Supplementation of Red Seaweed (*Chondrus crispus*) and Tasco® (*Ascophyllum nodosum*) in Laying Hen Diets

by

Cassandra Michelle Stupart

Submitted in partial fulfillment of the requirements

for the degree of Master of Science

at

Dalhousie University

Halifax, Nova Scotia

November 2019

I dedicate this work to my wonderful husband, Raymond, my incredible mother, Tracy, and my fantastic sister, Amanda, for their never-ending love and support

Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

This study evaluated the effect of dietary red seaweed (CC) and brown seaweed (Tasco®) on laying hens. Two commercial strains of hens [Lohmann LSL Lite (LL) and Lohmann Brown Lite (LB)] were assessed for production performance and egg quality from 34 to 70 weeks of age. The CC was included as a ground or extruded ingredient. After 70 weeks of age, birds were moved to controlled environment rooms where heat stress could be applied to half the birds for a 4-week period. As the birds aged, the two strains performed differently. LB hens had smaller eggs compared to LL hens, subsequently causing higher shell density, shell thickness and shell breaking strength in the LB hens. The LB hens were more reactive to heat stress conditions than LL. There is indication that for albumen height and shell density, extrusion of CC could reduce the impact of heat stress on LB hens.

LIST OF ABBREVIATIONS USED

ANOD	*Ascophyllum nodosum*
BW	Body Weight
CC	*Chondrus crispus*
ECC	Extruded *Chondrus crispus*
FCR	Feed Conversion Ratio
GCC	Ground *Chondrus crispus*
GIT	Gastrointestinal Tract
HTST	High Temperature Short Time
LL	Lohmann LSL Lite
LB	Lohmann Brown Lite
SG	Specific Gravity

ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge my supervisor, Dr. Bruce Rathgeber, for his endless support. Bruce, you've been there for me whenever I needed you, whether academic related or not. Your personality and willingness to provide for your students does not go unnoticed. I can't begin to tell you how much I appreciate you and how you've inspired me. Thank you, for everything you've done and everything you will do.

Next, I would like to thank Janice MacIssac, for her endless hours of assistance with data entry, data analysis, ordering supplies, and much more. Thank you for being there to answer my never-ending questions.

Next, I would like to thank my committee members, Stephanie Collins and Younes Miar, for their support throughout this whole process. Thank you for taking time out of your busy schedules to assist me.

I would of course like to thank my fellow graduate students, Holly Fisher, Nilakshi Abeysinghe, Xujie Li, Janessa Henry, and Kayla Graham, for all their help over the course of the trial.

I am grateful to the Atlantic Poultry Research Institute, Acadian Seaplants, Pan Atlantic and Egg Farmers of Nova Scotia for their financial support.

Finally, a huge thanks goes out to Sarah MacPherson and Ron Mekers of the Atlantic Poultry Research Centre for their assistance with feeding egg collection, sampling and general management of the birds. I'd like to give a huge thank you to Jamie Fraser as well, for her help with preparing all the diets for these trials.

CHAPTER 1: INTRODUCTION

Worldwide, the egg industry is moving toward cage-free production, posing a higher threat of disease compared to conventional caging systems (Egg Industry, 2018). Compared to broilers, laying hens are exposed to very few antibiotics in their lifetime (Van den Bogaard et al., 2002), as they are typically used for disease outbreak as opposed to preventative measures. With the directional movement toward antibiotic free production, antibiotics have become very seldom, if not completely absent for use in laying hen flocks in Canada. The use of them has faced scientific and consumer investigation as their use has been linked to an increase in antibiotic resistant strains of bacteria, inevitably posing a threat to the effective treatment of bacterial infections in humans. Ultimately, antibiotic use in all livestock production is negatively interpreted by the public and is linked with negative associations regarding their use. As a result, there is an increasing interest from both broiler and layer chicken producers to find natural gut enhancers to eliminate the need for antibiotics all together (Baurhoo et al., 2007).

Many natural alternatives have been evaluated and show promising results as potential feed additives in poultry. These include marine products, organic acids, probiotics, prebiotics, and herbal remedies (Venkitanarayanan et al., 2013). Prebiotics in specific have been shown to selectively increase the growth of beneficial microbes and inhibit pathogen colonization (O'Sullivan et al., 2010). Among prebiotics, seaweeds have become of interest as feed additives in poultry (Richmond, 2004). Seaweeds have been previously determined to enhance the immune system, modulate growth and positively influence microbial populations in pigs and ruminants (Evans and Critchley, 2014). However, their effects in

poultry have not been explored in depth, especially when considering laying hens. The effect of processing method on seaweed feed efficiency is also yet to be explored.

Red seaweeds are rich in unique carbohydrates, whereas brown seaweeds tend to have higher dietary fiber (Miscurcova et al., 2010) and a rich composition of phlorotannins (Zhang et al., 2018). Approximately 30% of seaweed biomass produced is used as animal feed (Richmond, 2004). The carbohydrates within seaweeds are considered prebiotic, and have the potential to reduce levels of pathogenic bacteria (Gudiel-Urbano and Goñi, 2002). In a study by MacArtain et al. (2007), dietary inclusion of seaweeds fed to commercial laying hens enhanced gut microbiota. Choi et al. (2014) demonstrated that seaweeds prime the immune system in birds. In another study, feeding seaweeds resulted in increased growth rate and nutrient uptake in chickens and ducks (El-Deek and Mervat Brikaa, 2009).

Over the past few decades, production performance of commercial laying hens has drastically improved. When investigating new feed ingredients in laying hen diets, it is important to consider the impact on production performance traits. Implementing new feed ingredients can pose many benefits, but if the additive is detrimental to egg production, egg quality or body conformation, a risk of loss of profit for the producer is possible. Carillo et al. (2008) found that supplementation of layer hen diets with the seaweed *Macrocystis pyrifera* resulted in elevated levels of n-3 fatty acids in the egg, indicating that including seaweeds in the diet of laying hens may have a positive effect on egg quality.

Revenue is optimized when a hen is at an optimal weight, is laying and eating at an efficient rate, and is laying eggs with superior egg quality. In order to ensure these traits are not compromised, when investigating new feed ingredient, procedures must be in place to track

these production performance traits. Measuring body weights and tracking daily egg production will help to monitor any changes that may occur during a feed trial. In addition, egg quality measurements should be performed. These include shell density of the egg, egg weight, shell breaking strength, yolk weight, albumen height, shell weight, yolk color, and shell thickness. In taking these measurements, it can be determined that a new feed ingredient is not adversely affecting egg quality and that the egg is kept up to consumer and grading center expectations.

Feed processing has the potential to improve bird feed efficiency, reduce costs and improves gut health and function (Kiarie and Mills, 2019). Extrusion is a combination of heat, shear and compressional forces. Utilization of this method causes starches to expand due to gelatinization and cross-linking of proteins within the matrix. The effect of processing method on seaweed feed efficiency is yet to be explored. Extruding poultry feeds has many advantages over basic pelleting and mashing. According to Peisker (1994), extrusion increases fat stability, allows additional fat to be added, increases metabolizable energy, decreases microbial contamination and increases soluble fiber.

The aim of this study is to investigate the effect of dietary inclusion of two seaweed species (i.e., *Chondrus crispus* and *Ascophyllum nodosum*) with two processing methods (ground and extruded) on laying hen productivity in both a standard temperature environment (short term and long term) and a heat stressed environment.

CHAPTER 2: LITERATURE REVIEW

A thorough literature review pertaining to the topics related to this project will be discussed in the following section. The idea behind why seaweed is an ideal feed ingredient in poultry, feed supplementation with seaweed, extrusion technology, laying hen performance traits and poultry in heat stressed environments will be discussed.

2.1 Why Feed Seaweed?

When studying a new potential feed ingredient, there is always the question of, "why do we want to feed this ingredient?" Seaweed has many positive nutritional properties, and there is an abundance of it along the coasts of Canada, making it an ideal candidate for livestock feed. Seaweeds are rich in vitamin and minerals and they exhibit prebiotic effects. These properties of seaweeds will be discussed in depth in this section.

2.1.1 History of Antibiotic Use in Poultry Production

The European Union banned the use of antibiotics as growth promoters in broiler chicken production as of January 1st, 2006 (Castanon, 2007). In North America, there has been an increased public awareness of the negative effects of antibiotics and as a result, there is strong directional movement into the development of alternatives (Yan et al., 2011). Ingredients with antimicrobial properties such as enzymes, peptides, bacteriophages, organic acids, plant extracts, probiotics, and prebiotics have been investigated for use in the broiler industry (Hinton and Mead, 1991; Joerger, 2003; Kiarie et al., 2013). When considering the laying hen industry, there are only few antibiotics still allowed by FDA for use. However, it is estimated that only a small percentage of flocks producing conventional eggs will ever receive antibiotics due to effective use of vaccines and other

management practices that minimize the need for antibiotics to treat illness (US Poultry and Egg Association, 2017). In Canada, antibiotics have been completely phased out in the laying hen industry in accordance with CFIA regulations. With the shift toward cage-free production, effective disease management will become more difficult.

2.1.2 Microbiota of the Chicken

The gastrointestinal tract (GIT) in the chicken houses a diverse population of bacteria, fungi and protozoa. Of these, bacteria are the most abundant organisms, consisting of both beneficial and harmful. *Lactobacillus* and *Bifidobacterium* are both considered beneficial and contribute to overall health of the animal and productivity in the GIT. They play a role in detoxification, modulation of the immune system and protection against pathogens. Beneficial bacteria secrete digestive enzymes including casein phosphatase, amylase and lipase that aid in nutrient digestion. On the other hand, *Clostridium* and *Salmonella* can be considered harmful at higher abundances, and produce toxins that lead to intestinal decomposition and infections (Gong et al., 2002). Aerobes are organisms that survive and grow in oxygenated environments whereas facultative anaerobes are organisms that can perform both anaerobic and aerobic respiration. In the chicken gut, aerobes and facultative anaerobes include *Escherichia*, *Lactobacillus*, and *Streptococcus*, and are the first bacterial species to colonize the GIT. Obligate anaerobes, such *Bacteroides*, *Eubacterium* and *Bifidobacterium*, thrive in non-oxygenated environments and are able to colonize the GIT after the aerobic bacteria. Obligate anaerobes perform most of the fermentation in the ceca, and are very abundant in the adult microflora (Dibner and Buttin, 2002).

Diversity of bacteria in the GIT increases from proximal to distal. Population of microbes among different parts of the GIT depends on the ability of the organism to bind to

enterocytes or the mucus layer, their tolerance to GIT environmental conditions and tolerance to the host immune system. Rate of passage of digesta, pH, nutrient availability, and the presence of antimicrobial substances also contributes greatly to microbial diversity (Apajalahti., 2005). The ileum, in specific, has a slow rate of digesta movement in the distal portion, which provides an ideal timeframe for both beneficial and harmful bacteria to bind to the wall, perform fermentation, and establish (Danicke et al., 1999).

2.1.3 Prebiotics as Alternatives to Antibiotics

Prebiotics are non-digestible food ingredients that selectively stimulate the growth and activity of bacteria in the gut (Gibson and Roberfroid, 1995). They help to improve gastrointestinal health by providing carbohydrates as a substrate for growth and establishment of beneficial bacteria (Cummings and Macfarlane, 2002). Carbohydrates include polysaccharides (pectins, hemicelluloses, gums, inulin and resistant starches), oligosaccharides (raffinose, stachyose, fructo-oligosaccharides, galactooligosaccharides and resistant dextrins), and sugars (lactulose, non-absorbed lactose and non-absorbed fructose). Most of the species of healthy bacteria, including *Bifidobacterium, Ruminococcus*, and *Lactobacillus* are able to utilize these carbohydrates (Gibson and Roberfroid, 1995). In a previous study, it was found that supplementation with fructooligosaccharide and galactooligosaccharide modulated the gut microbiota in broiler chickens by increasing the beneficial bacteria (*Bifidobacterium and lactobacilli)* and competitively reducing *Campylobacter jejuni* (Baffoni et al., 2012).

The prebiotic effects of oligosaccharides, including inulin, mannan-oligosaccharides, fructo-oligosaccharides, and galacto-oligosaccharides have been evaluated as potential feed additives in laying hens (Li et al., 2007; Ghasemian and Jahanian, 2016). Healthy

bacteria utilize these non-digestible carbohydrates to produce metabolites. Short chain fatty acids (SCFA) produced, such as acetic, butyric and propionic acids help to maintain mineral uptake and provide energy to the bird (Lan et al., 2005). Organic acids such as lactic acid and SCFA have the ability to lower gut pH and inhibit function and growth of acid sensitive pathogens, such as *Salmonella* (Donaldson et al., 2008). Finally, prebiotics help to enhance the activity of healthy microbiota, eventually aiding in the animal's ability to absorb minerals and vitamins in the intestines (Sako et al., 1999).

2.1.4 Seaweeds as Prebiotics

The prebiotic effects of seaweeds have been demonstrated in several studies. Gudiel-Urbano and Goni (2002) conducted a study in which rats were fed the red seaweed "nori" (*Porphyra tenera*) and the brown seaweed "wakame" (*Undaria pinnatifida*). The study showed alteration of composition and metabolic activity of microbes in the gut. Cecal pH was also increased (Gudiel-Urbano and Goni 2002). The brown seaweed *Laminaria digitata* has shown to increase acetic acid, propionic acid, and butyric acid concentrations in the large intestine of pigs (Hoebler et al., 2000). Seaweeds such as *Porphyra yezoensis, Undaria pinnatifida, Laminaria japonica, and Hizikia fusiformis* have demonstrated improved nutrient digestion as they bind to bile salts, inhibiting uptake of fats and lowering cholesterol (Wang et al., 2001). Finally, due to their fiber content, red and brown seaweeds have the ability to alter microbial activity, causing a decrease in enzymatic reactions that are associated with toxic enzymes, such as β-glucuronidase, nitroreductase, and azoreductase (Gudiel-Urbano and Goni 2002).

2.2 Feed Supplementation with Seaweed

Marine environments are a rich source of unique biological and chemical diversity. Many marine products, including seaweed, have been utilized to improve human and animal health. Seaweeds are classified into three groups: green algae (*Chlorophyta*), brown algae (*Phaeophyta*) and red algae *(Rhodophyta*) (Garson, 1989). Each group of seaweed has associated pigments, giving them their characteristic colours (Guiry and Guiry, 2015). Seaweeds are rich in dietary fibers and carbohydrates, allowing them to reach the lower GIT largely undigested and act as substrate for bacterial fermentation in the intestines, ceca and colon (MacArtain et al., 2007). Seaweeds also contain minerals, vitamins, proteins, polyphenols, and carotenoids (Ventura et al., 1994). The composition of seaweed differs slightly depending on type, but almost all algal fibers are soluble anionic polysaccharides that contain sugars unique to seaweeds (Lahaye et al., 1993). Red seaweeds are rich in unique carbohydrates, whereas brown seaweeds tend to have higher dietary fiber at around 40% dry matter (Miscurcova et al., 2010) and are rich in phlorotannins (Zhang et al., 2018). Minerals within seaweeds include Fe^{+2}, Cu^{+2}, Zn^{+2}, and Ca^{+2} (MacArtain et al., 2007).

2.2.1 Seaweeds in Livestock Production

Seaweeds and seaweed extracts are widely used as animal feed in a number of countries. Approximately 30% of seaweed biomass produced is used as animal feed (Richmond, 2004). The carbohydrates within seaweeds are considered prebiotic, and have the potential to reduce levels of pathogenic bacteria in the GIT (Gudiel-Urbano and Goñi, 2002). Studies with broilers showed that dietary inclusion of seaweed enhanced the health and productivity by increasing the growth of beneficial gut-microbiota in the lower GIT (Abudabos et al., 2013). In a study by MacArtain et al. (2007), dietary inclusion of

seaweeds fed to commercial laying hens enhanced gut microbiota. Choi et al. (2014) demonstrated that seaweeds prime the immune system in birds. Gudiel-Urbano and Goñi (2002) found that both red and brown seaweeds altered the metabolic activity of beneficial microbiota and reduced the number of pathogenic bacteria in rats, demonstrating prebiotic effects. Carrillo et al. (2008) found that supplementation of layer hen diets with the seaweed *Macrocystis pyrifera* resulted in elevated levels of n-3 fatty acids in the egg. In another study, feeding seaweeds resulted in increased growth rate and nutrient uptake in chickens and ducks (El-Deek and Mervat Brikaa, 2009).

2.2.2 *Ascophyllum nodosum* and Tasco®

Ascophyllum nodosum (ANOD) is a species of brown seaweed containing 1.3% insoluble fiber (MacArtain et al., 2007) and various bioactive polysaccharides such as laminarin, fucose containing polysaccharide (FCP), and alginates that are beneficial to health and growth (Lynch et al., 2010). Tasco® is a commercially produced, sun-dried, ground ANOD produced my Acadian Seaplants Ltd in Dartmouth, Nova Scotia. Many studies utilizing dietary inclusion of Tasco® have been conducted in agricultural animals. Most have researched its effects in ruminants, although a few have studied Tasco® in swine (Wiseman et al., 2012). In a study by Bach et al. (2008), steers were inoculated with *E. coli* O157:H7 and fed Tasco® at levels of 1% and 2% for 14 days, and 2.0% for 7 days in comparison to a negative control. *E. Coli* detection and concentrations were less in samples obtained from steers fed Tasco®, regardless of inclusion level. When Bach et al. (2008) fed Tasco® to lambs, *E. coli* populations were decreased when Tasco® was fed at 1.0% for 28 days. In another study involving broilers carried out by Wiseman et al. (2012), low levels of Tasco® (0.25% and 0.5%) were consistently effective at enhancing growth. It is

suggested that Tasco® plays a role in bioavailability of trace minerals, vitamins, and/or antioxidants, and alteration of digestibility (Fike et al., 2001).

2.2.3 *Chondrus Crispus*

Red seaweeds (i.e., *Chondrus crispus*, *Palmaria palmata*, *Porphyra* sp., and *Mastocarpus stellatus*) are commercially harvested along Pacific and Atlantic coasts and selected strains, such as *C. crispus*, are grown on land (Hafting et al., 2012). Red seaweeds are a good source of non-digestible carbohydrates, minerals, vitamins, carotenoids, amino acids, and several health-promoting compounds (Holdt and Kraan, 2011). Red seaweeds are rich in unique carbohydrates, such as floridean starch, sulfated galactans, agar, carrageenans and uronic acid. Carrageenans and sulphated galactans represent the main polysaccharides (Bouhlal et al., 2011). Due to the proportion of unique, non-digestible carbohydrates, there is potential that red seaweed *C. crispus* could make an effective prebiotic feed additive in livestock.

Research pertaining to the specific seaweed *C. Crispus* in livestock is quite limited. In one study, Kulshreshtha et al. (2017) supplemented laying hens with *C. crispus* and found improved feed conversion per g of egg, increased yolk weight, higher egg weight, greater villus height and greater villus surface area. They also found an increase in abundance of beneficial bacteria (*B. longum, S.salicarius*) and reduction in harmful bacteria (*C. perfringens*) in the gut of the hens. Additionally, the concentration of short-chain fatty acids (acetic acid, propionic acid, and butyric acid) was significantly higher. Kulshreshtha et al. (2017) concluded that inclusion of red seaweed could act as a potential prebiotic to improve performance, egg quality and overall gut health in laying hens.

2.3 Extrusion Technology

Extrusion is a combination of heat, shear and compressional forces. Utilization of this method causes expansion of starches due to increased gelatinization and cross-linking of proteins within the matrix. The result is a strongly bound, but porous pellet. Ileal digestibility of extruded feed is dependent on polysaccharide profile and processing variables (Glencross, 2016). Extrusion technology is considered the most economic and is utilized by the cereal and snack food industries (Harper and Clark, 1979). Foods that are rich in cereals, starches and vegetable proteins give texture, structure, mouth feel, bulk and other characteristics that provide functional properties such as expansion index, bulk density, water absorption and solubility indices, and viscosity. Therefore, these types of foods are generally the most frequently used for extrusion (Oikonomou and Krokida, 2011).

Extrusion technology is considered a high temperature short time (HTST) heat treatment that modifies raw ingredients into finished products (Reddy and Reddy, 2015). Extrusion cooking influences the nature of feed components by changing physical (particle size), chemical (starch gelatinization, inactivation of antinutrients) and nutritional (nutrient digestibility) properties (Diaz et al., 2006). During the extrusion cooking process, moistened, expandable feed materials are plasticized in a tube via moisture, pressure, heat and mechanical shear forces. The ingredient is loaded into a holding tank with a mixing cylinder and slowly fed into a series of pipes where the ingredient is heated via steam. The ingredient makes its way to an extrusion barrel where the actual extrusion process takes place. The extrusion barrel has a number of locks, dies and orifices with increasing restriction from beginning to end. The ingredient undergoes increasing pressure, friction

and attrition while passing through the extruder barrel whereby the ingredient reaches temperatures up to 200°C in 30 seconds or more. The ingredient is then forced through a die and exits the extrusion barrel, where there is a sudden drop in pressure that causes an expansion due to steam escaping the product. The loss of steam reduces moisture content by up to 50 percent depending on the original moisture content. The resulted product creates desirable changes, including shearing and gelatinization of starches, denaturation and shearing of protein, destruction of microorganisms and some toxicants, restructuring of tactile components and dehydration (Reddy and Reddy, 2015).

Camire (2000) stated five general physicochemical changes that may occur during extrusion technology. These include binding, cleavage, loss of native conformation, recombination of fragments and thermal degradation. As a result, composition of the feed materials could be altered by physical losses occurring due to leakage of fat, evaporation of water and volatile compounds at the dye. The extent to which an ingredient willchange is dependent on many factors, including type of ingredient or diet, particle size, type of extruder, and type of reactants present, such as water, lipids, carbohydrate and proteins. The extruder conditions, such as moisture content, screw speed, barrel temperature, die diameter, feed rate, screw compression ratio, residence time, torque, pressure, energy input and pH, also contribute greatly to ingredient changes (Anguita et al., 2006). Higher extrusion barrel temperature increases the expansion and reduces the hardness of the resulting extrudates (Sebio and Chang 2000).

There is a wide range of research investigating the effects of extrusion conditions and feed compositions on functional properties of cereal and pulse based products (Seth and

(Rajamanickam, 2012). Many of these studies found that when feed moisture content is increased, the bulk density, water solubility index, water absorption index and hardness increase as a result, and the expansion ratio decreases (Kirjoranta et al., 2012).

2.3.1 Effect of Extrusion Processing on Poultry Performance

Extruding poultry feeds has many advantages over basic pelleting and mashing. According to Peisker (1994), extrusion increases fat stability, allows additional fat to be added, increases metabolizable energy, decreases microbial contamination and increases soluble fibre. Jones et al. (1995), found that broilers fed crumbled starter rations extruded prior to crumbling weighed more than broilers fed crumbled starter rations that were pelleted prior to feeding. Previous research has shown that increased amino acid bioavailability could occur when corn or soybean meal is expanded under various pressures. In a study by Kidd et al. (2005), extrusion cooking of feed resulted in improved broiler feed body weight and carcass weight in comparison to birds fed the same diets in the non-extruded mash form. In another study, extrusion of whole feeds enhanced gastrointestinal digestion and increased the AME and AME_n by 1.5 and 3.5% ($P < 0.05$). Extrusion or expansion of wheat or barley and their addition to basal diets increased apparent metabolizable energy of the diets by 1.5–2.5%. The study concluded that high temperature short time extrusion and expansion processes appeared to enhance energy of common feeds for broilers (Plavnik and Sklan, 1995).

In terms of the effect of extrusion on egg quality parameters, only minimal research is available. However, there have been studies that observe the effect of other processing effects on egg quality. In a study by Deaton et al. (1989), corn based diets milled with hammer or roller mills showed no effect on average hen day and eggshell breaking strength.

In another study, hens fed with a barley diet ground using a roller mill had higher egg weight compared to hens fed a barely diet ground using a hammer mill.

However, in another study, hens fed a barley diet ground by a roller mill had reduced feed intake and average hen day compared to maize and wheat diets, yet no differences were observed for those hens fed a barley diet with the hammer mill (P´erez-Bonilla et al., 2014). In a study by Hamilton and Proudfoot (1995), thermal treating feed had no affect on egg quality parameters such as egg weight, Haugh unit and occurrence of blood spots. Another study found that Leghorn hens fed a barley based diet that was flame roasted had higher Haugh unit scores compared with hens fed the non roasted diets, which may have been due to improved feed conversion in the hens fed the flame roasted diets.

2.3.2 Processing of Seaweed

There is little to no research investigating the effects of processing on feed efficiency and palatability of seaweeds in livestock feed. Feed processing involves mixing, cooling, drying, separation, pelleting, cooking, vacuum coating, steam exploding and extruding (Church, 1991). These processes are performed in order to reduce cost and improve digestibility (Firkens et al., 2001) and feed efficiency (Owens et al., 1997). These improvements are established through elevating the feed stability and hygiene, affecting the chemical and physical characteristics of the constitutive ingredients. This in turn elevates nutrient bioavailability and improves feed efficiency and animal growth performance (Behnke, 1996).

2.4 Laying Hen Performance Traits

In Canada, as of 2018 there were over 25.2 million hens in production at 1,152 farms across the country (Egg Farmers of Canada Annual Report, 2018). More than 729 million dozen eggs are produced each year. Ninety-one percent of Canadians say that they trust the quality standards of foods from Canadian farmers (Egg Farmers of Canada Annual Report, 2018). With the egg industry being such an important contribution to the Canadian economy, it is crucial that high standards of egg quality are maintained.

When investigating new feed ingredients in laying hen diets, it is important to consider the ingredient's impact on production performance traits. Implementing new feed ingredients can pose many benefits, but if the additive is detrimental to egg production, egg quality or body conformation, than there is a risk of loss of profit for the producer. Over the past few decades, production performance of commercial laying hens has drastically improved.

Revenue is optimized when a hen is at an ideal body weight, is laying and eating at an efficient rate, and is laying eggs with superior egg quality. In addition, it is important that a flocks mortality percentages stay in the normal range. In order to ensure these traits are not compromised, when investigating new feed ingredients, procedures must be in place to track these production performance traits. Measuring body weights weekly or monthly, and tracking daily egg production per bird or cage will help to monitor any changes that may occur during a feed trial. In addition, egg quality measurements should be performed. These include shell density of the egg, egg weight, shell breaking strength, yolk weight, albumen height, shell weight, yolk colour, and shell thickness. In taking these

measurements it can be determined that a new feed ingredient is not adversely affecting egg quality and that the egg is kept up to consumer and grading center expectations.

2.4.1 Body Weight

Body weight is considered an important indicator of overall bird health for several reasons. An underweight or overweight hen may exhibit negative downstream health outcomes. The importance of maintaining proper body weight in terms of industry consideration relates to egg quality. Body weight has been found to correlate with egg size. During rearing, pullets are raised to a targeted weight and age that corresponds with breeder recommendations. Layer performance following the onset of lay is directly related to pullet development. Body weight directly influences egg production (egg weight in particular) and feed intake (Harms, et al., 1982; Bish et al., 1985). In a study by Lacin et al. (2008), hen body weight significantly affected several egg quality measures, such as shape index, yolk colour, albumin index, and Haugh Unit, but did not significantly influence shell strength, shell thickness and yolk index. In the same study, the hens that were not in the overweight group had higher egg production, Haugh Unit and improved feed conversion ratios.

The body weight of a hen is an important factor in managing on-farm performance in the egg industry. Homogeneous nutrient requirements ensure that there is less variability in body weight. All nutrients should be formulated and included in sufficient amounts that meet the requirements for optimal growth. This not only reduces feed costs but also improves a hens laying performance (Madsen and Pedersen, 2010). If the optimal body weight is not maintained, a hen can suffer from resulting health conditions. Larger body weights are associated with increased fat accumulation, early onset of sexual maturity, accelerated ovarian follicular development, and multiple ovulations (double yolks) (De

Beer and Coon, 2007). Measuring body weight of a hen can be achieved using many methods, such as a pan balance with live weight function and a hanging shackle, or a pan balance and large tarred container. More expensive and complex equipment is also available on the market that can be implemented in cages such that body weight is measured automatically.

2.4.2 Feed Consumption

The nutritional content of a laying hen diet is dependent on age, with each age phase posing separate requirements. Feeding of laying hens is very specific starting at hatch and ending at depopulation, which generally occurs around the 80-week mark in Canada. The breeder provides suggested nutrient requirements for hens at different age ranges, specified as phases. Lohmann provides suggested nutritional requirements up to what is referred to as Phase 4. Phase 4 is fed until the egg mass per hen per day is 56.3g. It is at this point that a new flock switch over is suggested. Lohmann suggests that crude protein, methionine and linoleic acid content be specifically noted when considered nutritional factors. As a bird ages, each diet phase change is associated with higher calcium levels and lower phosphorus levels (Lohmann LSL Lite Management Guide, 2019).

With any feed trial, it is important to track the feed consumed to ensure that the hens are not over eating or under eating as a result of a new feed ingredient. Over eating is both costly and can lead to subsequent health and egg problems, such as large eggs. Under eating is even more likely to cause subsequent health problems and problems with egg quality, such as cracked eggs or small eggs. Factors affecting feed consumption include timing of feeding, feed texture, controlled feeding, feed level in troughs and frequency of feeding (Lhomann LSL Lite Management Guide). Implementing new feed ingredients could also

affect feed intake, as the taste of the ingredient could be a deterrent or a stimulant.

2.4.3 Feed Conversion

Feed conversion ratio (FCR) in laying hens is not calculated based on grams of feed consumed per gram of body weight gain, as it is in broiler production. Instead, because the product of interest in laying hen production is the egg, feed conversion is based on grams of feed consumed per gram of egg produced. The formula for calculating FCR in laying hens is as follows:

$$FCR = \frac{Feed\ Consumed\ (g)}{Avg\ Egg\ Weight\ (g) * Total\ \#\ of\ Eggs}$$

In order to calculate FCR, the feed consumed, average egg weight and total number of eggs laid must be known. FCR is generally calculated on a cage basis in poultry production as opposed to an individual basis. FCR is an important measurement as it is an indication of how well a hen utilizing their feed consumed. With rising feed costs, the target goal is to produce more eggs with less feed. Thus, lower FCR is ideal.

2.4.4 Egg Production

The reproductive tract of a laying hen is comprised of the ovary and oviduct. Only the left ovary and oviduct is functional in the adult as the right ovary and oviduct regress during development (Scanes, et al., 2004). The structure of the ovary and oviduct is shown in *figure 1*.

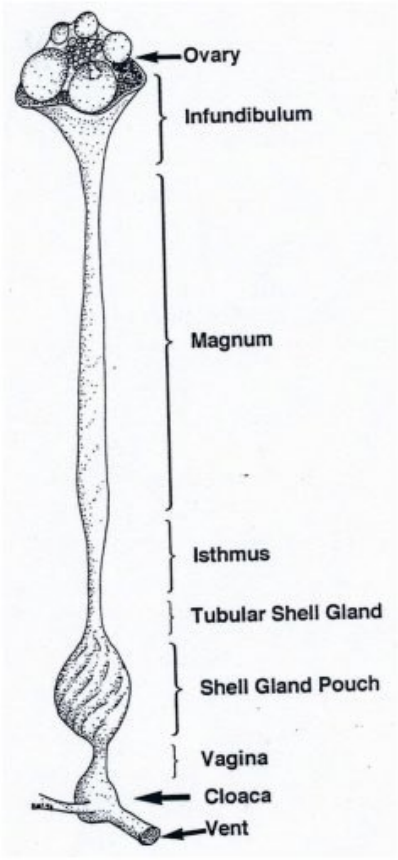

Figure 1. Structure of the Oviduct (source: Roberts and Brackpool, 1995)

In an immature bird, the ovary contains a mass of small ova, where 2000 or more are visible to the human eye. Only a small portion (250-500) of these ova will reach maturity and ovulate within the life span of most domesticated species (Johnson, 2015). Once an ovum, also known as the yolk, becomes mature, it is released from the follicle by rupture along a line called the stigma (Scanes et al., 2004). The left oviduct of a hen undergoes a quick development following 16 weeks of age, where it becomes fully functional just before the hen begins to lay eggs. This process occurs at approximately 20 weeks of age. There are five distinct regions of the oviduct. These are the infundibulum, magnum, isthmus, tubular shell gland, and the vagina (shown in *Figure 1*). Each region plays a particular role in egg formation.

When the ovum is released, it is engulfed by the infundibulum, where it resides for approximately 18 minutes. Next, the ovum travels to the largest portion of the oviduct- the magnum- which functions to produce albumen. Here, the ovum will remain for approximately 3 hrs. Next, the developing egg passes into the isthmus, where fibers are generated that makes up the inner and outer shell membranes. This process takes approximately one hour. The tubular shell gland is the next destination in the egg formation process, where water and electrolytes are added to the albumen. This process is referred to as "plumping", and takes about 5 hrs (Roberts, 2004). Calcite crystals begin to form at this location (Gautron and Nys, 2006; Dacke et al., 2015). Next, the egg moves to the shell gland pouch where it remains for a minimum of 15 hrs and the process of shell formation occurs (Roberts, 2004). Calcite growth continues in the shell gland pouch and calcium carbonate is deposited outward, forming the mammillary and palisade layers of the shell (Gautron and Nys, 2006). This mineralization stops 1 ½ hrs before oviposition (laying of the egg) and a thin, non-calcium based layer is deposited on the eggshell. Finally, the egg is laid through the vagina and cloaca (Nys et al., 2004; Roberts, 2004; Dacke et al., 2015). No longer than 30 minutes after oviposition, another ovum is released and the process starts over again. With this repeated process, an egg is laid approximately every 24 hours (Scanes et al., 2004).

2.4.5 Egg Quality

Egg quality is an important factor to consider when introducing a new feed ingredient to laying hens. Egg quality not only ensures that an egg makes it from farm to table, but also plays a significant role in consumer appeal. The overall appearance of an eggshell, yolk, and albumen is a determining factor for acceptance of an egg by a consumer. In addition,

there are standards that an egg must pass at a grading station in order to make it to market. There are a number of factors that influence egg quality. These include strain of hen (Tůmová et al., 2009), housing system (Đukić-Stojčić et al., 2009; Tůmová et al., 2009), age of the laying hens (Roberts and Ball, 2003; Silversides et al., 2006), nutrition (Świątkiewicz et al., 2010), disease (Berg et al., 1947), environmental conditions (Sarica et al., 2008) and stress (Roberts, 2004). *Figure 2* shows a schematic diagram of the egg structure. Each of the shown components will be discussed in detail in this section.

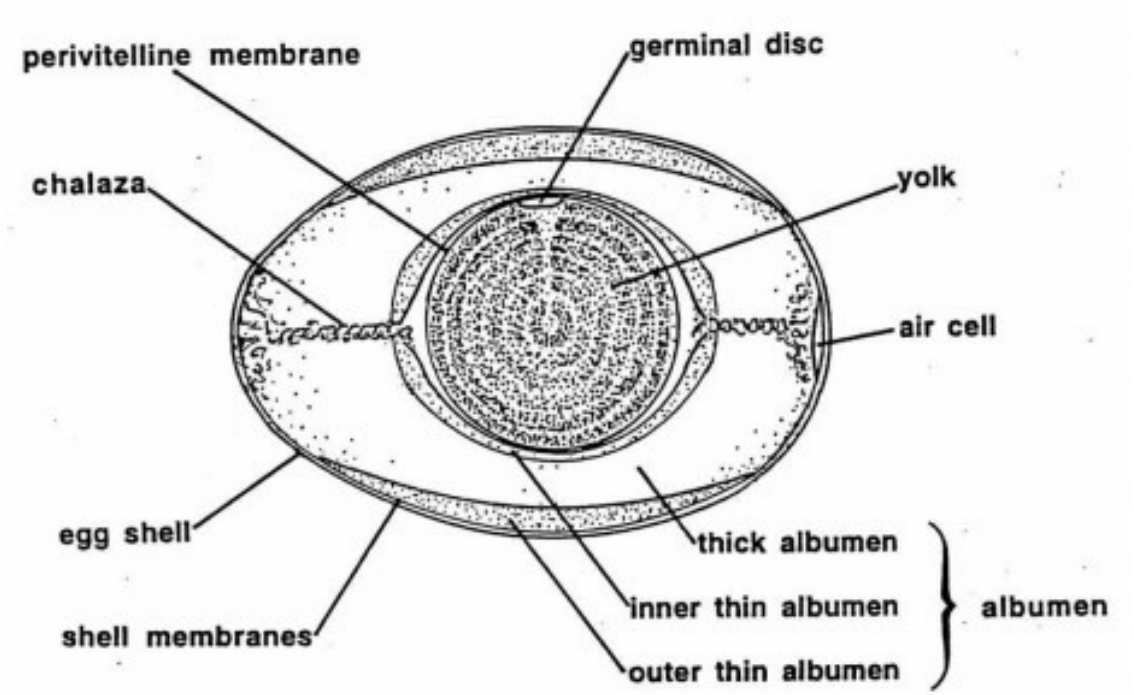

Figure 2. Schematic Diagram of Egg Structure (source: Roberts and Brackpool, 1995)

An egg is comprised of a central yolk that is surrounded by the perivitelline membrane, albumen, eggshell membranes, calcified eggshell and cuticle (Roberts, 2004; Mikšík et al., 2010). Generally, the yolk accounts for 32% of the entire egg, while the albumen accounts for 57% and the shell, 11% (Johnson, 2000). When performing egg quality measurements,

the percentage of each component can be determined by measuring the weight of the whole egg, the yolk weight, albumen weight and shell weight. The shell must be rinsed of any albumen residues using water and left out to dry before being weighed. Percentages of yolk, albumen and shell can be determined from these values.

