# EVALUATION OF PERFORMANCE OF HY-LINE BROWN LAYING HENS FED SOYBEAN AND SOYBEAN-FREE DIETS USING CAGE AND FREE-RANGE REARING SYSTEMS

A Dissertation

by

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## ABSTRACT

This study was conducted to evaluate the performance of Hy-line Brown laying hens reared in caged or free-range facilities and fed two different diets: soybean meal (SBM) or soybean meal free (SBMF) with cottonseed meal (CSM) and distillers dried grains with solubles (DDGS). The objectives were to: 1) evaluate laying hen early production performance and egg quality for hens fed both SBM or SBMF diets, using either caged or free-range rearing systems; 2) evaluate overall consumer preference regarding egg flavor, texture, odor, and color based on samples of scrambled and hard cooked eggs from both diets and systems; 3) evaluate the influence of yeast cell wall (YCW) on post peak performance of caged Hy-line Brown layers on egg production, egg quality, and ileum digestibility; and 4) evaluate the influence of YCW based on gene expression profile of metabolic pathways in caged hens.

Results from the first experiment indicated that free-range production (87.97± 2.52%) is more variable than the traditional cage system (92.40 ± 1.63%), and a SBMF diet can be used in both caged and free-range production systems without negative impact other than significantly lower egg weight (SBM 59.85 ± 0.59 versus SBMF 56.48 ± 0.60 g). From the consumers' perspective, flavor did not differ, but texture preference was higher for scrambled eggs from the SBMF diet versus scrambled eggs from the SBM diet. For hard cooked eggs, the consumer panel preferred the flavor of eggs from the caged rearing system versus eggs from the free-range system, and consumers liked the texture of eggs collected from hens fed SBM (6.91 ± 1.85) versus eggs hens fed SBMF (6.30 ± 2.01).

Data related to the use of YCW in the diet indicated that egg production was greatest for hens fed the SBM diet with 250 ppm YCW (93.6 ± 0.6%), and egg weight was greater for the SBM (63.5 ± 0.3 g) versus SBMF (61.2 ± 0.2 g) diet. Yeast cell wall supplementation improved apparent ileal amino acid digestibility of lysine in the SBM diet (83.9 ± 3.8 versus 71.2 ± 8.9%) but not the SBMF diet. Prebiotic YCW increased the expression of the liver tissue BAK gene for both the SBM and SBMF diets. With respect to splenic tissue, the combination of YCW with the SBMF diet increased the POR gene over 6 log fold. In the presence of YCW, the SBMF diet upregulated ($P<0.01$) CYP3A4 and MCL1 genes in the liver and BIK and POR genes in the spleen. The CYP1A2 gene was downregulated over 9 log fold in the liver.

## DEDICATION

I dedicate this dissertation to all the people that I love…

To my mom, Dr. Najwa Al-Nakash, for all the finances, faith, advice, and love she gave me through this difficult time in which I have had to obtain the knowledge I need in order to be a productive person to serve the community;

To my brothers, Mr. Muhsin Al-Ajeeli and Dr. Maytham Al-Ajeeli for their prayers and support;

To my children Reem and Rami for their love and patience while I pursue this degree.

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## NOMENCLATURE

| | |
|---|---|
| AH | Albumin height |
| BS | Breaking strength |
| CS | Cage soy |
| CSF | Cage soy-free |
| CGM | Corn gluten meal |
| CSM | Cottonseed meal |
| DDGS | Distillers dried grains with solubles |
| EP | Egg production |
| EWT | Egg weight |
| FCR | Feed conversion ratio |
| FDE | Feed per dozen eggs |
| HD% | Hen day production |
| HU | Haugh unit |
| SBM | Soybean meal |
| SBMF | Soybean meal-free |
| ST | Shell thickness |
| MOS | Mannan-oligosaccharide |
| YCW | Yeast cell wall |

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# CHAPTER I

# INTRODUCTION AND LITERATURE REVIEW

## INTRODUCTION

The poultry industry contributes significantly to economic activity in many countries, and the conditions under which poultry are produced greatly affects the amount of this economic impact. Recently, there has been widespread debate regarding how the increased use of cage-free facilities would affect this economic activity with respect to food safety, increased disease, higher flock mortality, and increased cannibalism and injuries as birds are housed together rather than in separate cages (Lay, et al., 2011).

Egg buyers have become increasingly vocal with respect to human health and the environment, as well as animal welfare. In addition, consumer demand for organic, free-range, and speciality eggs (such as Omega-3 or vitamin-enriched) also has increased (Loke, et al., 2016). The proliferation of the internet and mass media outlets have led to increased buyer awareness of the effects of soy, gluten, and other ingredients, as well as the "organic" origins of the foods they consume.

Approximately 94% of laying hens in the United States are housed in raised wire caged (Vizzier, et al., 2016). In the United States, hen housing has become one of the questionable chicken welfare topics, especially after California established new animal welfare laws on January 1, 2015, Proposition 2 (Standards for Confining Farm Animals). This regulation requires more space for laying hens which would result reduction in number of birds per area unit and thus increase production cost.

As early as the 1960s, European leaders addressed animal welfare, including the movement of laying hens within cages, and how this type of rearing system impacts certain behaviors (Dikmen, et al., 2016). Maintaining hen health in cage-free rearing systems can be a significant challenge, as these systems may increase the prevalence of several pathogens that affect birds and eggs. Chickens that have access to the outdoors are exposed to different microorganisms that may make them more susceptible to transmitting diseases such as salmonellosis (AVMA, 2012). The risk of manure contamination, which affects egg quality and safety, also is higher in these systems (Dailey, et al., 2016).

Feed and water systems also affect the economic impact of the poultry industry, since a majority of the cost of poultry production is associated with feed ingredients. Diet is an important aspect of poultry health, and feed must meet all nutritional requirements, including essential amino acids, vitamins, and trace minerals. The typical U.S. poultry diet consists of cereal grains, SBM, fat, animal by-products, vitamins, and minerals (NRC, 1994). The most common ingredients in the United States are corn for energy and SBM for protein. Properly processed SBM has the best nutrient profile of all the common oi-seed meals and dominates the animal feed industry as a source of vegetable protein. However, some consumers have become concerned with SBM as most of the soybeans in the United States are genetically modified GMO (Lappé, et al., 1998). Niche markets have grown in recent years for cage-free, soy-free, organic eggs, Omega-3 eggs with low cholesterol that are gluten-free, and so on. These concerns have pushed some producers to find alternatives ingredients to be used in the poultry feed that do not rely on SBM as a primary source of protein.

In addition to issues previously mentioned, perhaps the biggest issue is the use of antibiotics in feed which has produced consumer demand for antibiotic free production, and alternatives such as probiotics (use of living organisms), and prebiotics. The use of yeast cell wall (YCW) products as a prebiotic in poultry feed is one example. These yeast products are recognized as safe replacements for in-feed antibiotics (M'Sadeq, et al., 2015). Prebiotic YCW products have been demonstrated to increased egg weight and liquid egg yield in early production laying hens, especially when fed at 250 ppm (Hashim, et al., 2013).

The goals of this project are to evaluate alternatives for SBM as a source of protein in laying hen feed while having no or minimal impact on egg production and quality parameters. The objectives of this research are the following: 1) evaluate phase one or peak laying hen performance of Hy-line Brown layers fed SBM and soybean-meal-free SBMF diets, subjected to caged and free-range rearing systems based on hen performance, and egg quality parameters including albumen height, eggshell thickness, egg breaking strength, and Haugh unit; 2) conduct research to evaluate overall consumer favorability regarding egg flavor, texture, odor, and color, based on samples of scrambled and hard cooked eggs; 3) evaluate the influence of YCW prebiotic supplementation in both SBM and SBMF diets for caged Hy-line Brown laying hens during the latter half of their egg production cycle based on egg production, egg quality characteristics, and ileum amino acid digestibility; and 4) evaluate selected liver and spleen tissue gene expression profiles of these late production hens. For this dissertation we are defining free-range egg production as those eggs produced from cage-free hens with voluntary access to the

outside ambient environment from a sheltered area containing feeders, drinkers and nesting boxes.

## LITERATURE REVIEW

### Housing Considerations for Laying Hens in the United States

In the United States, one of the most influential regional regulations related to layers' housing is the Standards for Confining Farm Animals, passed in California in 2008, which effectively restricted or altered the use of conventional cage systems in California beginning in early 2015 (Vizzier et al., 2016). Similar legislation that either modified or limited the use of this type of cage also has been enacted in other states, including Michigan, Ohio, Oregon, and Washington (Vizzier et al., 2016), and very recently in November, 2016, in Massachusetts, as well. In the last 20 years, much research has been conducted to improve methods of optimizing hen health and welfare, even though this type of research is difficult, and many issues still need to be addressed (Lay, et al., 2011).

Since California announced the adoption of the Standards for Confining Farm Animals legislation, effective January 1, 2015, animal rights activists have been vocal in criticizing and publicizing cage systems that restrict the natural behavior of the hens, such as perching, nesting, and dust-bathing. Major food retailers and food service outlets have announced their pledges to sell or serve only eggs that are cage-free by 2025-2030. In order to meet these commitments and provide sufficient supply, egg producers must take significant action to abandon most (or perhaps all) of the conventional cage systems in use today. This somewhat drastic measure would lead to lower egg production per square feet

of production facility area and likely increase production cost. In addition to increased cost associated with housing feed cost will likely remain the single most expensive aspect of egg production. A properly balanced diet is the most important factor for egg production (nutrition = eggs) as well as body maintenance requirements for hens, so the diet must contain nutritional values that promote all aspects of bird health, including all energy requirements, protein (essential amino acids), vitamins, and trace minerals. Lighting also plays an important role in the life cycle of a laying hen, and has a direct impact on total profitability, including egg production and egg size.

Researchers have found that lighting intensity can impact bird development (Ozkan, et al., 2012). For decades, extensive research has assessed the effects of light intensity on different aspects of poultry production, including physiology, behavior, and welfare for broilers, laying hens, and turkeys (Manser, 1996).

Council directive 1999/74/EC for laying hens (CEU, 1999) requires all poultry buildings to have a lighting system that is sufficient to allow the birds to see clearly and engage at normal activity levels. After the first day of conditioning, the light regime also should be available to prevent any health or behavioral problems (Kristensen, 2008). It has been reported that hens are able to sustain production in light intensity above 5 lux, a level that also allows them to jump between perches (Kristensen, 2008). Natural light can be either direct sunlight or diffuse light reflected off clouds or other surfaces (Prescott, et al., 2003).

Artificial lighting programs are a critical aspect of poultry management (Blatchford, et al., 2009). In poultry houses, light programs are usually quite dim: 5 to 30 lux for broiler and hen houses, and 1 to 5 lux for turkey houses. These low illuminances

control cannibalism and pecking damage, as well as reduce energy costs (Prescott, et al., 2003). Several studies have examined general environmental conditions, such as temperature and humidity. The research to date on the effect of light programs on animal welfare, however, is limited (Archer, et al., 2009).

Poultry experience several types of stress (Mitchell and Kettlewell, 2009) such as heat stress due to thermal challenges such as hot weather throughout the growing period. Humidity also impacts bird health. Birds eat less as the weather gets hotter, so changing the diet composition can be an option to enhance bird tolerance for heat stress. The most common current rearing system for laying hens is a cage system where the environmental temperature is controlled with, power ventilation, a specific light program and mechanical feeding (Horne and Achterbosch, 2008). As the industry moves toward cage-free facilities, particularly those free-range systems with access to the outdoors complete control of the environment becomes more problematic with respect to minimal impact on egg production, egg quality parameters, and general health behavior.

Animal welfare regulations have been expanded in recent years to stipulate innate behavior while focusing on keeping the birds safe, comfortable, well nourished, and perhaps most importantly, disease-free. Many food service organizations and businesses have announced their commitment to cage-free rearing systems. In addition, animal welfare guidelines in the United States have increased as consumer awareness of animal health, especially with poultry, has grown. Each laying hen housing system has different considerations with regard to human health and safety for its employees and people living close to operation facilities, but to date, research on these topics has been scarce (Mench, et al., 2011).

Laying hen housing systems play a significant role in egg production overall, and recently, several types have been developed to meet consumer demand while maintaining high egg production. Each has several advantages and disadvantages with respect to bird welfare and egg production. This literature review explores the science behind these newer rearing facilities and educates consumers about the different housing characteristics that affect egg production while also providing safe and cost-effective conditions that promote bird welfare.

**Conventional Cage System**

The conventional cage system used in the United States today was developed in the 1930s (Dikmen, et al., 2016) and still represents approximately 94% of total laying hen housing facilities (Vizzier, et al., 2016). The modern cage production system consists of multiple tiers in environmentally controlled poultry houses (Samiullah, et al., 2016). This system began to be phased out in Europe beginning in 2012 and replaced with a variety of modified cage or non-cage facilities so that hens can practice their more innate natural behaviors (Tactacan, et. al., 2009).

The conventional cage system has several advantages and disadvantages. On the positive side these fully automated system provide better disease control with greater hen livability because the birds sit in relatively small cages, which allows for lower infection rates, easier management, and lower production costs (Duncan, 2001) in addition to improved egg hygiene, (Hannah, et al., 2011).

On the other hand, this type of rearing system prevents hens from expressing innate behaviors such as extended exercise, which could potentially lead to metabolic disorders and other movement restriction disorders (Dikmen, et al., 2016). Concerns over

hen welfare have been heavily publicized since the 1960s following the publication of Ruth Harrison's book "Animal Machines" and the Brambell Report in the United Kingdom (Mench, et al., 2011a).

Another factor that has reportedly led to the development of alternative rearing systems was the increased prevalence of osteoporosis in caged hens (Regmi, et al., 2016), as battery cages may hinder tibia bone strength during the laying phase. The cage system also is associated with other problems, such as manure handling and fly control, although any rearing system is likely to experience these particular issues.

Researchers have shown that the cage system produces higher egg weights (g), eggs with more protoporphrin (mainly within the calcareous part of the shell), and darker shell color compared to the barn-type system that described as fully slatted provided with nest boxes, and perch (Samiullah, et al., 2016).

Economic studies have indicated that the cage system is more cost effective, as labor and production costs and total capital investments per hen are significantly lower compared to the aviary or enriched house (Matthews and Sumner, 2015). The automation of feeding, watering, egg collection, and improved environment variables found in this system allow it to be economically efficient (Mench, et al., 2011).

With respect to egg safety, a study conducted by Jones, et al. (2016) to evaluate pathogen levels in commercial hen housing systems reported that *Salmonella* and *Campylobacter* were the most common pathogens associated with laying hens. In this regard, the main advantage of the cage system is in the reduction of microbial contamination because hens and eggs are separated from manure (Mench, et al., 2011).

**Cage-Free System**

In the United States, the conventional cage rearing system or eggs from caged hens is most common, despite increasing concerns about animal welfare (Zhao, et al., 2015). While the egg industry is exploring different housing system alternatives for laying hens in an effort to improve the housing and related environmental conditions, this research is still limited (Karcher, et al., 2015).

One alternative is the cage-free rearing system where the hens are allowed more freedom to move around, such as an aviary with a small covered floor area, or paddock (Hannah, et al., 2011). This approach has been reported to have several advantages: hens are able to walk, exercise, stretch their legs and wings, and express other natural behaviors, such as dust-bathing and foraging (AVMA, 2012). However, non-cage or enriched facilities can affect egg safety and quality, since eggs can be altered microbiologically by pathogens such as *Salmonella* Enteritidis, or chemically, due to contamination from pesticides or heavy metals (Holt, et al., 2011). Scientists have found that bacteria levels on washed and unwashed eggs were higher for hens raised with shaving and slat conditions, compared to hens raised in traditional cages (Hannah, et al., 2011).

One study, for example, compared three hen-rearing facilities, conventional cage, enriched colony cage, and cage-free aviary, with differing environment conditions on including egg safety, worker safety, and general hen health (Jones, et al., 2015). The study found that egg safety was enhanced with the use of nest boxes, and floor eggs had higher levels of human pathogens.

Hen housing can impact not only egg quality and safety, but also production costs. The few studies that have examined the costs of different rearing systems associated with commercial egg production have found that aviary costs are much higher than costs for conventional barns and enriched colonies, with higher feed costs and labor costs as well (Matthews and Sumner, 2015). Therefore, there should be more in depth consideration regarding sustainability issues before making any decision that may negatively impact one or more of the sustainability components (Mench, et al., 2011). This can be a disadvantage of cage-free production as it affects the economy in general.

**Free-Range System**

One of the housing systems for laying hens in the United States and other countries that mirrors the backyard system that was predominant before the invention of the raised cage system in the early 20th century (Dikmen, et al., 2016) is the cage-free design using barns or aviaries that provide outdoor access through regulated openings (Miao, et al., 2005). The primary differecne between cage-free rearing and free-range rearing is access to the outside ambient environment. Gates or pop-up holes can be closed in the evenings, or be fitted with bars to limit access by predators. Similarly, wire mesh grates could be placed in front of openings to reduce the amount of dirt carried back into the shed.

There are several animal welfare concerns associated with this system, however, including increased risk of disease and exposure to toxins and parasites, as well as feather pecking. In addition, cannibalism has been associated with free-range flocks in the United Kingdom, especially when large numbers of hens stay indoors because of inclement weather or limited vegetation (Lay, et al., 2011).

Just as we saw with the cage-free systems hen performance and efficiency are usually reduced under the free-range system. A decline in production results from several factors that vary over time, most notably, fluctuations in weather. Unlike hens in a caged system, free-range hens can be vulnerable to seasonal changes in an uncontrolled environment (Miao, et al., 2005). Moreover, studies have shown that free-range hens have higher mortality, lower production rates, lower egg weights, and lower body weights over 18 to 40 weeks of age (Glatz, et al., 2005).

Feed conversion ratio was found to be significantly higher in the free-range system than in the conventional or enriched cage system (Dikmen, et al., 2016). Samiullah, et al. (2016) reported that eggs produced by young hens reared in free-range systems were lighter in color. However, no significant differences were reported in egg albumen, yolk, and shell perecentages between eggs from conventional or free-range systems (Lordelo, et al., 2016). Also, it was reported that the costs of free-range production in Europe were higher than for other housing systems, with variable costs about 22% higher and total costs 45% higher than conventional cages (Mench, et al., 2011).

Researechers have found the move to non-cage system may impact both egg quality and food safety (Holt, et al., 2011). Free-range hens are expected to have additional sources of nutrients due to the opportunity for outdoor forage that provides vegetation as well as insects. However, these forage opportunites may vary greatly from one location to another. Researchers also compared the fatty acid composition of eggs from free-range and cage systems, and found no differences in polyunsaturated fatty acids, including Omega-3, between eggs from free-range and cage systems (Lordelo, et al., 2016). However, they reported that eggs from free-range hens had lower short-fatty acids

(SFA) and monounsaturated fatty acids (MOFA) wich could potentially affect consumer health. This apparent contradiction could be a result of different forage in differeent regions, because the dietary composition provided to the conventional cage hens was unreported (Holt, et al., 2011).

In a survey of farmers, researchers found that labor costs all were higher with the free-range system (Stadig, et al., 2016). Also, the free-range system requires more labor due to system complexity and the uncontrolled environment (Miao, et al., 2005).

**Pasture Housing System**

This system is still not recognized by the USDA because no standards have yet been established (AEB, 2016). As the name indicates, hens in pasture-rearing farms spend most of their lives in a pasture, with free foraging and ranging throughout the day (Ellis, 2015). In addition, shelter must be provided to protect hens from predators and inclement weather, and to give them sufficient opportunities for rest and egg laying. Pasture housing can be fixed, with a coop in the middle of large pasture area, or more commonly, mobile, with a coop that can be easily moved around the farm (Ellis, 2015). It has been reported that pasture poultry can affect soil quality due to overgrazing, and also destroy vegetation and increased the risk with respect to water quality (Ellis, 2015).

Scientists have found that integrating poultry into a pasture rotation system or a traditional crop can positively affect bird health and control poultry diseases, as well as weeds (Glatz, et al., 2005). In addition, pasture area affects egg quality by altering egg characteristics and composition. Moreover, the amount of available pasture also can impact productivity (Miao, et al., 2005). A study that compared three types of hen housing (conventional, organic with natural grazing at 4 $m^2$/bird, and organic “plus” with natural

grazing in a large pasture area at10 $m^2$/bird) found that eggs from the organic "plus" system produced eggs with higher eggshell weight, darker yolk color, and higher α-tocopherol, carotenoid, and polyphenols content (Mugnai, et al., 2016).

Public opinion supports the idea that pasture-raised hens produce eggs with healthier nutritional composition than eggs produced by traditional caged systems, a point that they promote heavily in marketing efforts. Ultimately the nutritional composition of the eggs is a function of what the hens consume. It is well accepted that feed composition can change egg nutrient profile. With respect to environmental impact, the pasture system has been reported to improve weed control, reduce chemical input into the soil, and improve soil fertility and crop yield, as well as change consumer perceptions of poultry production (Glatz, et al., 2005). On the other hand, pastured poultry can damage a pasture's health, most noticeably with the fixed-type system or when birds graze on wet pastures.

**Organic System**

The organic poultry production system refers to hen housing that meets several quality of life requirements defined by the USDA. The regulations define husbandry conditions, with certified organic grounds, organic feed, natural health treatments, and access to the outdoors. It is critical to understand that the topic of organic production has expanded to include cage-free and free-range production methods, as well (Anderson, 2009). Birds raised in "organic" conditions must have access to outdoor areas during rearing (Janczak and Riber, 2015). These additional requirements make the organic rearng system the most expensive of the cage-free options.

