THE EFFECT OF A *SACCHAROMYCES CEREVISIAE* FERMENTATION PRODUCT ON EGG PRODUCTION, COMPONENT YIELD AND COMPOSITION IN LAYING HENS

A Thesis

by

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ABSTRACT

Research undertakings have indicated that the hen's diet has an impact on the quality of the eggs she produces. Healthier birds generally result in greater production numbers, which benefits the producer in terms of profit, and the consumer in relation to cost. The use of feed additives, such as probiotics/direct-fed microbials and yeast supplementation, aim to provide a more "natural" way to uphold bird health and performance. The purpose of this study was to examine the effects of a yeast fermentation product on egg quality and laying hen performance. The experimentation was designed to evaluate the effects of a *Saccharomyces cerevisiae* fermentation product on laying hens' egg component yield and composition. The experiment was conducted using standard layer rations supplemented with yeast fermentation product levels ranging from 0.625 kg/metric ton to 1.25 kg/metric ton.

Hen-day egg mass and feed conversion for hens fed the low yeast fermentation product diet were significantly lower than hen-day egg mass and feed conversion for the control group and high yeast fermentation product. Feed consumption was significantly greater for hens fed the high YFP diet. The percentage of jumbo-sized eggs was lowest for the control group hens and highest for the hens fed the diet containing the low amount of yeast fermentation product. Yolk weight was significantly larger for the hens fed the high yeast fermentation product-containing diet. The percentage of albumen yield decreased with increasing amounts of yeast fermentation product, and the

percentage of yolk yield increased with increasing amounts of yeast fermentation product. The percentage of yolk solids was greatest in eggs from hens fed the diet containing high amounts of yeast fermentation product and lowest in eggs from hens fed the diet containing low amounts of yeast fermentation product. The percentage of yolk nitrogen was significantly larger for hens fed the control and low yeast fermentation product diets. The percentage of albumen nitrogen was significantly larger in eggs from hens fed the high yeast fermentation product-containing diet. The eggs from hens fed the control and low yeast fermentation product-containing diets were similar with regard to percentage of albumen nitrogen.

DEDICATION

This thesis is dedicated to God, my family, and my friends. Thank you for your support and encouragement along the way.

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NOMENCLATURE

ANOVA	Analysis of variance
C-S	Corn-soybean meal
CVS	Common vetch seed
DFM	Direct-fed microbials
FCR	Feed conversion ratio
GALT	Gut-associated lymphoid tissue
HD	Hen-day (per live hen per day)
LEP	Liquid egg products
ME	Metabolizable energy
n	Number of observations used
N	Nitrogen
PC	Positive control
SAS	Statistical Analysis System
SBM	Soybean meal
SE	Standard error
SEM	Standard Error Mean
TAMUPRC	Texas A&M University Poultry Research, Teaching and Extension Center
TSAA	Total sulfur amino acids

USFDA	United States Food and Drug Administration
USDA	United States Department of Agriculture
Wt.	Weight
YFP	Yeast fermentation product

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CHAPTER I

INTRODUCTION

Eggs are recognized as both nutritious and functional substances (Jin *et al*., 2014). Eggs are healthy and utilized by many cultures (Tang *et al*., 2015). According to Hasler (2000), eggs contain important nutrients and are a source of carotenoids, which have been linked to reducing the risk of macular degeneration. Eggs are used in many food products, including bread, desserts, and pasta (Jones, 2007). Eggs and egg products, which include eggs in liquid, dried, and frozen forms, offer consumers with benefits such as affordability, convenience, and simplicity. In recent times, the consumption of egg products has increased (American Egg Board, n.d.). As a result, greater attention is given to the quality of the egg, especially in terms of the internal egg contents (Ahn *et al*., 1997).

According to Shafer *et al*. (1998), valued products include the egg's albumen and yolk. Liquid egg products (LEP) are evaluated and marketed by factors such as percentage yolk and albumen solids (Shafer *et al*., 1998). The egg's yolk has a higher market value than the albumen, and contains protein used by the body (Fletcher *et al*., 1981 and Ahn *et al*., 1997). The eggshell is an important structure that protects the internal egg contents and acts as a defense against bacteria and pathogens (Hunton, P., 2005). Egg quality depends on suitability to the consumer and is essential for consumer appeal (Stadelman and Cotterill, 1995 and Anyaegbu *et al*., 2016**).** An increased liquid egg component yield enables processors to produce more liquid mass from the same number of eggs, and sufficient egg shell strength results in less cracked eggs (Shafer *et al*., 1998 and Caner *et al*., 2015).

Laying hen efficiency is influenced by nutrition, according to Figueiredo *et al*. (2012). The hen's distribution of nutrition to the egg can be gauged by the evaluation of egg size and composition (Li *et al*., 2011). The use of probiotics/direct-fed microbials as supplements in animal feeds has been increasing as a result of the aspiration for a more "natural" product (Katoch *et al*., 2013). *Lactobacilli*, *Streptococci*, *Bifidobacterium* and Yeast (*saccharomyces*) are commonly used microbes in the making of probiotics/direct-fed microbials (Katoch *et al*., 2013). Yeasts have a buffering effect in the digestive tract, and proper feed additives in chicken diets can improve the birds' digestive efficiency (Katoch *et al.*, 2013). Improvements in nutrient absorption and intestinal health have been past results of the utilization of *Saccharomyces cerevisiae* products similar to the one used in this study (Kidd *et al*., 2013).

Continuing to improve the understanding of the effects that layer nutrition has on the egg is ideal, considering the increased demand for liquid egg products (Prochaska, 1994). This experiment assessed the effects of a *Saccharomyces cerevisiae* fermentation product (YFP) on egg composition, component yield, quality and laying hen performance and the source of the YFP was Diamond V's Original XPC™.

