EVALUATION OF YEAST CELL WALL ON EARLY PRODUCTION LAYING HEN PERFORMANCE

A Thesis

by

MOHAMMED MALIK HASHIM HASHIM

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Approved by:

Chair of Committee,	Christopher Bailey
Committee Members,	David Caldwell
	James Byrd
Head of Department,	John Carey

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ABSTRACT

The influence of two levels of yeast cell wall on phase one laying hen performance was investigated in this study. A total of 75 Lohmann W-36 replacement pullets, 17-weeks-old, were distributed among 75 laying hen cages (1 bird per pen). A total of 3 treatments were sequentially assigned to pens and each treatment had 25 replicates. Feeds were prepared according to the management guide for those birds and a single basal diet was divided into three treatments. First was the control basal diet only with no feed additives. The second was the basal diet supplemented with 250 ppm of yeast cell wall (YCW 250) and the third treatment was the basal diet supplemented with 500 ppm of yeast cell wall (YCW 500). Individual birds per cage served as the experimental unit for this study. Feed and water were offered *ad libitum*. Data were collected when birds were 21 weeks old and hen day egg production was > 90%.

Treatment YCW 250 resulted in significantly higher egg weight than the control and YCW 500 treatment in the first and second production period and was higher than YCW 500 in the third and fourth production period. Feed consumed per dozen eggs was significantly lower in treatment YCW 500 versus treatment YCW 250, but not significantly lower than the control for the first production period and all treatments were not different from each other for the rest of the study. Average feed consumed per bird per day, and monthly cumulative egg production was not different between treatments. Period feed conversion ratios were lower for the second and fourth period (P = 0.15 and 0.18 respectively). There was no treatment effect on interior egg quality

except for yolk color for the YCW 500 treatment in the fourth period which had significantly higher Roche color scores than the YCW 250 treatment. Specific gravity, egg shell thickness, egg shell weight, and percent shell weight were significantly higher in hens fed YCW 500 versus YCW 250. Egg shell breaking force was significantly higher in hens fed YCW 250 versus the control group. Overall, feeding a diet supplemented with yeast cell wall improved laying hen performance and 250 ppm YCW had the most significant influences, particularly with respect to increasing egg weight in early production laying hens.

DEDICATION

I dedicate this work to my mother and late father for inspiring me to obtain knowledge and be productive in the society. Also, I dedicate this thesis, to my family and dearest friend who always supports me.

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NOMENCLATURE

A	Albumin
AH	Albumen Height (mm)
E	Egg
EP	Egg Production
EWT	Egg Weight (g)
F	Feed
FC	Feed Consumed per bird per day (g)
FCR	Feed Conversion Ratio
FDE	Feed consumed per Dozen Eggs
H	Height
RCS	Roche Color Score
S	Egg Shell
SBF	Egg shell Breaking Force (kg)
SG	Specific Gravity
ST	Egg shell Thickness (μm)
SWT	Egg shell Weight (g)
WT	Weight
Y	Yolk
YCW	Yeast Cell Wall

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Antibiotics have been widely used in animal industries as antibiotic growth promoters (AGPs) fed at sub-therapeutic levels. This practice has been prevalent in farm animal production for over fifty years in the US and other countries (Dibner and Richards, 2005; Niewold, 2007). Most of these antibiotics have also been used at therapeutic concentrations for humans to help the body fight many species of pathogenic bacteria.

Some pathogenic bacteria have developed drug resistance to antibiotics, and this resistance is thought to have been caused, in part, by the administration of antimicrobial medicines in food producing animals (Witte, 1998; Threlfall et al., 2000; Butaye et al., 2003; Johansson et al., 2004; Mathur and Singh, 2005; Castanon, 2007). Some of these pathogens such as *Salmonella* and *E. coli* are the causative agents for zoonotic diseases and are major contributors to carcass condemnation (Witte, 1998; Threlfall et al., 2000). Thus, the European Union barred the use of antibiotics such as avoparcin and virginiamycin as growth promoters in animal feeds starting in 1997 and 1999 respectively (Casewell et al., 2003) and later in 2006, the European Union banned the use of all antibiotic growth promoters (Castanon, 2007). Little regulatory action has been taken in the United States regarding the European Union AGPs ban (Dibner and Richards, 2005). However, the European Union ban has increased the demand for

antibiotic alternative growth promoters such as prebiotics, which can have growth promotion effects similar to those seen using AGPs.

Hundreds of types of microbes exist in the gastrointestinal tract of chickens and their quantities are varied depending on the specific region of the gut. These microbes can be either beneficial or harmful to the host. Naturally occurring bacteria affect several vital physiological and nutritional processes in the body. The recognition of the vital role of the gut microflora is increasing because of the pivotal role these microorganisms have inducing and maintaining the health and productivity of poultry (Hume et al., 2011). Most of the intestinal microbial community reside in the ceca of adult chickens because it is considered a more stable environment for these microflora than other regions of the gastrointestinal tract (Jacobs, 2011).

Interest in gut microbiology and the dietary use of prebiotics started in the late 1800s and the beginning of the twenty century (Patterson and Burkholder, 2003). In 1995, prebiotics were defined as nondigestible food ingredients by the host that beneficially affect the host by selectively inducing the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus improving health (Gibson and Roberfroid, 1995). They also concluded that a dietary ingredient can be classified as a prebiotic if it is not hydrolyzed in the upper gastro-intestinal tract, is a selective substrate for single or multiple types of colonic bacteria, changes the gut microflora to a more healthy composition, and stimulates luminal or systemic effects that are advantageous to the host health status (Gibson and Roberfroid, 1995). Prebiotics tend

to be undigested carbohydrates that may be fermented by intestinal microbes (Bauer et al., 2006).

In this paper, we review the use of prebiotics and their influence on the poultry industry with regards to productivity and health status of poultry. More specifically, the objectives of this study are to evaluate the influence of yeast cell wall on the performance of early production laying hens.