Organic poultry housing in the United States is required to follow the regulations of the United States Department of Agriculture National Organic Progam regarding the substances that are allowed and disallowed, and must follow a three-year transition process to become certified organic. Moreover, feed, including all the major ingredients found in feed, must be "organic", with no synthetic amino acids allowed except specific levels of DL-Methionine (Burley, et al., 2016), and L-Lysine HCL, the first and second limiting amino acids in the typical U.S. poultry diet.

Organic production has come about over the last few years as consumers have become more interested in knowing where their food comes from, and this includes questions for poultry producers regarding hen health and safety. With respect to egg nutrition and quality parameters, Lordelo, et al., 2016, investigated different characteristics of eggs in terms of cages, barns, free-range, organic eggs, and eggs enriched eggs with n-3 polysacchraides fatty acids (PUFA), and reported that organic eggs had higher Haugh units than eggs from caged hens. They also indicated in their sudy that eggs from caged hens also have lighter yolks and lower levels of albumen protein than organic eggs. It is doubful that these results are repeatble as stated earlier, egg quality is primarily a function of diet, not rearing system.

Consumer concerns regarding egg safety is another major issue. Salmonellosis has been identified as a public health concern worldwide (Whiley and Ross, 2015). In the United States, egg contamination represents approximately 53% of all cases of *Salmonella* reported to the Center for Disease Control and Prevention between 1985 and 2002 (Food and Drug Administration, 2009). A microbiological survey investigated the prevalence of the *Salmonella* serotype Enteritidis in eggs produced at organic farm and conventional

cage systems in South Korea, and found 26 Salmonellae as a *Salmonella* Gallinarum from 7,000 eggs, with higher levels in the organic eggs compared to those from conventional cage facilities (Lee, et al., 2013).

In summary, various hen housing systems used in the United States were described. Currently, the conventional cage rearing system is the most common. This system can provide better environmental control, lower the incidence of infection, is easy to manage, and has lower production costs because it is fully automated (Duncan, 2001).

On the other hand, animal welfare activists, and to some degree, consumers, have voiced concerns regarding the conventional cage system because in it, hens are unable to practice natural thy behaviors such as spreading their wings and legs, nesting, and dust-bathing. As a result, many egg producers are switching to cage-free systems which are perceived to result in better animal well-being than the conventional cage system'

Consumers today have to pay more for eggs produced by non-caged laying hens. This cost is even higher with specialty eggs, such as organic or Omega-3 enriched eggs. In order to meet the potential demand of food retailers and food services outlets for cage-free eggs, eggs producers, especially major suppliers, have to switch from conventional caged housing systems, or at least plan to make all future housing facilities to be cage-free.

This is going to be quite a challenge, not only for producers, but also for the birds, because the systems focusing primarily on animal welfare issues requires better management, more labor, more disease control, more sanitization strategies for pathogen reduction, and more work overall. While there may be benefits, this overhaul is not without costs and economic impact in general, and on egg producers in the United States

in particular. Diet composition will also play a significant role in the egg industry evolves to cage-free production systems.

**Soybean Meal**

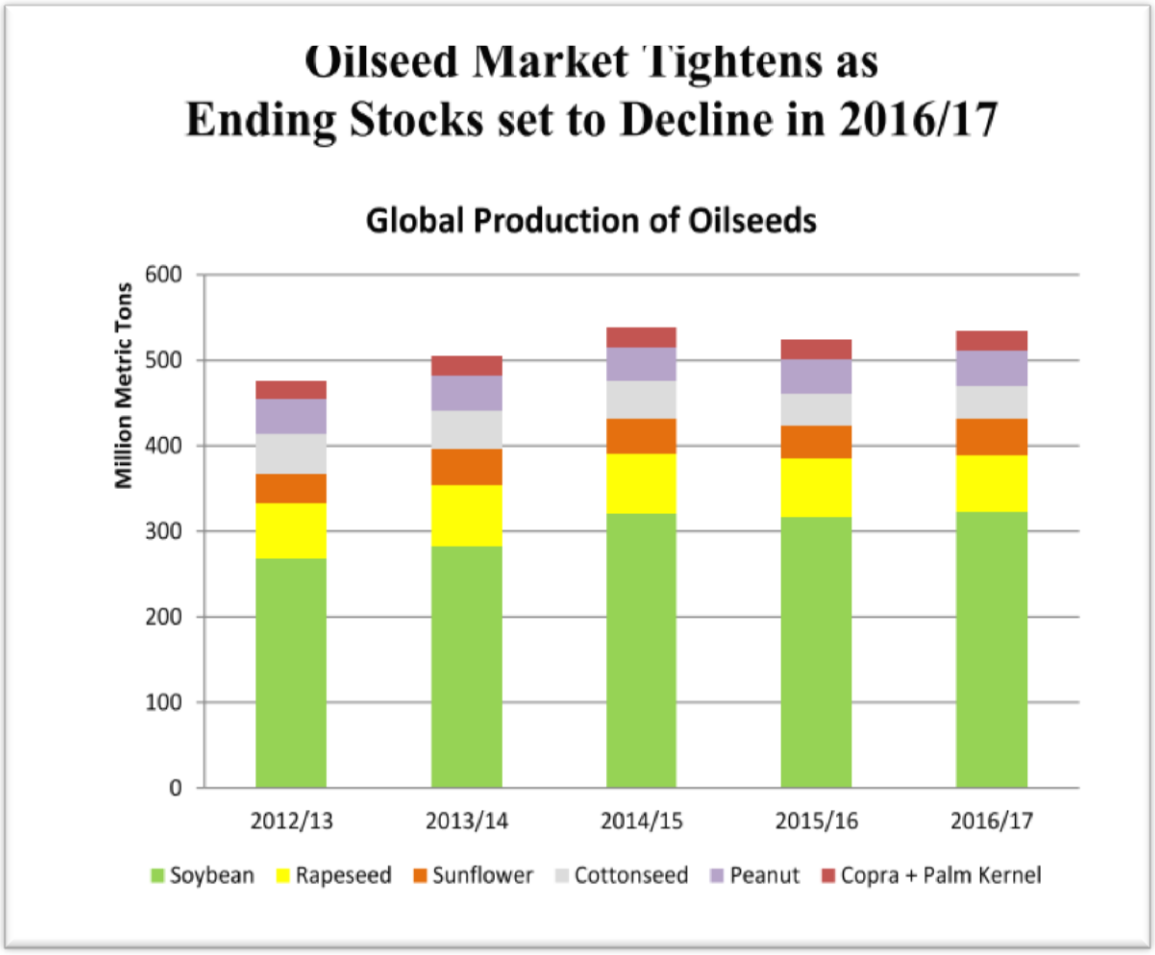
Soybean meal is one of the major oilseed ingredients used in animal feed. For poultry, it provides a complete protein source, as well as all essential amino acids. Soybean meal comes from the extraction of the soybean meal oil. It is considered a great source of energy (Nahashon, et al., 1994) as well as a means of balancing dietary amino acid levels with other ingredient (Park, et al., 2002). Since just after World War II, it has replaced crops like clover in crop rotations in the American Midwest (Dale, 1996). Soybean meal use has increased from 48 million tons in 1985 to 106 million tons in 2004 (Dalgaard, et al., 2007). It is by far the best oilseed meal available to animal nutritionist as an economic source of vegetable protein in the United States and dominates all other oilseed meals.

According to the (USDA) Foreign Agricultural Service, global oilseed supplies in general are up as much as 1% in both 2015/16 and 2016/17 (Figure 1.1). The production of SBM is expected to increase another 2% in response to demand in countries such as Brazil and China, as the crop in India rebounds (USDA, 2016). Recent data show that total protein meal consumption is growing at about 3% as all other meals except rapeseed meal have disappeared. The main driver in this expanding trade is growing demand for SBM, which is about 70% of total global consumption (USDA, 2016).

In the United States, more than 50% of SBM is used for poultry feed, with 26% for swine feed and the rest used for beef, dairy, and pet food. It is popular because of its unique composition of essential amino acids (Stein, et al., 2008). The protein in SBM is

considered high quality for poultry feed and is an especially good source for lysine and tryptophan (Baker, 2000). In addition, the required amount of digestible lysine in SBM exceeds the requirements of lysine for chicks (per unit of protein) compared to other oilseeds used for poultry (Baker, 2000). Park et al. (2002) found that SBM contains a large amount of lysine and has a good amino acid profile and good bioavailability; so that it usually is used to balance dietary amino acids levels with other ingredients in poultry feed (Park, et al., 2002).

**Figure 1.1 Oilseed market, reprinted from the Foreign Agricultural Service/USDA, 2017**



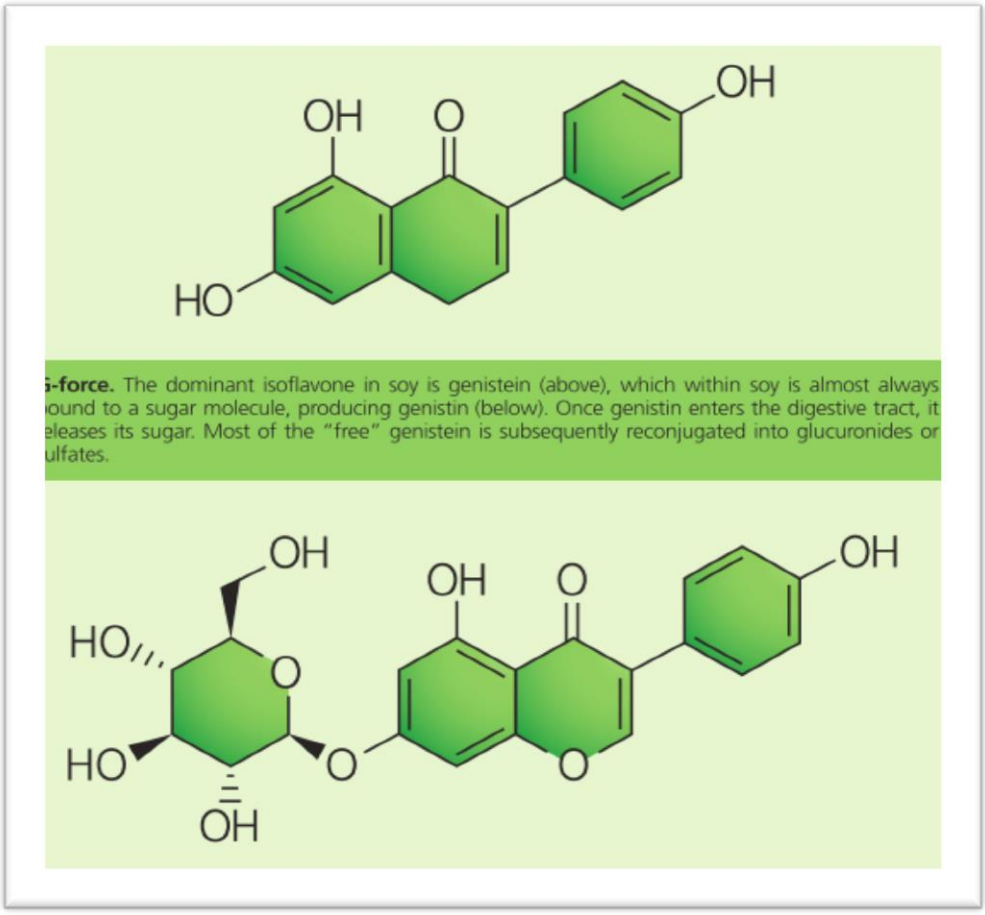
Soybean meal utilization is associated with the processing methods of the oil extraction and the animal's ability how to utilize this source of protein properly to fully benefit from the nutrient contents (Powell, et al., 2011). The quantity of the protein depends on aspects such as soy cultivar, moisture diluting effects, and flow agents (Dale, 1996). The quality of the SBM itself depends mainly on the processing methods used, and its origin (Park, et al., 2002). Other factors affecting quality are the process degree required to maintain hexane recapturing and to denature anti-nutritional proteins such as trypsin inhibitor, which may impact growth and performance, especially in young birds (Dale, 1996). Solvent-extracted SBM is broadly used and Expeller-extruded SBM is an alternative mainly used in organic poultry feed (Powell, et al., 2011).

Researchers have found that dehulled SBM (48% protein) has higher metabolic energy, and less fiber and ash (about 4%) compared to 44% protein non-dehulled SBM (Swick, 1995). The dehulled SBM, plus the excellent protein quality and amino acid digestibility found in feed used in the United States, benefits economic production and the performance of both layers and broilers (Park, et al., 2002).

As with any ingredient the price feed producers pay for it is variable. SBM has become extremely expensive over the last decade (H. Wall 2010; Shi, et al., 2012), just as consumers have become more demanding about the ingredients in their food, and their food's origin. In the United States, this concern includes the use of genetically modified organisms (GMO) in the production of SBM (Hermes, 2010). Most SBM available in the United States is genetically manipulated to increase its resistance to the herbicide Roundup (Hermes, 2010). Consumers also are concerned about anti-nutrient factors (ANF). Soybean meal has several of these factors, such as protease inhibitors, saponins,

phytic acid and iso-flavone phytoestrogens. (Figure 1.2). These concerns have created an opportunity for niche markets to develop for specialty eggs produced from hens not fed traditional corn – SBM based diets. For these specialty markets feed manufacturers must rely on other sources of vegetable protein to economically meet animal nutrient requirements.

**Figure 1.2 Isoflavone in soy, reprinted from Environmental Health Perspectives, 2006**



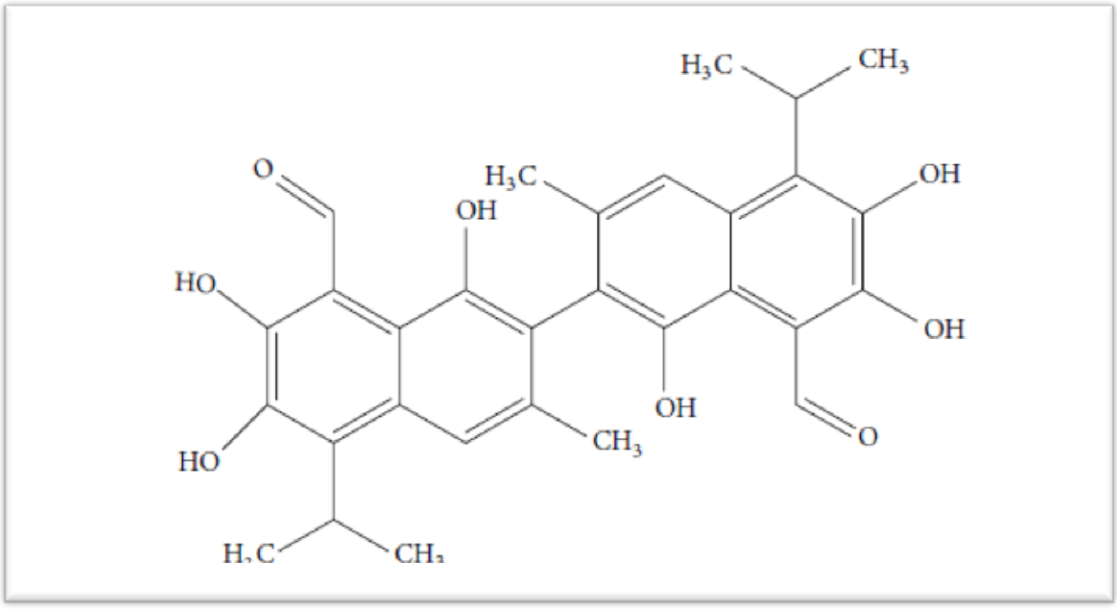
**Cottonseed Meal**

Cottonseed meal (CSM) is a relatively inexpensive co-product of cottonseed oil production and can be potentially used as a protein source alternative to SBM in poultry diets (Zeng, et al., 2015). It is derived from the residue created in cottonseed oil extraction (Nagalakshmi, et al., 2007). The cotton genus (*Gossypium*) belongs to the family of *Malvaceae*, and is common in temperate to tropical regions around the world (Nagalakshmi, et al., 2007). Cottonseed meal contains approximately 41% protein, 13.6% crude fiber, and 0.5% crude fat (NRC, 1994). Its disadvantages are high fiber, poor lysine digestibility (Zeng, et al., 2015) and low quantities of cysteine and methionine (Nzekwe and Olomu, 1982).

The primary concern regarding the use of CSM in poultry feed is the gossypol content (Gamboa, et al., 2001). Gossypol is the polyphenolic compounds found in the pigment glands of the cotton plant (Lordelo, et al., 2005), and its chemical formula is $C_{30}H_{30}O_8$ (Figure 1.3) (Gadelha, et al., 2014; Soto-Blanco, 2008).

Gossypol has two naphthalene rings that can rotate to surround the bond and connect the ring, resulting into two identical cell structures, with positive (+) and negative (-) enantiomers (Huang, et al., 1987). During the extraction process, the free gossypol binds to the epsilon group of lysine, thereby reducing the availability of this essential amino acid (Nagalakshmi, et al., 2007).

**Figure 1.3 Chemical structure of gossypol. Reprinted from Gadelha et al. (2014) and Soto-Blanco (2008)**




The amount of free gossypol and quality of the protein are the most important factors in evaluating CSM (Gamboa, et al., 2001). These factors can be modified through the processing methods used for oil extraction, and the specific variety of the cottonseed cultivar (Nagalakshmi, et al., 2007). The major issue with gossypol is that these toxic compounds inhibit the pepsin and trypsin enzymes in the alimentary tract and interfere with protein digestion (Tyani, et al., 1986). Free gossypol can also affect the reproductive system, heart, and liver of monogastric animals (Nagalakshmi, et al., 2007) in addition to decreasing overall poultry performance (Lordelo, et al., 2005).

Cottonseed meal has other toxic effects whereby free gossypol can combine with ferric ion in the bloodstream and affect erythrocyte oxygen-carrying (Brocas, et al., 1997).

All CSM produced in the United States contains gossypol (Lordelo, et al., 2005), and most CSM is fed to adult ruminants, which tolerate gossypol (Mandhania, et al., 2016). Generally, laying hens are more susceptible than other poultry to free gossypol, and gossypol produces several negative impacts on egg quality parameters when used in excess. Laying hens are more sensitive to free gossypol ingestion and the cyclopropenoid fatty acids that could be found in CSM (Davis, et al., 2002).

These cyclopropenoid fatty acids and the free gossypol can impact the egg quality, resulting in pink albumen and brownish green yolks (Heywang, et al., 1955). The yolk discoloration results from the combination of gossypol and released $Fe^{+}$ from the yolk protein (Kemmerr, et al., 1966). In addition, too much CSM in the diet of starter chicks can lead to lower body weight and increased feed: gain ratio (Hermes, et al., 1983). Many studies have evaluated the optimum level of CSM used in poultry diets without affecting layers (He, et al., 2015a). It is generally agreed that if the free gossypol content of the diet does not exceed 50 ppm for layers and 100 ppm for broilers it can be safely fed to poultry.

Several processing methods have improved the nutrient value of CSM and reduce the free gossypol level in the poultry diet. Unprocessed, whole ground CSM contains high levels of free gossypol and cyclopropenoid fatty acids (Davis, et al., 2002). High temperatures and high pressure could make CSM and oil less toxic compared to the solvent extraction method (Jones, 1981). Researchers have found that production of glandless cottonseed through genetic modification (*Bacillus thuringiensis* cotton) can reduce the content of free gossypol (Nagalakshmi, et al., 2007). The potential concentration of cyclopropenoid fatty acids and free gossypol is lower in the modern expander-solvent CSM because this technique leaves less residual oil compared to

extraction from older plants (Davis, et al., 2002). Therefore, CSM can be a substitute source of protein in the diet of layers. Although the usage level and processing method may need some modification to ensure that the free gossypol does not reach dietary concentrations that could affect egg production or quality.

**Distillers Dried Grains with Solubles**

Another partial substitute ingredient for SBM in layer feed is distillers dried grains with solubles (DDGS). This ingredient is a co-product of corn fermentation from the ethanol industry and has been historically used in poultry feed (Shin, et al., 2016) as a source of so-called unidentified growth factors (Batal and Dale, 2005). While it is a relatively inexpensive source of protein, as well as xanthophyll, its nutrient content varies and it can lead to digestibility issues resulting from the manufacturing process and variability between suppliers when used in excess (Spiehs, et al., 2002).

In the past, DDGS originated from the fermentation of different grains used by the beverage industry, but today they are generated exclusively from corn fermentation during ethanol production (Batal and Dale, 2005). Production of DDGS has increased six-fold in the United States in the past decade due to the expansion of dry-grind ethanol (Trupia, et al., 2016) making DDGS an economical source of nutrients for poultry feed (Purdum, et al., 2014).

Distillers dried grains with soluble can be an excellent source of protein for feedlot cattle (Klopfenstein, et al., 2008) as well as for poultry (Shin, et al., 2016). Early studies found that 5 to 10% DGGS could be used in layers' diets without a negative impact on egg production or egg weight (Harms, et al., 1969; Jensen, et al., 1974; Matterson, et al.,

1966). In addition, replacement pullets have shown a preference for diets containing up to 20% DDGS (Alenier and Combs, 1981).