2.4.5.1 The Yolk

The yolk accounts for up to 31% of a total egg and is the major source of vitamins and minerals within the egg. These include iron, vitamin A, vitamin D, vitamin E, vitamin B12, folate, selenium, choline, lutein and zeaxathin. The yolk contains approximately 33% lipids, 17% protein and 1% free carbohydrates and inorganic elements (Johnson, 2000). The function of the yolk is for nourishment of a growing embryo. Formation of the yolk occurs 10-12 days prior to oviposition. During ovulation, the yolk is released from the yolk sac at the upper, open end of the infundibulum. The vitelline membrane, known as the yolk membrane, is the outermost layer that holds the yolk together, while the laterba, germinal disk, and concentric layers of light and dark are in the middle (Stadelman et al., 1995). The germinal disk is a small white spot that lies on the yolk and is visible by the human eye. The germinal disk is approximately 2mm in diameter (Jacob et al., 2000).

The yolk is made up of 50% solids, whereas the albumen only contains 12% solids. Therefore, eggs with larger yolks will have larger total solids content. The percentage solids in a whole egg is dependent on factors such as ratio of yolk to albumen as well as total solids content in the yolk and albumen (Washburn, 1979). The yolk to albumen ratio varies depending on the size of the egg (Marion et al., 1964). Factors such as age and stain of hens, as well as storage conditions can affect the total solids content of a yolk. In

addition, it is likely that genetics plays a role in total solids content as a result of intense genetic selection for egg size and egg production.

Studies have shown that yolk weight increases as a hen ages (Fletcher et al., 1983). Yolk content has been shown to be lesser in smaller sized eggs compared to larger ones (Kaminska and Skraba, 1991). Measuring yolk weight is an important consideration as the yolk holds the majority of the whole egg's value. Thus, measuring yolk weight is an excellent quality control measure when performing feed trials and also gives an indication of declining quality as a hen ages. To measure yolk weight, the yolk must be separated from albumen and simply placed on a pan balance.

2.4.5.2 Eggshell Quality

The eggshell is comprised of calcium carbonate and functions to keep the egg intact and prevent penetration of bacteria and other contaminants. The eggshell is comprised of several membranes (limiting membrane, outer membrane and inner membrane), the mammillary core, a surrounding organic matrix, and finally, an outer coating, called the cuticle. The eggshell can be divided into three layers, called the mammillary layer (innermost), palisade layer (middle), and the surface crystal layer (outermost) (Roberts and Brackpool, 1995). These components are all shown in *Figure 3*. The eggshell is comprised of both organic and inorganic components. The organic components account for 5% of the eggshell, while the inorganic components account for 95% (Fernandez et al., 2003).

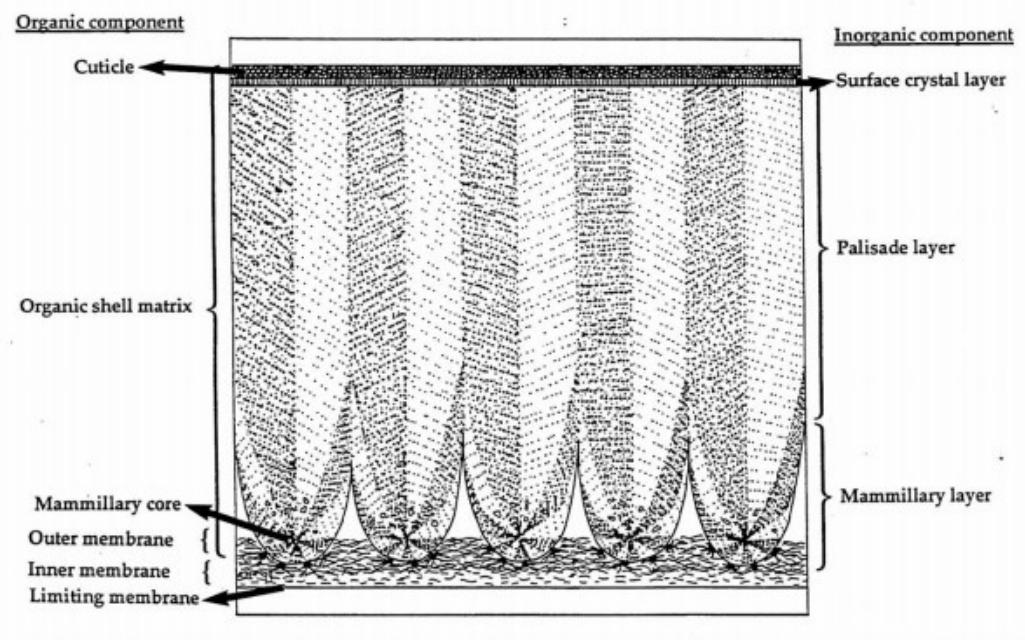

Figure 3. Structure and Layers of the Eggshell (Source: Roberts and Brackpool, 1995)

It is important that an egg remains intact through the transportation process from farm, to wash, to grading, and finally, to the grocery store. Considering eggshell quality is important in terms of consumer appeal, as it is common that a consumer will inspect a carton of eggs for visible cracks and will likely reject any cracked eggs that they discover (Roberts and Ball, 2003). In order to access eggshell quality, four measurements can be made. First, eggshell weight indicates what percentage of the egg's weight is taken up by the shell. The percentage of eggshell in the whole egg is approximately 11% (Johnson, 2000). Second, eggshell thickness indicates an egg's susceptibility to breakage, and can also be an indicator of deterioration in layer performance at the end of the laying cycle. As a bird ages, eggs become larger and a hens ability to absorb calcium decreases. Thus, the resulting shell becomes thinner (Cordts et al., 2002; Roberts et al., 2013). Determining

eggshell thickness can be performed either with the cuticle still intact, or after removing the cuticle. The measurement can be achieved by using a set of callipers, or a machine such as a texture analyser (Stable Micro Systems, model TA.XT Plus).

Thirdly, shell density is another important quality measurement that determines the approximate density of the eggshell. Density is strongly correlated with shell thickness, in which thicker shells generally have a higher density, and thinner shells have a lower density. Density measurements can range from 0.072g/cm^3 to 1.104 g/cm^3, with 0.090 g/cm^3 being the average at peak production. To measure density, specific combinations of salt and water can be created at each density of interest. Eggs are then submerged in these solutions and the density of any egg that floats to the top corresponds with that solutions specific gravity. That is, if an egg floats to the surface of the water with a 0.086g/cm^3 specific gravity concentration, the density of the egg is 0.086g/cm^3.

Lastly, egg-breaking strength determines the amount of force (kg) required to crack an eggshell. Measuring egg-breaking strength can be determined using a texture analyser (Stable Micro Systems, model TA.XT Plus), similar to measuring shell thickness, but with a different attachment probe. Egg breaking strength is a good indicator of shell quality as it helps determine the likelihood of an egg making it through transfer and packaging whilst subject to circumstances that could cause cracking. Kemps et al. (2006) viewed egg-breaking strength in two separate categories: material strength and structural strength. Material strength is dependent on the relationship between the mineral and organic components within the shell, whereas structural strength is dependent on factors such as size, shape, thickness, and distribution of the shell components (Kemps et al., 2006).

Measuring shell weight, shell thickness and shell density will give a strong indication of eggshell quality.

2.4.5.3 Albumen

The albumen is a clear, jellylike substance that surrounds the yolk and is generally referred to as the egg whites by consumers. The albumen accounts for approximately 60% of the whole egg weight (Stadelman et al., 1995) and contains 88% water, 9-10% protein, 0.4-0.9% carbohydrates, 0.5-0.6% minerals and a small fraction (0.03%) of lipids. The albumen encompasses four layers in the egg. These chalaziferous layer accounts for 2.7% and is attached to the yolk. The inner thin layer accounts for 6.8%, the outer thick layer accounts for 57.3%, and finally, the outer thin layer accounts for 23.2% (*Figure 2*) (Johnson, 2000). There are several proteins in the albumen, some of the most abundant being ovalbumin (54%), ovotransferrin (13%), ovomucoid (11%), ovoglobulins (8%), lysozyme (3.5%) and ovomucin (2%). Ovotrasnferrin binds to iron, zinc and copper, while ovomucoid inhibits protease. Ovoglobulin produces antibodies while ovomucin is antimicrobial. Lysozyme is an enzyme that lyses bacteria (Scanes et al., 2004).

To access quality of albumen, two common measurements can be performed. These are albumen height and Haugh unit. Albumen height can be measured with specialized devices such as the QCH micrometer albumen height gauge made by TSS (TSS, Technical Services and Supplies). The Haugh unit is a measure of internal egg quality. Raymond Haugh developed the Haugh unit in 1937 as a measure of egg protein as determined by the height of the albumen and the weight of the egg. To measure Haugh units, the height of the albumen must be determined as well as the egg weight. The resulting value ranges from 0 to 130, with higher numbers representing fresher, higher quality eggs with thicker

albumen. Haugh units are utilized at grading stations as a measure of egg quality and shelf life. A value above 67 is considered a Grade A egg. Factors that affect albumen quality and Haugh unit include age, breed, time between collection and cooling post lay and length of storage time. Previous research has shown that albumen quality is rarely influenced by nutrition, barn environment or housing (Egg Farmers of Alberta, 2019).

2.4.5.4 Factors Affecting Egg Quality

Ensuring that egg quality is not compromised is most important when considering economical aspects of egg production. In countries such as the United States of America, producers profit solely on the output of eggs. Therefore, the more eggs produced, the more money they generate, making egg quality of upmost importance. In Canada, egg farming is a supply-managed commodity, meaning that farmers are only able to produce their allotted quota of eggs. Egg quality is still important in this case, as it would be detrimental to fail to meet quota due to damaged or unqualified eggs. Eggshell quality is generally the most important aspect of egg quality, as cracked shells represent the highest losses (Zita et al., 2009). Frequency of defective eggs can increase up to 4% during the laying, collecting and packing stages of egg transfer (Ravan et al., 2010). It is rare that a problem related to egg quality is caused by a single factor. Factors that could potentially influence egg quality include nutrition (Leek, 2015), stress (Banga-Mboko, et al., 2010), hen age (Rodriguez-Navarro et al., 2002; Zita et al., 2009), flock density (Benyi et al., 2006; Hegelund et al., 2006), housing systems (Clerici et al., 2006; Singh et al., 2009; Sekeroglu et al., 2010), genetic strain (Silversides et al., 2006; Zita et al., 2009) and disease (De Reu et al., 2008).

2.5 Poultry in Heat Stressed Environments

Poultry producers will face issues related to heat stress due to the impending climate crisis that is slowly increasing global temperature and lengthening the hot seasons (Hansen et al., 2010). Exposure to high temperatures leads to loss of profit, reduced bird welfare, and increased mortality. The response of birds to increased temperatures is altered behaviour and physiological homeostasis in an attempt to thermoregulate and decrease internal body temperature. The response of different types of birds is generally very similar, although some individual variation will be exhibited. Under heat stressed conditions, birds may spend less time feeding and more time drinking, more time panting and raising their wings, less time moving around and more time resting (Mack et al., 2013). In response to high temperatures, birds display increased radiant, convective and evaporative heat loss through vasodilation and perspiration (Mustof et al., 2003). In addition, through panting, the air sacs promote air circulation on surfaces, resulting in an increase in gas exchange in the air and evaporative loss of heat (Feede, 1998).

Both egg production and egg quality can be negatively influenced by high ambient temperatures (Lin et al., 2004; Mashaly et al., 2004; Franco-Jimenez et al., 2007). Heat stress causes a decrease in feed consumption, limiting the intake of dietary calcium. Moreover, blood calcium concentration is further reduce by panting (Richards, 1970). Due to this panting, the partial pressure of carbon dioxide in the blood decreases, and blood pH increases in response, leading to a decrease in ionic calcium available for egg production (Usavran et al., 2001).

Some factors may influence a hen's variation in response to heat stress. These include

limited housing space, insufficient ventilation, social interactions and previous experiences (Hemsworth, 2003; Boissy et al., 2007). There has been increasing evidence showing that genetics play a key role in hen varying response to heat stress (Soleimani et al., 2011; Felver-Grant et al., 2012; Mack et al., 2013), whereby birds of smaller body size will respond better to heat stress (Sharifi et al., 2010).

2.6 Objectives

The overall objective of this experiment is to evaluate the use of dietary seaweed in laying hens at the end of their laying cycle, over the entire production period, and during heat stressed conditions. Sub-objectives are as follows:

1. To evaluate the effect of red seaweed (*C. crispus*) and brown seaweed, Tasco® (*A. nodosum*) on layer performance traits, including body weight, egg production and feed consumption.
2. To evaluate the effect of red seaweed (*C. crispus*) and brown seaweed, Tasco® (*A. nodosum*) on egg quality variables – shell density, egg weight, egg breaking strength, albumen height, yolk weight, eggshell weight, eggshell thickness, and yolk colour.
3. To determine the effect of heat processing of *C. crispus* on feed efficiency.
4. To evaluate *C. crispus* and Tasco® as a dietary additive in a heat stressed environment.

2.7 Hypothesis

The general hypothesis is that *Chondrus crispus* and *Ascophyllum nodosum* will have no negative affect on layer performance. Specific sub-hypotheses relevant to the sub-objectives are as follows:

1. Red seaweed (*C. crispus*) and brown seaweed, Tasco® (*A. nodosum*) will not negatively effect layer performance traits, including body weight, egg production and feed consumption.
2. Red seaweed (*C. crispus*) and brown seaweed, Tasco® (*A. nodosum*) will not negatively influence egg quality variables – shell density, egg weight, egg breaking strength, albumen height, yolk weight, eggshell weight, eggshell thickness, and yolk colour.
3. Heat processing of *C. crispus* will improve feed conversion.

CHAPTER 3: PRELIMINARY SHORT TERM EVALUATION OF *CHONDRUS CRISPUS* AS A FEED ADDITIVE IN LAYING HEN DIETS

3.1 Abstract

The aim of this study was to evaluate the effect of short-term inclusion of red seaweed (*Chondrus crispus*) in 70-week-old laying hen diets. Parameters monitored were egg production, body weight change, and egg quality, using two commercial strains of laying hens (Lohmann LSL Lite and Lohmann Brown Lite). A total of 150 birds were used with two processing methods: 1) raw ground and 2) extruded. Hens were randomly assigned to 6 diet groups. Control hens were fed a 0% seaweed while remaining hens were fed one of the following diets: 1% extruded *Chondrus crispus* (ECC), 2% ECC, 3% ECC, 4% ECC and 4% ground *Chondrus crispus* (GCC). There was a significant difference ($P<0.05$) in initial and final results (age) for shell density, whereby the shell density was reduced from 70 weeks of age (1.090g/cm^3) compared to 73 weeks of age (1.087g/cm^3). A significant difference ($P<0.05$) was detected for both diet and age of hen for albumen height. For age, albumen height was reduced ($P<0.05$), from 7.8 to 7.1 mm over the three week trial. For the diet effect, birds fed the 3% ECC had significantly higher ($P<0.05$) albumen height (7.8) compared to birds fed 4% ECC (7.0). However, the 4% ECC was not different from any other diet. When considering the ground versus extruded diets, there were no significant differences observed between 4% ECC and 4% GCC, indicating that the extrusion process had no positive effects on performance and egg quality traits.

3.2 Introduction

Worldwide, the egg industry is moving toward incorporating natural feed ingredients that positively influence gut microbiota. This has become a particular concept of interest due to the directional movement toward completely eliminating antibiotic use. Marine products, organic acids, probiotics, prebiotics, and herbal remedies have all been evaluated as potential beneficial feed ingredients (Venkitanarayanan et al., 2013). Prebiotics in specific have been shown to selectively increase the growth of beneficial microbes and inhibit pathogen colonization (O'Sullivan et al., 2010). Among prebiotics, seaweeds have become of interest as feed additives in poultry (Richmond, 2004). Seaweeds have been previously determined to enhance the immune system, modulate growth and positively influence microbial populations in pigs and ruminants (Evans and Critchley, 2014), but their effects in poultry have not been explored in depth, especially when considering laying hens and the potential impact on layer performance and egg quality.

When implementing new feed ingredients in laying hen diets, it is important to identify whether the ingredient has an impact on layer performance traits such as body weight, egg production and feed consumption. It is also important to explore whether egg quality is affected, as profit margins can be drastically reduced with compromised eggs.

Feed processing has the potential to improve bird feed efficiency and reduce costs. Extrusion is a combination of heat, shear and compressional forces. Utilization of this method results in expansion of starches from increased gelatinization and cross-linking of proteins. The result is a strongly bound, but porous pellet. The effect of processing method on seaweed feed efficiency is yet to be explored.

The objective of this study is to investigate the effect of dietary inclusion of red seaweed, *Chondrus crispus* with two processing methods (ground and extruded) on laying hen performance and egg quality.

3.3 Materials and Methods

The short-term preliminary trial took place from August 9th, 2017 to August 30th, 2017. Birds were housed at the Atlantic Poultry Research Institute of the Dalhousie Agricultural Campus.

3.3.1 Birds and Housing

One strain of commercial laying hen was used in the study. The strain of hen was Lohmann LSL Lite, hatched in New Brunswick, Canada. A total of 150 hens at 70 weeks of age were used and had been in production for 53 weeks. All birds were randomly assigned to one of 30 wire cages in the middle tiers, of a two-sided, 3-tier battery conventional cage system with 5 birds per cage. Water was available *ad libitum* throughout the trial. A controlled environment was established with 16 hrs of light per day and a temperature of 25°C. All experimental procedures were carried out in accordance with the Canadian Council of Animal Care guidelines (CCAC, 2009).

3.3.2 Seaweed Supplemented Diets

Six dietary treatments with red seaweed (*Chondrus crispus*) were utilized. Seaweed was obtained from Acadian Seaplants Limited, located in Dartmouth, NS, Canada. Two processing methods of red seaweed (*Chondrus crispus*) were utilized to determine any differences in feed efficiency: 1) Ground seaweed that was extruded and then reground (ECC), and 2) ground seaweed without extrusion (GCC). Dietary inclusion levels were as

follows: 0% *C. Crispus*, 1% ECC, 2% ECC, 3% ECC and 4% ECC, and 4% GCC. Diets were formulated based off commercial requirements for Lohmann LSL Lite provided by Lohmann Tierzucht GmbH. Formulations were created to meet the metabolizable energy, protein, calcium, available phosphorus, methionine and sodium requirements. Formulations were adjusted to account for the salt content with the seaweed.

3.3.3 Preparation of Seaweed Supplemented Diets

Two tonnes of cultivated *Chondrus crispus* was obtained from Acadian Seaplants Limited, Dartmouth, NS, Canada. The seaweed was grown on land artificially in salt water. In an environmentally controlled room at the Atlantic Poultry Research Centre at, the CC was dried at room temperature for 48 hrs and manually turned every few hrs to allow for uniform drying. Following drying, the seaweed was further processed at the Chute Centre for Animal Nutrition. The seaweed was ground to a powder (mesh size, 0.4 mm) using a micro Wiley mill, standard model 3 (Arthur H Thomas Co, Philadelphia, PA, USA). Half of the ground seaweed was further processed using extrusion, while the remaining half was set aside to be later mixed into the diet. A Kahl OEE8 extruder with a barrel temperature set at 100^{o}C was used to extrude the remaining half of the seaweed (Amandus Kahl GmbH and Co. KG). After extrusion, the extruded feed was then dried for 4 hrs at 60^{o}C using a convection oven. Following drying of the extruded seaweed, the feed was ground to a powder (mesh size, 0.4 mm) using a micro Wiley mill, standard model 3 (Arthur H Thomas Co, Philadelphia, PA, USA). Feed prepared in the mash form. Proximate analysis was performed on all diets to ensure that there were no large deviations from the calculated composition. Extrusion of CC resulted in a decrease in NDF percentage (Appendix Table A10).

Table 1. Diet Formulation (g/kg) and calculated composition (as fed basis) of the Preliminary *Chondrus crispus* Layer Diets

Feed Ingredient	Diet					
	C	1% Extruded CC	2% Extruded CC	3% Extruded CC	4% Extruded CC	4% Ground CC
Ground Corn	547.48	536.59	525.76	514.86	503.96	502.65
Canola Meal	124.77	124.77	124.47	124.47	124.47	124.47
Wheat	100.00	100.00	100.00	100.00	100.00	100.00
Soybean Meal	90.74	88.57	86.66	84.49	82.31	82.79
Limestone	50.00	49.95	49.88	49.81	49.75	49.79
Shell Mix	25.02	24.97	24.94	24.91	24.87	24.90
Oyster Shell	25.00	24.97	24.94	24.91	24.87	24.90
Animal/Vegetable Fat	16.00	20.15	24.27	28.43	32.58	32.95
Dicalcium Phosphate	10.08	10.14	10.21	10.27	10.33	10.34
MCL8	5.00	5.00	5.00	5.00	5.00	5.00
Salt	4.17	3.13	2.09	1.04	0	0.37
Methionine Premix	1.73	1.76	1.79	1.82	1.85	1.84
Extruded *Chondrus crispus*	0	10.00	20.00	30.00	40.00	0
Ground *Chondrus crispus*	0	0	0	0	0	40.00
Total	1000	1000	1000	1000	1000	1000
Calculated Composition (%)						
Metabolizable Energy (kcal/kg)	2800.03	2800.03	2800.03	2800.03	2800.03	2800.03
Protein	14.73	14.73	14.73	14.73	14.73	14.73
Calcium	4.09	4.09	4.09	4.09	4.09	4.09
Available Phosphorus	0.37	0.37	0.37	0.37	0.37	0.37
Sodium	0.18	0.18	0.18	0.18	0.18	0.18

[1]Diet group: C: control; 1% ECC: contains 1% of extruded *Chondrus crispus*; 2% ECC: contains 2% of extruded *Chondrus crispus;* 3% ECC: contains 3% of extruded *Chondrus crispus;* 4% ECC: contains 4% of extruded *Chondrus crispus*; 4% GCC: contains 4% of ground *Chondrus crispus*

[2]Vitamin and Mineral mixture (g/kg of premix): vitamin A (retinol), 1.56 g; vitamin D3 (cholecalciferol), 480.00 g; vitamin E (dl-alpha tocopheryl acetate), 8.00 g; vitamin K (menadione sodium bisulphate), 1.80 g; thiamine, 0.40 g; riboflavin, 1.90 g; pantothenic acid (as DL-calcium pantothenate), 3.20 g; biotin, 32.00 g; folic acid, 4.40 g; vitamin B12, 2.30 g; niacin, 6.16 g; pyridoxine, 0.80 g; manganous oxide, 23.40 g; zinc oxide, 22.22 g; copper sulphate, 20.00 g; selenium premix, 14.86 g; ethoxyquin, 16.66 g; ground corn, 46.66 g; limestone, 100 g.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

3.3.4 Production Performance

The following production parameters were monitored:

1. **Daily Feed Consumption**: The feed consumption per cage was weighed and recorded daily. Feeders were removed and weighed at the beginning and end of the study.
2. **Feed Conversion Ratio**: The grams of feed consumed per gram of egg produced will be calculated as an indicator of feed utilization. The following formula will be employed:

$$FCR = \frac{Feed\ Consumed\ (g)}{Avg\ Egg\ Weight * Total\ \#\ of\ Eggs\ (g)}$$

3. **Body Weight Change:** Average body weight per cage was measured once at the beginning of the study and once at the end of the trial. The difference was determined by subtracting the final weight from the initial weight.
4. **Daily Egg Production**: Number of eggs laid per cage was recorded daily. Any soft shelled, weak shelled, small, large, or cracked eggs were noted. To determine laying performance, the hen day production calculation was used. Hen day production is calculated as follows:

$$\%\ Hen\ Day = \frac{(Total\ Eggs\ Laid\ Per\ Cage/Number\ of\ days\ on\ Trial\ )}{\#\ Birds\ Per\ Cage} * 100$$

The hen day (%) was calculated on a per cage basis over the entire trial period. The total eggs laid was divided by the number of days on trial to give the number of eggs laid per day. The resulting value was then divided by the number of birds per in that

cage. The resulting value was then multiplied by 100 to give a percentage hen day production.

5. **Mortality**: All mortalities that occurred over the span of the trial were recorded with the time of death, weight, and feed weigh back at time of death. Deceased birds were accounted for in the feed consumption and egg production data analysis.

3.3.5 Egg Quality

Three eggs per cage were collected at the start of the trial, and at the end of the trial. Eggs underwent the following measurements:

1. **Shell Density**: Eggs were floated in salt water ranging from specific gravities of 1.074g/cm^3 to 1.106g/cm^3. Salt solutions were prepared at the APRC whereby 2126g, 2246g, 2364g, 2486g, 2606g, 2726g, 2846g, 2966g and 3086g were added to 20L of water to create specific gravities of 1.070g/cm^3, 1.074g/cm^3, 1.078g/cm^3, 1.082g/cm^3, 1.086g/cm^3, 1.090g/cm^3, 1.094g/cm^3, 1.098g/cm^3 and 1.102g/cm^3, respectively. The salt/water solutions were stirred vigorously directly following salt addition. To prevent any salt from settling, the solutions were stirred each morning following creation for one week.
2. **Egg Weight**: Eggs were weighed using an egg holder and scale.
3. **Albumen Height**: A QCH albumen height gauge from Technical Services and Supplies, York, UK was used to determine height of albumen in mm.
4. **Yolk Weight:** Yolks were separated from albumen manually (with hands) and weighed on a scale.
5. **Shell Weight:** Shells were washed, dried overnight and weighed with membrane still intact. Shell weights were determined using a scale.

6. **Shell Thickness**: The TA.xt Plus Texture Analyzer with a 5kg load cell was used to determine height of the eggshell in mm.

3.3.6 Statistical Analysis

A completely randomized design, with 6 dietary treatments as the main factors was utilized. Each cage of 5 birds was considered an experimental unit, with 5 replicates per treatment combination. The results were analyzed using the Proc Mixed procedure of SAS (SAS, 2018). All effects (inclusion level, age, processing method) were considered fixed. The assumptions of normal distribution equal variance were tested. Tukey-Kramer test was utilized to determine differences among means. The calculated probability value was 0.05, whereby all main effects and interactions that had a P-value less than 0.05 were considered statistically significant. Standard error of the mean was reported with the mean.

3.4 Results and Discussion

Table 2. Effect of Red Seaweed Supplementation on Layer Performance Traits for Short-Term Feeding Trial

Measurement		Feed Intake	Body Weight (g)	Body Weight Change (g)	FCR	Hen Day (%)
Diet[1]	0% CC	123.75 ± 1.81	1849^{a} ± 21.6	-2.04^{b} ± 0.56	1.85 ± 0.07	92.00 ± 1.45
	1% ECC	123.10 ± 1.81	1769^{ab} ± 21.6	-1.03^{ab} ± 0.56	1.83 ± 0.07	94.82 ± 1.45
	2% ECC	124.35 ± 1.81	1839^{a} ± 21.6	-0.28^{ab} ± 0.56	1.90 ± 0.07	92.94 ± 1.45
	3% ECC	122.58 ± 1.81	1830^{ab} ± 21.6	$+0.48^{a}$ ± 0.56	1.96 ± 0.07	90.12 ± 1.4
	4% ECC	124.03 ± 1.81	1743^{b} ± 21.6	$+0.04^{ab}$ ± 0.56	1.87 ± 0.07	90.35 ± 1.45
	4% GCC	122.27 ± 1.81	1778^{ab} ± 21.66	$+0.88^{a}$ ± 0.56	1.84 ± 0.07	89.88 ± 1.45
P-*value*	Diet	0.9547	0.0030	0.0138	0.7710	0.1471
	Age	N/A	0.7444	N/A	N/A	N/A

[1]Diet group: C: control; 1% ECC: contains 1% of extruded *Chondrus crispus*; 2% ECC: contains 2% of extruded *Chondrus crispus;* 3% ECC*:* contains 3% of extruded *Chondrus crispus;* 4% ECC: contains 4% of extruded *Chondrus crispus*; 4% GCC: contains 4% of ground *Chondrus crispus*

Feed intake, egg production, body weight change and feed conversion ratio (FCR) was averaged over the three-week trial; therefore no repeated measures for age were assessed. No difference was observed between diets for feed intake ($P>0.05$). For body weight, data displayed a quadratic response. A significant difference was detected for diet ($P<0.05$), whereby birds fed the control and the 2% extruded seaweed had significantly higher body weights than birds fed the 4% extruded seaweed (Table 2), Body weight data displayed a quadratic response. Age (measurement at beginning of trial compared to the end) showed no difference for all measurements, and no interaction effect was observed ($P>0.05$). When assessing body weight as weight lost/gained over the three weeks, the results follow more of a clear trend. Birds fed the control diet lost the most weight. Weight loss was reduced as seaweed level increased. At 3% inclusion and above, regardless of processing, birds gained weight, although a very small amount. Since these differences in body weight change are so small (2g and under), it is likely that the significant difference is due to external factors, such as stage of egg development, last excretion, and last meal, as opposed to diet. No difference in feed conversion ratio or egg production ($P>0.05$) for diet was detected.

Table 3. Effect of Red Seaweed Supplementation on Layer Egg Quality for Short-Term Feeding Trial

Measurement		Shell Density (g/cm^3)	Egg Weight (g)	Yolk Weight (g)	Egg Albumen Height (mm)	Shell Thickness (mm)	Shell Weight (g)
Diet[1]	0% CC	1.089 ± 0.0008	63.36 ± 0.15	17.93 ± 0.28	7.2^{ab} ± 0.59	0.50 ± 0.007	6.26 ± 0.12
	1% ECC	1.089 ± 0.0008	62.37 ± 0.15	17.30 ± 0.28	7.7^{ab} ± 0.59	0.50 ± 0.007	6.23 ± 0.12
	2% ECC	1.089 ± 0.0008	62.06 ± 0.15	17.64 ± 0.28	7.4^{ab} ± 0.59	0.51 ± 0.007	6.21 ± 0.12
	3% ECC	1.089 ± 0.0008	64.05 ± 0.15	17.87 ± 0.28	7.8^{a} ± 0.59	0.50 ± 0.007	6.29 ± 0.12
	4% ECC	1.090 ± 0.0008	63.57 ± 0.15	17.27 ± 0.28	7.0^{b} ± 0.59	0.52 ± 0.007	6.44 ± 0.12
	4% GCC	1.087 ± 0.0008	63.62 ± 0.15	18.01 ± 0.28	7.5^{ab} ± 0.59	0.49 ± 0.007	6.25 ± 0.12
Age	Start (70 weeks)	1.090 ± 0.0005	63.62 ± 0.09	17.80 ± 0.16	7.8 ± 0.34	N/A	N/A
	End (73 weeks)	1.087 ± 0.0005	62.73 ± 0.09	17.54 ± 0.16	7.1 ± 0.34	N/A	N/A
P-*value*	Diet	0.1262	0.1377	0.2576	0.0072	0.2035	0.8033
	Age	0.0003	0.0689	0.2571	<0.0001	N/A	N/A

[1]Diet group: C: control; 1% ECC: contains 1% of extruded *Chondrus crispus*; 2% ECC: contains 2% of extruded *Chondrus crispus;* 3% ECC*:* contains 3% of extruded *Chondrus crispus;* 4% ECC: contains 4% of extruded *Chondrus crispus*; 4% GCC: contains 4% of ground *Chondrus crispus.*

Egg weight and yolk weight showed no diet or age effect. Shell weight and shell thickness showed no diet effect. The effect of age could not be determined because an initial measurement was not obtained. There was a significant difference in age ($P<0.05$) for shell density, whereby the shell density was reduced from 70 weeks of age (1.090 g/cm^3) compared to 73 weeks of age (1.087g/cm^3). The reduction in shell density is consistent with previous research whereby eggshell quality declines with increased hen age (Roland, 1979; Sokolowicz et al., 2018). The decline in shell density is generally associated with an increase in egg weight. Egg size increases as a hen becomes older, with no increase in shell deposition, causing shell quality to decline (Roland, 1979). In this study however, there was no increase in egg weight or decrease in shell thickness associated with the decline in shell density. It is likely that if egg weight were examined over the entire production cycle, that it would be clear that there was indeed an increase in egg weight. A significant difference was detected for both diet and age for albumen height ($P<0.05$). For age, albumen height was reduced to 7.1mm at 73 weeks of age from 7.8mm at 70 weeks or age. Albumen height has also been shown to reduce with age (Zita et al., 2009). For the diet effect, birds fed the 3% ECC had significantly higher albumen height (7.8mm) compared to the birds fed the 4% ECC (7.0mm). However, the 4% ECC was not different from any other diet, indicating that the significant difference may not be directly reflective of a diet effect, but perhaps due to other external factors. Such external factors include poor egg quality from one hen in the diet group, or an egg that was laid earlier than other eggs collected for measurement. These types of external factors may not show up as an outlier but would influence the results.

When considering the ground versus extruded diets, there were no significant differences observed between the 4% ECC and the 4% GCC, indicating that the extrusion process had no positive effects on performance and egg quality traits. Therefore, the added cost of extrusion may be an unnecessary process where the same effect can be achieved through a simple grinding.

3.5 Conclusion

Although significant differences were observed for albumen height, body weight and body weight change, it is likely that these differences are not due to a direct diet effect, but due to external factors. Age had a significant effect on shell density and albumen height, which was to be expected based on previous research. Further exploration is needed to assess *Chondrus crispus* as a dietary additive. Inclusion of various levels for each processing method, and long term feeding would be advantageous in ongoing research investigating this seaweed in layer hens.

CHAPTER 4: LONG TERM EVALUATION OF *CHONDRUS CRISPUS* AND *ASCOPHYLLUM NODOSUM* AS A FEED ADDITIVE IN LAYING HEN DIETS

4.1 Abstract

The aim of this study was to evaluate the effect of inclusion of red seaweed (*Chondrus crispus*) and brown seaweed (*Ascopyllum nodosum*, or Tasco®, as it's trademarked name) in standard laying hen diets. Parameters monitored were feed consumption, feed conversion, egg production, body weight change and egg quality using two commercial strains of laying hens; Lohmann LSL Lite and Lohmann Brown Lite, and two processing methods: ground and extruded. Hens were randomly assigned to ten treatment groups. Control hens were fed a 0% seaweed inclusion diet while remaining hens were fed one of the following diets: 0.5% extruded *Chondrus crispus* (ECC), 1.75% ECC, 3% ECC, 0.5% ground *Chondrus crispus* (GCC), 1.75% GCC, 3% GCC, 0.25% Tasco® and 0.5% Tasco®. Age of hen and strain were highly influential when considering all parameters measured. Shell quality declined with age. LB birds had smaller eggs, denser shells, thicker shells, greater breaking strength, higher feed conversion, larger body weights, lower hen day production, higher feed consumption, smaller yolks and lower shell weights compared to LL birds. Processing had no effect on egg quality or production parameters, indicating that extruding is likely unnecessary when feeding *Chondrus crispus*. Hens fed the 0.5% inclusion level of CC had the lowest shell thickness ($P<0.05$). There were no other significant differences in level detected. CC had no negative influence on parameters measured, but also showed no improvements in egg quality, egg production or feed efficiency when fed at the levels utilized in this experiment.

