3-2017

The Effects of Xylanase or an Algal Sourced Zinc Polysaccharide Complex on the Performance of Laying Hens Fed a Corn and Soybean Diet

Ashley Bigge
University of Nebraska-Lincoln, ashley.bigge@huskers.unl.edu

THE EFFECTS OF XYLANASE OR AN ALGAL SOURCED ZINC POLYSACCHARIDE COMPLEX ON THE PERFORMANCE OF LAYING HENS FED A CORN AND SOYBEAN DIET

by

Ashley E. Bigge

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor Sheila E. Purdum

Lincoln, NE

March, 2017

THE EFFECTS OF XYLANASE OR AN ALGAL SOURCED ZINC POLYSACCHARIDE COMPLEX ON THE PERFORMANCE OF LAYING HENS FED A CORN AND SOYBEAN DIET

Ashley Elizabeth Bigge, M.S.

University of Nebraska, 2017

Advisor: Sheila E. Purdum

Two studies were conducted to determine the effects of two different feed additives on the performance of laying hens. Study 1 examined the effects of non-starch polysaccharide degrading enzyme inclusion in a low energy diet. Study 2 examined the effects of supplementing a paramylon zinc polysaccharide complex at increasing concentrations. Both studies utilized a randomized complete block design for treatment assignment, and data were analyzed using the Glimmix procedure in SAS 9.4 for windows.

The first study took place July 2014 to March 2015 and consisted of two phases. Each phase tested a positive control, negative control with lower ME and nutrient density, and two different xylanases as supplements to the negative control diet. There were 72 cages with 3 Bovan White Leghorns per cage used for this study. Data for percent egg production, feed intake, bodyweight, egg mass, and feed conversion were collected. Significant differences were seen for all parameters ($p \leq 0.05$) except egg production (phase 1: $p=0.47$; phase 2: $p=0.54$) and baseline bodyweight ($p=0.63$). The enzymes did not improve the performance of the birds fed the low ME negative control diet up to the level of those fed the positive control.

The second study occurred from June to October 2015 and used 36 cages with 3 Hy-Line Brown Leghorn hens per cage. There were 4 treatments tested, including a control and three diets with increasing dosage of Algamune^{TM} ZPC (at 100, 200, and 300 g/ton), a paramylon zinc polysaccharide complex. Recorded parameters included percent egg production, feed intake, egg weights and components, yolk color, egg mass, feed conversion, bodyweight, eggshell breaking strength, and egg nutrient analysis. There were no significant differences among treatments ($p \leq 0.05$), however egg omega-3 fatty acid content was approaching significance comparing the low and high levels of paramylon supplementation ($p=0.0688$). The Algamune^{TM} ZPC did not negatively affect performance and may be considered safe to supplement as a source of omega-3 and Zn to poultry at the levels tested in this study.

ACKNOWLEDGMENTS

I would like to thank my committee members Dr. Purdum, Dr. Burkey, and Dr. Hanford for reviewing and editing my thesis. They have provided great insight and helped me to learn how to be a better scientist and writer. Of course I still have much to learn!

I also need to thank my fellow students and past graduates in the poultry program, as well as my mom, dad, sister, and brother. Everyone has been so supportive throughout my masters program and I look forward to working on my PhD!

TABLE OF CONTENTS

Chapter 1: Literature Review

I. Enzymes in poultry nutrition

Enzymes are specialized protein molecules that function as catalysts in chemical reactions. Virtually every biochemical reaction necessary for life has an enzyme or class of enzymes associated with it. Enzymes are increasing in popularity as feed additives in livestock production. The goals of enzyme use in animal agriculture is to increase the available nutrients of feed ingredients to improve feed efficiency, save money on ingredient costs, and decrease the potential negative impacts of animal agriculture on the environment (Barletta, 2010). Enzyme supplementation can also allow producers to use alternative feed ingredients that do not have as much energy as non-cereal grains such as corn (Paloheimo et al., 2010). It is estimated that swine and poultry cannot digest up to 25% of their feed due to anti-nutritional factors and lack of endogenous enzymes (Barletta, 2010).

History of exogenous enzyme use in the poultry industry

Scientific research into the effects of enzyme inclusion in poultry diets has been conducted for nearly a century, with one of the first studies presented in 1925 by Clickner and Follwell, and published in the 5th volume of *Poultry Science* in 1926. The product examined in this study was "Protozyme," a cocktail containing enzymes such as amylase and protease, derived from the fungus *Asperigillus orizae* (Clickner and Follwell, 1926). Today this fungus is often used as an enzyme source for many fermented foods, especially those in Asian cuisine that utilize soy or rice as an ingredient (Shurtleff and

Aoyagi, 2012). When used in the 1926 study "Protozyme" was observed to improve productivity and nutrient performance to chickens in a diet containing whole wheat and cracked corn (Clickner and Follwell, 1926). The researchers noted decreased mortality in birds fed the enzyme cocktail, as well as increased egg production and body weight gain. The preliminary data collected in this study helped pave the way for further research into the effectiveness of enzymes in poultry diets. Further research in the 1940's and 1950's tested an amylase, which seemed to improve the performance of birds fed a barley based diet. However it is now known that chickens produce amylase endogenously so these studies likely had impurities in the amylase, perhaps including β-glucanase activity, which were the cause of the improvement of the barley based diets (Choct 2006).

Since then enzymes have become increasingly popular as feed additives, with xylanases and glucanases being introduced as recently as the 1980s (Cowieson and Bedford, 2009). A substantial amount of nutritional research has been conducted in just the past few decades, though there is still much that needs to be done in order to fully understand the effects of enzymes, how they interact with ingredients in the diet, and how they are metabolized to elicit changes in the utilization of the feed by the bird. Much of the motivation for this sudden increase in enzyme usage stems from the need to increase efficiency of feed ingredients to decrease costs, as well as increased ethanol production reducing availability of whole corn to be used for animal agriculture (O'Neill et al., 2012). Use of plant protein sources also creates a need for exogenous enzyme supplementation, as non-starch polysaccharides can decrease the bird's ability to utilize protein and energy from these sources efficiently (Choct, 2006). Through enzyme

supplementation producers may now be able to use feed ingredients that, on their own, would normally be poor in apparent metabolizable energy.

Enzymes provide a variety of functions assisting birds in the digestion of different ingredients. One class of enzymes are non-starch polysaccharide (NSP) degrading enzymes. These enzymes are found in bacterial species and hydrolyze glycosidic bonds between sugars in carbohydrates such as xylans and glucans, common components of the cell wall making about 2-7% of the starchy endosperm in plant seeds (Davies and Henrissat, 1995; Saulnier et al., 2012). Because of the increase in energy released from oligosaccharides not normally available to them, the NSP degrading enzymes can be assigned an energy value for use in least cost diet formulation (O'Neill et al., 2012). The effectiveness of NSP degrading enzymes can vary depending on which ingredients are used in the diet, as well as variability in NSP and starch content in these ingredients due to factors such as cultivation, harvest conditions, and digestibility parameters (Mirzaie et al., 2012; Cowieson and Bedford, 2009). Most NSP degrading enzymes are targeted toward cereal grains (Paloheimo et al., 2010). Birds in the United States are fed a diet consisting primarily of corn, while European birds are fed more small grains such as barley and rye (Bedford, 1995). In young birds fed a corn-based diet, NSP degrading enzymes can increase body weight gain, feed conversion ratio, and apparent metabolizable energy, especially if the corn used is finely ground (Kaczmarek et al., 2014). Consumption of non-starch polysaccharides can decrease the viscosity of the digesta which increases the time spent in the digestive tract (Buchanan et al., 2007). Increased viscosity can also impair fat digestion, which may be improved by NSP degrading enzymes and other carbohydrases (Meng et al., 2004). Decreasing digesta

viscosity through the use of enzymes can also have unique benefits to poultry as it can reduce the number of dirty eggs and enhance yolk color (Paloheimo et al., 2010).

Xylanase

Xylanase is a common NSP degrading enzyme added to poultry diets that hydrolyzes bonds between sugars in xylan molecules, including arabinoxylans (Davies and Henrissat, 1995). Xylans are the main component of hemicellulose in plant cells. A xylan molecule is composed of xylose sugar polymers with varied structure and multiple branch points. There are many other factors that can influence the properties of xylans including substituent groups on the xylose units, genetics and enzymes of the particular plant, and environmental conditions. Rye, wheat, and sorghum have higher concentrations of arabinoxylans than other grains, digestion of which can benefit from xylanase activity (Saulnier et al., 2012). The structure of the xylanase enzyme allows multiple sugars to be bound randomly due to an open cleft active site. The hydrolysis of the glycosidic bond occurs via acid catalysis which utilizes a water molecule to break the bond (Davies and Henrissat, 1995). Xylanase generally improves digestibility of amino acids of the indigestible fraction of an ingredient by about 16% when added to poultry diets, however the effect can vary depending on the chosen ingredient. Wheat middlings and dried distillers grains and solubles (DDGS), for example, will only have 5% improvement in digestibility of the undigested amino acid fraction by xylanase due to high amounts of insoluble fiber, whereas digestibility of fractions in rye or barley are more greatly improved at 30% due to high soluble fiber. The improvement of digestibility by xylanase activity may be due to its effect on lowering intestinal viscosity (Cowieson and Bedford, 2009).

Xylanase not only seems to affect the digesta, but also the gastrointestinal tract itself. O'Neill and others (2012) conducted a study looking at the effect of xylanase and fat supplementation in broilers fed a low energy corn and soybean meal diet. The effect of xylanase was consistent and it had the most improvement on the feed conversion ratio of the birds fed low energy diets during the later phases of the study. They found no interaction between the enzyme and added fat. Birds fed lower energy diets tend to have poorer feed conversion ratios since they need to eat more in order to get energy needed for growth, maintenance, and/or production of eggs (O'Neill et al., 2012).

It is hypothesized that xylanase may also affect gut microflora. Choct and others (2006) determined that supplementing xylanase to broilers fed a wheat-based diet not only improved bird performance, but also reduced variability in apparent metabolizable energy between individual birds and decreased the number of *Clostridium perfringens*, a harmful bacteria that can cause necrotic enteritis, in the ileum and ceca (Choct et al., 2006). Xylanase may also enhance the fermentative capability of the ceca as the birds age (O'Neill et al., 2012). The ceca is a blind sac that is connected to the main digestive tract where the ileum and colon meet. Chickens have two ceca, though some types of birds do not have any at all. The function of the ceca is still not well understood, and removal does not seem to produce significant effects on nutrient utilization. In wild birds, the ceca are adapt to accommodate differences in diet depending on the season. Such adaptations have been observed to occur in domestic broiler chickens and turkeys over a period ranging from a few weeks to over four months respectively after a diet change. The ceca contents remain for a long period of time (upwards of 24 hours) before being emptied, supporting its currently known function as a site of mixing and fermentation (Svihus et al., 2013).

Laying hens can also benefit from xylanase supplementation in feed. Mathlouthi and others (2002) determined that the enzyme can improve egg production, egg mass, and feed conversion in layers fed low energy wheat and wheat-barley based diets. They also calculated that 1400 IU/kg of xylanase added to the diet resulted in at least 100 kcal/kg of increased energy (Mathlouthi et al., 2002). Mirzaie and others (2012) also reported increased egg production and egg mass, improved feed conversion ratio, and decreased digesta viscosity due to xylanase supplementation in diets containing wheat.

It is not uncommon to see xylanase in combination with other enzymes as feed additives, especially as the poultry industry moves more toward improving efficiency through the use of exogenous enzymes. There are many enzyme complexes available to poultry producers which can include any combination of carbohydrases, proteases, amylases, and other enzymes. Overall, enzyme supplementation has been observed to be effective in improving the digestion of feed, but the benefit may decelerate as more enzymes are mixed (Cowieson and Bedford, 2009).

Xylanase with NSP degrading enzymes

Other non-starch polysaccharide degrading enzymes may be added along with xylanase to a poultry diet. These can include cellulases, glucanases, and galactosidases.

The class of NSP degrading enzymes that utilize glucans as a substrate are called glucanases. β-glucans are found in many ingredients, with barley and oats having particularly high quantities (Saulnier et al., 2012). β-1,3- and β-1,6-glucans often have branching points and are not as rigid as cellulose, but are still difficult for poultry to digest (Paloheimo et al., 2010). Hydrolysis of the glycosidic bonds in glucans by

glucanase, and the general structure of the glucanase enzyme itself, is fairly similar to that of xylanase, meaning it has multiple sites in an open cleft to bind sugars and cleave glycosidic linkages via acid catalysis using water (Davies and Henrissat, 1995). A study by Choct and others (1995) examined the effect of glucanase in an NSP-rich wheat diet fed to broilers. The enzyme supplementation significantly decreased digesta viscosity and increased weight gain, feed conversion, and apparent metabolizable energy. They also found significantly higher volatile fatty acid concentrations in the ceca of birds supplemented with glucanase, as well as decreased fermentation in the ileum (Choct et al., 1995). Mathlouthi and others (2011) found that broilers fed a corn, wheat, and barley diet supplemented with β-glucanase had higher body weights, improved feed conversion, and reduced small intestine weight. A study by Cowieson and others (2010) determined that much of the benefits of xylanase and glucanase in a corn/soy diet were due to the individual effects of each enzyme, but that they did have some additivity when improving feed conversion, nutrient digestibility, ileal digestibility of nitrogen and phosphate, and ileal amino acid digestibility before the finisher phase. Xylanase and glucanase together improved the diet 25% more than either enzyme alone, though there was no observable interaction (Cowieson et al., 2010). Xylanase and glucanase mixtures have also been reported to improve body weight and feather condition, and reduce mortality in turkeys (Odetallah et al., 2002). Supplementing arabinoxylanase and β-glucanase from *Aspergillus niger* in a corn/soybean/wheat diet resulted in improved performance of broilers (Hajati et al., 2009). Xylanase has a greater effect on lowering viscosity in the jejunum for wheat than for corn when combined with β-glucanase in the diet (Munyaka et al., 2016).

Galactosidases may be added to poultry diets, which can aid in the digestion of α and β-galactosides. Unlike xylanases and glucanases, the active site of a galactosidase enzyme is shaped like a crater to be able to accommodate substrates with many different chain ends (Davies and Henrissat, 1995). Alpha-galactosidases are used in non-ruminants due to the indigestibility of α-galactosides which are found in high concentrations in legumes. Chickens are unable to digest these carbohydrates, specifically raffinose and stachyose, due to a lack of endogenous α-1,6-galactosidase (Brenes et al., 1989; Parsons et al., 2000). This can pose a problem in the poultry industry as diets in the United States are typically formulated using soybean meal as one of the primary ingredients. When supplemented to broilers fed a corn-soybean meal diet, α-galactosidase improved metabolizable energy and nutrient utilization including increased digestibility of calcium and phosphorus, and increased availability of methionine and cysteine (Wang et al., 2005). Galactosidases are not typically complexed solely with xylanase, but may appear in multi-enzyme products. Enzyme mixtures containing xylanases, β-glucanases, α-galactosidases, other hemicellulases, pectinases, proteases, and inulinase can be beneficial in diets with a high lupin content. Lupins are seeds that may be used as a protein substitute for soybeans (Marquardt et al., 1996). Meng and Slominski (2005) found that a multiple enzyme preparation including xylanase, glucanase, pectinase, cellulose, mannanase, and galactanase can improve NSP digestibility for corn, soybean meal, canola meal, and peas. However, the corn-based diet was the only one that showed an improved feed conversion ratio as a result of the enzymes.