More recent studies have found that using DDGS at the level of 20% in layers' diets can improve the color of egg yolk without any impact on laying hen performance or other egg quality characteristics (Shin, et al., 2016). The improvement in the yolk color is due to the concentration of the xanthophyll, which can make yolks a deeper yellow (Masa'deh, et al., 2011; Salim, et al., 2010). In addition, DDGS in laying hen diets also can increase several beneficial lipophilic nutrients in the yolk, with no ill effects (Trupia, et al., 2016), and using 94 to 96% of DDGs in molting diets did not affect subsequent egg production (Bland, et al., 2014).

One study found that feeding young Bovan laying hens from 20 to 33 weeks of age a diet with 20% DDGS that differed in oil content resulted in different production characteristics (Purdum, et al., 2014). Hens fed a low 5.2% oil DDGS did not affect short term egg production, egg weight, egg mass, or hen weight gain comparing those fed the high oil (7.3 and 10.3%) DDGS. In short, DDGS can be used as an alternate source of both protein and xanthophyll in laying hen diets without a negative impact on egg quality parameters or other economic factors.

**Corn Gluten Meal**

Corn gluten meal (CGM) is a co-product of the wet corn milling industry (Castanon, et al., 1990). It is a good source of xanthophyll pigments, which are important for egg yolk color and can contain as much as 60% protein making it potentially useful for designing a SBM free diet. In addition, CGM is suitable in molting diets when used in high concentrations (Castanon, et al., 1990). Corn gluten feed production has increased

from 1.9 million metric tons to approximately 4 million metric tons in 1984 (Jones, 1987). On disadvantage to corn gluten meal is it's relatively low lysine concentration which can limit its use without supplemental crystalline lysine.

Although there may be many ways of creating laying hen diets that do not utilize SBM, alternative ingredients such as CSM, DDGS, or CGM can easily be obtained throughout the U.S. at a relatively low cost conducive to least-cost formulation of SBMF laying hen diets.

**Yeast Cell Wall as an Antibiotic Alternative**

The use of antibiotics at sub-therapeutic levels to improve bird performance and feed utilization has been very controversial over the past several years. Numerous studies have sought alternative growth promotors for the poultry industry (Hashim, et al., 2013). In addition, concerns about antimicrobial resistance to both animal and human antibiotics also have increased demand for alternatives.

The recent adoption of the Veterinary Feed Directive (VFD) is one approach to regulating the use of medically important drugs in animal industry. The VFD, implemented January 1, 2017, mandates that all antibiotic growth promoters previously approved for laying hens except Bacitracin Methylene Disalicylate be prescribed by a veterinarian. As a consequence, several prebiotics have been introduced to improve the health and performance of laying hens.

Prebiotics are non-digestible food ingredients that are considered beneficial for the host in selectively stimulating the growth and activity of one (or a limited number of) bacteria present in the colon, improving the host's health (Gibson and Roberfroid, 1995b). Yeast cell wall (YCW) is one of the antibiotic alternatives widely used in the poultry

industry as a prebiotic. It originates from the species *Saccharomyces cerevisiae* (Ballou, 1982), and generally makes up 25-35% of the dry weight of this species (Hashim, et al., 2013).

Yeast Cell Wall is mainly composed of three components: glucan, mannoprotein, and chitin (Ballou, 1982). Specifically, it is approximately 30 to 60% polysaccharides, 15 to 30% proteins, 5 to 20% lipids (Hashim, et al., 2013) and also contains 15 to 30% ß-glucan, and 15 to 30% mannan oligosaccharides (MOS) (Northcote and Horne, 1952). It has been shown that MOS has the ability to bind pathogens which can decrease these organisms level from the intestine and also improve the host immune system (Spring, et al., 2000). In addition, it has been reported that these oligosaccharides can inhibit bacteria with Type-1 fimbriae like, *Salmonella* and *E.coli,* from freely colonizing to the intestinal mucosa (Spring, et al., 2000).

Yeast cell wall products are now widely used as prebiotics in feed for layers, broilers, and other poultry. Supplementation of YCW-MOS in layer diets has been found to significantly improve the feed and the caloric conversion ratio (Hassan and Ragab, 2007). In addition, Hassan and Ragab found that the supplement also improved egg quality parameters such as shell and yolk percentages, yolk index, and egg weight. A study conducted during the summer season to evaluate layer hen performance using the supplement of 1 gm/kg YCW-MOS as an alternative to the antibiotic feed additive (avilamycin) indicated that YCW-MOS increased egg production, decreased the ratio of cracked or broken eggs, and reduced the mortality rate for layers at the age of 54 weeks (Çabuk, et al., 2006).

In addition, the use of YCW at 250 ppm in early production laying hens had a positive impact on general performance and improved egg weight (and liquid egg yield specifically) while the use at a 500 ppm concentration improved shell quality (Hashim, et al., 2013). At the level of 500 ppm, YCW resulted in higher percentages of egg shell thickness and weight compared to hens fed YCW at 250 ppm.

Yeast cell wall products are used as feed additives for broilers as well, with the result of increased villus height of jejunal mucosa (Morales-López, et al., 2009). Studies also have found that the use of *Saccharomyces cerevisiae* cell wall in broiler diets improved weight gain in broiler chickens (Santin, et al., 2001). This improvement was thought to be due to the additive's trophic effect regarding the intestinal mucosa, as it increases villus height, especially during the broilers' first week of life.

Gao, et al., 2008, documented that the use of yeast culture in broiler diets can improve growth performance, immune response, and increase calcium and phosphorous digestibility. This study found that the optimum growth performance was obtained when feeding 2.5 g of yeast culture per kg of diet.

It is clear that the egg industry in the United States is changing the way it houses hens. Commercial battery cages are being replaced with furnished cages (enriched), or cage-free or free-range facilities or aviaries to address animal welfare concerns. In addition, alternative feed ingredients, as well as prebiotics, have been found to improve overall hen performance and human health with no negative impact on hen egg production or welfare.

The experiments presented in this dissertation explore egg production of peaking laying hens utilizing a conventional single hen cage system and a free-range rearing

system whereby the hens have access to the outdoor ambient environment. It also explores the use of a SBM free diet using easily obtained alternative sources of protein. Consumer panels were set up to evaluate preference for hard cooked and scrambled eggs obtained from the experimental hens. A separate study explores the effectiveness of a YCW prebiotic (Saffmannan$^{TM}$) in older caged hens fed SBM and SBMF diets. The final experiment explores the up- and down-regulation of several genes extracted from both liver and spleen tissues as a function of YCW or a SBMF diet.

# CHAPTER II

# AN EVALUATION OF THE PERFORMANCE OF HY-LINE BROWN LAYING HENS FED SOYBEAN OR SOYBEAN-FREE DIETS USING CAGE OR FREE-RANGE REARING SYSTEMS

## INTRODUCTION

The conventional cage system is the most common rearing system for laying hens in the United States, and continues to be a topic of discussion across the nation (Jones, et al., 2016) as several concerns have increased with respect to the animal welfare. Major food retailers and food service outlets have pledged over the next few years to sell or serve only eggs that are produced within larger cage-free aviaries, free-range or even pasture-raised production systems which provide greater access to move about the production system. These facilities utilize a floor, rather than raised cage system with free access to the outdoors allowing the hens to express their natural behavior (Mench, et. al., 2011).

In order to meet these commitments and provide sufficient supply, egg producers must take significant action with respect to future management and feeding of laying hens. In addition to animal welfare concerns, a niche market has recently developed for consumers seeking USDA labeled Organic Eggs or other specialty eggs enriched with omega -3 fatty acids are obtained from hens not fed GMO ingredients such as soybeans. Even though properly processed SBM is known to be one of the best oilseed meals ever used in the animal feed industry, health concerns have been raised due to several anti-nutrient factors (ANF) such as trypsin inhibitors, lectins, saponins, and stachyose and raffinose non-starch polysaccharides associated with soybeans.

Alternative oil seed meals such as cottonseed meal (CSM) have been used to substitute for at least a portion of the SBM in laying hen diets (Qi, et al., 2016). Cottonseed meal however, has poor lysine digestibility and the presence of free gossypol (FG), limits its incorporation in poultry feed (Zeng, et al., 2015). Generally, laying hens are more susceptible to FG than other poultry. Free gossypol causes yolk discoloration due to the combination of its chemical makeup with the Fe+ released from yolk protein (Kemmerr, et al., 1966). A study has been conducted to determine the optimal level of CSM in the laying hen diet without affecting laying hen performance (He, et al., 2015). It is well known that feeding laying hens FG (+) can cause severe egg yolk discoloration (Lordelo, et al., 2007). It has been reported that when feeding laying hens a diet with a high concentration (20 or 30%) of CSM, the egg yolk color changes, and brown discoloration is observed (Davis, et al., 2002).

The purpose of this experiment is to evaluate the performance of Hy-Line Brown laying hens that fed SBM or soybean meal-free (SBMF) diets while using a traditional cage or free-range rearing system that provides both indoor area with nesting boxes and free access to the outdoors.

## MATERIALS AND METHODS

### Birds, Diets, and Management

This study was conducted at the Texas A&M University Poultry Research Center, and received approval from the university's Animal Care and Use Committee (IACUC 2017-0072). A total of 246 Hy-Line Brown pullets, 11 wk of age, were placed in floor pens and fed a mash form of a typical corn/soybean diet that met this breed's nutritional requirements for their appropriate age. At 20 wk of age, the hens were separated into their treatment groups and placed in 2 respective laying facilities in a split-plot design.

A total of 120 laying hens were randomly assigned to 2 dietary treatments and distributed in 3 blocks from east to west throughout a traditional tunnel ventilated caged hen house. Each block represented a group of 20 cages and each individual hen was kept in a 50.8 W × 30.5 L × 30.5 H cm cage (1,549 cm² per hen) with 1 nipple drinker for every two cages. Each cage had access to individual trough feeders (30.5 cm feeder space/hen).

For the free-range system, a total of 126 hens were randomly assigned into 6 pens (182.9 W × 365.8 L cm covered) and distributed in 3 blocks (2 dietary treatments per block) from east to west. Each pen enclosure was equipped with 9 nest boxes (2,791 $cm^2$ indoor floor area) and contained 21 hens that had free access to an uncovered outdoor area (182.9 W × 731.5 L cm) which was fully enclosed with wire mesh. The total surface was 9,509 cm² per hen. Additionally, all pens had 11 nipple drinkers (6 outdoor area & 5 inside area) and 1 circular hanging feeder occupying 30 $cm^2$ of indoor floor space.

Treatments were SBM and SBMF diets containing CSM, corn distillers dried grains with solubles DDGS, corn gluten meal CGM, and wheat middlings. Diets were formulated based on the recommendations of the management guidelines for Hy-Line Brown laying hens. All diets were formulated to have equal calculated nutritional content (based on crude protein, ME, calcium, available phosphorous, standardized ileal digestible amino acids, xanthophyll, sodium, and electrolytes) and were provided in mash form (Table 2.1). Lighting timers were set to provide 16 hr of light for each rearing system. Feed and water were provided ad libitum.

**Table 2.1 Composition and nutrient levels of corn/soybean SBM and corn/soybean-free SBMF diets from 19 to 55 weeks of age**

| | 19 to 38 wk | | 39 to 44 wk | |
|---|---|---|---|---|
| **Ingredients** | **SBM%** | **SBMF%** | **SBM%** | **SBMF%** |
| Corn | 64.11 | 46.99 | 65.25 | 42.85 |
| Dehulled Soybean Meal | 20.04 | - | 21.07 | - |
| Cottonseed Meal | - | 14.94 | - | 15.00 |
| Corn Gluten Meal | 0.38 | 1.38 | 0.31 | 1.63 |
| Corn Distiller Dried Grains with Solubles | - | 15.00 | - | 15.00 |
| Wheat Midds | - | 3.59 | - | 8.65 |
| DL-Methionine 98% | 0.35 | 0.36 | 0.17 | 0.18 |
| L-Threonine 98% | - | 0.03 | - | 0.05 |
| Lysine HCL | 0.24 | 0.66 | - | 0.42 |
| Fat - Animal-Vegetable Blend[5] | 2.75 | 4.73 | 1.76 | 4.75 |
| Limestone | 9.76 | 9.92 | 9.45 | 9.62 |
| Mono-Dical PO4 | 1.66 | 1.65 | 1.32 | 1.27 |
| Salt | 0.29 | - | 0.38 | 0.06 |
| Sodium Bicarbonate | 0.12 | 0.44 | - | 0.23 |
| Trace Minerals[1] | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamins[2] | 0.25 | 0.25 | 0.25 | 0.25 |
| **Calculated Nutrient Composition (%)** | | | | |
| ME(kcal/kg) | 2911 | 2911 | 2867 | 2867 |
| Crude Protein | 16.5 (15.5)[4] | 16.5 (16.2)[4] | 16.75(14.96)[4] | 16.75(17.16)[4] |
| Crude Fat | 4.58 | 7.36 | 3.62 | 7.43 |
| Crude Fiber | 1.71 (2.6)[4] | 4.18 (5.7)[4] | 1.76(3.06)[4] | 4.51(8.56)[4] |
| Calcium | 4.08 | 4.08 | 3.91 | 3.91 |
| Phosphorous | 0.68 | 0.78 | 0.62 | 0.73 |
| Available Phosphate | 0.45 | 0.45 | 0.38 | 0.38 |
| Digestible Methionine[3] | 0.58 | 0.58 | 0.41 | 0.40 |
| Digestible Lysine[3] | 0.90 (1.0)[4] | 0.90 (0.99)[4] | 0.74(0.79)[4] | 0.73(0.90)[4] |
| Digestible TSAA[3] | 0.80 | 0.80 | 0.64 | 0.63 |
| Xanthophyll (mg/kg) | 12 | 12 | 12 | 12 |
| Electrolytes (meq/kg) | 170 | 170 | 170 | 170 |
| Sodium | 0.17 | 0.20 | 0.20 | 0.20 |
| Chloride | 0.26 | 0.18 | 0.17 | 0.17 |

1Trace minerals premix added at this rate yields (mg/kg): zinc, 60.0; manganese, 60.0; iron, 60.0; copper, 7.0; iodine, 0.4.

2 Vitamin premix added at this rate yields (per kg): vitamin A, 11 IU; vitamin D3, 3,850 IU; vitamin E, 45.8 IU; menadione, 1.5 mg; B12, 0.017 mg; biotin, 0.55 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; B6, 7.15 mg; niacin,45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

3 Standardized digestibility Coefficients for cottonseed Methionine, Lysine, and TSAA were 0.73, 0.67 and 0.73 respectively.

4 Nutrient analysis was performed by Experiment Station Chemical Laboratory, University of Missouri.

5 Griffin Industries, Bastrop TX.

**Data Collection**

Egg production was recorded daily for both the cage and free-range rearing systems. Hen mortality was accessed daily and hen body weight was recorded every 28 day. Feed consumption and egg quality parameters assessments were bi-weekly. Feed offered was weighed at the beginning and feed retained was weighed at the end of every two weeks to calculate the bi-weekly feed consumption. When offered feed was consumed before the end of the two weeks, additional feed was weighed, recorded, and added into the feeders. Feed conversion ratio (FCR) was calculated using the bi-weekly feed consumed (gram) divided by total bi-weekly egg weight (gram).

The feed per dozen of eggs (FDE) was calculated as: bi-weekly feed consumption (gram) / bi-weekly dozens of eggs produced. Subsets of 10 fresh eggs per treatment block were collected from each rearing facility at weekly intervals to evaluate egg quality parameters. Albumen height was measured using a tripod micrometer (Ames S-6428; B. C. Ames Co.), and Haugh unit was calculated using the method of Haugh, 1937. Egg shell thickness was determined using the Ames No. 25M (masters of measurement), and eggshell strength was measured with a texture analyzer (TA-XT plus; Texture Technologies Corp.) using a 5 kg load cell according to the procedure of Jones et al. 2010.

**Statistical Analysis**

For data analysis, the two rearing systems were considered whole plots while the 3 location blocks (east to west) were considered sub-plots with diet type as an additional factor. For every 28 d period, data whole plots and sub-plots and were subjected to a split plot design ANOVA. Additionally, the cumulative data were subjected to two-way ANOVA. Both analyses were performed using SPSS Software V22. Any significant

interactions between rearing system and diet were reanalyzed as a one-way ANOVA and means separated using Tukey's HSD procedure when F-tests were significant. When the main effects of rearing system were significant the effects of diet were analyzed independently for dietary effects within the rearing system. Means were considered statistically different at $P < 0.05$.

**RESULTS AND DISCUSSION**

Egg production and egg weight for both cage and free-range facilities are presented in Figures 2.1 and 2.2, respectively. Figure 2.1 shows that onset of egg production in the free-range rearing facility was about a week behind the cage system. It should be noted that these flocks would be considered out-of-season since they began egg production in the late summer and early fall, as day length was decreasing, although the indoor light time clock helped counter act any day length effect. There was also likely more environmental stress associated with the birds moving from the fully enclosed pullet rearing pens to the free-range system with continual access to the outdoors. The dip in egg production between 28 to 32 wk (September) for the free-range hens consuming the SMBF diet was likely weather related although other possibilities such as the presence of predators cannot be overlooked. Weather temperature was measured throughout this time and it was ranged between (77.0- 94.0 °F).

The second dip in egg production between 39 and 40 wk was likely due to predation as two hens completely disappeared during that time frame. There was higher variability associated with the free-range environment versus the traditional fully housed cage system as our standard errors were consistently higher for all variables in the free-range system.

Egg weights were generally lower for birds receiving the SBMF diet irrespective of rearing system, particularly noticeable during the last 3 periods of data collection (Figure 2.2).

**Figure 2.1 Weekly hen day egg production in cage-soy diet (C-S), cage-soy free diet (C-SF), free-range soy diet (FR-S), and free-range-soy free diet (FR-SF)**

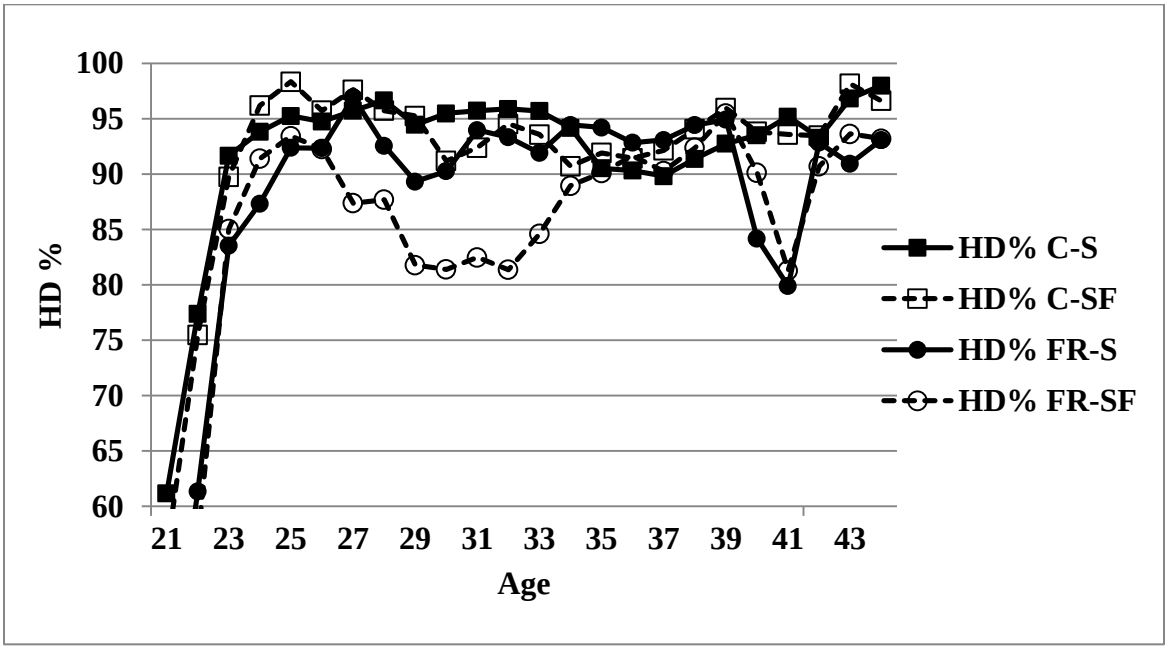
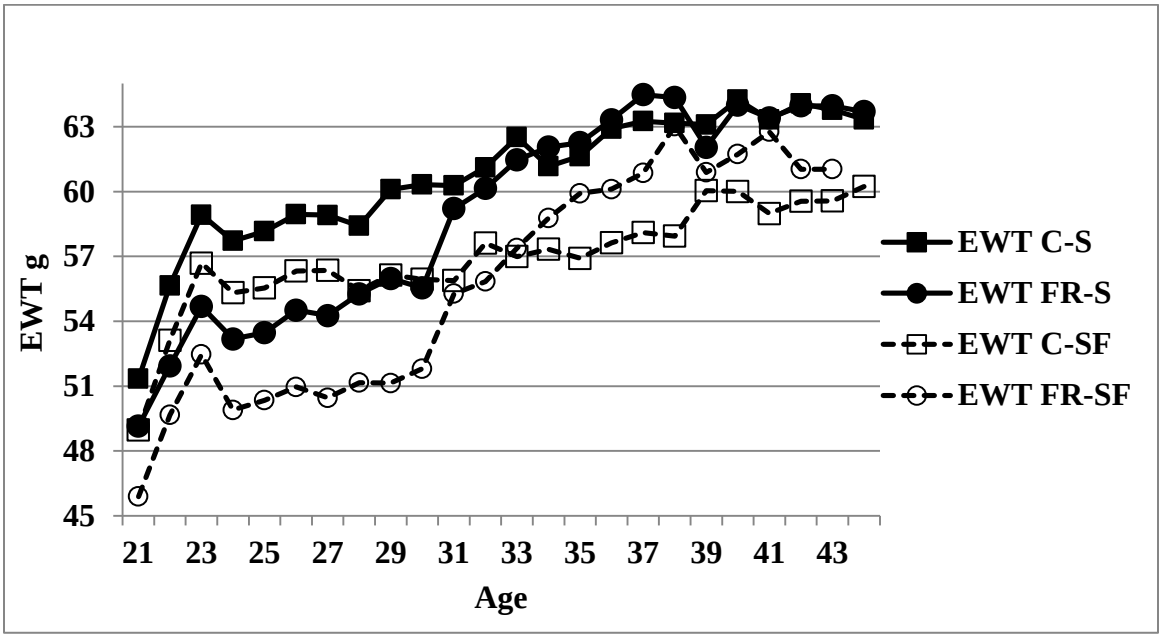


**Figure 2.2 Weekly egg weights in cage-soy diet (C-S), free-range-soy diet (FR-S), cage-soy free diet (C-SF), and free-range soy free diet (CF-SF)**



The results of laying hen production analyzed as a split plot over six 28-d production periods (wk 21 to 44) are presented in Table 2.2. There were numerous significant differences ($P<0.05$) observed between treatments for egg production (EP %), egg weight (EWT), feed per dozen eggs (FDE) and gram of feed to gram of egg ratio (FCR) with several interactions throughout the six periods of data collection. High pooled SE (PSEM) for egg production observed during the first and last production periods were due to the dips in EP% discussed previously with respect to Figure 2.1.