4.2 Introduction

Worldwide, the egg industry is moving toward incorporating natural feed ingredients that positively influence gut microbiota as the poultry industry has almost completely eliminated antibiotic use. Marine products, organic acids, probiotics, prebiotics, and herbal remedies have all been evaluated as potential feed ingredients (Venkitanarayanan et al., 2013). Prebiotics in specific have been shown to selectively increase the growth of beneficial microbes and inhibit pathogen colonization (O'Sullivan et al., 2010). Among prebiotics, seaweeds have become of interest as feed additives in poultry (Richmond, 2004). Seaweeds have been previously determined to enhance the immune system, modulate growth and positively influence microbial populations in pigs and ruminants (Evans and Critchley., 2014), but their effects in poultry have not been explored in depth, especially when considering laying hens and the potential impact on layer performance and egg quality.

When implementing new feed ingredients in laying hen diets, it is important to identify whether the ingredient has an impact on layer performance traits such as body weight, egg production and feed consumption. It is also important to explore whether egg quality is affected, as profit margins can be drastically reduced with compromised eggs.

Feed processing has the potential to improve bird feed efficiency and reduces costs. Extrusion is a combination of heat, shear and compressional forces. Utilization of this method results in expansion of starches from increased gelatinization and cross-linking of proteins within the matrix. The result is a strongly bound, but porous pellet. The effect of processing method on seaweed feed efficiency is yet to be explored.

The aim of this study is to investigate the effect of dietary inclusion of the red seaweed, *Chondrus crispus* and the brown seaweed *Ascophyllum nodosum*, or Tasco® as it's trademarked name, with two processing methods (ground and extruded) on laying hen performance and egg quality.

4.3 Materials and Methods

The primary study took place from January 3rd, 2018 to September 12th, 2018. Birds were housed at the Atlantic Poultry Research Institute of the Dalhousie Agricultural Campus. The trial followed a 28-day cycle, with feed weigh backs, body weights and egg quality measured every 4 weeks.

4.3.1 Birds and Housing

Two strains of commercial laying hens were used in the primary study. The two strains of hens were Lohmann LSL Lite and Lohmann LSL Brown, hatched in New Brunswick, Canada. A total of 400 hens at 34 weeks of age were used for a 3 week trial. These birds had been in production for 17 weeks prior to starting the feeding trial. All birds were randomly assigned to one of 80 wire cages in the middle tiers, of a two-sided, 3-tier battery conventional cage system in stocking densities of 5 birds per cage. Water was available *ad libitum* throughout the trial. A controlled environment was established with 16 hrs of light per day and a temperature of 25°C. All experimental procedures were carried out in accordance with the Canadian Council of Animal Care guidelines (CCAC, 2009).

4.3.2 Seaweed Supplemented Treatments

Ten dietary treatments with *Chondrus Crispus* or Tasco® were prepared and used. Both seaweed species were obtained from Acadian Seaplants Limited in Dartmouth, NS,

Canada. Two processing methods of red seaweed (*C. Crispus*) were utilized to determine any differences in feed efficiency: 1) Ground seaweed that was extruded and then reground (ECC), and 2) ground seaweed without extrusion (GCC). The treatments were as following: 0% GCC, 0.5% GCC, 1.75% GCC, 3% GCC, 0% ECC, 0.5% ECC, 1.75% ECC, 3% ECC, 0.25% Tasco®, and 0.5% Tasco®. Tasco® inclusion levels were determined based off of recommended feeding levels provided by Acadian Seaplants. Diets were formulated based off commercial requirements for Lohmann LSL Lite provided by Lohmann Tierzucht GmbH. Four diets phases were prepared over the course of the trial, with reduced protein and increased calcium at each phase change. Formulations were created to meet the metabolizable energy, protein, calcium, available phosphorus, methionine and sodium requirements. Formulations were adjusted to account for the salt content with the seaweed.

4.3.2 Experimental Design

Each seaweed type was associated with an individual experimental design and statistical analyses was performed separately. Each treatment combination had 4 replicates.

4.3.2.1 Chondrus crispus Experimental Design

For *Chondrus crispus*, the following factorial arrangement was utilized:

4 x 2 x 2 x 9

with the main effects of;

- Inclusion level (0, 0.5, 1.75 and 3%)
- Strain (Lohmann LSL Lite and Lohmann Brown Lite)
- Processing method (ground and extruded)
- Age (nine 28-day periods)

4.3.2.2 Tasco® Experimental Design

For Tasco®, the following factorial arrangement was utilized:

3 x 2 x 9

with the main effects of;

- Inclusion level (0, 0.25 and 05%)
- Strain (Lohmann LSL Lite and Lohmann Brown Lite)
- Age (nine 28-day periods)

4.3.3 Preparation of Seaweed Supplemented Treatments

Two tonnes of cultivated *Chondrus crispus* was obtained from Acadian Seaplants Limited, Dartmouth, NS, Canada. In an environmentally controlled room at the Atlantic Poultry Research Centre, the CC was dried at room temperature for 48 hrs and manually turned every few hrs to allow for uniform drying. Following drying, the seaweed was further processed at the Chute Centre for Animal Nutrition. Seaweed was ground to a powder (mesh size, 0.4 mm) using a micro Wiley mill, standard model 3 (Arthur H Thomas Co, Philadelphia, PA, USA). Half of the ground seaweed was further processed using extrusion, while the remaining half was set aside to be mixed into the diet. A Kahl OEE8 extruder with a barrel temperature set at 100^{o}C was used to extrude half of the seaweed (Amandus Kahl GmbH and Co. KG). After extrusion, the extruded feed was then dried for 4 hrs at 60^{o}C using a convection oven. Following drying of the extruded seaweed, the feed was ground to a powder (mesh size, 0.4 mm) using a micro Wiley mill, standard model 3 (Arthur H Thomas Co, Philadelphia, PA, USA). Feed was prepared in the mash form.

Proximate analysis was performed on all diets to ensure that there were no large deviations from the calculated composition. Extrusion of CC resulted in a decrease in NDF percentage (Appendix Table A10).

4.3.3.2 Phase 1 Diets

Table 4. Diet Formulation (g/kg) and calculated composition (as fed basis) of the Phase 1 *Chondrus crispus* Layer Diets

Ingredient	Diet[1]						
	C	0.5% GCC	1.75% GCC	3% GCC	0.5% ECC	1.75% ECC	3% ECC
Ground Corn	532.56	526.71	512.09	497.47	526.77	512.30	497.12
Canola Meal	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Wheat	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Soybean Meal	143.59	142.43	139.54	136.64	142.40	139.43	136.31
Limestone	45.15	45.13	45.05	44.96	45.13	45.05	44.97
Shell Mix	22.58	22.56	22.53	22.49	22.56	22.53	22.49
Oyster Shell	22.58	22.56	22.53	22.49	22.56	22.52	22.28
Animal/Vegetable Fat	11.82	14.43	20.95	27.47	14.42	20.90	27.70
Dicalcium Phosphate	11.18	11.22	11.31	11.39	11.22	11.30	11.39
MCL9[2]	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Salt	3.73	3.14	1.64	0.14	3.12	1.60	0
Methionine Premix[3]	1.80	1.82	1.87	1.91	1.82	1.87	1.92
Extruded *Chondrus crispus*	0	0	0	0	5.00	17.50	30.61
Ground *Chondrus crispus*	0	5.00	17.50	30.00	0	0	0
Total	1000	1000	1000	1000	1000	1000	1000
Calculated Composition (%)							
Metabolizable Energy (kCal/kg)	2800.03	2800.03	2800.03	2800.03	2800.03	2800.03	2800.03
Protein (%)	16.04	16.04	16.04	16.04	16.04	16.04	16.04
Calcium (%)	3.73	3.73	3.73	3.73	3.73	3.73	3.73
Available Phosphorus (%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium (%)	0.17	0.17	0.17	0.17	0.17	0.17	0.17

[1]Treatment group: C: control; 0.5% GCC: contains 0.5% of ground *Chondrus crispus*; 1.75% GCC: contains 1.75%% of ground *Chondrus crispus;* 3% GCC: contains 3% of ground Chondrus crispus*;* 0.5% ECC: contains 0.5% of extruded *Chondrus crispus*; 1.75% ECC: contains 1.75%% of extruded *Chondrus crispus*; 3% ECC: contains 3% of extruded *Chondrus crispus.*

[2]Vitamin and Mineral mixture (g/kg of premix): vitamin A (retinol), 1.56 g; vitamin D3 (cholecalciferol), 480.00 g; vitamin E (dl-alpha tocopheryl acetate), 8.00 g; vitamin K (menadione sodium bisulphate), 1.80 g; thiamine, 0.40 g; riboflavin, 1.90 g; pantothenic acid (as DL-calcium pantothenate), 3.20 g; biotin, 32.00 g; folic acid, 4.40 g; vitamin B12, 2.30 g; niacin, 6.16 g; pyridoxine, 0.80 g; manganous oxide, 23.40 g; zinc oxide, 22.22 g; copper sulphate, 20.00 g; selenium premix, 14.86 g; ethoxyquin, 16.66 g; ground corn, 46.66 g; limestone, 100 g.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

Table 5. Diet Formulation (g/kg) and calculated composition (as fed basis) of the Phase 1 Tasco® Layer Diets

	Diet[1]	
Ingredient	0.25% Tasco	0.5% Tasco
Ground Corn	528.57	524.58
Canola Meal	100.00	100.00
Wheat	100.00	100.00
Soybean Meal	143.94	144.28
Limestone	45.11	45.07
Shell Mix	22.56	22.53
Oyster Shell	22.55	22.53
Animal/Vegetable Fat	13.26	14.70
Dicalcium Phosphate	11.20	11.21
MCL9[2]	5.00	5.00
Salt	3.53	3.33
Methionine Premix[3]	1.78	1.75
Tasco	2.50	5.00
Total	1000	1000
Calculated Composition (%)		
Metabolizable Energy (kCal/kg)	2800.03	2800.03
Protein (%)	16.04	16.04
Calcium (%)	3.73	3.73
Available Phosphorus (%)	0.40	0.40
Sodium (%)	0.17	0.17

[1]Treatment group: 0.25% Tasco®: contains 0.25% of sundried, ground *Ascophyllum nodosum*; 3%; 0.5% Tasco®: contains 0.5% of sundried, ground *Ascophyllum nodosum*

[2] Vitamin and Mineral mixture (g/kg of premix): vitamin A (retinol), 1.56 g; vitamin D3 (cholecalciferol), 480.00 g; vitamin E (dl-alpha tocopheryl acetate), 8.00 g; vitamin K (menadione sodium bisulphate), 1.80 g; thiamine, 0.40 g; riboflavin, 1.90 g; pantothenic acid (as DL-calcium pantothenate), 3.20 g; biotin, 32.00 g; folic acid, 4.40 g; vitamin B12, 2.30 g; niacin, 6.16 g; pyridoxine, 0.80 g; manganous oxide, 23.40 g; zinc oxide, 22.22 g; copper sulphate, 20.00 g; selenium premix, 14.86 g; ethoxyquin, 16.66 g; ground corn, 46.66 g; limestone, 100 g.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

4.3.3.3 Phase 2 Diets

Table 6. Diet Formulation (g/kg) and calculated composition (as fed basis) of the Phase 2 *Chondrus crispus* Layer Diets

	Diet[1]						
Ingredient	C	0.5% GCC	1.75% GCC	3% GCC	0.5% ECC	1.75% ECC	3% ECC
Ground Corn	538.45	532.60	517.98	503.37	532.66	518.19	503.62
Canola Meal	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Wheat	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Soybean Meal	135.57	131.41	131.51	126.62	134.38	131.41	128.45
Limestone	46.43	46.41	46.33	46.26	46.41	46.33	46.25
Shell Mix	23.23	23.20	23.17	23.13	23.20	23.17	23.13
Oyster Shell	23.21	23.20	23.17	23.13	23.20	23.16	23.12
Animal/Vegetable Fat	11.93	14.54	21.06	27.57	14.52	21.01	27.53
Dicalcium Phosphate	10.80	10.83	10.92	11.01	10.83	10.92	11.00
MCL9[2]	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Salt	3.61	3.02	1.532	0.02	3.00	1.48	0.01
Methionine Premix[3]	1.77	1.79	1.84	1.88	1.79	1.84	1.89
Extruded *Chondrus crispus*	0	0	0	0	5.00	17.50	30.00
Ground *Chondrus crispus*	0	5.00	17.50	30.00	0	0	0
Total	1000	1000	1000	1000	1000	1000	1000
Calculated Composition (%)							
Metabolizable Energy (kCal/kg)	2800.03	2800.03	2800.03	2800.03	2800.03	2800.03	2800.03
Protein (%)	15.71	15.71	15.71	15.71	15.71	15.71	15.71
Calcium (%)	3.82	3.82	3.82	3.82	3.82	3.82	3.82
Available Phosphorus (%)	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16

[1]Treatment group: C: control; 0.5% GCC: contains 0.5% of ground *Chondrus crispus*; 1.75% GCC: contains 1.75%% of ground *Chondrus crispus;* 3% GCC: contains 3% of ground Chondrus crispus*;* 0.5% ECC: contains 0.5% of extruded *Chondrus crispus*; 1.75% ECC: contains 1.75%% of extruded *Chondrus crispus*; 3% ECC: contains 3% of extruded *Chondrus crispus.*

[2]Vitamin and Mineral mixture (g/kg of premix): vitamin A (retinol), 1.56 g; vitamin D3 (cholecalciferol), 480.00 g; vitamin E (dl-alpha tocopheryl acetate), 8.00 g; vitamin K (menadione sodium bisulphate), 1.80 g; thiamine, 0.40 g; riboflavin, 1.90 g; pantothenic acid (as DL-calcium pantothenate), 3.20 g; biotin, 32.00 g; folic acid, 4.40 g; vitamin B12, 2.30 g; niacin, 6.16 g; pyridoxine, 0.80 g; manganous oxide, 23.40 g; zinc oxide, 22.22 g; copper sulphate, 20.00 g; selenium premix, 14.86 g; ethoxyquin, 16.66 g; ground corn, 46.66 g; limestone, 100 g.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

Table 7. Diet Formulation (g/kg) and calculated composition (as fed basis) of the Phase 2 Tasco® Layer Diets

	Diet[1]	
Ingredient	0.25% Tasco	0.5% Tasco
Ground Corn	534.46	530.47
Canola Meal	100.00	100.00
Wheat	100.00	100.00
Soybean Meal	135.91	136.26
Limestone	46.39	46.35
Shell Mix	23.30	23.17
Oyster Shell	23.19	23.17
Animal/Vegetable Fat	13.37	14.81
Dicalcium Phosphate	10.81	10.83
MCL9[2]	5.00	5.00
Salt	3.41	3.21
Methionine Premix[3]	1.75	1.72
Tasco	2.50	5.00
Total	1000	1000
Calculated Composition (%)		
Metabolizable Energy (kCal/kg)	2800.03	2800.03
Protein (%)	15.71	15.71
Calcium (%)	3.82	3.82
Available Phosphorus (%)	0.39	0.39
Sodium (%)	0.16	0.16

[1]Treatment group: 0.25% Tasco®: contains 0.25% of sundried, ground *Ascophyllum nodosum*; 3%; 0.5% Tasco®: contains 0.5% of sundried, ground *Ascophyllum nodosum*

[2] Vitamin and Mineral mixture (g/kg of premix): vitamin A (retinol), 1.56 g; vitamin D3 (cholecalciferol), 480.00 g; vitamin E (dl-alpha tocopheryl acetate), 8.00 g; vitamin K (menadione sodium bisulphate), 1.80 g; thiamine, 0.40 g; riboflavin, 1.90 g; pantothenic acid (as DL-calcium pantothenate), 3.20 g; biotin, 32.00 g; folic acid, 4.40 g; vitamin B12, 2.30 g; niacin, 6.16 g; pyridoxine, 0.80 g; manganous oxide, 23.40 g; zinc oxide, 22.22 g; copper sulphate, 20.00 g; selenium premix, 14.86 g; ethoxyquin, 16.66 g; ground corn, 46.66 g; limestone, 100 g.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

4.3.3.4 Phase 3 Diets

Table 8. Diet Formulation (g/kg) and calculated composition (as fed basis) of the Phase 3 *Chondrus crispus* Layer Diets

Ingredient	Diet[1]						
	C	0.5% GCC	1.75% GCC	3% GCC	0.5% ECC	1.75% ECC	3% ECC
Ground Corn	549.13	543.26	528.64	513.17	543.27	528.80	514.26
Canola Meal	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Wheat	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Soybean Meal	123.35	122.20	119.30	116.42	122.17	119.20	116.24
Limestone	47.70	47.68	47.61	47.55	47.69	47.61	47.53
Shell Mix	23.85	23.84	23.80	23.77	23.85	23.81	23.77
Oyster Shell	23.85	23.83	23.80	23.77	23.84	23.80	23.77
Animal/Vegetable Fat	11.42	14.04	20.56	27.09	14.04	20.53	27.04
Dicalcium Phosphate	10.44	10.48	10.57	10.66	10.48	10.57	10.65
MCL9[2]	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Salt	3.63	3.03	1.53	0.04	3.02	1.49	0
Methionine Premix[3]	1.62	1.64	1.69	1.74	1.64	1.69	1.74
Extruded *Chondrus crispus*	0	0	0	0	5.00	17.50	30.00
Ground *Chondrus crispus*	5.00	17.50	30.00	0	0	0	
Total	1000	1000	1000	1000	1000	1000	1000
Calculated Composition (%)							
Metabolizable Energy (kCal/kg)	2800.00	2800.00	2800.00	2800.00	2800.00	2800.00	2800.00
Protein (%)	15.22	15.22	15.22	15.22	15.22	15.22	15.22
Calcium (%)	3.91	3.91	3.91	3.91	3.91	3.91	3.91
Available Phosphorus (%)	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16

[1]Treatment group: C: control; 0.5% GCC: contains 0.5% of ground *Chondrus crispus*; 1.75% GCC: contains 1.75%% of ground *Chondrus crispus;* 3% GCC: contains 3% of ground Chondrus crispus*;* 0.5% ECC: contains 0.5% of extruded *Chondrus crispus*; 1.75% ECC: contains 1.75%% of extruded *Chondrus crispus*; 3% ECC: contains 3% of extruded *Chondrus crispus.*

[2]Vitamin and Mineral mixture (g/kg of premix): vitamin A (retinol), 1.56 g; vitamin D3 (cholecalciferol), 480.00 g; vitamin E (dl-alpha tocopheryl acetate), 8.00 g; vitamin K (menadione sodium bisulphate), 1.80 g; thiamine, 0.40 g; riboflavin, 1.90 g; pantothenic acid (as DL-calcium pantothenate), 3.20 g; biotin, 32.00 g; folic acid, 4.40 g; vitamin B12, 2.30 g; niacin, 6.16 g; pyridoxine, 0.80 g; manganous oxide, 23.40 g; zinc oxide, 22.22 g; copper sulphate, 20.00 g; selenium premix, 14.86 g; ethoxyquin, 16.66 g; ground corn, 46.66 g; limestone, 100 g.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

Table 9. Diet Formulation (g/kg) and calculated composition (as fed basis) of the Phase 3 Tasco® Layer Diets

	Diet[1]	
Feed Ingredient	0.25% Tasco®	0.5% Tasco®
Ground Corn	545.12	541.13
Canola Meal	100.00	100.00
Wheat	100.00	100.00
Soybean Meal	123.70	124.05
Limestone	47.66	47.61
Shell Mix	23.83	23.81
Oyster Shell	23.83	23.81
Animal/Vegetable Fat	12.87	14.31
Dicalcium Phosphate	10.46	10.48
MCL9[2]	5.00	5.00
Salt	3.42	3.22
Methionine Premix[3]	1.60	1.58
Tasco	2.50	5.00
Total	1000	1000
Calculate Composition (%)		
Metabolizable Energy (kCal/kg)	2800.00	2800.00
Protein	15.22	15.22
Calcium	3.91	3.91
Available Phosphorus	0.38	0.38
Sodium	0.16	0.16

[1]Treatment group: 0.25% Tasco®: contains 0.25% of sundried, ground *Ascophyllum nodosum*; 3%; 0.5% Tasco®: contains 0.5% of sundried, ground *Ascophyllum nodosum*

[2] Vitamin and Mineral mixture (g/kg of premix): vitamin A (retinol), 1.56 g; vitamin D3 (cholecalciferol), 480.00 g; vitamin E (dl-alpha tocopheryl acetate), 8.00 g; vitamin K (menadione sodium bisulphate), 1.80 g; thiamine, 0.40 g; riboflavin, 1.90 g; pantothenic acid (as DL-calcium pantothenate), 3.20 g; biotin, 32.00 g; folic acid, 4.40 g; vitamin B12, 2.30 g; niacin, 6.16 g; pyridoxine, 0.80 g; manganous oxide, 23.40 g; zinc oxide, 22.22 g; copper sulphate, 20.00 g; selenium premix, 14.86 g; ethoxyquin, 16.66 g; ground corn, 46.66 g; limestone, 100 g.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

4.3.4 Production Performance

The following production parameters were monitored:

1. **Daily Feed Consumption**: The feed consumption per cage was weighed and recorded daily. Feeders were removed and weighed at the beginning and end of the study.
2. **Feed Conversion Ratio**: The grams of feed consumed per gram of egg produced will be calculated as an indicator of feed utilization. The following formula will be employed:

$$FCR = \frac{Feed\ Consumed\ (g)}{Avg\ Egg\ Weight * Total\ \#\ of\ Eggs\ (g)}$$

3. **Body Weight:** Average body weight per cage was measured once at the beginning of the study and once at the end.
4. **Daily Egg Production**: Number of eggs laid per cage was recorded daily. Any soft shelled, weak shelled, small, large, or cracked eggs were noted. To determine laying performance, the hen day production calculation was used. Hen day production is calculated as follows:

$$\%\ Hen\ Day = \frac{(Total\ Eggs\ Laid\ Per\ Cage/28\ )}{\#\ Birds\ Per\ Cage} * 100$$

The hen day (%) was calculated on a per cage basis over each 28-day period. The total eggs laid were divided by 28 days to give the number of eggs laid per day. The resulting value was then divided by the number of birds per cage. The resulting value was then multiplied by 100 to give a percentage hen day production.

5. **Mortality**: All mortalities that occurred over the span of the trial were recorded with the time of death, weight, and feed weigh back at time of death. Deceased birds were accounted for in the feed consumption and egg production data analysis.

4.3.5 Egg Quality

Three eggs per cage were collected at the start of the trial, and every 28 days following. Eggs underwent the following egg quality measurements:

1. **Shell Density**: Eggs were floated in salt water ranging from specific gravities of 1.074g/cm^3 to 1.106g/cm^3. Salt solutions were prepared at the APRC whereby 2126g, 2246g, 2364g, 2486g, 2606g, 2726g, 2846g, 2966g and 3086g were added to 20L of water to create specific gravities of 1.070g/cm^3, 1.074g/cm^3, 1.078g/cm^3, 1.082g/cm^3, 1.086g/cm^3, 1.090g/cm^3, 1.094g/cm^3, 1.098g/cm^3 and 1.102g/cm^3, respectively. The salt/water solutions were stirred vigorously directly following salt addition. To prevent any salt from settling, the solutions were stirred each morning following creation for one week.
2. **Shell Breaking Strength:** A TA.xt Plus Texture Analyzer from Texture Technologies Corp, New York, NY, USA with a 50kg load cell was used to determine total force required to crack the top of the eggshell.
3. **Egg Weight**: Eggs were weighed using an egg holder and scale.
4. **Albumen Height**: A QCH albumen height gauge from Technical Services and Supplies, York, UK was used to determine height of albumen in mm.
5. **Yolk Weight:** Yolks were separated from albumen manually (with hands) and weighed on a scale.

6. **Shell Weight:** Shells were washed, dried overnight and weighed with membrane still intact. Shell weights were determined using a scale.
7. **Shell Thickness**: The TA.xt Plus Texture Analyzer with a 5kg load cell was used to determine height of the eggshell in mm.

4.3.6 Statistical Analysis

A completely randomized design was utilized, with 8 dietary treatments for the CC trial and 3 dietary treatments for the Tasco® trial. Each cage of 5 birds was considered an experimental unit, with 4 replicates per treatment combination. The results from both the CC and Tasco® were analyzed using the Proc Mixed procedure of SAS (SAS, 2018). All effects (inclusion level, age, processing method and bird strain) were considered fixed. The assumptions of normal distribution equal variance were tested. Variables were measured using repeated measures at the end of each identified age period. The Tukey-Kramer test was utilized to determine differences among means. Slicing was utilized to perform partitioned analysis of the LS-means for an interaction. To create main effects plots and interaction plots, Minitab was utilized (Minitab, 2018). The calculated probability value was 0.05, whereby all main effects and interactions that had a P-value less than or equal to 0.05 (to two decimal places) were considered statistically significant. Standard error of the mean was reported with the mean.

4.4 Results and Discussion

Tables 10 through 15 show the effects of dietary seaweed inclusion level, bird strain, bird age and processing method for all measured variables. Main effect means and associated standard error are included in Tables 10 to 15. P-values for main effects, 2-way, 3-way and

4-way interactions are also included in Tables 10 to 15. Any significant interactions detected are discussed through text and figures following the main effects tables. All main and interaction effects were deemed significant if the p-value was less than or equal to 0.05 (to two decimal places).

4.4.1 *Chondrus crispus*

Table 10. Effect of Inclusion Level, Strain, Processing Method and Age on Layer Performance Traits for Hens fed *Chondrus crispus* during the Long-Term Feeding Trial

			Feed Intake (g)	Hen Day (%)	FCR	BW (g)
Main Effect Means	Inclusion Level[1]	0%	117 ± 0.58	95.25 ± 0.45	1.86 ± 0.01	1982 ± 19.21
		0.5%	117 ± 0.58	95.55 ± 0.45	1.87 ± 0.01	1899 ± 19.25
		1.75%	116 ± 0.58	94.60 ± 0.46	1.86 ± 0.01	1890 ± 19.20
		3%	117 ± 0.58	95.79 ± 0.45	1.84 ± 0.01	1879 ± 19.20
	Strain[2]	LL	117 ± 0.41	96.03 ± 0.32	1.84^{b} ± 0.01	1758 ± 13.60
		LB	117 ± 0.41	94.57 ± 0.32	1.88^{a} ± 0.01	2035 ± 13.58
	Processing Method	Extruded	117 ± 0.41	95.46 ± 0.32	1.86 ± 0.01	1889 ± 13.58
		Ground	116 ± 0.41	95.14 ± 0.32	1.86 ± 0.01	1904 ± 13.560
	Age	34-37 weeks	114 ± 0.44	97.13 ± 0.35	1.86^{bc} ± 0.01	1855 ± 10.63
		38-41 weeks	115 ± 0.45	96.02 ± 0.35	1.86^{c} ± 0.01	1852 ± 10.66
		42-45 weeks	112 ± 0.43	96.86 ± 0.35	1.79^{d} ± 0.01	1664 ± 10.69
		46-49 weeks	112 ± 0.43	95.02 ± 0.35	1.77^{d} ± 0.01	1905 ± 10.66
		50-53 weeks	117 ± 0.45	95.37 ± 0.35	1.88^{abc} ± 0.01	1913 ± 10.69
		54-57 weeks	118 ± 0.44	94.68 ± 0.35	1.88^{abc} ± 0.01	1917 ± 10.66
		58-61 weeks	119 ± 0.44	94.98 ± 0.36	1.90^{ab} ± 0.01	1914 ± 10.76
		62-65 weeks	121 ± 0.44	93.99 ± 0.35	1.90^{abc} ± 0.01	1955 ± 10.63
		66-69 weeks	121 ± 0.44	93.64 ± 0.36	1.91 a ± 0.01	1955 ± 10.66
P-Value	Main Effects	Strain	0.5999	0.0024	0.0002	<0.0001
		Level	0.6022	0.2935	0.3079	0.5105
		Age	<0.0001	<0.0001	<0.0001	<0.0001
		Processing	0.4589	0.4806	0.7092	0.4442
	2-Way Interaction	Level*Strain	0.1666	0.4045	0.4264	0.5417
		Level*Age	0.5709	0.2522	0.6501	0.0030
		Level*Processing	0.4566	0.6907	0.0815	0.7325
		Strain*Age	0.0028	0.0005	0.0445	<0.0001
		Strain*Processing	0.4376	0.7754	0.4773	0.4553

			Feed Intake (g)	Hen Day (%)	FCR	BW (g)
P-Value	2- Way Interaction	Age*Processing	0.6670	0.0891	0.1587	0.8798
	3- Way Interaction	Level*Age*Strain	0.8732	0.3922	0.2318	0.1155
		Level*Age*Processing	0.4765	0.6564	0.4628	0.8606
		Level*Processing*Strain	0.1266	0.6871	0.9358	0.6291
		Processing*Age*Strain	0.6284	0.9532	0.7718	0.0062
	4-Way Interaction	Level*Age*Strain*Processing	0.3085	0.3612	0.9358	0.0674

[1]Inclusion Level: 0%: control; 0.5%: contains 0.5% of *Chondrus crispus*; 1.75%: contains 1.75%% of *Chondrus crispus;* 3%: contains 3% of *Chondrus crispus.*

[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

4.4.1.1 Feed Consumption

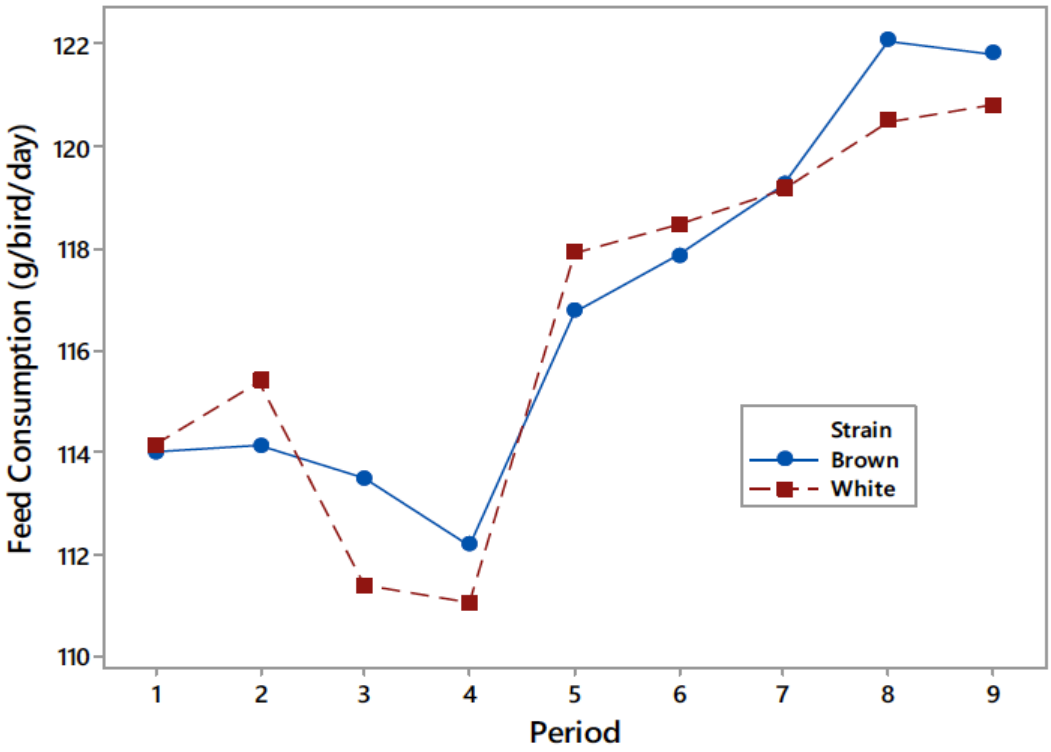

Figure 4. Interaction Plot Between Age and Strain for Feed Consumption (g/bird/day) of Hens fed *Chondrus crispus* during the Long Term Feeding Trial

Legend

Period	Age Range
0 (baseline)	34 weeks
1	34-37 weeks
2	38-41 weeks
3	42-45 weeks
4	46-49 weeks
5	50-53 weeks
6	54-57 weeks
7	58-61 weeks
8	62-65 weeks
9	66-69 weeks

Both the LB and LL birds followed had similar feed consumption (~114 g/bird/day for LB and ~114-116g/bird/day for LL) for periods 1 and 2, but feed consumption dropped during periods 3 and 4. The first feed change occurred at the start of period 3, where the birds were switched from phase 1 to phase 2. The LL birds appeared to be more influenced by the phase change, as their feed consumption dropped from 116g/bird/day to 112g/bird/day. With the implementation of the new diet, the amount of corn, methionine and calcium components increased (limestone, shell mix, oyster shells) while soybean meal decreased. This allowed for a decrease in percentage protein and an increase in percentage calcium. During period 5, feed consumption increased for both strains. Phase 5 also happened to be when the next phase change occurred. A similar increase/decrease in feed ingredients occurred as that of phase 1 to 2 (increased corn and calcium sources, decreased soy). Protein levels continued to decrease while calcium increased. The last phase change occurred at period 9, where the birds were switched over to their final, phase 4 diets for the remainder of the trial. Feed consumption stayed consistent for the switch over from phase 3 to 4, although the LB feed consumption decreased slightly.

In observing this decline in feed consumption during the phase 2 feeding, it is clear that the birds did not prefer this particular diet. There was no drop in energy or increase in temperature in their environment during these periods. Thus, the decrease in feed consumption in response to the phase 2 diet is likely a reflection of something other than the energy level, temperature, or varying formulations. A possible explanation could be that the particular batch of corn, wheat, canola or soybean was less desirable due to an anti-nutrient present, such as beta-glucans, arabinoxylans, or L-canavanin. Further research

should be performed to identify why this particular diet caused the decrease in feed consumption. It should be noted, however, that although the feed consumption dropped, the feed intake was still within the normal levels identified by Lohmann (Lohmann LSL Lite Management Guide, 2018). Regardless of strain, other than the decrease in feed consumption from period 3 to 4, the feed consumption increased as the birds aged. Feed wastage was not accounted for in this trial, which could have contributed to the increase in feed consumption. However, feed consumption did not exceed the average feed intake identified by Lohmann producers (Lohmann LSL Lite Management Guide, 2018) .

4.4.1.2 Egg Production

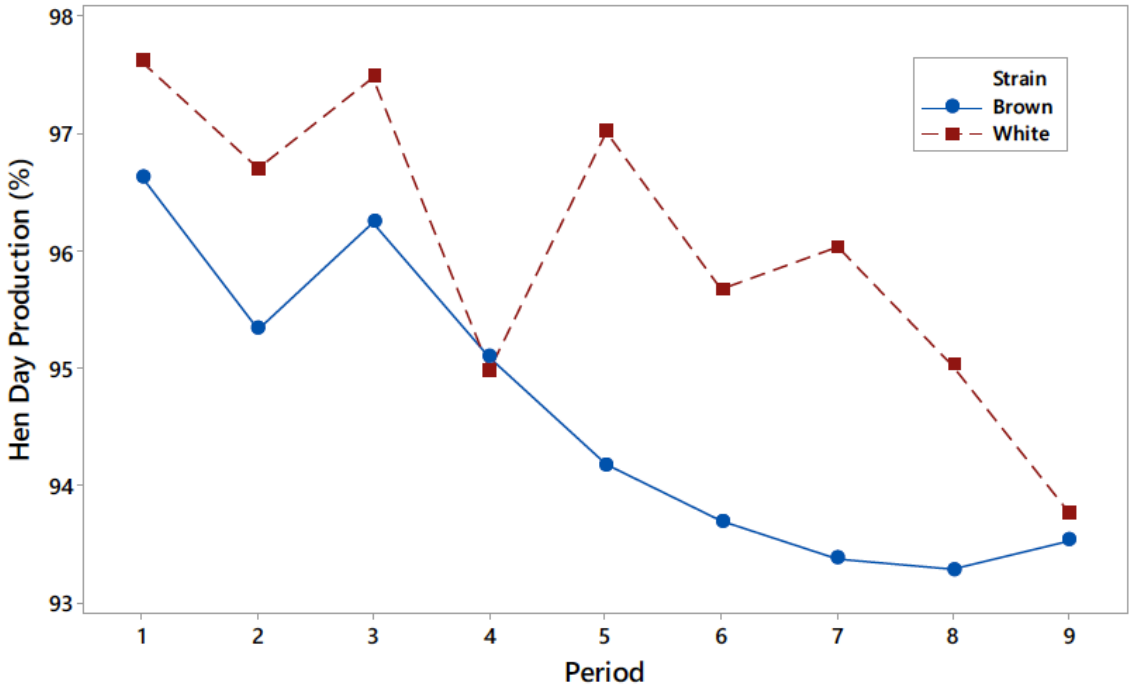

Figure 5. Interaction Plot Between Age and Strain for Egg Production (%Hen Day Production) of Hens fed *Chondrus crispus* during the Long Term Feeding Trial

Legend

White	Lohmann LSL Lite (LL)
Brown	Lohmann Brown Lite (LB)

Egg production decreased as the birds aged. These results are consistent with previously reported findings (Joyner et al., 1987; Silversides and Scott, 2001). A typical egg production curve for a flock will increase quickly during the first 8 to 9 weeks of production and then decrease at a constant rate for the remainder of production until a flock switch over (North and Bell, 1990). The LL hens displayed a consistently higher egg production for the entirety of the trial compared to the LB birds. Both strains ended the trial around 93.50% hen day. In a study by Bish et al. (1985), hen day production was not effected by bird body size. This is inconsistent with the results of this trial. The diets were formulated to the requirements of the LL birds, which could account for the reduced egg production in the LB birds. The significant interaction was detected due to the period 4 drop in egg production displayed by the LL birds. This is the only period where LL hen day production was less than that of the LB birds. Period 3 was the second period where birds were fed the phase 2 diets, so it is possible that egg production suffered in period 4 from the reduction in feed consumption during periods 3 and 4.