Xylanase with phytase

Phytase is a very common exogenous enzyme in the poultry industry and it has been a focus of nutritional research since the 1990s. This enzyme has importance to both nutrition and environmental concerns. Phytase acts by releasing phosphates from phytate. Phosphate is crucial for cellular function and metabolism and is present in key molecules such as DNA and ATP. It is also important for skeletal structure and strength. Phosphates that are tied up in phytate become unavailable to the bird and are excreted in the feces, which can have a negative effect on the environment. Excess phosphate in freshwater can cause algal blooms, killing fish (Selle et al., 2010). When supplemented with phytase, xylanase can increase egg weight and albumin height with no differences between brown and white laying hens fed a diet high in wheat (Silversides et al., 2006). The combined effectiveness of xylanase and phytase in corn/soybean diets is not yet consistent in the literature. Gehring and others (2013) found that there was no interaction between xylanase and phytase in corn/soybean diets when fed to broilers, and that the effectiveness of the enzymes may be influenced by the age of the birds. However a recent study by Schramm and others (2016) reported improvements in the effects of xylanase when supplemented with phytase in a corn/soybean diet. They also investigated these effects in a purely corn-based diet which did not yield significant results.

Xylanase with other enzymes

There are a number of enzymes that may be added to break down cellulose, hemicellulose, and starch in animal feed. To help with cellulose digestion a cellulose may be added. The glucans in cellulose are linear and arranged in microfibrils in the cell walls of plants which are stabilized by hydrogen bonding. This crystalline structure is what is

targeted by cellobiohydrolases to break down cellulose (Paloheimo et al., 2010). Cellulase can decrease the viscosity of the digesta and is resistant to the effects of extrusion, meaning that the enzyme will remain functional after the feed has been pelleted (Spring et al., 1996). Bacterial species that produce cellulase and xylanase have been shown to be effective at reducing viscosity and digesting NSPs. Proliferation of *Clostridium perfringens* in *in vitro* models of the avian digestive system has also been shown to be reduced by directly supplementing these enzyme producing bacteria into the system (Latorre et al., 2015).

Amylase breaks down starch by hydrolyzing α-1,4 linkages in carbohydrates. While it is supposedly produced in sufficient quantities by chickens, research is continuing to work on developing enzyme complexes that include amylase to further improve energy utilization and performance (Vieira et al., 2015). In chicks, amylase does not affect performance and apparent metabolizable energy when supplemented in a conventional corn-based diet, but may improve these parameters when used with finely ground corn (Kaczmarek et al., 2014). Lipase breaks down fat. Endogenous lipase production increases as chicks age, though addition of lipase does not affect digestion and utilization of nutrients. However there was an interaction for fat digestibility between a mix of carbohydrases (xylanase, glucanase, cellulase, and other enzymes) and the type of fat added to the diet, with tallow being the fat type that was improved the most in terms of fat digestibility (Meng et al., 2004).

Protease is another enzyme that is commonly available as part of a complex with NSP degrading enzymes, including xylanase. Protease on its own increases the digestibility of nitrogen in the diet by hydrolyzing peptide bonds in proteins, though it

may also have more complex effects on nutrient digestibility and the microflora in the intestines which are not well understood. The magnitude of the improvement in digestibility by protease can be increased when it is used alongside other enzymes (Olukosi et al., 2015). Addition of an enzyme complex containing xylanase, glucanase, amylase, protease, and invertase increased performance of broilers fed a triticale (a wheat hybrid) based diet (Oryschak et al., 2010). Xylanase, amylase, and protease cocktails can improve the apparent metabolizable energy of a corn/soybean diet in broiler chicks (Dourado et al., 2009).

II. β-glucan use in poultry nutrition

Sources and method of action

The general structure of β-glucans varies depending on the source, but can be classified by the β-glycosidic linkages which connect the chains and branches that make up the saccharide polymers. They are components of cell walls and serve as energy stores for plants and single-celled organisms (Stier et al., 2014). β-glucan activity is determined by the number of branching points, conformation, and the size (molecular weight) of the molecule (Zeković et al., 2005).

Paramylon produced by *Euglena gracilis* consists of insoluble β-1,3-glucan (Soltanian et al., 2009). The wild-type of this species of green algae is photosynthetic, but can be cultivated heterotrophically for paramylon production using glucose and corn steep solid media (Barsanti et al., 2001; Ivušić and Šantek, 2015). The non-

photosynthetic mutant strain of *E. gracilis* produces much more paramylon than the wild-type. The paramylon is made and stored in many granules throughout the cytoplasm of the single-celled algae. (Barsanti et al., 2001). Before more was discovered about *Euglena* paramylon, it was *Saccharomyces cerevisiae* (baker's yeast) that was used as a source of β-glucan (Barsanti et al., 2001). Fungi, yeast, and lichen produce β-1,3/1,6-glucans that can have many branching points. *S. cerevisiae* β-glucan can inhibit tumor growth and bind mycotoxins, making them unavailable and preventing toxicity from contaminated feed (Zeković et al., 2005).

β-1,3-glucans are of specific interest to livestock health and production for their immunostimulatory properties (Zeković et al., 2005; Stier et al., 2014). The mechanism by which β-1,3-glucans achieve their effects has not been precisely identified, mainly due to the wide variety of sources used when conducting research (Zeković et al., 2005). Not all β-glucans exhibit immunostimulatory effects, such as cellulose, a β-1,4-glucan, which serves mainly as a rigid structural component of cell walls (Stier et al., 2014). β-glucan receptors exist on several types of cells that make up the immune system including monocytes, neutrophils, and natural killer cells, as well as cells that make up other parts of the body such as epithelial cells and fibroblasts. One of these receptors is Dectin-1 which is a type-II transmembrane glycoprotein. It can recognize β-1,3/1,6-glucan and its solubility, determining specificity (Zeković et al., 2005). Other receptors include CR3, lactosylceramide, scavenger receptors, and Toll-like receptors 2 and 6. The response stimulated by activation of these receptors is for immune cells to secrete cytokines and kill pathogens (Soltanian et al., 2009).

Use of β-glucan in poultry production

Research on the use of algae and algal products in poultry is limited, however β-glucans overall have been investigated for their role in gut health and the immune response. Breed and sex of the birds can affect their response to β-glucan supplementation (Redmond et al., 2010). β-1,3/1,6-glucans can help the mucosa of the intestines against *Salmonella* Typhimurium in broilers. The mucosa consists of epithelial cells that line the inner portion of the intestine, protecting against harmful bacteria and other pathogens by acting as a barrier, and is the site of nutrient absorption. Broilers supplemented with β-1,3/1,6-glucan had a greater villus height:crypt depth ratio in the jejunum than control birds (Morales-Lopez et al., 2009; Shao et al., 2013). They also had higher density of goblet cells (which secrete mucus) and significantly lower *Salmonella* levels in the ceca and liver than the birds without the supplement. This shows that the β-glucan could help protect and relieve the mucosa from damage by *Salmonella* (Shao et al., 2013). Supplementation of β-1,3-1,6-glucan may be beneficial as protection from *Salmonella* Enteritidis in chicks. In vitro tests of β-glucan treatment of abdominal macrophages from β-glucan-free chicks resulted in increased engulfment of *Salmonella* (Chen et al., 2008). Some improvement was also seen in broilers challenged with *Escherichia coli*, but β-glucan may not be as beneficial for unchallenged flocks and may lead to decreased production due to increased stimulation of the immune system (Huff et al., 2006).

β-1,3-1,6-glucan, called Sophy β-glucan, is produced by the AFO-202 strain of *Aureobasidium pullulan*, a species of black yeast. This β-glucan was fed to Peking ducks to determine its effectiveness as a supplement compared to using bacitracin zinc (an

antibiotic), and whether it could be effectively used for antibiotic-free production. The β-glucan did not improve growth or performance of the ducks, but it did increase proliferation of mononuclear cells and increase nonspecific cellular immunity (Tang et al., 2011).

Use of β-glucan in other species

Research of algal polysaccharides has been conducted in a wide variety of other species ranging from domestic livestock to aquaculture. Immune benefits and increased performance have been reported in many of these studies. In mice, β-glucan has been shown to aid the immune response. Paramylon-treated mice produce more IL-1 (in vitro) and IL-6 (transiently in blood) as a response to lipopolysaccharide (Kondo et al., 1992). It has also been shown to have antitumor activity, specifically against colon cancer in mice (Watanabe et al., 2013). β-glucans have been shown to stimulate the immune response in swine, and may be an option for antibiotic-free production. Sonck and others determined that β-glucans can have significant effects on cell cultures and neutrophils, which are dependent on the structure and source of the β-glucan. *Euglena gracilis* β-glucan performed very well in stimulating the proliferation of lymphocytes, production of reactive oxygen species, and release of cytokines such as interleukins, IFN-γ, and TNF-α in swine (Sonck et al., 2010).

III. Zinc polysaccharide

Zinc is considered an essential mineral and is an important part of the diet. Minerals are often bound to other compounds for feed sources in poultry diets. Organic

sources include chelates where the metal ion, such as zinc, is bound to protein, amino acids (such as methionine), or carbohydrates. One carbohydrate source for organic zinc is β-glucan. Inorganic sources include phosphates, sulfates, and oxides. Mineral oxides and sulfates are generally kept at a minimum in poultry diets due to the risk of oxidizing vitamins and other important nutrients (Park et al., 2004). Organic zinc sources, such as zinc carbohydrates, have been shown to provide high zinc availability to the bird (Cao et al., 2000).

Zinc has an important role in bone and feather development. It is also a cofactor for many different metabolic pathways and enzymes, notably carbonic anhydrase which is a metalloenzyme that catalyzes the reaction between carbon dioxide and water to bicarbonate. Hens use this enzyme to produce bicarbonate which will combine with calcium and be deposited as calcium carbonate during eggshell formation (Nys et al., 1999). Zinc is also known to have a role in immune health. Zinc methionine can promote nonspecific cellular immunity, improve phagocytosis by macrophages, and increase *E. coli* survivability in older birds (Park et al., 2004). Once infection by a pathogen occurs the bird rapidly uses up zinc to facilitate the increased need for immune cell proliferation. Too little zinc can reduce lymphocyte proliferation and cause abnormal development of T lymphocytes (Kidd et al., 1996). The presence of zinc in the gut may also prevent proliferation of the bacteria, and the immune benefits of zinc mentioned earlier may help the bird stay healthy as well (Park et al., 2004).

IV. Use of omega-3 fatty acids in poultry production

Fat is a key nutrient in livestock diets. It is an important source of energy, increases palatability, and prevents feed from becoming dusty (Omidi et al., 2015). Modifications of the fatty acid composition of the diet can affect fat deposition in animal products, such as eggs. Laying hens of all ages and breeds are required to have at least 1% fat, in the form of linoleic acid, per kilogram of feed (NRC, 1994). Linoleic acid is an omega-6 fatty acid, an unsaturated fatty acid with the first double bond at the sixth carbon from the methyl end. Omega-3 fatty acids have been the focus of research lately due to benefits for both animals and humans. The defining feature of an omega-3 fatty acid is similar to an omega-6, except the first double bond is on the third carbon from the methyl end. A study conducted in 2015 showed that Omega-3 supplementation may have benefits to the hen by increasing bone strength. However, high levels of long chain omega-3 fatty acids in the diet may also cause negative effects such as decreased body weight gain, egg production, and egg size (Toscano et al., 2015).

The human diet changed drastically during the past century due to advances in agriculture and changes in production needs. One of the effects of this was a change in the fatty acid content of consumers' diets. The use of grains as animal feeds, for example, introduced more omega-6 fatty acids into the diets of livestock, and subsequently human consumers. This can lead to an imbalance of omega-6:omega-3 fatty acids, of which the ideal ratio near 1. Increasing consumption of omega-3 fatty acids may help lower the risk of certain cancers, cardiovascular disease, and other chronic illnesses (Simopoulos, 2002). Fish is a common source for omega-3 fatty acids, but other sources are becoming more available to consumers. One such option is enriched chicken meat by feeding green

microalgae, a byproduct of biofuel production (Gatrell et al., 2015). Another option is omega-3 enriched eggs. Increasing the omega-3 content of the egg can be accomplished by supplementing the diet of the layer hen in several ways, typically by adding oils rich in omega-3 fatty acids to the diet. Research has been conducted using various oils including fish, soy, canola, olive, and grapeseed. Fish oil seems to be the most effective supplement as it also decreases omega-6 fatty acids, helping to bring the omega fatty acid ratio closer to 1, as mentioned earlier (Omidi, 2015). Linseed oil is another source that can facilitate deposition of omega-3 fatty acids in the egg yolk (Bourre and Galea, 2006). Algal sources of omega-3 fatty acids have become considered, though the rigidity of the algal cell wall can affect the availability of the fat to the bird, so the species chosen for supplementation should be considered carefully (Lemahieu et al., 2016).

Literature cited

Barletta, A., M. Paloheimo, J. Piironen, J. Vehmaanpera, P. H. Selle, V. Ravindran, A. J. Cowieson, and M. R. Bedford. 2010. Enzymes in Farm Animal Nutrition (M Bedford and G Partridge, Eds.). 2nd ed. CAB International.

Barsanti, L., R. Vismara, V. Passarelli, and P. Gualtieri. 2001. Paramylon (β-1,3-glucan) content in wild type and WZSL mutant of *Euglena gracilis*. Effects of growth conditions. Journal of Applied Phycology 13:59-65.

Bedford, M. R., 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. Animal Feed Science and Technology 53:145-155.

Bourre, J. M. and F. Galea. 2006. An important source of omega-3 fatty acids, vitamins D and E, carotenoids, iodine and selenium: A new natural multi-enriched egg. The Journal of Nutrition, Health & Aging 10:371-376.

Brenes, A., J. Trevino, C. Centeno, and P. Yuste. 1989. The influence of alpha-galactosides extracted from lupin seed (Lupinus albus) on the digestion of dietary starch by growing chicks. Recent advances in research in antinutritional factors in legume seeds. (J. Huisman, T. F. B. van der Poel, and I. E. Liener, Eds.). 374-377.

Buchanan, N. P., L. B. Kimbler, A. S. Parsons, G. E. Seidel, W. B. Bryan, E. E. D. Felton, and J. S. Moritz. 2007. The effects of nonstarch polysaccharide enzyme addition and dietary energy restriction on performance and carcass quality of organic broiler chickens. J. Appl. Poult. Res. 16:1-12.

Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J. Anim. Sci. 78:2039-2054.

Chen, K. L., B. C. Weng, M. T. Chang, Y. H. Liao, T. T. Chen, and C. Chu. 2008. Direct enhancement of the phagocytic and bactericidal capability of abdominal macrophage of chicks by β-1,3–1,6-glucan. Poultry Science 87:2242-2249.

Choct, M. 2006. Enzymes for the feed industry: past, present and future. World's Poultry Science Journal 62:5-15.