Literature Review

Prebiotic mechanisms of action

The mechanisms in which prebiotics work in the digestive tract have been studied by several scholars both in vitro and in vivo (Ballou, 1970; Bailey et al., 1991; Gibson and Fuller, 2000; Corrigan et al., 2011; Charalampopoulos and Rastall, 2012; Tako and Glahn, 2012; Xiao et al., 2012). Gastrointestinal tract microbiota compete with the host for other nutrients, induce quick turnover of absorptive epithelial cells, cause an increased rate of mucus secretion by intestinal goblet cells, and induce inflammatory reactions by the immune system (Dibner and Richards, 2005). On the other hand, intestinal microbiota help the host by the fermentation of poorly digestible dietary ingredients and production of short chain fatty acids (Gibson and Fuller, 2000) which lower the gut pH and create environmental conditions that are capable of deterring

the growth of some bacterial pathogens in the intestine (Niba et al., 2009). Also, short chain fatty acid production of acetate, propionate, and butyrate from non-hydrolyzable oligo and polysaccharides increases the proliferation of the gut epithelial cells, thus increasing intestinal tissue weight, with changes in the overall morphology of intestinal mucosa (Niba et al., 2009; Bonos et al., 2011; Reisinger et al., 2012). Villi length increased when broiler chickens were fed a diet containing 0.2% yeast cell wall and this increase in the lengthening of the villi is thought to increase the absorption surface of the intestine (Reisinger et al., 2012). The density of goblet cells was also higher when yeast cell wall was supplemented in the diet leading to increased intestinal mucin, which is very important for the elimination of intestinal pathogens (Reisinger et al., 2012).

Another mode of action for prebiotics is by inhibiting the colonization of pathogenic bacteria (Takeda et al., 1995; Spring et al., 2000; Haldar et al., 2011). These studies have found that the concentration of enteric bacteria in the ceca changed significantly when birds were fed a diet containing prebiotic which caused *Salmonella* numbers to decrease in samples of pooled digesta and excreta. When prebiotics adhere to pathogenic bacteria having type -1 fimbriae, they can decrease the ability of the bacteria to colonize and multiply within the digestive tract mucosa (Baurhoo et al., 2007b; Bonos et al., 2011; Jacobs, 2011).

Types of prebiotics

Most identified prebiotics are classified as carbohydrate oligosaccharides with differing molecular structure that are normally presence in most animal diets. All dietary

fibers are candidate prebiotics, but the most capable are short chain nondigestible oligosaccharides (Gaggia et al., 2010). Many compounds may function as a prebiotic, and some of them have been widely investigated both in vivo, and in vitro (Gibson and Roberfroid, 1995; Gibson, 1998; Gibson and Fuller, 2000; Spring et al., 2000; Douglas et al., 2003; Hofacre et al., 2003b; Donalson et al., 2007; Ghosh et al., 2007; Hassan and Ragab, 2007; McReynolds et al., 2007; Baurhoo et al., 2007b; Gao et al., 2008; Niba et al., 2009; Stringfellow et al., 2009; Zakeri et al., 2009; Bonos et al., 2011; Haldar et al., 2011; Jacobs, 2011; Zakeri and Kashefi, 2011).

Fructooligosaccharides, which include all nondigestible oligosaccharide compounds with fructose and glucose monomers, are perhaps the most studied prebiotics (Swanson et al., 2002). Fructooligosaccharides are found naturally in onions and some types of cereal crops such as wheat, barley, and rye (Bailey et al., 1991). It is proposed that feeding of fructooligosaccharides increases short chain fatty acid concentrations which in turn favor the growth of *Lactobacilli* and *Bifidobacteria*. These short chain fatty acids decrease pH leading to reductions in anaerobic pathogens such as *Clostridia*, *Fusobacteria* and *Bacteroides* (Gibson and Roberfroid, 1995; Patterson et al., 1997; Ziemer and Gibson, 1998; Gibson and Fuller, 2000; Baurhoo et al., 2007b).

Lignin effects on the microbial community and morphology of the intestine was also investigated in poultry. It was reported that when broilers were fed a diet containing 1.25% lignin, those birds had longer villi on day 42, and higher number of goblet cells per villus on day 28 compared with birds that were fed a diet containing antibiotic growth promoter (Baurhoo et al., 2007a). They also concluded that *Lactobacillus* had

significantly higher population in the ceca at 42-days while *Bifidobacteria* was not different than the control diet. Their results showed that *E. coli* populations in the litter were significantly lower in broilers that were fed diet supplemented with 2.5% lignin versus birds that were fed diets containing no antibiotic growth promoter and were not different than birds that were fed diets containing antibiotic growth promoter. Another study concluded that feeding broiler chicks a diet containing 1.25% lignin resulted in significantly higher population logs of *Lactobacillus* and *Bifidobacteria* in the ceca on day 28 and 38 compared with birds fed a diet supplemented with antibiotic growth promoter (Baurhoo et al., 2007b). However, the same study reported that adding 1.25% lignin to the diet had insignificantly lower *E. coli* population in the ceca than control groups with or without antibiotic growth promoter on day 3, 6, and 9 of the challenge with *E. coli*, and significantly lower *E. coli* population log than both control groups (with or without AGP) when lignin was fed at 2.5% on day 3 and 6.

Glucooligosaccharides are synthesized from the transfer of glucose molecules from sucrose to maltose by the enzymatic action of glucosyltransferase, which is derived from *Leuconostoc mesenteroides*, while Galactooligosaccharides are typically generated from lactose by the enzymatic action of β-galactosidase, an enzyme synthesized by *Aspergillus orizae* (Kan and Matsumoto., 1989; Vallete et al., 1993; Jacobs, 2011). These compounds produce short chain fatty acids in similar concentration to fructooligosaccharides, and they positively affect beneficial types of intestinal bacteria, including *Bifidobacteria* and *Lactobacilli* (Jacobs, 2011).

Mannanoligosaccharides (MOS), complex components derived from the outer layer of the yeast cell wall (Yang et al., 2009), are another type of oligosaccharide that may beneficially affect microbial populations and immune function in the gut (Ferket, 2004). The primary carbohydrate monomer of mannanoligosaccharides is mannose which is known to minimize colonization of pathogenic bacteria; however, high cost of pure mannose limits its use in commercial poultry production. Fortunately, mannose-based carbohydrates occur naturally in many reasonable priced products like yeast cell walls and certain gums (Spring et al., 2000). *Salmonella* strains expressing Type-1 fimbriae, such as *Salmonella typhimurium*, have been shown to be reduced in young chicks when dietary mannanoligosaccharides were used under laboratory conditions (Spring et al., 2000). It was proposed that when birds were fed mannanoligosaccharides derived from the cell walls of yeast, it may stop the colonization of *Clostridium perfringens* bacteria, and thus yeast cell wall could be used with competitive exclusive cultures as a substitute to the use of growth promoter antibiotics for the prevention of necrotic enteritis (Hofacre et al., 2003b).