Egg quality data are presented as analyzed using the split plot design in Table 2.3. There was a significant rearing system by diet interaction ($P<0.01$) for shell thickness during the 2nd period of production with the shell thickness averaging 41.1 µm from birds in the free-range system receiving SBM and 39.47 µm from birds receiving SBM in the caged rearing system (Table 2.3).

**Table 2.2 Effects of rearing system and soybean-free diets on egg production (EP%) egg weight (EWT) feed per dozen eggs (FDE) and feed conversion ratio (FCR) over 6 periods of lay**

| Age (wk) | Dependent Variable | Caged Rearing | | | Free-range Rearing | | Pooled SEM |
|---|---|---|---|---|---|---|---|
| | | SBM | SBMF | | SBM | SBMF | |
| 21 to 24 | [1]EP% | 81.1 | 79.4 | | 68.4 | 67.1 | 7.14 |
| | [1]EWT g | 55.9 | 53.5 | | 52.2 | 49.5 | 1.04 |
| | FDE kg | 1.57 | 1.77 | | 1.75 | 2.07 | 0.09 |
| | FCR | 2.42 | 2.86 | | 2.81 | 3.52 | 0.17 |
| 25 to 28 | [2]EP% | 95.8$^{x}$ | 97.0$^{x}$ | | 93.6$^{xy}$ | 90.2$^{y}$ | 0.59 |
| | [1]EWT g | 58.6$^{a}$ | 55.9$^{b}$ | | 54.4$^{a}$ | 50.7$^{b}$ | 0.14 |
| | [2]FDE kg | 1.34$^{x}$ | 1.50$^{x}$ | | 1.37$^{x}$ | 1.86$^{y}$ | 0.02 |
| | [2]FCR | 1.91$^{x}$ | 2.24$^{y}$ | | 2.10$^{xy}$ | 3.05$^{z}$ | 0.03 |
| 29 to 32 | [2]EP% | 95.4$^{x}$ | 93.3$^{xy}$ | | 91.7$^{y}$ | 81.8$^{z}$ | 0.42 |
| | [1]EWT g | 60.5$^{a}$ | 56.4$^{b}$ | | 57.7$^{a}$ | 53.5$^{b}$ | 0.49 |
| | [2]FDE kg | 1.33$^{x}$ | 1.43$^{x}$ | | 1.54$^{x}$ | 2.35$^{y}$ | 0.04 |
| | [2]FCR | 1.88$^{x}$ | 2.15$^{x}$ | | 2.26$^{x}$ | 3.68$^{y}$ | 0.07 |
| 33 to 36 | [1]EP% | 92.7$^{a}$ | 91.9$^{b}$ | | 93.3$^{a}$ | 88.8$^{b}$ | 0.42 |
| | [1]EWT g | 62.1$^{a}$ | 57.2$^{b}$ | | 62.3$^{a}$ | 59.0$^{b}$ | 0.24 |
| | [1]FDE kg | 1.34 | 1.39 | | 1.97 | 1.94 | 0.08 |
| | FCR | 1.91 | 2.06 | | 2.66 | 2.74 | 0.14 |
| 37 to 40 | EP% | 91.9 | 94.0 | | 91.6 | 92.2 | 0.75 |
| | [1]EWT g | 63.4$^{a}$ | 59.0$^{b}$ | | 63.7$^{a}$ | 61.6$^{b}$ | 0.23 |
| | [2]FDE kg | 1.34$^{x}$ | 1.42$^{x}$ | | 2.15$^{y}$ | 1.91$^{y}$ | 0.03 |
| | [2]FCR | 1.85$^{x}$ | 2.10$^{x}$ | | 2.82$^{y}$ | 2.60$^{y}$ | 0.04 |
| 41 to 44 | [1]EP% | 97.5 | 97.2 | | 89.2 | 89.7 | 1.61 |
| | [2]EWT g | 63.6$^{x}$ | 59.6$^{z}$ | | 63.8$^{x}$ | 61.8$^{y}$ | 0.14 |
| | [1]FDE kg | 1.41$^{a}$ | 1.61$^{b}$ | | 1.81$^{a}$ | 2.01$^{b}$ | 0.03 |
| | [1]FCR | 1.94$^{a}$ | 2.36$^{b}$ | | 2.36$^{a}$ | 2.70$^{b}$ | 0.04 |

[1] Rearing system (whole plot) results in a significantly different response $P < 0.05$.
[a-b] Means within the row (for each facility) with different letters differ at $P < 0.05$.
[2] Significant rearing system by diet interaction $P < 0.05$.
[x-z] Means within the row (across diet and facility) with different letters differ at $P < 0.05$.

**Table 2.3 Effects of rearing system and soybean-free diets on albumen height (AH) Haugh unit (HU) shell thickness (ST) and breaking strength (BS) using cage and free-range rearing systems over 5 periods of lay**

| Age (wk) | Dependent Variable | Caged Rearing | | | Free-range Rearing | | Pooled SEM |
|---|---|---|---|---|---|---|---|
| | | SBM | SBMF | | SBM | SBMF | |
| 25 to 28 | [1]AH mm | 11.20 | 11.52 | | 12.31 | 12.12 | 0.16 |
| | [1]HU | 104.5 | 106.2 | | 109.3 | 109.0 | 0.49 |
| | [1]ST µm | 40.55 | 40.67 | | 39.77 | 39.15 | 0.39 |
| | BS kg | 4.63 | 4.73 | | 4.63 | 4.43 | 0.05 |
| 29 to 32 | AH mm | 10.17 | 10.27 | | 10.69 | 10.92 | 0.34 |
| | HU | 99.7 | 100.5 | | 102.9 | 103.6 | 1.45 |
| | [2]ST µm | $39.47^{y}$ | $39.83^{xy}$ | | $41.10^{x}$ | $39.83^{xy}$ | 0.30 |
| | BS kg | $4.68^{a}$ | $4.52^{b}$ | | $4.58^{a}$ | $4.13^{b}$ | 0.07 |
| 33 to 36 | AH mm | 9.43 | 9.62 | | 8.20 | 9.30 | 0.20 |
| | [1]HU | $96.2^{b}$ | $97.8^{a}$ | | $90.3^{b}$ | $95.5^{a}$ | 0.53 |
| | ST µm | 40.53 | 40.38 | | 41.47 | 39.35 | 0.41 |
| | BS kg | 4.67 | 4.50 | | 4.68 | 4.67 | 0.05 |
| 37 to 40 | [1]AH mm | 8.95 | 9.30 | | 8.47 | 8.67 | 0.15 |
| | 1HU | 93.4 | 95.9 | | 90.9 | 92.8 | 0.70 |
| | ST µm | 40.18 | 40.85 | | 41.30 | 41.05 | 0.18 |
| | BS kg | 4.47 | 4.63 | | 4.48 | 4.50 | 0.07 |
| 41 to 44 | [1]AH mm | $8.43^{b}$ | $8.85^{a}$ | | $8.77^{b}$ | $9.12^{a}$ | 0.08 |
| | HU | $90.8^{b}$ | $93.8^{a}$ | | $92.2^{b}$ | $94.9^{a}$ | 0.39 |
| | ST µm | 39.77 | 40.07 | | 40.22 | 39.93 | 0.14 |
| | BS kg | 4.50 | 4.43 | | 4.29 | 4.52 | 0.06 |

[1] Rearing system (whole plot) results in a significantly different response $P \leq 0.05$.
[a-b] Means within the row (for each facility) with different letters differ at $P \leq 0.05$.
[2] Significant rearing system by diet interaction $P \leq 0.05$. Tukey's means separation did not detect significant differences.
[x-z] Means within the row (across diet and facility) with different letters differ at $P < 0.05$.

To simplify the presentation of the key results learned from this study, the cumulative production data has been presented in Table 2.4 as a 2 x2 factorial. There was a difference (P=0.01) in cumulative EP% based on rearing system with hen day EP averaging 92.28±1.23% for the conventional cage system vs. 86.46±1.84% for the free-range system (Table 2.4).

Diet type did not significantly affect cumulative EP% (P>0.05). With respect to average cumulative egg weight we observed that eggs produced from hens fed the SBMF diets weighed less (P<0.01) than those eggs produced from hens fed the SBM diets averaging 56.48±0.60 and 59.85±0.59 g respectively. Main effects for both rearing system and diet were significantly different with respect to both average cumulative feed per dozen eggs and average cumulative feed conversion ratio in favor of the more traditional caged rearing system and SBM diets (Table 2.4).

Effects of rearing system and soy diets on cumulative albumen height, Haugh unit, shell thickness, and breaking strength using cage and free-range rearing systems over five periods of lay, presented as a 2x2 factorial in (Table 2.5). The rearing system by diet interaction for cumulative shell thickness was significant (P<0.02), averaging 40.77±0.19 µm for birds receiving SBM and 39.86±0.31 µm for hens receiving SBMF diets in the free-range system (Table 2.5). However, both systems and diets produced egg shell thickness above 33 µm which the minimum shell thickness to be within normal incidence of broken eggs.

**Table 2.4 Effects of rearing system and soybean-free diets on cumulative egg production, egg weight, feed per dozen eggs, and feed conversion ratio over 5 periods of lay presented as a 2x2 factorial**

| Rearing System | Diet | | |
|---|---|---|---|
| | SBM | SBMF | Main Effects |
| **Egg Production (%)** | | | |
| **Caged** | 92.40±1.63 | 92.16±1.87 | 92.28±1.23[a] |
| **Free-range** | 87.97±2.52 | 84.96±2.69 | 86.46±1.84[b] |
| **Main Effects** | 90.18±1.52 | 88.56±1.70 | |
| **Egg Weight (g)** | | | |
| **Caged** | 60.68±0.63 | 56.94±0.50 | 58.81±0.48 |
| **Free-range** | 59.02±0.99 | 56.03±1.09 | 57.52±0.76 |
| **Main Effects** | 59.85±0.59[a] | 56.48±0.60[b] | |
| **Feed per Dozen Eggs kg** | | | |
| **Caged** | 1.39±0.02 | 1.52±0.03 | 1.45±0.02[a] |
| **Free-range** | 1.76±0.07 | 2.02±0.06 | 1.89±0.05[b] |
| **Main Effects** | 1.57±0.04[a] | 1.77±0.05[b] | |
| **Feed Conversion Ratio** | | | |
| **Caged** | 1.98±0.04 | 2.29±0.05 | 2.14±0.04[a] |
| **Free-range** | 2.50±0.01 | 3.05±0.12 | 2.77±0.08[b] |
| **Main Effects** | 2.24±0.06[a] | 2.67±0.08[b] | |

[a,b] Means ± standard error within a main effects grouping with no common superscripts differ significantly ($P<0.05$).

**Table 2.5 Effects of rearing system and soybean-free diets on cumulative albumen height, Haugh unit, shell thickness, and breaking strength using cage and free-range rearing systems over 5 periods of lay, presented as a 2x2 factorial**

| Rearing System | Diet | | |
|---|---|---|---|
| | SBM | SBMF | Main Effects |
| **Albumen Height (mm)** | | | |
| **Caged** | 9.64±0.22 | 9.91±0.21 | 9.77±0.15 |
| **Free-range** | 9.69±0.32 | 10.02±0.32 | 9.85±0.23 |
| **Main Effects** | 9.66±0.19 | 9.97±0.19 | |
| **Haugh Unit** | | | |
| **Caged** | 96.92±1.05 | 98.82±0.94 | 97.87±0.71 |
| **Free-range** | 97.10±1.54 | 99.16±1.36 | 98.13±1.02 |
| **Main Effects** | 97.01±0.92 | 98.99±0.82 | |
| **[2]Shell Thickness ( µm)** | | | |
| **Caged** | 40.10±0.24[ab] | 40.36±0.20[ab] | 40.23±0.16 |
| **Free-range** | 40.77±0.19[a] | 39.86±0.3[b] | 40.32±0.19 |
| **Main Effects** | 40.44±0.16 | 40.11±0.18 | |
| **Breaking Strength (kg)** | | | |
| **Caged** | 4.59±0.06 | 4.56±0.05 | 4.58±0.04 |
| **Free-range** | 4.53±0.05 | 4.45±0.07 | 4.49±0.04 |
| **Main Effects** | 4.56±0.04 | 4.51±0.04 | |

[2] Significant rearing system by diet interaction $P \leq 0.05$.
[a,b] Means ± standard error within a grouping with no common superscripts differ significantly ($P<0.05$).

Conventional cage systems used by the poultry industry have both advantages and disadvantages. On a positive side, because modern systems are fully automated, with the birds sitting in relatively limited size cages, it allows for better disease control, lower infection rates, easier management, and lower production costs (Duncan, 2001). In addition, this system improves egg hygiene, with greater hen livability (Hannah, et al., 2011). On the negative side, this system can prevent hens from expressing innate behaviors, which may lead to metabolic disorders and other movement restriction disorders (Dikmen, et al., 2016).

Concerns over hen welfare have been heavily publicized since the 1960s, following the publication of Ruth Harrison's book "Animal Machines", and the Brambell Report in the United Kingdom (Britain and Brambell, 1965; Harrison, 2013). An example of animal welfare that have been associated with cage system is freedom of movement (Mench, et al., 2011). Another factor that led to the development of alternative rearing systems was the increasing incidence of osteoporosis in hens (Regmi, et al., 2016), because conventional cages restrict movement and impact tibia properties during the laying phase. Other problems, such as manure handling and fly control can be more difficult to alleviate with this limited cage rearing systems (Lay, et al., 2011).

Cage-free, free-range, and pasture-raised rearing systems are alternatives to the conventional cage rearing systems. These approaches are reported to have several advantages: hens are able to walk, exercise, stretch their legs and wings, and express other natural behaviors, such as dust-bathing and foraging (AVMA, 2012). However, non-cage or enriched facilities can negatively affect egg safety and quality, since eggs can be altered microbiologically by pathogens such as *Salmonella Enteritidis*, or chemically, due to contamination from pesticides or heavy metals (Holt, et al., 2011).

Scientists have found that bacteria levels on washed and unwashed eggs were higher for hens raised on shavings and slat conditions, compared to hens raised in cages (Hannah, et al., 2011). One study, for example, compared 3 hen-rearing facilities (conventional cage, enriched colony cage, and free-range aviary) on several environment conditions, including egg safety, worker safety, and general hen health (Jones, et al., 2015). The study found that egg safety was enhanced with the use of nest boxes, and floor eggs had higher levels of human pathogens. The total aerobes (5.3 – 7.5 log cfu/mL) and total coliform count (1.6 – 4.0 log cfu/mL) were generally higher in free-range aviary system compare to conventional cage system (4.8 and 2.3 log cfu/Ml, respectively), and enriched colony cage (4.7 – 5.6 and 1.7 - 3.8 log cfu/mL respectively). The prevalence of *Salmonella* spp. in the environmental swabs was less in enriched colony cage wire and nest box (16% of the total swabs for both locations) compare to the same locations of the aviary system (18% and 28%, respectively).

The typical corn/SBM diet is the most common diet for laying hens across the United States. However, consumers have grown increasingly concerned about the presence of GMO ingredients used in animal diets. This has created new opportunities for non-traditional diets formulated with non-GMO and/or organic labeled ingredients other than SBM.

Our SBMF diet contained 15% DDGS, and 14.94% CSM (Table 2.1). We did not observe any unacceptable pigmentation from hens fed the SBMF diet containing CSM. Additionally, corn gluten meal (CGM) can provide additional protein although its lysine concentration is rather low. Corn gluten meal is also a good source of xanthophyll pigments important in maintaining egg yolk color. Our SBMF diet contained average of 1.5% CGM for both 19 to 38 and 39 to 44 wk.

In conclusion, the traditional cage system resulted in improved cumulative egg production, feed per dozen eggs and feed conversion (g feed/g egg) compared with the free-range system. The results also suggest free-range production is more variable than traditional closed house cage systems with standard errors sometimes twice that of the cage system. Cumulative egg production, feed per dozen eggs and feed conversion ratio (g feed/g egg) were 92±1.23 and 86±1.84%, 1.45±0.02 and 1.89±0.05 kg, 2.14±0.04 and 2.77±0.08 ($P$ <0.05), respectively, for the caged versus free-range rearing systems. Cumulative egg weight, feed per dozen eggs and feed conversion ratio were 59.9±0.59 and 56.5±0.60 g, 1.57±0.04 and 1.77±0.05 kg, 2.24±0.06 and 2.67±0.08 kg ($P$ <0.05) for SBM and SBMF diets, respectively. Diet did not affect cumulative egg production ($P$ >0.05).

With respect to egg quality, there were no differences in cumulative albumen height, Haugh unit or breaking strength but there was a significant rearing system by diet interaction for shell thickness with the free-range hens averaging 40.77±0.19 and 39.86±0.31 μm ($P$ < 0.05), respectively, for the hens fed SBM vs. SBMF diets. In addition, SBMF diets containing $\leq$ 15% CSM can be used in both caged and free-range production systems without affecting egg production, although would expect lower egg weights.

# CHAPTER III

# SENSORY EVALUATION AND CONSUMER ACCEPTANCE OF EGGS FROM HY-LINE BROWN LAYERS FED SOYBEAN AND SOYBEAN MEAL FREE DIETS USING CAGE AND FREE-RANGE REARING SYSTEMS

## INTRODUCTION

The poultry industry has undergone significant changes with respect to moving toward cage-free rearing systems recently, and housing space for layers has become a common topic of research and discussion now in the United States. Several food retailers and food service chains, such as Wal-Mart and McDonalds, have announced their pledges to only sell or use eggs produced only by cage-free laying hens. This move would require a change from the current conventional cage facilities to cage-free systems in order to meet the increased demand for cage-free eggs in the near future. This change may affect the table egg price and also increase consumers' concern about whether to buy cage versus cage-free.

The popular press is full of articles suggesting that eggs taste better from free-range hens and are also better for both consumer health and the birds as well. This of course has much more to do with the hens' diet rather than the rearing environment, but they are intertwined. As all ingredients may have both positive and negative impacts on egg properties, it is important to understand all the factors that can affect the layers` performance and the egg quality. Sensory analysis is a beneficial procedure that is used to assess consumer acceptance or preference of products using human senses such as smell, sight, and taste (Meilgaard, et al., 2007) and can provide scientific evidence with respect to the claims. A smaller segment of the population also has concerns about the use of

SBM in poultry feeds primarily because most the SBM used in the U.S. are genetically modified GMO. The goal of this study was to evaluate consumer acceptance of eggs from cage and free-range rearing systems fed SBM and SBMF diets utilizing CSM, and DDGS through scrambled and hard cooked sensory analysis.

## MATERIALS AND METHODS

### Laying Hens' Diets and Housing Environment

This study was conducted at the Texas A & M University (TAMU) Poultry Research Center and received approval from the university's Animal Care and Use Committee (IACUC 2014-0030). Over a period of 2 days eggs were collected from 36 week old Hy-line Brown hens that had been previously assigned to 2 diets from the onset of their egg production: SBM and SBMF diets made up of CSM, DDGS, and wheat midds.

Diets (Table 3.1) were formulated based on the recommendations of the management guidelines for Hy-line Brown laying hens. Two rearing facilities were used in this study to house the hens; individual laying hen cages 50.8 W × 30.5 L × 30.5 H cm cage (1,549 $cm^2$/hen), and free-range system consisting of a 182.9 W × 365.8 L cm indoor area and (182.9 W × 731.5 L cm) outdoor area located at the Poultry Research Center on the campus of Texas A&M University (College Station, TX). Each free-range enclosure housed 21 hens and was equipped with 6 nesting boxes within the indoor area.