Choct, M., R. J. Hughes, R. P. Trimble, K. Angkanaporn, and G. Annison. 1995. Non-starch polysaccharide-degrading enzymes increase the performance of broiler chickens fed wheat of low apparent metabolizable energy. The Journal of Nutrition 125:485-492.

Choct, M., M. Sinlae, R. A. M. Al-Jassim, and D. Pettersson. 2006. Effects of xylanase supplementation on between-bird variation in energy metabolism and the number of *Clostridium perfringens* in broilers fed a wheat-based diet. Australian Journal of Agricultural Research 57:1017-1021.

Clickner, F. H., and E. H. Follwell. 1926. Application of "Protozyme" (Aspergillus orizae) to Poultry Feeding. Poultry Science 5:241–247.

Cowieson, A. J., and M. R. Bedford. 2009. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: complimentary mode of action? World's Poultry Science Journal 65:609-624.

Cowieson, A. J., M. R. Bedford and V. Ravindran. 2010. Interactions between xylanase and glucanase in maize-soy-based diets for broilers. British Poultry Science 51:246-257.

Davies, G., and B. Henrissat. 1995. Structures and mechanisms of glycosyl hydrolases. Structure 3:853-859.

Dourado, L. R. B., N. K. Sakomura, N. A. A. Barbosa, M. A. Bonato, I. M. Kawuauchi, J. B. K. Fernandes, F. G. P. Costa. 2009. Corn and soybean meal metabolizable energy with the addition of exogenous enzymes for poultry. Brazilian Journal of Poultry Science 11:51-55.

Gatrell, S. K., J. Kim, T. J. Derksen, E. V. O'Neil, and X. G. Lei. 2015. Creating ω-3 Fatty-Acid-Enriched Chicken Using Defatted Green Microalgal Biomass. J Agric Food Chem 63:9315:9322.

Gehring, C. K., M. R. Bedford, and W. A. Dozier III. 2013. Extra-phosphoric effects of phytase with and without xylanase in corn-soybean meal-based diets fed to broilers. Poultry Science 92:979-991.

Gehring, C. K., M. R. Bedford, and W. A. Dozier III. 2013. Interactive effects of phytase and xylanase supplementation with extractable salt-soluble protein content of corn in diets with adequate calcium and nonphytate phosphorus fed to broilers. Poultry Science 92:1858-1869.

Hajati, H., M. Rezaei and H. Sayyahzadeh. 2009. The effects of enzyme supplementation on performance, carcass characteristics and some blood parameters of broilers fed on corn-soybean meal-wheat diets. International Journal of Poultry Science 8:1199-1205.

Huff, G. R., W. E. Huff, N. C. Rath, and G. Tellez. 2006. Limited treatment with *β*-1,3/1,6-glucan improves production values of broiler chickens challenged with *Escherichia coli*. Poultry Science 85:613-618.

Ivušić, F, and B. Šantek. 2015. Optimization of complex medium composition for heterotrophic cultivation of Euglena gracilis and paramylon production. Bioprocess Biosyst Eng. 38:1103-1112.

Kaczmarek, S. A., A. Rogiewicz, M. Mogielnicka, A. Rutkowski, R. O. Jones, and B. A. Slominski. 2014. The effect of protease, amylase, and nonstarch polysaccharide-degrading enzyme supplementation on nutrient utilization and growth performance of broiler chickens fed corn-soybean meal-based diets. Poultry Science 93:1745-1753.

Kidd, M. T., P. R. Ferket, and M. A. Qureshi. 1996. Zinc metabolism with special reference to its role in immunitv. World's Poultry Science Journal 52:309-324.

Kondo, Y., A. Kato, H. Hojo, S. Nozoe, M. Takeuchi, and K. Ochi. 1992. Cytokine-related immunopotentiating activities of paramylon, a beta-(1-->3)-D-glucan from Euglena gracilis. J Pharmacobiodyn. 15:617-621.

Latorre, J. D., X. Hernandez-Velasco, V. A. Kuttappan, R. E. Wolfenden, J. L. Vicente, A. D. Wolfenden, L. R. Bielke, O. F. Prado-Rebolledo, E. Morales, B. M. Hargis, and G. Tellez. 2015. Selection of *Bacillus* spp. for cellulase and xylanase production as direct-fed microbials to reduce digesta viscosity and *Clostridium perfringens* proliferation using an in vitro digestive model in different poultry diets. Front. Vet. Sci. 2:1-8 doi: 10.3389/fvets.2015.00025

Lemahieu, C., C. Bruneel, C. Dejonghe, J. Buyse, and I. Foubert. 2016. The cell wall of autotrophic microalgae influences the enrichment of long chain omega-3 fatty acids in the egg. Algal Research 16:209-215.

Marquardt, R. R., A. Brenes, Z. Zhang, and D. Boros. 1996. Use of enzymes to improve nutrient availability in poultry feedstuffs. Animal Feed Science Technology 60:321-330.

Mathlouthi, N., M. Larbier, M. A. Mohamed, and M. Lessire. 2002. Performance of laying hens fed wheat, wheat-barley or wheat-barley-wheat bran based diets supplemented with xylanase. Can. J. Anim. Sci. 82: 193–199.

Mathlouthi N. and M. Larbier. 2011. Influence of beta-glucanase supplementation on growth performances and digestive organs weights of broiler chickens fed corn, wheat and barley-based diet. International Journal of Poultry Science 10:157-159.

Meng, X., B. A. Slominski, and W. Guenter. 2004. The effect of fat type, carbohydrase, and lipase addition on growth performance and nutrient utilization of young broilers fed wheat-based diets. Poultry Science 83:1718-1727.

Meng, X. and B. A. Slominski. 2005. Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. Poultry Science 84:1242-1251.

Mirzae, S., M. Zaghari, S. Aminzadeh, M. Shivazad, and G. G. Mateos. 2012. Effects of wheat inclusion and xylanase supplementation of the diet on productive performance, nutrient retention, and endogenous intestinal enzyme activity of laying hens. Poultry Science 91:413-425.

Morales-Lopez, R., E. Auclair, F. Garcia, E. Esteve-Garcia, and J. Brufau. 2009. Use of yeast cell walls; β-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. Poultry Science 88:601-607.

Munyaka, P. M., N. K. Nandha, E. Kiarie, C. M. Nyachoti, and E. Khafipour. 2016. Impact of combined β-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. Poultry Science 95:528-540.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th ed. National Academies Press, Washington, D.C.

Nys, Y., M. T. Hincke, J. L. Arias, J. M. Garcia-Ruiz, and S. E. Solomon. 1999. Avian eggshell mineralization. Poultry and Avian Biology Reviews 10:143-166.

Odetallah, N. H., C. W. Parks, and P. R. Ferket. 2002. Effect of wheat enzyme preparation on the performance characteristics of tom turkeys fed wheat-based rations. Poultry Science 81:987-994.

O'Neill, H. V. M., G. Mathis, B. S. Lumpkins, and M. R. Bedford. 2012. The effect of reduced calorie diets, with and without fat, and the use of xylanase on performance characteristics of broilers between 0 and 42 days. Poultry Science 91:1356-1360.

Olukosi, O. A., L. A. Beeson, K. Englyst, and L. F. Romero. 2015. Effects of exogenous proteases without or with carbohydrases on nutrient digestibility and disappearance of on-starch polysaccharides in broiler chickens. Poultry Science 94:2662-2669.

Omidi, M., S. Rahimi, and M. A. K. Torshizi. 2015. Modification of egg yolk fatty acids profile by using different oil sources. Vet Res Forum 6:137-141.

Oryschak, M., D. Korver, M. Zuidhof, and E. Beltranena. 2010. Nutritive value of single-screw extruded and nonextruded triticale distillers dried grains with solubles, with and without an enzyme complex, for broilers. Poultry Science 89:1411-1423.

Park, S. Y., S. G. Birkhold, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2004. Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. Biological Trace Element Research 101:147-163.

Parsons, C. M., Y. Zhang, and M. Araba. 2000. Nutritional Evaluation of Soybean Meals Varying in Oligosaccharide Content. Poultry Science 79:1127-1131.

Redmond, S. B., R. M. Tell, D. Coble, C. Mueller, D. Palić, C. B. Andreasen, and S. J. Lamont. 2010 Differential splenic cytokine responses to dietary immune modulation by diverse chicken lines. Poultry Science 89:1635-1641.

Saulnier, L., F. Guillon, and A. Chateigner-Boutin. 2012. Cell wall deposition and metabolism in wheat grain. Journal of Cereal Science 56:91-108.

Schramm, V. G., J. F. Durau, L. N. E. Barrilli, J. O. B. Sorbara, A. J. Cowieson, A. P. Félix, and A. Maiorka. 2016. Interaction between xylanase and phytase on the digestibility of corn and a corn/soy diet for broiler chickens. Poultry Science 0:1-8.

Shao, Y., Y. Guo, and Z. Wang. 2013. β-1,3/1,6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella enterica* serovar Typhimurium. Poultry Science 92:1764-1773.

Shurtleff, W., and A. Aoyagi. 2012. History of Koji-Grains and/or Soybeans Enrobed with a Mold Culture (300 BCE to 2012). Soyinfo Center, Lafayette, CA.

Silversides, F. G., T. A. Scott, D. R. Korver, M. Afsharmanesh, and M. Hruby. 2006. A study on the interaction of xylanase and phytase enzymes in wheat-based diets fed to commercial white and brown egg laying hens. Poultry Science 85:297-305.

Simopoulos, A. P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56:365-379.

Soltanian, S., E. Stuyven, E. Cox, P. Sorgeloos and P. Bossier. 2009. Beta-glucans as immunostimulant in vertebrates and invertebrates. Critical Reviews in Microbiology. 35:109-138.

Sonck, E., E. Stuyven, B. Goddeeris, and E. Cox. 2010. The effect of beta-glucans on porcine leukocytes. Vet Immunol Immunopathol 135:199-207.

Spring, P., K. E. Newman, C. Wenk, R. Messikommer, and M. Vukic Vranjes. 1996. Effect of pelleting temperature on the activity of different enzymes. Poultry Science 75:357-361.

Stier, H., V. Ebbeskotte, and J. Gruenwald. 2014. Immune-modulatory effects of dietary yeast beta-1,3/1,6-D-glucan. Nutrition Journal 13:1-9.

Svihus, B., M. Choct, and H. L. Classen. 2013. Function and nutritional roles of the avian caeca: a review. World's Poultry Science 69:249-263.

Tang, X. Y., J. S. Gao, F. Yuan, W. X. Zhang, Y. J. Shao, F. Sakurai, and Z. D. Li. 2011. Effects of Sophy β-glucan on growth performance, carcass traits, meat composition, and immunological responses of Peking ducks. Poultry Science 90:737-745.

Toscano, M. J., F. Booth, L. J. Wilkins, N. C. Avery, S. B. Brown, G. Richards, and J. F. Tarlton. 2015. The effects of long (C20/22) and short (C18) chain omega-3 fatty acids on keel bone fractures, bone biomechanics, behavior, and egg production in free-range laying hens. Poultry Science 94:823-835.

Vieira, S. L,, C. Stefanello, H. V. Rios, N. Serafini, R. G. Hermes, and J. O. B. Sorbara. 2015. Efficacy and metabolizable energy equivalence of an α-amylase-β-glucanase complex for broilers. Brazilian Journal of Poultry Science 17:227-236.

Wang, C. L., W. Q. Lu, D. F. Li, and J. J. Xing. 2005. Effects of alpha-galactosidase supplementation to corn-soybean meal diets on nutrient utilization, performance, serum indices, and organ weight in broilers. Asian-Aust J. Anim. Sci. 18:1761-1768.

Watanabe, T., R. Shimada, A. Matsuyama, M. Yuasa, H. Sawamura, E. Yoshida, and K. Suzuki. 2013. Antitumor activity of the β-glucan paramylon from Euglena against preneoplastic colonic aberrant crypt foci in mice. Food Funct. 4:1685-1690.

Zeković, D. B., S. Kwiatkowski, M. M. Virvć, D. Jakovljević, and C. A. Moran. 2005. Natural and modified (1→3)-*β*-D-glucans in health promotion and disease alleviation. Critical Reviews in Biotechnology 25:205-230.

Chapter 2: Effect of NSP degrading enzymes on the performance of laying hens.

INTRODUCTION

Non-starch polysaccharide (NSP) degradable enzymes are one of the tools available to producers in the poultry industry to help increase bird performance by improving digestibility of fibrous materials in the diet. Cellulose and hemicellulose are not digested by the bird and can affect digesta viscosity, movement, and mixing in the intestines. Xylanase and β-glucanase are two common enzymes added to poultry diets, though cellulase, galactosidase, and others can be included. These enzymes are also often complexed with amylase and/or protease. The effects of these enzymes on performance has been noted in areas where poultry diets consists largely of cereal ingredients. Birds fed corn and soybean diets do not always benefit from NSP degrading enzymes due to the different amount and type of NSPs present, which should be considered by producers when choosing enzyme products (Slominski, 2011).

Corn contains a variety of xylans and arabinoxylans with differing viscosities. Over 35% of the corn hull is comprised of these carbohydrates (Hromádková and Ebringerová, 1995). Much of the NSP content in a soybean is in the hull, which is comprised of about 50% hemicellulose. The xylan in this hemicellulose fraction is mostly linear and contains β-1,4-D-glycosidic linkages. (Aspinall et al., 1966; Karr-Lilienthal et al., 2005). The amount of xylan present can change during soybean meal processing, decreasing as a percentage of dry matter after toasting or extrusion (Karr-Lilienthal, 2005).

Much of the research focusing on the effectiveness of xylanase has been with broiler chickens, while the literature for its effects on laying hen performance and production is not as extensive. Much of the benefit of xylanase occurs in diets where the primary ingredients have a higher NSP content, such as wheat and barley (Mathlouthi et al., 2002; Mirzaie et al., 2012). The effects of xylanase or xylanase-containing enzyme complexes on the digestibility of corn or corn/soybean based diets are not as consistent or definitive. In broilers, enzyme cocktails containing xylanase, amylase, and protease have been shown to improve crude protein digestibility, bodyweight gain, and feed conversion when added to a corn/soybean meal diet (Zanella et al., 1999) Studies of laying hens have shown that enzyme addition to diets with corn distiller's grains with solubles (CDDGS) did not affect performance, but did decrease nitrogen and phosphorus content in the manure (Deniz, et al., 2013).

The purpose of this experiment was to test whether addition of exogenous xylanase enzymes to a low ME corn/soybean diet results in equivalent performance compared to layer hens fed a normal ME corn/soybean diet. It was hypothesized that layer performance and production would vary based on enzyme inclusion and ME.

MATERIALS AND METHODS

Birds and Housing

This study used 72 cages, containing three 23-week-old Bovan White (ISA North America, Ontario, Canada) leghorn hens each, which were assigned 1 of 4 dietary treatments using a complete randomized block design. Each block comprised of 12 cages contained in a Chore-Time® cage system that had two sides containing 3 rows of wire

cages separated vertically by manure belts (CTB, Inc., Milford, IN). This resulted in 3 replicates per block, and 18 replicates total for each treatment. There was 688.17cm^2 of floor space per hen in each cage with removable feeder troughs. The hens were fed 110 grams per hen per day, which is the amount calculated to provide the correct energy level according to the diet formulation, from buckets containing a pre-weighed amount of feed for each week corresponding to the number of hens in each cage. The hens also had ad libitum water accessible via nipple drinkers. The housing unit was in a windowless, climate controlled room with a 16-hour light:8-hour dark photoperiod. It was located in the Animal Science Complex on the University of Nebraska-Lincoln's East Campus.