Although lactose cannot be considered by definition as a true prebiotic, it fits with the prebiotic concept for poultry because birds cannot digest it because they lack the ability to produce the enzyme lactase (Denbow, 2000). Lactose is thus available as a substrate for microflora in the hindgut (Jacobs, 2011). Low levels of lactose have been reported to increase the growth of commercial broiler chicks (Douglas et al., 2003).

Lactose was studied by several scholars to examine its effects on the health status of the birds by reducing the intestinal colonization of pathogenic bacteria such as

Clostridium perfringens and decreasing the incidence of the intestinal lesions (Takeda et al., 1995; McReynolds et al., 2007; Stringfellow et al., 2009). Significant reduction in the intestinal pH and necrotic lesion development were noticed when broiler chicks were fed a diet containing 2.5% lactose and 100 ppm of bismuth citrate when those chicks were challenged with *Clostridium perfringens* compared with the birds that had positive challenge, and no lactose and bismuth citrate added to their diet (Stringfellow et al., 2009). In addition, when lactose was fed alone to broiler chickens infected with necrotic enteritis caused by *Clostridium perfringens*, it significantly diminished the incidence of intestinal lesions (McReynolds et al., 2007; Stringfellow et al., 2009). Lactose has also been shown to have positive effects on weight gain by reducing the number of aerobes and coliform in the ceca of broiler chickens (Orban et al., 1997). Mortality and intestinal lesion scores were significantly lower when broiler chicks were fed diets supplemented with 2.5% lactose and challenged with *Clostridium perfringens* (McReynolds et al., 2007).

Effects of prebiotics on productivity

The influence of prebiotics on bird productivity during different phases of productions and under variable environment conditions has been investigated in several studies (Benites et al., 2008; Gao et al., 2008; Baurhoo et al., 2009; El-Sheikh et al., 2009; Radu-Rusu and Pop, 2009; Aghaei et al., 2010; Gurbuz et al., 2011; Haldar et al., 2011; Kim et al., 2011; Zakeri and Kashefi, 2011; Faber et al., 2012; Houshmand et al., 2012; Reisinger et al., 2012; Shanmugasundaram and Selvaraj, 2012; Sohail et al., 2012;

Bozkurt et al., 2012b) . When broilers were fed a diet containing 0.1% yeast cell wall derivative, body weight and daily weight gain were significantly improved (Reisinger et al., 2012). They concluded that this increased weight gain was due to improved feed efficiency. In another study it was found that body weight gain was improved and feed conversion ratio was decreased when broilers were fed a diet supplemented with mannanoligosaccharides and then subjected to heat stress (Sohail et al., 2012). They assumed that mannanoligosaccharides improved the absorption of nutrients from the intestine and counterbalanced the deleterious effect of heat stress. It was noted that feeding a diet containing 0.05% mannanoligosaccharides or 0.25% fructooligosaccharides improved the performance of broiler chickens when these prebiotics were supplemented in the diet for periods greater than 28 days (Kim et al., 2011). In another study with Japanese quail, feed conversion ratio and performance index were significantly increased when the quail were fed a diet containing organic acid salts in combination with mannanoligosaccharide (Ghosh et al., 2007). Adding 2 - 4% of lactose to the diet of 1 to 21-day-old broiler chicks increased weight gain ($P<0.08$) (Douglas et al., 2003). Also, adding sucrose thermal oligosaccharide caramel to the broiler chicken diet significantly increased weight gain ($P < 0.001$), feed intake, and improved feed conversion (Orban et al., 1997).

Prebiotics have also been investigated in laying hen diets. Zhang (2004) found that a molt induced by feeding relatively high concentrations of guar meal (20%) as a source of prebiotic galactomannans improves the resistance to *Salmonella* infection of laying hens, and the improvement may be enhanced by the inclusion of β-mannanase

(Hemicell)^{TM} (Zhang, 2004). When 57- week-old laying hens were fed a diet containing 1.0% oligofructose-type commercial prebiotic or 1.0% (w/w) inulin (an archetypal or prototype prebiotic found in many dietary plants) there was significant improvement ($P<0.05$) in egg production, feed conversion, and intestine length (Chen et al., 2005). Feed conversion, crude protein conversion, caloric conversion ratio, egg weight, shell %, yolk %, and yolk index (the flattening of the yolk of an egg) were significantly higher when 49-week-old laying hens fed diets containing 0.1% mannanoligosaccharide (Hassan and Ragab, 2007). Another study found that adding mannanoligosaccharides at 1 g/kg of diet increased eggshell weights ($P < 0.01$), eggshell thickness ($P = 0.10$), and shell breaking strength ($P < 0.09$) for laying hens between 36 and 51 weeks old (Bozkurt et al., 2012b). They reported that these enhancements in egg quality might be because of the influence of the prebiotic on the metabolic activity of the intestinal microflora which improves the absorption rate of the minerals, specifically Mg^{2+} and Ca^{2+} (Roberfroid, 2000). It was also stated that yeast cell wall improved egg production and final body weight when it was supplemented to the diet of 48-week-old laying hens, although egg weight and specific gravity did not improve (Gurbuz et al., 2011). They suggested that these positive influences in egg production and body weight might be as a result of increased feed intake and villi width.

Effects of prebiotics on the health status of poultry

Maintaining a healthy flock is very important for profitable poultry production and providing safe poultry products to consumers. There are several foodborne pathogens such as *Salmonella* that can be found in poultry products and these pathogens can cause serious human disease (Scallan et al., 2011). Intestinal microbiota can be a dietary liability in fast growing broiler chickens (Dibner and Richards, 2005) because they may lead to increased energy requirement for maintenance and decreased nutrient utilization efficiency (Yang et al., 2009). The modulation of beneficial microbiota growth and the suppression of pathogenic bacteria have been achieved by supplementing the diet with different types of feed additives such antibiotic growth promoters, probiotics, prebiotics, and symbiotics (Miles et al., 1983; Stutz and Lawton, 1984; Patterson et al., 1997; Gaskins et al., 2002; Roberfroid et al., 2010; Bonos et al., 2011; Corrigan et al., 2011; Hume et al., 2011; Jacobs, 2011; Kim et al., 2011). A symbiotic is a combination of both prebiotics and probiotics where by the probiotic microbes use the prebiotic as a fermentable substrate to extend their lives and thus favor the health status of the host (Collins and Gibson, 1999; Yang et al., 2009).