4.4.1.3 Feed Conversion

There was a significant interaction between strain and age ($P<0.05$) on feed conversion. Lohmann Brown Lite hens were significantly heavier than the Lohmann LSL Lite hens. The trend of feed conversion is highly relevant to the trend observed for feed consumption.

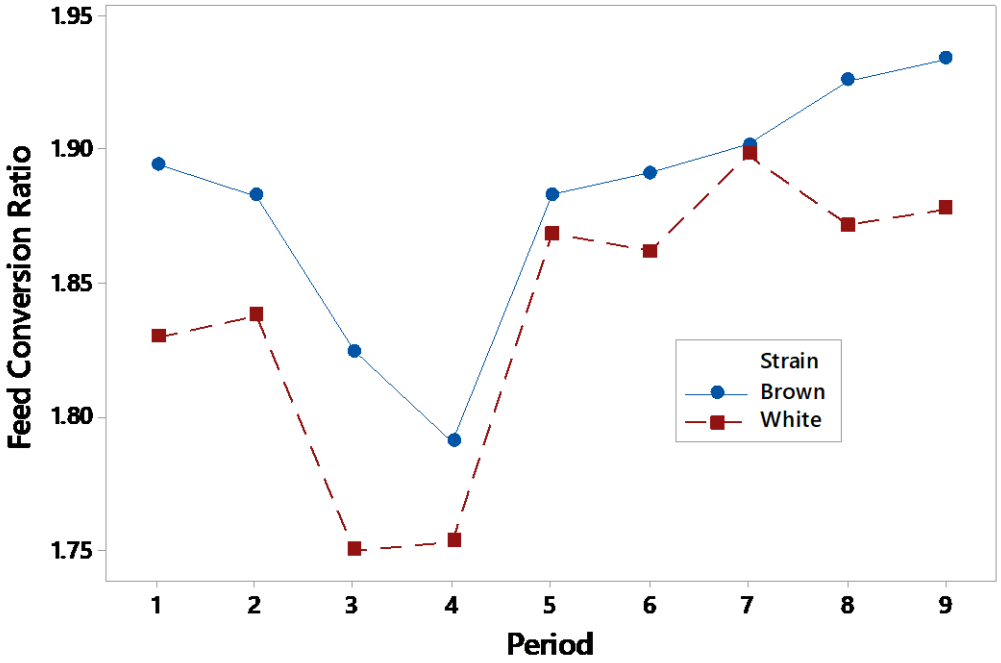

Figure 6. Interaction Plot Between Age and Strain for Feed Conversion Ratio of Hens fed *Chondrus crispus* during the Long Term Feeding Trial

The amount of feed consumed was much less during periods 3 and 4, but the egg production stayed consistent and the egg weight increased (discussed in the following section). Thus, the birds were still producing the same amount of eggs, and larger eggs, while consuming less feed. This made the feed conversion significantly better during period 3 and 4. From period 5 onward, the feed conversion stayed consistent, similar to the feed consumption trend. For strain, the Lohmann LSL lite hens had significantly lower feed conversion compared to the Lohmann Brown Lite. This is because the eggs from the LB hens were significantly lighter (discussed in next section), compared with the LL birds. In addition, as previously discussed, egg production was higher in the LL birds for the majority of the trial. Thus, although both strains were consuming a similar amount of feed, the LL birds were producing larger eggs at a higher quantity, making their feed conversion much better

than the LB birds. In a previous study, birds with larger body size consumed more feed per dozen than medium and light birds (Bish et al., 1985).

4.4.1.4 Body Weight

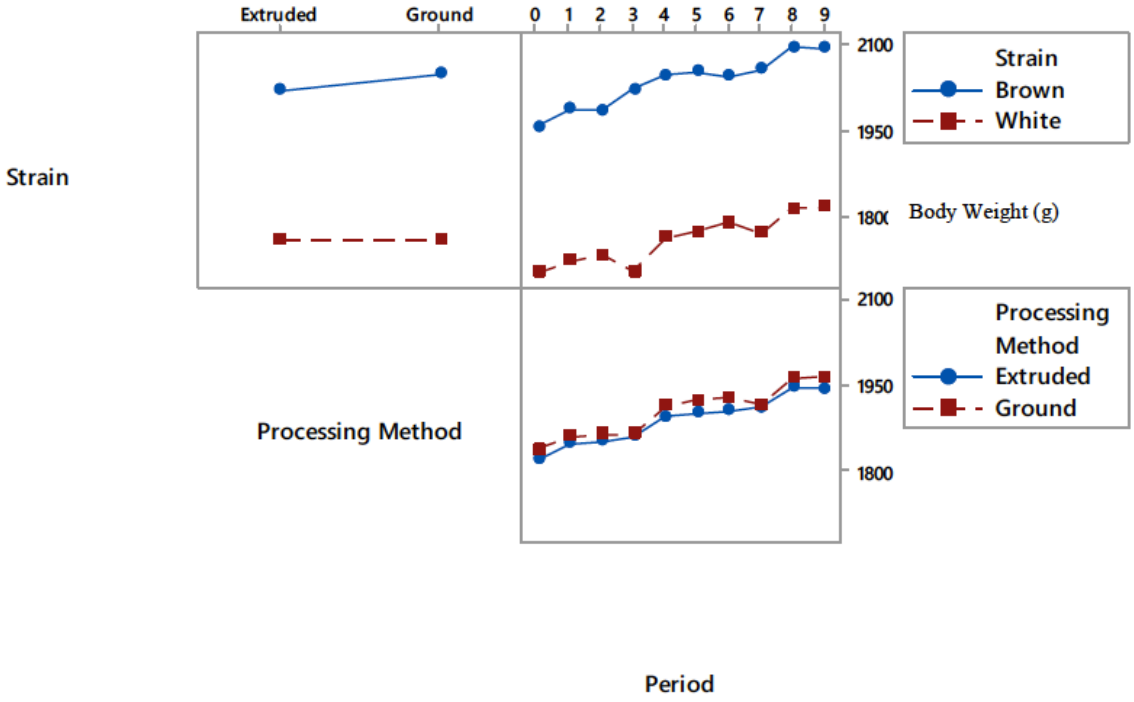

Figure 7. Interaction Plot Between Strain, Age and Processing Method for Body Weight (g) of Hens fed *Chondrus crispus* during the Long Term Feeding Trial

Lohmann Brown Lite hens were significantly heavier than the Lohmann LSL Lite hens. For both strains, and both processing treatments, the overall trend was that body weight increased over the trial, with some fluctuations. LB birds fed the ground treatment were heavier compared to LB birds fed the extruded treatment, likely as a result of better feed utilization of the extruded feed. LL birds had similar body weights for both the extruded and ground diets. For the LL birds, the hens fed ground CC had similar body weights to hens fed extruded CC. The largest deviation occurred during period 3, where the LL birds had the lowest body weights. This is a direct reflection of their drop in feed consumption.

Table 11. Effect of Inclusion Level, Strain, Processing Method and Age on Internal Layer Egg Quality for Hens fed *Chondrus crispus* during the Long Term Feeding Trial

			Egg Weight (g)	Yolk Weight (g)	Albumen Height (mm)
Main Effect Means	Inclusion Level[1]	0%	62.32 ± 0.25	16.47 ± 0.10	6.7 ± 0.09
		0.5%	62.65 ± 0.25	16.74 ± 0.10	6.8 ± 0.09
		1.75%	62.32 ± 0.25	16.42 ± 0.10	6.8 ± 0.09
		3%	63.39 ± 0.25	16.76 ± 0.10	6.7 ± 0.09
	Strain[2]	LL	63.40 ± 0.17	17.26 ± 0.07	7.2[a] ± 0.07
		LB	61.02 ± 0.17	15.94 ± 0.07	6.3[b] ± 0.07
	Processing Method	Extruded	62.74 ± 0.18	16.67 ± 0.07	6.7 ± 0.07
		Ground	62.68 ± 0.17	16.52 ± 0.07	6.8 ± 0.07
	Age	34-37 weeks	61.33 ± 0.25	15.67 ± 0.01	7.2 ± 0.06
		38-41 weeks	61.82 ± 0.25	16.07 ± 0.01	-
		42-45 weeks	62.97 ± 0.24	16.56 ± 0.01	-
		46-49 weeks	62.97 ± 0.25	16.94 ± 0.01	-
		50-53 weeks	62.47 ± 0.25	16.73 ± 0.01	-
		54-57 weeks	62.90 ± 0.25	17.05 ± 0.01	-
		58-61 weeks	62.65 ± 0.25	17.08 ± 0.01	-
		62-65 weeks	63.83 ± 0.25	17.43 ± 0.01	-
		66-69 weeks	63.44 ± 0.25	17.43 ± 0.01	5.9 ± 0.06
P-Value	Main Effects	Strain	<0.0001	<0.0001	<0.0001
		Age	<0.0001	<0.0001	<0.0001
		Processing	0.8242	0.1402	0.4405
		Level	0.0168	0.0398	0.4937
	2-Way Interaction	Level*Strain	0.3601	0.2265	0.2486
		Level*Age	0.6266	0.6249	0.1275
		Level*Processing	0.0258	0.7537	0.0719
		Strain*Age	0.0136	0.0086	0.1362
		Strain*Processing	0.9675	0.6670	0.4428
		Age*Processing	0.2855	0.6923	0.7325

			Egg Weight (g)	Yolk Weight (g)	Albumen Height (mm)
P-Value	3- Way Interactions	Level*Age*Strain	0.0642	0.5524	0.3397
		Level*Age*Processing	0.2339	0.7522	0.1857
		Level*Processing*Strain	0.0385	0.4147	0.8053
		Processing*Age*Strain	0.2123	0.4011	0.4604
	4-Way Interaction	Level*Age*Strain*Processing	0.1696	0.8693	0.1737

[1]Inclusion Level: 0%: control; 0.5%: contains 0.5% of *Chondrus crispus*; 1.75%: contains 1.75%% of *Chondrus crispus;* 3%: contains 3% of *Chondrus crispus.*

[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

4.4.1.5 Egg Weight

There was a significant effect of age ($P<0.05$) on egg weight. An increase in egg weight as a response to age has been frequently reported in previous research (Roland, 1979; Nys, 1986; Sokolowicz et al., 2018). From Table 11, it is clear that there is an increase in egg size from period 1 to 9. Average egg weight increased from 61.33g in period 1 to 63.44g in period 9. A 3-way interaction was also detected for egg weight (level by process by strain). This is shown in Table 12.

Table 12. Interaction for Process by Strain by Level for Egg Weight (g)

Strain[2]	Process	Inclusion Level[1]			
		0 %	0.5 %	1.75 %	3 %
LB	Ground	61.34^{c}	62.83^{abc}	61.58^{bc}	62.23^{bc}
	Extruded	62.34^{bc}	61.47^{bc}	61.93^{bc}	62.42^{abc}
LL	Ground	62.33^{bc}	62.80^{abc}	62.96^{abc}	65.39^{a}
	Extruded	63.89^{ab}	63.49^{abc}	62.82^{abc}	63.54^{abc}

[1]Inclusion Level: 0%: control; 0.5%: contains 0.5% of *Chondrus crispus*; 1.75%: contains 1.75%% of *Chondrus crispus;* 3%: contains 3% of *Chondrus crispus.*
[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

The LL birds fed the 3% ground CC had the largest average egg weight of 65.39g. This value was significantly different than almost all LB egg weights, with the exception of the 3% extruded CC group and the 0.5% ground CC group. For the LL birds, the only egg weight values that were significantly different from one another were the 3% ground compared to the 0% ground. The 3% ground group had significantly larger eggs compared

to the 0% ground group. For the LB birds, none of the egg weights were significantly different from each other.

In addition, the LB birds fed the 0% ground CC had significantly lower egg weight compared to the LL birds fed the 0% extruded CC. Both groups were fed the exact same control diet, therefore it is unknown as to why this difference was observed.

The significant three-way interaction was a reflection of the large average egg weight value for the LL 3% extruded birds as well as the very low average egg weight for the LB ground control birds. The LB birds had significantly smaller eggs overall ($P<0.05$) in comparison to the LL birds, but the 61.34g average egg weight displayed by the LB ground control birds was much lower than the 65.39g and 63.89g values displayed by the LL fed 3% ground and 3% extruded, respectively (largest average egg weights overall). Ultimately, this three-way interaction is meaningless and the main effect of strain is really the key component for egg weight. Inconsistent with the results of this trial, Bish et al. (1985) found that heavier birds produced larger egg weights. Because this was an older study, it is likely that the progression in genetic selection may have an influence that accounts for the difference in results.

4.4.1.6 Yolk Weight

Although the P-value for level was less than 0.05, the Tukey-Kramer test determined that there were no significant differences among yolk weight values between levels. There was however, a significant interaction effect ($P<0.05$) for strain and age, shown in *Figure 8.* The yolk weights for the LL birds were significantly higher than the yolk weights for the

brown birds. This was due to the fact that LL egg weights were larger. In a study by Suk and Park. (2001), yolk, albumen and shell weight were positively (P<0.001) associated with egg weight. This stayed consistent for the whole trial. The yolk weight increased as the hens aged. Because the egg weight increased, the yolk weight increased in response as the birds aged.

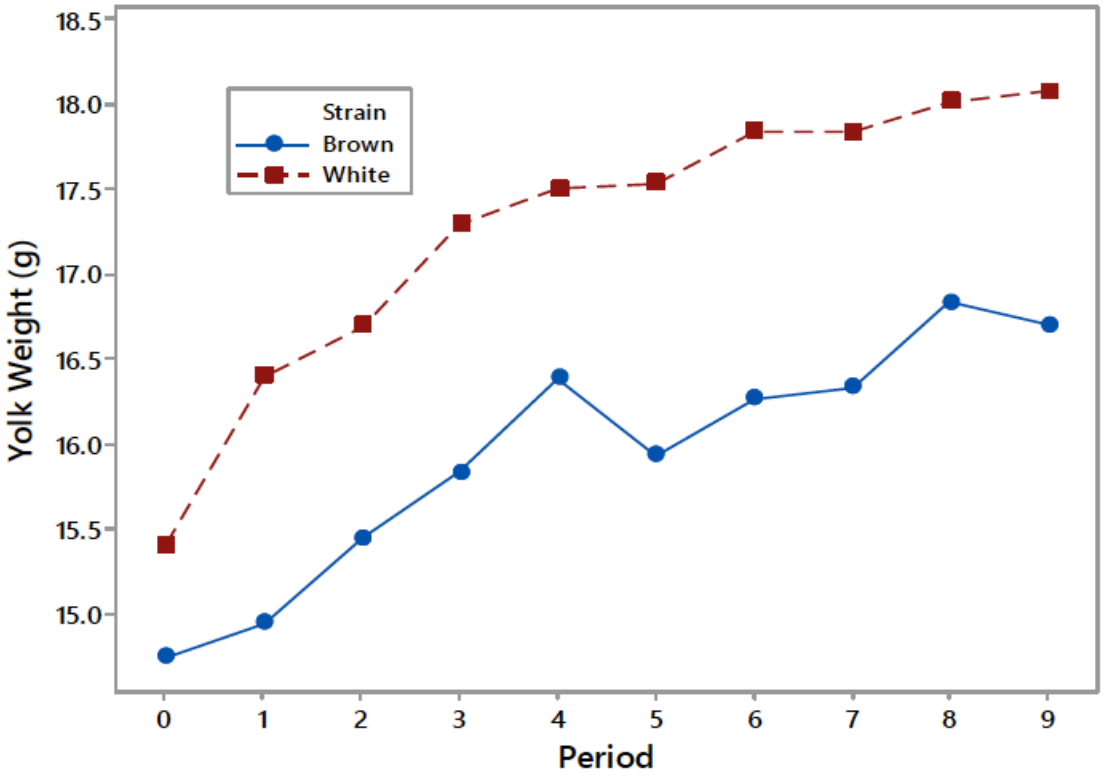

Figure 8. Interaction Plot Between Strain and Age for Yolk Weight (g) of Hens fed *Chondrus crispus* during the Long Term Feeding Trial

4.4.1.7 Albumen Height

Both a strain effect and age effect were detected for albumen height (P<0.05). For strain, LL hens had significantly taller albumen height (7.2mm) compared to LB hens (6.3). For age, albumen height decreased in period 9 (5.9mm) compared to period 1 (7.2mm). Albumen height measures were only taken at the beginning and end of the trial. This is due

to equipment malfunction where the albumen height gauge had to be sent off for repair during periods 2 to 8. The age of the hens is important when considering albumen height because albumen quality declines with bird age (Baker and Vadehra, 1970; Roberts and Ball, 2004; Silversides and Scott, 2001). In addition, albumen quality is affected by the strain of bird and genetic selection (Scott and Silversides, 2000; Tharrington et al., 1999; Toussant and Latshaw, 1999).

Table 13. Effect of Inclusion Level, Strain, Processing Method and Age on Layer Shell Quality for Hens fed *Chondrus crispus* during the Long Term Feeding Trial

			Shell Density (g/cm^3)	Shell Weight (g)	Shell Thickness (mm)	Breaking Strength (g)
Main Effect Means	Inclusion Level[1]	0%	$1.090^{a} \pm 0.0004$	6.23 ± 0.03	$0.434^{a} \pm 0.002$	5495 ± 70.718
		0.5%	$1.090^{ab} \pm 0.0004$	6.11 ± 0.04	$0.424^{b} \pm 0.002$	5277 ± 70.838
		1.75%	$1.089^{ab} \pm 0.0004$	6.20 ± 0.04	$0.430^{ab} \pm 0.002$	5368 ± 70.718
		3%	$1.088^{b} \pm 0.0004$	6.23 ± 0.04	$0.429^{ab} \pm 0.002$	5453 ± 70.838
	Strain[2]	LL	1.088 ± 0.0003	6.15 ± 0.03	0.427 ± 0.001	5205 ± 50.090
		LB	1.091 ± 0.0003	6.24 ± 0.03	0.432 ± 0.001	5591 ± 50.005
	Processing Method	Extruded	1.089 ± 0.0003	6.20 ± 0.03	0.429 ± 0.001	5337 ± 50.005
		Ground	1.089 ± 0.0003	6.18 ± 0.03	0.429 ± 0.001	5460 ± 50.090
	Age	34-37 weeks	1.088 ± 0.0004	-	0.423 ± 0.002	5755 ± 68.284
		38-41 weeks	1.091 ± 0.0004	6.22 ± 0.03	0.415 ± 0.002	5717 ± 68.536
		42-45 weeks	1.089 ± 0.0004	6.28 ± 0.03	0.417 ± 0.002	5633 ± 68.284
		46-49 weeks	1.090 ± 0.0004	6.23 ± 0.03	0.420 ± 0.002	5279 ± 68.284
		50-53 weeks	1.089 ± 0.0004	6.25 ± 0.03	0.423 ± 0.002	5450 ± 68.284
		54-57 weeks	1.089 ± 0.0004	6.27 ± 0.03	0.428 ± 0.002	5333 ± 68.284
		58-61 weeks	1.089 ± 0.0004	6.23 ± 0.03	0.431 ± 0.002	5149 ± 68.284
		62-65 weeks	1.089 ± 0.0004	6.20 ± 0.03	0.430 ± 0.002	5082 ± 68.284
		66-69 weeks	1.087 ± 0.0004	6.12 ± 0.03	0.419 ± 0.002	5087 ± 68.284
P-Value	Main Effects	Strain	<0.0001	0.0358	0.0030	<0.0001
		Age	<0.0001	<0.0001	<0.0001	<0.0001
		Processing	0.8846	0.6611	0.9774	0.0895
		Level	0.0114	0.1318	0.0056	0.1490
	2-Way Interaction	Level*Strain	0.3386	0.6943	0.2971	0.7616
		Level*Age	0.3044	0.4939	0.2704	0.4324
		Level*Processing	0.4666	0.5108	0.6732	0.9690
		Strain*Age	<0.0001	<0.0001	<0.0001	0.0005
		Strain*Processing	0.4341	0.9402	0.6286	0.9966

			Shell Density (g/cm^3)	Shell Weight (g)	Shell Thickness (mm)	Breaking Strength (g)
P-Value	3- Way Interaction	Age*Processing	0.7830	0.6082	0.1996	0.7503
		Level*Age*Strain	0.3557	0.0554	0.0567	0.3546
		Level*Age*Processing	0.9138	0.2602	0.4645	0.8298
		Level*Processing*Strain	0.3038	0.9821	0.8084	0.2413
		Processing*Age*Strain	0.3985	0.1010	0.7818	0.2688
	4-Way Interaction	Level*Age*Strain*Processing	0.3609	0.7614	0.2457	0.6223

[1]Inclusion Level: 0%: control; 0.5%: contains 0.5% of *Chondrus crispus*; 1.75%: contains 1.75%% of *Chondrus crispus;* 3%: contains 3% of *Chondrus crispus.*

[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

4.4.1.8 Shell Density

The main effect of level showed significant differences ($P<0.05$) for shell density. The hens fed the control had significantly higher shell density (1.090g/cm^3) compared to hens fed the 3% red seaweed (1.088g/cm^3). Hens fed the 0.5% and 1.75% were not significantly different from the control birds or the birds fed the 3% inclusion level. An interaction effect was observed between age and strain ($P<0.05$). The interaction effect is presented in *Figure 9*.

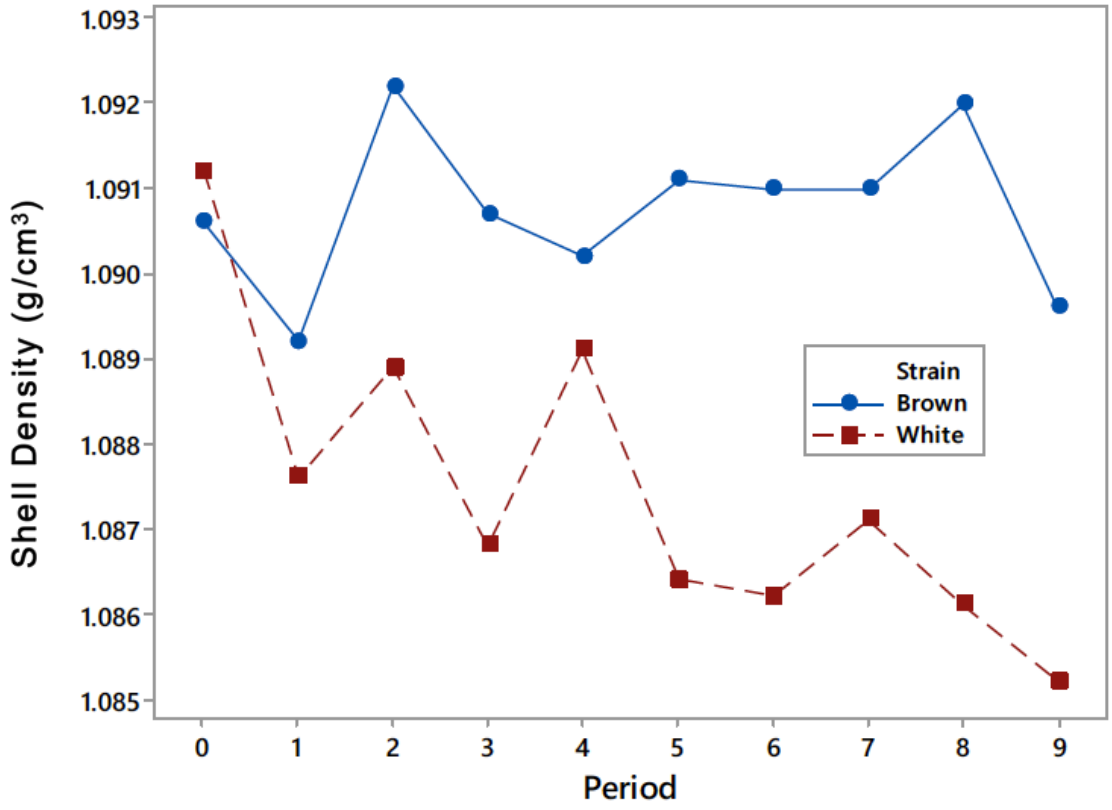

Figure 9. Interaction Plot Between Age and Strain for Shell Density (g/cm^3) of Hens fed *Chondrus crispus* during the Long Term Feeding Trial

There is a clear tend showing that eggshell density decreased as the birds aged, especially with the LL birds. There was a spike in density during period 2 and 4 for LL birds, but then density declined for the rest of the trial. LB birds showed a spike in density during periods 2 and 8, and values stayed consistent in between these two spikes. The LB shell density was higher than the LL birds for all periods, except period 1. This finding corresponds with shell weight, breaking strength and shell thickness (discussed below). The LL birds followed a pattern similar to previous research whereby shell quality, including density of eggshells, decreases with age (Roland, 1979; Sokolowicz et al., 2018). The reason that the LB bird shell density did not decline is likely due to the smaller egg weights. The more devastating decline in shell density for LL birds compared to the LB birds is likely reflective of the higher production output and higher genetic selection in the LL strain.

4.4.1.9 Shell Weight

There is a clear trend showing that shell weight decreased with hen age for LL birds. For LB birds, shell weight remained constant until period 7. After period 7, shell weight declined for LB birds. Although shells become thinner with hen age, there is a lack of consistent literature proving that the eggshell weight increases in response. With the increase in egg size, there is no corresponding increase in shell deposition (Roland, 1979), causing the eggshells to become thinner. Thus, the expected result would be that shell weight would remain consistent. Previous studies have reported that while egg size increases, eggshell weight stays the same, causing a decrease in shell quality (Nys, 1986). Declining shell quality corresponds with a reduction of the attachment force and breaking strength of the shell membranes (Kemps et al., 2006). In this study, it was found that shell weight decreased for LL birds, but remained the same until period 7 for LB birds. A

possible explanation may be that the organic matrix of the shell undergoes changes as a hen ages, affecting the microstructure of the shell and causing older hens to show more variability in structural properties compared to younger hens (Rodriguez-Navarro et al., 2002). The significant interaction detected between age and strain occurred due to period 2. Period 2 was the only timeframe in which LB hens had lighter shells when compared to LL hens. After period 2, the LB hens had consistently heavier shells for the entirety of the trial. Unfortunately, a period 1 shell weight was not obtained, but a similar trend was observed between strain and age for shell thickness during period 1.

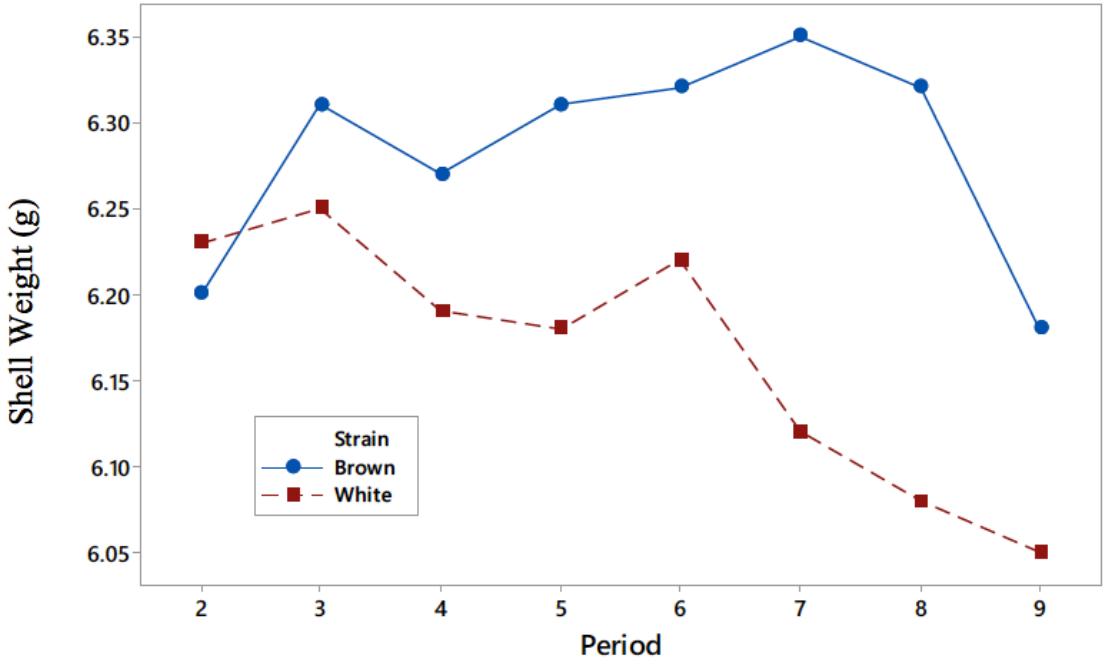

Figure 10. Interaction Plot Between Age and Strain for Shell Weight (g) of Hens fed *Chondrus crispus* during the Long Term Feeding Trial

4.4.1.10 Shell Thickness

Shell thickness followed an interesting and unexpected trend. Because eggs become larger with hen age, with no proportionate increase in shell deposition (Roland, 1979; Roberts,

2004), it would be expected that shell thickness would decrease as the hens aged. The LL (white) birds started out with thicker shells in period 1, but quickly declined in period 2. After period 2, the LB (brown) birds had consistently thicker shells compared to the LL birds for the entirety of the trial. After period 7, LL shells began to decrease in thickness, while LB shells began to decrease after period 8.

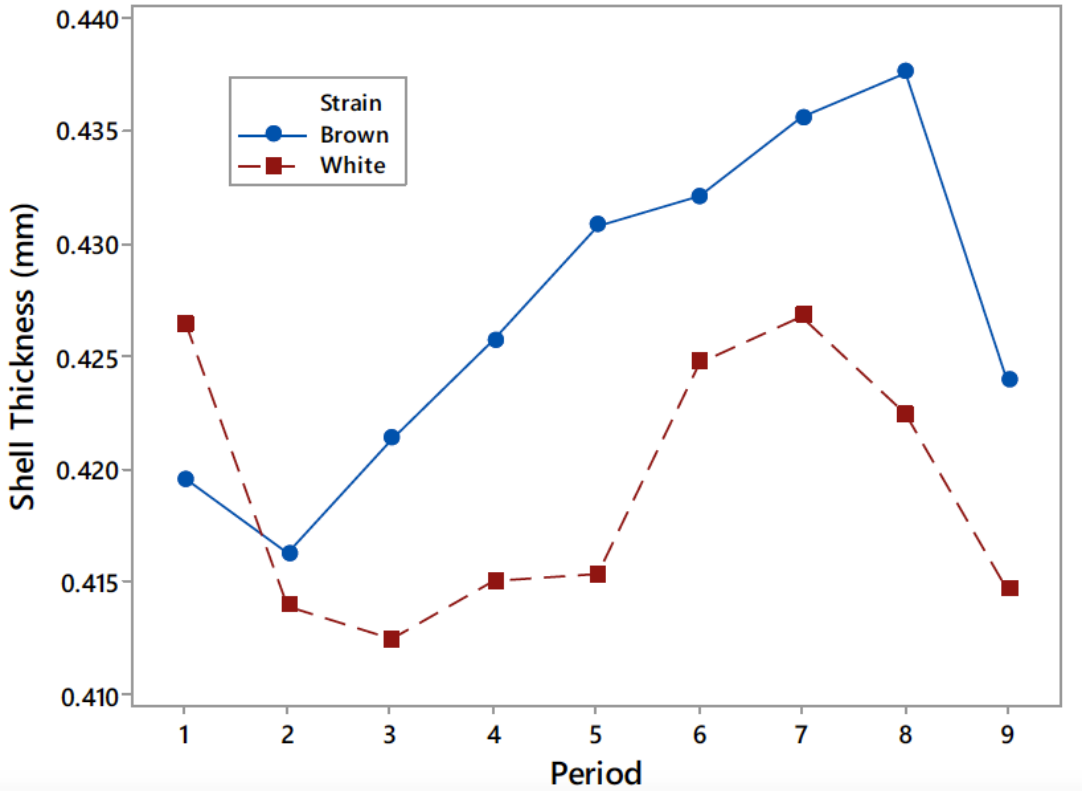

Figure 11. Interaction Plot Between Age and Inclusion Level for Shell Thickness (mm) of Hens fed *Chondrus crispus* during the Long Term Feeding Trial

A significant ($P<0.05$) effect of level was also observed for shell thickness. Hens fed the control diet had the thickest shells at 0.434 mm. The control was significantly different than the 0.5% inclusion level (0.424 mm) but was not significantly different from the 1.75% (0.430 mm) or the 3% inclusion level (0.429 mm). The birds fed the 0.5% CC had

the lowest shell thickness, lowest breaking strength and lowest shell weight, indicating that the 0.5% level may pose issues with shell quality.

4.4.1.11 Breaking Strength

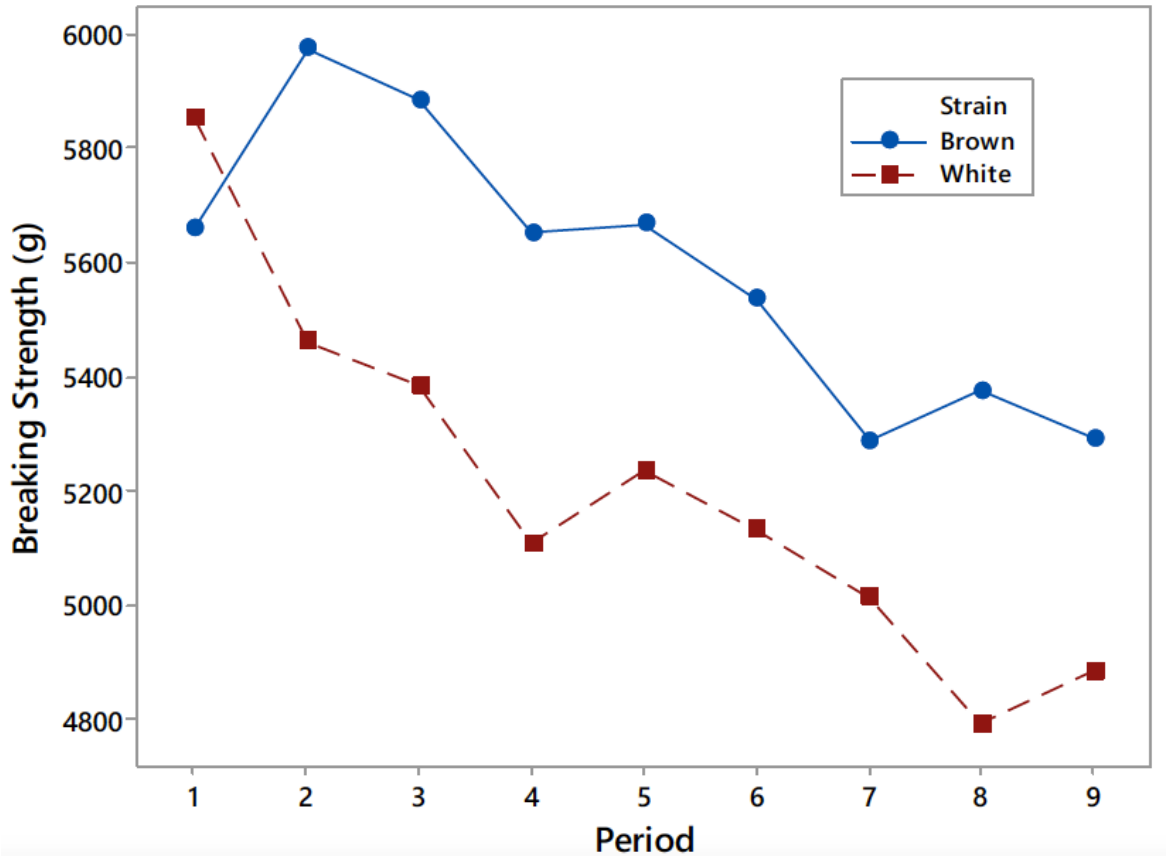

Figure 12. Interaction Plot Between Age and Inclusion Level for Shell Weight (g) of Hens fed *Chondrus crispus* during the Long Term Feeding Trial

Breaking strength showed a very clear, expected trend. For both strains, the general trend was a declining breaking strength with the exception of the LB layers. LB hens had an increase in breaking strength from period 1 to 2, but then proceeded to decline in strength from period 2 to period 9. The interaction effect was detected due to period 1, which was the only instance when LB hens had lower breaking strength compared to LL hens. Due to the declining quality of the shell, breaking strength has been proven to decline with

age. As hens approach the end of their laying cycle (approximately 80 weeks in Canada), there is an unmistakable decrease in shell strength (Hamilton et al., 1979; Potts and Washburn, 1983). Some studies have reported changes in the organic matrix component of the eggshells from older hens as a cause (Fraser et al., 1998; Panheleux et al., 2000).