Diets

The treatments tested in this study consisted of a positive control diet, a negative control with lower ME and amino acid density, and two diets with xylanases, labeled enzyme A and enzyme B supplemented to the negative control. There were 18 replicates for each treatment within the 72 cages used for this study. All diets were corn/soybean meal based with 10% dried distillers grains and solubles (DDGS). The lower ME in the negative control was achieved by diluting the diet with soy hulls and decreasing fat content. The full diet composition and key digestible amino acids can be viewed in Table 2.1, and overall amino acid composition for the phase 1 diets in Table 2.2. There were two phases in this experiment, and feed samples were collected for each. Phase 1 was 23-43 weeks of age and phase 2 was 43-58 weeks. The ME of the negative control and diets 3 and 4 were decreased further during phase 2 to determine if the enzyme supplementation would have more pronounced effects than found during phase 1. Enzyme A was added at 181.6g/ton (trt 3), and enzyme B was added at 340.5g/ton (trt 4)

and feed was mixed at the University of Nebraska-Lincoln's feed mill (Ithaca, NE) prior to the start of each phase of the study. Both xylanases were provided by Kerry Ingredients (Beloit, WI). The enzymes were supplemented at the same level for both phase 1 and phase 2. Feed sample analysis showed enzyme B had a higher xylanase activity (Analysis by Kerry Global Technology and Innovation Centre, Kildare, Ireland) than enzyme A, and the activity of both enzymes increased in the phase 2 diets (Table 2.3). This increase in enzyme activity was likely due to an increase in soy hulls, which increases the xylan content of the diet. This increase in substrate would lead to increased enzyme activity.

Measurements

Procedures for data collection were approved by the University of Nebraska IACUC. Egg production was recorded daily. Feed intake (FI) was determined weekly and calculated by subtracting the amount of feed remaining from the feed given then divided by the number of hens in each cage. Average egg weights (EW) were measured biweekly using a sample of two eggs per cage. A baseline average body weight (BW) per cage was determined at the beginning of the study and subsequent BWs were measured at 10, 20, and 35 weeks. Percent egg production (EP) was calculated by dividing the number of eggs collected by the number of hen day eggs expected, egg mass (EM) by multiplying average EW by EP per cage, and feed conversion ratio (FC) by dividing FI by EM. All calculated data used biweekly periods, and statistical analysis also used biweekly data.

Statistical Analysis

The data were analyzed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, 2015) All of the response variables, except body weight were analyzed using a repeated measures model including random block effect and fixed effects of time, treatment and their interaction. Various error covariance structures were evaluated for each response variable to determine the one that fit the best. Body weight response was analyzed using a model with a random block effect and fixed treatment effect.

RESULTS AND DISCUSSION

The results for egg percent production, egg weight, and egg mass are found in table 2.4 and figures 2.1 and 2.2. Egg production was not affected by the lower ME diets during both phases 1 and 2 ($p \geq 0.05$). This contradicts a previous study that found significantly decreased egg production in birds fed a lower energy diet (Suresh et al., 2014). The lack of differences in egg production could be due to the young age of the birds as they were early into their first egg laying cycle, and producing very well. If this study were conducted with older hens more differences and variability may be observed in egg production. No differences in egg production between the negative control and the two negative diets with enzymes may also be due to lack of enzyme activity.

There was an overall treatment effect for egg weight for phase 1 and phase 2. The hens fed the positive control laid heavier eggs in phase 1 compared to the hens fed the negative control diet with enzyme B ($p=0.019$). In phase 2 the hens fed the positive control laid heavier eggs than all other treatments ($p=0.036$). Egg mass during phase 1 was significantly different ($p=0.01$) between the positive control and the negative control

with enzyme B, while there was no significant treatment effect ($p=0.12$) on egg mass during phase 2. Egg mass is calculated using percent egg production and egg weight so it is likely that the lack of differences in percent egg production and the less significant differences in egg weight during phase 2 were why no differences were seen for egg mass in phase 2.

The results for body weight, feed intake, and feed conversion are in table 2.5 and figures 2.3, 2.4, and 2.5. Overall, body weight and feed intake were the most significant results seen in this study. There was no significant treatment effect for body weight at the beginning of the study ($p=0.63$). While all hens were slightly underweight compared to the breed management guidelines at the beginning, which has a recommended weight of 1550g at 23 weeks of age (Hendrix-ISA LLC, 2015), the hens fed the positive control gained significantly more weight than all other treatment groups ($p<0.05$), and were closer to the standard. Hens on the positive control diet consumed significantly less feed during both phases ($p=0.0001$) yet gained more weight (phase 1: $p=0.01$; phase 2: $p=0.03$) than hens fed other treatments. This shows that the higher energy in the positive control diet allowed the hens to consume less feed to obtain the energy necessary for growth. The decrease in feed intake during the last period may have influenced a similar decrease in percent egg production as lower intake provides less nutrients for the hen to make the egg.

There was a significant effect of treatment on feed conversion for both phases ($p\leq0.0001$). Hens fed the positive control diet had the most efficient feed conversion with a feed intake:egg mass ratio of 1.962 for phase 1, and 1.944 for phase 2. The higher feed intake by hens on the negative diet and with enzyme supplementation had the greatest

effect on this parameter. As mentioned earlier the hens fed the positive control laid heavier eggs in phase 1 compared to the hens fed the negative control diet with enzyme B, and in phase 2 the positive control hens produced heavier eggs than all other treatment groups. In phase 2 the hens fed the positive control laid heavier eggs than all other treatments ($p=0.036$). Eating more, yet laying smaller eggs causes the feed conversion ratio to increase, showing that hens are less efficient at converting feed into a product (eggs). Feed conversion can also affect body weight since the hens will need to consume more feed not only to produce eggs, but also to grow and eventually maintain body weight.

The differences seen in the study were caused by the decrease in metabolizable energy and amino acid density in the negative control diet. There was no interaction between time and treatment for the parameters measured in this study. The enzyme supplementation to the negative control diet did not support bodyweight or egg weight equivalent to hens on the positive control diet. As expected the enzyme activity increased numerically in the lower ME diet due to the higher NSP content from the soy hulls, however the activity was still quite low compared to similar studies. Olukosi and others (2007) conducted a study of a xylanase, amylase, and protease complex that was dosed to provide 650 U/kg of feed of xylanase in a corn/soybean diet. While there were some significant results, the researchers attributed much of this to phytase instead of the xylanase in the XAP complex (Olukosi et al., 2007). Another study used a much higher dosage which resulted in 5,500 U/kg of feed of xylanase in a wheat-based diet which yielded a significant decrease in feed conversion ratio, and an increase in apparent metabolizable energy when compared to the control diet (Guo et al., 2014). The highest

xylanase activity in this study was 669 U/kg of feed by enzyme B during phase 2. The results indicate that this activity level was still not high enough to improve the negative diet to where the hens performed at the same level as those on the positive control, especially since this higher activity was achieved by further decreasing the energy of the diet with soy hulls which caused an increase in substrate concentration for the enzyme. As mentioned earlier the young age of the hens in this study meant that they were highly productive, which may be why no differences were seen for egg production despite the effects on bodyweight and feed intake. Repeating this study using older laying hens may help to elucidate the effects of xylanase inclusion in low energy diets.

Literature cited

Aspinall, G. O., K. Hunt, and I. M. Morrison. 1966. Polysaccharides of soy beans. Part II. Fractionation of hull cell-wall polysaccharides and the structure of a xylan. J. Chem. Soc. 21:1945-1949.

Deniz, G., H. Gencoglu, S. S. Gezen, I. I. Turkmen, A. Orman, and C. Kara. 2013. Effects of feeding corn distiller's dried grains with solubleswith and without enzyme cocktail supplementation to laying hens on performance, egg quality, selected manure parameters, and feed cost. Livestock Science 152:174-181.

Guo, S., D. Liu, X. Zhao, C. Li, and Y. Guo. 2014. Xylanase supplementation of a wheat-based diet improved nutrient digestion and mRNA expression of intestinal nutrient transporters in broiler chickens infected with *Clostridium perfringens*. Poultry Science 93:94-103.

Hromádková, Z. and A. Ebringerová. 1995. Isolation and characterization of corn hulls. Chem. Papers 49:97-101

Hendrix-ISA LLC. 2015. Bovans White Commercial Management Guide. Available at http://www.hendrix-isa.com/en/products/bovans-white/

Karr-Lilienthal, L. K., C. T. Kadzere, C. M. Grieshop, and G. C. Fahey Jr. 2005. Chemical and nutritional properties of soybean carbohydrates as related to nonruminants: A review. Livestock Production Science 97:1-12.

Mathlouthi, N., M. Larbier, M. A. Mohamed, and M. Lessire. 2002. Performance of laying hens fed wheat, wheat-barley or wheat-barley-wheat bran based diets supplemented with xylanase. Can. J. Anim. Sci. 82: 193–199.

Mirzae, S., M. Zaghari, S. Aminzadeh, M. Shivazad, and G. G. Mateos. 2012. Effects of wheat inclusion and xylanase supplementation of the diet on productive performance, nutrient retention, and endogenous intestinal enzyme activity of laying hens. Poultry Science 91:413-425.

Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2007. Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. Poultry Science 86:77-86.

Slominski, B. A. 2011. Recent advances in research on enzymes for poultry diets. Poultry Science 90:2013-2023.

Suresh, B. N., B. V. Reddy, R. G. Gloridoss, B. C. Umashankar, and K. C. Basavanta. 2014. Effect of Low Nutrient Density Diets and Certain Feed Additives on Performance of Laying Hens. Animal Nutrition and Feed Technology 14:399-404.

Zanella, I., N. K. Sakomura, F. G. Silversides, A. Fiqueirdo, and M. Pack. 1999. Effect of enzymes supplementation of broiler diets based on corn and soybeans. Poultry Science 78:561-568.

Table 2.1 Diet Composition

Ingredient (%)	Phase 1		Phase 2	
	Positive Control	Negative Control	Positive Control	Negative Control
Corn	56.90	58.50	55.48	57.18
Soybean Meal	19.10	18.30	19.70	17.90
Soy Hulls		0.50		2.15
DDGS	10.00	10.00	10.00	10.00
Vegetable Oil	2.20	0.50	2.57	0.50
Limestone	9.64	9.98	10.24	10.22
Dical Phosphate	1.28	1.29	1.16	1.18
Salt	0.40	0.40	0.36	0.38
Lysine	0.14	0.15	0.13	0.14
Methionine	0.18	0.18	0.19	0.17
Threonine	0.03	0.02	0.03	0.03
Premix**	0.20	0.20	0.20	0.20

Analyzed Nutrient Composition

Nutrient	Phase 1		Phase 2	
	Positive Control	Negative Control	Positive Control	Negative Control
ME (kcal/kg)	2866	2756	2866	2712
CP (%)	16.53	16.33	16.45	15.93
Ca (%)	4.10	4.23	4.30	4.30
Avail. Phos. (%)	0.46	0.46	0.54	0.54
Linoleic Acid (%)	2.60	1.70	2.79	1.68
Dig. Arginine (%)	0.89	0.874	0.898	0.859
Dig. Lysine (%)	0.79	0.78	0.79	0.771
Dig. Methionine (%)	0.419	0.41	0.422	0.396
Dig. TSAA (%)	0.66	0.65	0.66	0.63
Dig. Threonine (%)	0.553	0.535	0.553	0.53

*Analysis by Midwest Laboratories, Omaha, NE

**Poultrymate® Pinnacle Premix (Nutra Blend LLC, Neosho, MO)

Table 2.2 Amino acid analysis of diets (phase 1)

Amino Acid (%)	Positive Control	Negative Control
Protein	16.80	16.40
Threonine	0.68	0.68
Alanine	1.02	1.03
Cystine	0.25	0.27
Methionine	0.58	0.49
Valine	0.66	0.66
Isoleucine	0.70	0.74
Leucine	1.37	1.45
Tyrosine	0.66	0.59
Phenylalanine	0.99	0.80
Lysine	0.96	0.99
Arginine	1.27	1.24
Tryptophan	0.19	0.23

*Analysis by Midwest Laboratories, Omaha, NE

Table 2.3 Enzyme activity

Xylanase	Phase 1	Phase 2
Enzyme A		
Concentration (grams/ton)	181.6	181.6
Activity (U/kg)	68	102
Enzyme B		
Concentration (grams/ton)	340.5	340.5
Activity (U/kg)	432	669

*Analysis by Kerry Global Technology and Innovation Centre, Kildare, Ireland

Table 2.4 Effect of treatment on egg percent production, egg weight, and egg mass

Egg production (%)

Treatment	Phase 1	Phase 2
Positive Control	92.1	89.4
Negative Control	91.9	90.0
Neg. Ctl +Enzyme A	91.5	89.2
Neg. Ctl +Enzyme B	91.0	88.5
SEM[1]	0.547	0.735

P Values

Treatment	0.47	0.54
Time	0.006	≤0.0001
Trt*time	NS	NS

Egg weight (g), egg mass (g/day)

	Phase 1		Phase 2	
Treatment	EW	EM	EW	EM
Positive Control	58.0[a]	53.97[a]	60.98[a]	54.74
Negative Control	58.4[a]	54.10[a]	59.65[b]	54.21
Neg. Ctl +Enzyme A	57.6[ab]	53.22[ab]	59.66[b]	53.48
Neg. Ctl +Enzyme B	57.0[b]	52.33[b]	59.70[b]	53.27
SEM[1]	0.326	0.417	0.392	0.481

P Values

Treatment	0.019	0.01	0.036	0.12
Time	≤0.0001	≤0.0001	0.016	0.36
Trt*time	NS	NS	NS	NS

*means within a column with differing superscripts are significantly different (p≤0.05)
[1]standard error of the mean

Table 2.5 Effect of treatment on bodyweight, feed intake, and feed conversion

Body weight (g)

	Baseline	Phase 1		Phase 2
Treatment	Week 0	Week 10	Week 20	Week 35
Positive Control	1510	1603[a]	1628[a]	1639[a]
Negative Control	1486	1533[b]	1562[b]	1572[b]
Neg. Ctl +Enzyme A	1487	1542[b]	1567[b]	1570[b]
Neg. Ctl +Enzyme B	1511	1550[b]	1564[b]	1574[b]
SEM[1]	18.082	15.901	15.980	18.690
P Values				
Treatment	0.63	0.01	0.01	0.03

Feed intake (g/bird/day), feed conversion (g feed:g egg)

	Phase 1		Phase 2	
Treatment	FI	FC	FI	FC
Positive Control	95.3[a]	1.962[a]	104.8[a]	1.944[a]
Negative Control	97.2[bc]	1.986[ab]	107.7[b]	2.010[b]
Neg. Ctl +Enzyme A	96.7[b]	2.011[b]	107.8[b]	2.035[b]
Neg. Ctl +Enzyme B	97.9[bc]	2.070[c]	108.9[b]	2.061[b]
SEM[1]	0.300	0.166	0.6312	0.0179
P Values				
Treatment	0.0001	0.0001	0.0001	0.0001
Time	≤0.0001	0.13	≤0.0001	0.33
Trt*time	NS	NS	NS	NS