Prebiotics have been shown to significantly improve the immune status of birds by regulating the enteric microbiota and diminishing pathogenic microbial load in the intestinal tract thus improving the health status of the birds (Takeda et al., 1995; Orban et al., 1997; Patterson et al., 1997; Spring et al., 2000; Hofacre et al., 2003b; Tuohy et al., 2003; Dahiya et al., 2006; Donalson et al., 2007; Ghosh et al., 2007; Hassan and Ragab, 2007; McReynolds et al., 2007; Baurhoo et al., 2007b; Gao et al., 2008;

Shoaf-Sweeney and Hutkins, 2008; Stringfellow et al., 2009; Zakeri et al., 2009; Bonos et al., 2011; Zakeri and Kashefi, 2011). There are many research studies that have been conducted to assess the influence of different types of prebiotics at various levels on the effects of microbiota in the gut of the animal and intestinal histology (Fernandez et al., 2002; Tuohy et al., 2003; Sims et al., 2004; Sun et al., 2005; Bauer et al., 2006; Juskiewicz et al., 2006; Biggs et al., 2007; De los Santos et al., 2007; Donalson et al., 2007; Ghosh et al., 2007; Lenoir-Wijnkoop et al., 2007; Baurhoo et al., 2007a; Baurhoo et al., 2007b; Baurhoo et al., 2009; Kim et al., 2009; Yang et al., 2009; Aghaei et al., 2010; Bonos et al., 2011; Corrigan et al., 2011; Jacobs, 2011; Kim et al., 2011; Reisinger et al., 2012)

Sucrose thermal oligosaccharide caramel significantly increased ($P<0.03$) cecal *Bifidobacterial* numbers in the ceca of broiler chickens compared with birds fed basal control diet (Orban et al., 1997). Fructooligosaccharides have been used in the diet of 1-day-old broiler chickens at 0.25% and led to decreased *Clostridium perfringens* and *E. coli* populations and increased *Lactobacilli* in the intestine (Kim et al., 2011). Fructooligosaccharides also increased *Lactobacillus spp.* and decreased *Clostridium perfringens* and *Escherichia coli* in 27-week-old laying hens when it was added to the diet at 0.25% (Kim et al., 2009). Another study found that feeding laying hens a diet containing 0.375% fructooligosaccharides during molt significantly decreased the *Salmonella enteritidis* in ovaries and liver versus laying hens subjected to feed withdrawal (Donalson et al., 2008). Also, they found that cecal microbial count was significantly lower in laying hens fed the fructooligosaccharides diet. When broiler

chicks were fed a diet supplemented with 0.75% fructooligosaccharides and stressed by feed deprivation then challenged with Salmonella, they had significantly lower *Salmonella* positive birds and four times lower *Salmonella* per gram of cecal content than control chicks at age 21 days (Bailey et al., 1991).

Mannanoligosaccharide can improve the immunity status of birds by activating macrophages via mannose specific receptors and thus allowing the macrophage to become more active with respect to destroying pathogenic bacteria (Zakeri and Kashefi, 2011). These authors found that 25-day-old broiler chicks had significant increases ($P<0.05$) in serum antibody titer against Newcastle vaccine when chicks were fed a diet containing 1000 mg/kg mannanoligosaccharide. In addition, mannanoligosaccharides had significant effects on the secondary immune response and cutaneous basophil hypersensitivity against sheep red blood cells in 49-week-old laying hens fed a diet containing 0.1% mannanoligosaccharide (Hassan and Ragab, 2007). A study has found that adding 0.025% or 0.05% mannanoligosaccharides to broiler diets increased the population log of *Lactobacillus* over the control treatment or avilamycin treatment while both mannanoligosaccharide treatments had lower population logs of *E. coli* than the control treatment, and 0.05% mannanoligosaccharides had lower *Clostridium perfringens* than the control treatment (Kim et al., 2011). When mannanoligosaccharides was added to broiler diets at 1 or 2 kg/metric ton, significant changes were noticed in the bacterial community structure of the cecal content, and these changes were in favor of animal health although no significant changes were noted on animal performance (Corrigan et al., 2011). They related the insignificant changes in broiler performance

between treatments to the ideal environment and management conditions that were maintained in their trial (Geier et al., 2009). Another study found that villi height and goblet cell number per villus were significantly higher when broilers were fed diets containing 0.2 or 0.5% mannanoligosaccharides over broilers fed diets containing virginiamycin or bacitracin at 14, 24, and 34 days of age (Baurhoo et al., 2009). They also found that population log of *Bifidobacteria* were significantly higher in both mannanoligosaccharide treatments over the antibiotic growth promoter treatments for the entire study while *Lactobacilli* population log was significantly higher in the low mannanoligosaccharides treatment than the bacitracin treatment after 24 days. The high mannanoligosaccharides treatment had significantly higher *Lactobacilli* population logs than the bacitracin treatment at 34 days of age.

Another study has found that treating broilers with a defined lactic acid bacterial competitive exclusion culture at day one and feeding a diet containing 2 grams of mannanoligosaccharide per ton decreased the mortality caused by necrotic enteritis (Hofacre et al., 2003a). The cecal *Salmonella* concentration in 13-day-old chicks was significantly lower ($P<0.05$) when those birds were fed a diet containing 4000 ppm of mannanoligosaccharides and challenged with *Salmonella* at 3-days of age (Spring et al., 2000). They also found significantly ($P<0.05$) less *Salmonella Dublin* positive chicks after 10 days of the challenge when fed 4000 ppm mannanoligosaccharides.

CHAPTER II

EFFECTS OF YEAST CELL WALL EARLY PRODUCTION LAYING HEN PERFORMANCE

Introduction

The use of antibiotic growth promoters (AGP) has been widely adapted within the animal feed industry to improve animal resistance to pathogens and increase animal productivity (Stutz and Lawton, 1984; Gaskins et al., 2002; Butaye et al., 2003). Concerns that antibiotic growth promoters may lead to microbial resistance when fed at therapeutic levels has led to a ban in the European Union (Witte, 1998; Threlfall et al., 2000; Witte, 2000; Butaye et al., 2003; Castanon, 2007). The ban of antibiotic growth promoters in the European Union and concerns of expanding this ban to other countries, have led to increased demand for alternative growth promoters.