From reading previous literature, it is fairly consistent that in most cases, a linear relationship exists between the strength of the shell and its thickness and/or density (Romanoff and Romanoff, 1949; Tyler, 1961). Although the shell thickness inclined and then declined, the shell density showed a clear declining trend, similar to the breaking strength, further justifying that shell quality declined as the trial went on.

4.4.2 Tasco®

Table 14. Effect of Inclusion Level, Strain, and Age on Layer Performance Traits for Hens fed Tasco® during the Long-Term Feeding Trial

			Feed Intake (g/bird/day)	FCR	Hen Day (%)	BW (g)
Main Effect Means	Inclusion Level[1]	0%	116 ± 0.97	1.84 ± 0.02	95.45 ± 0.70	1876 ± 19
		0.25%	118 ± 0.97	1.85 ± 0.02	94.44 ± 0.70	1911 ± 19
		0.5%	116 ± 0.97	1.88 ± 0.02	96.70 ± 0.71	1918 ± 19
	Strain[2]	LL	117 ± 0.79	1.83 b ± 0.02	95.15 ± 0.57	1781 ± 16
		LB	117 ± 0.79	1.89 a ± 0.02	94.91 ± 0.57	2024 ± 16
	Age	34-37 weeks	115^{de} ± 0.82	1.88 ± 0.02	97.50^{a} ± 0.50	1855 ± 13
		38-41 weeks	116^{cd} ± 0.82	1.87^{a} ± 0.02	96.04^{abc} ± 0.35	1861 ± 13
		42-45 weeks	113^{e} ± 0.82	1.79^{ab} ± 0.02	96.59^{ab} ± 0.59	1882 ± 14
		46-49 weeks	112^{e} ± 0.82	1.80^{c} ± 0.02	94.90^{bcd} ± 0.56	1903 ± 13
		50-53 weeks	118^{abc} ± 0.83	1.87^{bc} ± 0.02	96.44^{ab} ± 0.56	1921 ± 13
		54-57 weeks	118^{bcd} ± 0.83	1.87^{a} ± 0.02	95.26^{bcd} ± 0.56	1935 ± 13
		58-61 weeks	119^{ab} ± 0.83	1.89^{ab} ± 0.02	94.51^{cd} ± 0.58	1924 ± 13
		62-65 weeks	121^{a} ± 0.82	1.90^{a} ± 0.02	94.77^{bcd} ± 0.57	1969 ± 13
		66-69 weeks	120^{ab} ± 0.84	1.87^{a} ± 0.02	93.75^{d} ± 0.57	1954 ± 13
P-Value	Main Effects	Strain	0.5575	0.0260	0.1452	<0.0001
		Age	<0.0001	<0.0001	<0.0001	<0.0001
		Level	0.4587	0.3535	0.1001	0.2820
	2-Way Interaction	Level*Strain	0.5625	0.6527	0.8635	0.0123
		Level*Age	0.8152	0.7591	0.5114	0.0461
		Age* Strain	0.1457	0.2370	0.5317	0.0965
	3- Way Interaction	Level*Age *Strain	0.1572	0.1237	0.3012	0.0219

[1]Inclusion Level: 0.25%: contains 0.25% of sundried, ground *Ascophyllum nodosum*; 3%; 0.5%: contains 0.5% of sundried, ground *Ascophyllum nodosum*

[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

4.4.2.1 Feed Consumption

The same trend for feed consumption as birds fed the CC diets was observed in birds fed the Tasco® diets. Feed consumption increased from period 1 to 2, but then drastically decreased from period 3 to 4. Feed consumption was significantly less ($P<0.05$) in periods 3 and 4 compared to all other periods, except for period 1 (32). The first feed change occurred at the start of period 3, where the birds were switched from phase 1 to phase 2. This caused a drop in feed consumption from 116 g/bird/day to 113 g/bird/day. With the implementation of the new diet, the amount of corn, methionine and calcium components increased (limestone, shell mix, oyster shells) while soybean meal decreased. This allowed for a decrease in percentage protein and an increase in percentage calcium. During period 5, feed consumption increased drastically again Phase 5 also happened to be when the next phase change occurred. A similar increase/decrease in feed ingredients occurred from phase 2 to 3 as that of phase 1 to 2 (increased corn and calcium sources, decreased soy). Protein levels continued to decrease while calcium increased. The last phase change occurred at period 9, where the birds were switched over to their final, phase 4 diets for the remainder of the trial. There also seemed to be a decline in feed consumption for this switch over.

In observing the decline in feed consumption during the phase 2 feeding, it is clear that the birds did not prefer this particular diet. There was no drop in energy or increase in temperature in their environment during these periods. Thus, the decrease in feed consumption in response to the phase 2 diet is likely a reflection of something other than the energy level, temperature, or varying formulations. A possible explanation could be

that the particular batch of corn, wheat, canola or soybean was less desirable due to an anti-nutrient present, such as beta-glucans, arabinoxylans, or L-canavanin. Further research should be performed to identify why this particular diet caused the decrease in feed consumption. It should be noted, however, that although the feed consumption dropped, the feed intake was still within the normal levels identified by Lohmann (Lohmann LSL Lite Management Guide, 2018). With the exception of periods 3 and 4, and the small drop in period 9, the feed consumption increased as the birds aged. Between periods 5 and 6 there is a slight drop in feed consumption, likely reflective of the birds overeating the new, phase 3 diet in period 5 and the leveling out their consumption again for period 6.

4.4.2.2 Feed Conversion Ratio

There was a significant main effect of strain ($P<0.05$) on feed conversion. Consistent with the hens receiving the CC diets, the Lohmann LSL lite hens had significantly lower feed conversion compared to the Lohmann Brown Lite hens (Table 14). This is because the eggs from the LB hens were significantly lighter (discussed in next section), compared with the LL birds. Thus, although both strains were consuming a similar amount of feed and had comparable hen day production, the LL birds were producing larger, making their feed conversion much better than the LB birds. In a previous study, birds with larger body size consumed more feed per dozen than medium and light birds (Bish et al., 1985).

There was also a significant main effect of age ($P<0.05$) on feed conversion (Table 14). The trend of feed conversion is highly relevant to the trend observed for feed consumption. The amount of feed consumed was much less during periods 3 and 4, but the egg production stayed consistent and the egg weight increased (discussed in the following section). Thus,

the birds were still producing the same amount of eggs, and larger eggs, while consuming less feed. This made the feed conversion significantly better during period 3 and 4. From period 5 on ward, the feed conversion stayed consistent, similar to the feed consumption trend.

4.4.2.3 Body Weight

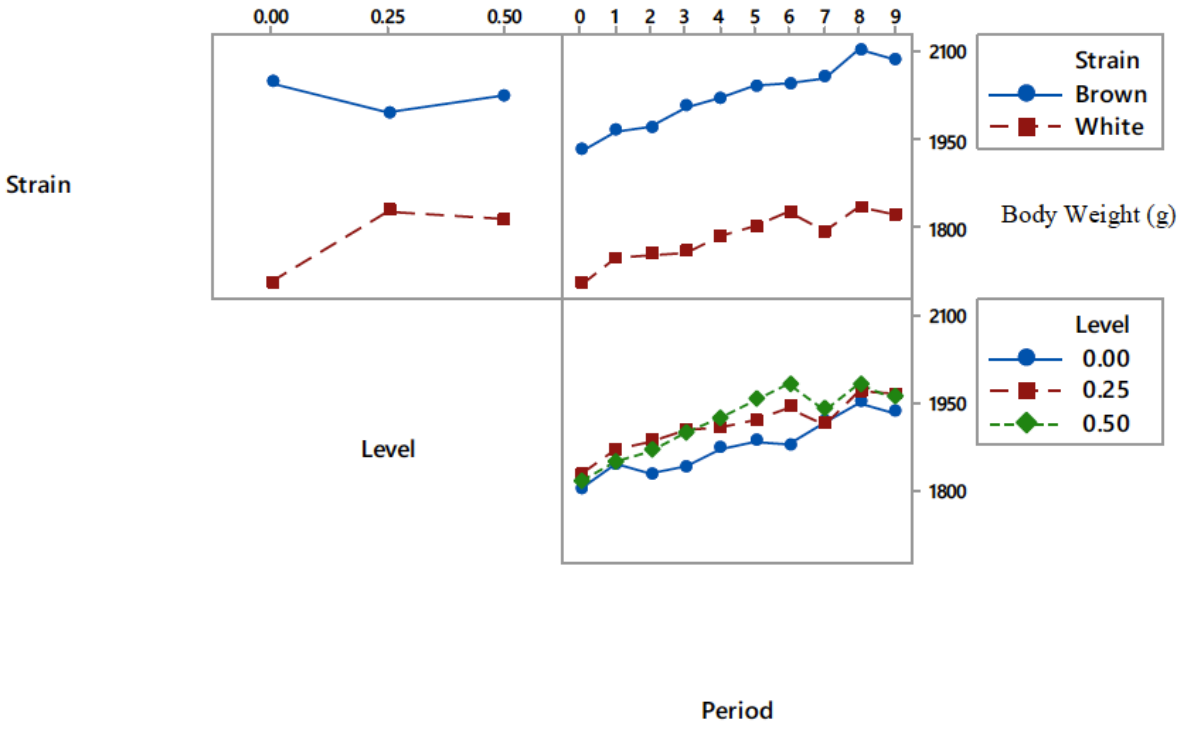

Figure 13. Interaction Plot Between Age, Inclusion Level and Strain for Body Weight (g) of Hens fed Tasco® during the Long Term Feeding Trial

A significant 3-way interaction was detected for body weight of hens fed Tasco®. The LB hens were significantly larger across all inclusion levels. The body weight of both LB and LL hens increased with age. The hens from each level group also followed the same trend, where their body weight increased with age. The significant interaction stems from the difference in body weight observed within the 0% and 0.25% inclusion levels. For LB

birds, the 0.5% inclusion level presented the lowest body weights while the control diet presented the highest body weights. The opposite was seen for the LL birds. For body weight by age, both the LL and LB had very similar trends, but for inclusion level, LL and LB birds had very different responses for body weight.

4.4.2.4 Egg Production

There was a significant effect of age ($P<0.05$) on egg production, whereby hen day production decreased as the birds aged (Table 14). These results are consistent with previously reported findings (Joyner et al., 1987; Silversides and Scott, 2001). A typical egg production curve for a flock will increase quickly during the first 8 to 9 weeks of production and then decrease at a constant rate for the remainder of production until a flock switch over (North and Bell, 1990).

Table 15. Effect of Inclusion Level, Strain, and Age on Layer Internal Egg Quality for Hens fed Tasco® during the Long Term Feeding Trial

			Egg Weight	Yolk Weight	Albumen Height
Main Effect Means	Inclusion Level[1]	0%	63.11^{ab} ± 0.45	16.61 ± 0.16	6.50 ± 0.16
		0.25%	63.82^{a} ± 0.45	17.17 ± 0.16	6.67 ± 0.16
		0.5%	62.04^{b} ± 0.45	16.94 ± 0.16	6.80 ± 0.16
	Strain[2]	LL	63.81 ± 0.37	17.70^{a} ± 0.13	6.94^{a} ± 0.13
		LB	62.17 ± 0.37	16.11^{b} ± 0.13	6.38^{b} ± 0.13
	Age	34-37 weeks	61.42 ± 0.45	15.62^{d} ± 0.17	7.32^{a} ± 0.12
		38-41 weeks	62.00 ± 0.45	16.37^{c} ± 0.17	-
		42-45 weeks	63.00 ± 0.45	16.69^{bc} ± 0.18	-
		46-49 weeks	62.57 ± 0.45	16.84^{abc} ± 0.17	-
		50-53 weeks	63.34 ± 0.45	17.11^{ab} ± 0.17	-
		54-57 weeks	63.57 ± 0.45	17.35^{ab} ± 0.17	-
		58-61 weeks	63.24 ± 0.45	17.37^{a} ± 0.17	-
		62-65 weeks	63.82 ± 0.45	17.38^{a} ± 0.17	-
		66-69 weeks	63.96 ± 0.45	17.43^{a} ± 0.17	5.99^{b} ± 0.12
P-Value	Main Effects	Strain	0.0057	<0.0001	0.0068
		Age	<0.0001	<0.0001	<0.0001
		Level	0.0376	0.0747	0.4193
	2-Way Interaction	Level*Strain	0.9787	04599	0.4260
		Level*Age	0.7561	0.8637	0.3681
		Age* Strain	0.0161	0.3592	0.3382
	3- Way Interaction	Level*Age *Strain	0.1311	0.8904	0.2214

[1]Inclusion Level: 0.25%: contains 0.25% of sundried, ground *Ascophyllum nodosum*; 3%; 0.5%: contains 0.5% of sundried, ground *Ascophyllum nodosum*

[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

4.4.2.5 Egg Weight

A significant effect of level for egg weight was observed (P<0.05). Birds fed the 0.25% inclusion had the highest egg weight (63.82g) and the 0.5% inclusion level had the lowest egg weights (62.04g).

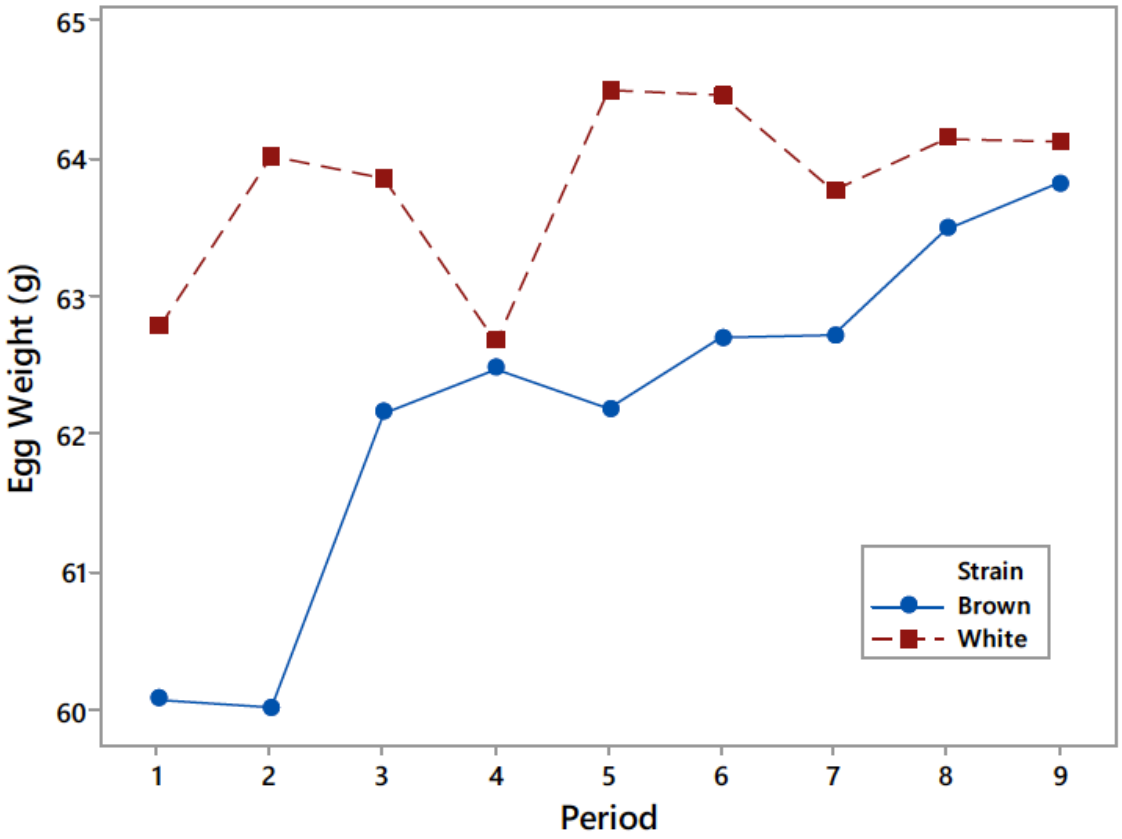

Figure 14. Interaction Plot Between Age and Strain for Egg Weight (g) of Hens fed Tasco® during the Long Term Feeding Trial

A strain by age interaction effect (*Figure 13*) was also observed for egg weight (P<0.05). LL birds had consistently higher egg weights for the entirety of the trial. There was an increase in egg weight as a response to bird age for the LB birds. An increase in egg weight as a response to age has been frequently reported in previous research (Roland,

1979; Nys, 1986; Sokolowicz et al., 2018). From *Figure 13*, it is visible that there is a clear increase in egg size from period 1 to 9 for the LB birds. However, this trend was not seen for the LL birds. The LL birds had a higher egg weight in period 9 compared to period 1, but there were many fluctuations in egg weight in between. There was over a gram decrease in egg weight during period 4, likely due to the drop in feed consumption during period 3 and 4. The LL birds fed the CC diets were more influenced by the change to the phase 2 diets. Although strain was not significant when considering feed consumption in the birds fed Tasco, it is likely that the LL birds again had a greater response to the switch to phase 2. A decrease in feed consumption would cause a decrease in egg weight.

4.4.2.6 Yolk Weight

Age had a significant effect on yolk weight ($P<0.05$), whereby yolk weight increase with age. Period 1 yolks had an average weight of 15.62g, while period 9 yolks had an average weight of 17.43g. In addition, strain had a significant effect on yolk weight. LL birds had significantly higher ($P<0.05$) yolk weight in comparison to LB birds. This is likely a reflection of LL birds having larger egg weights. In a study by Suk and Park (2001), yolk, albumen and shell weight was positively ($P<0.001$) associated with egg weight. This stayed consistent for the whole trial. The yolk weight increased as the trial moved forward. Because the egg weight increased, the yolk weight increased in response as the birds aged.

4.4.2.7 Albumen Height

A strain effect was observed for albumen height ($P<0.05$), whereby LL birds had significantly higher albumen compared to LL birds (Table 15). Although research involving albumen height comparing these specific two strains (Lohmann LSL Lite and Lohmann Brown Lite) is limited, albumen quality is affected by the strain of bird and genetic selection (Scott and Silversides, 2000; Tharrington et al., 1999; Toussant and Latshaw, 1999).

Albumen height was also effected by age ($P<0.05$). Similar to the birds fed the CC diets, albumen height measures were only taken at the beginning and end of the trial. This is due to equipment malfunction where the albumen height gauge had to be sent off for repair during periods 2 to 8. The age of the hens is important when considering albumen height because albumen quality declines with bird age (Baker and Vadehra, 1970; Roberts and Ball, 2004; Silversides and Scott, 2001). There was a clear decline in albumen height from period 1 to period 9, consistent with previous literature (Table 15).

Table 16. Effect of Inclusion Level, Strain, and Age on Layer Eggshell Quality for Hens fed Tasco® during the Long Term Feeding Trial

			Shell Density (g/cm^3)	Shell Weight (g)	Shell Thickness (mm)	Breaking Strength (g)
Main Effect Means	Inclusion Level[1]	0%	1.090 ± 0.0006	6.31^{a} ± 0.04	0.433 ± 0.003	5480 ± 94
		0.25%	1.089 ± 0.0006	6.34^{a} ± 0.04	0.429 ± 0.003	5457 ± 94
		0.5%	1.089 ± 0.0006	6.13^{b} ± 0.04	0.427 ± 0.003	5413 ± 94
	Strain[2]	LL	1.087 ± 0.0005	6.19 ± 0.04	0.426 ± 0.002	5292^{b} ± 78
		LB	1.092 ± 0.0005	6.33 ± 0.04	0.434 ± 0.002	5608^{a} ± 78
	Age	34-37 weeks	1.090 ± 0.0006	-	0.420 ± 0.004	5507^{bcd} ± 115
		38-41 weeks	1.091 ± 0.0006	6.23 ± 0.05	0.415 ± 0.004	5767^{ab} ± 115
		42-45 weeks	1.089 ± 0.0006	6.24 ± 0.05	0.418 ± 0.004	5635^{abc} ± 121
		46-49 weeks	1.090 ± 0.0006	6.25 ± 0.05	0.420 ± 0.004	5271^{bcd} ± 121
		50-53 weeks	1.090 ± 0.0006	6.36 ± 0.05	0.428 ± 0.004	5419^{bcd} ± 115
		54-57 weeks	1.089 ± 0.0006	6.31 ± 0.05	0.430 ± 0.004	5308^{bcd} ± 115
		58-61 weeks	1.089 ± 0.0006	6.26 ± 0.05	0.431 ± 0.004	5158^{cd} ± 115
		62-65 weeks	1.090 ± 0.0006	6.21 ± 0.05	0.434 ± 0.004	5092^{d} ± 115
		66-69 weeks	1.088 ± 0.0006	6.23 ± 0.05	0.421 ± 0.004	5225^{cd} ± 123
P-Value	Main Effects	Strain	<0.0001	0.0167	0.0160	0.0100
		Age	0.0047	0.3382	<0.0001	<0.0001
		Level	0.7891	0.0055	0.3145	0.8803
	2-Way Interaction	Level*Strain	0.9744	0.9165	0.7038	0.2688
		Level*Age	0.1611	0.1086	0.7041	0.4777
		Age* Strain	0.0185	0.0477	0.0280	0.3293
	3- Way Interaction	Level*Age *Strain	0.9677	0.8032	0.5389	0.4460

[1]Inclusion Level: 0.25%: contains 0.25% of sundried, ground *Ascophyllum nodosum*; 3%; 0.5%: contains 0.5% of sundried, ground *Ascophyllum nodosum*

[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

4.4.2.8 Shell Density

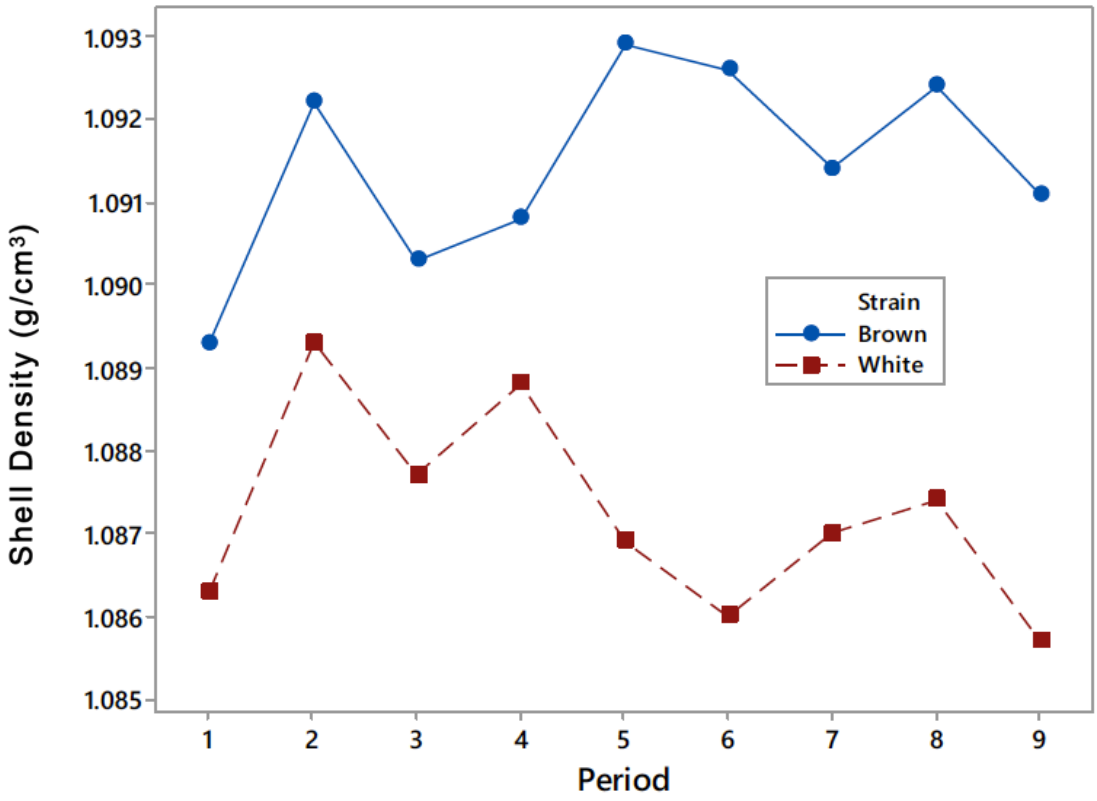

Figure 15. Interaction Plot Between Age and Strain for Shell Density (g/cm³) *of Hens fed Tasco® during the Long Term Feeding Trial*

The shell density results were much different when compared to the birds fed *Chondrus crispus*. It is expected that shell density decrease with age because shell quality, including density of eggshells, decreases with age (Roland, 1979; Sokolowicz et al., 2018). However, for the hens in the Tasco® design, the shell density remained fairly constant for the entirety of the trial. In fact, the LB birds completed the trial with a higher shell density than the start of the trial. The LB birds had significantly higher shell density than the LL birds for the entirety of the trial. This is likely a reflection of the LL birds having larger eggs, causing thinner shells with lower density. In period 2, the shell density for both LL and LB birds

increased by approximately 0.003, but dropped again in period 3. It is unclear as to why the shell density remained constant for the Tasco® birds, but a possible explanation is that were only 24 experimental units. It is likely that if the sample size were larger, the expected decline in shell density would have been evident.

4.4.2.9 Shell Weight

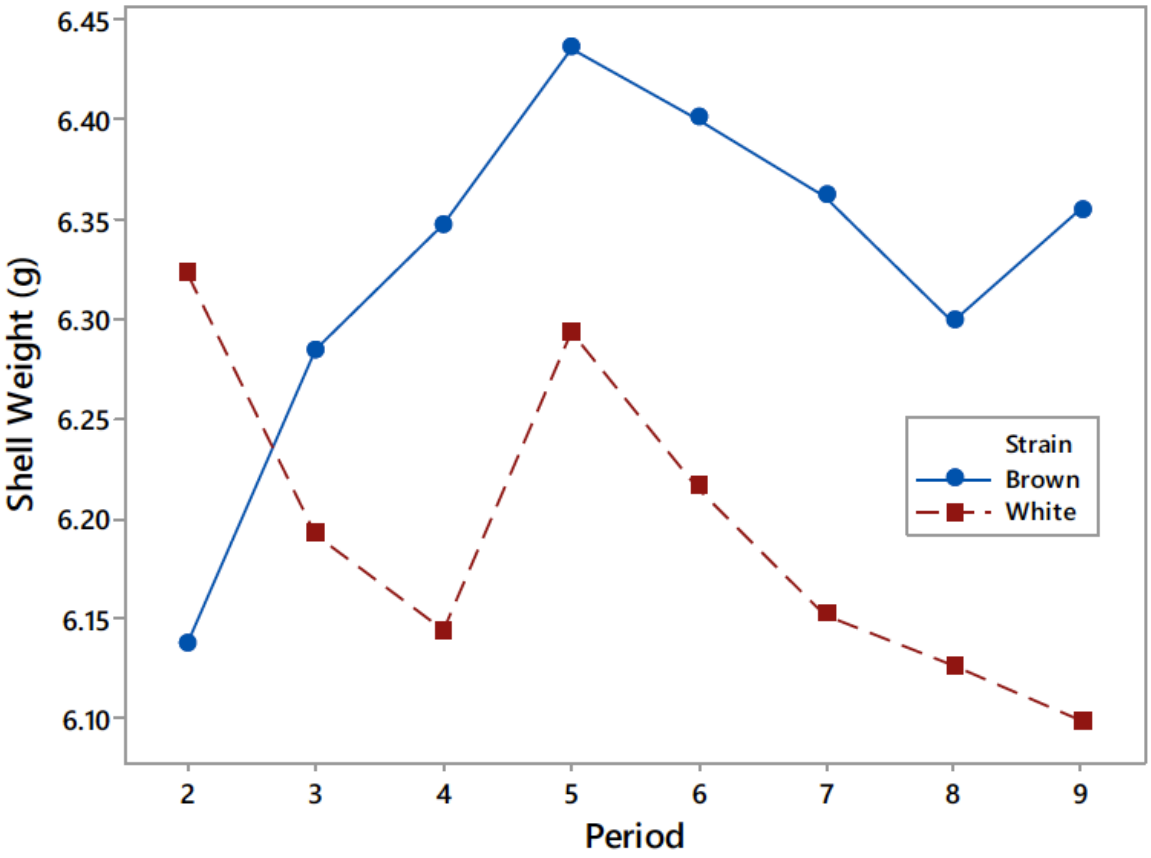

Figure 16. Interaction Plot Between Age and Strain for Shell Weight (g) of Hens fed Tasco® during the Long Term Feeding Trial

From *Figure 16*, it is clear that shell weight increased over the trial for LB birds, whereas the shell weight decreased over the trial for the LL birds. Regardless, both strains demonstrated a peak in shell weight during period 5. Period 5 is when the birds were

switched to phase 3 diets and when their feed consumption increased after the drop in feed consumption during phase 2 feeding. There is a clear trend showing that shell weight decreased with hen age for LL birds. Although shells become thinner with hen age, there is a lack of consistent literature proving that the eggshell weight increases in response. With the increase in egg size, there is no corresponding increase in shell deposition (Roland, 1979), causing the eggshells to become thinner. Thus, the expected result would be that shell weight would remain consistent. Previous studies have reported that while egg size increases, eggshell weight stays the same, causing a decrease in shell quality (Nys, 1986). Declining shell quality is corresponds with a reduction of the attachment force and breaking strength of the shell membranes (Kemps et al., 2006). In this study, it was found that shell weight decreased for LL birds. This is inconsistent with previous literature. A possible explanation may be that the organic matrix of the shell undergoes changes as a hen ages, affecting the microstructure of the shell and causing older hens to show more variability in structural properties compared to younger hens (Rodriguez-Navarro et al., 2002).

The shell weight of the LB birds showed the opposite effect. There was an increase in shell weight as the birds aged, with period 9 shell weights being higher than period 1 shell weights. Since the LB diets were less effected by the change to phase 2 diets, their shell weights were not influenced to the extent that the LL bird shells were. The shell weight of LB birds increased up until period 5, and then decreased until period 8. From period 8 to period 9, the shell weight increased again. A large contributing factor to these unexplainable results is the low sample size. Only 12 LB cages were fed Tasco® diets for

the heat stress trial. The significant interaction detected between age and strain occurred due to period 2. Period 2 was the only timeframe in which LB hens had lighter shells when compared to LL hens. After period 2, the LB hens had consistently heavier shells for the entirety of the trial. Unfortunately, a period 1 shell weight was not obtained.

A significant effect of level for shell weight was also observed ($P<0.05$). Birds fed the 0.25% inclusion had the highest shell weight (6.34g) and the 0.5% inclusion level had the lowest egg weights (6.13g). This is consistent with the level effect for egg weight, in which birds fed the 0.25% Tasco® had the highest egg weight while birds fed the 0.5% had the lowest egg weight. For both egg weight and shell weight, the control values fell in between the 0.25% and the 0.5% levels.

4.4.2.10 Shell Thickness

Shell thickness followed an interesting and unexpected trend similar to the birds fed the CC diets. Because eggs become larger with hen age, with no proportionate increase in shell deposition (Roland, 1979; Roberts, 2004), it would be expected that shell thickness would decrease as the hens aged. The LB birds started out with thicker shells than the LL birds, but in period 2 the LB shell thickness dropped below that of the LL birds. After period 2, the LB (brown) birds had consistently thicker shells compared to the LL birds for the entirety of the trial. After period 7, LB shells began to decrease in thickness, while LL shells began to decrease after period 8.

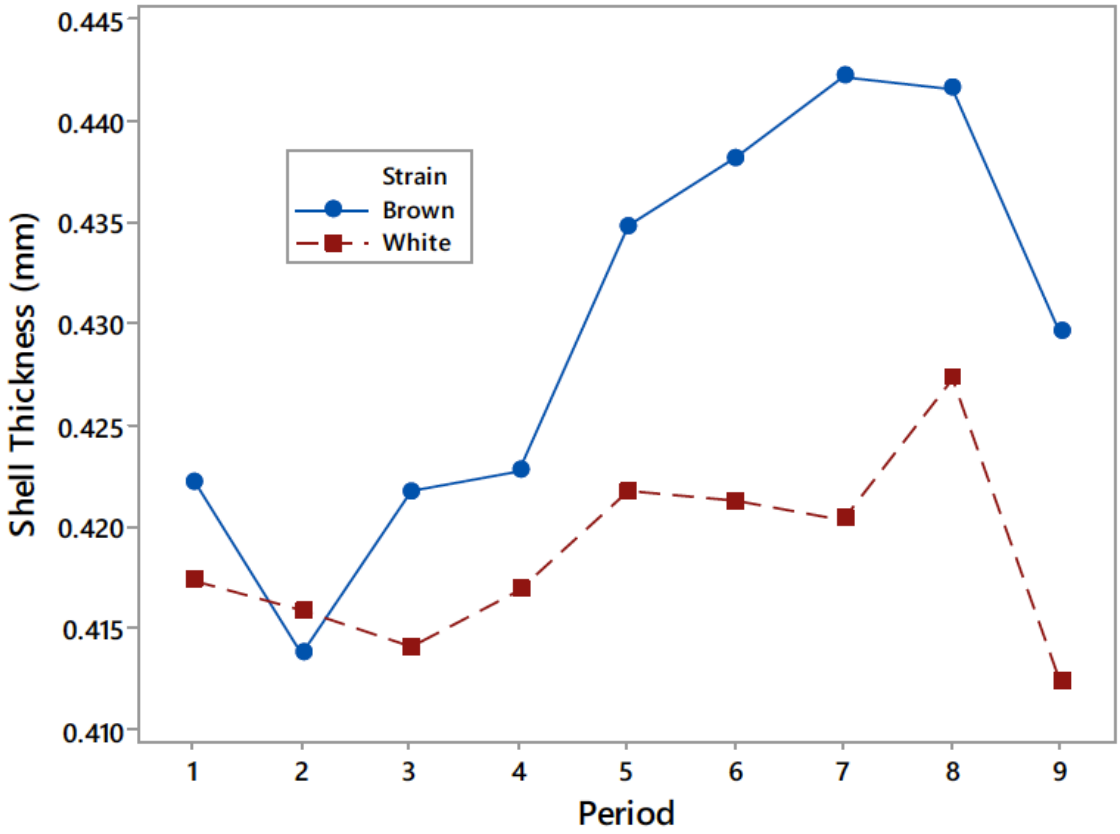

Figure 17. Interaction Plot Between Age and Strain for Shell Thickness (mm) of Hens fed Tasco® during the Long Term Feeding Trial

4.4.2.11 Breaking Strength

Both the main effects of strain and age significantly influenced breaking strength ($P<0.05$). These values are shown in Table 16. The LB birds had higher breaking strength compared to LL birds. This is due to the fact that LB hen had lower egg weights, higher shell thickness, and higher shell density, making their eggs stronger. Breaking strength showed a very clear, expected trend of declining breaking strength (Table 16). There was an increase in breaking strength from period 1 to 2, but then the breaking strength declined until period 5. The increase in breaking strength from period 4 to period 5 was again likely reflective of the switch off of the unpopular phase 2 diets, to the phase 3 diets. Due to the

declining quality of the shell, breaking strength has been proven to decline with age. As hens approach the end of their laying cycle (approximately 80 weeks in Canada), there is an unmistakable decrease in shell strength (Hamilton et al., 1979; Potts and Washburn, 1983). Some studies have reported changes in the organic matrix component of the eggshells from older hens as a cause (Fraser et al., 1998; Panheleux et al., 2000).