*means within a column with differing superscripts are significantly different ($p \leq 0.05$)
[1]standard error of the mean

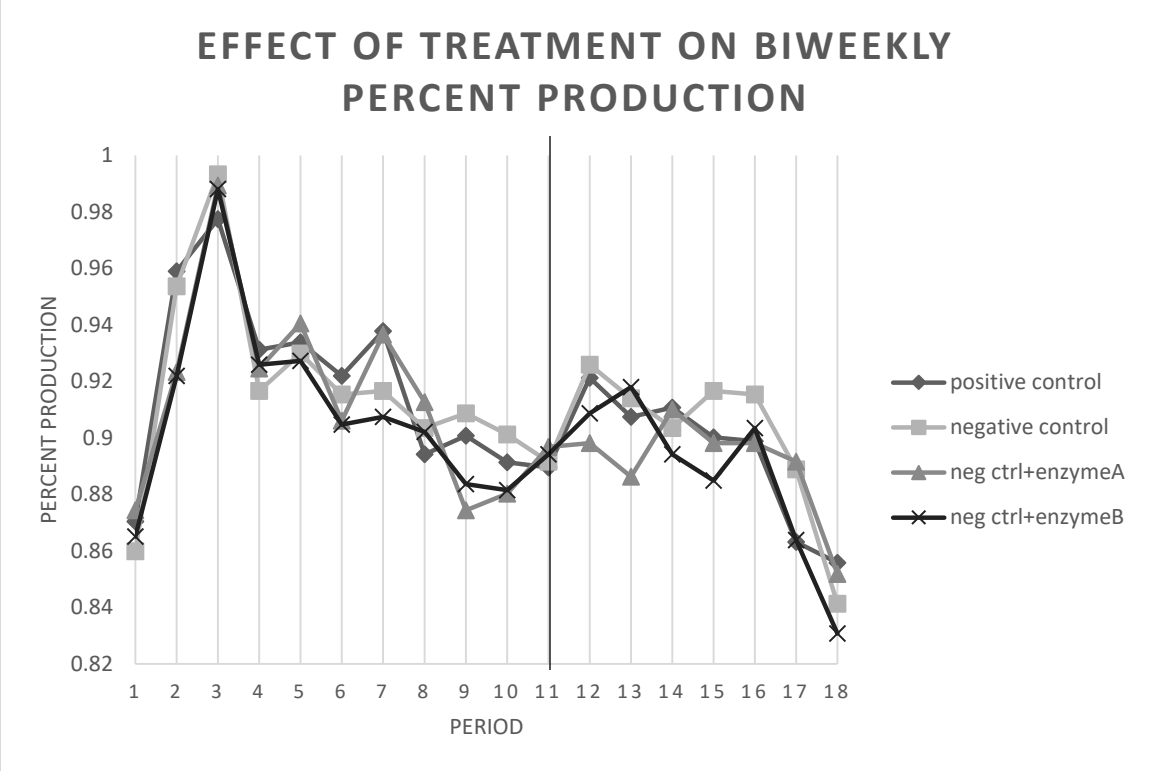

Figure 2.1 Egg percent production.
*vertical line indicates beginning of phase 2, each period is equal to 2 weeks

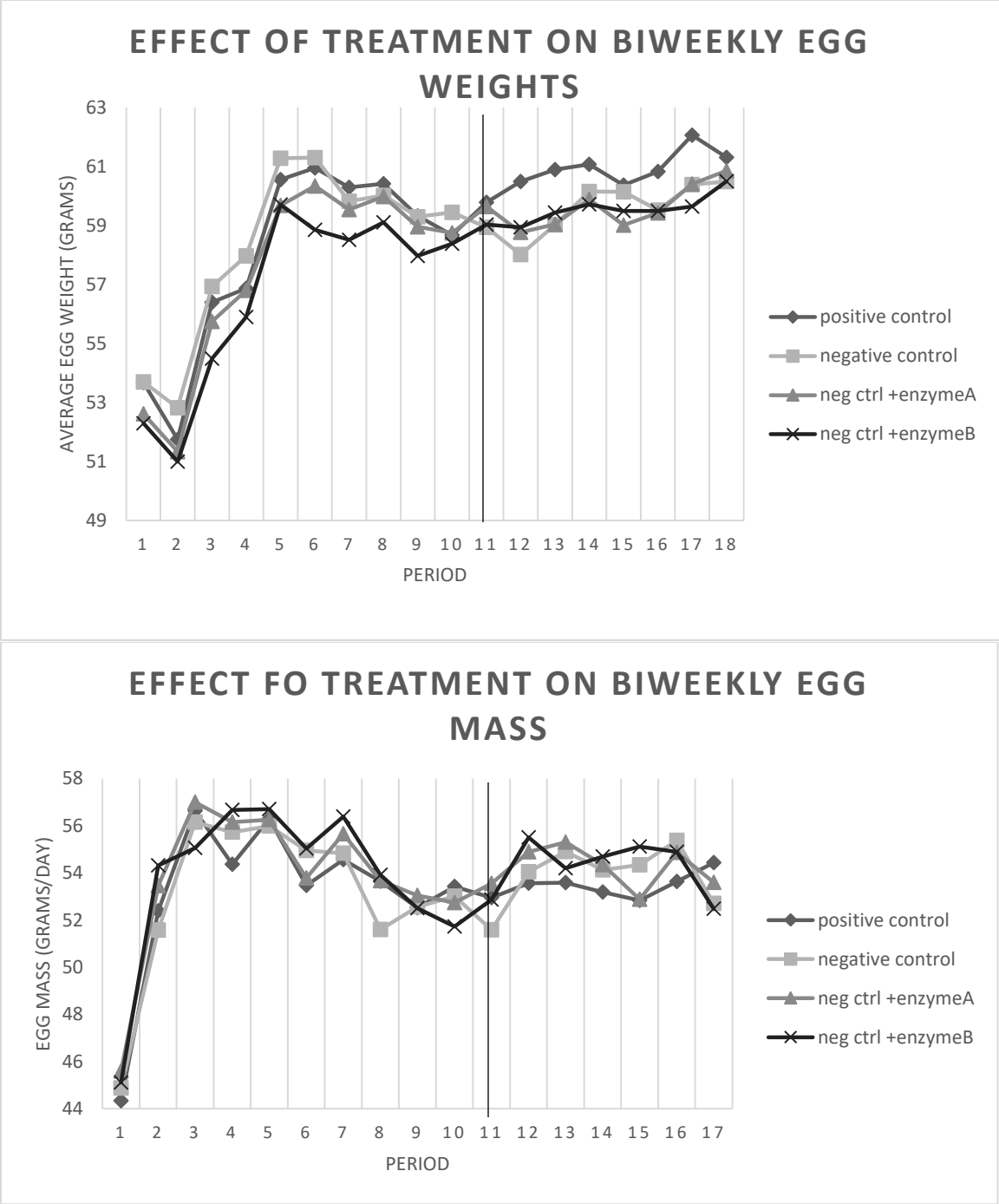

Figure 2.2 Egg weight and egg mass
*vertical line indicates beginning of phase 2, each period is equal to 2 weeks

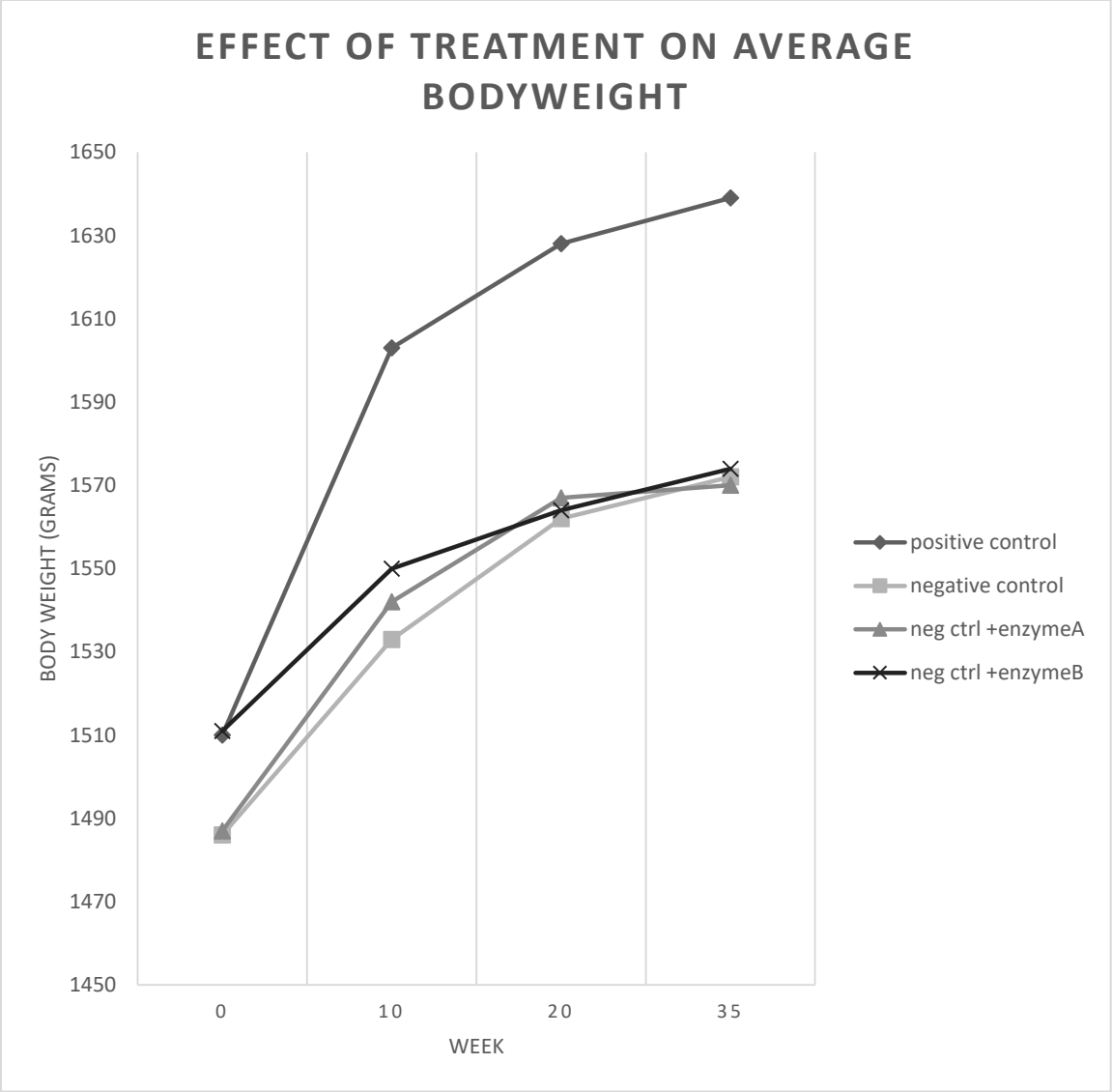

Figure 2.3 Bodyweight
*phase 2 begins at week 20

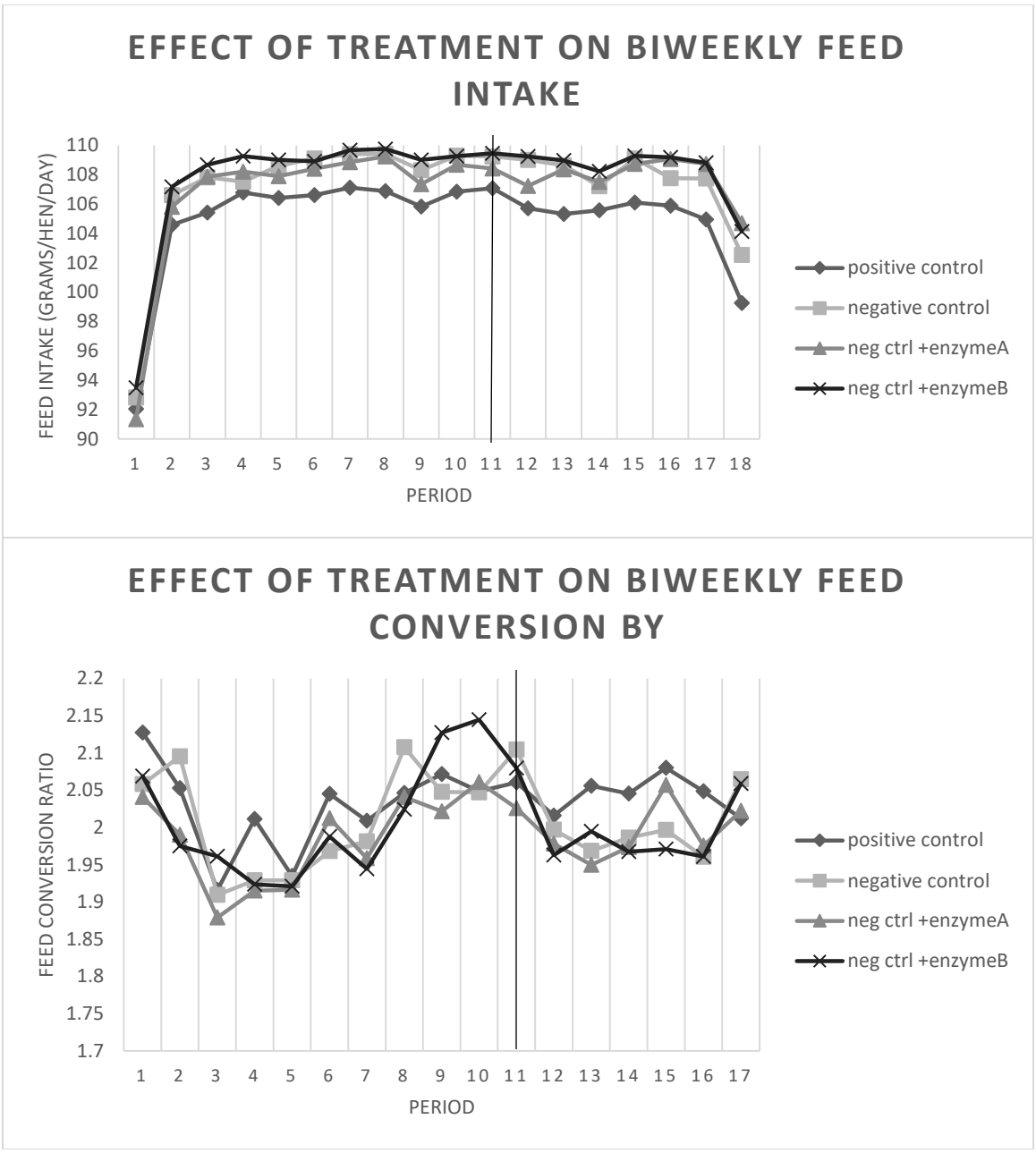

Figure 2.4 Feed intake and feed conversion
*vertical line indicates beginning of phase 2, each period is equal to 2 weeks

Chapter 3: Effect of paramylon zinc polysaccharide complex derived from *Euglena gracilis* on the performance of laying hens.

