Adisodium	3.00	0.150			0.1500		
45	L-THREONINE	2.45	0.123		5.9177			
78	BIOAVAIL TRACE	1.50	0.075			0.0750		
63	CHOLINE LIQ.	1.36	0.068		51.1009			
166	Opti Bac S/L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500		
177	NICARB 25%	0.900	0.045			0.0450	0.0450	
60	BROILER VITAMI	0.500	0.025			0.0250		
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
775	Hemicell Dry	0.400	0.020			0.0200	0.0200	
72	LIQ ETHOXYQUIN	0.250	0.013			0.0125		
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
12	SODIUM	PCT	0.02 PCT
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name		Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed		0.0150	0.0150	146.1
293	Hostazym X WSP	Suppressed		0.0100	0.0100	82.20
46	BIOLYS	Suppressed				1.98

Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT	0.99	0.9994	1.01
2	PROTEIN		22.71	
3	FAT		5.29	
4	FIBER		2.52	
5	MOISTURE		12.61	
6	ASH		4.91	
7	CALCIUM	0.90	0.9060	0.93
8	TOTAL PHOS.		0.5924	
9	AVAIL. PHOS.	0.45	0.4499	
10	SALT		0.3073	
11	CHLORIDE	0.21	0.2100	0.28
12	SODIUM	0.19	0.2204	0.22
13	POTASSIUM	0.65	0.9024	
14	MAGNESIUM		0.1747	
15	MANGANESE		94.49	
18	COPPER		155.86	
21	SULFUR		0.2853	
22	LINOLEIC ACID		1.74	
23	XANT. ACTIVITY		5.28	
36	CHOLINE	825.00	829.70	
37	ME; POULTRY	1375.00	1381.36	
53	AVAIL. ARGININ		1.43	
54	AVAIL. LYSINE	1.25	1.26	
55	AVAIL. METHION		0.6372	
56	AVAIL. METH+CY		0.9502	
57	AVAIL. TRYPTOP		0.2477	
58	AVAIL. ISOLEUC		0.9011	
59	AVAIL.VALINE		0.9687	
61	AVAIL. CYSTINE		0.6505	
62	AVAIL. THREONI		0.8879	
69	ANALYZED CALCI		0.7494	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		95.87	
81	Potassium mEq/		230.78	
82	Chloride mEq/K		59.23	
83	DEB mEq/Kg	160.00	267.42	
84	DEB+S		105.50	
100	Nacogdoches		90.98	

APPENDIX C

Diet # 4: Pilgrim's Broiler Starter Positive Control (PC) + Protease.

Date Printed: 05/17/17
Date Optimized: 02/16/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 4
Page: 1

Plant: 27 Nacogdoches
Product: 101.4 Broiler Starter + Protease w/ Matrix
Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Pct	Market $/Lb	Range Low	Range High	Restriction Min Pct	Restriction Max Pct	Rcost
101	CORN, Fine	1054.11	52.705	0.0779		0.1402			
111	SOYBEAN MEAL	765.52	38.276	0.1640	0.0879				
143	Soy Oil	66.06	3.303	0.2892	0.1157		0.5000		
153	DISTILLER'S GR	40.00	2.000	0.0745			2.0000	2.0000	
28	LIMESTONE FINE	23.56	1.178	0.0245		1.0742			
299	NEXPHOS MONO-D	19.07	0.953	0.2291		10.9765	0.2500		
41	ALIMET	7.94	0.397	1.1158		7.9681			
36	SALT PLAIN	5.22	0.261	0.0352		1.4862			
13	LIQ LYSINE50%	5.21	0.261	0.3608		1.9294			
919	Adisodium	3.00	0.150	0.1300			0.1500		
45	L-THREONINE	2.45	0.123	0.8617		5.9177			
78	BIOAVAIL TRACE	1.50	0.075	1.0330			0.0750		
63	CHOLINE LIQ.	1.36	0.068	0.4095		51.1009			
166	Opti Bac S/L	1.00	0.050	1.8500			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050	0.9030			0.0500		
177	NICARB 25%	0.900	0.045	4.4666			0.0450	0.0450	
60	BROILER VITAMI	0.500	0.025	3.3976			0.0250		
642	Optiphos 6000P	0.500	0.025	0.0000			0.0250	0.0250	
775	Hemicell Dry	0.400	0.020	3.4948			0.0200	0.0200	
289	Poultry Grow 2	0.250	0.013	5.1445			0.0125	0.0125	
72	LIQ ETHOXYQUIN	0.250	0.013	3.1993			0.0125		
282	Hostazyme X dr	0.200	0.010	0.0000			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT	0.99	0.9994	1.01
2	PROTEIN		22.71	
3	FAT		5.29	
4	FIBER		2.52	
5	MOISTURE		12.61	
6	ASH		4.91	
7	CALCIUM	0.90	0.9060	0.93
8	TOTAL PHOS.		0.5924	
9	AVAIL. PHOS.	0.45	0.4499	
10	SALT		0.3073	
11	CHLORIDE	0.21	0.2100	0.28
12	SODIUM	0.19	0.2204	0.22
13	POTASSIUM	0.65	0.9024	
14	MAGNESIUM		0.1747	
15	MANGANESE		94.49	
18	COPPER		155.86	
21	SULFUR		0.2853	
22	LINOLEIC ACID		1.74	
23	XANT. ACTIVITY		5.28	
36	CHOLINE	825.00	829.70	
37	ME; POULTRY	1375.00	1381.36	
53	AVAIL. ARGININ		1.43	
54	AVAIL. LYSINE	1.25	1.26	
55	AVAIL. METHION		0.6372	
56	AVAIL. METH+CY		0.9502	
57	AVAIL. TRYPTOP		0.2477	
58	AVAIL. ISOLEUC		0.9011	
59	AVAIL.VALINE		0.9687	
61	AVAIL. CYSTINE		0.6505	
62	AVAIL. THREONI		0.8879	
69	ANALYZED CALCI		0.7494	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		95.87	
81	Potassium mEq/		230.78	
82	Chloride mEq/K		59.23	
83	DEB mEq/Kg	160.00	267.42	
84	DEB+S		105.50	
100	Nacogdoches		90.98	