4.5 Conclusion

The interaction between age and strain was the clear influencing factor for most egg quality traits in this study. When considering age, egg weight increased with age, while shell quality declined. Shell thickness, shell density and breaking strength decreased as the trial moved forward. When considering strain, LB birds had smaller eggs, denser shells, thicker shells, greater breaking strength, higher feed conversion, larger body weights, lower hen day production, higher feed consumption, smaller yolks and lower shell weights compared to LL birds. Processing had no effect on egg quality or production parameters, indicating that extruding is likely unnecessary when feeding *Chondrus crispus*. Because extrusion is an added cost, producers and feed companies would benefit from minimal processing (simple grinding). Hens fed the 0.5% inclusion level had the significantly lowest shell thickness and although not significant, had the lightest shell weight and lowest breaking strength, indicating that red seaweed may pose shell quality issues at the 0.5% inclusion rate. Birds fed the 3% level had the largest egg weights, however, there were no other significant differences caused by the 3% level. Because the CC is a pricey product, feeding at such a high level may not be necessary. The 0.5% inclusion level for Tasco® also seemed to cause some issues, such as lower egg and shell weights. The recommendation levels for

Tasco® fed to laying hens is 0.25-0.5%, according to Acadian Seaplants. Birds fed the 0.5% level had the lowest egg weights and lowest shell weights. However, these differences were not drastic enough to cause concern. Tasco® had no highly influential negative effects on egg quality or production parameters. Therefore, either the low or high level of Tasco® could be utilized.

CHAPTER 5: EVALUATION OF *CHONDRUS CRISPUS* AND *ASCOPHYLLUM NODOSUM* AS A FEED ADDITIVE IN LAYING HENS CHALLENGED WITH HEAT

5.1 Abstract

Seaweed is considered a prebiotic with beneficial micronutrients that have shown to positively influence gut function and performance in laying hens. The purpose of this study was to monitor production performance of heat stressed laying hens fed *Chondrus crispus* (CC) and *Ascophyllum nodosum* (Tasco®). The experiment was a 2 x 3 x 2 x 2 factorial in a completely randomized design with processing method of the CC [Ground and Extruded], inclusion level [0, 0.5, and 3%], strain of hen [Lohmann Lite-LSL White (LL) and Lohmann Lite Brown (LB)] and heat challenge [Heat and No Heat] as the main effects. Birds at 70 weeks of age were kept in the heat stressed environment and challenged with rising heat levels from 11AM to 6PM, where temperatures rose from 24°C to 33°C for 4 weeks. At 6pm, the temperature dropped back to 23-24°C. For hens fed CC, there was a significant effect of strain ($P<0.05$) for body weight, feed consumption, shell thickness, and shell weight. LB birds were heavier (2056g) and ate more (116g/bird/day) compared to LL birds who weighed 1767g and ate 112.16g/bird/day. LL hens had thinner eggshells (0.411mm) and lighter shells (5.56g), while LB birds had thicker shells (0.427mm) and heavier shells (6.18g). For hens fed Tasco®, there was an effect of strain on egg production, feed conversion and body weight ($P<0.05$). LL hens had higher hen day production (96.16%) compared to LB birds (90.63%), lower feed conversion (1.82) compared to LB hens (2.00). LL hens had larger eggs and heavier yolks. There was no negative influence of heat on production parameters. The largest contributing factor was strain.

5.2 Introduction

The response of birds to increased temperatures is altered behaviour and physiological homeostasis in an attempt to thermoregulate and decrease internal body temperature. Under heat stressed conditions, birds may spend less time feeding and more time drinking, more time panting and raising their wings, less time moving around and more time resting (Mack et al., 2013). In response to high temperatures, birds display increased radiant, convective and evaporative heat loss through vasodilation and perspiration (Mustof et al., 2003). Increased panting leads to an increase in carbon dioxide levels and higher blood pH and increases organic acid availability, thereby reducing blood bicarbonate availability for eggshell mineralization. Thus, it is important to avoid heat stress in laying hen production as it can affect egg quality. Overall, heat stress affects egg production by altering the neuroendocrine profile through decreasing feed intake and activating the hypothalamic-pituitary-adrenal axis (Marder and Arad, 1989).

Altering feed ingredients have the potential to aid in the physiological response to heat stress. In specific, prebiotics have been shown to aid in alleviating some of the detrimental effects of heat stress on microstructure of the broiler gut (Ashraf et al., 2013). Prebiotics have been shown to selectively increase the growth of beneficial microbes and inhibit pathogen colonization (O'Sullivan et al., 2010). Among prebiotics, seaweeds have become of interest as feed additives in poultry (Richmond, 2004). Seaweeds have been previously determined to enhance the immune system, modulate growth and positively influence microbial populations in pigs and ruminants (Evans and Critchley., 2014), but their effects

in poultry have not been explored in depth, especially when considering laying hens and the potential impact on layer performance and egg quality.

When implementing new feed ingredients in laying hen diets, especially when under heat stresses conditions, it is important to identify whether the ingredient has an impact on layer performance traits such as body weight, egg production and feed consumption, It is also important to explore whether egg quality is affected, as profit margins can be drastically reduced with compromised eggs.

Feed processing has the potential to improve bird feed efficiency and reduces costs. Extrusion is a combination of heat, shear and compressional forces. Utilization of this method results in expansion of starches from increased gelatinization and cross-linking of proteins within the matrix. The result is a strongly bound, but porous pellet. The effect of processing method on seaweed feed efficiency is yet to be explored.

The aim of this study is to investigate the effect of dietary inclusion of the red seaweed, *Chondrus crispus* and the brown seaweed Ascophyllum nodosum, or Tasco® as it's trademarked name, with two processing methods (ground and extruded) on laying hen performance and egg quality under heat stressed conditions.

5.3 Materials and Methods

The heat stress trial took place from September 18th, 2018 to October 16th, 2018. Birds were housed at the Atlantic Poultry Research Institute of the Dalhousie Agricultural Campus.

5.3.1 Birds and Housing

Two strains of commercial laying hens were used in the heat stress study. The two strains of hens were Lohmann LSL Lite and Lohmann LSL Brown, hatched in New Brunswick, Canada. Birds used in this trial were previously a part of the main trial described above. Birds fed the 1.75% inclusion level in the main trial were dropped from continuation into the heat stress trial due to space restraints in the controlled environment suites. A total of 247 hens at 70 weeks of age were on trial for 4 weeks. All birds were randomly assigned to one of 128 wire cages in, of a one-sided, 2-tier 8-cage mobile battery conventional cage unit with 2 birds per cage. Of the four remaining birds per cage from the main trial, two birds were placed in a heat stress room, and the other two birds were placed in a standard temperature environment. Birds remained on the same diet as in the previous experiment. Water was available *ad libitum* throughout the trial. 16 hrs of light was provided per day. Half of the birds were challenged with rising heat levels, where temperatures gradually started to rise from 23-24°C, starting at 11am, to 33°C. The highest temperature was reached at approximately 6pm each day. Heat stress temperatures were determined based off previous research performed at the Atlantic Poultry Research Centre (Abeysinghe et al., 2019). The remaining half of the birds were kept at a temperature maintained at 23-

24°C. All experimental procedures were carried out in accordance with the Canadian Council of Animal Care guidelines (CCAC, 2009).

5.3.2 Seaweed Supplemented Treatments

Eight dietary treatments with *Chondrus Crispus* or Tasco® were prepared and used. Both seaweed species were obtained from Acadian Seaplants Limited in Dartmouth, NS, Canada. Two processing methods of red seaweed (*C. Crispus*) were utilized to determine any differences in feed efficiency: 1) Ground seaweed that was extruded and then reground (ECC), and 2) ground seaweed without extrusion (GCC). The treatments were as following: 0% GCC, 0.5% GCC, 3% GCC, 0% ECC, 0.5% ECC, 3% ECC, 0.25% Tasco®, and 0.5% Tasco®. Tasco® inclusion levels were determined based off of recommended feeding levels provided by Acadian Seaplants Diets were formulated based off commercial requirements for Lohmann LSL Lites provided by Lohmann Tierzucht GmbH. Four diets phases were prepared over the course of the trial, with reduced protein and increased calcium at each phase change. Formulations were created to meet the metabolizable energy, protein, calcium, available phosphorus, methionine and sodium requirements. Formulations were adjusted to account for the salt content with the seaweed.

5.3.3 Experimental Design

Each seaweed type was associated with an individual experimental design and statistical analyses were performed separately.

5.3.3.1 Chondrus crispus Experimental Design

For *Chondrus crispus*, the following factorial arrangement was utilized for all measurements without repeated measurements (feed consumption, feed conversion, egg production):

3 x 2 x 2 x 2

with the main effects of;

- Inclusion level (0, 0.5 and 3%)
- Strain (Lohmann LSL Lite and Lohmann Brown Lite)
- Processing method (ground and extruded)
- Environment (heat stress and standard temperature)

These measurements involved averages over the entire month of the trial as opposed to initial and final measurements.

The following factorial arrangement was utilized for all measurements with repeated measurements (Egg quality measurements, body weight):

3 x 2 x 2 x 2 x 2

with the main effects of;

- Inclusion level (0, 0.5 and 3%)
- Strain (Lohmann LSL Lite and Lohmann Brown Lite)
- Processing method (ground and extruded)
- Environment (heat stress and standard temperature)
- Age (initial and final)

These measurements involved an initial measurement at 70 weeks of age and a final measurement at 73 weeks of age

5.3.3.2 Tasco® Experimental Design

For Tasco®, the following factorial arrangement was utilized for all measurements without repeated measurements (feed consumption, feed conversion, egg production):

3 x 2 x 2

with the main effecs of;

- Inclusion level (0, 0.25 and 05%)
- Strain (Lohmann LSL Lite and Lohmann Brown Lite)
- Environment (heat stress and standard temperature)

These measurements involved averages over the entire month of the trial as opposed to initial and final measurements.

The following factorial arrangement was utilized for all measurements with repeated measurements (Egg quality measurements, body weight):

3 x 2 x 2 x 2

with the main effects of;

- Inclusion level (0, 0.25 and 05%)
- Strain (Lohmann LSL Lite and Lohmann Brown Lite)
- Environment (heat stress and standard temperature)
- Age (initial and final)

These measurements involved an initial measurement at 70 weeks of age and a final measurement at 73 weeks of age.

5.3.4 Preparation of Seaweed Supplemented Treatments

Two tonnes of cultivated *Chondrus crispus* was obtained from Acadian Seaplants Limited, Dartmouth, NS, Canada. The seaweed was grown on land artificially in salt water. In an environmentally controlled room at the Atlantic Poultry Research Centre, the CC was dried at room temperature for 48 hrs and manually turned every few hrs to allow for uniform drying. Following drying, the seaweed was ground to a powder (mesh size, 0.4 mm) using a micro Wiley mill, standard model 3 (Arthur H Thomas Co, Philadelphia, PA, USA). Half of the ground seaweed was further processed using extrusion, while the remaining half was set aside to be later mixed into the diet A Kahl OEE8 extruder with a barrel temperature set at 100ºC was used to extrude the remaining half of the seaweed (Amandus Kahl GmbH and Co. KG). After extrusion, the extruded feed was then dried for 4 hrs at 60ºC using a convection oven. Following drying of the extruded seaweed, the feed was ground to a powder (mesh size, 0.4 mm) using a micro Wiley mill, standard model 3 (Arthur H Thomas Co, Philadelphia, PA, USA). Feed was perpared in the mash form. Proximate analysis was performed on all diets to ensure that there were no large deviations from the calculated composition. Extrusion of CC resulted in a decrease in NDF percentage (Appendix Table A10).

Table 17. Diet Formulation (g/kg) and calculated composition (as fed basis) of the Heat Stress *Chondrus crispus* Layer Diets

Ingredient	Diet[1]				
	C	0.5% GCC	3% GCC	0.5% ECC	3% ECC
Ground Corn	554.50	548.88	520.82	549.05	519.73
Canola Meal	100.00	100.00	100.00	100.00	100.00
Wheat	100.00	100.00	100.00	100.00	100.00
Soybean Meal	111.97	110.94	105.81	110.85	104.85
Limestone	50.18	50.15	50.02	50.15	50.00
Shell Mix	25.09	25.08	25.01	25.08	25.00
Oyster Shell	25.09	25.08	25.00	25.07	25.00
Animal/Vegetable Fat	12.73	14.85	25.47	14.81	28.31
Dicalcium Phosphate	10.11	10.15	10.31	10.15	10.32
MCL9[2]	5.00	5.00	5.00	5.00	5.00
Salt	3.64	3.16	0.78	3.12	0
Methionine Premix[3]	1.69	1.70	1.77	1.70	1.80
Extruded *Chondrus crispus*	0	0	0	5.00	30.00
Ground *Chondrus crispus*	0	5.00	30.00	0	0
Total	1000	1000	1000	1000	1000
Calculated Composition (%)					
Metabolizable Energy (kCal/kg)	2800.00	2800.00	2800.00	2800.00	2800.00
Protein	14.73	14.73	14.73	14.73	14.73
Calcium	4.09	4.09	4.09	4.09	4.09
Available Phosphorus	0.37	0.37	0.37	0.37	0.37
Sodium	0.16	0.16	0.16	0.16	0.16

[1]Treatment group: C: control; 0.5% GCC: contains 0.5% of ground *Chondrus crispus*; 3% GCC: contains 3% of ground *Chondrus crispus; 0.5% ECC: contains 0.5% of extruded Chondrus crispus; 3% ECC: contains 3% of extruded Chondrus crispus.*

[2]Vitamin and Mineral mixture (g/kg of premix): vitamin A (retinol), 1.56 g; vitamin D3 (cholecalciferol), 480.00 g; vitamin E (dl-alpha tocopheryl acetate), 8.00 g; vitamin K (menadione sodium bisulphate), 1.80 g; thiamine, 0.40 g; riboflavin, 1.90 g; pantothenic acid (as DL-calcium pantothenate), 3.20 g; biotin, 32.00 g; folic acid, 4.40 g; vitamin B12, 2.30 g; niacin, 6.16 g; pyridoxine, 0.80 g; manganous oxide, 23.40 g; zinc oxide, 22.22 g; copper sulphate, 20.00 g; selenium premix, 14.86 g; ethoxyquin, 16.66 g; ground corn, 46.66 g; limestone, 100 g.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

Table 18. Diet Formulation (g/kg) and calculated composition (as fed basis) of the Heat Stress Tasco® Layer Diets

	Diet[1]	
Feed Ingredient	0.25% Tasco®	0.5% Tasco®
Ground Corn	550.51	546.52
Canola Meal	100.00	100.00
Wheat	100.00	100.00
Soybean Meal	112.32	112.66
Limestone	50.13	50.09
Shell Mix	25.07	25.05
Oyster Shell	25.07	25.04
Animal/Vegetable Fat	14.17	15.61
Dicalcium Phosphate	10.13	10.15
MCL9[2]	5.00	5.00
Salt	3.44	3.24
Methionine Premix[3]	1.66	1.64
Tasco	2.50	5.00
Total	1000	1000
Calculated Composition (%)		
Metabolizable Energy (kCal/kg)	2800.00	2800.00
Protein	14.73	14.73
Calcium	4.09	4.09
Available Phosphorus	0.37	0.37
Sodium	0.16	0.16

[1] Treatment group: 0.25% Tasco®: contains 0.25% of sundried, ground *Ascophyllum nodosum*; 3%; 0.5% Tasco®: contains 0.5% of sundried, ground *Ascophyllum nodosum*

[2] Vitamin and Mineral mixture (g/kg of premix): vitamin A (retinol), 1.56 g; vitamin D3 (cholecalciferol), 480.00 g; vitamin E (dl-alpha tocopheryl acetate), 8.00 g; vitamin K (menadione sodium bisulphate), 1.80 g; thiamine, 0.40 g; riboflavin, 1.90 g; pantothenic acid (as DL-calcium pantothenate), 3.20 g; biotin, 32.00 g; folic acid, 4.40 g; vitamin B12, 2.30 g; niacin, 6.16 g; pyridoxine, 0.80 g; manganous oxide, 23.40 g; zinc oxide, 22.22 g; copper sulphate, 20.00 g; selenium premix, 14.86 g; ethoxyquin, 16.66 g; ground corn, 46.66 g; limestone, 100 g.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

5.3.5 Production Performance

The following production parameters were monitored:

1. **Daily Feed Consumption**: The feed consumption per cage was weighed and recorded daily. Feeders were removed and weighed at the beginning and end of the study.
2. **Feed Conversion Ratio**: The grams of feed consumed per gram of egg produced will be calculated as an indicator of feed utilization. The following formula will be employed:

$$FCR = \frac{Feed\ Consumed\ (g)}{Avg\ Egg\ Weight * Total\ \#\ of\ Eggs\ (g)}$$

3. **Body Weight:** Average body weight per cage was measured once at the beginning of the study and once at the end.
4. **Daily Egg Production**: Number of eggs laid per cage was recorded daily. Any soft shelled, weak shelled, small, large, or cracked eggs were noted. To determine laying performance, the hen day production calculation was used. Hen day production is calculated as follows:

$$\%\ Hen\ Day = \frac{(Total\ Eggs\ Laid\ Per\ Cage/Number\ of\ days\ on\ Trial\ )}{\#\ Birds\ Per\ Cage} * 100$$

The hen day (%) was calculated on a per cage basis over the entire trial period. The total eggs laid was divided by the number of days on trial to give the number of eggs laid per day. The resulting value was then divided by the number of birds per cage

(generally 2). The resulting value was then multiplied by 100 to give a percentage hen day production.

5. **Mortality**: All mortalities that occurred over the span of the trial were recorded with the time of death, weight, and feed weigh back at time of death. Deceased birds were accounted for in the feed consumption and egg production data analysis.

5.3.6 Egg Quality

Two eggs per cage were collected at the start of the trial, and over 3 days prior to the end of the trial following. Eggs underwent the following egg quality measurements:

1. **Shell Density**: Eggs were floated in salt water ranging from specific gravitess of 1.074g/cm^3 to 1.106g/cm^3. Salt solutions were prepared at the APRC whereby 2126g, 2246g, 2364g, 2486g, 2606g, 2726g, 2846g, 2966g and 3086g were added to 20L of water to create specific gravities of 1.070g/cm^3, 1.074g/cm^3, 1.078g/cm^3, 1.082g/cm^3, 1.086g/cm^3, 1.090g/cm^3, 1.094g/cm^3, 1.098g/cm^3 and 1.102g/cm^3, respectively. The salt/water solutions were stirred vigorously directly following salt addition. To prevent any salt from settling, the solutions were stirred each morning following creation for one week.
2. **Shell Breaking Strength:** A TA.xt Plus Texture Analyzer from Texture Technologies Corp, New York, NY, USA with a 50kg load cell was used to determine total force required to crack the top of the eggshell.
3. **Egg Weight**: Eggs were weighed using an egg holder and scale.
4. **Albumen Height**: A QCH albumen height gauge from Technical Services and Supplies, York, UK was used to determine height of albumen in mm.

5. **Yolk Weight:** Yolks were separated from albumen manually (with hands) and weighed on a scale.
6. **Shell Weight:** Shells were washed, dried overnight and weighed with membrane still intact. Shell weights were determined using a scale.
7. **Shell Thickness**: The TA.xt Plus Texture Analyzer with a 5kg load cell was used to determine height of the eggshell in mm.

5.3.7 Statistical Analysis

A completely randomized design was utilized, with 8 dietary treatments for the CC trial, and 3 dietary treatments for the Tasco® trial. Each cage of 5 birds was considered an experimental unit, with 4 replicates per treatment combination. The results from both the CC and Tasco® treatements were analyzed using the Proc Mixed procedure of SAS (SAS, 2018). All effects (inclusion level, age, processing method, heat and bird strain) were considered fixed. The assumptions of normal distribution equal variance were tested. The Tukey-Kramer test was utilized to determine differences among means. To create main effects plots and interaction plots, Minitab was utilized (Minitab, 2018). The calculated probability value was 0.05, whereby all main effects and interactions that had a P-value less than or equal to 0.05 (to two decimal places) were considered statistically significant. Standard error of the mean was reported with the mean.

5.4 Results and Discussion

Tables 19 through 23 show the effects of Inclusion Level, Strain and Environment for all measured variables. Main effect means and associated standard error are shown in Tables 19 to 23. P-values for main effects, 2-way, 3-way and 4-way interactions are also included

in tables 19 to 23. Any interaction effects detected are discussed through text and figures following each table. Any of the measurements with repeated measures (all internal egg and shell quality measures, and body weight) were analyzed for an age effect. The main effect of age is not included in the main effects tables, but instead, discussed afterwards. All main and interaction effects were deemed significant if the p-value was less than or equal to 0.05 (to two decimal places).

5.4.1 *Chondrus crispus*

Table 19. Effect of Inclusion Level, Strain, Processing Method and Environment on Layer Performance Traits for Hens fed *Chondrus crispus* during the Heat Stress Trial

			FCR	Egg Production	Feed Intake	Body Weight
Main Effect Means	Inclusion Level[1]	0%	1.93 ± 0.03	93.14 ± 0.71	113.45 ± 1.5	1934 ± 26.32
		0.5%	1.95 ± 0.03	93.90 ± 0.80	115.86 ± 1.5	1921 ± 26.32
		3%	1.91 ± 0.03	93.34 ± 0.77	112.82 ± 1.5	1894 ± 26.32
	Strain[2]	LL	1.82 ± 0.03	95.29 ± 0.60	112.16 ± 1.2	1767^{b} ± 21.49
		LB	2.03 ± 0.03	91.63 ± 0.64	115.93 ± 1.2	2065^{a} ± 21.49
	Processing Method	Extruded	1.91 ± 0.03	93.92 ± 0.61	114.02 ± 1.2	1903 ± 21.49
		Ground	1.95 ± 0.03	92.99 ± 0.63	114.06 ± 1.2	1929 ± 21.49
	Environment	Heat	1.90 ± 0.03	94.79 ± 0.60	112.98 ± 1.2	1935 ± 21.49
		No Heat	1.96 ± 0.03	92.13 ± 0.64	115.11 ± 1.2	1898 ± 21.49
P-Value	Main Effects	Strain	<0.0001	<0.0001	0.0312	<0.0001
		Environment	0.0804	0.0034	0.2186	0.2303
		Level	0.6785	0.7671	0.3156	0.5446
P Value	2-Way Interaction	Processing	0.2620	0.2924	0.9817	0.3961
		Level*Strain	0.5179	0.5182	0.1128	0.1811
		Level*Environment	0.6498	0.4735	0.5299	0.9216
		Level*Processing	0.1961	0.7876	0.6865	0.6176
		Strain*Environment	0.0247	0.0159	0.1264	0.9952
		Strain*Processing	0.5273	0.0197	0.0748	0.8017
		Environment*Processing	0.5560	0.8990	0.7821	0.7360
	3- Way Interactions	Level*Environment*Strain	0.4850	0.7800	0.6828	0.7801
		Level*Environment*Processing	0.3631	0.0267	0.5741	0.3831
		Level*Processing*Strain	0.7559	0.2118	0.1394	0.1972
		Processing*Environment*Strain	0.8694	0.6022	0.7652	0.3448
	4-Way Interaction	Level*Environment*Strain*Processing	0.9756	0.0033	0.2097	0.8593

[1]Treatment group: C: control; 0.5% GCC: contains 0.5% of ground *Chondrus crispus*; 3% GCC: contains 3% of ground *Chondrus crispus; 0.5% ECC: contains 0.5% of extruded Chondrus crispus; 3% ECC: contains 3% of extruded Chondrus crispus.*
[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

Table 20. Effect of Inclusion Level, Strain, Processing Method and Environment on Layer Internal Egg Quality for Hens fed *Chondrus crispus* during the Heat Stress Trial

			Egg Weight	Yolk Weight	Egg Albumen Height
Main Effect Means	Inclusion Level[1]	0%	62.94 ± 0.29	16.92 ± 0.11	5.7 ± 0.07
		0.5%	63.40 ± 0.29	17.27 ± 0.11	5.7 ± 0.08
		3%	63.63 ± 0.29	17.27 ± 0.11	5.7 ± 0.07
	Strain[2]	LL	63.76 ± 0.24	17.89 ± 0.09	6.2 ± 0.06
		LB	62.89 ± 0.24	16.52 ± 0.09	5.2 ± 0.06
	Processing Method	Extruded	63.50 ± 0.24	17.08 ± 0.09	5.7 ± 0.06
		Ground	63.14 ± 0.24	17.22 ± 0.09	5.7 ± 0.06
	Environment	Heat	63.24 ± 0.24	17.20 ± 0.09	5.7 ± 0.06
		No Heat	63.41 ± 0.24	17.11 ± 0.09	5.8 ± 0.06
P-Value	Main Effects	Strain	0.0116	<0.0001	<0.0001
		Environment	0.6174	0.5088	0.2768
		Level	0.2469	0.0385	0.9807
		Processing	0.2834	0.2842	0.8616
	2-Way Interaction	Level*Strain	0.1918	0.0505	0.0334
		Level*Environment	0.7983	0.6916	0.4980
		Level*Processing	0.0162	0.7398	0.5982
		Strain*Environment	0.4436	0.7643	0.0332
		Strain*Processing	0.0149	0.1709	0.2397
		Environment*Processing	0.3265	0.8445	0.3261
	3- Way Interaction	Level*Environment*Strain	0.7418	0.6161	0.4142
		Level*Environment*Processing	0.8731	0.9511	0.4142
		Level*Processing*Strain	0.0024	0.1143	0.6208
		Processing*Environment*Strain	0.3641	0.8092	0.8942
	4-Way Interaction	Level*Environment*Strain*Processing	0.5276	0.7743	0.3936

[1]Treatment group: C: control; 0.5% GCC: contains 0.5% of ground *Chondrus crispus*; 3% GCC: contains 3% of ground *Chondrus crispus; 0.5% ECC: contains 0.5% of extruded Chondrus crispus; 3% ECC: contains 3% of extruded Chondrus crispus.*

[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

Table 21. Effect of Inclusion Level, Strain, Processing Method and Environment on Layer Eggshell Quality for Hens fed *Chondrus crispus* during the Heat Stress Trial

			Shell Density (g/cm^3)	Shell Thickness (mm)	Shell Weight (g)	Breaking Strength (g)
Main Effect Means	Inclusion Level[1]	0%	1.087 ± 0.0005	0.425^{a} ± 0.003	6.10 ± 0.04	5035 ± 73
		0.5%	1.085 ± 0.0005	0.411^{b} ± 0.003	6.00 ± 0.04	4911 ± 77
		3%	1.086 ± 0.0005	0.421^{ab} ± 0.003	6.11 ± 0.04	4959 ± 72
	Strain[2]	LL	1.083 ± 0.0004	0.411^{b} ± 0.002	5.96^{b} ± 0.03	4854 ± 61
		LB	1.088 ± 0.0004	0.427^{a} ± 0.002	6.18^{a} ± 0.03	5082 ± 60
	Processing Method	Extruded	1.086 ± 0.0004	0.420 ± 0.002	6.12^{a} ± 0.03	4919 ± 60
		Ground	1.086 ± 0.0004	0.417 ± 0.002	6.02^{b} ± 0.03	5017 ± 60
	Environment	Heat	1.086 ± 0.0004	0.418 ± 0.002	6.04 ± 0.03	5079 ± 61
		No Heat	1.086 ± 0.0004	0.420 ± 0.002	6.09 ± 0.03	4857 ± 59
P-Value	Main Effects	Strain	<0.0001	<0.0001	<0.0001	0.0092
		Environment	0.1105	0.6596	0.3080	0.0111
		Level	0.0040	0.0043	0.0974	0.4997
		Processing	0.2431	0.3651	0.0273	0.2543
	2-Way Interaction	Level*Strain	0.7691	0.6928	0.3385	0.0903
		Level*Environment	0.7848	0.8455	0.4598	0.0383
		Level*Processing	0.9588	0.6826	0.2173	0.0782
		Strain*Environment	0.5200	0.8449	0.9648	0.5485
		Strain*Processing	0.0688	0.1455	0.8771	0.0676
		Environment*Processing	0.4292	0.4630	0.2891	0.7037
	3- Way Interactions	Level*Environment*Strain	0.9802	0.5218	0.8002	0.8480
		Level*Environment*Processing	0.4754	0.9749	0.6479	0.9703
		Level*Processing*Strain	0.0349	0.0724	0.9995	0.0523
		Processing*Environment*Strain	0.5590	0.5052	0.8630	0.3637
	4-Way Interaction	Level*Environment*Strain*Processing	0.8347	0.9919	0.7102	0.8177

[1]Treatment group: C: control; 0.5% GCC: contains 0.5% of ground *Chondrus crispus*; 3% GCC: contains 3% of ground *Chondrus crispus; 0.5% ECC: contains 0.5% of extruded Chondrus crispus; 3% ECC: contains 3% of extruded Chondrus crispus.*

[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

There have been many studies investigating the effect of high environmental temperature on the performance of various poultry species, including turkeys (Kohne and Jones, 1976; McKee and Sams, 1997), young chickens (Henken et al., 1983), broilers (Cooper and Washburn, 1998), broiler breeders (McDaniel et al., 1995), and laying hens (Emery et al., 1984; Muiruri and Harrison, 1991; Whitehead et al., 1998). Results of this research have reported that high environmental temperatures cause a decline in productive performance.

In laying hens, heat stress decreases body weight (Scott and Balnave, 1988), egg production (Muiruri and Harrison, 1991; Whitehead et al., 1998), egg weight (Balnave and Muheereza, 1997), and shell quality (Emery et al., 1984; Mahmoud et al., 1996). Additionally, with heat stressed environments, feed intake is reduced, which is likely a contributing factor to the decline in production. Larbier et al. (1993) reported that chronic heat exposure significantly decreased protein digestion and Bonnet et al. (1997) found that the feed digestibility of proteins, fats, and starch decreased with exposure of broiler chickens to high temperatures.

Surprisingly, there was no effect of heat on any of the egg quality parameters, with the exception of a level by environment interaction for breaking strength, and a strain by environment interaction for both feed conversion ratio and albumen height. These figures are shown below.

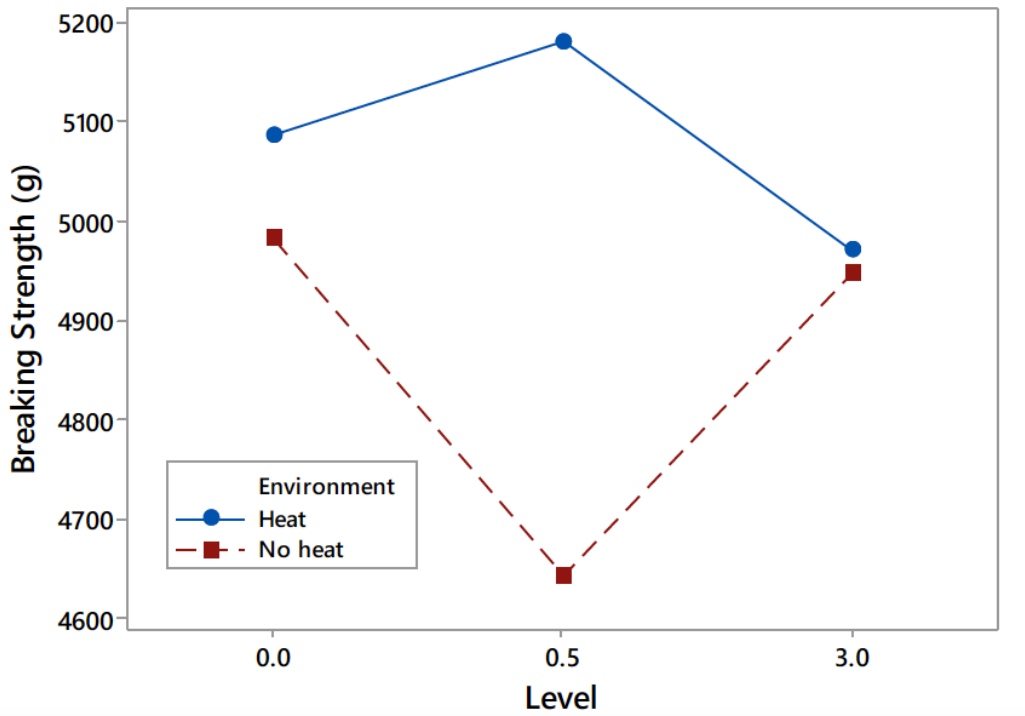

Figure 18. Interaction Plot Between Inclusion Level and Environment for Breaking Strength (g) of Hens fed *Chondrus crispus* during the Heat Stress Trial

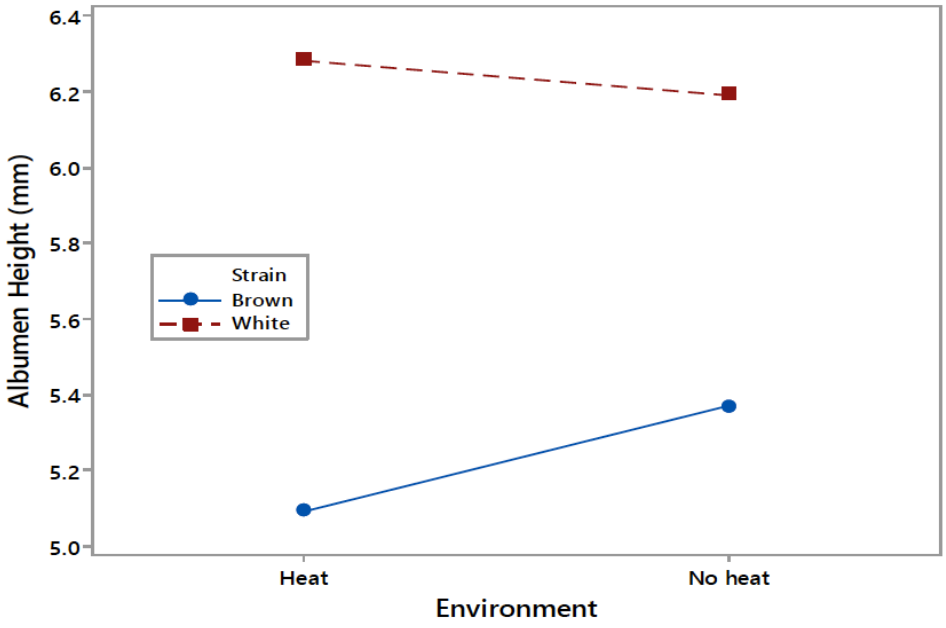

Figure 19. Interaction Plot Between Strain and Environment for Albumen Height (mm) of Hens fed *Chondrus crispus* during the Heat Stress Trial

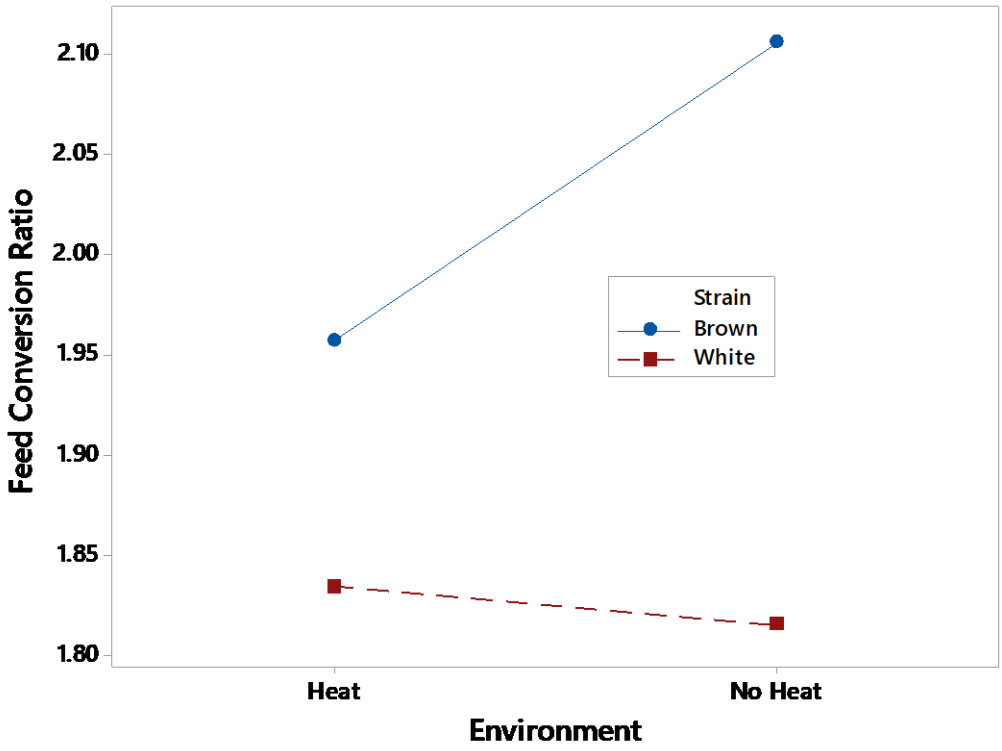

Figure 20. Interaction Plot Between Inclusion Level and Environment for Feed Conversion Ratio (g) of Hens fed *Chondrus crispus* during the Heat Stress Trial

Legend

Heat	Heat Stress Environment
Noheat	Standard Temperature Environment

In the heat environment, breaking strength was highest for birds fed the 0.5% inclusion level. The opposite was detected for birds in the standard temperature environment. Breaking strength was similar in both environments among birds fed the 3% level. For albumen height, LL birds had much higher albumen height than LB birds. Albumen height

was reduced for LB birds, as expected. However, albumen height was not reduced with heat for LL birds.