INTRODUCTION

Algal products have been gaining popularity recently for various purposes, including animal agriculture. Feeding red algae to poultry may affect fatty acid and cholesterol levels in eggs, specifically the egg yolk (Ginzberg et al., 2000). However directly feeding algae may only increase fatty acid deposition in the egg if there is sufficient availability. Rigidity of the algal cell wall may inhibit digestion and absorption of beneficial fatty acids by the bird (Lemahieu et al., 2016). One type of product that is being considered for use in poultry production is algal β-glucan, specifically paramylon (a β-1,3-glucan) produced by *Euglena gracilis*, a species of green algae. Paramylon has been known for some time for its immmunostimulatory properties in mice and swine (Kondo et al., 1992; Sonck et al., 2010). Its use in poultry is not as common, but is being explored more as a feed additive due to the increase in organic and antiobiotic-free production.

β-glucans are identified by the β-glycosidic linkages between glucose polymers, and the type and number of branch points within the polysaccharide (Zeković et al., 2005). β-1,3/1,6-glucans are typically produced by fungi such as *Saccharomyces cerevisiae* (baker's yeast), and β-1,3-glucan is produced by *Euglena* (Barsanti et al., 2001). In poultry, β-glucans can aid in protection against harmful bacterial infection by increasing the density of mucus-producing goblet cells and increasing villus crypt depth in the small intestine (Morales-Lopez et al., 2009; Shao et al., 2013). It also primes the immune response by increasing proliferation and activity of immune cells (Chen et al.,

2008; Tang et al., 2011). The use of paramylon has not been extensively studied for safety of consumption, but a recent study found that there were no significant negative health effects when fed in high concentrations over time to mice (Simon et al., 2016).

Algamune™ ZPC is an organic-certified, dried, paramylon zinc polysaccharide complex produced by Algal Scientific Corporation (Plymouth, MI). It contains over 50% β-1,3-glucan out of 60% total carbohydrates, and 2% zinc (Table 3.1). The product is in the form of a fine gold-colored powder that is free-flowing and easily mixed into a premix or nutritionally complete diet. The goal of this product is to provide an alternative to antibiotics by priming the bird's immune system.

The zinc in this product could also be useful to the bird. Zinc can be included in the diet in a variety of ways, but is typically bound to other compounds and is classified as organic or inorganic based on what it is bound to. In this case the source is considered organic since the zinc is bound to a carbohydrate forming a polysaccharide complex. Zinc polysaccharide and other organic sources can provide high zinc availability to the bird, which is important for utilization of this mineral (Cao et al., 2000). Zinc is an essential mineral for poultry as it has an important role in bone, feather, and eggshell formation. It is a common cofactor for metabolic pathways and enzymes, such as carbonic anhydrase which helps facilitate eggshell mineralization by catalyzing the reaction to create bicarbonate from carbon dioxide and water.

The objective of this study was to determine the effects of Algamune™ ZPC supplementation on the production and performance of laying hens. It was hypothesized that supplementing a nutritionally complete diet with different concentrations of

Algamune™ ZPC at 100g/ton, 200g/ton, and 300g/ton of feed would result in differences in laying hen performance and production.

MATERIALS AND METHODS

Birds and Housing

This study used 108 Hy-Line Brown (Hy-Line, Des Moines, IA) Leghorn hens housed in 36 cages containing 3 hens each. The unit that these hens were housed in was a Chore-Time® cage system with 3 rows of 12 wire cages separated vertically by manure belts (CTB, Inc., Milford, IN) with removable feeder troughs on each cage, and 688.17cm^2 of floor space per hen. The hens in this study were fed 110 grams per hen per day, which is the amount calculated to provide the correct energy level according to the diet formulation, from buckets containing a pre-weighed amount of feed for each week corresponding to the number of hens in each cage. It also helps to minimize feed wastage due to the small feed troughs. Water was provided ad libitum via a nipple drinker in each cage. The housing unit was located in a windowless climate-controlled room maintained on a 16-hour light:8-hour dark photoperiod. The study ran from June to October 2015, and treatment diets were fed from 19 to 39 weeks of age. To help reduce variability the housing unit was divided into 3 blocks where each block was a row of 12 cages. There were four treatments that were assigned randomly within each block, resulting in a total of 9 replicates per treatment.

Diets

The experimental diets used for this study consisted of a control diet and three treatment diets containing different levels of Algamune^{TM} ZPC (Algal Scientific Corporation, Plymouth, MI) supplemented at 100, 200, or 300 g/ton of feed. The product was a paramylon β-1,3-glucan zinc polysaccharide complex derived from *Euglena gracilis*, a species of green algae (Table 3.1). The basal diet was a complete peak layer diet (Table 3.2) that was mixed at the University of Nebraska-Lincoln's feed mill (Ithaca, NE). The Algamune^{TM} ZPC was added to the basal diet for each treatment and mixed in 200lb batches onsite at the University of Nebraska-Lincoln.

Measurements

The birds did not undergo an immunological challenge during this study so only production and performance data were collected to determine differences between the treatments. Measurements for this study included egg production, feed intake, egg weight and components, yolk color, egg mass, feed conversion, bodyweights, and eggshell breaking strength. Six eggs per treatment were also randomly collected at the end of the study for egg nutrient analysis. The room the hens were housed in was heated above 80°F from July 13-17 and 22-25, as well as October 15-17 and 20-24 by increasing the setting of the room's thermostat as well as placement of space heaters throughout the room. This was done in response to reports of avian influenza in the Midwest United States and was used as a preventative measure against viral contamination of the facilities. This is seen as an effective method because the avian influenza virus has a more difficult time surviving in an environment with a higher temperature and higher relative humidity (Guan et al., 2016). Procedures were approved by the University of Nebraska IACUC.

Egg production was recorded daily for each cage, and calculated as biweekly percent production (EP). Feed intake was recorded weekly by subtracting the remaining feed from the total amount allotted. The average feed intake (FI) per bird per day was calculated biweekly. Egg weight (EW) was recorded biweekly using a sample of two average-sized eggs per cage. Egg components were also measured from the egg samples to determine percent shell (PS), percent yolk (PY), and percent albumen (PA). PS was determined by weighing the shell after scraping excess albumen from the inside, and dividing the weight by the total EW. PY was determined by carefully separating the yolk from the albumen using a plastic egg separator, removing the chalazae, and dividing the resulting weight by the total EW. PA was determined by subtracting the yolk and shell weights from the total EW to estimate the albumen weight. Yolk color (YC) was measured using a 2007 Kemin yolk color fan (Kemin Industries, Inc., Des Moines, IA) where a higher number indicated a darker, more orange yolk. Egg mass (EM) was calculated by multiplying average biweekly EW by EP. Feed conversion ratio (FC) was calculated by dividing biweekly FI by EM. Body weights (BW) were measured monthly for each individual bird and averaged per cage.

Eggshell breaking strength (SS) was determined during the final two weeks of the study. A sample of two average-sized eggs per cage were collected each week. The strength of the shell was measured using a Texture Analyzer (TA.XTPlus, Texture Technologies Corporation, Scarsdale, NY) which used a compression test of 1g of force at 10mm/sec (Anderson et al., 2004). The peak force required to break the eggshell, in newtons, was the value of measurement. This was measured by exponent software

(Stable Micro Systems LTD., Surrey, UK). Each egg that was measured for shell strength was also weighed to determine if EW as a covariate affected SS.

Egg nutrients were measured at the end of the study using one egg from 6 randomly chosen cages per treatment. The nutrients analyzed included percent protein, total fat, saturated fat, polyunsaturated fat, and monounsaturated fat. Specific fatty acids that were measured were oleic, linoleic, palmitic, α-linolenic, DHA, omega-3, omega-6, and omega-9. Cholesterol was also measured for each egg. Egg nutrient analysis for each egg was conducted by Midwest Labs (Midwest Laboratories, Omaha, NE).

Statistical Analysis

The data were analyzed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, 2015) All of the response variables were analyzed using a repeated measures model including random block effect and fixed effects of time, treatment and their interaction. Various error covariance structures were evaluated for each response variable to determine the one that fit the best. Eggshell breaking strength also used egg weight as a covariate.

RESULTS AND DISCUSSION

Table 3.3 shows the results of egg percent production, feed intake, egg weight, egg components, and yolk color. There were no significant differences seen in egg components or yolk color. Again, this shows that the product did not negatively impact the hens. The consistency of egg component weights including shell, yolk, and albumen as a percentage of the total egg weight is important for producers and consumers. Yolk color is also an important factor for consumers. The darkness of the yolk is mainly

attributed to deposition of pigments such as carotenoids. Yolk color is easily manipulated by ingredients in the diet (Nogareda et al., 2015). The product used in this study was a light golden powder, but the pigmentation of the product did not significantly affect the yolk color compared to the control.

Egg mass, feed conversion, and body weight results are shown in table 3.4 and figures 3.4, 3.5, and 3.6. EM and FC were also not significantly different among the treatments. This is consistent with the lack of differences for EP, FI, and EW since EM and FC are calculated using these parameters.

The hens performed very well and were close to the expected standards for their breed, with the exception of bodyweight which was lower than what was expected for hens at 32 weeks of age (Hy-Line International, 2014). This could be due to the decreases in feed intake at 27 weeks of age and at the end of the study (weeks 8-9, and week 20 of the study respectively). These decreases coincide with the regimen of increasing ambient temperatures above 80°F. The increased temperature was utilized as a preventative measure against avian influenza in the hen facilities. Due to the possible heat stress experienced by the hens, the effect of treatment on feed intake was determined for weeks 8 and 20. Comparing treatment means for feed intake within these weeks showed no significant differences between treatments, with the exception of week 8 where there was a difference between 100g/ton and 300g/ton treatments ($p=0.0314$). Week 20 of the study showed a numerical difference between the control and other treatments (Table 3.5). This is not direct evidence to claim that supplementation of Algamune^{TM} ZPC to a corn/soybean diet may help alleviate the negative effects of heat stress on feed intake. However, it is still interesting to note and may be a focus of further study. β-glucan is

well known for its immunostimulative properties and this is likely the component of the product that may provide the stress alleviation, however further research is necessary to determine its effects on laying hens during heat stress.

The results of eggshell breaking strength are shown in table 3.6. Eggshell breaking strength showed not only a lack of significant differences among the different treatments, but there was also no interaction between eggshell strength and egg weight as a covariate. This is supported by a previous study that examined the relationships between egg weight and eggshell strength in different breeds of laying hens (De Ketelaere et al., 2002).

The results for egg nutrients and fatty acid profile are shown in table 3.7. Differences between treatments for a few of the egg fatty acid content analyzed at the end of the study approached significance ($p<0.07$), but overall there was no significant effect due to treatment. These included saturated fat, DHA, and omega-3 fatty acid content in the eggs. The following are numerical differences. Saturated fat was lower in eggs from birds fed the diet supplemented with 100g/ton of Algamune^{TM} ZPC compared to those fed 300g/ton ($p=0.1986$). DHA was lower for birds fed 100g/ton compared to 200g/ton ($p=0.1737$). Omega-3 fatty acids were lower for birds fed 100g/ton compared to the control and the 200g/ton supplemented diet ($p=0.0688$). The fatty acid content of an egg may be important as it is an ideal way to enrich the egg for consumers. Research has indicated that the amount of omega-3 fatty acids has decreased in animal products as well as in the human diet due to increased consumption of cereal grains and other plants, while omega-6 consumption has increased. Bringing the ratio of omega-6:omega-3 fatty acids closer to 1 is thought to be beneficial, especially for prevention and management of

chronic diseases (Simopoulos, 2002). The variability in nutrient deposition, particularly fatty acids, in the yolk when birds are fed different types of algae needs to be researched further.

Supplementation of Algamune^{TM} ZPC, even at the high level of 300g/ton, did not have significant negative effects on the production and performance of brown Leghorn hens. There were no significant treatment differences for EP, FI, and EW. While paramylon supplementation did not improve EP and EW, it also did not negatively affect them which may show that the levels of supplementation used in this trial are safe for the hens. The lack of differences among treatments for feed intake also implies that palatability should not be a problem for this product. If it adversely affected palatability of the feed the hens would have eaten less. The small range of concentrations of Algamune^{TM} ZPC used in this study may also explain why no differences were seen among the treatments, as the high level (300g/ton) was only three times the concentration of the low level (100g/ton).

There is potential for paramylon to increase fatty acid content of eggs and to alleviate heat stress. More research into the use of algal derivatives in poultry during times of stress should be conducted in order to determine the specific effects of this type of feed additive when supplemented to corn/soybean based diets, and whether there is a consistent pattern to how laying hens respond both physiologically and in terms of productivity. This type of product may also be an effective means of supplementing fatty acids and zinc to the hens, as well as enriching eggs with omega-3 fatty acids. However, it is important to note that the vitamin and mineral premix used in the control diet contained the necessary amount of zinc to meet the hens' nutritional needs. As the

Algamune^{TM} ZPC only contains 2% zinc, it is unlikely that it had a significant impact at the levels of supplementation used in this study.

Literature cited

Anderson, K. E., J. B. Tharrington, P. A. Curtis, and F. T. Jones. 2004. Shell characteristics of eggs from historic strains of single comb white leghorn chickens and the relationship of egg shape to shell strength. International Journal of Poultry Science 3:17-19.

Barsanti, L., R. Vismara, V. Passarelli, and P. Gualtieri. 2001. Paramylon (*β*-1,3-glucan) content in wild type and WZSL mutant of *Euglena gracilis*. Effects of growth conditions. Journal of Applied Phycology 13:59-65.

Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J. Anim. Sci. 78:2039-2054.

Chen, K. L., B. C. Weng, M. T. Chang, Y. H. Liao, T. T. Chen, and C. Chu. 2008. Direct enhancement of the phagocytic and bactericidal capability of abdominal macrophage of chicks by β-1,3–1,6-glucan. Poultry Science 87:2242-2249.

De Ketelaere, B., T. Govaertz, P. Coucke, E, Dewil, J. Visscher, E. Decuypere, and J. De Baerdemaeker. 2002. Measuring the eggshell strength of 6 different genetic strains of laying hens: techniques and comparisons. British Poultry Science 43:238-244.

Ginzberg, A., M. Cohen, U. A. Sod-Moriah, S. Shany, A. Rosenshtrauch, and S. Arad. 2000. Chickens fed with the biomass of the red microalga *Porphyridium* sp. Havereduced blood cholesterol level and modified fatty acid composition in egg yolk. Journal of Applied Phycology 12:325-330.

Guan, J., M. Chan, and A. VanderZaag. 2016. Inactivation of avian influenza viruses on porous and non-porous surfaces is enhanced by elevating absolute humidity. Transbound Emerg Dis. Available at http://onlinelibrary.wiley.com/doi/10.1111/tbed.12499/abstract.

Hy-Line International. 2014. Hy-Line® Brown Performance Summary. Available at http://www.hyline.com/userdocs/pages/BRN_PERF_ENG.pdf.

Kondo, Y., A. Kato, H. Hojo, S. Nozoe, M. Takeuchi, and K. Ochi. 1992. Cytokine-related immunopotentiating activities of paramylon, a beta-(1-->3)-D-glucan from Euglena gracilis. J Pharmacobiodyn. 15:617-621.

Lemahieu, C., C. Bruneel, C. Dejonghe, J. Buyse, and I. Foubert. 2016. The cell wall of autotrophic microalgae influences the enrichment of long chain omega-3 fatty acids in the egg. Algal Research 16:209-215.