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
12	SODIUM	PCT	0.02 PCT
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name		Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed		0.0150	0.0150	146.1
293	Hostazym X WSP	Suppressed		0.0100	0.0100	82.20
46	BIOLYS	Suppressed				1.98

APPENDIX D

Diet # 3: Pilgrim's Broiler Starter Negative Control (NC) + Protease.

Date Printed: 05/17/17
Date Optimized: 02/16/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 3
Page: 1

Plant: 27 Nacogdoches
Product: 101.3 Broiler Starter + Protease No Matrix

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Pct	Range Low	Range High	Restriction Min Pct	Restriction Max Pct	Rcost
101	CORN, Fine	1018.12	50.906		0.1402			
111	SOYBEAN MEAL	796.10	39.805	0.0879				
143	Soy Oil	71.75	3.588	0.1157		0.5000		
153	DISTILLER'S GR	40.00	2.000			2.0000	2.0000	
28	LIMESTONE FINE	23.43	1.171		1.0742			
299	NEXPHOS MONO-D	18.86	0.943		10.9765	0.2500		
41	ALIMET	8.21	0.411		7.9681			
36	SALT PLAIN	5.23	0.262		1.4862			
13	LIQ LYSINE50%	5.03	0.251		1.9294			
919	Adisodium	3.00	0.150			0.1500		
45	L-THREONINE	2.45	0.122		5.9177			
78	BIOAVAIL TRACE	1.50	0.075			0.0750		
63	CHOLINE LIQ.	1.32	0.066		51.1009			
166	Opti Bac S/L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500		
177	NICARB 25%	0.900	0.045			0.0450	0.0450	
60	BROILER VITAMI	0.500	0.025			0.0250		
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
775	Hemicell Dry	0.400	0.020			0.0200	0.0200	
288	Poultry Grow 2	0.250	0.013			0.0125	0.0125	
72	LIQ ETHOXYQUIN	0.250	0.013			0.0125		
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT	0.99	0.9995	1.01
2	PROTEIN		23.27	
3	FAT		5.52	
4	FIBER		2.54	
5	MOISTURE		12.53	
6	ASH		4.96	
7	CALCIUM	0.90	0.9060	0.93
8	TOTAL PHOS.		0.5968	
9	AVAIL. PHOS.	0.45	0.4499	
10	SALT		0.3086	
11	CHLORIDE	0.21	0.2100	0.28
12	SODIUM	0.19	0.2204	0.22
13	POTASSIUM	0.65	0.9262	
14	MAGNESIUM		0.1767	
15	MANGANESE		94.80	
18	COPPER		156.70	
21	SULFUR		0.2897	
22	LINOLEIC ACID		1.78	
23	XANT. ACTIVITY		5.10	
36	CHOLINE	825.00	838.45	
37	ME; POULTRY	1375.00	1381.47	
53	AVAIL. ARGININ		1.44	
54	AVAIL. LYSINE	1.25	1.26	
55	AVAIL. METHION		0.6402	
56	AVAIL. METH+CY		0.9496	
57	AVAIL. TRYPTOP		0.2502	
58	AVAIL. ISOLEUC		0.9044	
59	AVAIL.VALINE		0.9678	
61	AVAIL. CYSTINE		0.6688	
62	AVAIL. THREONI		0.8873	
69	ANALYZED CALCI		0.7496	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		95.87	
81	Potassium mEq/		236.87	
82	Chloride mEq/K		59.23	
83	DEB mEq/Kg	160.00	273.51	
84	DEB+S		108.61	
100	Nacogdoches		90.71	

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
12	SODIUM	PCT	0.02 PCT
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name		Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed		0.0150	0.0150	146.1
293	Hostazym X WSP	Suppressed		0.0100	0.0100	82.20
46	BIOLYS	Suppressed				1.98

APPENDIX E

Diet # 1: Pilgrim's Broiler Grower Positive Control (PC).