Numerous studies have been conducted regarding this matter, and several antibiotic alternatives have emerged. One of the antibiotic growth promoter alternatives that is of major interest and is being investigated by many scholars across the animal feed industry is the use of dietary prebiotics. Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health (Gibson and Roberfroid, 1995). Fructooligosaccharides are among the prebiotics that have been studied in poultry. When broilers were fed a diet containing 0.25% fructooligosaccharides, overall body weight gains were significantly higher (Kim et al.,

2011). It was also noted that feeding 27-week-old laying hens a diet containing 0.25% fructooligosaccharides significantly improved egg shell thickness (Kim et al., 2009).

Inulin is a naturally occurring plant polysaccharide prebiotic that selectively induces the growth and activity of the intestinal microflora (Ziemer and Gibson, 1998; Gibson and Fuller, 2000; Chen et al., 2005; Tako and Glahn, 2012). Inulin significantly increased the level of iron in the blood of broiler chicks when it was administered into the amniotic fluid of 17-day-old embryos or supplemented in the diet after hatching and thus helped to improve the iron-deficient status of those broilers (Tako and Glahn, 2012). It was also reported that feeding 57-week-old laying hens a diet containing 1% inulin significantly increased weekly egg production, cumulative weekly egg weight per bird, and improved feed conversion ratio (Chen et al., 2005).

The effects of yeast cell wall components, such as mannanoligosaccharides, on poultry production have been investigated by many scholars (Zaghini et al., 2005; Biggs et al., 2007; Benites et al., 2008; Kim et al., 2009; Radu-Rusu and Pop, 2009; Gurbuz et al., 2011; Haldar et al., 2011; Kim et al., 2011; Zakeri and Kashefi, 2011; Houshmand et al., 2012; Reisinger et al., 2012; Shanmugasundaram and Selvaraj, 2012; Sohail et al., 2012; Bozkurt et al., 2012a; Bozkurt et al., 2012b). Broiler chicks that were fed a diet supplemented with 0.5% of mannanoligosaccharides had significantly higher body weight gain and lower feed conversion ratio when subjected to heat stress (Sohail et al., 2012). Body weight and daily weight gain were significantly higher when broilers were fed a diet containing 1 kg/tonne yeast derivative, which contains yeast cell wall fragments and yeast extract derived from *Saccharomyces cerevisiae* (Reisinger et al.,

2012). In another study, when 1-day-old broiler chicks were fed a diet supplemented with either 0.025% or 0.05% mannanoligosaccharides for 4 weeks, the overall body weight gain was significantly higher (Kim et al., 2011).

Influences of mannanoligosaccharides on performance of laying hens were also studied (Bozkurt et al., 2012a; Bozkurt et al., 2012b). It was concluded that egg production rate was significant increased ($P<0.01$) when 52-week-old laying hens were fed a diet containing 0.1% mannanoligosaccharides for 10 weeks; however, other productive performance parameters such as egg weight, egg mass, feed consumption, feed conversion were not significantly different (Bozkurt et al., 2012a). Egg shell weight ($P<0.01$), egg shell thickness ($P=0.1$), and shell breaking strength ($P=0.09$) were increased when 36 to 51-week-old laying hens were fed a diet containing 0.1% mannanoligosaccharides (Bozkurt et al., 2012b). Another study reported that egg production, feed consumption, and final body weight were significantly higher when 48-week-old laying hens were fed a diet supplemented with 500 mg/kg yeast cell walls for 90 days, although egg weight and specific gravity were not significantly different (Gurbuz et al., 2011). Hen-day and hen-house egg production were statistically higher when 27-week-old laying hens were feed a diet containing 0.025% mannanoligosaccharides while feed intake, egg weight, egg shell strength, egg yolk color, and Haugh unit were not significantly affected (Kim et al., 2009).

It was reported that when 49-week-old laying hens were fed a diet supplemented with 0.1% mannanoligosaccharides, they had significant improvements in feed conversion, crude protein conversion, and caloric conversion ratio (Hassan and Ragab, 2007). These authors also indicated that egg weight, shell %, yolk %, and yolk index were significantly higher. The effects of feeding a diet supplemented with 0.1% mannanoligosaccharides during the hot summer season was investigated in another study and showed significant increases in egg production and decreases in cracked-broken egg shell, and mortality of 54-week-old layers (Çabuk et al., 2006).

There is lack of scientific knowledge regarding the influence of yeast cell wall during the early phase of laying hens production. It is hypothesized that yeast cell wall improves the performance of laying hens in the early stage of production. The objectives of this study are to evaluate the prebiotic effects of using the yeast cell wall derivative (Safmannan)^{TM} on phase one laying hen performance (hens entering peak performance for four production periods).

Materials and Methods

Animal and husbandry

A total of 75 Lohmann Hy-Line W-36, replacement pullets (17-week-old) were distributed among 75 laying hen cages (1 bird per pen) in an open sided hen house located at the Texas A&M Poultry Research Center. Individual birds/cages served as the experimental unit for this study. A total of three treatments were sequentially assigned to pens, and each treatment had 25 birds. Birds were fed an industry type phase 1 laying hen diet prepared according to the Hy-Line W-36 commercial management guide. Two diets were mixed, first one was prepared for the hens between 21 and 32 weeks of age (Table 2.1) and the second one was prepared for hens 33 to 36-weeks-old (Table 2.2). The basal diets were divided into three equally sized batches and supplemented with yeast cell wall at 0, 250, 500 ppm. The yeast cell wall was premixed in 2.27 kg of basal carrier, then added to the basal diet and mixed in a horizontal feed mill for five minutes at the Texas A&M Poultry Research Center. Birds were fed daily on an individual basis. Feed and water were provided *ad libitum* and diets were fed in mash form. The average hen weight at the initiation of the experiment was 1270 grams. When the hens reached 21-weeks-old, their egg production was more than 90% and this was considered the first day of the initial 28-day production period.