Emery et al. (1984) reported that heat stress did not affect egg production. In addition, Muiruri and Harrison (1991) found that heat stress did not significantly affect egg weight or feed conversion. In another study, nighttime heat exposure had no significantly affect on egg weight or albumen weights (Wolfenson et al., 1979). Koelkebeck et al. (1998) indicated that acute heat stress had no adverse effects on dietary amino acid digestibility in laying hens. Ultimately, there are varying results regarding heat stress and it's effect on production parameters in laying hens. These varying results could be due to the production facility, temperature, or strain of birds used.

In terms of the effect of heat on production performance (body weight, feed consumption, feed conversion and egg production), there was a strain by environment interaction ($P<0.05$) for feed conversion ratio. This interaction is shown in *Figure 20*. The LB birds had lower feed conversion in the heat stress environment, while LL birds were not affected by the heated environment.

A 4-way interaction ($P<0.05$) was detected for egg production (level by process by strain by environment), shown in *Figure 21*. This interaction was quite complex. Egg production was highest for both strains in the heat environment. The difference in egg production for LB birds between the two environments is higher than that of the LL birds. The LB birds were more negatively impacted by heat stress due to larger body size. Egg production was highest for LL birds fed the extruded diet and lowest for LL birds fed the ground diet. The

opposite was observed for the LB birds. Regardless of strain or environment, egg production was highest for birds fed the extruded diet. Egg production was similar for both the LL and LB hens fed the control. However, egg production was very low for the LB birds fed the 3% level. Egg production was similar for all levels in the standard temperature environment, but was highest at the 0.5% level for birds in the heat stress environment. Finally, egg production was similar within all level groups for extruded versus ground.

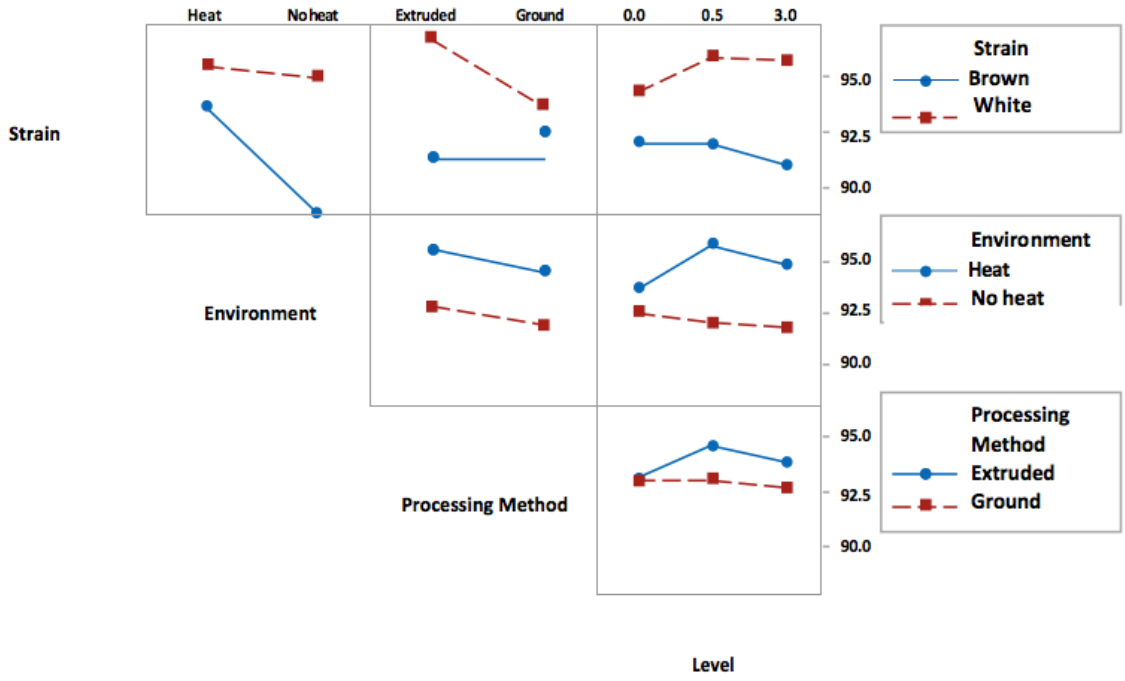

Figure 21. Interaction Plot Between Strain, Environment, Process and Inclusion Level for Egg Production (% Hen Day Production) of Hens fed *Chondrus crispus* during the Heat Stress Trial

For albumen height, in addition to the strain by environment interaction, there was a strain by level interaction; albumen height was highest among birds fed the 0.5% level, yet the opposite was observed for LB hens. This effect is shown in *Figure 22*.

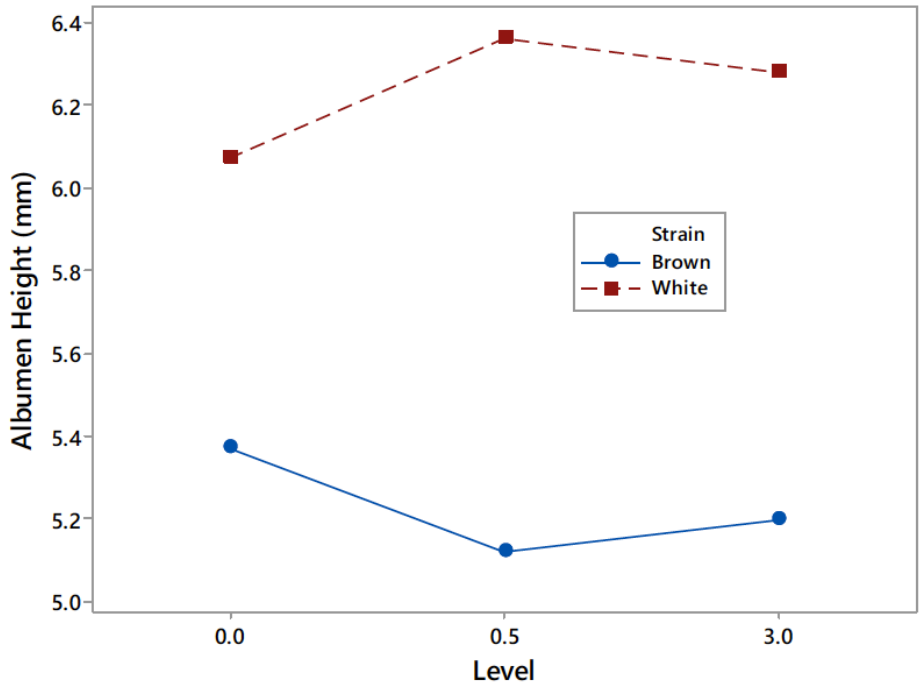

Figure 22. Interaction Plot Between Inclusion Level and Strain for Albumen Height (mm) of Hens fed *Chondrus crispus* during the Heat Stress Trial

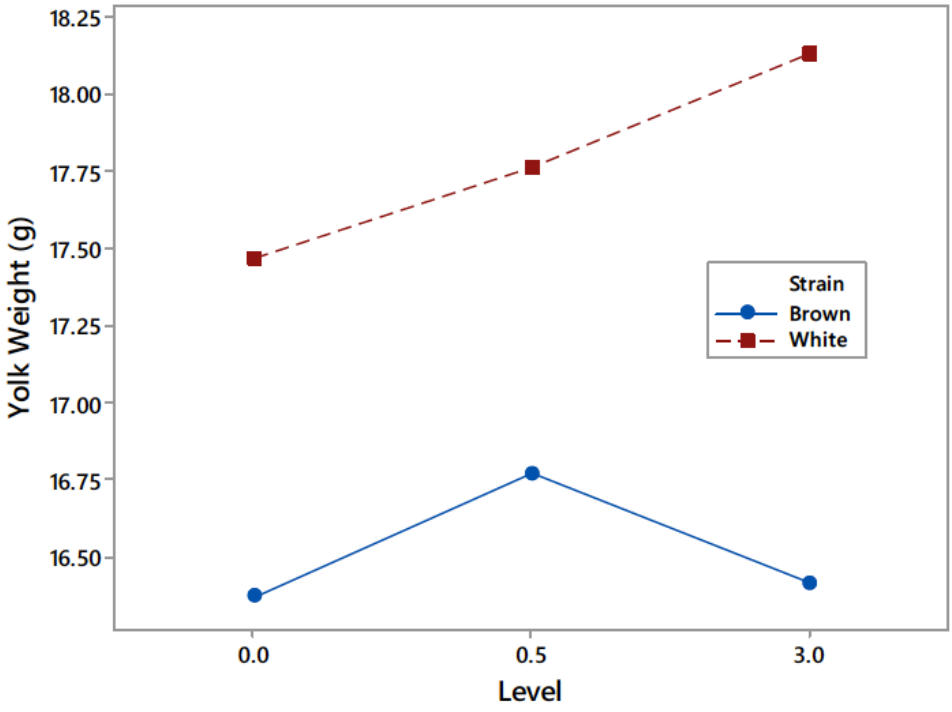

Figure 23. Interaction Plot Between Strain and Inclusion Level for Yolk Weight (g) of Hens fed *Chondrus crispus* during the Heat Stress Trial

Figure 23 shows the interaction of strain and level for yolk weight. For LL birds, yolk weight increased in correspondence to the increasing levels of seaweed, whereas for LB birds, the yolk weight was similar for the control and 3% level, but highest for birds fed the 0.5% level.

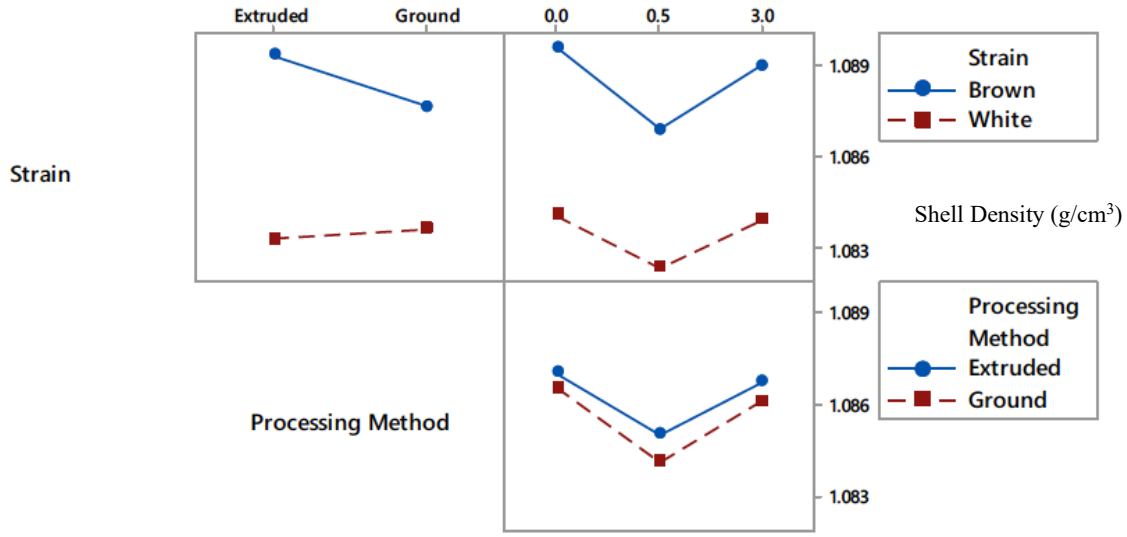

Figure 24. Interaction Plot Between Processing, Strain and Level for Shell Density (g/cm^3) of Hens fed *Chondrus crispus* during the Heat Stress Trial

A 3-way interaction was detected for shell density ($P<0.05$). LB birds had higher shell density compared to LL birds, regardless of processing method or level. LB birds had lower shell density when fed the ground CC versus the extruded CC, whereas LL had similar shell density for both processing methods. The shell density was the lowest for both strains when fed the 0.5% level, regardless of processing method. The 0.5% level has proven to cause the most shell quality issues in both the main trial and heat stress trial. The 3-way interaction is shown in *Figure 24.*

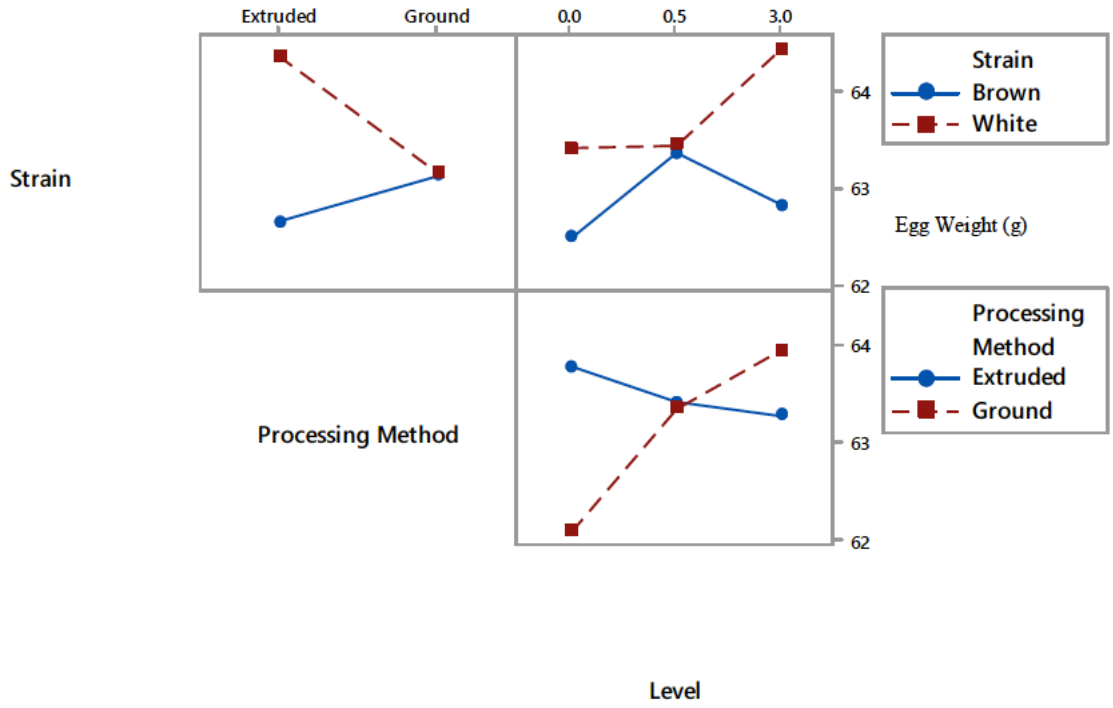

Figure 24. Interaction Plot Between Strain, Process and Level for Egg Weight (g) of Hens fed *Chondrus crispus* during the Heat Stress Trial

A 3-way interaction effect for strain by process by level was observed for egg weight ($P<0.05$). For LB birds, egg weights were higher for birds fed the ground CC compared to the extruded CC. The opposite was seen for the LL birds. Egg weights were highest among LL birds that were fed the 3% inclusion level, whereas for the LB birds, egg weights were highest among the 0.5% level. Egg weights were lowest for the control for both strains. There was a difference among hens fed the two control diets. This is likely the cause of the 3-way interaction. Similar to the previous trial, it is likely that there was a bird among the extruded control group was larger. Larger body size is associated with larger eggs (Bish et al., 1985), and thus, with such a small sample size (8 extruded control cages), one bird laying larger eggs could influence the entire extruded control group. The 3-way interaction is shown in *Figure 24*.

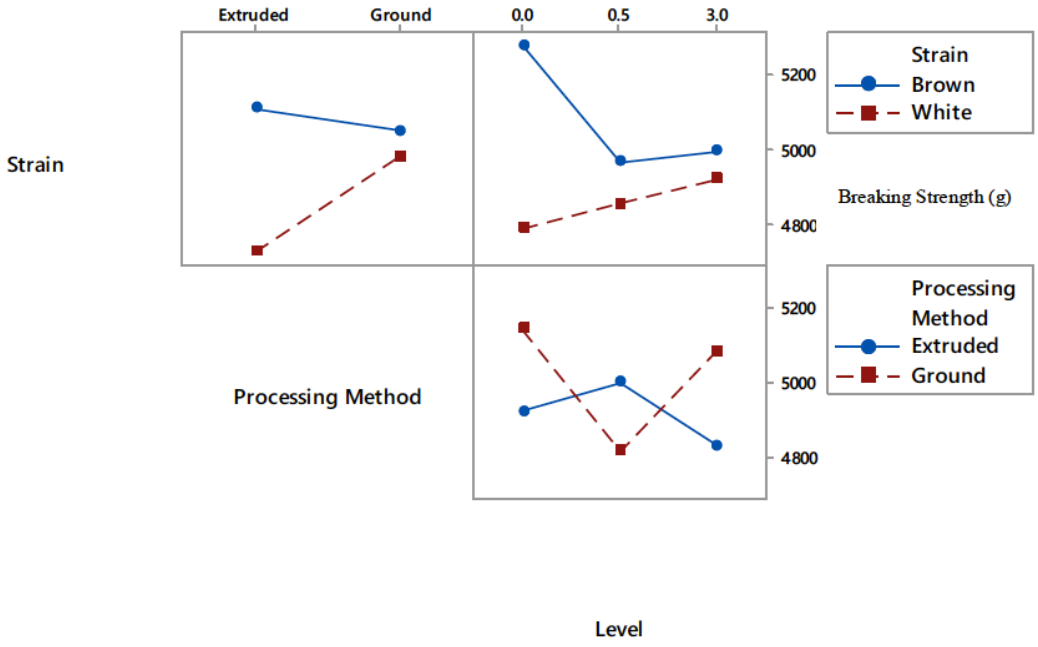

Figure 25. Interaction Plot Between Strain, Process and Inclusion Level for Breaking Strength (g) of Hens fed *Chondrus crispus* during the Heat Stress Trial

For breaking strength, a 3-way interaction effect was observed for strain by level by process (shown in *Figure 25*). A similar trend to the level by environment interaction was observed for the process by level interaction. Birds fed the extruded CC had the highest breaking strength at the 0.5% level, whereas birds fed the ground CC had the lowest breaking strength at the 0.5% level. In fact, for the processing methods, the trend is the opposite for each level. Where breaking strength is the highest among birds fed the extruded CC, breaking strength is the lowest among birds fed the ground CC. For strain by level, the LB hens fed the control had the highest breaking strength, whereas LL hens fed the control had the lowest breaking strength. The breaking strength for 0.5% and 3% were similar for both strains. LL birds had much lower breaking strength than LB hens when fed the extruded diet, but the breaking strength was similar for both strains when fed the ground diet.

When considering the main effects, there was a significant effect of strain ($P<0.05$) for body weight. LB birds were heavier (2056g) and ate more (116g/bird/day) compared to LL birds who weighed 1767g and ate 112.16g/bird/day. A main effect of strain was also detected for shell thickness and shell weight ($P<0.05$). LL hens had thinner eggshells (0.411mm) and lighter shells (5.56g), while LB birds had thicker shells (0.427mm) and heavier shells (6.18g). There was also a level effect for shell thickness ($P<0.05$) and a processing effect for shell weight ($P<0.05$). Hens fed the control diet had significantly thicker shells (0.425mm) compared to hens fed the 0.5% inclusion level (0.411mm), while birds fed the 3% level were intermediate in between (0.421mm). Hens fed the extruded CC had heavier shells (6.12g) compared to birds fed the ground CC (6.02g)

There was no effect of age for egg weight. There was an effect of age for yolk weight, whereby by the initial yolks were significantly smaller (17.4) compared to final yolks (16.9). There was an effect of age on albumen height, with initial albumen height being larger (5.9mm) compared to final albumen height (5.6mm). There was a significant effect of age on body weight, whereby initial body weights were higher (1959) compared to final body weights (1873).

There was as significant effect of age for shell density (g/cm^3), whereby initial shell density was higher ($1.087g/cm^3$) compared to final shell density ($1.085g/cm^3$). There was an effect of age on breaking strength, whereby initial breaking strength was 4836.29 and final breaking strength was 5100.49. There was no effect of age for shell thickness. There was no effect of age on shell weight.

5.4.2 Tasco®

Table 22. Effect of Inclusion Level, Strain, Processing Method and Environment on Layer Performance Traits for Hens fed Tasco® during the Heat Stress Trial

	Main and Interaction Effects		Feed Intake (g/bird/day)	Egg Production (%)	FCR	Body Weight (g)
Main Effect Means	Inclusion Level[1]	0%	113.46 ± 1.56	93.19 ± 1.36	1.92 ± 0.04	1934 ± 25
		0.25%	113.34 ± 1.69	95.74 ± 1.36	1.88 ± 0.04	1931 ± 36
		0.5%	114.46 ± 1.75	95.24 ± 1.42	1.93 ± 0.04	1952 ± 36
	Strain[2]	LL	112.77 ± 1.34	96.16[a] ± 1.15	1.82 ± 0.03	1801 ± 27
		LB	114.73 ± 1.38	90.63[b] ± 1.11	2.00 ± 0.03	2077 ± 27
	Environment	Heat	112.00 ± 1.38	93.63 ± 1.15	1.89 ± 0.03	1946 ± 27
		No Heat	115.50 ± 1.34	93.15 ± 1.11	1.93 ± 0.03	1932 ± 27
P-Value	Main Effects	Level	0.8801	0.2191	0.5674	0.8964
		Strain	0.3152	0.0014	0.0003	<0.0001
		Environment	0.0785	0.7697	0.2812	0.6962
	2-Way Interaction	Level x Strain	0.1364	0.3173	0.9400	0.2109
		Level x Environment	0.2752	0.8036	0.9303	0.9068
		Environment x Strain	0.3204	0.9141	0.8266	0.7523
	3- Way Interaction	Level x Environment x Strain	0.0573	0.2637	0.0901	0.3586

[1]Inclusion Level: 0.25%: contains 0.25% of sundried, ground *Ascophyllum nodosum*; 3%; 0.5%: contains 0.5% of sundried, ground *Ascophyllum nodosum*

[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

Table 23. Effect of Inclusion Level, Strain, Processing Method and Environment on Layer Egg Quality for Hens fed Tasco® during the Heat Stress Trial

			Shell Density (g/cm^3)	Egg Weight (g)	Yolk Weight (g)	Egg Albumen Height (mm)	Shell Thickness (mm)	Shell Weight (g)	Breaking Strength (g)
Main Effect Means	Inclusion Level[1]	0%	1.0862 ± 0.0005	62.9^a ± 0.31	16.91^a ± 0.11	5.7 ± 0.07	0.425 ± 0.004	6.10^a ± 0.04	5009 ± 122
		0.25%	1.0874 ± 0.0005	64.5^b ± 0.44	17.49^b ± 0.17	5.7 ± 0.09	0.425 ± 0.004	6.35^b ± 0.06	5189 ± 122
		0.5%	1.0877 ± 0.0005	62.2^a ± 0.44	17.30^b ± 0.16	5.7 ± 0.09	0.413 ± 0.004	6.00^a ± 0.06	5098 ± 122
	Strain[2]	LL	1.084 b ± 0.0005 b	63.8^a ± 0.33	17.88^a ± 0.12	6.1 a ± 0.07	0.408 b ± 0.003 a	6.03^b ± 0.05	5248^a ± 91
		LB	1.090 a ± 0.0005 a	62.7^b ± 0.33	16.63^b ± 0.12	5.3 b ± 0.07	0.433 a ± 0.003 b	6.27^a ± 0.05	4949^b ± 91
Main Effect Means	Environment	Heat	1.087 ± 0.0005	63.0 ± 0.33	17.24 ± 0.12	5.7 ± 0.07	0.421 ± 0.0003	6.11 ± 0.005	5184 ± 91
		No Heat	1.087 ± 0.0005	63.4 ± 0.33	17.27 ± 0.12	5.7 ± 0.07	0.421 ± 0.0003	6.187 ± 0.005	5013 ± 91
P-value	Main Effects	Level	0.2314	0.0009	0.0102	0.9558	0.0609	0.0005	0.4807
		Strain	<0.0001	0.0233	<0.0001	<0.0001	<0.0001	0.0007	0.0218
		Environment	0.2853	0.4308	0.3915	0.6732	0.9952	0.2650	0.1852
	2-Way Interaction	Level x Strain	0.3051	0.9325	0.7990	0.1229	0.7282	0.6514	0.3273
		Level x Environment	0.7893	0.3271	0.8112	0.5027	0.9078	0.4030	0.7193
		Environment x Strain	0.3839	0.8731	0.8531	0.2708	0.9132	0.3538	0.7521
P-Value	3- Way Interaction	Level x Environment x Strain	0.6432	0.2193	0.6104	0.1424	0.7928	0.5144	0.4072

[1]Inclusion Level: 0.25%: contains 0.25% of sundried, ground *Ascophyllum nodosum*; 3%; 0.5%: contains 0.5% of sundried, ground *Ascophyllum nodosum*

[2]Strain: LL: Lohmann LSL Lite; LB: Lohmann Brown Lite

There was a significant effect of strain on egg production, feed conversion and body weight ($P<0.05$). For egg production, LL hens had higher hen day production (96.16%) compared to LB birds (90.63%). For feed conversion, LL hens had lower feed conversion (1.82) compared to LB hens (2.00). For body weight, LB hens were significantly heavier (2077g) compared to LL hens (1801g).

For egg quality, strain had a significant influence on egg shell density, where LB hens had higher shell density compared to LL hens (Table 23). For egg weight, both a strain and level effect were observed ($P<0.05$) (Table 23). LL hens had significantly larger eggs compared to LB. Hens fed the 0.25% Tasco® had significantly larger eggs compared to the hens fed the control and 0.5% Tasco®. For shell weight, both a strain effect and a level effect were detected (Table 23). LB hens had significantly ($P<0.05$) heavier shell weights compared with LL hens. Hens fed the 0.25% level had significantly ($P<0.05$) heavier shells compared to hens fed the control and 0.5% level. For yolk weight, a strain effect and level effect were found (Table 23). LB hens had significantly ($P<0.05$) lighter yolks compared to LL. Hens fed the 0% control had significantly ($P<0.05$) lighter yolks compared to hens fed both the 0.25% and 0.5% levels. For breaking strain, LB birds had significantly ($P<0.05$) higher breaking strength compared with LL birds (Table 23).

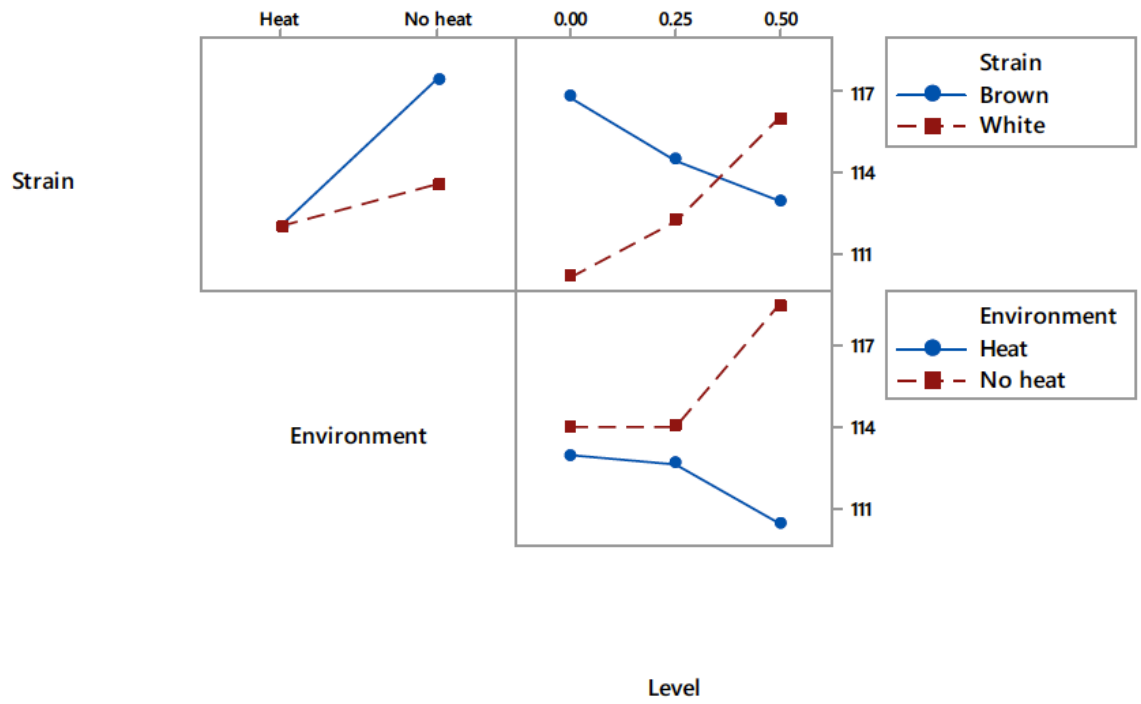

Figure 26. Interaction Plot Between Strain, Environment and Inclusion Level for Feed Consumption (g/bird/day) of Hens fed Tasco® during the Heat Stress Trial

A strain by environment by level interaction was found for feed consumption. The birds consumed significantly more feed in the standard temperature environment when fed the 0.5% level. Regardless of level, the birds in the heat stress environment consumed less feed. The birds fed the control and 0.25% level had similar feed consumption in both environments. The LB hens consumed more feed when fed the control, and the least amount of feed when fed the 0.5% inclusion level. The opposite trend was observed for LL birds. The browns consumed more feed in the standard temperature environment compared to the LL birds, but the feed consumption was the same for both strains in the heat stress environment.

An age effect was observed for yolk weight ($P<0.05$), with initial yolks weighing more (17.45) than final yolks (17.04). A similar trend was seen for shell density. Initial shell

density readings were significantly ($P<0.05$) higher (1.088g/cm^3) than final readings (1.086g/cm^3). It is expected that egg quality decline as a hen ages, especially shell density. For albumen height, an age effect was observed ($P<0.0001$) whereby final albumen height was lower (5.0) than initial albumen height (5.9). Decreasing albumen height is typical of aging hens. A strain effect was also observed where LB hens had significantly lower albumen height compared to LL hens (Table 23).

For shell thickness, an age by strain interaction effect was observed (P=0093), and hence the significant strain effect was ignored. LB hens at the beginning (70 weeks) of the trial had thinner shells (0.426mm) compared to at the end (74 weeks) of the trial (0.437). The opposite was seen for the LL birds, with a thickness of 0.417 at the start of the trial and 0.404 at the end. The final (end of trial) shell thickness measurements for LB were significantly different ($P<0.05$) than LL final shell thickness. The initial (beginning of trial) shell thickness measurements were significantly different between LB and LL. The expected trend would be for shell thickness to decrease over the entirety of the trial due to bird aging. However, only LL birds followed this trend, whereas LB birds performed the opposite.

5.5 Conclusion

There was no negative influence of heat on production parameters, as previous literature would predict. The most influential main effect for this trial was strain. For hens fed CC, there was a significant effect of strain ($P<0.05$) for body weight, feed consumption, shell thickness, and shell weight. LB birds were heavier and ate more compared to LL birds. LL hens had thinner eggshells and lighter eggshells compared to LB birds. For hens fed Tasco®, there was an effect of strain on egg production, feed conversion and body weight

($P<0.05$). LL hens had higher hen day production and lower feed conversion compared to LB hens. LL hens had larger eggs and heavier yolks. Because the heat stress had no negative impact on production parameters of egg quality, it is difficult to determine the effectiveness of *Chondrus crispus* or Tasco® in a heat stress environment. Results of this trial are very similar to the previous, long-term trial in that the largest contributing factor was strain. When considering the next direction of this research, a longer heat stress trial would be advantageous to access the effectiveness of both seaweeds long term.

CHAPTER 6: CONCLUSION

6.1 Summary and Conclusion

Age and strain were the clear influencing factor for most egg quality traits in this study. When considering age, egg weight increased with age, while shell quality declined. Shell thickness, shell density and breaking strength decreased as the trial moved forward. When considering strain, LB birds had smaller eggs, denser shells, thicker shells, greater breaking strength, higher feed conversion, larger body weights, lower hen day production, higher feed consumption, smaller yolks and lower shell weights compared to LL birds. Processing had no effect on egg quality or production parameters, indicating that extruding is likely unnecessary when feeding *Chondrus crispus*. Because extrusion is an added cost, producers and feed companies would benefit from minimal processing. Hens fed the 0.5% inclusion level had the lowest consistent shell density, indicating that red seaweed may pose shell quality issues at the 0.5% inclusion rate. Therefore, it is recommended that *Chondrus crispus* not be fed at the 0.5% level, but instead at the 1-2% range. Birds fed the 3% level had the largest egg weights, however, there were no other significant differences caused by the 3% level. Because CC is an expensive product in comparison to other widely available feed ingredients, feeding at such a high level may not be necessary. The 0.5% inclusion level for Tasco® also seemed to cause some issues, such as lower egg and shell weights. The recommendation levels for Tasco® fed to laying hens is 0.25-0.5%, according to Acadian Seaplants. Birds fed the 0.5% level had the lowest egg weights and lowest shell weights. However, these differences were not drastic enough to cause concern. Generally, Tasco® is included in the diet as a prebiotic. Tasco® had no highly influential negative

effects on egg quality or production parameters. Therefore, either the low or high level of Tasco® could be utilized.

6.2 Future Prospective of Research

With the directional movement toward cage free egg production, it is important for producers to be able to utilize natural feed ingredients in function of the role that antibiotics once played. With the limited research showing the capability of red seaweeds in poultry, this study serves to bridge that gap. Seaweed has shown to serve as an effective prebiotic by stimulating healthy gut bacteria, resulting in reduction of pathogenic bacteria. Aside from the 0.5% inclusion level, *Chrondus cripsus* had no severe negative impacts on production performance or egg quality. Therefore, if the prebiotic effectiveness were explored, CC may have the potential to be a very valuable and effective feed additive. The effectiveness of Tasco® as a prebiotic has been verified through many previous studies (Wiseman, 2012; Kandasamy et al., 2012; Evans et al., 2014). Very few of these studies looked at the effect of Tasco® in laying hens. Thus, this study will be of value for producers in the future when considering Tasco® for laying hens.

6.3 Next Steps

This research proves to show researchers and producers that *Chondrus crispus* and Tasco® can be fed to laying hens without negatively influencing production performance and egg quality. The next step in terms of further research would be to evaluate *Chondrus crispus* as a prebiotic through evaluation of the microbiome in the small intestine. This would allow for a more informed decision as to the effectiveness of CC as a dietary additive.

REFERENCES

Abeysinghe, N. (2019). *Adaptation to the pullet-rearing environment by providing lighting during embryo development*. Master of Science. Dalhousie University, Halifax, NS, Canada.

Apajalahti J. (2005). Comparative gut microflora, metabolic challenges, and potential opportunities. *Journal of Applied Poultry Research,* 14, 444-453.

Ashraf, S., Zaneb, H., Yousaf, M. S., Ijaz, A., Sohail, M. U., Muti, S., ... and Rehman, H. (2013). Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. *Journal of Animal Physiology and Animal Nutrition*, *97*, 68-73.

Bach, S. J., Wang, Y., and McAllister, T. A. (2008). Effect of feeding sun-dried seaweed (Ascophyllum nodosum) on fecal shedding of Escherichia coli O157:H7 by feedlot cattle and on growth performance of lambs. *Animal Feed Science and Technology,*142, 17–32.