CFC/Concept5

Date Printed: 05/17/17
Date Optimized: 02/16/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 3
Page: 1

Plant: 27 Nacogdoches
Product: 102.1 Broiler Grower Positive Control

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Pct	Range Low	Range High	Restriction Min Pct	Restriction Max Pct	Rcost
101	CORN, Fine	1107.21	55.360	0.0158	0.1390			
111	SOYBEAN MEAL	650.82	32.541	0.0906				
153	DISTILLER'S GR	100.00	5.000		0.1099		5.0000	-0.71
143	Soy Oil	71.42	3.571	0.1190	1.0601	0.5000		
28	LIMESTONE FINE	23.58	1.179		0.6414			
299	NEXPHOS MONO-D	16.36	0.818		10.8581	0.2500		
41	ALIMET	7.20	0.360		7.8958			
13	LIQ LYSINE50%	6.52	0.326		1.9136			
36	SALT PLAIN	4.50	0.225		1.4862			
919	Adisodium	3.00	0.150			0.1500		
45	L-THREONINE	2.33	0.117		5.8658			
78	BIOAVAIL TRACE	1.50	0.075			0.0750		
63	CHOLINE LIQ.	1.19	0.060		24.0149			
166	Opti Bac S/L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500		
177	NICARB 25%	0.900	0.045			0.0450	0.0450	
60	BROILER VITAMI	0.500	0.025			0.0250		
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
72	LIQ ETHOXYQUIN	0.250	0.013			0.0125		
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs at 249.68 $/Ton 12.484 $/100Lb 0.1248 $/Lb

Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT	0.99	0.9996	1.01
2	PROTEIN		21.11	
3	FAT		5.82	
4	FIBER		2.57	
5	MOISTURE		12.67	
6	ASH		4.64	
7	CALCIUM	0.86	0.8672	0.89
8	TOTAL PHOS.		0.5581	
9	AVAIL. PHOS.	0.43	0.4297	0.46
10	SALT		0.2734	
11	CHLORIDE	0.19	0.1900	0.28
12	SODIUM	0.18	0.2049	0.22
13	POTASSIUM	0.60	0.8335	
14	MAGNESIUM		0.1684	
15	MANGANESE		94.10	
18	COPPER		152.64	
22	LINOLEIC ACID		1.88	
23	XANT. ACTIVITY		5.57	
36	CHOLINE	770.00	770.00	
37	ME; POULTRY	1400.00	1395.51	
53	AVAIL. ARGININ		1.25	
54	AVAIL. LYSINE	1.12	1.13	
55	AVAIL. METHION		0.5783	
56	AVAIL. METH+CY		0.8637	
57	AVAIL. TRYPTOP		0.2171	
58	AVAIL. ISOLEUC		0.7980	
59	AVAIL.VALINE		0.8699	
61	AVAIL. CYSTINE		0.6005	
62	AVAIL. THREONI		0.7971	
69	ANALYZED CALCI		0.7104	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		89.12	
81	Potassium mEq/		213.17	
82	Chloride mEq/K		53.59	
83	DEB mEq/Kg	160.00	248.70	
84	DEB+S		89.92	
100	Nacogdoches		87.90	

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
36	CHOLINE	MG/LB	0.10 MG/LB
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name		Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed		0.0150	0.0150	146.1
293	Hostazym X WSP	Suppressed		0.0100	0.0100	82.20
288	Poultry Grow 2	Suppressed		0.0125	0.0125	102.9
46	BIOLYS	Suppressed				2.00
19	S-CARB	Suppressed		0.1500		3.12

APPENDIX F

Diet # 2: Pilgrim's Broiler Grower Negative Control (NC).

Date Printed: 05/17/17
Date Optimized: 02/16/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 5
Page: 1

Nacogdoches
Broiler Grower Negative Control

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Pct	-- Range -- Low	High	-- Restriction --- Min Pct	Max Pct	Rcost
101	CORN, Fine	1142.62	57.131	0.0158	0.1390			
111	SOYBEAN MEAL	620.32	31.016	0.0906				
153	DISTILLER'S GR	100.00	5.000		0.1099		5.0000	-0.71
143	Soy Oil	65.94	3.297	0.1190	1.0601	0.5000		
28	LIMESTONE FINE	23.72	1.186		0.6414			
299	NEXPHOS MONO-D	16.57	0.828		10.8581	0.2500		
41	ALIMET	6.93	0.346		7.8958			
13	LIQ LYSINE50%	6.71	0.335		1.9136			
36	SALT PLAIN	4.47	0.224		1.4862			
919	Adisodium	3.00	0.150			0.1500		
45	L-THREONINE	2.34	0.117		5.8658			
78	BIOAVAIL TRACE	1.50	0.075			0.0750		
63	CHOLINE LIQ.	1.29	0.064		24.0149			
166	Opti Bac S/L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500		
177	NICARB 25%	0.900	0.045			0.0450	0.0450	
60	BROILER VITAMI	0.500	0.025			0.0250		
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
72	LIQ ETHOXYQUIN	0.250	0.012			0.0125		
289	Poultry Grow 2	0.250	0.012			0.0125	0.0125	
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
36	CHOLINE	MG/LB	0.10 MG/LB
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name	Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed	0.0150	0.0150	146.1
293	Hostazym X WSP	Suppressed	0.0100	0.0100	82.20
46	BIOLYS	Suppressed			2.00
19	S-CARB	Suppressed	0.1500		3.12

Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT	0.99	0.9997	1.01
2	PROTEIN		20.55	
3	FAT		5.59	
4	FIBER		2.55	
5	MOISTURE		12.75	
6	ASH		4.59	
7	CALCIUM	0.86	0.8672	0.89
8	TOTAL PHOS.		0.5536	
9	AVAIL. PHOS.	0.43	0.4297	0.46
10	SALT		0.2712	
11	CHLORIDE	0.19	0.1900	0.28
12	SODIUM	0.18	0.2045	0.22
13	POTASSIUM	0.60	0.8097	
14	MAGNESIUM		0.1665	
15	MANGANESE		93.79	
18	COPPER		151.80	
22	LINOLEIC ACID		1.84	
23	XANT. ACTIVITY		5.75	
36	CHOLINE	770.00	770.00	
37	ME; POULTRY	1400.00	1395.40	
53	AVAIL. ARGININ		1.24	
54	AVAIL. LYSINE	1.12	1.13	
55	AVAIL. METHION		0.5753	
56	AVAIL. METH+CY		0.8643	
57	AVAIL. TRYPTOP		0.2145	
58	AVAIL. ISOLEUC		0.7948	
59	AVAIL.VALINE		0.8707	
61	AVAIL. CYSTINE		0.5822	
62	AVAIL. THREONI		0.7977	
69	ANALYZED CALCI		0.7102	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		88.96	
81	Potassium mEq/		207.07	
82	Chloride mEq/K		53.59	
83	DEB mEq/Kg	160.00	242.44	
84	DEB+S		86.81	
100	Nacogdoches		88.15	

APPENDIX G

Diet # 3: Pilgrim's Broiler Grower Positive Control (NC) + Protease.

Date Printed: 05/17/17
Date Optimized: 02/16/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 2
Page: 1

Nacogdoches
Broiler Grower + Protease w/ Matrix

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Pct	Range Low	Range High	Restriction Min Pct	Restriction Max Pct	Rcost
101	CORN, Fine	1142.62	57.131	0.0158	0.1390			
111	SOYBEAN MEAL	620.32	31.016	0.0906				
153	DISTILLER'S GR	100.00	5.000		0.1099		5.0000	-0.71
143	Soy Oil	65.94	3.297	0.1190	1.0601	0.5000		
28	LIMESTONE FINE	23.72	1.186		0.6414			
299	NEXPHOS MONO-D	16.57	0.828		10.8581	0.2500		
41	ALIMET	6.93	0.346		7.8958			
13	LIQ LYSINE50%	6.71	0.335		1.9136			
36	SALT PLAIN	4.47	0.224		1.4862			
919	Adisodium	3.00	0.150			0.1500		
45	L-THREONINE	2.34	0.117		5.8658			
78	BIOAVAIL TRACE	1.50	0.075			0.0750		
63	CHOLINE LIQ.	1.29	0.064		24.0149			
166	Opti Bac S/L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500		
177	NICARB 25%	0.900	0.045			0.0450	0.0450	
60	BROILER VITAMI	0.500	0.025			0.0250		
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
72	LIQ ETHOXYQUIN	0.250	0.012			0.0125		
289	Poultry Grow 2	0.250	0.012			0.0125	0.0125	
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT	0.99	0.9997	1.01
2	PROTEIN		20.55	
3	FAT		5.59	
4	FIBER		2.55	
5	MOISTURE		12.75	
6	ASH		4.59	
7	CALCIUM	0.86	0.8672	0.89
8	TOTAL PHOS.		0.5536	
9	AVAIL. PHOS.	0.43	0.4297	0.46
10	SALT		0.2712	
11	CHLORIDE	0.19	0.1900	0.28
12	SODIUM	0.18	0.2045	0.22
13	POTASSIUM	0.60	0.8097	
14	MAGNESIUM		0.1665	
15	MANGANESE		93.79	
18	COPPER		151.80	
22	LINOLEIC ACID		1.84	
23	XANT. ACTIVITY		5.75	
36	CHOLINE	770.00	770.00	
37	ME; POULTRY	1400.00	1395.40	
53	AVAIL. ARGININ		1.24	
54	AVAIL. LYSINE	1.12	1.13	
55	AVAIL. METHION		0.5753	
56	AVAIL. METH+CY		0.8643	
57	AVAIL. TRYPTOP		0.2145	
58	AVAIL. ISOLEUC		0.7948	
59	AVAIL.VALINE		0.8707	
61	AVAIL. CYSTINE		0.5822	
62	AVAIL. THREONI		0.7977	
69	ANALYZED CALCI		0.7102	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		88.96	
81	Potassium mEq/		207.07	
82	Chloride mEq/K		53.59	
83	DEB mEq/Kg	160.00	242.44	
84	DEB+S		86.81	
100	Nacogdoches		88.15	

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
36	CHOLINE	MG/LB	0.10 MG/LB
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LE
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name		Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed		0.0150	0.0150	146.1
293	Hostazym X WSP	Suppressed		0.0100	0.0100	82.20
46	BIOLYS	Suppressed				2.00
19	S-CARB	Suppressed		0.1500		3.12

APPENDIX H

Diet # 4: Pilgrim's Broiler Grower Positive Control (NC) + Protease.