Morales-Lopez, R., E. Auclair, F. Garcia, E. Esteve-Garcia, and J. Brufau. 2009. Use of yeast cell walls; β-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. Poultry Science 88:601-607.

Nogareda, C., J. A. Moreno, E. Angulo, G. Sandmann, M. Portero, T. Capell, C. Zhu, and P. Christou. 2015. Carotenoid-enriched transgenic corn delivers bioavailable carotenoids to poultry and protects them against coccidiosis. Plant Biotechnology Journal 1-9.

Shao, Y., Y. Guo, and Z. Wang. 2013. β-1,3/1,6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella enterica* serovar Typhimurium. Poultry Science 92:1764-1773.

Simopoulos, A. P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56:365-379.

Simon, R. R., T. D. Vo, and R. Levine. 2016. Genotoxicity and subchronic toxicity evaluation of dried *Euglena gracilis* ATCC PTA-123017. Regulatory Toxicology and Pharmacology 80:71-81.

Sonck, E., E. Stuyven, B. Goddeeris, and E. Cox. 2010. The effect of beta-glucans on porcine leukocytes. Vet Immunol Immunopathol 135:199-207.

Tang, X. Y., J. S. Gao, F. Yuan, W. X. Zhang, Y. J. Shao, F. Sakurai, and Z. D. Li. 2011. Effects of Sophy β-glucan on growth performance, carcass traits, meat composition, and immunological responses of Peking ducks. Poultry Science 90:737-745.

Zeković, D. B., S. Kwiatkowski, M. M. Virvć, D. Jakovljević, and C. A. Moran. 2005. Natural and modified (1→3)-*β*-D-glucans in health promotion and disease alleviation. Critical Reviews in Biotechnology 25:205-230.

Table 3.1 Algamune™ ZPC product parameters

Nutrient	(%)
Moisture	4.78
Crude Protein	25.3
Crude Fat	2.58
Total Carbohydrates (calculated)	60.28
Ash	7.06
Beta Glucan	53.2
Zinc	2.0

*Lot analysis provided by Algal Scientific Corporation, Plymouth, MI

Fatty Acid Profile	
Crude Fiber (%)	15.9
Crude Fat (%)	2.93

Fatty Acid	(g/100g)
Saturated Fat	1.33
Polyunsat. Fat	1.03
Monounsat. Fat	0.56
Oleic	0.42
Linoleic	0.12
Palmitic	0.54
Alpha-linolenic	0.06
DHA	0.01
Omega-3	0.15
Omega-6	0.88
Omega-9	0.43

*Product sample analysis by Midwest Laboratories, Omaha, NE

Table 3.2 Diet Composition

Ingredient	(%)
Corn	63.13
Soybean Meal	16.42
DDGS	5.08
Vegetable Oil	2.40
Dical Phosphate	0.90
Limestone	5.65
Shell&Bone Builder	5.65
Salt	0.40
Lysine	0.09
Methionine	0.08
Pinnacle Premix	0.20

Nutrient Composition	(%)
Moisture	12.26
Dry Matter	87.74
	dry weight (%)
Crude Protein	17.3
Phosphorous (total)	0.70
Calcium (total)	4.90

*Analysis by Midwest Laboratories, Omaha, NE

Table 3.3 Effect of treatment on egg percent production, feed intake, egg weight, and egg components

Treatment	EP (%)	FI (g/hen/day)	EW (grams)
Control	91.43±0.15	103.93±0.75	54.01±0.65
100g/ton	89.49±0.16	104.86±0.79	53.86±0.65
200g/ton	89.40±0.16	103.27±0.79	54.59±0.65
300g/ton	92.41±0.16	104.19±0.75	53.99±0.65

P Values			
Treatment	0.4388	0.5667	0.8624
Time	≤0.0001	≤0.0001	≤0.0001
Trt*Time	0.9306	0.5138	0.4580

Egg components

Treatment	% shell	% yolk	% albumen	Yolk color
Control	13.81±0.17	25.97±0.33	60.21±0.38	9.106±0.11
100g/ton	13.86±0.17	26.31±0.33	59.83±0.38	8.844±0.11
200g/ton	13.55±0.17	26.09±0.33	60.35±0.38	8.894±0.11
300g/ton	13.61±0.17	26.22±0.33	60.17±0.38	8.972±0.11

P Values				
Treatment	0.5107	0.8922	0.7945	0.3864
Time	≤0.0001	≤0.0001	≤0.0001	≤0.0001
Trt*time	0.7983	0.6348	0.7554	0.1933

*means within a column with differing superscripts are significantly different (p≤0.05)
**standard error of the mean is signified by ±value

Table 3.4 Effect of treatment on egg mass, feed conversion, and bodyweight

Treatment	EM (g/day)	FC (g feed:g egg)	BW (grams)
Control	49.45±0.93	2.135±0.56	1684±30.38
100g/ton	48.49±0.98	2.204±0.58	1660±30.38
200g/ton	48.96±0.98	2.163±0.58	1683±30.38
300g/ton	49.96±0.93	2.105±0.56	1682±30.38
P Values			
Treatment	0.7020	0.5391	0.9312
Time	≤0.0001	≤0.0001	≤0.0001
Trt*time	0.9948	0.8893	0.2985

*means within a column with differing superscripts are significantly different ($p \leq 0.05$)
**standard error of the mean is signified by ±value

Table 3.5 Feed intake during increasing heat regimen of specific weeks

FI (g/hen/day) during weeks 8 and 20		
Treatment	Week 8	Week 20
Control (trt1)	105.67±1.8109	95.55±2.2656
100g/ton (trt2)	104.85±1.9207	100.18±2.4031
200g/ton (trt3)	99.98±1.9207	100.05±2.4031
300g/ton (trt4)	102.59±1.8109	100.21±2.2656
P Values		
Trt 1 vs trt 2	0.7543	0.1612
Trt 1 vs trt 3	0.0314	0.1733
Trt 1 vs trt 4	0.2297	0.1458
Overall treatment effect p value	0.5667	

*significance indicated by $p \leq 0.05$

**standard error of the mean is signified by ±value

Table 3.6 Effect of treatment on eggshell breaking strength (SS)

Treatment	SS (newtons)
Control	53.77±2.59
100g/ton	54.30±2.61
200g/ton	56.51±2.54
300g/ton	57.18±2.61

P Values	
Treatment	0.5777
Trt*time	0.5662
Time	0.0204
Egg weight	0.1278

*means within a column with differing superscripts are significantly different ($p \leq 0.05$)
**egg weight was used as a covariate
***standard error of the mean is signified by ±value

Table 3.7 Effect of treatment on egg nutrients and fatty acid profile

		Algamune™ ZPC				trt
Nutrient (%)	Control	100g/ton	200g/ton	300g/ton	SEM[1]	P Value
Protein	12.80	12.37	12.44	12.77	0.21	0.3202
Total Fat	9.30	8.64	9.14	9.42	0.42	0.5051
Saturated Fat	3.20	2.94	3.15	3.31	0.13	0.1986
Polyunsat. Fat	2.34	2.09	2.43	2.30	0.12	0.2581
Monounsat. Fat	3.68	3.54	3.49	3.73	0.21	0.7536
Nutrient (g/100g)						
Oleic	3.49	3.35	3.29	3.53	0.19	0.7172
Linoleic	1.99	1.77	2.07	1.97	0.11	0.2730
Palmitic	2.27	2.09	2.24	2.35	0.11	0.3341
Alpha-linolenic	0.02804	0.02505	0.02843	0.02515	0.0031	0.7903
DHA	0.048	0.043	0.052	0.050	0.003	0.1737
Omega-3	0.087	0.075	0.089	0.082	0.044	0.0688
Omega-6	2.23	2.00	2.33	2.20	0.12	0.2725
Omega-9	3.48	3.35	3.29	3.53	0.19	0.7236
Nutrient (mg/100g)						
Cholesterol	353.69	355.67	381.98	369.67	15.0	0.531

[1]standard error of the mean

*means within a row with differing superscripts are significantly different ($p \leq 0.05$)

**Analysis by Midwest Laboratories, Omaha, NE

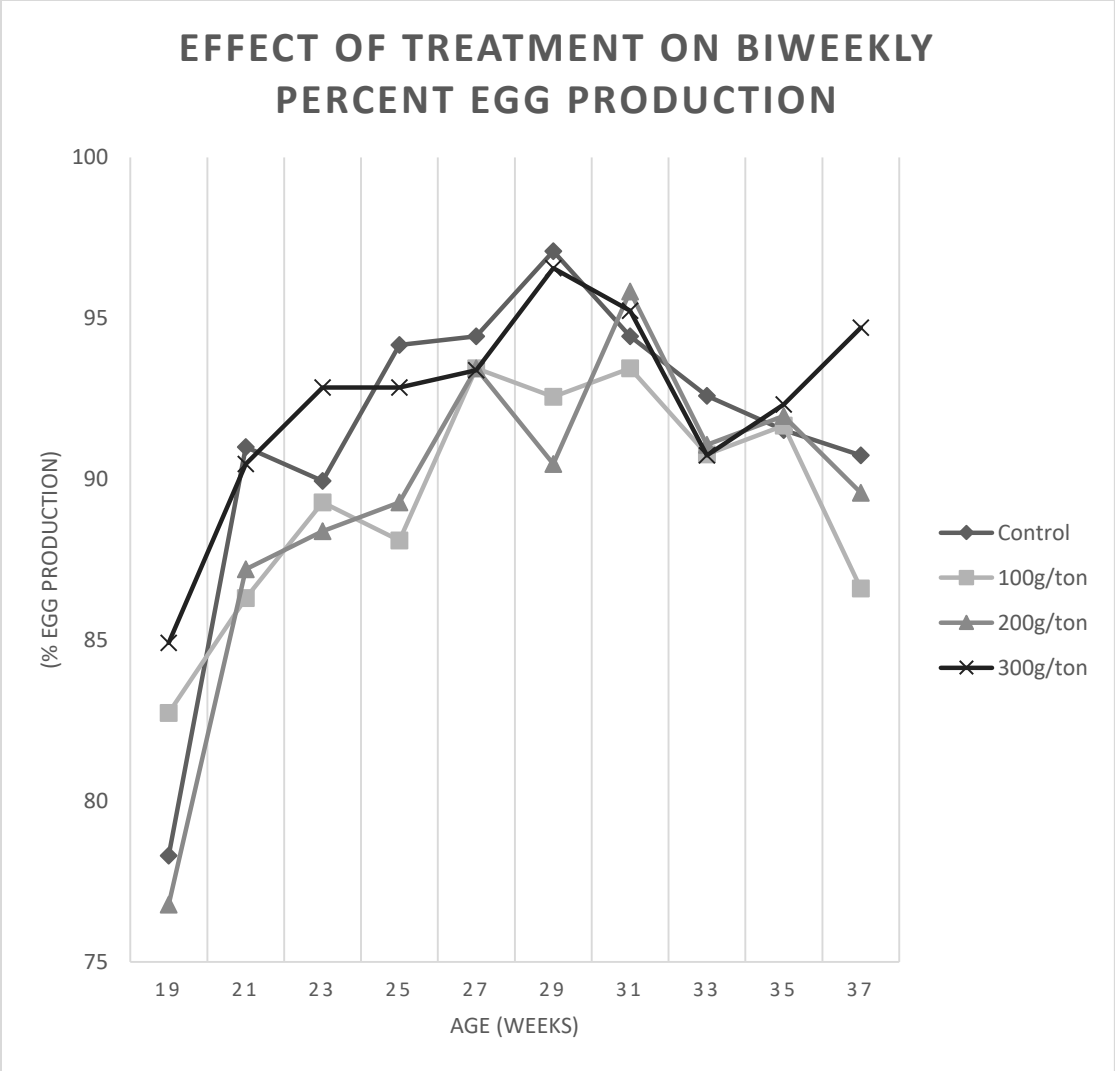

Figure 3.1 Egg percent production

*temperatures above 80°F occurred between 26-27 weeks of age, and at 37 weeks of age

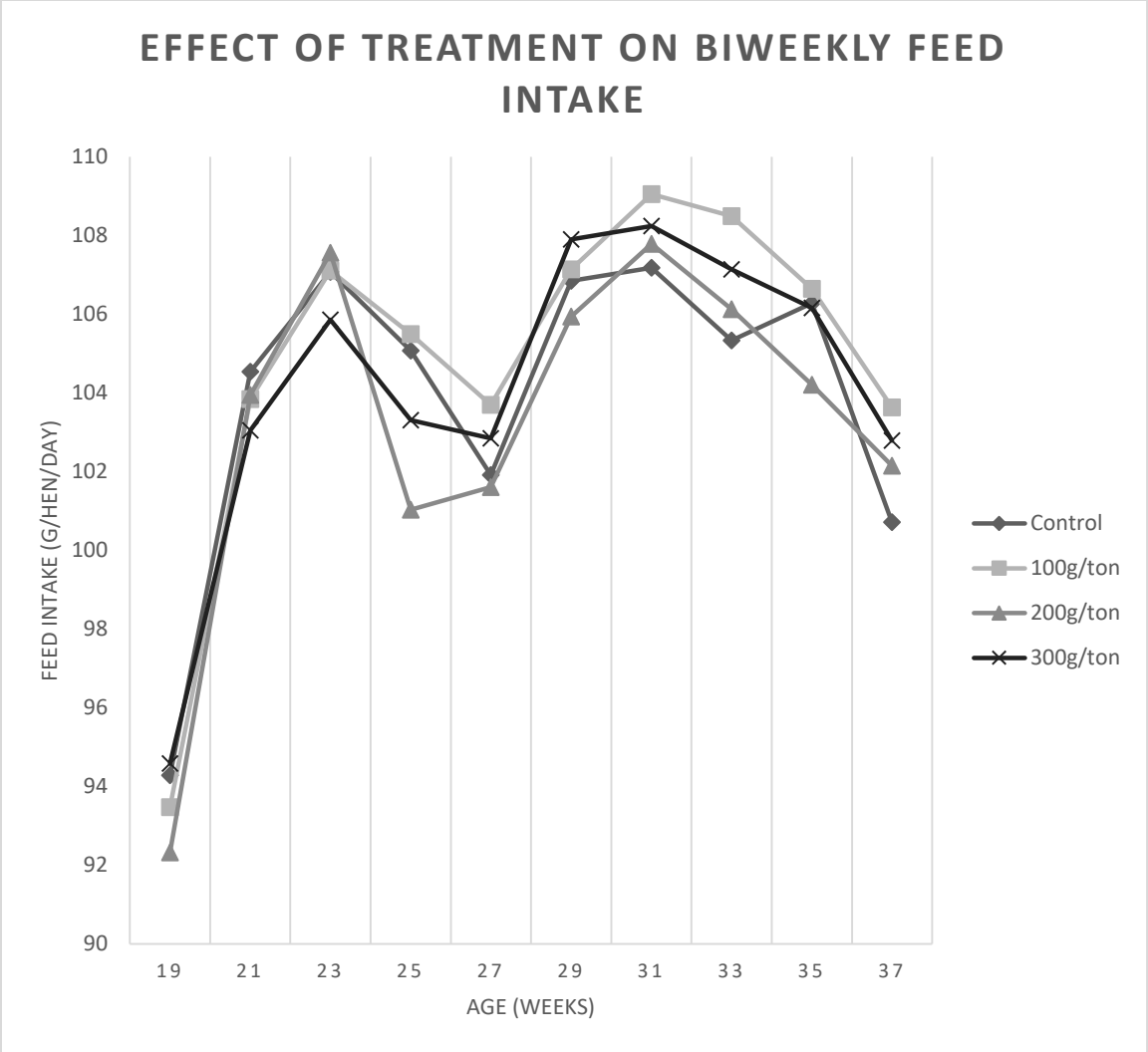

Figure 3.2 Feed intake

*temperatures above 80°F occurred between 26-27 weeks of age, and at 37 weeks of age