**Table 3.1 Composition and nutrient levels of SBM and SBMF diets**

| | 19 to 38 wk | |
|---|---|---|
| **Ingredients** | **SBM %** | **SBMF %** |
| **Corn** | 64.11 | 46.99 |
| **Dehulled Soybean Meal** | 20.04 | - |
| **Cottonseed Meal** | - | 14.94 |
| **Corn Gluten Meal** | 0.38 | 1.38 |
| **Distillers Dried Grains with Solubles** | - | 15.00 |
| **Wheat Midds** | - | 3.59 |
| **DL-Methionine 98** | 0.35 | 0.36 |
| **L-Threonine 98** | - | 0.03 |
| **Lysine HCL** | 0.24 | 0.66 |
| **AV Fat Blend** | 2.75 | 4.73 |
| **Limestone** | 9.76 | 9.92 |
| **Mono-Dical PO4** | 1.66 | 1.65 |
| **Salt** | 0.29 | - |
| **Sodium Bicarbonate** | 0.12 | 0.44 |
| **Trace Minerals**[1] | 0.05 | 0.05 |
| **Vitamins**[2] | 0.25 | 0.25 |
| **Calculated nutrient composition (%)** | | |
| **ME(kcal/kg)** | 2911 | 2911 |
| **Crude Protein** | 16.50 | 16.50 |
| **Crude Fat** | 4.58 | 7.36 |
| **Crude Fiber** | 1.71 | 4.18 |
| **Calcium** | 4.08 | 4.08 |
| **Phosphorous** | 0.68 | 0.78 |
| **AV Phosphate** | 0.45 | 0.45 |
| **AV Methionine** | 0.58 | 0.58 |
| **AV Lysine** | 0.90 | 0.90 |
| **AV TSAA** | 0.80 | 0.80 |
| **Xanthophyll (mg/kg)** | 12 | 12 |

[1]Trace minerals premix added at this rate yields (mg/kg): zinc, 60; manganese, 60; iron, 60.0; copper, 7.0; iodine, 0.4.

[2]Vitamin premix added at this rate yields (per kg): vitamin A, 11 KIU; vitamin $D_{3,}$ 3,850 IU; vitamin E, 45.8 IU; menadione, 1.5 mg; $B_{12,}$ 0.017 mg; biotin, 0.55 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; $B_{6,}$ 7.15 mg; niacin,45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

**Sensory Panel**

Consumer acceptability trials were conducted to determine consumers` acceptance of scrambled and hard cooked eggs from hens fed SBM and SBMF diets using cage and free-range rearing systems. Consumers (n=60) made up of TAMU students, faculty and staff ages 18 to 50 were recruited to evaluate egg samples. The sensory tests were approved by the TAMU Office of Research Compliance and Bio-safety Institutional Review Board (IRB) for the use of Human Subjects in Research (IRB2015-0547M). After 16 wk of production a total of 60 eggs per treatment were collected for each test. All eggs were stored in a food cooler (4 °C) at the sensory lab for a day prior to each test.

Based on the procedure for sensory evaluation of scrambled eggs (Al-Ajeeli, et al. (2016), eggs were beaten in four separate bowls by treatment for 2 minutes to ensure a homogenous mixture and then scrambled) by treatment using four separate pans (Rival model CKRVSK11 Skillet, Boca Raton, FL 33431). Canola oil spray was used to coat the bottom of the pan prior to cooking. The same concentration of spray was used to ensure identical cooking methods. All scrambled eggs were cooked to the same endpoint temperature of 350°F (176.7°C). Samples were placed into four separate stainless steel containers with lids under the heat from heat lamps to maintain the temperature and make sure the samples were presented warm and served within 15 m of cooking.

Consumers were seated in separate booths, and provided with one tablespoon of cooked eggs per treatment. Samples were served in clear, plastic soufflé cups and consumers were presented with unsalted saltine crackers and a cup of distilled deionizer water as palate cleansers between samples. Samples were placed in separate weigh boats labeled with 3 random digit codes to avoid visual bias. Consumers evaluated the four

treatments using 9-point hedonic scales for liking and disliking for flavor and texture of scrambled eggs and flavor, texture, color, and odor liking and disliking were evaluated for hard cooked eggs.

For the hard cooked eggs, eggs were cooked in an egg cooker (Dash go Model DEC005, Storebound, LLC, NY) at the same time for 12-15 minutes to ensure consistency of the time, and were served under white light so panelists could recognize if there were any differences in yolk color between samples.

**Statistical Analysis**

The sensory data were analyzed by ANOVA as a 2x2 factorial with the main effects being cage or cage-free rearing system and SBM or SBMF diets using an alpha $<0.05$ for main effect means of ANOVA unless otherwise indicated. No significant interactions were observed.

**RESULTS AND DISCUSSION**

Currently, the multiple wire pen cage system is the most common type of laying hen rearing system in the United States. This continues to be a topic for much discussion across the country (Jones, et al., 2016) as a movement toward cage-free rearing systems is underway. However, the lack of laying hen system variety is considered a deficiency of US commercial-scale research (Swanson, et al., 2015).

Many food service retailers and suppliers have pledged to provide eggs produced by cage-free laying hens. The metabolic requirements for cage-free hens is different from those of caged hens because of different allocation of maintenance energy due to differences in locomotion activity (Goldstein, 1988). This increase in energy requirements is also associated

with increased demand for available nutrients. As a result, laying hens' egg production and egg quality can be affected by a shift to cage-free systems.

For this study, identical diets were fed to hens for both the cage and free-range rearing system. Birds maintained within the free-range system had access to insects and vegetation growing within the free-range area. However, after 4 months of production the free-range area was mostly devoid of the vegetation that covered the area at the onset of the production study.

Consumers did not detect a difference in flavor of the scrambled eggs (*P>0.05;* Table 3.2). However, consumers did prefer the texture of the scrambled eggs from the free-range system (*P*=0.064). It's not clear why rearing system would affect texture.

**Table 3.2 Overall mean consumer scores of flavor and texture for scrambled eggs from hens fed SBM and SBMF diets using cage and free-range rearing systems[1]**

| | Flavor[2] | | |
|---|---|---|---|
| | Cage | Free-range | Mean |
| SBM | 6.53±1.92 | 6.32±1.80 | 6.43±1.85 |
| SBMF | 6.67±1.73 | 6.72±1.74 | 6.69±1.73 |
| Mean | 6.60±1.82 | 6.52±1.77 | |
| | Texture[1] | | |
| SBM | 6.91±1.86 | 6.39±1.88 | 6.65±1.88[b] |
| SBMF | 7.12±1.76 | 7.05±1.59 | 7.08±1.67[a] |
| Mean | 7.02±1.80 | 6.72±1.77 | |

[1]Hens had fed the test feeds 16 wk prior to sensory evaluation.
[2] Means ± Standard Deviation
[ab]Texture means within the column are significantly different (*P*=0.064)
n=60

When asked to evaluate flavor of the hard cooked eggs, consumer preference favored ($P$= 0.014) the eggs from the raised wire individual bird caging system, 7.11 vs 6.60 for the free-range rearing system (Table 3.3). With respect to texture, consumers preferred the texture of the SBM diet (6.91) compared to the SBMF diet (6.30). It is of interest to note that this is the opposite of what we found for the scrambled eggs. No other significant differences were detected for any of the sensory variables evaluated.

**Table 3.3 Overall mean consumer scores of flavor, texture, color and odor for hard cooked eggs from hens fed SBM and SBMF diets using cage and free-range rearing systems[1]**

| | Flavor[2] | | |
|---|---|---|---|
| | Cage | Free-range | Mean |
| SBM | 7.17±1.44 | 6.88±1.54 | 7.02±1.49 |
| SBMF | 7.05±1.48 | 6.32±1.85 | 6.68±1.71 |
| Mean | 7.11±1.45[a] | 6.60±1.72[b] | |
| | Texture[1] | | |
| SBM | 7.02±1.87 | 6.80±1.84 | 6.91±1.85[a] |
| SBMF | 6.57±1.91 | 6.03±2.25 | 6.30±2.01[b] |
| Mean | 6.79±1.90 | 6.42±2.08 | |
| | Color[1] | | |
| SBM | 6.55±1.73 | 6.88±1.65 | 6.72±1.69 |
| SBMF | 6.70±1.75 | 6.60±1.96 | 6.65±1.85 |
| Mean | 6.63±1.73 | 6.74±1.81 | |
| | Odor[1] | | |
| SBM | 6.95±1.44 | 6.67±1.66 | 6.81±1.56 |
| SBMF | 6.75±1.51 | 6.50±1.65 | 6.63±1.58 |
| Mean | 6.85±1.48 | 6.58±1.65 | |

[1]Hens had fed on the test feeds 16 wk prior to sensory evaluation.
[2] Means ± Standard Deviation
[ab] Main effect means within the row or column are significantly different ($P \leq 0.05$)
n=60

In addition to animal welfare concerns, some consumers are concerned about the use of SBM as a major ingredient in layer feed and niche markets have developed for eggs produced from hens never receiving soybean products in their diets. For this study we created a SBMF diet using 15% CSM and 15% DDGS along with some wheat midds and corn gluten meal in place of dehulled SBM (Table 3.1).

With respect to scrambled eggs, we observed a main effect of diet ($P$=0.064) for consumer scores on texture and consumers like of the texture of eggs from the SBMF diet (7.08 and 6.65 for SBMF and SBM diets, respectively; Table 2.2). It was interesting to note that with respect to hard cooked eggs, consumers preferred ($P$=0.018) the texture of eggs originating from hens fed the SBM diet, averaging 6.91 and 6.30 for the SBM and SBMF diets, respectively. It would be expecting that the method of cooking (scrambling versus boiling) can affect consumer preference.

From this study, it can be concluded that consumers are more likely to detect flavor differences in hard cooked eggs versus scrambled eggs. In contrast to much of the public press, our consumer panel preferred hard cooked eggs from the caged rearing system over the free-range system. It was also interesting to note that diet affected consumer scores for texture in favor of the SBMF diet for scrambled eggs and SBM for the hard cooked eggs.

# CHAPTER IV

# EVALUATION OF YEAST CELL WALL PRODUCT IN SOYBEAN AND SOYBEAN-FREE DIETS FED TO HY-LINE BROWN LAYING HENS BASED ON EGG PRODUCTION, EGG QUALITY, AND ILEUM DIGESTIBILITY

## INTRODUCTION

The use of antibiotic growth promotors has historically been adapted by the poultry industry to enhance feed conversion ratio, animal growth, and to reduce morbidity and mortality that can occur due to clinical diseases (Butaye, et al., 2003). Concerns of antimicrobial resistance and transference of antibiotic resistant genes from animal to human microbiota resulted in the European Union ban in 2006 (Castanon, 2007). In the United States, there has also been increasing interest in developing new feed additives to replace traditional antibiotics for poultry production. This issue has motivated the poultry industry to find alternative products such as prebiotics and probiotics that can be used in poultry diets without any harm or risk to both animal and human health.

Prebiotics and probiotics have been used and consumed for centuries either as natural foods or processed as fermented products (Patterson and Burkholder, 2003). Prebiotics are defined as non-digestible food ingredients that benefit the host by selectively stimulating the growth and activity of one type or a limited number of bacteria in the colon, resulting in improvement of the host's health (Gibson and Roberfroid, 1995).

The term probiotic is based on the meaning of the word "life" in the Greek language, and the concept is based on the use of microorganisms that beneficially affect the host and improve the intestinal microbial balance (Schrezenmeir and de Vrese, 2001).

Poultry productivity is primarily associated with achieving maximum efficiency of nutrient utilization at a given cost. Yeast cell wall (YCW) is a prebiotic that has been widely used for poultry feed to enhance nutrient digestibility. It consists primarily of three components: glucan, mannoprotein, and chitin (Ballou, 1982). This product is produced by the autolysis of yeast and the separation of the insoluble cell wall from the soluble portion of the yeast cell by centrifugation (Hashim, et al., 2013). Yeast cell wall has been demonstrated to have a positive impact on egg production, feed conversion ratio (FCR), and egg quality parameters. Specifically, researchers have found that supplementation with YCW improved the relative economic efficiency of laying hens compared to hens not fed this supplementation (Hassan and Ragab, 2007).

A study conducted at Texas A&M University indicated that Hy-line W-36 laying hens fed YCW at levels of 250 ppm had egg weights higher than those eggs coming from hens fed a diet not supplemented with YCW during the first 8 weeks of initial egg production (Hashim, et al., 2013). The authors stated that liquid egg yolk was improved by feeding the hens YCW at 250 ppm and specific gravity, egg shell thickness, egg shell weight, and shell weight percentages were higher when hens were fed YCW at 500 ppm versus 250 ppm at 36 weeks of age. Hassanein and Soliman (2010) reported that feeding Hy-line W-36 hens active live yeast at levels of 0.4 or 0.8% (4000 or 8000 ppm) enhanced productive performance and nutrient utilization. They indicated that this live yeast supplementation positively affected FCR; comparing the intestinal microflora make-up, they found that the ileal content pH was lower in the live yeast supplemented birds than in the control group.

Although soybean meal (SBM) is the primary oil seed meal used in the United States as a source of vegetable protein in animal feeds, there are several anti-nutrient factors

associated with it such as trypsin inhibitors, lectins, saponins and non-starch polysaccharides. There are also concerns that most the SBM in the United States are genetically modified GMO. A segment of our population is thus interested in food products not associated with SBM.

To the best of our knowledge, research related with the use of YCW in SBMF diets (SBMF) is limited, if not non-existent. Also, the effects of YCW on egg production, egg quality characteristics, and ileum digestibility in post peak Hy-line Brown laying hens has not been studied. The aim of this experiment was to investigate the influence of YCW prebiotic supplementation in both SBM and SBMF diets for Hy-line Brown laying hens during the latter half of their egg production cycle. The influence was evaluated based on egg production, egg quality characteristics, and ileum digestibility.

## MATERIALS AND METHODS

### Birds, Diets, and Management

This experiment was conducted at the Texas A&M University Poultry Research Center, and received approval from the University`s Animal Care and Use Committee (IACUC 2017-0072). This experiment was performed to evaluate a prebiotic YCW product in Hy-line Brown commercial layers fed SBM and SBMF diets based on egg production, and egg quality, and ileum digestibility over four 28-day periods from 47 to 62 week old. Using a randomized block design a total of 120 layers at 43 weeks old were distributed in wire cages (30.5 W x 30.5 L x 50.8 H cm) with an individual hen per cage. For this experiment, the YCW treatment (0, 250 ppm) was overlaid with the SBM, and SBMF dietary treatments to create a 2 x 2 factorial arrangement of 3 randomized complete pen location blocks throughout the hen house (10 hens per treatment block).

Treatments were SBM control, SBM + YCW, SBMF control, and SBMF + YCW. Corn-based diets were formulated based on the nutrient requirements suggested by the 2014 Hy-line Brown management guide. The SBMF diet utilized CSM, CGM, DDGS, and wheat middlings in place of soybean meal. Feed and water were offered ad libitum. All treatments were formulated to have equal calculated nutritional content (based on standardized ileal digestible amino acids) and were provided in mash form (Table 4.1). The SBM and SBMF diets were fed for 4 wk and YCW for 2 wk prior the beginning of data collection at 47 weeks of age.

**Table 4.1 Composition and nutrient levels of SBM and SBMF diets with or without 250 ppm YCW from 47 to 62 wk of age**

| | 47 to 55 wk | | 55 to 62 wk | |
|---|---|---|---|---|
| **Ingredients** | **SBM%** | **SBMF %** | **SBM%** | **SBMF%** |
| **Corn** | 64.17 | 42.22 | 65.67 | 51.19 |
| **Dehulled Soybean Meal** | 21.17 | - | 19.56 | - |
| **Cottonseed Meal** | - | 15.00 | - | 15.00 |
| **Corn Gluten Meal** | 0.38 | 1.66 | 0.29 | 1.14 |
| **Dried Distiller Grains** | - | 15.00 | - | 15.00 |
| **Wheat Midds** | - | 8.31 | - | 1.08 |
| **DL-Methionine 98** | 0.17 | 0.22 | 0.15 | 0.16 |
| **L-Threonine 98** | - | 0.06 | - | - |
| **Lysine HCL** | - | 0.48 | 0.02 | 0.43 |
| **AV Fat Blend** | 2.12 | 5.00 | 1.27 | 2.74 |
| **Limestone** | 9.91 | 10.1 | 11.0 | 11.1 |
| **Mono-Dical PO4** | 1.42 | 1.37 | 1.43 | 1.44 |
| **Salt** | 0.38 | 0.04 | 0.33 | 0.04 |
| **Sodium Bicarbonate** | - | 0.27 | 0.03 | 0.37 |
| **Trace Minerals**[1] | 0.05 | 0.05 | 0.05 | 0.05 |
| **Vitamins**[2] | 0.25 | 0.25 | 0.25 | 0.25 |
| **Calculated Nutrient Composition (%)** | | | | |
| **ME(kcal/kg)** | 2867 | 2867 | 2800 | 2800 |
| **Crude Protein** | 16.75 | 16.75 | 16.00 | 16.00 |
| **Crude Fat** | 3.95 | 7.66 | 3.13 | 5.40 |
| **Crude Fiber** | 1.75 | 4.47 | 1.71 | 4.05 |
| **Calcium** | 4.10 | 4.10 | 4.50 | 4.50 |
| **Phosphorous** | 0.64 | 0.75 | 0.63 | 0.73 |
| **AV Phosphate** | 0.40 | 0.40 | 0.40 | 0.40 |
| **[3]AV Methionine** | 0.41 | 0.44 | 0.38 | 0.38 |
| **[3]AV Lysine** | 0.74 | 0.77 | 0.71 | 0.71 |
| **[3]AV TSAA** | 0.64 | 0.67 | 0.60 | 0.60 |
| **Xanthophyll (mg/kg)** | 12 | 12 | 12 | 12 |

[1]Trace minerals premix added at this rate yields (mg/kg): zinc, 60.0; manganese, 60.0; iron, 60.0; copper, 7.0; iodine, 0.4.

[2]Vitamin premix added at this rate yields (per kg): vitamin A, 11 IU; vitamin $D_{3,}$ 3,850 IU; vitamin E, 45.8 IU; menadione, 1.5 mg; $B_{12,}$ 0.017 mg; biotin, 0.55 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; $B_{6,}$ 7.15 mg; niacin,45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

[3]Standardized digestibility Coefficients for cottonseed Methionine, Lysine, and TSAA were 0.73, 0.67 and 0.73.

**Data Collection**

Egg production and hen mortality were assessed daily. Hen body weight was recorded every 28 days to ensure that hens were not under or over weight. Feed consumption was recorded every 2 weeks. Eggs were collected daily for egg production evaluation, and egg weight was recorded weekly. For egg quality parameters, a subset of 3 eggs were evaluated every 2 weeks, as described in Chapter II, to assess albumen height, shell thickness, eggshell strength, and Haugh unit.

**Ileal Digestibility Samples**

At the end of the study (62 wk of age), ileal digesta procedures were performed on 6 birds per treatment. Titanium dioxide was added to the feed as a marker for 2 days prior to the sample collection. Ileal digesta samples were taken from the lower half of the ileum, about 2 cm above the ileal-cecal junction towards Meckel`s diverticulum. These samples were placed in 50 ml plastic centrifuge tubes and stored immediately at -20°C. Then, samples were freeze-dried (Thermovac) and ground (Mr. Coffee, Sunbeam Products Inc., Boca Raton, FL) for further analysis.

At the same time, diet samples were collected for the analysis as well. Both ileal digesta (3 pooled samples per treatment) and the 4 diet samples were sent to a laboratory of the University of Missouri (Agriculture Experiment Station Chemical Laboratories, University of Missouri, Colombia, MO) for essential amino acid and titanium analysis to determine apparent and standardized ileal digestibility.

**Statistical Analysis**

The YCW supplementation was overlaid on the 2 dietary treatments in a randomized block design to create a 2 X 2 factorial arrangement of 3 pen location blocks throughout the hen house. All data were analyzed using General Linear Model Procedures within IBM SPSS software. Average cumulative data was analyzed as 2 X 2 full factorial and production period and Ileal digestibility data were analyzed as a one-way ANOVA, and means were separated using Tukey's HSD procedure at $P \leq 0.05$.

**RESULTS AND DISCUSSION**

Egg production (EP%), egg weight (EWT), feed per dozen eggs (FDE), and feed conversion ratio (FCR) over 4 different periods of lay are presented in Table 4.2 based on One-Way ANOVA.

For egg production, during the first (47-50 wk) and the second (51-54 wk) periods, the supplementation of YCW improved egg production percentage for the SBM diet significantly compared to the other treatments (Table 4.2). In both periods, laying hens that were fed SBM with YCW had significantly higher egg production, approximately 95, and 93%, respectively while other treatments of the SBM control, SBMF control, and SBMF with YCW were less than 90%. According to the Hy-line Brown management guide, egg production percentage would be expected to be just under 90% at this age. This indicates that YCW supplementation has a positive impact in corn/SBM diets.