Date Printed: 05/17/17
Date Optimized: 02/16/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 3
Page: 1

Nacogdoches
Broiler Grower + Protease No Matrix

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Pct	-- Range -- Low	High	-- Restriction -- Min Pct	Max Pct	Rcost
101	CORN, Fine	1106.71	55.336	0.0158	0.1390			
111	SOYBEAN MEAL	650.89	32.544	0.0906				
153	DISTILLER'S GR	100.00	5.000		0.1099		5.0000	-0.71
143	Soy Oil	71.60	3.580	0.1190	1.0601	0.5000		
28	LIMESTONE FINE	23.58	1.179		0.6414			
299	NEXPHOS MONO-D	16.36	0.818		10.8581	0.2500		
41	ALIMET	7.20	0.360		7.8958			
13	LIQ LYSINE50%	6.52	0.326		1.9136			
36	SALT PLAIN	4.50	0.225		1.4862			
919	Adisodium	3.00	0.150			0.1500		
45	L-THREONINE	2.33	0.117		5.8658			
78	BIOAVAIL TRACE	1.50	0.075			0.0750		
63	CHOLINE LIQ.	1.20	0.060		24.0149			
166	Opti Bac S/L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500		
177	NICARB 25%	0.900	0.045			0.0450	0.0450	
60	BROILER VITAMI	0.500	0.025			0.0250		
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
288	Poultry Grow 2	0.250	0.013			0.0125	0.0125	
72	LIQ ETHOXYQUIN	0.250	0.013			0.0125		
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT	0.99	0.9997	1.01
2	PROTEIN		21.11	
3	FAT		5.83	
4	FIBER		2.57	
5	MOISTURE		12.67	
6	ASH		4.64	
7	CALCIUM	0.86	0.8672	0.89
8	TOTAL PHOS.		0.5581	
9	AVAIL. PHOS.	0.43	0.4297	0.46
10	SALT		0.2734	
11	CHLORIDE	0.19	0.1900	0.28
12	SODIUM	0.18	0.2049	0.22
13	POTASSIUM	0.60	0.8335	
14	MAGNESIUM		0.1684	
15	MANGANESE		94.10	
18	COPPER		152.64	
22	LINOLEIC ACID		1.88	
23	XANT. ACTIVITY		5.57	
36	CHOLINE	770.00	770.00	
37	ME; POULTRY	1400.00	1395.51	
53	AVAIL. ARGININ		1.25	
54	AVAIL. LYSINE	1.12	1.13	
55	AVAIL. METHION		0.5783	
56	AVAIL. METH+CY		0.8637	
57	AVAIL. TRYPTOP		0.2171	
58	AVAIL. ISOLEUC		0.7980	
59	AVAIL.VALINE		0.8699	
61	AVAIL. CYSTINE		0.6005	
62	AVAIL. THREONI		0.7971	
69	ANALYZED CALCI		0.7104	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		89.13	
81	Potassium mEq/		213.17	
82	Chloride mEq/K		53.59	
83	DEB mEq/Kg	160.00	248.70	
84	DEB+S		89.92	
100	Nacogdoches		87.88	

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
36	CHOLINE	MG/LB	0.10 MG/LB
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name		Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed		0.0150	0.0150	146.1
293	Hostazym X WSP	Suppressed		0.0100	0.0100	82.20
46	BIOLYS	Suppressed				2.00
19	S-CARB	Suppressed		0.1500		3.12

APPENDIX I

Diet # 1: Pilgrim's Broiler Finisher (Withdrawal) Positive Control (PC).

Date Printed: 05/17/17
Date Optimized: 03/24/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 5
Page: 1