Table 2.1. Composition of basal diets from 21-32 weeks of age

Ingredients	Percentage
Corn	52.25
Dehulled Soybean meal	27.89
DL-Methionine 98	0.31
Lysine HCL	0.05
Fat	5.21
Limestone	11.18
BioFos 16/21P	2.33
Salt	0.48
Trace minerals	0.05
Vitamins	0.25
Calculated composition	
AME_n (kcal/kg)	2,900
Crude fat	6.76
Crude protein	19.05
Lysine	1.05
Methionine	0.60
TSAA	0.91
Tryptophan	0.23
Threonine	0.71
Calcium	4.76
Phosphorus	0.84
Nonphytate phosphorus	0.60
Sodium	0.21
Linoleic acid	2.33

Table 2.2. Composition of basal diets from 33 -36 weeks of age

Ingredients	Percentage
Corn	59.99
Dehulled Soybean meal	23.75
DL-Methionine 98	0.18
Fat	2.93
Limestone	10.50
BioFos 16/21P	1.92
Salt	0.43
Trace minerals	0.05
Vitamins	0.25
Calculated composition	
AME_n (kcal/kg)	2,850
Crude fat	4.66
Crude protein	17.48
Lysine	0.89
Methionine	0.45
TSAA	0.75
Tryptophan	0.20
Threonine	0.65
Calcium	4.42
Phosphorus	0.75
Nonphytate phosphorus	0.51
Sodium	0.91
Linoleic acid	1.14

Data collection

Data were collected on body weight, feed consumption, hen-day egg production, and daily exterior egg quality (checks, cracks shell-less eggs, etc.). Hens were weighted every 28 days and followed through peak production over four 28-day production periods. Egg weight was determined for all eggs laid on a single day (one egg per bird) on a weekly basis using a Mettler Toledo scale (AG285). Interior egg shell quality (Haugh units, yolk color, and albumen height) were determined for all eggs laid on a single day (one egg per bird) once during each production period using an Egg Analyzer (Model 05-UM-001, Version B, Orka Food Tech.Ltd). Egg shell thickness and breaking strength were measured for all eggs laid on a single day (one egg per bird) once during the first and second production periods. Egg shell breaking strength was measured using an Instron machine (Model 1011, Instron Corp., Norwood, MA). The Instron machine was set up with a 50 kg load cell, a load range of 10 kg, and a cross head speed of 50 mm/min. Egg shell thickness was measured using a micrometer at three different locations, top, middle and bottom of the egg. Then, these three measurements were averaged to determine overall egg shell thickness.

At the end of the study (36-weeks-old), specific gravity was measured for all eggs laid on a single day (one egg per bird) for three consecutive days, and egg weight, specific gravity, shell weight, shell thickness and percent shell were also taken. Seven solutions of varying specific gravity were prepared. Those solutions had specific gravities of 1.070, 1.075, 1.077, 1.080, 1.083, 1.085, and 1.090. The approximate initial amount of salt was 211, 237, 250, 263, 266, 270, 276 gram/liter consecutively and 11

liters were prepared for each solution. A hydrometer was used to adjust the final specific gravity for those solutions until the desired reading was obtained. After the specific gravity was determined, eggs were cracked at the middle of the egg and albumen and yolk were removed. Then, egg shells were washed carefully and dried in the oven at 90°C until full dryness was verified. After that, egg shell was weighed, and egg shell thickness was measured using a micrometer as previously described.

Statistical analysis

Data were analyzed for normality using the Shapiro-Wilk test (SPSS, 2012). Outliers affecting normality were identified by box-plot analysis and excluded from further analysis. Data were analyzed as a One Way Analysis of Variance using the general linear model procedure of SPSS software (SPSS, 2012). Duncan's multiple range test (SPSS, 2012) was used to compare differences in parameter among treatment groups if they were significantly different by ANOVA.

Results and Discussion

Productivity measurements

Laying hen productivity for the four production periods is shown in Table 2.3. Monthly cumulative egg production was not significantly different between treatments over the entire study. However, the hen day egg production percentage was higher in hens fed yeast cell wall at 250 ppm than the control and birds fed yeast cell wall at 500 ppm in the second, third and fourth production period. In addition, hens that fed 250 ppm of yeast cell wall had higher hen day egg production in the fourth period ($P = 0.18$) than other birds. Figure 2.1 shows the weekly egg production for 19-week-old laying hens, two weeks before the birds reached over 90% of production. The dip in hen day egg production observed in 29-week-old hens may be due to the sharp increase in ambient temperature during the preceding 2-3 weeks. Feed per dozen eggs was significantly higher in birds fed yeast cell wall at 250 ppm (1.6 kg/dozen) than birds fed 500 ppm yeast cell (1.4 kg/dozen) and numerically higher than the control (1.5 kg/dozen) for the first production period (21 to 24-weeks-old). There were no significant differences between treatments in feed consumption per dozen eggs other than the first period. There were no significant differences between treatments in the average feed consumption per bird per day over the entire study. Period feed conversion ratio was calculated based on monthly feed consumption per bird and the estimated average monthly egg weight based on weekly egg weight sampling. There were no significant differences for period feed conversion ratio between treatments.

However, the phase feed conversion ratio was numerically better in birds fed yeast cell wall at 250 ppm on the second, third, and fourth period of the study and the p value = 0.15 and 0.18 at the second and fourth periods, respectively. Egg weight was significantly higher in hens fed yeast cell wall at 250 ppm (57.0 g) than the control (55.0 g) and 500 ppm treatment (54.3 g) during the first and second period. During the third and fourth period, egg weight was significantly higher in hens fed yeast cell wall at 250 ppm (59.1 g, 60.6 g) than in hens fed yeast cell wall at 500 ppm (56.8 g, 58.1 g) and numerically higher than the control treatment (57.9 g, 59.8 g). These results can explain the higher feed consumption per dozen eggs in hens fed yeast cell wall at 250 ppm as hens consuming greater quantity of feed for any given body weight usually produce larger eggs. Heavier egg weight could also be due to an increase in feeding efficiency and feed utilization when birds were fed the diet supplemented with yeast cell wall (Ferket et al., 2002; Ghosh et al., 2007; Hassan and Ragab, 2007; Reisinger et al., 2012).

Table 2.3. Laying hen productivity from 1 to 4 production periods. CEP = cumulative egg production; FDE = feed consumption per dozen of eggs; EWT = egg weight (g); FC = average period feed consumed per bird per day (g); FCR = period feed conversion ratio (based on estimated period egg weight); EP = period egg production percentage.