CHAPTER II

LITERATURE REVIEW

The Importance of Eggs

For many centuries, humans have consumed hen eggs (Tang *et al*., 2015). The egg industry is important: eggs are one of the few foods used throughout the world (Stadelman and Cotterill, 1995). Eggs are nutrient dense: they contain high quality protein, lipids, several important nutrients, including vitamins and minerals, and all nine essential amino acids, which are needed for optimal health (Tang *et al*., 2015; Caner and Muhammed, 2015). According to Hasler (2000), a functional food provides health benefits beyond basic nutrition, and eggs have been classified as nature's original functional food. Eggs provide protein that is of a high biological value, and supply the body's nutrient needs during growth periods (Stadelman and Cotterill, 1995). According to the USDA National Nutrient Database (2015), one large egg provides 6.28 grams of protein. Eggs are also a versatile food (Caner and Muhammed, 2015). According to Jones (2007), eggs are commonly used as an ingredient in many food items, and have the ability to create emulsification, leavening, smoothness, color, and flavor in food products. Consumers are able to choose eggs and liquid, dried, or frozen egg products because they provide benefits such as affordability, convenience, and simplicity.

Liquid Egg Products

In some instances, laying hens are placed solely to produce eggs intended for liquid egg products, with little to none of the hens' eggs sold as table eggs (Ahn *et al*., 1997). Eggs that have been removed from the shell, pasteurized, and packaged in liquid, frozen, or dried forms are referred to as processed egg products (USDA Food Safety and Inspection Service, 2015). According to Ahn *et al*. (1997), the eggs are broken out for liquid whole egg, liquid white, liquid yolk, and dried egg products. Processed egg products are available in grocery stores, and food manufacturing and foodservice companies utilize numerous egg products (USDA Economic Research Service, 2015). According to Shafer *et al*. (1996), the consumption of liquid eggs in hotel, restaurant, and institutional settings is substantial. From 1984 to 2012, consumption of egg products increased from 25.6 million cases to 70.4 million cases of shell eggs broken into egg products (American Egg Board, n.d.). In 2009, thirty percent of the 76.2 billion eggs consumed were in the form of egg products (USDA Food Safety and Inspection Service, 2015). According to Ahn *et al*. (1997), the escalation in liquid egg products has resulted in heightened concern for both the processor and purchaser with regard to the quality of the egg solid contents.

Internal Egg Contents

The egg's interior consists of 65% albumen or white and 35% yolk; the egg albumen is mainly water, and the egg yolk is 32% lipid and 16% protein (Ahn *et al*., 1997). Valued products include the egg's albumen and yolk (Shafer *et al*., 1998). Component yield, including the egg's yolk, albumen, and shell, is a factor taken into account when evaluating liquid egg production (Shafer *et al*., 1998). Liquid egg products are evaluated and marketed by standard parameters such as percentage yolk and albumen solids; percentage, or dried, solids is the nonaqueous component remaining after water removal (Shafer *et al*., 1998). According to Stadelman and Cotterill (1995), egg quality relies largely on the egg's acceptability to the consumer. For consumers, the internal egg quality (albumen and yolk) is very important, and egg quality is key for consumer appeal (Anyaegbu *et al*., 2016**).** Therefore, factors that affect egg component yields and solids content are significant (Fletcher *et al*., 1981).

Another reason liquid egg component yield is important is because an increased yield enables processors to produce more liquid mass from the same number of eggs (Shafer *et al*., 1998). Percent solids is the main mechanism used to evaluate the commercial value of liquid egg albumen and yolk (Prochaska, 1994). Egg yolk is typically sold on a solids content basis or in dried form, and has a higher market value than the albumen (Fletcher *et al*., 1981). In a study by Fletcher *et al*. (1981), it was determined that egg weight, dry shell weight, deformation, and percent yolk increased as the hens aged; percent shell, percent albumen, and percent albumen solids decreased with the hens' age; finally, percent yolk solids did not show a consistent pattern. In another study, Fletcher *et al*. (1983) found that increasing yolk yields was equally dependent on both increasing egg weight and flock age. Moghaddam *et al*. (2012)

established that as the hen ages, egg size generally increases, which can be a reason for reduced eggshell quality because the same amount of shell is being dispersed over a larger sized egg.

With regard to the yolk in particular, the avian egg is the result of biological synthesis, transport, and deposition (Burley *et al*., 1993). The liver of the layer is the site of egg yolk synthesis. Studies have shown that feed additives can have an influence on specific parameters, such as egg yolk cholesterol. Fujiwara *et al*., (2008) recorded that natto supplementation resulted in a decrease of yolk cholesterol for laying hens. In a study by Haddadin *et al*., (1996), yolk cholesterol values also decreased with the use of a *Lactobacillus acidophilus* feed additive. Yalçin *et al*. (2008) found that egg yolk cholesterol significantly decreased with the addition of a yeast culture feed supplement in the diet of Lohmann Brown laying hens. The use of a probiotic supplement caused a significant decrease in laying hen egg cholesterol levels (Mahdavi *et al*., 2005).

Eggshell Quality

The quality of the eggshell is noteworthy. The eggshell consists of around 97% calcium carbonate and provides protection for the contents located inside, whether that be a developing embryo or food contents in the form of albumen and yolk (Hunton, P., 2005). The eggshell defends against bacteria and other pathogens (Hunton, P., 2005). In the case of a fertilized egg, the eggshell allows gas exchange to occur and serves as a support structure for the developing chick (Hunton, P., 2005). The egg intended for food consumption relies on the eggshell to shelter the albumen and yolk inside (Hunton, P., 2005). In both cases of the hatching egg and the food-product egg, the egg's worth is diminished if the shell fails (Hunton, P., 2005). It is ideal that the industry experience a low number of cracked eggs by preserving the egg's shell strength and overall quality (Caner *et al*., 2015).