Nacogdoches
Broiler Withdrawal 1 Positive Control

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Pct	Range Low	Range High	Restriction Min Pct	Restriction Max Pct	Rcost
101	CORN, Fine	1355.40	67.770	0.0646				
111	SOYBEAN MEAL	482.44	24.122	0.1008				
153	DISTILLER'S GR	77.75	3.887		0.0791		9.0000	
143	Soy Oil	29.12	1.456		0.3811	0.5000	1.5000	-1.98
28	LIMESTONE FINE	16.12	0.806		0.2971			
31	PHOS DEFL	11.56	0.578			0.2500		
46	BIOLYS	7.20	0.360		2.1969			
41	ALIMET	5.44	0.272		22.8747			
36	SALT PLAIN	4.46	0.223		1.6752			
919	Adisodium	2.91	0.146			0.1500		
45	L-THREONINE	1.76	0.088		8.8050			
78	BIOAVAIL TRACE	1.21	0.061			0.0625		
165	Optibac L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500	0.0500	
63	CHOLINE LIQ.	0.794	0.040		76.4123			
85	Magni Phi	0.500	0.025			0.0250	0.0250	
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
60	BROILER VITAMI	0.388	0.019			0.0200		
72	LIQ ETHOXYQUIN	0.243	0.012			0.0125		
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Optimum Weight Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT		0.9996	
2	PROTEIN		17.91	
3	FAT		3.96	
4	FIBER		2.45	
5	MOISTURE		13.12	
6	ASH		3.79	
7	CALCIUM	0.75	0.7325	0.78
8	TOTAL PHOS.		0.4688	
9	AVAIL. PHOS.	0.36	0.3536	0.39
10	SALT		0.2663	
11	CHLORIDE	0.19	0.1845	0.28
12	SODIUM	0.19	0.1877	0.22
13	POTASSIUM	0.56	0.6976	
14	MAGNESIUM		0.1571	
15	MANGANESE		77.58	
18	COPPER		147.23	
22	LINOLEIC ACID		1.54	
23	XANT. ACTIVITY		6.80	
36	CHOLINE	650.00	631.04	
37	ME; POULTRY	1440.00	1398.58	
53	AVAIL. ARGININ		1.02	
54	AVAIL. LYSINE	0.98	0.9514	
55	AVAIL. METHION		0.4727	
56	AVAIL. METH+CY		0.7231	
57	AVAIL. TRYPTOP		0.1746	
58	AVAIL. ISOLEUC		0.6557	
59	AVAIL.VALINE		0.7329	
61	AVAIL. CYSTINE		0.4882	
62	AVAIL. THREONI		0.6565	
69	ANALYZED CALCI		0.5850	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		81.63	
81	Potassium mEq/		178.42	
82	Chloride mEq/K		52.03	
83	DEB mEq/Kg	175.00	208.02	
84	DEB+S		66.79	
100	Nacogdoches		91.89	

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
36	CHOLINE	MG/LB	0.10 MG/LB
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name	Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed	0.0300	0.0300	54.46
293	Hostazym X WSP	Suppressed	0.0100	0.0100	79.87
141	Intellibond C2	Suppressed	0.0368	0.0368	70.58
288	Poultry Grow 2	Suppressed	0.0125	0.0125	101.4
13	LIQ LYSINE50%	Suppressed			-1.66

APPENDIX J

Diet # 2: Pilgrim's Broiler Finisher (Withdrawal) Negative Control (NC).

Date Printed: 05/17/17
Date Optimized: 03/24/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 7
Page: 1

Nacogdoches
Broiler Withdrawal 1 Negative control

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Unrounded Pct	Range Low	Range High	Restriction Min Pct	Restriction Max Pct	Rcost
101	CORN, Fine	1342.72	67.136	0.0646				
111	SOYBEAN MEAL	452.03	22.601	0.1008				
153	DISTILLER'S GR	121.47	6.073		0.0791		9.0000	
143	Soy Oil	29.12	1.456		0.3811	0.5000	1.5000	-1.98
28	LIMESTONE FINE	17.06	0.853	-0.1911	0.2971			
31	PHOS DEFL	10.61	0.530	-0.0087		0.2500		
46	BIOLYS	7.18	0.359		2.1969			
41	ALIMET	4.87	0.244	-0.1158	22.8747			
36	SALT PLAIN	4.38	0.219	-0.1557	1.6752			
919	Adisodium	2.91	0.146	-0.1597		0.1500		5.78
45	L-THREONINE	1.54	0.077	-0.1395	8.8050			
78	BIOAVAIL TRACE	1.21	0.061	-0.1545		0.0625		24.04
165	Optibac L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500	0.0500	
63	CHOLINE LIQ.	0.797	0.040	-0.0939	76.4123			
85	Magni Phi	0.500	0.025			0.0250	0.0250	
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
60	BROILER VITAMI	0.388	0.019	-0.1361		0.0200		66.31
289	Poultry Grow 2	0.250	0.013			0.0125	0.0125	
72	LIQ ETHOXYQUIN	0.243	0.012	-0.1597		0.0125		67.19
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
36	CHOLINE	MG/LB	0.10 MG/LB
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name		Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed		0.0300	0.0300	54.46
293	Hostazym X WSP	Suppressed		0.0100	0.0100	79.87
141	Intellibond C2	Suppressed		0.0368	0.0368	70.58
288	Poultry Grow 2	Suppressed		0.0125	0.0125	101.4
13	LIQ LYSINE50%	Suppressed	7.4000			-1.66

Optimum Weight Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT		0.9997	
2	PROTEIN		17.74	
3	FAT		4.10	
4	FIBER		2.52	
5	MOISTURE		13.09	
6	ASH		3.80	
7	CALCIUM	0.75	0.7325	0.78
8	TOTAL PHOS.		0.4664	
9	AVAIL. PHOS.	0.36	0.3536	0.39
10	SALT		0.2649	
11	CHLORIDE	0.19	0.1845	0.28
12	SODIUM	0.19	0.1881	0.22
13	POTASSIUM	0.56	0.6905	
14	MAGNESIUM		0.1568	
15	MANGANESE		77.90	
18	COPPER		146.38	
22	LINOLEIC ACID		1.57	
23	XANT. ACTIVITY		6.76	
36	CHOLINE	650.00	631.04	
37	ME; POULTRY	1440.00	1398.57	
53	AVAIL. ARGININ		1.02	
54	AVAIL. LYSINE	0.98	0.9523	
55	AVAIL. METHION		0.4648	
56	AVAIL. METH+CY		0.7238	
57	AVAIL. TRYPTOP		0.1747	
58	AVAIL. ISOLEUC		0.6647	
59	AVAIL.VALINE		0.7495	
61	AVAIL. CYSTINE		0.4623	
62	AVAIL. THREONI		0.6571	
69	ANALYZED CALCI		0.5842	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		81.82	
81	Potassium mEq/		176.60	
82	Chloride mEq/K		52.03	
83	DEB mEq/Kg	175.00	206.39	
84	DEB+S		62.36	
100	Nacogdoches		89.74	