Period		CONTROL	YCW 250	YCW 500
First period 21-24 weeks	CEP	33.8±5.60	32.8±7.80	33.5±6.00
	FDE	1.5±0.19 [ab]	1.6±0.17 [a]	1.4±0.12 [b]
	EWT	55.0±4.6 [b]	57.0±4.1 [a]	54.3±4.3 [b]
	FC	89.3±11	90.2±8	86.2±9
	FCR	2.28±0.28	2.29±0.23	2.20±0.18
	EP	95.14±1.2	93.86±1.2	96.71±1.2
Second period 25-28 weeks	CEP	60.9±5.9	60.0±8.2	60.5±5.9
	FDE	1.17±0.11	1.18±0.10	1.18±0.13
	EWT	57.0±4.5 b	59.5±3.4 a	56.6±5.1b
	FC	94.1±9	95.1±8	94.2±10
	FCR[1]	1.71±0.16	1.65±0.14	1.73±0.16
	EP	96.57±0.7	97.00±0.7	96.29±0.7
Third period 29-32 weeks	CEP	87.4±6.0	86.5±8.1	86.2±6.5
	FDE	1.19±0.16	1.18±0.12	1.15±0.28
	EWT	57.9±4.5 [ab]	59.1±3.8 [a]	56.8±3.9 [b]
	FC	93.9±13	93.3±9	92.6±12

Table 2.3 continued

Period		CONTROL	YCW 250	YCW 500
Fourth period 33-36 weeks	FCR	1.71±0.22	1.67±0.16	1.76±0.19
	EP	94.57±0.9	94.71±0.9	92.93±0.9
	CEP	113.8±6.4	113.0±8.9	111.2±8.1
	FDE	1.23±0.16	1.20±0.13	1.17±0.27
	EWT	59.8±4.4 [a]	60.6±4.4 [a]	58.1±3.4 [b]
	FC	96.9±11	94.1±9	93.8±10
	FCR[2]	1.72±0.21	1.65±0.18	1.75±0.14
	EP[2]	94.43±0.9	95.24±0.9	93.01±0.9

[a,b] Means within a row without common superscript differ significantly ($P < 0.05$)

[1] P value = 0.15

[2] P value = 0.18

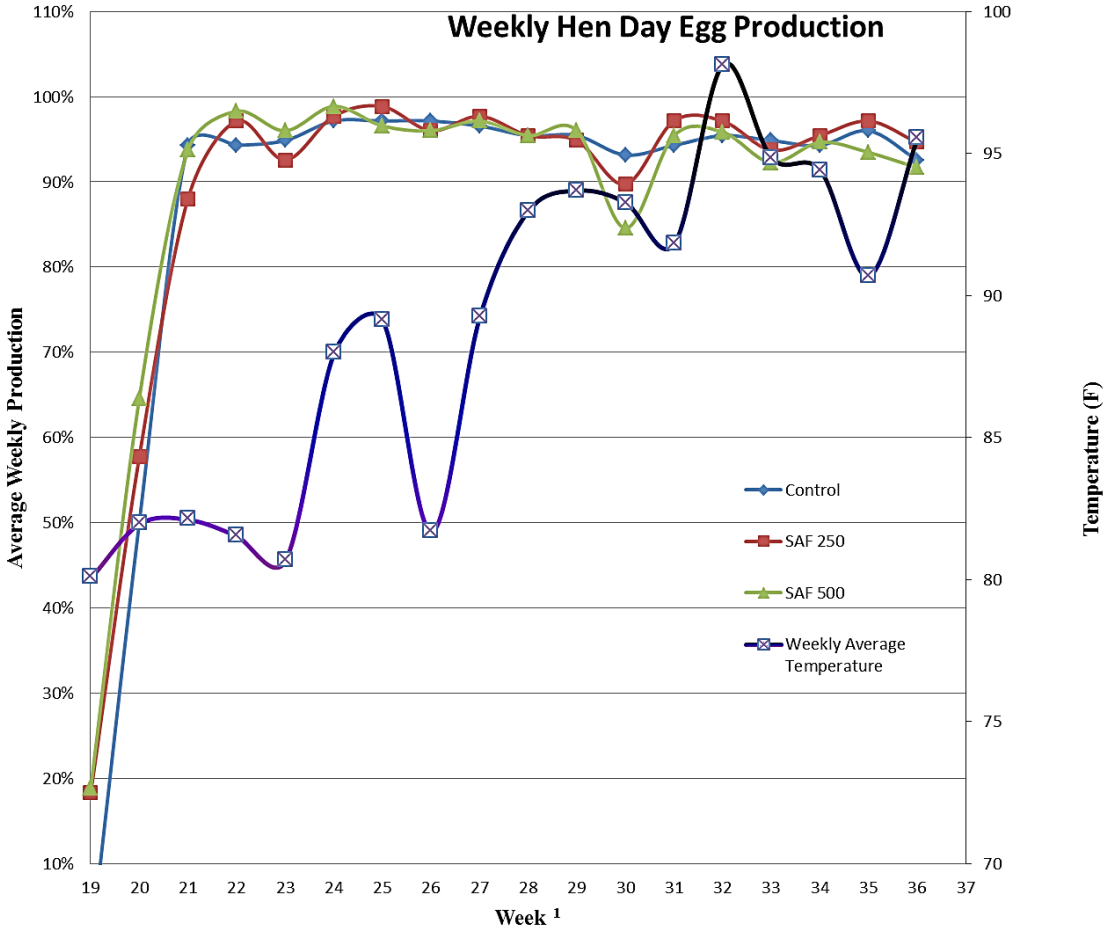

Figure 2.1. Weekly hen day egg production. Temperature data were obtained from College Station airport through uweather.com. This weather station is located less than one mile from the Poultry Science Research Center at Texas A&M University.

[1] week 21-24 (first period), week 25-28 (second period), week 29-32 (third period), and week 33-36 (fourth period).

Internal egg quality

Internal egg quality for the four production periods is shown in Table 2.4. Albumin height, Haugh unit, and yolk color were measured monthly over the four production periods. The albumen height and Haugh unit are two important parameters of internal egg quality. There were no significant differences between treatments in all of the internal quality parameters almost over the entire study and these results agree with the results by other scholars (Chen et al., 2005; Kim et al., 2009; Radu-Rusu and Pop, 2009; Aghaei et al., 2010). However, during the fourth period, yolk color score was significantly higher in hens fed yeast cell wall at 500 ppm than in hens fed yeast cell wall at 250 ppm, but not significantly higher than the control.

Table 2.4. Internal egg quality for 1 to 4 production periods. AH = albumen height (μm); Haugh = Haugh unit; RCS = calculated Roche Color Score.