Hen Nutrition

Feed is one of the largest costs associated with commercial poultry production (Lowman and Ashwell, 2016). According to Kidd *et al*., (2013), further assessment of feed supplements has transpired due to increases in diet costs. There have been many studies performed which conclude that the hen's nutrition does have an effect on the egg that is produced. For a fertile egg, the hen's nutrition has an impact on the chick's immune status, stamina, and size, and the internal contents of the egg supply a developing embryo and newly hatched chick with the nutrients needed for proper growth and development (Kenny, M. and Kemp, C., n.d.). According to Figueiredo *et al*. (2012), the egg yolk, egg albumen, internal egg quality, total solids percentage, and egg size are impacted by the diet's essential amino acid levels. Methionine is the first limiting amino acid and lysine is the second limiting amino acid in poultry diets (Novak et al., 2004). The amount, configuration, and digestibility of amino acids in laying hen diets are fundamental for proper protein use by the bird (Narváez-Solarte, *et al*., 2005). According to Figueiredo *et al*. (2012), improved nutrition and genetics are responsible for increased productivity in laying hens. Avian egg size and composition are qualities that can be assessed to determine the hen's distribution of nutrition to the egg (Li *et al.*, 2011). In terms of hen performance, there is a definite association between hen feed intake and egg quality (Topcu *et al.*, 2014). In a study by Li *et al.* (2011), a significant interaction was found between the hen's dietary intake and line for several parameters, such as percentage yolk, yolk/albumen ratio, and the cholesterol content of the egg yolk. Nutritionally well-balanced feeding and better feed efficiency are components that can ensure economically successful poultry production (Katoch, S., 2013).

The term probiotics is comparatively new, means "for life", and can be defined as a live microbial supplement which positively affects the host by improving the intestinal microbial balance (FAO/WHO, 2006; Heyman and Ménard, 2002). The appropriate balance of microorganisms helps to ensure a well-functioning gastrointestinal tract (Katoch *et al*., 2013). According to a joint report from FAO/WHO (2006), probiotics are significant in immunological and digestive functions, and could have an effect on the improvement of infectious diseases. Factors that have an impact on overall flock performance include the birds' physiological status as well as the probiotic's vitality, livability, dose, and treatment length (Gallazzi *et al*., 2008). Probiotics can also be referred to as direct-fed microbials, which the United States Food and Drug Administration (USFDA) defines as: products that are assumed to contain live microorganisms, such as bacteria and/or yeast (Quigley, J., 2011). Prebiotics, not living organisms, stimulate the growth of gut bacteria and selectively enhance the activity of some groups of beneficial bacteria (Al-Sheraji *et al.*, 2013; Quigley, J., 2011). In terms of the production of poultry, probiotics/direct-fed microbials are growing in prominence as feed additives (Katoch *et al*., 2013).

Feed Supplementation

In reaction to consumers' drive for the use of a more "natural" way to support bird growth, the use of direct-fed microbials and other nontraditional feed additives has been increasing (Katoch *et al*., 2013). A key use for direct-fed microbials is improving livestock health (Waititu *et al*., 2014). Fuller (1989) listed features of good probiotics which includes: benefits the host animal, non-pathogenic, non-toxic, stable and able to survive in the gut environment. The groups of microbes frequently used in the production of probiotics/direct-fed microbials include *Lactobacilli*, *Streptococci*, *Bifidobacterium* and Yeast (*saccharomyces*) (Katoch *et al*., 2013). Yeasts, unicellular fungi known for their fermentative ability, have a buffering effect in the digestive tract (Katoch *et al.*, 2013). Appropriate feed additives in chicken diets can improve the birds' digestive efficiency (Katoch *et al.*, 2013).

Ezema (2013) concluded that probiotic use stimulated digestion and nutrient utilization in poultry, which contributed to increased productive performance. Gallazzi *et al*. (2008), assessed hen performance and egg quality using a probiotic (*Lactobacillus acidophilus*). In the study, Gallazzi *et al*. (2008) found that the use of the probiotic improved feed conversion ratio (FCR), overall egg production, egg specific gravity, and albumen viscosity. Nahashon *et al*. (1994 b) established that egg weight, egg mass, and egg size were considerably improved with direct-fed microbial (*Lactobacillus)* supplementation in a corn-soybean meal (C-S) diet for Single Comb White Leghorn layers. In another study, Nahashon *et al*. (1994 a) found that feeding 1,100 parts per million *Lactobacillus*, as a direct-fed microbial (DFM), motivated the laying hens' appetites, as well as improved egg production, egg mass, egg weight, egg size, and feed conversion. The commercial fermentation products, Vigofac® and Fermacto®, were evaluated by Waldroup *et al.* (1972). In the study by Waldroup *et al.* (1972), Vigofac® in a corn-soy diet maintained a significantly increased rate of hatchability. In a study by Ding *et al*. (2016), a yeast diet supplementation with selenium and vitamin E in partridge parents' diets significantly influenced chick quality, and in increase in eggshell and embryo relative weight was observed. Kidd *et al*. (2013) stated that, "feeding hens yeast fermentation products to affect progeny is an under-researched area". In the study by Kidd *et al*., (2013), it was determined that a *Saccharomyces cerevisiae* fermentation product reduced egg contamination and improved hatchability of fertile eggs. It was also reported that laying hens fed *Saccharomyces cerevisiae* had offspring with better feed conversion ratios and improved breast meat yield (Kidd *et al*., 2013).