**Table 4.2 Effect of YCW product in SBM and SBMF diets based on egg production (EP), egg weight (EWT), feed per dozen eggs (FDE) and feed conversion ratio (FCR) over 4 periods of lay**

| Age (week) | Dependent Variable | SBM Control | SBM + YCW | SBMF Control | SBMF + YCW |
|---|---|---|---|---|---|
| | **EP%** | $88.4^{b} \pm 1.76$ | $95.6^{a} \pm 1.64$ | $86.2^{b} \pm 1.76$ | $86.3^{b} \pm 1.92$ |
| **47-50** | **EWT g** | $63.6^{a} \pm 0.47$ | $63.3^{a} \pm 0.76$ | $62.1^{ab} \pm 0.41$ | $60.1^{b} \pm 0.44$ |
| **wk** | **FDE kg** | $1.58 \pm 0.05$ | $1.47 \pm 0.05$ | $1.63 \pm 0.05$ | $1.63 \pm 0.06$ |
| | **FCR** | $2.07^{ab} \pm 0.07$ | $1.94^{a} \pm 0.03$ | $2.19^{b} \pm 0.07$ | $2.26^{b} \pm 0.07$ |
| | **EP%** | $85.8^{b} \pm 2.76$ | $93.9^{a} \pm 1.05$ | $87.8^{ab} \pm 1.76$ | $88.2^{ab} \pm 1.76$ |
| **51-54** | **EWT g** | $63.7 \pm 0.63$ | $63.1 \pm 0.80$ | $61.5 \pm 0.49$ | $61.3 \pm 0.68$ |
| **wk** | **FDE kg** | $1.57^{a} \pm 0.04$ | $1.52^{a} \pm 0.04$ | $1.79^{ab} \pm 0.11$ | $2.09^{b} \pm 0.17$ |
| | **FCR** | $2.06^{a} \pm 0.05$ | $2.01^{a} \pm 0.04$ | $2.42^{ab} \pm 0.13$ | $2.84^{b} \pm 0.22$ |
| | **EP%** | $81.0^{c} \pm 2.02$ | $92.2^{a} \pm 1.17$ | $84.7^{bc} \pm 2.43$ | $90.3^{ab} \pm 1.32$ |
| **55-58** | **EWT g** | $63.9^{a} \pm 0.60$ | $64.0^{a} \pm 0.73$ | $61.2^{b} \pm 0.45$ | $60.2^{b} \pm 0.49$ |
| **wk** | **FDE kg** | $1.77^{ab} \pm 0.04$ | $1.64^{a} \pm 0.05$ | $1.98^{b} \pm 0.12$ | $1.93^{ab} \pm 0.10$ |
| | **FCR** | $2.32^{ab} \pm 0.04$ | $2.13^{a} \pm 0.05$ | $2.69^{b} \pm 0.16$ | $2.67^{b} \pm 0.14$ |
| | **EP%** | $82.4^{b} \pm 1.86$ | $92.5^{a} \pm 0.86$ | $79.2^{b} \pm 2.35$ | $82.8^{b} \pm 2.14$ |
| **59-62** | **EWT g** | $64.0^{a} \pm 1.03$ | $62.8^{ab} \pm 0.78$ | $62.3^{ab} \pm 0.14$ | $60.7^{b} \pm 0.47$ |
| **wk** | **FDE kg** | $1.77^{ab} \pm 0.08$ | $1.62^{a} \pm 0.05$ | $2.10^{b} \pm 0.15$ | $1.96^{ab} \pm 0.09$ |
| | **FCR** | $2.30^{ab} \pm 0.10$ | $2.15^{a} \pm 0.06$ | $2.80^{c} \pm 0.20$ | $2.68^{bc} \pm 0.11$ |

[a-c] Means within the row with different letters differ at $P \leq 0.05$.

For the second period of the study (51-54 wk), there was a significant difference between the SBM with YCW (93.9%) vs the control group (85.8%), and no significant difference in both SBMF control and SBMF with YCW treatments. In period 3 (55-58 wk), the SBM control diet had the lowest egg production at 81.0% with the YCW supplementation in both SBM and SBMF diets at 92.2% and 90.3% respectively. This it appears that YCW supplementation was effect during those months regardless of diet type, SBM or SBMF.

The cumulative effects of YCW supplementation on average EP, EWT, FDE, and FCR over all 16 weeks of the study is presented in Tables 4.3, 4.4, 4.5, and 4.6 respectively as a 2x2 factorial arrangement to address both main effects and interactions between YCW inclusion and diet type.

There was an interaction observed ($P<0.05$) between diet type and YCW supplementation on average EP% analyzed as a 2x2 factorial over the entire 16 weeks of the study (Table 4.3). Hens fed the SBM diet with YCW prebiotic had significantly higher EP% at 93.6% hen day production versus all other treatment combinations.

Egg weights from hens receiving the SBMF diets were consistently lower than those from the hens fed the SBM diet until the final period of data collection where egg weights from birds fed the SBMF diet and not receiving the YCW prebiotic were not lower ($P>0.05$) than either SBM treatment (Table 4.2). When average egg weight over the entire 16 wk of the experiment was analyzed as a 2x2 factorial, a significant main effect differences for both diet type and YCW treatment was found (Table 4.4). Hens not receiving the YCW prebiotic produced average egg weights significantly more (62.8 g) than those fed 250 ppm YCW (61.9 g). Hens fed the SBM diets produced average egg

weights significantly more (63.5 g) than those fed the SBMF diets (61.2 g). This is in contrast to Hashim et.al, (2013) who reported Hy-Line W-36 hens fed YCW produced heavier eggs than the control birds not receiving YCW. It should be noted the egg production was higher in the YCW supplemented birds which perhaps why egg weights were a little lower in this experiment. The YCW treatment did result in higher overall egg weight yields due the higher rate of egg production.

**Table 4.3 Effect of YCW supplementation on average egg production (EP%) over all 16 weeks of the study**

| Diet | Yeast Cell Wall Inclusion (ppm) | | |
|---|---|---|---|
| | **0** | **250** | **Total-Diet** |
| **SBM** | 84.4 ± 1.1[b] | 93.6 ± 0.6[a] | 89.0 ± 0.7 |
| **SBMF** | 84.5 ± 1.5[b] | 86.9 ± 0.9[b] | 85.7 ± 0.7 |
| **Total-YCW** | 84.5 ± 0.8 | 90.2 ± 0.6 | 87.3 ± 0.5 |

[a-b] Significant Interaction ($P \leq 0.05$)
± Standard Error

**Table 4.4 Effect of YCW supplementation on average egg weight (EWT g) over all 16 weeks of the study**

| Diet | Yeast Cell Wall Inclusion (ppm) | | |
|---|---|---|---|
| | **0** | **250** | **Total-Diet** |
| **SBM** | 63.8±0.4 | 63.3±0.4 | 63.5±0.3[a] |
| **SBMF** | 61.8±0.2 | 60.6±0.3 | 61.2±0.2[b] |
| **Total-YCW** | 62.8±0.2[a] | 61.9±0.3[b] | 62.4±0.2 |

[a-b] Significant Main Effect ($P \leq 0.05$)
± Standard Error

Feed per dozen eggs from hens receiving the SBM diets were consistently lower than those from the hens fed the SBMF diet throughout the 4 periods of data collection. During the final period of data collection, FDE from birds fed the SBM diet and receiving the YCW prebiotic was lower ($P<0.05$) than SBMF treatment not receiving YCW (Table 4.2). When average FDE over the entire 16 wk of the experiment was analyzed as a 2x2 factorial, a significant main effect difference based on diet type was found (Table 4.5). Hens fed the SBM diets produced a FDE average significantly lower (1.62 kg) than those fed the SBMF diets (1.89 kg).

Feed Conversion ratio (total wt of eggs produced divided by total wt of feed consumed) from hens receiving the SBM diets were consistently lower than those from the hens fed the SBMF diet throughout the 4 periods of data collection. During the final period of data collection FCR from birds fed the SBM diet and receiving the YCW prebiotic was lower ($P<0.05$) than both SBMF treatments (Table 4.2). When average FCR over the entire 16 wk of the study was analyzed as a 2x2 factorial, a significant main effect difference based on diet type was found (Table 4.6). Hens fed the SBM diets produced a FCR average significantly better (2.12) than those fed the SBMF diets (2.57). An interaction ($P= 0.068$) occurred between diet type and YCW. Hens receiving the SBM diet with 250 ppm YCW had the best FCR at 2.06 versus 2.53 for the SBMF diet without YCW and 2.61 for the hens receiving the SBMF diet with YCW (Table 4.6).

**Table 4.5 Effect of YCW supplementation on average feed per dozen eggs (FDE kg) over all 16 weeks of the study**

| Diet | Yeast Cell Wall Inclusion (ppm) | | |
|---|---|---|---|
| | **0** | **250** | **Total-Diet** |
| **SBM** | 1.67±0.03 | 1.56±0.02 | 1.62±0.02[a] |
| **SBMF** | 1.87±0.06 | 1.90±0.06 | 1.89±0.4[b] |
| **Total-YCW** | 1.77±0.04 | 1.73±0.04 | 1.75±0.02 |

[a-b] Significant Main Effect ($P \leq 0.05$)
± Standard Error

**Table 4.6 Effect of YCW supplementation on average feed conversion ratio (FCR) over all 16 weeks of the study**

| Diet | Yeast Cell Wall Inclusion (ppm) | | |
|---|---|---|---|
| | **0** | **250** | **Total-Diet** |
| **SBM** | 2.19±0.04[ab] | 2.06±0.02[a] | 2.12±0.02[x] |
| **SBMF** | 2.53±0.08[b] | 2.61±0.08[b] | 2.57±0.06[y] |
| **Total-YCW** | 2.36±0.05 | 2.33±0.05 | 2.35±0.05 |

[a-b] Significant Interaction ($P = 0.068$)
[x-y] Significant Main Effect ($P \leq 0.05$)
± Standard Error

Effects of YCW in both SBM and SBMF diets on albumen height (AH), Haugh unit (HU), shell thickness (ST), and breaking strength (BS) over four periods of lay are presented in Table 4.7. Results indicated that there was no significant interaction and no treatment effect was observed over any of the four periods of lay (Table 4.7).

**Table 4.7 Effects of YCW on SBM and SBMF diets on albumen height (AH), Haugh unit (HU), shell thickness (ST), and breaking strength (BS) over 4 periods of lay**

| Age (week) | Dependent Variable | SBM Control | SBM+ YCW | SBMF Control | SBMF+ YCW |
|---|---|---|---|---|---|
| 47-50 wk | AH mm | 7.8 ± 0.7 | 8.0 ± 1.0 | 8.0 ± 0.83 | 8.0 ± 0.58 |
| | HU | 87.3 ± 4.0 | 88.5 ± 5.1 | 88.8 ± 4.6 | 89.2 ± 4.0 |
| | ST mm | 39.0 ± 1.4 | 39.1 ± 1.0 | 38.6 ± 1.2 | 39.5 ± 1.3 |
| | BT kg | 4.3 ± 0.6 | 4.2 ± 0.4 | 4.2± 0.5 | 4.6 ± 0.6 |
| 51-54 wk | AH mm | 7.5 ± 0.7 | 7.4 ± 0.7 | 7.5 ± 0.7 | 7.4 ± 0.7 |
| | HU | 84.8 ± 4.8 | 85.2 ± 4.0 | 86.1 ± 4.6 | 86.1 ± 5.7 |
| | ST mm | 40.1 ± 1.6 | 39.6 ± 1.7 | 40.1 ± 1.9 | 39.9 ± 1.8 |
| | BT kg | 3.9 ± 0.4 | 4.1 ± 0.7 | 3.9 ± 0.6 | 3.9 ± 0.6 |
| 55-58 wk | AH mm | 7.3 ± 0.6 | 7.2 ± 0.6 | 7.4 ± 0.8 | 7.3 ± 0.5 |
| | HU | 84.7 ± 3.4 | 83.3 ± 4.0 | 85.6 ± 4.4 | 84.8 ± 3.7 |
| | ST mm | 39.7 ± 2.3 | 38.1 ±1.7 | 38.6 ± 2.5 | 40.0 ± 1.7 |
| | BT kg | 4.0 ± 0.4 | 4.1 ± 0.8 | 4.1 ± 0.5 | 4.0 ± 0.6 |
| 59-62 wk | AH mm | 7.4 ± 0.7 | 7.5 ± 0.8 | 8.9 ± 5.6 | 7.3 ± 0.8 |
| | HU | 84.9 ± 4.1 | 85.7 ± 5.2 | 87.2 ± 10.1 | 85.1 ±5.4 |
| | ST mm | 39.0 ± 1.7 | 38.1 ± 1.8 | 39.7 ± 1.2 | 40.2 ± 1.2 |
| | BT kg | 3.8 ± 0.3 | 3.6 ± 0.6 | 3.9 ± 0.5 | 4.0 ± 0.5 |

The ileal amino acid digestibility coefficients (apparent and standardized) for all treatments are presented in Table 4.8 and Table 4.9, respectively. As shown in Table 4.8, for the apparent ileal amino acid digestibility coefficient percentages**,** data indicated that the apparent digestibility of lysine for the SBM+YCW diet was significantly higher at 83.9% compared to 71.2%, 68.2%, and 68.9% for the SBM control, SBMF control and SBMF+YCW, respectively. Similarly, apparent ileal arginine digestibility improved for the SBM+YCW treatment at 90.5% versus the SBMF+YCW treatment at 80.3%. There was no significant difference between the control groups of both diets.

**Table 4.8 Apparent ileal amino acid digestibility coefficient percentages for laying hens fed YCW with SBM and SBMF diets**

| Amino acid | SBM control% | SBM+YCW% | SBMF control% | SBMF+YCW% |
|---|---|---|---|---|
| **Lysine** | 71.2±8.9[b] | 83.9±3.8[a] | 68.2±5.2[b] | 68.9±5.1[b] |
| **Methionine** | 85.4±3.3 | 91.6±1.9 | 88.2±2.5 | 85.6±4.8 |
| **Threonine** | 55.1±9.0 | 62.9±9.6 | 54.7±8.5 | 57.0±8.7 |
| **Isoleucine** | 71.0±7.9 | 75.5±7.8 | 66.9±7.3 | 71.5±6.6 |
| **Leucine** | 74.3±6.8 | 81.6±6.6 | 75.8±5.0 | 73.4±9.1 |
| **Valine** | 65.9±7.7 | 75.4±6.7 | 65.3±7.2 | 65.6±7.8 |
| **Histidine** | 74.8±6.8 | 81.7±5.9 | 71.6±3.6 | 69.1±7.0 |
| **Phenylalanine** | 73.9±7.1 | 81.4±6.2 | 75.4±5.0 | 73.9±7.7 |
| **Serine** | 66.6±5.7 | 72.9±7.1 | 70.2±6.5 | 65.0±9.1 |
| **Proline** | 72.4±3.8 | 77.4±6.7 | 71.1±3.9 | 70.5±9.1 |
| **Glycine** | 62.8±8.7 | 72.3±7.4 | 62.7±7.0 | 64.2±6.8 |
| **Alanine** | 70.3±7.1 | 81.1±6.1 | 71.7±7.3 | 69.7±9.3 |
| **Arginine** | 83.4±4.6[ab] | 90.5±2.0[a] | 82.8[ab]±2.1 | 80.3±4.4[b] |
| **Tyrosine** | 73.4±7.0 | 79.9±6.2 | 75.6±4.8 | 75.1±6.2 |
| **Glutamic acid** | 79.6±5.3 | 86.1±4.8 | 80.9±3.9 | 78.8±7.0 |
| **Aspartic acid** | 70.3±7.2 | 72.6±7.4 | 64.8±8.0 | 73.7±5.3 |

[a-b] Means within the row with different letter differ at P value = 0.05.

The standardized ileal amino acid digestibility data, which is corrected for endogenous ileal nitrogen losses, is shown in Table 4.9. As expected, the treatment relationships were similar to the previously mentioned apparent amino acid digestibility coefficients, just higher after the adjustment for endogenous losses. Standardized ileal digestibility for lysine was 86.0% for hens receiving SBM diet with YCW. This was significantly better than all other treatments and may explain the higher hen day egg production seen with this treatment.

**Table 4.9 Standardized ileal amino acid digestibility coefficient percentages for laying hens fed YCW with SBM and SBMF diets**

| Amino acid | SBM Control% | SBM+YCW % | SBMF Control% | SBMF+YCW % |
|---|---|---|---|---|
| **Lysine** | 73.8±9.0[b] | 86.0±3.8[a] | 70.5±5.2[b] | 71.3±5.1[b] |
| **Methionine** | 87.3±3.3 | 93.4±1.9 | 89.9±2.5 | 87.4±4.4 |
| **Threonine** | 65.0±9.0 | 73.0±9.6 | 64.6±8.5 | 66.4±8.7 |
| **Isoleucine** | 76.6±7.9 | 81.9±7.8 | 73.1±7.3 | 76.9±6.6 |
| **Leucine** | 77.0±6.9 | 84.1±6.6 | 78.4±5.0 | 76.1±9.1 |
| **Valine** | 71.5±7.7 | 80.8±6.7 | 70.8±7.2 | 71.0±7.8 |
| **Histidine** | 79.9±6.8 | 86.4±5.9 | 76.4±3.6 | 74.0±7.0 |
| **Phenylalanine** | 76.9±7.1 | 84.1±6.2 | 78.2±5.0 | 76.7±7.7 |

[a-b] Means within the row for each diet with different letters differ at $P \leq 0.05$.

Lysine is one of the most limiting essential amino acids necessary for meeting the bird's daily requirement for egg production. It is a basic amino acid, ketogenic, and supplemented as a crystalline powder (Lysine HCl) in poultry diets. It is interesting to note that the YCW prebiotic significantly improved lysine's ileal digestibility in the corn-SMB diet but the effect was not as great for the non SBM diet. This suggests the specific ingredients making up the diet can affect or interact with the YCW prebiotic. Fowler et al, 2015 conducted a study evaluating YCW on broilers fed simple corn-soy diets versus more complex diets using a variety of ingredients to meet the birds nutrient requirements and found that the use of YCW at a level of 250 ppm improved broiler performance and feed conversion ratio, especially in the starter phase of production.

It has been stated that YCW supplementation is beneficial for layer chicks with respect to growth performance and intestinal histology during the growing period (1-60 days) (Gurbuz, et al., 2011a). It has been concluded that the use of YCW in older laying hen diets (48 weeks old) improves egg production and final body weight (Gurbuz, et al., 2011b). These authors also have indicated that YCW supplementation increases feed intake and villi width.

A study by Hashim et al. (2013) reported that feeding laying hens a diet supplemented with YCW at a level of 250 ppm in the early stages of production improved egg weight and liquid egg yield. They also found that specific gravity, egg shell thickness, egg shell weight, and percent shell weight were higher when they used YCW at the level of 500 ppm versus 250 ppm at 36 weeks of age.

The use of mannan-oligosaccharide (MOS) type prebiotics has become more common due to the antibiotic growth promotor (AGP) restriction in animal feed. It has

been shown that supplementation of MOS type products increases egg production rate as well as eggshell weight but has no effect on egg quality parameters (Bozkurt, et al., 2012). This is similar to our results, where YCW product improved hen day egg production in the SBM diet when it was added at a level of 250 ppm.

Ghasemian & Jahanian (2016) documented that dietary MOS type supplementation has a positive impact on 68-week old laying hens by increasing egg production and feed efficiency when it is used at levels of 1-1.5 g/kg. These authors indicated that this improvement could be attributed to increased ileal nutrient digestibility and pathogen reduction. In addition, (Xiao, et al., 2012) have reported that addition of YCW-derived MOS to young broiler chicks' diet improved general bird performance. They have indicated that MOS product supplementation improved body weight gain by approximately 4% and led to a 3% enhancement in feed-gain ratio.

Similarly, researchers have found that yeast extract can increase bird performance by favoring intestinal mucosal improvement (Morales-Lopez, et al., 2010). Moreover, a different study was conducted by Ghosh, et al. (2012) to assess YCW products as replacements for antibiotic growth promotors in broiler diets – this study indicated that YCW improves feed efficiency when it is compared to growth promotors such as bacitracin methylene disalicylate (BMD). These researchers also found that YCW performed better as a protector against pathogen colonization than did BMD.

With respect to pathogen challenge, researchers have found that the use of prebiotic YCW supplementation in starter broilers improved body weight and feed conversion ratio when birds were challenged with *Clostridia perfringens* (Fowler, et al.,

2015). It has been stated that the use of whole yeast culture product can reduce the coccidial infection in both growing pullets and laying hens (Markazi, et al., 2017).

In this study, it can be concluded that supplementation of YCW at the level of 250 ppm in post peak laying hen diets improved egg production percentage in the corn-SBM diet. Laying hens who were fed SBM+YCW had the greater egg production over all four periods of lay. With respect to the diet type, average egg weight over the entire 16 weeks of the study was greater for the SBM than the SBMF diet. Feed per dozen eggs and FCR improved more for hens receiving the SBM diet than for the SBMF diet.

In addition, YCW product improved ileal apparent and standardized ileal amino acid digestibility coefficients for lysine over the control groups for both diet types. These results have indicated that YCW supplementation has a beneficial effect on laying hens' performance in post peak production, and thus could be a viable additive for use in poultry feeds.

# CHAPTER V

# DIFFERENTIAL GENE EXPRESSION OF CAGE LAYERS SUPPLEMENTED WITH COTTONSEED MEAL AND YEAST CELL WALL PRODUCT

## INTRODUCTION

One of the major economic factors that the poultry industry faces is feed cost. The most common poultry diet in the United States is composed of corn and soybean meal (SBM). Properly processed SBM is perhaps the best oil seed meal ever discover as a valuable source of protein for animal feeds. However, the cost of soybean meal is volatile and can increase feed costs dramatically (Shi et al., 2012) and raw soybeans have several anti-nutrient factors (ANF) such as trypsin inhibitors, lectins, saponins, and stachyose and raffinose non-starch polysaccharides. As a result, a niche market has developed based on some consumer demand for food products not associated with SBM. One alternative to SMB is cottonseed meal (Zeng, et al., 2015), but it too has antinutritional compounds that limit its use.