Period		CONTROL	YCW 250	YCW 500
First period 21-24 weeks	AH	7.14±1.94	7.33±1.95	7.48±0.85
	Haugh	82.48±19.74	81.71±25.99	87.66±5.52
	RCS	2.85±0.59	3.04±0.64	3.04±0.55
Second period 25-28 weeks	AH	6.21±2.15	6.48±2.37	6.45±1.84
	Haugh	75.08±23.11	75.52±24.09	79.37±19.64
	RCS	2.13±0.45	2.38±0.58	2.28±0.54
Third period 29-32 weeks	AH	6.57±1.99	6.86±1.56	6.26±1.55

Table 2.4 continued

Period		CONTROL	YCW 250	YCW 500
	Haugh	77.76±22.20	81.43±13.61	78.19±16.93
	RCS	1.83±0.38	2.08±0.58	2.04±0.48
Fourth period	AH	6.99±0.90	6.93±0.78	6.65±0.98
33-36 weeks	Haugh	83.20±5.50	82.75±5.10	81.85±5.62
	RCS	2.00±0.31[ab]	1.79±0.42 [b]	2.13±0.45 [a]

[a,b] Means within a row without common superscript differ significantly ($P < 0.05$)

External egg quality

Egg shell breaking strength and shell thickness are destructive methods to measure external egg shell quality. They were measured only for the first and second period of the study because the Instron device had un-repairable technical issues. External quality results are shown in Table 2.5. There was no statistical significant difference in shell thickness between treatments. This finding is in agreement with the results of other studies (Kim et al., 2009; Radu-Rusu and Pop, 2009; Aghaei et al., 2010; Bozkurt et al., 2012a; Bozkurt et al., 2012b). For the first period, egg shell breaking force was statistically higher in hens fed yeast cell wall at 250 ppm (3.53 kg) than control (3.06 kg), but insignificantly higher than hens fed yeast cell wall at 500 ppm (3.42 kg). This trend was not constant as during the second month birds fed yeast cell wall at 250 ppm was not significantly higher (3.59 kg) than the control (3.47 kg) or birds fed yeast cell wall at 500 ppm (3.45 kg). Egg weight was numerically higher during the first period and statistically higher during the second period in hens fed yeast cell wall at 250 ppm.

Table 2.5. External egg quality for 1 to 2 production periods. EWT = egg weight (g); SBF = egg shell breaking force (kg); ST = egg shell thickness (μm).

Period		CONTROL	YCW 250	YCW 500
First period 21-24 weeks	EWT	52.97±11.63	56.70±3.62	54.52±3.16
	SBF	3.06±0.79 [b]	3.53±0.56 [a]	3.42±0.68 [ab]
	ST	0.37±0.08	0.39±0.02	0.39±0.02
Second period 25-28 weeks	EWT	57.47[b]±3.96	60.00[a]±3.15	56.99[b]±3.93
	SBF	3.47±0.49	3.59±0.52	3.46±0.54
	ST	0.38±0.02	0.38±0.018	0.38±0.02

[a,b] Means within a row without common superscript differ significantly ($P < 0.05$)

Specific gravity is a non-destructive measurement of the exterior quality of eggs and it is correlated to percent shell and shell thickness. Results for specific gravity are shown in Table 2.6. In this study, specific gravity was significantly lower in hens fed yeast cell wall at 250 ppm. For those eggs that were used to measure specific gravity, egg shell weight, percent shell, and shell thickness were all significantly lower for hens fed 250 ppm yeast cell wall versus the control and 500 ppm yeast cell wall treatment. However, egg weight was numerically higher in birds fed yeast cell wall at 250 ppm (59.78 g) than the control (59.33 g) and 500 ppm yeast cell wall treatment (58.41 g). It is generally agreed that egg shell quality is inversely related to egg weight. These results are in agreement with the results from two other studies (Aghaei et al., 2010; Bozkurt et al., 2012a) that showed feeding mannanoligosaccharides did not significantly increase shell weight and shell thickness.

Table 2.6. Specific gravity of eggs sampled from 36 week old laying hens.

	CONTROL	YCW 250	YCW 500
Egg weight (g)	59.33±3.80	59.78±4.79	58.41±3.87
Specific Gravity	1.082±0.0045 [a]	1.077±0.0037 [b]	1.082±0.0043 [a]
Egg Shell weight (g)	4.96±0.42 [a]	4.54±0.47 [b]	4.85±0.38 [a]
Egg Shell Thick (μm)	0.35±0.022 [a]	0.33±0.015 [b]	0.34±0.019 [a]
Percent Shell	8.38±0.10 [a]	7.60±0.10 [b]	8.32±0.10 [a]

[a,b] Means within a row without common superscript differ significantly ($P < 0.05$).

CHAPTER III

CONCLUSION

Finding suitable substitutes for antibiotic growth promoters now banned in the European Union is a high priority for poultry producers. These substitutes are required to enhance the performance of the birds without fear of developing antimicrobial resistance. Incorporation of dietary prebiotics is thought to be one type of alternative having positive influences on productivity and health of poultry.

Yeast cell wall feed additives have been shown to positively influence multiple characteristics of bird performance like feed efficiency, feed conversion, body weight, and feed consumption. However, there is lack of research on the effects of yeast cell wall on laying hen performance and more specifically on the early phase of production when birds are undergoing the stress of peak production. This study evaluated the effect of yeast cell wall on performance of phase one laying hens by assessing several aspects of production including both internal and external measures of egg quality.

The overall results showed significant increases in egg weight for hens fed yeast cell wall at 250 ppm. Period feed conversion ratio was improved in the 250 ppm yeast cell wall treatment during the second ($P = 0.15$) and fourth production period ($P = 0.18$) of the study. Period egg production percentage was also numerically higher ($P = 0.18$) in the 250 ppm treatment for the fourth period of the experiment.

For internal egg quality, only yolk color score was significantly increased in the 500 ppm yeast cell wall treatment for the last period of the study. All other internal quality traits such as albumen height and Haugh unit were not significantly different.

External egg quality measures such as egg shell thickness and egg shell breaking force, and egg weight were also examined in this study. Egg shell breaking force was significantly higher for the 250 ppm treatment for the first period of the study. However, it was only numerically higher during the second period. Egg weight was significantly greater in the second month of the study for the 250 ppm yeast cell wall treatment.

Specific gravity, egg shell weight, egg shell thickness, and percent shell were significantly lower for hens fed 250 ppm yeast cell wall. Egg weight was numerically greater for these same hens. When yeast cell wall was fed at 500 ppm, shell quality was not different from the control treatment.

The overall results suggest significant improvements in egg weight of young laying hens may be obtained as a result of feeding 250 ppm yeast cell wall. Internal egg quality such as Haugh unit and yolk color was generally better when yeast cell wall was fed at 500 ppm versus 250 ppm.

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