In a study by Akbari Moghaddam Kakhki *et al*. (2016), significant improvements were observed for egg production, egg weight, egg mass, Haugh units, and feed conversion ratio in laying hens with increased lysine intake. Egg components, specific gravity, eggshell thickness, and protein components of eggs were not affected by the dietary lysine concentration (Akbari Moghaddam Kakhki *et al*., 2016). In a study by Figueiredo *et al*. (2012), a relationship between eggshell percentage and Haugh units in fresh eggs with the diet's digestible lysine and threonine levels was observed, using Hy-Line W36 laying hens. Figueiredo *et al*. (2012) concluded that the best results for digestible lysine levels in the diet were 0.754%, 0.772%, and 0.795%, respectively. In a study by Narváez-Solarte, *et al.* (2005), the lowest egg production numbers were recorded for hens fed without methionine supplementation. The goal of a study by Topcu *et al*. (2014) was to define the optimal levels of common vetch seed (CVS) required to aid in peak laying hen performance and maximum eggshell quality. CVS (*Vicia Sativa L.*) is a source of dietary protein and energy (Topcu *et al*., 2014). Calcium, an instrumental part of the eggshell, has the ability to affect laying hen performance and eggshell quality (Narváez-Solarte *et al*., 2006). In this study, Narváez-Solarte *et al.* (2006) found that an increase in dietary calcium improved laying hen performance, and determined the dietary calcium requirement for white laying hens to be 3.56% or 4.02 grams of calcium per hen daily.

Research by Gao *et al*. (2008) involving broilers, determined that a supplemental yeast culture improved growth performance, feed conversion and also affected immune functions. Van der Sluis (2007) noted that feed nutritionist, Dr. Roch, observed improvements in growth performance of broilers fed selenium yeast in the diet. In a study by Mohan *et al*. (1996), it was reported that probiotic supplementation in broiler diets improved both weight gain and feed efficiency. Katoch *et al*. (2013), concluded that broiler birds offered a calcium and phosphorus deficient diet overcame this adversity with the help of direct-fed microbials, which have the potential to inhibit the growth of pathogenic microorganisms, maintain beneficial microflora in the gastrointestinal tract, and increase nutrient utilization through improved intestinal enzyme activity and nutrient availability. Direct-fed microbial use to aid in nutrient retention in Ross 308 male broilers was evaluated by Angel *et al*. (2005). In both experiments, Angel *et al*. (2005) concluded that the broilers experienced increased nutrient retention because of the addition of dietary direct-fed microbials. In a study by Waititu *et al*. (2014), it was found that direct-fed microbials caused an anti-inflammatory effect in the ileum of broilers; the direct-fed microbials with a blend of three *Bacillus* strains affected both local and systemic immunity in male broiler chickens. Lei *et al*. (2015), conducted a study to determine if a *Bacillus amyloliquefaciens*-based direct-fed microbial supplement had an effect on broiler chickens. It was concluded that the supplementation of 30 mg/kg and 60 mg/kg of *Bacillus amyloliquefaciens*-based direct-fed microbial in the plant protein-based diet improved the growth performance of broilers, and was associated with positive effects on nutrient utilization, intestinal morphology, and cecal microflora (Lei *et al*., 2015). In a study by Latorre *et al*. (2015), a multiple enzyme-producing *Bacillus*-based direct-fed microbial was tested on broiler chickens fed a rye-based diet in order to determine if growth performance and other factors would be effected. Latorre *et al*. (2015) found that the *Bacillus*-based direct-fed microbial enhanced the feed conversion ratio, as well as bird body weights and bone quality measurements. It was concluded by Latorre *et al*. (2015) that the intestinal integrity of the birds and their ability to absorb nutrients improved, causing an increase in the production performance.

The Gut's Role in Health and Performance

The gut serves as the interface between the metabolic events which sustain life and the diet (Salminen, *et al*., 1998). A large immune organ, gut-associated lymphoid tissue (GALT), is located in the digestive tract. The immune system's principal function is to locate and remove pathogens (Waititu *et al*., 2014). An imbalance in intestinal microflora often accompanies a lowering of the body's defense mechanisms (Hosseini *et al*., 2006). The proper balance of microorganisms is an important feature of a well-functioning gastrointestinal tract (Katoch *et al*. (2013). The addition of microorganisms that contribute to the appropriate microbial balance of the intestinal microflora can be an advantage to the host (Haddadin *et al*., 1996). Fermentation supplementation in the feed improves nutrient deficiencies and overall performance by adjusting the intestinal microflora (Grimes, *et al.* 1997). According to Salminen *et al*. (1998), "one of the most promising areas for the development of functional foods lies in modification of the activity of the gastrointestinal tract by use of probiotics, prebiotics and synbiotics."

The objective of this study was to evaluate the effects of a *Saccharomyces cerevisiae* fermentation product (YFP) on egg quality and laying hen performance. The source of the YFP was Diamond V's Original XPC^{TM}. Supplemental information about Diamond V's Original XPC^{TM} product can be found in the Appendix section.

CHAPTER III

MATERIALS AND METHODS

Study Design and Animal Information

Three hundred one-day-old Hy-Line W36 pullets were obtained from a local commercial source and transported to the Texas A&M University Poultry Research, Teaching and Extension Center (TAMUPRC). At fifteen weeks of age, the pullets were randomly assigned to groups of three consecutive cages (seventeen groups per treatment) and assigned to one of three treatments: diets with no YFP (Control) and YFP at either 0.625 kg/metric ton YFP (Low YFP) or 1.25 kg/metric ton (High YFP). The experimental diets were fed for twelve weeks (18 – 30 weeks of age) prior to the initiation of egg analyses and thereafter throughout the experiment. The twelve week period was used to assure that the impact of the treatments on the egg yolks would be fully incorporated into the samples utilized for further analysis.