APPENDIX K

Diet # 3: Pilgrim's Broiler Finisher (Withdrawal) Positive Control (PC) + Protease.

Date Printed: 05/17/17
Date Optimized: 03/24/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 7
Page: 1

Nacogdoches
Broiler Withdrawal 1 + Protease with mat

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Pct	-- Range -- Low	High	-- Restriction --- Min Pct	Max Pct	Rcost
101	CORN, Fine	1342.72	67.136	0.0646				
111	SOYBEAN MEAL	452.03	22.601	0.1008				
153	DISTILLER'S GR	121.47	6.073		0.0791		9.0000	
143	Soy Oil	29.12	1.456		0.3811	0.5000	1.5000	-1.98
28	LIMESTONE FINE	17.06	0.853	-0.1911	0.2971			
31	PHOS DEFL	10.61	0.530	-0.0087		0.2500		
46	BIOLYS	7.18	0.359		2.1969			
41	ALIMET	4.87	0.244	-0.1158	22.8747			
36	SALT PLAIN	4.38	0.219	-0.1557	1.6752			
919	Adisodium	2.91	0.146	-0.1597		0.1500		5.78
45	L-THREONINE	1.54	0.077	-0.1395	8.8050			
78	BIOAVAIL TRACE	1.21	0.061	-0.1545		0.0625		24.04
165	Optibac L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500	0.0500	
63	CHOLINE LIQ.	0.797	0.040	-0.0939	76.4123			
85	Magni Phi	0.500	0.025			0.0250	0.0250	
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
60	BROILER VITAMI	0.388	0.019	-0.1361		0.0200		66.31
289	Poultry Grow 2	0.250	0.013			0.0125	0.0125	
72	LIQ ETHOXYQUIN	0.243	0.012	-0.1597		0.0125		67.19
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
36	CHOLINE	MG/LB	0.10 MG/LB
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name		Would Use	Minimum Pct	Maximum Pct	Rcost
621	Optiphos IC .	Suppressed		0.0300	0.0300	54.46
293	Hostazym X WSP	Suppressed		0.0100	0.0100	79.87
141	Intellibond C2	Suppressed		0.0368	0.0368	70.58
288	Poultry Grow 2	Suppressed		0.0125	0.0125	101.4
13	LIQ LYSINE50%	Suppressed	7.4000			-1.66

Optimum Weight Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT		0.9997	
2	PROTEIN		17.74	
3	FAT		4.10	
4	FIBER		2.52	
5	MOISTURE		13.09	
6	ASH		3.80	
7	CALCIUM	0.75	0.7325	0.78
8	TOTAL PHOS.		0.4664	
9	AVAIL. PHOS.	0.36	0.3536	0.39
10	SALT		0.2649	
11	CHLORIDE	0.19	0.1845	0.28
12	SODIUM	0.19	0.1881	0.22
13	POTASSIUM	0.56	0.6905	
14	MAGNESIUM		0.1568	
15	MANGANESE		77.90	
18	COPPER		146.38	
22	LINOLEIC ACID		1.57	
23	XANT. ACTIVITY		6.76	
36	CHOLINE	650.00	631.04	
37	ME; POULTRY	1440.00	1398.57	
53	AVAIL. ARGININ		1.02	
54	AVAIL. LYSINE	0.98	0.9523	
55	AVAIL. METHION		0.4648	
56	AVAIL. METH+CY		0.7238	
57	AVAIL. TRYPTOP		0.1747	
58	AVAIL. ISOLEUC		0.6647	
59	AVAIL.VALINE		0.7495	
61	AVAIL. CYSTINE		0.4623	
62	AVAIL. THREONI		0.6571	
69	ANALYZED CALCI		0.5842	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		81.82	
81	Potassium mEq/		176.60	
82	Chloride mEq/K		52.03	
83	DEB mEq/Kg	175.00	206.39	
84	DEB+S		62.36	
100	Nacogdoches		89.74	

APPENDIX L

Diet # 4: Pilgrim's Broiler Finisher (Withdrawal) Negative Control (PC) + Protease.