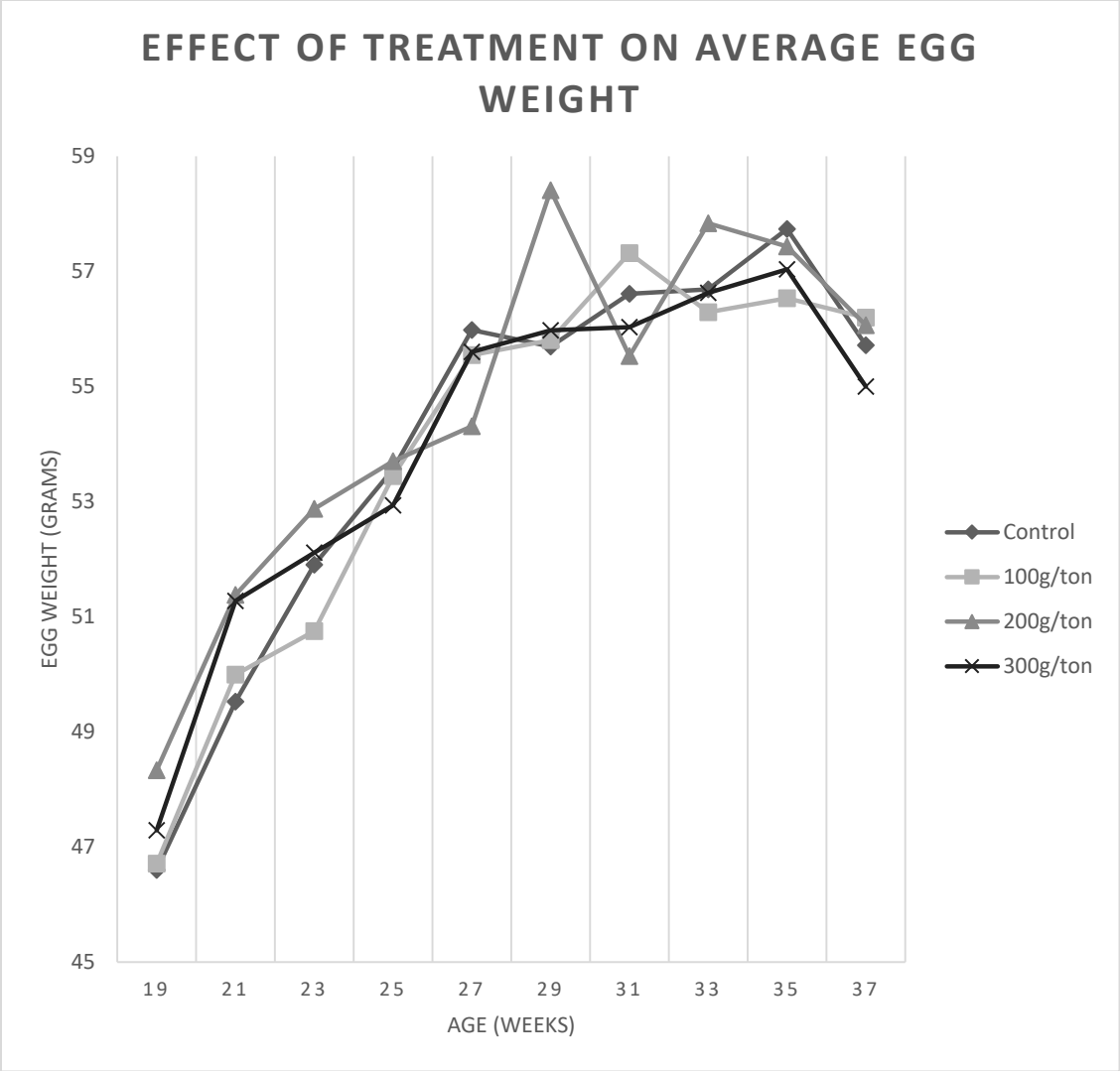

Figure 3.3 Egg weight

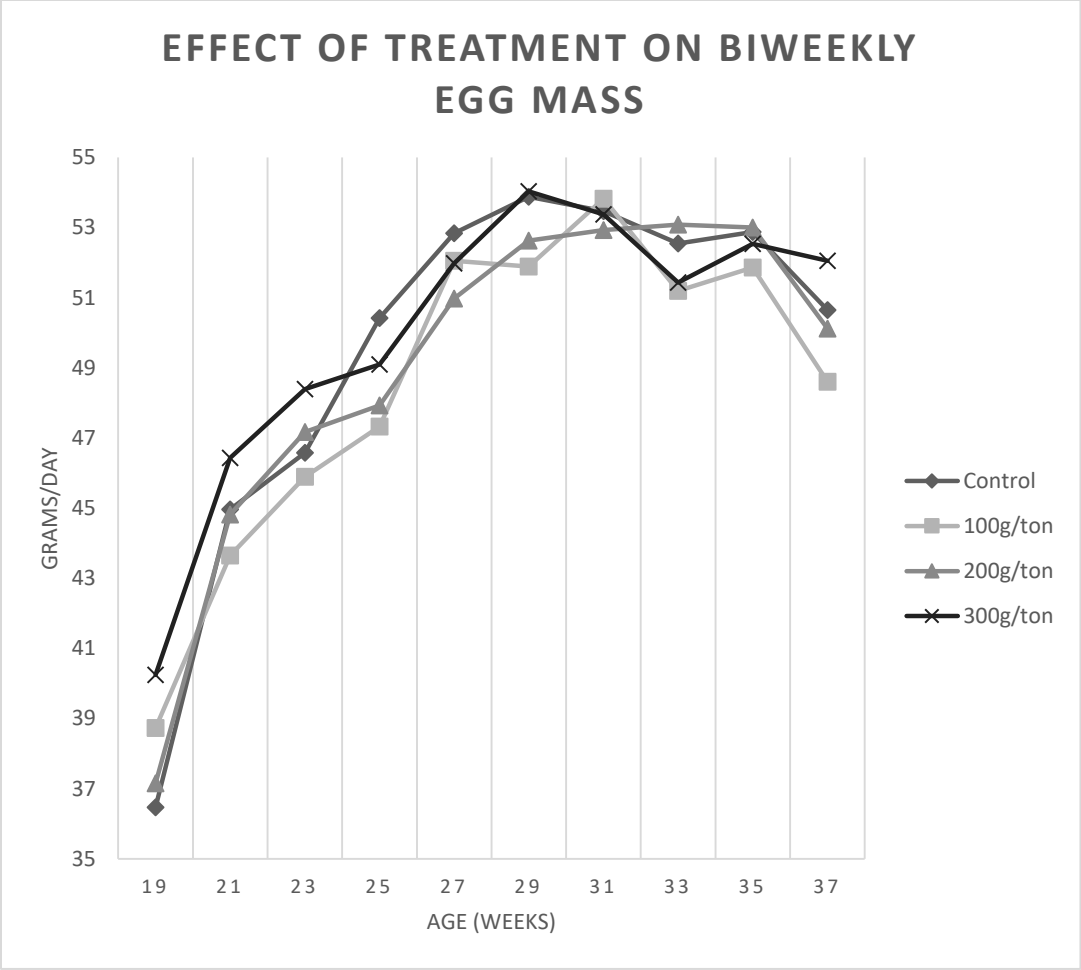

Figure 3.4 Egg mass

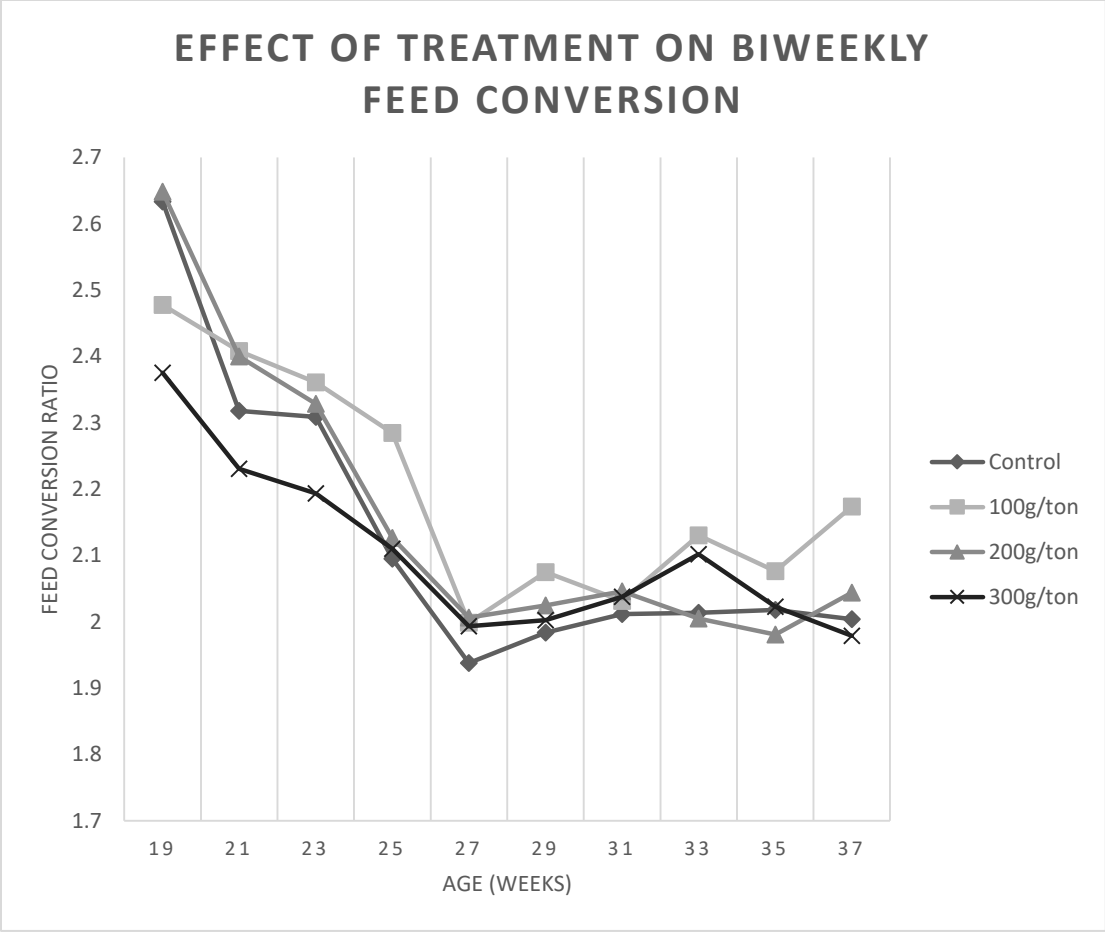

Figure 3.5 Feed conversion

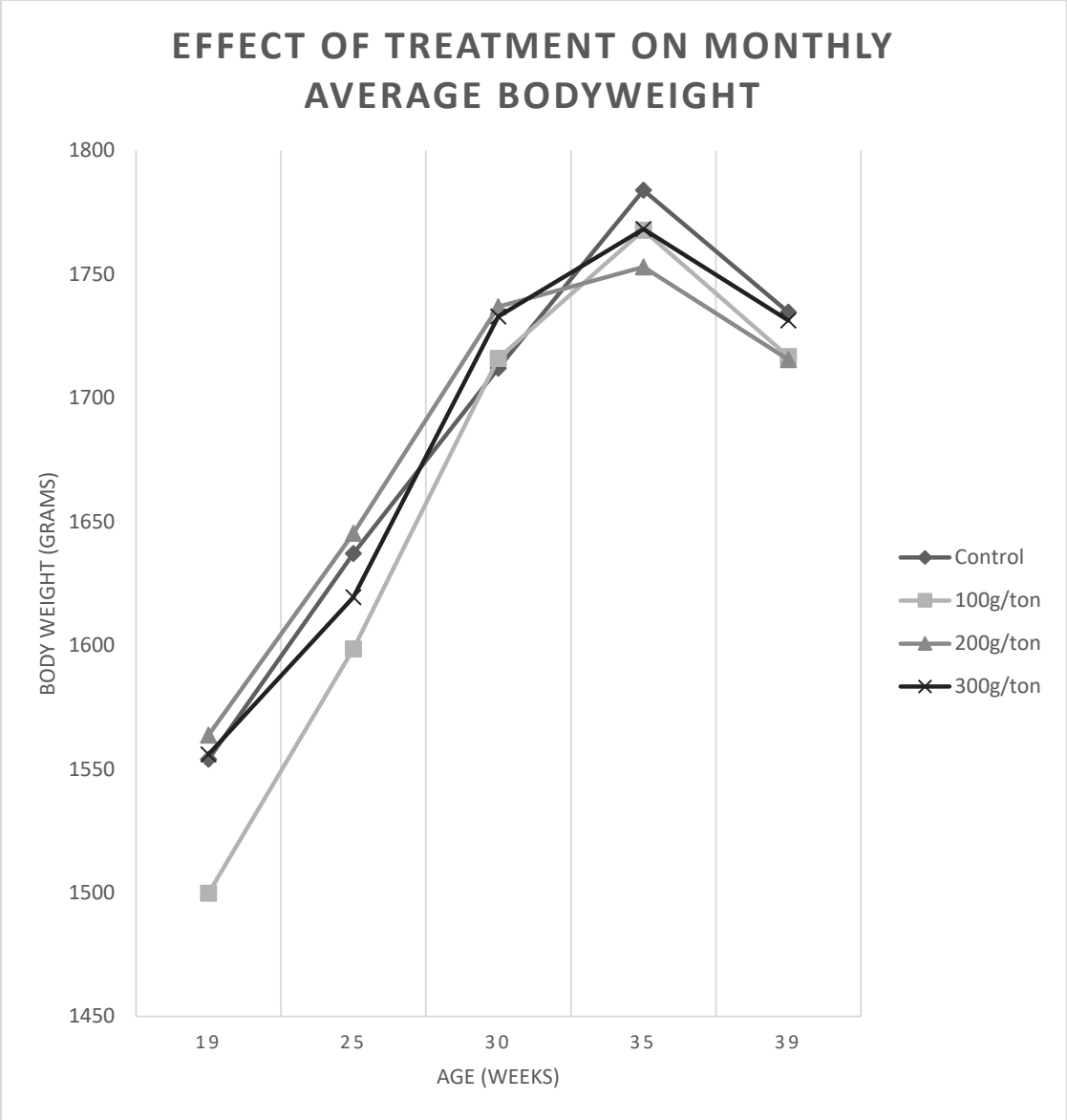

Figure 3.6 Bodyweight