All diets were formulated to meet breeder recommendations. Feed and water were provided *ad libitum*. Hens were housed in individual cages in a tunnel ventilated laying facility with groups of three hens sharing access to a common feed trough, and 51 hens per treatment group were utilized. At thirty weeks of age, a twenty week experiment evaluating egg parameters was conducted.

The hens were individually weighed at the initiation of the study (15 weeks of age) and after termination of the experiment (62 weeks of age). Hen-day egg production, feed consumption, egg mass, feed efficiency (g egg/ g feed), eggshell thickness, eggshell breaking strength, specific gravity (Archimedes Principle), and Haugh units were calculated every 28 days. Average egg production, feed consumption, and feed conversion were also calculated overall (18-53 weeks of age) for each group.

From 31 – 53 weeks of age, eggs were sampled weekly (twenty sample days). For each experimental unit, average egg weight, egg component weight, egg component yield, egg component solids, and egg component nitrogen was determined. All eggs were manually separated using a plastic egg separator. The yolk was tamped with a damp paper towel, to remove chalaza or adhering albumen, and then weighed. To calculate albumen yield, the weight of the yolk and shell with shell membranes intact was subtracted from the whole egg weight. Egg shells were allowed to air dry at room temperature for a minimum of 24 hours prior to shell thickness measurement. Measurement of shell thickness with membranes was conducted weekly, by averaging three thickness measurements of samples collected from the equator of the egg using an AMES thickness gauge (B.C. AMES Co, Waltham, Mass.)

Liquid albumen and yolk were pooled by treatment. Pooled liquid albumen and yolk were homogenized using an upright, hand-held household blender for eight, five-second pulses to reduce froth. Solids were determined by drying fifty aliquots of yolk (5mL) and albumen (10 mL) per treatment on each sampling day at approximately 100°C for 24 hours. After drying, samples were taken from each dried yolk (~85 mg, 24/treatment) and albumen (~35 mg, 12/treatment) aliquot for determination of protein content using an Elementar Nitrogen analyzer.

Housing

The chickens were housed at the TAMUPRC, as described above. The birds were provided with an *ad libitum* supply of feed and water. A continuous feed trough was located at the front of the cages and there was one nipple drinker per two cages. The temperature was controlled by supplemental furnaces and evaporative cooling pads, as appropriate; target temperature was between 65 and 80^{o}F. The lighting program under which the pullets were raised was sustained and the maximum photoperiod during lay was 15.5 hours.

Experimental Diets and Treatments

Diets. All diets contained a corn/soybean meal (SBM) base (Table 1). Complete diets were manufactured at the TAMUPRC feed mill. Feed was stored in plastic-lined thirty gallon barrels labeled with the date, diet, and treatment code. A 3x batch of the control diet was mixed and split into three equal parts. The control feed was placed in barrels. The treated feed was returned to the mixer and an appropriate amount of YFP was added and allowed to mix for ten minutes, prior to storage in barrels. Composite feed samples from each feed mixing were collected and stored at -20^{o}C until submitted for proximate analysis. Feed added and feed remaining during each 28-day period was monitored.

Management

Disease Control. No concomitant drug therapy was used during the study.

Monitoring. All birds were monitored twice daily for general flock condition, temperature, lighting, water, feed, and unanticipated house conditions/events. Findings were documented on pen sheets or room temperature log sheets. The Sponsor was notified of any abnormal conditions or behavior that may affect the study.

Morbidity and Mortality. Mortality was monitored on a daily basis. Birds will only be culled to relieve suffering. Injured or crippled birds were euthanized by approved euthanasia techniques.

Table 1. Control diet formulation and nutrient composition (15 to 53 weeks of age).

Ingredients (%)	PC fed from 15 weeks of age to first egg	PC fed from first egg to post peak 90%	PC fed below 90%
Corn	68.35	61.98	64.60
Soybean meal	22.85	21.84	20.00
Blended fat	0.55	3.40	2.30
Limestone	5.63	10.10	10.54
Dicalcium phosphate	1.78	1.82	1.73
NaCl	0.36	0.36	0.35
DL-Methionine	0.17	0.18	0.16
L-Lysine	0.003	0.02	0.02
Vitamin/mineral premix[1]	0.30	0.30	0.30
Nutrient composition			
ME (kcal/kg)	2,900	2,900	2,850
Crude Protein (%)	17.00	18.00	16.0
Methionine (%)	0.44	0.48	0.40
TSAA (%)	0.74	0.80	0.71
Lysine (%)	0.85	1.0	0.82
Calcium (%)	2.5	4.5	4.5
Total Phosphorus (%)	0.71	0.70	0.67
Nonphytate Phosphorus (%)	0.48	0.48	0.46

[1] Trace mineral premix added at this rate yields 149.6 mg manganese, 55.0 mg zinc, 26.4 mg iron, 4.4 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil. Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

Bird and Feed Disposition. All birds will be euthanized at the end of the study. Carcasses will be disposed of by incineration at TAMUPRC. All remaining feed will also be incinerated. Records of disposition will be included in the source data.

Data Analysis

All data were subjected to ANOVA utilizing the General Linear Models procedure of SAS (1990) statistical analysis software program, Version 6.04, with the main effects being diet, date, and replication. Mean differences were separated via the PDIFF option, which uses pairwise *t* tests, of the General Linear Model option. Least squares means and SE were determined.