Date Printed: 05/17/17
Date Optimized: 03/24/2017
Optimized By: DM
Trial Version: 1
Prod'n Version: 7
Page: 1

Nacogdoches
Broiler Withdrawal 1 + protease no matri

Formulated By: Single Product Formulation

Used Ingredients

Ingr Code	Ingredient Name	Unrounded Lbs	Pct	Range Low	Range High	Restriction Min Pct	Restriction Max Pct	Rcost
101	CORN, Fine	1356.50	67.825	0.0271				
111	SOYBEAN MEAL	482.95	24.148	0.0950				
153	DISTILLER'S GR	75.88	3.794		0.0996		9.0000	
143	Soy Oil	29.12	1.456		0.7570	0.5000	1.5000	-9.50
28	LIMESTONE FINE	16.08	0.804	-0.5827	0.3666			
31	PHOS DEFL	11.61	0.581	-0.0645		0.2500		
46	BIOLYS	7.18	0.359		2.1969			
41	ALIMET	5.45	0.272	-0.4019	22.8747			
36	SALT PLAIN	4.47	0.223	-0.4246	2.3243			
919	Adisodium	2.91	0.146	-0.4381		0.1500		11.35
45	L-THREONINE	1.76	0.088	-0.4580	8.8050			
78	BIOAVAIL TRACE	1.21	0.061	-0.4252		0.0625		29.46
165	Optibac L	1.00	0.050			0.0500	0.0500	
35	COPPER SULFATE	1.00	0.050			0.0500	0.0500	
63	CHOLINE LIQ.	0.796	0.040	-0.2844	76.4123			
85	Magni Phi	0.500	0.025			0.0250	0.0250	
642	Optiphos 6000P	0.500	0.025			0.0250	0.0250	
60	BROILER VITAMI	0.388	0.019	-0.3790		0.0200		71.17
288	Poultry Grow 2	0.250	0.013			0.0125	0.0125	
72	LIQ ETHOXYQUIN	0.243	0.012	-0.4381		0.0125		72.76
282	Hostazyme X dr	0.200	0.010			0.0100	0.0100	

Total Batch: 2000.00 Lbs

Binding Nutrients

Nutr No	Nutrient Name	Unit of Measure	Increment Change
7	CALCIUM	PCT	0.02 PCT
9	AVAIL. PHOS.	PCT	0.02 PCT
11	CHLORIDE	PCT	0.02 PCT
36	CHOLINE	MG/LB	0.10 MG/LB
37	ME; POULTRY	KCAL/LB	10.00 KCAL/LB
54	AVAIL. LYSINE	PCT	0.01 PCT

Unused Ingredients

Ingr Code	Ingredient Name		Would Use	Minimum Pct	Maximum Pct	Rcost
289	Poultry Grow 2	Suppressed		0.0125	0.0125	101.4
621	Optiphos IC .	Suppressed		0.0300	0.0300	54.46
293	Hostazym X WSP	Suppressed		0.0100	0.0100	79.87
141	Intellibond C2	Suppressed		0.0368	0.0368	70.58
13	LIQ LYSINE50%	Suppressed	7.2000			-0.73

Optimum Weight Nutrient Solution (Class 5)

Nutr No	Nutrient	Minimum	Actual	Maximum
1	WEIGHT		0.9997	
2	PROTEIN		17.90	
3	FAT		3.96	
4	FIBER		2.44	
5	MOISTURE		13.12	
6	ASH		3.79	
7	CALCIUM	0.75	0.7325	0.78
8	TOTAL PHOS.		0.4688	
9	AVAIL. PHOS.	0.36	0.3536	0.39
10	SALT		0.2663	
11	CHLORIDE	0.19	0.1845	0.28
12	SODIUM	0.19	0.1876	0.22
13	POTASSIUM	0.56	0.6973	
14	MAGNESIUM		0.1571	
15	MANGANESE		77.56	
18	COPPER		147.25	
22	LINOLEIC ACID		1.54	
23	XANT. ACTIVITY		6.81	
36	CHOLINE	650.00	631.04	
37	ME; POULTRY	1440.00	1398.57	
53	AVAIL. ARGININ		1.02	
54	AVAIL. LYSINE	0.98	0.9514	
55	AVAIL. METHION		0.4728	
56	AVAIL. METH+CY		0.7231	
57	AVAIL. TRYPTOP		0.1746	
58	AVAIL. ISOLEUC		0.6555	
59	AVAIL.VALINE		0.7326	
61	AVAIL. CYSTINE		0.4885	
62	AVAIL. THREONI		0.6565	
69	ANALYZED CALCI		0.5850	
73	Feather Meal		0.0000	
80	Sodium mEq/Kg		81.62	
81	Potassium mEq/		178.32	
82	Chloride mEq/K		52.03	
83	DEB mEq/Kg	175.00	207.92	
84	DEB+S		66.91	
100	Nacogdoches		91.97	

VITA

The Author, Jawad Al-juboori, graduated from University of Baghdad, College of Agriculture, Animal Science Department in July 1996. After 16 years of working experience, the author moved to USA, and was accepted into Stephen F. Austin State University, in January 2016 in a Master of Science in Agriculture program with thesis in poultry nutrition. He intends to complete his Master of Science in Agriculture by August 2017, after which, pursue a Ph.D. in the Poultry Science.

Permanent Address: 327 West College St. Apt. 120

Nacogdoches, Texas, 75965.

The APA style Manual of the American Psychological Association 6th edition was used for the reference style in this thesis.

This thesis was typed by Jawad Al-juboori