Cottonseed meal (SCM) contains about 41% protein, 13.6% crude fiber, and 0.5% crude fat (NRC, 1994). It is derived from the residue created in cottonseed oil extraction (Nagalakshmi, et al. (2007). In addition, CSM is usually less expensive than SBM. However, concerns regarding gossypol and cyclopropenoid fatty acids limit the use of this product in all poultry diets (Gadelha et al., 2014). The main anti-nutritional factor of CSM is free gossypol which lowers protein digestibility and affects the reproductive system, heart, and liver in monogastric animals (Nagalakshmi et al., 2007) leading to decreased poultry performance (Lordelo et al., 2005).

Generally, laying hens are more susceptible to free gossypol than other poultry, and there are several negative impacts on egg quality parameters from ingestion of free gossypol and cyclopropenoid fatty acids (Davis et al., 2002) as well as an overall reduction in performance (Lordelo et al., 2007). Researchers have found that free gossypol causes discoloration in the yolk due to chemical combination between ferric irons Fe+ that are released from yolk protein, and the gossypol compound (He et al., 2015). Dietary levels of free gossypol up to 50 ppm may be safely fed without egg yolk discoloration (Smith, 1970).

In addition, consumer demand for so called antibiotic-free chicken has increased the need to find alternative products such as prebiotics that can be used in poultry feed to enhance bird performance and feed utilization. Prebiotics are defined as non-digestible ingredients that benefit the host by selectively stimulating the growth of one type or a limited number of bacteria in the colon, resulting in improvement of the host`s health (Gibson and Roberfroid, 1995). Prebiotics can substitute for the use of antibiotics in poultry feed, and they are considered beneficial for hens with respect to general gut health and feed utilization. It has been reported that prebiotics improve body weight and decrease the feed conversion ratio (Hooge and Connolly, 2011). However, there are some concerns in regards to prebiotic use including gastric acidic environment resistance, and absorption across intestinal epithelium (Hume, 2011).

Although many prebiotics have been shown to improve poultry performance, particularly under stressful rearing conditions, their specific modes of action are not yet clearly understood. New methods of analyzing gene expression may allow us to gain a better perspective of how these prebiotics may function.

The objective of this research was to obtain a greater understanding of how a YCW prebiotic (Saffmannan$^{TM}$) may affect gene expression of caged laying hens fed SBM or SBMF diets. A transcriptomics approach was used to evaluate this supplementation on the gene expression profile of several genes from liver and spleen of laying hens. This was based on identifying the functional pathways of specific genes in laying hens that or upregulated or downregulated under the conditions of this study.

**MATERIALS AND METHODS**

To test the hypothesis that YCW is an effective prebiotic as well as the suitability of CSM as a replacement protein source for SBM, metabolic pathways that were expected to be influenced by these diets were identified and investigated through analysis of differences in gene expression. A total of ten genes of interest were determined based on the current literature. Some of these genes involve pathways related to the metabolism of gossypol. For relative quantification of differential gene expression, two reference genes (AHR and GAPDH) were also selected from a literature review of qPCR investigations of chicken gene expression. Lists of these genes are shown in Table 5.1 and Table 5.2.

**Table 5.1 List of reference genes used, including descriptions and NCBI sequence accession numbers**

| Category | Gene | Function | NCBI Accession N0. |
|---|---|---|---|
| Ref | AHR | Cell-cycle regulation, and tissue development | NM – 20411.82 |
| Ref | GAPDH | Glycolysis, RNA transport, and DNA replication | NM – 204305.1 |

**Table 5.2 List of functional genes investigated, including descriptions and NCBI sequence accession numbers**

| Gene | Function | NCBI Accession N0. |
|---|---|---|
| BAK | Apoptosis regulation, mitochondria energy metabolism regulation | NM - 001030920.1 |
| BIK | Programmed cell death accelerator via apoptosis | NM - 001278058.1 |
| BCL2 | Apoptosis suppressor, cell death regulator | NM - 205339.2 |
| MCL1 | Anti-apoptotic protein, cell viability maintenance | NM - 001319309.1 |
| NLRP3 | Innate immunity | XM – 015286280.1 |
| POR | Oxidative metabolism of steroids, and carcinogens | NM – 001195796.1 |
| CYP1A1 | NADPH-dependent electron transport pathway | NM – 205147.1 |
| CYP1A2 | Xenobiotic metabolism, carcinogenic aromatic | NM – 205146.2 |
| CYP2C23A | Xenobiotic and drug metabolism | NM – 001001616.1 |
| CYP3A4 | Monooxygenase | NM – 001329508.1 |

**Tissue Collection and Storage**

This study was conducted at the Texas A&M University Poultry Research Center, and it received approval from the University`s Animal Care and Use Committee (IACUC 2017-0072). A total of four treatments were fed to the hens: SBM control, SBM+YCW, SBMF control, and SBMF+YCW. Corn based diets were formulated based on the nutrient requirements suggested by the 2014 Hy-line Brown management guide as described in Chapter 3. Feed and water were offered ad libitum. A total of 16 Hy-line Brown laying hens 63 wk of age; 4 per treatment) were euthanized using $CO_2$ gas at the conclusion of the previous study described in Chapter 4. From each hen, approximately two grams of both liver and spleen tissues were collected and stored in RNALater following manufacturer guidelines (ThermoFisher Scientific). These samples were then stored at 4°C for 24 hours before being removed from the RNALater and stored at -80°C until RNA isolation.

**RNA Extraction, Quality Analysis and Reverse Transcription**

RNA was isolated from 100 mg sections of liver and spleen tissue samples, using the TRIzol Reagent method (ThermoFisher Scientific), and samples were then quantified on a Nanodrop Spectrophotometer. The quality of the RNA isolates was checked using the Agilent Bioanalyzer 2100 with the RNA 6000 Nano Kit following the manufacturer's protocol.

Samples with RNA Integrity Numbers (RIN) above 7 were retained for further analyses. RNA isolates not meeting these quality criteria were re-extracted. Finally, reverse transcription reactions were performed according to the manufacturer's protocol

for the SuperScript VILO Master Mix (ThermoFisher Scientific). A portion of each cDNA sample was pooled for use as the template for primer testing.

**Primer Design and Testing**

Primers were designed using the NCBI Primer-BLAST tool freely available online. Amplicon size was set to 200 – 300 base pairs and primers were selected to span exon-exon junctions, to exclude the probability of DNA contamination in RNA isolates. Primers were ordered through Integrated DNA Technologies (IDT) and stored as specified. Primer testing was performed on a pooled cDNA sample according to the manufacturer specified protocol for the PowerUP SYBR Green Master Mix (ThermoFisher Scientific). Primer pairs yielding efficiency between 90 and 110% were accepted and all dissociation curves were visually assessed for evidence of amplification of unintended targets.

**Table 5.3 Primers used for real-time quantitative Polymerase Chain Reaction (qPCR)**

| Gene | Primers Sequence 5 `- 3 ` | |
|---|---|---|
| BAK | Forward Primer | ACGAGAGATCAATGCAGAGGAC |
| | Reverse Primer | ACTCGTAGGCGTTCTCCTTG |
| BIK | Forward Primer | TCTCCAGATACCCCAACGGA |
| | Reverse Primer | ACTGATAGCAACCCTGCGTG |
| BCL2 | Forward Primer | GGATGGGATGCCTTTGTGGAA |
| | Reverse Primer | TTAGCCAGGAAGTTGTTTTGCTC |
| MCL1 | Forward Primer | GAGGCTGGGAGGGCTTTGTT |
| | Reverse Primer | GGTGACTCAAGTCTGGCTGT |
| NLRP3 | Forward Primer | GTCACTAAACCTGGTGGGGC |
| | Reverse Primer | CCTGCGCTCTCCTGATCCAT |
| POR | Forward Primer | ACAAGGGAAGTGAGTGGAGTT |
| | Reverse Primer | ACTATGTTTCGGCCCGTCTT |
| CYP1A1 | Forward Primer | GCAGCACCCAAAGGTTCACT |
| | Reverse Primer | ATGGTCACCTCCATCACGTC |
| CYP1A2 | Forward Primer | ACACCACGCTTCCCCTTAGT |
| | Reverse Primer | TCCATCACGTCCCCGTATTT |
| CYP2C23A | Forward Primer | CCTTCAGTGGGAGAGGAATACTG |
| | Reverse Primer | TGAAAGGTTCCTCGTGTGTCTT |
| CYP3A4 | Forward Primer | ACACCACGCTTCCCCTTAGT |
| | Reverse Primer | TCCATCACGTCCCCGTATTT |
| AHR | Forward Primer | GTGCAGAAAATAGTAAAGCCATCT |
| | Reverse Primer | CCCCTCTCCAAGTTTTGCTGT |
| GAPDH | Forward Primer | TCGGAGTCAACGGATTTGGC |
| | Reverse Primer | GCCCATTTGATGTTGCTGGG |

**Real-Time Quantitative Polymerase Chain Reaction (qPCR)**

The qPCR reactions were set up as instructed by the PowerUP SYBR Green Master Mix manufacturer's protocol. Samples were run in duplicate and each gene was run with all samples on a single 384 well plate on an ABI 7900 HT (Applied Biosystems).

**Statistical Analysis**

Data generated by qPCR were analyzed in Excel through calculation of the log fold change of expression for each gene, using the ΔΔCT method. We also tested for statistical significance of gene expression among experimental groups using an ANOVA with the treatments as the independent variables and the ΔCT values as the dependent variables. Statistical comparisons that yield a *P*-value smaller than $\alpha=0.05$ were considered significantly different.

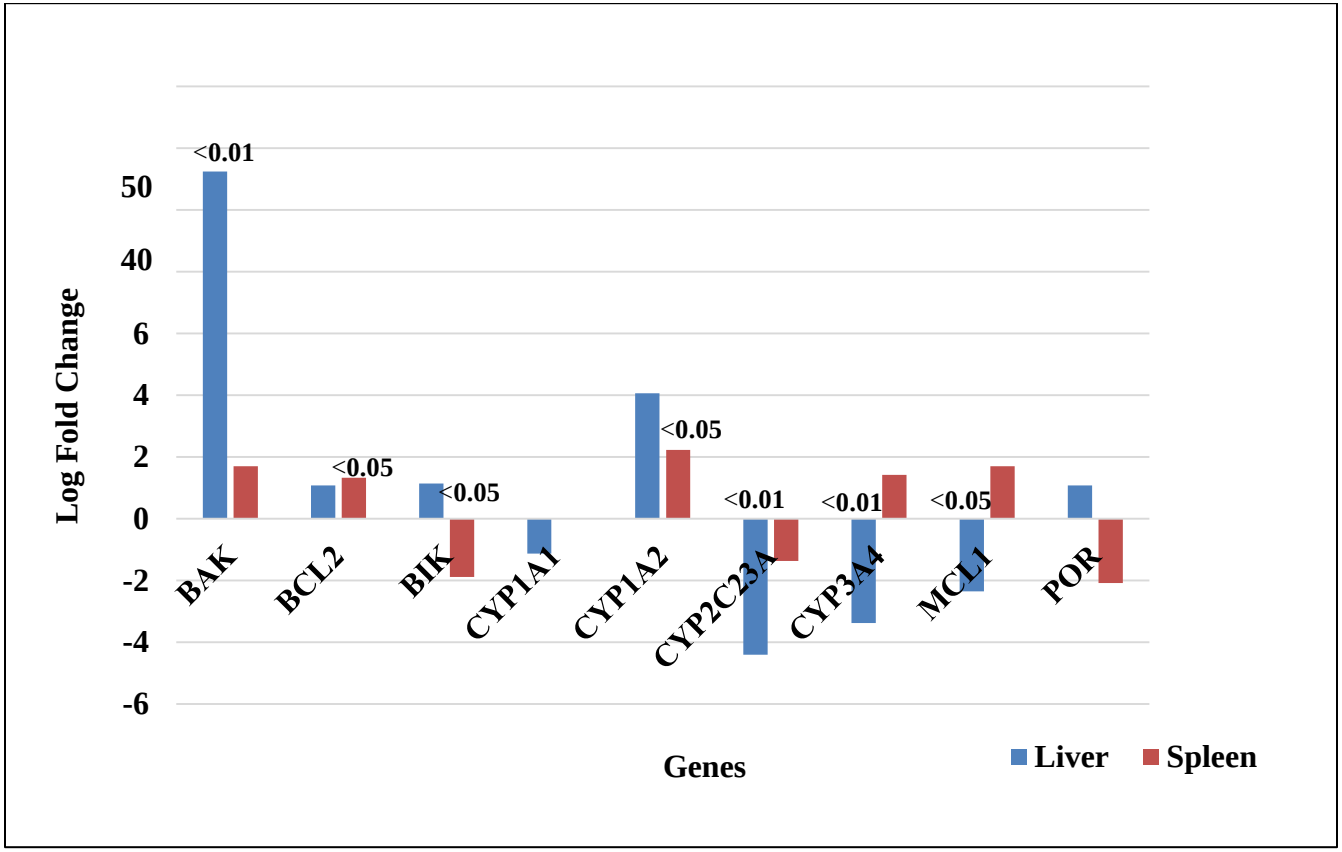
**RESULTS AND DISCUSSION**

Data for the inclusion of YCW product in the cage system of hens fed the SBM diet are presented in Figure 5.1. Bars representing gene expression above 0 represent increased expression and bars below 0 represent decreased expression. Our results indicated that the supplementation of YCW significantly upregulated the BAK gene in the liver samples.

It is of interest that the BAK gene which is responsible for apoptosis regulation and mitochondria energy metabolism regulation (Bleicken, et al., 2013) had a log fold upregulation of 51 for hens fed 250 ppm YCW versus those hens fed SBM without YCW.

This also correlates with the significant increase in egg production observed for this particular treatment in the experiment of Chapter 4. Specifically, the combination of SBM and YCW treatment had the greatest egg production (93.6%) comparing to the other treatments. The CYP2C23A, CYP3A4 (involved with xenobiotic metabolism) and MCL1 genes were downregulated in the liver tissues. The gene MCL1 involves in cell viability maintenance but not proliferation (Yang, et al., 1996). With respect to the spleen, BCL2 and CYP1A2 were significantly upregulated and the BIK was down regulated.

**Figure 5.1 Differential gene expression in caged hens fed SBM with YCW supplementation**

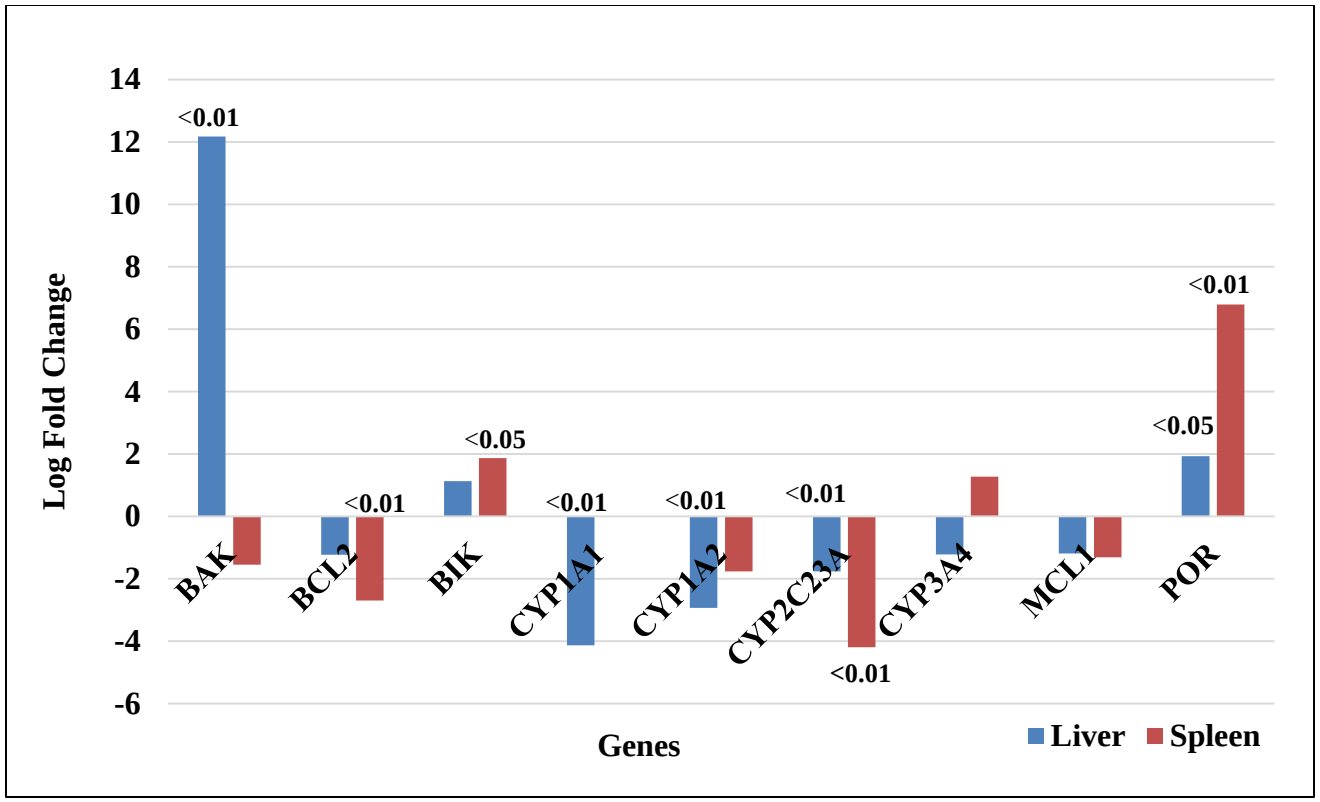


Data for the inclusion of YCW product in the cage system of hens fed the SBMF diet are presented in Figure 5.2. The BAK gene was also highly upregulated in liver tissue with a 12 log fold change for these hens. The POR was also significantly upregulated in liver tissue. The CYP1A1, CYP1A2 and CYP2C23A genes were downregulated in the liver tissues. These genes are involved in xenobiotic metabolism and cell maintenance activity.

Xenobiotics are defined as chemical compounds that can be found in an organism but are not produced naturally, and many xenobiotics can reach the toxicity concentration without metabolism (Croom, 2012). The CYP1A2 gene plays significant roles in metabolism of several drugs as well as carcinogen activation because of its higher level of expression in the liver (Gunes and Dahl, 2008). These genes also play an important role with NADPH- dependent electron transport pathway as CYP1A1 is considered a monooxygenase for xenobiotic and drug metabolism and CYP1A2 participates in the bio-activation of carcinogens.

Xenobiotic metabolism is considered an important factor, as it determines an animal's sensitivity to any chemical compound. The Cytochrome P450 (CYP) families 1-3 are recognized as the major xenobiotic-metabolizing enzymes that participate in the bioactivation or the inactivation of different xenobiotics compounds (Nebert & Russell, 2002). This Cytochrome P450 (CYP) is found primarily in the liver (Watanabe et al., 2013). For the splenic tissue, the BIK and POR genes were upregulated and the BCL2 and CYP2C23A were significantly downregulated. The BCL2 acts as a suppressor of apoptosis and a regulator of cell death by controlling mitochondrial membrane permeability.

**Figure 5.2 Differential gene expression in caged hens fed SBMF with YCW supplementation**



Results for liver and spleen gene expression samples related to the diet type, SBM versus SBMF not being fed YCW are presented in Figure 5.3. For this particular group feeding the SBMF diet did not cause upregulation of any of the genes versus the SBM diet for either liver or splenic tissue except CYP2C23A and POR genes were significantly downregulated for liver and spleen tissue respectively.

Results for liver and spleen gene expression samples related to the diet type, SBM versus SBMF fed YCW are presented in Figure 5.4. For this particular group feeding the YCW diet resulted in upregulation of CYP3A4 and MCL1 genes versus the SBM diet for liver tissue and BAK, BIK and POR genes for splenic tissue. We observed significant downregulation of liver tissue BAK, CYP1A1 and CYP1A2 and splenic tissue BCL2 and CYP1A2 genes.

Researchers have investigated the Cytochrome 450 CYP (1-3) isoforms in avian species using the available genomes for chicken, zebra finch, and turkey (Watanabe, et al., 2013). They found that CYP2C45 had the highest expressed isoform in chicken liver while the most induced gene by phenobarbital was CYP2C23b. These authors also indicated that CYP2C45 may have a dominant role in the chicken liver because of the constitutive high expression levels (Watanabe, et al., 2013).