CHAPTER IV

RESULTS AND DISCUSSION

In terms of production parameters, hen-day egg production was not significantly affected by YFP (Table 2). Haddadin *et al.* (1996) observed significantly higher levels of egg production for hens given feed with *Lactobacillus acidophilus* supplementation. Correspondingly, Harms and Miles, (1988) determined that the addition of Fermacto® in laying hen feed significantly increased egg production. A study by Mahdavi *et al*. (2005) showed that the *Bacillus*-based feed supplement tested did not significantly influence egg production for Hy-Line W-36 laying hens. Likewise, Hosseini *et al.*, (2006) concluded that *Saccharomyces cerevisiae* did not have a significant impact on layer egg production. Fujiwara *et al*. (2008) reported that the addition of the fermented soybean product, natto, supplemented in layer diets did not show improvements in egg production. Dizaji and Pirmohammadi (2009) reported that a *Saccharomyces cerevisiae* and *Bacillus* probiotic did not significantly affect laying hen egg production. Grimes *et al.*, (1997) also found that egg production in laying hens was not affected by the fermentation product Fermacto®. Clearly there are a number of factors that affect the impact of these feed additives on egg production. Number of eggs per hen was not significantly affected by YFP (Table 2). Waldroup *et al.* (1972) reported that Fermacto® significantly increased number of eggs per hen when used in a fish meal diet though Vigofac did not have a significant effect on number of eggs per hen. Hen-day egg mass was significantly affected by YFP (Table 2). Hen-day egg mass for hens fed the diet containing low yeast fermentation product was less than hen-day egg mass for hens given the control and high yeast fermentation diet. Harms and Miles, (1988) reported a significant increase in laying hen egg mass as a result of feeding Fermacto®. Hosseini *et al.*, (2006) determined that yeast supplementation increased egg mass, though not significantly. Research by Mahdavi *et al*. (2005) found that the *Bacillus* feed supplement used did not significantly affect egg mass. Dizaji and Pirmohammadi (2009) recorded that the use of a probiotic did not have a significant effect on egg mass for laying hens. Feed consumption for hens fed the high YFP diet was significantly greater than that of hens fed the control diet. Low YFP fed hens were not significantly different from either other treatment (Table 2). Grimes, *et al.* (1997) as well as Harms and Miles, (1988) also observed an increase in feed consumption as a result of a fermentation feed supplement. In a study by Hosseini *et al.*, (2006), laying hen feed intake was not impacted by yeast supplementation in the diet. Mahdavi *et al*. (2005) concluded that the probiotic feed supplement tested did not significantly affect laying hen feed consumption. Dizaji and Pirmohammadi (2009) reported that probiotic use did not have a significant effect on laying hen daily feed consumption. Feed conversion was significantly lower for hens fed the low YFP diet compared to hens fed control feed and the high YFP (Table 2). Grimes, *et al.* (1997) found that the inclusion of a fermentation product in the diet enhanced feed conversion for both young and old layer hens. Likewise, research by Haddadin *et al*. (2006) concluded that *Lactobacillus acidophilus* improved feed conversion in laying hens. In a study by Mahdavi *et al*. (2005), it was determined that a *Bacillus*-based probiotic feed supplement did not significantly alter feed conversion in Hy-Line layers. Fujiwara *et al*. (2008) reported that fermented product natto did not improve the feed conversion ratio for laying hens. Dizaji and Pirmohammadi (2009) recorded that probiotic use significantly increased feed conversion for laying hens. There were no significant differences among the treatments for body weight at 15 or 62 weeks of age or body weight gain (Table 2). Grimes, *et al.* (1997) reported increased body weights in hens fed with a fermentation supplement, and Yalçin *et al*. (2008) concluded that a yeast culture supplement in the feed increased laying hen body weight gain. Dizaji and Pirmohammadi (2009) reported that the use of probiotics did not significantly affect laying hen body weights. In a study by Waldroup *et al*. (1972), it was determined that hen body weight was not significantly influenced by Vigofac®. There is a general lack of consistent impacts of these feed additives on laying hen productive performance. A wide variety of factors including diet, strain, environment and age, among others all may influence laying hen performance and thus the impact of a feed additive.

Table 2. The influence of YFP treatments on production parameters (18 – 53 weeks of age).

	Control[1]	Low YFP	High YFP	SEM	n
Production Parameters					
HD Egg Production (%)	85.79	84.54	85.38	0.377	5820
Eggs/hen	214.1	213.7	213.6	2.01	161
HD Egg Mass (g/HD)	50.8^{A}	48.5^{B}	50.6^{A}	0.47	484
Feed Consumption (g/HD)	100.7^{b}	101.4^{ab}	102.7^{a}	0.53	486
Feed Conversion (egg:feed)	0.498^{A}	0.472^{B}	0.485^{B}	0.005	484
Body Wt. at 15 Weeks of Age (g)	1040	1050	1040	9.34	162
Body Wt. at 62 Weeks of Age (g)	1710	1690	1720	33.91	160
Body Wt. Gain (g)	660	630	680	30.59	160

[abc] Means within a row lacking a common superscript differ significantly ($P < 0.05$).
[A,B,C] Means within a row lacking a common superscript differ significantly ($P < 0.01$).
[1] Diets with no YFP (Control) and YFP at either 0.625 kg/metric ton YFP (Low YFP) or 1.25 kg/metric ton (High YFP).

Eggshell thickness was not significantly impacted by the treatments (Table 3). Mahdavi *et al*. (2005) similarly found that the inclusion of a *Bacillus*-based probiotic supplement did not have a significant effect on eggshell thickness. Fujiwara *et al*. (2008) recorded no improvement in eggshell thickness for laying hens fed diets with natto supplementation. Hosseini *et al.* (2006) also determined yeast supplementation to have no significant effect on eggshell thickness. Eggshell breaking strength was not significantly influenced by YFP in the diet (Table 3). Grimes *et al.* (1997) reported that eggshell strength was not affected by Fermacto® supplementation in laying hen diets. Hosseini *et al.* (2006) found that yeast supplementation did not impact eggshell breaking strength. Fujiwara *et al*. (2008) reported that the fermentation product natto did not improve eggshell strength. Egg specific gravity was consistent across all three treatments (Table 3). Hosseini *et al.* (2006) observed no positive effects on egg specific gravity as a result of the supplementation of *Saccharomyces cerevisiae*. There were no significant differences recorded for Haugh units with relation to the treatment groups (Table 3). In the same way, Mahdavi *et al*. (2005) did not find significance in Haugh units related to the probiotic feed additive tested. Fujiwara *et al*. (2008) recorded no improvements in Haugh units as a result of feeding laying hens the fermented product natto. A study by Hosseini *et al.*, (2006) concluded that the use of *Saccharomyces cerevisiae* was not effective on Haugh units.

For USDA Egg Size Distribution, as determined by egg weight, the percentage of jumbo-sized eggs was significantly influenced by YFP (Table 3). The percentage of jumbo-sized eggs was least for hens fed diets without YFP (Control) and greatest for hens fed the diet containing the Low YFP. A study by Waldroup *et al.* (1972) recognized that a fermentation feed additive significantly increased egg size when used in a corn-soy diet. Harms and Miles (1988) established that egg weight was significantly increased by the utilization of a fermented feed additive. The percentages of extra-large, large and medium eggs were not significantly influenced by the different treatments (Table 3). In a study by Grimes *et al.* (1997), it was determined that the fermentation product utilized increased the percentage of extra-large eggs laid by young hens. The fact that Jumbo sized eggs was influenced by the low YFP treatment may have been influenced by the number of double-yolked eggs produced by these hens, however these data were not collected.

Table 3. The influence of YFP on egg quality and USDA egg size distribution (31 – 53 weeks of age).

	Control[1]	Low YFP	High YFP	SEM	N
Egg Quality					
Eggshell Thickness (mm)	40.0	40.3	40.2	0.29	270
Eggshell Breaking Strength (g)	3562	3488	3564	82.97	269
Egg Specific Gravity	1.079	1.079	1.079	0.00071	270
Haugh Units	85.3	84.3	85.3	1.12	267
USDA Egg Size Distribution					
Jumbo (%)	1.9^{b}	5.9^{a}	3.6^{ab}	1.01	270
Extra Large (%)	31.0	29.6	36.6	2.74	270
Large (%)	61.7	57.1	52.4	3.03	270
Medium (%)	5.5	7.4	7.2	1.42	270

[abc] Means within a row lacking a common superscript differ significantly ($P < 0.05$).
[1] Diets with no YFP (Control) and YFP at either 0.625 kg/metric ton YFP (Low YFP) or 1.25 kg/metric ton (High YFP).

For egg components, whole egg weight showed no significance in this experiment (Table 4). In a study by Yalçin *et al*. (2008), increases in egg weights were noted as a result of yeast culture supplementation. (Hosseini *et al.* (2006) determined that the *Saccharomyces cerevisiae* yeast product tested did not affect average egg weight in commercial laying hens. Research by Mahdavi *et al*. (2005) recorded that the Bacillus-based probiotic did not significantly affect egg weight. Similarly, a study by Grimes *et al.* (1997) concluded that Fermacto® used in the diet did not affect egg weight. Waldroup *et al*., (1972) reported that Vigofac® did not significantly influence laying hen egg weight. Fujiwara *et al*. (2008) found that fermented natto did not improve egg weight in layers. Dizaji and Pirmohammadi (2009) recorded that the use of probiotics significantly decreased egg weights for laying hens. Albumen weight did not exhibit significance relative to YFP supplementation; yolk weight however was significantly influenced by diet (Table 4). The values recorded for yolk weight were larger for the hens fed the high yeast fermentation product-containing diet than those on the control and low yeast fermentation product diets (Table 4). Fujiwara *et al*. (2008) reported that the fermented soybean product natto did not improve yolk weights in laying hen eggs. Shell weight values increased numerically as the amount of yeast fermentation product in the laying hen diet increased (Table 4). Grimes, *et al.* (1997) concluded that Fermacto® did not affect shell weight values for laying hen eggs. There was a significant impact on both percentage of albumen and yolk by YFP (Table 4). The percentage of albumen yield decreased with increasing amounts of yeast fermentation product, whereas the percentage of yolk yield increased with increasing amounts of yeast fermentation product (Table 4). In this study, the percentage of shell yield was not significant, but exhibited a numerical increase with increasing amounts of YFP (Table 4). This is consistent with similar trends in eggshell thickness (Table 3) and eggshell weight.

For egg composition, YFP had a significant effect on yolk solids (Table 4). The percentage of yolk solids was greatest in eggs from hens fed the diet containing high amounts of yeast fermentation product and lowest in eggs from hens fed the diet containing low amounts of yeast fermentation product. In this experiment, the percentage of yolk nitrogen was significantly larger for hens fed the control and low yeast fermentation product diets (Table 4). The percentage of albumen solids recorded was equivalent for all three treatments (Table 4). The results of this study showed that the percentage of albumen nitrogen was significantly larger in eggs from hens fed the high yeast fermentation product-containing diet (Table 4). The eggs from hens fed the control and low yeast fermentation product-containing diets were similar with regard to percentage of albumen nitrogen.