**Figure 5.3 Differential gene expression in caged hens not fed YCW with SBMF**

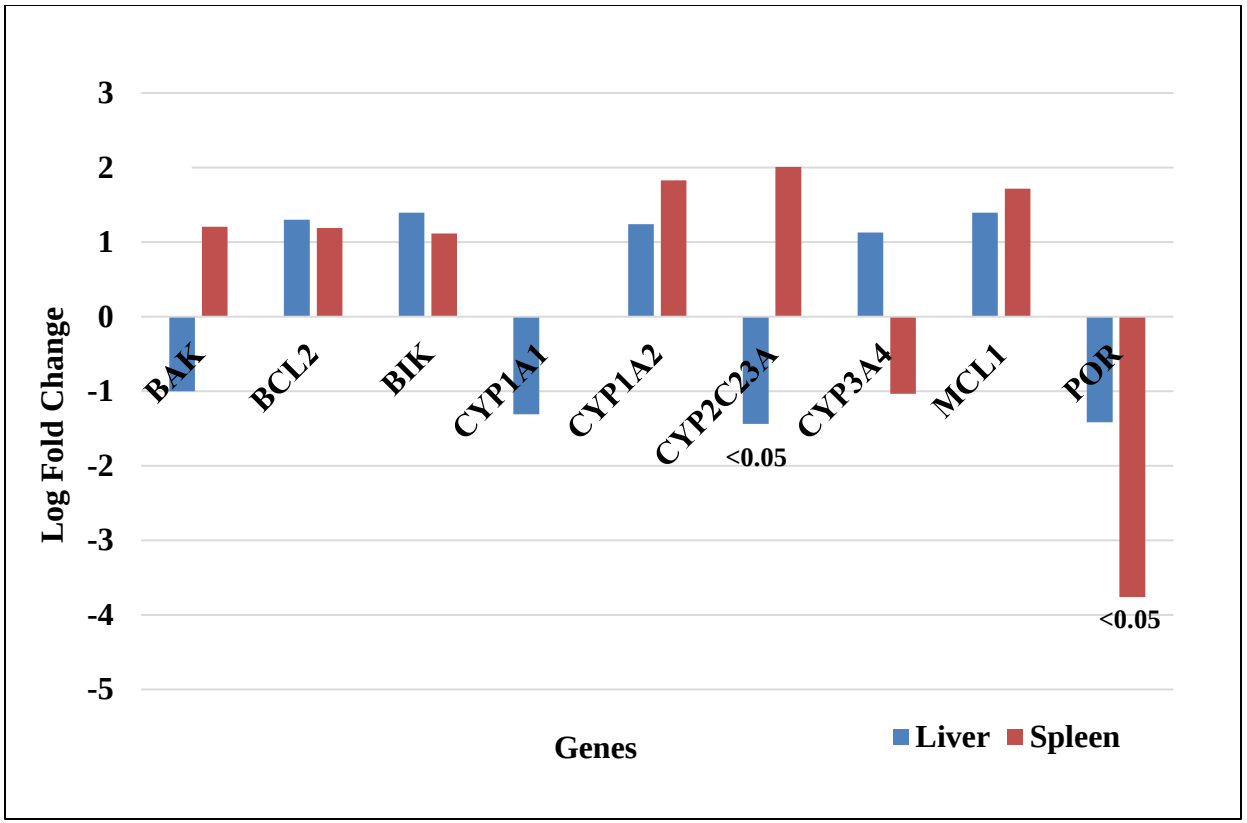
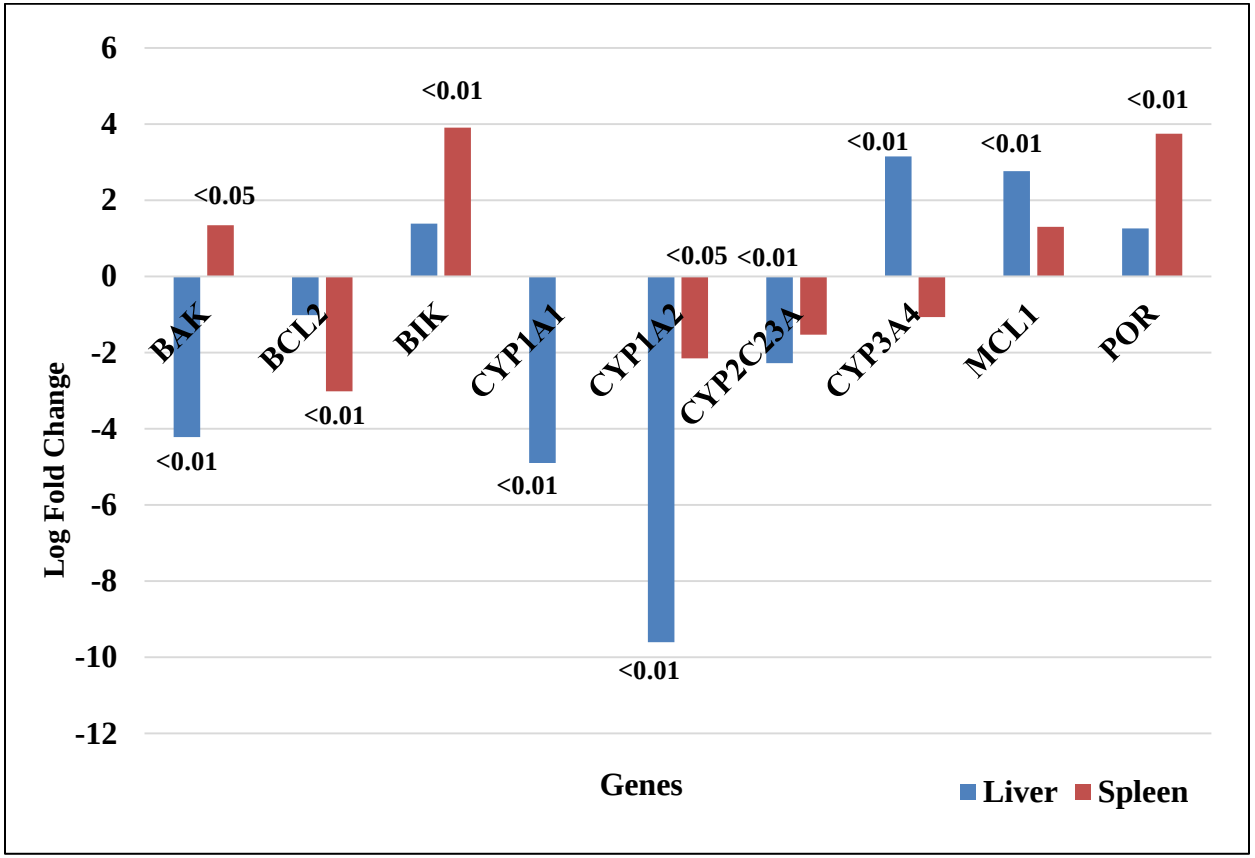


**Figure 5.4 Differential gene expression in caged hens fed YCW with SBMF**



The POR gene is a P450 oxidoreductase containing the flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) moieties that transfer electrons from NADPH to microsomal cytochrome P450 enzymes (Huang, et al., 2008). It is involved generally in the oxidative metabolism of steroids and carcinogens. A deficiency of POR can cause disordered steroidogenesis and severe mutation causing genital ambiguity in both sexes (Sahakitrungruang, et al., 2009). Researchers have examined the biological function of cytochrome P450 in mice small intestine and confirmed the relation or the mechanistic link between intestinal immunity and the POR-dependent enzymes (D'Agostino, et al., 2012).

The BIK gene is a pro-apoptotic BH3-only member of the BCL-2 family that targets the endoplasmic reticulum (ER) membrane (Mathai, et al., 2005). It is an accelerator of programed cell death, and this occurs via apoptosis. Apoptosis plays an important role in development regulation and tissue homeostasis (Lindsten, et al., 2000). Viedma-Rodriguez et al. (2013) reported that BIK has been used as a therapeutic molecule in gene therapy-based approaches to treat cancer, and researchers have found that this suppression of the gene enhances resistance to tamoxifen (TAM) in MCF-7 breast cancer cells. The BCL-2, BAX and BAK genes code for multi-functional proteins that play an important role in apoptosis regulation (Bleicken, et al. (2013).

The gene MCL1 was identified as an “early induction gene” and proved to be a member of the BCL2 gene family, as it has sequence similarity to BCL2 (Kozopas, et al., 1993). Also, it has been stated that this MCL1 gene is associated with cell viability maintenance but not proliferation (Yang, et al., 1996).

Studies have shown a reduction in succinate dehydrogenase and cytochrome oxidase activities in chick livers in response to gossypol (Abou-Donia and Dieckert, 1974). The mechanisms behind these observed effects are in relationship to reduced oxygen-carrying capacity of the blood and hemolysis of erythrocytes which occurs due to ingestion of gossypol (Abou-Donia and Dieckert, 1974).

Our results indicated that the use of a SBMF diet utilizing CSM caused significant changes in several genes for the liver and spleen samples. This could be related to the use of CSM and the level of gossypol in the diet. However, based on the Insect Control and Cotton Disease Research Unit/Sothern Plains ARS/USDA gossypol analysis of the SBMF diet, the free gossypol level was below 21.8 ppm which is not normally considered a toxic concentration with respect to performance (Smith, 1970).

In summary, based on these findings, the prebiotic YCW supplementation increased the expression of the liver tissue BAK gene for both the SBM and SBMF diets. The gene BAK is responsible for apoptosis regulation and mitochondria energy metabolism. With respect to splenic tissue, the combination of YCW with the SBMF diet increased the POR gene over 6 log fold. The gene POR is involved in oxidative metabolism of steroids. In the presence of YCW, the SBMF diet upregulated ($P<0.01$) CYP3A4 and MCL1 genes in the liver and BIK and POR genes in the spleen. The CYP1A2 gene was downregulated over 9 log fold in the liver.

# CHAPTER VI

# CONCLUSION

In recent years, egg quality characteristics, especially eggshell strength, have received great attention due to the movement toward cage-free rearing systems (Ni, et al., 2007). To meet the demand of egg production using these new rearing systems, new research must be conducted to fully understand the hens' needs including hen health, welfare, and production efficiency. Additionally, consumers' needs and demands must be taken into account with respect to welfare concerns and the historic use of certain feed additives in poultry feeds.

Several aspects need to be addressed to face this new movement without significantly increasing production cost, while providing the hens healthy environments to control the mortality that can occur due to clinical diseases. In addition, there also have been concerns about moving toward antibiotic-free feed involving different factors that affect the commercial poultry industry in general. These factors include but are not limited to bird health care and veterinary attention, feed and water system, and even transportation.

Recently, hen housing have become the primary chicken welfare topic in the United States, especially after California announced the adoption of a regulation banning the use of conventional cage housing, effective January 1, 2015. In addition, most food service outlets have announced their pledge to sell or serve only eggs that are cage-free over the next few years. In order to meet these commitments and provide sufficient supply, egg producers must take significant action to abandon most (or perhaps all) of the conventional cage systems in use today.

Using cage-free systems means that birds will have more space to move about, exercise, stretch their legs and wings, and express other natural behaviors, such as dust-bathing and foraging (AVMA, 2012). However, from a producer's standpoint, moving to cage-free facilities will likely have an economic effect because building new facilities will require more space, cost a lot of money, and likely also impact bird health by opening the door for several diseases associated with poultry, contamination with pathogens (Holt, et al., 2011). Higher mortality is also likely (Glatz, et al., 2005), and management problems will likely increase.

Generally, the greatest cost of poultry production is associated with purchasing feed ingredients and manufacturing the feed. The most common poultry diet ingredients in the United States primarily use corn and soybean meal as the primary ingredients making up the feeds. However, the use of corn has become a cost issue as a result of the bio-fuels initiatives. In addition there is a small segment of our population that is concerned with the use of SBM as the vast majority of soybeans grown in the United States is now genetically modified (GMO) (Lappé, et al., 1998) to help protect against insect predation. Consumers are increasingly aware of what they eat and what products are being sold at various grocery stores. Modern advertising and labeling highlighting traits like soy free, gluten free, organic, cage free, non-GMO and so on have made consumers more aware about their food choices.

Therefore, the aim of this project was to evaluate Hy-line Brown laying hens reared in cage and free-range facilities and fed two different diets (SBM and SBMF) utilizing a CSM substitute for SBM based on egg production and quality parameters. We also investigated overall consumer favorability regarding egg flavor, texture, odor, and

color, based on samples of scrambled and hard cooked eggs fed other ingredients besides SBM to meet the hen's needs for dietary protein. In addition, we evaluated three other factors: the influence of YCW as a prebiotic on post peak performance for these hens, how ileum digestibility compares to a diet without the prebiotic, and how gene expression for those hens can be altered due to different ingredients.

From the experiment in chapter two, it can be concluded that SBM can be a replaced using 15% CSM as a primary oilseed meal providing vegetable protein. This of course is not a new finding but does add to the existing literature. With respect to rearing system, the results suggested that free-range production is more variable than traditional closed house cage systems, with standard errors of production variables consistently higher in the free-range system and the SBMF diets containing CSM can be used in both caged and free-range production systems without any impact on egg production, although one might see lower egg weights.

For the egg quality parameters, no difference was found in albumen height, Haugh unit, or breaking strength. However, there was significant rearing system by diet interaction for shell thickness with the free-range hens averaging 40.77 and 39.86 μm ($P < 0.05$) respectively for the hens fed SBM versus SBMF diets and no significant difference in shell thickness observed in birds reared in the traditional cage system.

For the sensory evaluation and consumer acceptance of scrambled eggs, flavor did not differ, but texture preference was higher for scrambled eggs from the SBMF diet (7.08) versus scrambled eggs from the SBM diet (6.65). With respect to the hard cooked eggs, the consumer panel preferred the flavor of the eggs from the caged rearing system (7.11) versus eggs from the free-range system (6.60). Consumers liked the texture of

hard cooked eggs collected from hens fed SBM (6.91) versus eggs from hens fed the SBMF diet (6.30).

Results from the fourth chapter indicated that YCW supplementation to the SBM diet had a positive impact on laying hens in post peak production, and this can encourage the poultry industry to use it as an alternative to bacitracin antibiotics, as it enhances feed conversion ratio as well as general hen performance. We also found that supplementation of YCW at the level of 250 ppm in post peak laying hen diets improved egg production percentage for both SBM and SBMF diets. Laying hens who were fed SBM+YCW had higher egg production over the four periods of lay.

With respect to diet type, egg weight was greater for the SBM than the SBMF diet. Feed per dozen eggs and egg weight significantly improved for hens receiving the SBM diet than for the SBMF diet. In addition, YCW product improved ileal apparent and standardized ileal amino acid digestibility coefficients for lysine over the control groups for both diet types

For the gene expression profile, the prebiotic YCW supplementation increased the expression of the liver tissue BAK gene for both the SBM and SBMF diets. The gene BAK is responsible for apoptosis regulation and mitochondria energy metabolism. With respect to splenic tissue, the combination of YCW with the SBMF diet increased the POR gene over 6 log fold. The gene POR is involved in oxidative metabolism of steroids. In the presence of YCW, the SBMF diet upregulated ($P<0.01$) CYP3A4 and MCL1 genes in the liver and BIK and POR genes in the spleen. The CYP1A2 gene was downregulated over 9 log fold in the liver. To our knowledge this body of work is among

the first to link the addition of YCW to the up- or down-regulation of various genes in poultry tissues.

For this dissertation, it can be clearly understood that free-range rearing production is more variable than the traditional cage system, and the SBMF diet utilizing CSM can be an alternative diet for laying hens without any negative impact on egg production as well as egg quality characteristics. However, this diet may produce lower egg weight.

In addition, the inclusion of YCW at the level of 250 ppm in post peek laying hens' diets improved egg/day production percentage and improved amino acid ileal digestibility of lysine in caged hens. The use of YCW supplementation in the laying hen diet had indicated positive impacts when it was used in either the commercial corn-soybean meal diet or the alternative soy-free diet. The data suggest that a free-range system may affect the economic feasibility of commercial applications due to increased variability of the production data. Further researches on a large scale would be beneficial to identify the economic implications of free-range system.

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## APPENDIX A

## SCRAMBLED EGG SENSORY BALLOT USED IN THE SENSORY EVALUATION TEST BETWEEN THE TREATMENTS AND THE REARING SYSTEMS

**Instruction**

Thank you for your participation in this study. Your assistance is very much appreciated. The objective of this study is to evaluate Soybean and Soybean free diets using cages and cage free rearing systems. Please take your time and evaluate the samples given to you carefully. Please proceed at your own rate.

This sampling will take you about 15 minutes and you will be eating **total of 4 samples**. Please answer the following questions as completely as possible. If you have any questions, please ask the monitor for assistance.

1. Samples will be served one at a time.
2. Between samples please clear your palate with a bite of cracker followed by a sip of water.

Please take a bite of cracker followed by a sip of water prior to evaluating the sample.

Place a mark in the box that represents your answer for each of the following questions.

**Code _____**

1. Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **FLAVOR** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

2- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **TEXTURE** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

**Now taste the second sample**

**Code _____**

1- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **FLAVOR** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (2) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

2- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **TEXTURE** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (2) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

**Now taste the third sample:**

**Code _____**

1- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **FLAVOR** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (3) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

2- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **TEXTURE** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (3) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

**Now taste the fourth sample:**

**Code _____**

1- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **FLAVOR** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (4) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

2- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **TEXTURE** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (4) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

## APPENDIX B

## HARD COOKED EGG SENSORY BALLOT USED IN THE SENSORY EVALUATION TEST BETWEEN THE TREATMENTS AND THE REARING SYSTEMS

Please take a bite of cracker followed by a sip of water prior to evaluating the sample. Place a mark in the box that represents your answer for each of the following questions.

**Code** _____

1- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **FLAVOR** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

2- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **TEXTURE** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

3- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **COLOUR** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

4- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **ODOR** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

**Now taste the second sample**

**Code _____**

1- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **FLAVOR** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

2- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **TEXTURE** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

3- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **COLOUR** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

4- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **ODOR** (1:Dislike and 9: Like)

☐☐☐☐☐☐☐☐☐

Dislike Neither Like

| | | |
|---|---|---|
| Extremely | Like or Dislike | Extremely |
| (1) | | (9) |

**Now taste the third sample**

**Code** _____

1- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **FLAVOR** (1:Dislike and 9: Like)

□□□□□□□□□

| | | |
|---|---|---|
| Dislike Extremely | Neither Like or Dislike | Like Extremely |
| (1) | | (9) |

2- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **TEXTURE** (1:Dislike and 9: Like)

□□□□□□□□□

| | | |
|---|---|---|
| Dislike Extremely | Neither Like or Dislike | Like Extremely |
| (1) | | (9) |

3- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **COLOUR** (1:Dislike and 9: Like)

□□□□□□□□□

| | | |
|---|---|---|
| Dislike Extremely | Neither Like or Dislike | Like Extremely |
| (1) | | (9) |

4- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **ODOR** (1:Dislike and 9: Like)

□□□□□□□□□

| | | |
|---|---|---|
| Dislike Extremely | Neither Like or Dislike | Like Extremely |
| (1) | | (9) |

**Now taste the fourth sample**

**Code** _____

1- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **FLAVOR** (1:Dislike and 9: Like)

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

2- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **TEXTURE** (1:Dislike and 9: Like)

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

3- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **COLOUR** (1:Dislike and 9: Like)

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

4- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **ODOR** (1:Dislike and 9: Like)

| Dislike Extremely (1) | Neither Like or Dislike | Like Extremely (9) |
|---|---|---|

## APPENDIX C

## CONSENT FORM

## PROJECT TITLE: EVALUATION OF SOY AND SOY FREE DIETS USING CAGES AND CAGE FREE (FREE RANGE) FACILITIES BASED ON EGG QUALITY AND SENSORY ATTRIBUTE

You are invited to take part in a research study being conducted by Dr. Bailey, a researcher from Texas A&M University. The information in this form is provided to help you decide whether or not to take part. If you decide to take part in the study, you will be asked to sign this consent form. If you decide you do not want to participate, there will be no penalty to you, and you will not lose any benefits you normally would have.

Why Is This Study Being Done?

The purpose of this study is to determine any sensory differences in scrambled egg that come from laying hens fed Soy and Soy free diets.

Why Am I Being Asked To Be In This Study?

You are being asked to be in this study because you are an egg consumer.

How Many People Will Be Asked To Be In This Study?

50 people (participants) will be invited to participate in this study locally.

What Are the Alternatives to being in this study?

The alternative to being in the study is not to participate.

What Will I Be Asked To Do In This Study?

You will be asked to taste a set of egg samples and answer questions including texture, and flavor acceptability. Your participation in this study will last up to 15 minutes,

Are There Any Risks To Me?

There are no risks to you to be in this study.

Will There Be Any Costs To Me?

Aside from your time, there are no costs for taking part in the study.

Will I Be Paid To Be In This Study?

You will not be paid for being in this study.

Will Information From This Study Be Kept Private?

The records of this study will be kept private. No identifiers linking you to this study will be included in any sort of report that might be published. Information about you will be kept confidential to the extent permitted or required by law. People who have access to your information include the Principal Investigator and research study personnel. Representatives of regulatory agencies such as the office of Human Research Protection (OHRP) and entities such as the Texas A & M University Human Subjects Protection Program may access your records to make sure the study is being run correctly and that information is collected properly.

Who may I Contact for More Information?

You may contact the Principal Investigator, Dr. Christopher Bailey, to tell him about a concern or complaint about this research at 979-945-7537 or chris.bailey@ag.tamu.edu. For questions about your rights as a research participant; or if you have questions, complaints, or concerns about the research, you may call the Texas A & M University Human Subjects Protection Program office at (979) 458-4067, toll free at 1-855-795-8636, or by email at irb@tamu.edu.

What if I Change My Mind About Participating?

You have the choice whether or not to be in this research study. You may decide not to participate or stop participating at any time. If you choose not to be in this study, there will be no personal impact. You can stop being in this study at any time with no personal impact.

STATEMENT OF CONSENT

I agree to be in this study and know that I am not giving up any legal rights. The procedures, risks, and benefits have been explained to me, and my questions have been answered. I know that new information about this research study will be provided to me as it becomes available and that the researcher will tell me if I must be removed from the study. I can ask more questions if I want. A copy of this entire consent form will be given to me.

| | |
|---|---|
| Participant's Signature | Date |
| Printed Name | Date |

INVESTIGATOR'S AFFIDAVIT:

Either I have or my agent has carefully explained to the participant the nature of the above project. I hereby certify that to the best of my knowledge the person who signed this consent form was informed of the nature, demands, benefits, and risks involved in his/her participation.

| | |
|---|---|
| Signature of Presenter | Date |
| Printed Name | Date |