Table 4. The influence of YFP on egg components and composition (31 – 53 weeks of age).

	Control[1]	Low YFP	High YFP	SEM	n
Egg Components					
Egg Wt. (g)	61.9	61.6	62.6	0.35	2729
Albumen Wt. (g)	39.8	39.5	40.2	0.343	2711
Yolk Wt. (g)	16.0 ^{B}	16.1 ^{B}	16.3 ^{A}	0.041	2712
Shell Wt. (g)	6.07	6.09	6.14	0.043	2730
Albumen yield (%)	64.3 ^{A}	64.0 ^{B}	63.8 ^{C}	0.087	2711
Yolk yield (%)	25.9 ^{C}	26.1 ^{B}	26.3 ^{A}	0.053	2712
Shell yield (%)	9.83	9.89	9.92	0.066	2729
Egg Composition					
Yolk solids (%)	51.5 ^{B}	51.4 ^{C}	51.6 ^{A}	0.012	2851
Yolk N (%)	5.36 ^{A}	5.34 ^{A}	5.27 ^{B}	0.011	1284
Albumen solids (%)	12.4	12.4	12.4	0.008	2681
Albumen N (%)	14.01 ^{B}	14.02 ^{B}	14.08 ^{A}	0.013	640

^{A,B,C} Means within a row lacking a common superscript differ significantly ($P < 0.01$).
[1] Diets with no YFP (Control) and YFP at either 0.625 kg/metric ton YFP (Low YFP) or 1.25 kg/metric ton (High YFP).

Much can be gained from relating each measured parameter to one another. Hen-day egg mass and feed conversion was higher for the control and high YFP diet group. Yolk weight, albumen weight, percentage yolk yield, yolk solids, shell weight, shell yield, and egg weights were all highest for hens fed the high YFP diet. There were consistent trends in eggshell parameters that favored the inclusion of YFP in the diet. It can be surmised that the high YFP diet affected overall egg size, specifically in relation to the yolk. Yolk weight was increased in the high YFP hens potentially contributing to the numerical increase in egg weight and the significant increase in hen day egg mass for this treatment. Percentage albumen yield was highest for hens on the control diet, without YFP supplementation. There was increase recorded for percentage albumen nitrogen with the YFP supplement, while a decrease was noted for yolk nitrogen, which demonstrates that YFP influences egg nitrogen content.

CHAPTER VI

CONCLUSION

The consumption of eggs and liquid egg products is abundant; these commodities are healthy and generally reasonably priced. Feed supplementation as a mechanism to influence animal health and performance is being researched. Improvements in the areas of animal health and performance result in success for both the consumer and producer. Variability exists in previous research studies evaluating the efficacy of feed supplementation. Feed additives have the potential to help the host achieve optimum health and peak performance.

An increase in egg mass and size as a result of a feed supplement allows producers to produce greater amounts of liquid egg products. Enhancements in egg yolk weight and yield would also result in greater production. These modifications in egg component weights and yield can be important for food processors that use egg products with specific qualities. Egg albumen-yolk ratios have an impact on food products. An alteration in nitrogen percentages of the egg components affects the egg's protein content. The protein content of an egg is important for the consumer that relies on the egg for nutrition. Protein content also influences functional properties of the egg such as gelation and foaming. Improvements in hen feed conversion ratio provides economic benefits to the producers. Qualities that benefit producers economically also enable the product to be sold to consumers for a lower price.

There are many factors which should be considered when assessing the effectiveness of a feed supplement. Bird challenges and flock health can influence a product's effectiveness. The type of feed supplement and its features, such as strain and stability, should also be taken into account. Another aspect that can influence the outcome of a study includes the amount of supplement utilized and the duration of the study. Biosecurity and best management practices are significant for overall flock health and performance.

Future research ideas are centered on the formation of the egg yolk; including analysis of the laying hen liver, as this is the location of yolk synthesis. Information gathered from the collection of layer blood samples may provide beneficial data. Further investigation involving the internal egg contents, specifically yolk lipids, may be helpful in determining the effects of feed additives, such as YFP. Exploration of the hen's digestive system has also been considered. Continued research regarding the effects of feed supplements on poultry health and performance could be advantageous.

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APPENDIX

XPC™ by Diamond V

Diamond V's Original XPC™ is an all-natural fermentation product that contains bioactive components produced using proprietary anaerobic fermentation technology of *Saccharomyces cerevisiae*; it is composed of metabolites, beta-glucans, and mannans that promote animal health and performance (Diamond V Original XPC™, n.d. b; Diamond V Original XPC™, n.d. a). The metabolites in Original XPC ™ help to balance the gut microbiota in order to support optimum digestive health, and the antioxidant activity of XPC ™ ads immune function (Diamond V Original XPC™, n.d. a).

Research has shown that Original XPC ™ supports production performance for all poultry, specifying in egg production, quality and efficiency for layers. (Diamond V Original XPC™, n.d. b) According to (Diamond V Original XPC™, n.d. a), XPC™ is used in all classes of livestock, poultry, equine and pet diets. The recommended feeding rates of Original XPC ™ XPC for layer or breeder feeds are 3.0 lb/ton (pullet starter), 2.0 lb/ton (pullet grower) and 1.5 lb/ton (layer cycle/breeder) (Diamond V Original XPC™, n.d. a) The improved gastrointestinal health because of products such as XPC™ is apparent in enhanced bird performance, such as weight gain, egg production, hatchability, fertility, and feed efficiency (Diamond V Original XPC™, n.d. b).