Balnave, D., and Muheereza, S. K. (1997). Improving eggshell quality at high temperatures with dietary sodium bicarbonate. *Poultry Science*, *76*(4), 588-593.

Banga-Mboko, H., Mabas, J. S., and Adzona, P. P. (2010). Effect of Housing System (BatteryCages Versus Floor Pen) on Performance of Laying Hens under Tropical Conditions in Congo Brazzaville. *Research Journal of Poultry Sciences*, 3(1), 1-4.

Barrow P. A, Tucker J. F, and Simpson J. M. (1987). Inhibition of colonization of the chicken alimentary-tract with *Salmonella*-Typhimurium Gram-negative facultatively anaerobic- Bacteria. *Epidemiology Infect*ion 98, 311-322.

Baffoni L, Gaggia F, Di Gioia D, Santini C, Mogna L, and Biavati B. (2012). A Bifidobacterium-based synbiotic product to reduce the transmission of C. jejuni along the poultry food chain. *International Journal of Food Microbiology,* 157, 156-161.

Baker, R. C., and Vadehra, D. V. (1970). The influence of quantity of thick albumen on internal egg quality measurements. *Poultry Science*, *49*(2), 493-496.

Baurhoo B., Phillip L., and Ruiz-Feria C. (2007). Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. *Poultry Science,* 86, 1070-1078.

Behnke, K.C. (1996). Feed manufacturing technology: current issues and challenges. *Animal Feed Science and Technology,* 62, 49–57.

Benyi, K., Norris, D., and Tsatsinyane, P. M. (2006). Effects of stocking density and group size on the performance of white and brown Hyline layers in semi-arid conditions. *Tropical Animal Health and Production,* 38(7-8), 619-624.

Berg, L. R., Bearse, G. E., and Hamilton, C. M. (1947). The effect of Newcastle disease on egg production and egg quality. *Poultry Science*, *26*(6), 614-622.

Bish, C. L., Beane, W. L., Ruszler, P. L., and Cherry, J. A. (1985). Body weight influence on egg production. *Poultry Science*, 64(12), 2259-2262.

Boissy, A., Manteuffel, G.; Jensen, M.B., Moe, R.O., Spruijt, B., Keeling, L.J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I., and Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology and Behav*ior, 92, 375–397.

Bonnet, S., Geraert, P. A., Lessire, M., Carre, B., and Guillaumin, S. (1997). Effect of high ambient temperature on feed digestibility in broilers. *Poultry Science*, *76*(6), 857-863.

Bouhlal R, Haslin C, Chermann J, Colliec-Jouault S, Sinquin C, Simon G, Cerantola S, Riadi H, and Bourgougnon N. (2011). Antiviral activities of sulfated polysaccharides isolated from *Sphaerococcus coronopifolius* (Rhodophytha, gigartinales) and *Boergeseniella thuyoides* (Rhodophyta, ceramiales). *Marine Drugs,* 9, 1187-1209.

Canadian Council of Animal Care (CCAC). (2009). Guidelines on: The Care and Use of Farm Animals in Research, Teaching and Testing. Canadian Council on Animal Care, Ottawa, ON, Canada

Carrillo S, Lopez E, Casas M. M, Avila E, Castillo R. M, Carranco M. E, and Calvo C. (2008). Potential use of seaweeds in the laying hen ration to improve the quality of n-3 fatty acid enriched eggs. *Journal of Applied Phycology,* 20, 271-278.

Castanon J. I. R. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science,* 86, 2466-2471.

Chousalkar, K., Gole, V., Caraguel, C., and Rault, J. L. (2016). Chasing Salmonella Typhimurium in free range egg production system. *Veterinary Microbiology*, *192*, 67-72.

Church, D. (1991). *Livestock feeds and feeding* (3rd ed.). Englewood Cliffs, N.J.: Prentice Hall.

Clerici, F., Casiraghi, E., Hidalgo, A., and Rossi, M. (2006). Evaluation of eggshell quality characteristics in relation to the housing system of laying hens. Paper presented at the EPC 2006-12th European Poultry Conference, Verona, Italy, 10-14 September, 2006.

Commission Internationale de l'Eclaraige, *Colorimetry* (1986), 2nd edition, Publication No. 15.2. CIE, Vienna.

Cooper, M. A., and Washburn, K. W. (1998). The relationships of body temperature to weight gain, feed consumption, and feed utilization in broilers under heat stress. *Poultry Science*, *77*(2), 237-242.

Cordts, C., Schmutz, M., and Preisinger, R. (2002). New alternatives for improving eggshell stability through breeding. Lohmann Information, 26, 13-16.

Cummings J. H., and Macfarlane G. T. (2002). Gastrointestinal effects of prebiotics. *British Journal of Nutrition,* 87, S145-S151.

Dacke, C. G., Sugiyama, T., and Gay, C. V. (2015). The role of hormones in the regulation of bone turnover and eggshell calcification. In C. G. Scanes (Ed.), Sturkie's Avian Physiology (Sixth ed.): Academic Press, Elsevier.

Danicke S, Vahjen W, Simon O, and Jeroch H. (1999). Effects of dietary fat type and xylanase supplementation to rye-based broiler diets on selected bacterial groups adhering to the intestinal epithelium, on transit time of feed, and on nutrient digestibility. *Poultry Science,* 78, 1292-1299.

David, B., Mejdell, C., Michel, V., Lund, V., and Moe, R. O. (2015). Air quality in alternative housing systems may have an impact on laying hen welfare. Part II—Ammonia. *Animals*, *5*, 886-896.

De Beer, M., and Coon, C. N. (2007). The effect of different feed restriction programs on reproductive performance, efficiency, frame size, and uniformity in broiler breeder hens. *Poultry Science*, 86(9), 1927-1939.

De Reu, K., Messens, W., Heyndrickx, M., Rodenburg, T. B., Uyttendaele, M., and Herman, L. (2008). Bacterial contamination of table eggs and the influence of housing systems. *World's Poultry Science Journal*, 64(01), 5-19.

Dibner J. J., and Buttin P. (2002). Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *Journal of Applied Poultry Research,* 11, 453-463.

"Disease Challenges of Cage-free Egg Production." *Egg Industry* (2018): Egg Industry, Mar 2018. Web.

Donalson L. M, Kim W. K, Chalova V. I, Herrera P, McReynolds J. L, Gotcheva V. G, Vidanovic D, Woodward C. L, Kubena L. F, Nisbet D. J, and Ricke S. C. (2008). *In vitro* fermentation response of laying hen cecal bacteria to combinations of fructooligosaccharide prebiotics with alfalfa or a layer ration. *Poultry Science,* 87, 1263-1275.

Đukić-Stojčić, M., Perić, L., Bjedov, S., and Milošević, N. (2009). The quality of table eggs produced in different housing systems. *Biotechnology in Animal Husbandry*, 25(5-6- 2), 1103-1108.

Dvořák, P., Suchý, P., Straková, E., and Kopřiva, V. (2012). Possibilities of enhancing the colour of egg yolk. *Journal of the Science of Food and Agriculture, 92*(4), 853-856.

Ebeid, T.A., Suzuki, T., and Sugiyama, T. (2012). High temperature influences eggshell quality and calbindin-D28k localization of eggshell gland and all intestinal segments of laying hens. *Poultry Science,* 91, 2282–2287.

Egg Farmers of Canada Annual Report (2018). *Egg Farmers of Canada*. 21 Florence Street, Ottawa, Ontario, K2P 0W6

El-Deek A.A., and Brikaa M. A. (2009). Nutritional and biological evaluation of marine seaweed as a feedstuff and as a pellet binder in poultry diet. *International Journal of Poultry Science,* 9, 875–881. 169

Elnagar, S.A., Scheideler, S.E., and Beck, M.M. (2010). Reproductive hormones, hepatic deiodinase messenger ribonucleic acid, and vasoactive intestinal polypeptide-immunoreactive cells in hypothalamus in the heat stress-induced or chemically induced hypothyroid laying hen. *Poultry Science,* 89, 2001–2009.

Emery, D. A., Vohra, P., Ernst, R. A., and Morrison, S. R. (1984). The effect of cyclic and constant ambient temperatures on feed consumption, egg production, egg weight, and shell thickness of hens. *Poultry Science*, *63*(10), 2027-2035.

Evans F. D. and Critchley A. T. (2014). Seaweeds for animal production use. *Journal of Applied Phycology,* 26, 891-899.

Faber T. A, Dilger R. N, Iakiviak M, Hopkins A. C, Price N.P, and Fahey Jr G.C. (2012). Ingestion of a novel galactoglucomannan oligosaccharide-arabinoxylan (GGMO-AX) complex affected growth performance and fermentative and immunological characteristics of broiler chicks challenged with *Salmonella* Typhimurium. *Poultry Science,* 91, 2241- 2254.

Fedde, M.R. (1998). Relationship of structure and function of the avian respiratory system to disease susceptibility. *Poultry Science,* 77, 1130–1138.

Felver-Gant, J.N.; Mack, L.A.; Dennis, R.L.; Eicher, S.D.; and Cheng, H.W. (2012). Genetic variations alter physiological responses following heat stress in 2 strains of laying hens. *Poultry Science,* 91, 1542–1551

Fernendez, M. S., Escobar, C., Lavelin, I., Pines, M., and Arias, J. L. (2003). Localization of osteopontin in oviduct tissue and eggshell during different stages of the avian egg laying cycle. *Journal of Structural Biology*, *143*(3), 171-180.

Firkins J L, Eastridge M L, St-Pierre N R, and Noftsger S M. (2001). Effects of grain variability and processing on starch utilization by lactating dairy cattle. *Journal of Animal Science,* 79, E218-E238.

Fike, J. H., Allen, V. G., Schmidt, R. E., Zhang, X., Fontenot, J. P., Bagley, C. P., Ivy, R. L., Evans, R. R., Coelho, R. W., and Wester, D. B. (2001). Tasco-Forage: I. Influence of a seaweed extract on antioxidant activity in tall fescue and in ruminants. *Journal of Animal Science,* 79, 1011–1021.

Fletcher, D. L., Britton, W. M., Pesti, G. M., Rahn, A. P., and Savage, S. I. (1983). The relationship of layer flock age and egg weight on egg component yields and solids content. *Poultry Science*, *62*(9), 1800-1805.

Franco-Jimenez, D. J., Scheideler, S. E., Kittok, R. J., Brown-Brandl, T. M., Robeson, L. R., Taira, H., and Beck, M. M. (2007). Differential effects of heat stress in three strains of laying hens. *Journal of Applied Poultry Research*, *16*(4), 628-634.

Fraser, A. C., Bain, M. M., and Solomon, S. E. (1998). Organic matrix morphology and distribution in the palisade layer of eggshells sampled at selected periods during lay. *British Poultry Science*, *39*(2), 225-228.

Garson M. J. (1989). Biosynthetic-studies on marine natural-products. *Natural Product Report,* 6, 143-170.

Gautron, J., and Nys, Y. (2006). Eggshell matrix proteins and natural defenses of the egg. Paper presented at the Symposium COA/INRA Scientific Cooperation in Agriculture, November 7-10, Tainan (Taiwan, R.O.C.).

Ghasemian, M., and Jahanian, R. (2016). Dietary mannan-oligosaccharides supplementation could affect performance, immunocompetence, serum lipid metabolites, intestinal bacterial populations, and ileal nutrient digestibility in aged laying hens. *Animal Feed Science and Technology*, 81-89.

Gibson, G.R., and Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of Nutrition*, 125, 1401–1412.

Glencross, B. (2015). Understanding the nutritional and biological constraints of ingredients to optimize their application in aquaculture feeds. *Aquafeed Formulation*, 33-73.

Gong J. H, Forster R. J, Yu H, Chambers J. R, Wheatcroft R, Sabour P. M, and Chen S. (2002) Molecular analysis of bacterial populations in the ileum of broiler chickens and comparison with bacteria in the cecum. *FEMS Microbiology Ecology,* 41, 171-179.

Gudiel-Urbano M., and Goñi I. (2002). Effect of edible seaweeds (Undaria pinnatifida and Porphyra ternera) on the metabolic activities of intestinal microflora in rats. *Nutrition Research,* 22, 323-331.

Guiry M.D., and Guiry G.M. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org. Accessed March, 2018

Hafting J. T, Critchley A. T, Cornish M. L, Hubley S. A, and Archibald A. F. (2012). On-land cultivation of functional seaweed products for human usage. *Journal of Applied Phycology,* 24, 385-392.

Hamilton, R. M. G. (1982). Methods and factors that affect the measurement of egg shell quality. *Poultry Science*, *61*(10), 2022-2039.

Hansen, J., Ruedy, R., Sato, M., and Lo, K. (2010). Global surface temperature change. *Reviews of Geophysics*, *48*(4).

Harms, R. H., Costa, P. T., and Miles, R. D. (1982). Daily feed intake and performance of laying hens grouped according to their body weight. *Poultry Science*, 61(6), 1021-1024.

Hegelund, L., Sørensen, J. T., and Hermansen, J. E. (2006). Welfare and productivity of laying hens in commercial organic egg production systems in Denmark. *NJAS-Wageningen Journal of Life Sciences*, 54(2), 147-155.

Hemsworth, P.H. (2003). Human-animal interactions in livestock production. *Applied Animal Behavioral Sciences,* 81, 185–198.

Henken, A. M., Groote Schaarsberg, A. M. J., Nieuwland, M. G. B. (1983). The effect of environmental temperature on immune response and metabolism of the young chicken. 3. Effect of environmental temperature on the humoral immune response following injection of sheep red blood cells. *Poultry Science*, *62*(1), 51-58.

Hinton M., and Mead G. C. (1991). *Salmonella* control in poultry: the need for the satisfactory evaluation of probiotics for this purpose. *Letters in Applied Microbiology,* 13, 49-50.

Hoebler, C., Guillon, F., Fardet, A., Cherbut, C. and Barry, J. L. (1998). Gastrointestinal or simulated in vitro digestion changes dietary fibre properties and their fermentation. *Journal of the Science and Food and Agriculture*, 77, 327–333.

Holdt S., and Kraan S. (2011). Bioactive compounds in seaweed: Functional food applications and legislation. *Journal of Applied Phycology* 23, 543-597.

Informetrica Limited, The Economic Impact of the Poultry and Egg Industry. December 2011

Jacob, J. P., Miles, R. D., and Mather, F. B. (2000). Egg quality. Florida: Institute of Food and Agricultural Science-University of Florida.

Joerger R. D. (2003). Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. *Poultry Science,* 82, 640-647.

Johnson, A. L. (2000). Reproduction in female. In G. C. Whittow (Ed.), Sturkie's Avian physiology (fifth ed., pp. 569-596). London, UK: Academic Press.

Jones, F. T., Anderson, K. E., and Ferket, P. R. (1995). Effect of extrusion on feed characteristics and broiler chicken performance. *Journal of Applied Poultry Research*, *4*(3), 300-309.

Joyner, C. J., Peddie, M. J., and Taylor, T. G. (1987). The effect of age on egg production in the domestic hen. *General and Comparative Endocrinology*, *65*(3), 331-336.

Kaminska, B. Z., and Skraba, B. (1991). Analysis of hen types considering albumen: yolk ratio and its changes during the laying cycle. In *Proceedings of the 4th European Symposium on the Quality of Poultry Products,* (pp. 43-49).

Kandasamy, S., Khan, W., Evans, F., Critchley, A. T., and Prithiviraj, B. (2012). Tasco®: a product of Ascophyllum nodosum enhances immune response of Caenorhabditis elegans against Pseudomonas aeruginosa infection. *Marine Drugs*, *10*(1), 84-105.

Karunajeewa, H., Hughes, R. J., McDonald, M. W., and Shenstone, F. S. (1984). A review of factors influencing pigmentation of egg yolks. *World's Poultry Science Journal*, *40*(1), 52-65.

Kemps, B. J., Govaerts, T., De Ketelaere, B., Mertens, K., Bamelis, F. R., Bain, M. M., Decuypere, E. M., and De Baerdemaeker, J. G. (2006). The influence of line and laying period on the relationship between different eggshell and membrane strength parameters. *Poultry Science*, *85*(7), 1309-1317.

Kiarie, E. G., and Mills, A. (2019). Role of feed processing on gut health and function in pigs and poultry: Conundrum of optimal particle size and hydrothermal regimens. *Frontiers in Veterinary Science, 6*, 19.

Kiarie, E., Romero, L., and Nyachoti, C. (2013). The role of added feed enzymes in promoting gut health in swine and poultry. *Nutrition Research Reviews, 26*(1), 71-88.

Kidd, M. T., Corzo, A., Hill, S. M., Zumwalt, C. D., Robinson, E. H., and Dozier III, W. A. (2005). Growth and meat yield responses of broilers provided feed subjected to extrusion cooking. *Journal of Applied Poultry Research*, *14*(3), 536-541.

Kirjoranta, S., Solala, K., Suuronen, J. P., Penttilä, P., Peura, M., Serimaa, R., Tenkanen, M., Jouppila, K. (2012). Effects of process variables and addition of polydextrose and whey protein isolate on the properties of barley extrudates. *International Journal of Food Science and Technology*, *47*(6), 1165-1175.

Koelkebeck, K. W., Parsons, C. M., and Wang, X. (1998). Effect of acute heat stress on amino acid digestibility in laying hens. *Poultry Science*, *77*(9), 1393-1396.

Kohne, H. J., and Jones, J. E. (1976). The relationship of circulating levels of estrogens, corticosterone and calcium to production performance of adult turkey hens under conditions of increasing ambient temperature. *Poultry Science*, *55*(1), 277-285.

Kulshreshtha, G. (2017). *The use of selected red microalgae (seaweeds) for the reduction of salmonella enteritis in poultry*. Doctor of Philosophy, Dalhousie University, Halifax, NS, Canada.

Lacin, E., Yildiz, A., Esenbuga, N., and Macit, M. (2008). Effects of differences in the initial body weight of groups on laying performance and egg quality parameters of Lohmann laying hens. *Czech Journal of Animal Science*, 53, 466-471.

Lahaye, M., Michel, C., and Barry, J. L. (1993). Chemical, physiochemical, and in- vitro fermentation characteristics of dietary fibres from Palmaria palmata (L.) Kuntze. *Food Chemistry* 4, 39–36.

Lan Y, Verstegen M, Tamminga S, and Williams B. (2005). The role of the commensal gut microbial community in broiler chickens. *Worlds Poultry Science Journal,* 61, 95-104.

Larbier, M., Chagneau, A. M., and Geraert, P. A. (1993). Influence of ambient temperature on true digestibility of protein and amino acids of rapeseed and soybean meals in broilers. *Poultry Science*, *72*(2), 289-295.

Leek, A. B. G. (2015). Feeding for egg quality. Paper presented at the 26th Australian Poultry Science Symposium, Sydney, New South Wales, Australia, 9th -11th February 2015.

Li X., Liu L., Li K., Hao K., and Xu C. (2007). Effect of fructooligosaccharides and antibiotics on laying performance of chickens and cholesterol content of egg yolk. *British Poultry Science,* 48, 185-189.

Lin, H., Mertens, K., Kemps, B., Govaerts, T., De Ketelaere, B., De Baerdemaeker, J., Decuypere, E., and Buyse, J. (2004). New approach of testing the effect of heat stress on eggshell quality: mechanical and material properties of eggshell and membrane. *British Poultry Science*, *45*(4), 476-482.

Lohmann T. G. (2019). *Management Guide LSL Lite*, Cuxhaven Germany.

Lynch, M. B., Sweeney, T., Callan, J. J., O'Sullivan, J. T., and O'Doherty, J. V. (2010). The effect of dietary laminaria derived laminarin and fucoidan on nutrient digestibility, nitrogen utilisation, intestinal microflora and volatile fatty acid concentration in pigs. *Journal of the Science of Food and Agriculture,* 90, 430–437.

MacArtain P, Gill C. I. R, Brooks M, Campbell R, and Rowland I. R. (2007). Nutritional value of edible seaweeds. *Nutrition Reviews,* 65, 535-543.

Mack, L.A., Felver-Gant, J.N., Dennis, R.L., and Cheng, H.W. (2013) Genetic variation alter production and behavioral responses following heat stress in 2 strains of laying hens. *Poultry Science,* 92, 285–294.

Madsen, T. G., and Pedersen, J. R. (2010). Factors affecting broiler uniformity (Vol. DLMetionin, April): Evonik Degussa GmbH.

Mahmoud, K. Z., Beck, M. M., Scheideler, S. E., Forman, M. F., Anderson, K. P., and Kachman, S. D. (1996). Acute high environmental temperature and calcium-estrogen relationships in the hen. *Poultry Science*, *75*(12), 1555-1562.

Marder, J. and Arad, Z. (1989). Panting and acid-base regulation in heat stressed birds. *Comparative Biochemistry and Physiology* 94, 395–400.

Marion, W. W., Nordskog A. W., Tolman H. S., and Forsythe R. H. (1964). Egg composition as influenced by breeding, egg size, age and season. *Poultry Science,* 43, 255– 264.

Mashaly, M. M., Hendricks 3rd, G. L., Kalama, M. A., Gehad, A. E., Abbas, A. O., and Patterson, P. H. (2004). Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poultry Science*, *83*(6), 889-894.

McDaniel, C. D., Bramwell, R. K., Wilson, J. L., and Howarth J. R. (1995). Fertility of male and female broiler breeders following exposure to elevated ambient temperatures. *Poultry Science*, *74*(6), 1029-1038.

Mikšík, I., Sedláková, P., Lacinová, K., Pataridis, S., and Eckhardt, A. (2010). Determination of insoluble avian eggshell matrix proteins. *Analytical and Bioanalytical Chemistry,* 397(1), 205-214.

Miscurcova, L., Stanislav, K., Borivoj, K., and Vacek, J. (2010). Nitrogen content, dietary fiber, and digestibility in algal food products. *Czech Journal of Food Science,* 28, 27–35.

Muiruri, H. K., and Harrison, P. C. (1991). Effect of roost temperature on performance of chickens in hot ambient environments. *Poultry Science*, *70*(11), 2253-2258.

Mustaf, S., Kahraman, N.S., and Firat, M.Z. (2009). Intermittent partial surface wetting and its effect on body-surface temperatures and egg production of white brown domestic laying hens in Antalya (Turkey). *Poultry Science,* 50, 33–38.

North, M.O., and D.D.Bell, (1990) .Commercial Chicken Production Manual. 4th ed. Chapman & Hall, New York, NY

Nys, Y. (1986). Relationships between age, shell quality and individual rate and duration of shell formation in domestic hens. *British Poultry Science*, *27*(2), 253-259.

Nys, Y., Gautron, J., Garcia-Ruiz, J. M., and Hincke, M. T. (2004). Avian eggshell mineralization: biochemical and functional characterization of matrix proteins. *Comptes Rendus Palevol,* 3(6), 549-562.

O'Sullivan, L., Murphy, B., McLoughlin, P., Duggan, P., Lawlor, P. G., Hughes, H., and Gardiner, G. E. (2010). Prebiotics from marine macroalgae for human and animal health applications. *Marine Drugs,* 8, 2038-2064.

Owens F. N., Secrist D. S., Hill W. J., and Gill D. R. (1997). The effect of grain source and grain processing on performance of feedlot cattle: a review. *Journal of Animal Science,* 75, 868- 879.

Panheleux, M., Nys, Y., Williams, J., Gautron, J., Boldicke, T., and Hincke, M. T. (2000). Extraction and quantification by ELISA of eggshell organic matrix proteins (ovocleidin-17, ovalbumin, ovotransferrin) in shell from young and old hens. *Poultry Science*, *79*(4), 580-588

Peisker, M. (1994). Influence of expansion on feed components. *Feed Mix*, *2*(3), 26.

Plavnik, I., and Sklan, D. (1995). Nutritional effects of expansion and short time extrusion on feeds for broilers. *Animal Feed Science and Technology*, *55*(3-4), 247-251.

Ponsano, E. H. G., Pinto, M. F., Neto, M. G., and Lacava, P. M. (2004). Rhodocyclus gelatinosus biomass for egg yolk pigmentation. *Journal of Applied Poultry Research*, *13*(3), 421-425.

Potts Sr, P. L., and Washburn, K. W. (1983). The relationship of age, method of measuring, and strain on variation in shell strength. *Poultry Science*, *62*(2), 239-246.

Rayan, G. N., Galal, A., Fathi, M. M., and El-Attar, A. H. (2010). Impact of layer breeder flock age and strain on mechanical and ultrastructural properties of eggshell in chicken. *International Journal of Poultry Science*, 9(2), 139-147.

Richards, S. A. (1970). Physiology of thermal panting in birds. In *Annales de Biologie Animale Biochimie Biophysique,* (Vol. 10, No. Hors-série 2, pp. 151-168). EDP Sciences.

Richmond A. (2004). Handbook of Microalgal Culture: biotechnology and applied phycology. Oxford, OX, UK; Ames, Iowa, USA: Blackwell Science.

Roberts, J. R. (2004). Factors affecting egg internal quality and eggshell quality in laying hens. *The Journal of Poultry Science* 41(3), 161-177.

Roberts, J. R., and Ball, W. (2003). Egg and eggshell quality guidelines for the Australian egg industry. Paper presented at the *Proceedings of the Australian Poultry Science Symposium.*

Roberts, J. R., and Brackpool, C. (1995). Eggshell ultrastructure and the assessment of eggshell quality. Armidale: University of New England.

Roberts, J. R., Choulsalkar, K., and Samiullah. (2013). Egg quality and age of laying hens: implications for product safety. *Animal Production Science,* 53, 1291-1297.

Rodriguez-Navarro, A., Kalin, O., Nys, Y., and Garcia-Ruiz, J. M. (2002). Influence of the microstructure on the shell strength of eggs laid by hens of different ages. *British Poultry Science,* 43(3), 395-403.

Romanoff, A. L., and Romanoff, A. J. (1949). The avian egg. *The avian egg.*

Roland Sr, D. A. (1979). Factors influencing shell quality of aging hens. *Poultry Science*, *58*(4), 774-777.

Sako T., Matsumoto K, and Tanaka R. (1999). Recent progress on research and applications of non-digestible galacto-oligosaccharides. *International Dairy Journal,* 9, 69-80.

Sams, A. R. (1997). The effect of seasonal heat stress on rigor development and the incidence of pale, exudative turkey meat. *Poultry Science*, *76*(11), 1616-1620.

Sarica, M., Boga, S., and Yamak, U. S. (2008). The effects of space allowance on egg yield, egg quality and plumage condition of laying hens in battery cages. *Czech Journal of Animal Science*, *53*(8), 346-353.

Scanes, C. G., Brant, G., and Ensminger, M. E. (2004). Poultry Science 4th ed. Upper Sadle River, New Jersey, 07458: Pearson Education, Inc.

Scott, T. A., and Balnave., D. (1988). Comparison between concentrated complete diets and self-selection for feeding sexually maturing pullets at hot and cold temperatures. *British Poultry Science*, 29:613–625.

Scott, T., and Silversides, F. (2000). The effect of storage and strain of hen on egg quality. *Poultry Science, 79*(12), 1725-1729.

Sekeroglu, A., Sarica, M., Demir, E., Ulutas, Z., Tilki, M., Saatci, M., and Omed, H. (2010). Effects of different housing systems on some performance traits and egg qualities of laying hens. *Journal of Animal and Veterinary Advances,* 9(12), 1739-1744.

Silversides, F. G., Korver, D. R., and Budgell, K. L. (2006). Effect of strain of layer and age at photostimulation on egg production, egg quality, and bone strength. *Poultry Science,* 85(7), 1136-1144.

Silversides, F. G., and Scott, A. T. (2001). Effect of storage and layer age on quality of eggs from two lines of hens. *Poultry Science*, *80*(8), 1240-1245.

Singh, R., Cheng, K. M., and Silversides, F. G. (2009). Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. *Poultry Science,* 88(2), 256-264.

Sirri, F., Iaffaldano, N., Minelli, G., Meluzzi, A., Rosato, M. P., and Franchini, A. (2007). Comparative pigmentation efficiency of high dietary levels of apo-ester and marigold extract on quality traits of whole liquid egg of two strains of laying hens. *Journal of Applied Poultry Research*, *16*(3), 429-437.

Soleimani, A.F., Zulkifli, I., Omar, A.R., and Raha, A.R. (2011). Physiological responses of 3 chicken breeds to acute heat stress. *Poultry Science,* 90, 1435–1440.

Sokołowicz, Z., Krawczyk, J., and Dykiel, M. (2018). The Effect of the Type of Alternative Housing System, Genotype and Age of Laying Hens on Egg Quality. *Annals of Animal Science, 18*(2), 541-556.

Stadelman, W. J., Newkirk, D., and Newby, L. (1995). Egg science and technology (4th ed.): The Haworth Press, Inc. USA.

Świątkiewicz, S., Koreleski, J., and Arczewska, A. (2010). Laying performance and eggshell quality in laying hens fed diets supplemented with prebiotics and organic acids. *Czech Journal of Animal Science*, *55*(7), 294-306.

Tharrington, J., Curtis, P., Jones, F., and Anderson, K. (1999). Comparison of physical quality and composition of eggs from historic strains of single comb White Leghorn chickens. *Poultry Science, 78*(4), 591-594.

Toussant, M. J., and Latshaw, J. D. (1999). Ovomucin content and composition in chicken eggs with different interior quality. *Journal of the Science of Food and Agriculture*, *79*(12), 1666-1670.

Tůmová, E., and Ledvinka, Z. (2009). The effect of time of oviposition and age on egg weight, egg components weight and eggshell quality. *Archiv für Geflügelkunde*, 73(2), 110-115.

Tůmová, E., Skřivan, M., Englmaierová, M., and Zita, L. (2009). The effect of genotype, housing system and egg collection time on egg quality in egg type hens. *Czech Journal of Animal Science,* 54(2009), 17-23.

Tyler, C. (1961). Shell strength: its measurement and its relationship to other factors. *British Poultry Science*, *2*(1-3), 3-19.

US Poultry and Egg Association. Frequently Asked Questions, Statistics, Facts and Information." *Industry Economic Data, Consumption, Exports, Processing, Production: Uspoultry.org*, www.uspoultry.org/faq/faq.cfm. Accessed April, 2018.

Usayran, N., Farran, M. T., Awadallah, H. H., Al-Hawi, I. R., Asmar, R. J., and Ashkarian, V. M. (2001). Effects of added dietary fat and phosphorus on the performance and egg quality of laying hens subjected to a constant high environmental temperature. *Poultry Science*, *80*(12), 1695-1701.

Van den Bogaard, A., Willems, R., London, N., Top, J., and Stobberingh, E. (2002). Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy, 49*(3), 497-505.

Venkitanarayanan K, Kollanoor-Johny A, Darre M. J, Donoghue A. M, and Donoghue D. J. (2013). Use of plant-derived antimicrobials for improving the safety of poultry products. *Poultry Science,* 92, 493-501.

Ventura M. R, Castañon J. I. R, and McNab J. M. (1994). Nutritional value of seaweed (*Ulva rigida*) for poultry. *Animal Feed Science and Technology,* 49, 87-92.

Wang, Onnagawa, Yoshie, Y. and Suzuki, T. (2001). Binding of bile salts to soluble and insoluble dietary fibers of seaweeds. *Fisheries Science,* 67(6), 1169–1173.

Washburn, K. W., (1979). Genetic variation in the chemical composition of the egg. *Poultry Science* 58, 529–535

Whitehead, C. C., Bollengier-Lee, S., Mitchell, M. A., and Williams, P. E. V. (1998). Alleviation of depression in egg production in heat stressed laying hens by vitamin E. Pages 576– 578 in *Proceedings of 10th European Poultry Conference, Jerusalem, Israel*

Wiseman, M. (2012). Evaluation of Tasco® as a candidate prebiotic in broiler chickens. Master of Science. Dalhousie University, Halifax, NS, Canada

Wolfenson, D., Frei, Y. F., Snapir, N., and Berman, A. (1979). Effect of diurnal or nocturnal heat stress on egg formation. *British Poultry Science*, *20*(2), 167-174.

Yan G., Guo Y., Yuan J., Liu D., and Zhang, B. (2011). Sodium alginate oligosaccharides from brown algae inhibit Salmonella Enteritidis colonization in broiler chickens. *Poultry Science,* 90, 1441-1448.

Zita, L., Tůmová, E., and Štolc, L. (2009). Effects of genotype, age and their interaction on egg quality in brown-egg laying hens. *Acta Veterinaria Brno*, *78*(1), 85-91.

APPENDIX

Table A1 Recommendations for Nutrient Levels for LOHMANN LSL-LITE Pullets

(Source: Lohmann LSL-Lite Management Guide, 2019)

Diet type*	**Starter****		**Grower**	**Developer**	**Pre-Layer**
Nutrient	**1.–3. Week**		**4.–8. Week**	**9.–16. Week**	**17.Week–5% Production**
Metabol. Energy	kcal / kg	2900	2800	2800	2800
	kcal / lbs	1315	1275	1275	1275
Minimum	MJ / kg	12.00	11.70	11.70	11.70
Crude Protein	%	20.00	18.50	15.00	17.00
Methionine	%	0.48	0.40	0.34	0.36
Dig. Methionine	%	0.39	0.33	0.28	0.29
Methionine/Cystine	%	0.83	0.70	0.60	0.68
Digestible M./C.	%	0.68	0.57	0.50	0.56
Lysine	%	1.20	1.00	0.70	0.85
Digestible Lysine	%	0.98	0.82	0.57	0.70
Valine	%	0.89	0.75	0.53	0.64
Dig. Valine	%	0.76	0.64	0.46	0.55
Tryptophan	%	0.23	0.21	0.16	0.20
Dig. Tryptophan	%	0.19	0.17	0.13	0.16
Threonine	%	0.80	0.70	0.50	0.60
Dig. Threonine	%	0.65	0.57	0.40	0.49
Isoleucine	%	0.83	0.75	0.60	0.74
Dig. Isoleucine	%	0.68	0.62	0.50	0.61
Calcium	%	1.05	1.00	0.90	2.50
Phosphorus total	%	0.75	0.70	0.58	0.65
Phosphorus available	%	0.48	0.45	0.37	0.45
Sodium	%	0.18	0.17	0.16	0.16
Chlorine	%	0.20	0.19	0.16	0.16
Linoleic Acid	%	2.00	1.40	1.00	1.00

Table A2 Recommended Nutrient Levels for LOHMANN LSL-LITE Layers for Different Daily Feed Consumptions: Pre-Peak (~ 18 weeks to 50 % Production) (Source: Lohmann LSL-Lite Management Guide, 2019)

Nutrient		**Daily Feed Consumption /Hen**			
		90 g (19.8 lbs./100 birds)	**95 g** (20.9 lbs./100 birds)	**100 g*** (22.0 lbs./100 birds)	**105 g** (23.2 lbs./100 birds)
Protein	%	20.00	18.95	18.00	17.14
Calcium**	%	4.22	4.00	3.80	3.62
Phosphorus***	%	0.71	0.68	0.64	0.61
Av. Phosphorus	%	0.50	0.47	0.45	0.43
Sodium	%	0.20	0.19	0.18	0.17
Chlorine	%	0.20	0.19	0.18	0.17
Lysine	%	0.93	0.89	0.84	0.80
Dig. Lysine	%	0.77	0.73	0.69	0.66
Methionine	%	0.46	0.44	0.41	0.39
Dig. Methionine	%	0.38	0.36	0.34	0.32
Meth./Cyst.	%	0.84	0.80	0.76	0.72
Dig. M/C	%	0.69	0.65	0.62	0.59
Arginine	%	0.96	0.91	0.87	0.82
Dig. Arginine	%	0.79	0.75	0.71	0.68
Valine	%	0.78	0.74	0.71	0.67
Dig. Valine	%	0.67	0.63	0.60	0.57
Tryptophan	%	0.20	0.19	0.18	0.17
Dig. Tryptophan	%	0.17	0.16	0.15	0.14
Threonine	%	0.65	0.62	0.59	0.56
Dig. Threonine	%	0.53	0.51	0.48	0.46
Isoleucine	%	0.75	0.71	0.67	0.64
Dig. Isoleucine	%	0.61	0.58	0.55	0.52
Linoleic Acid	%	2.44	2.32	2.20	2.10

Table A3. Recommended Nutrient Levels for LOHMANN LSL-LITE Layers in Phase 1 for Different Daily Feed Consumptions (50 % Production to 40 weeks ~ up to 59.4 g Egg Mass/Hen/Day) (Source: Lohmann LSL-Lite Management Guide, 2019)

Nutrient		**Daily Feed Consumption /Hen**			
		95 g (20.9 lbs./100 birds)	**100 g*** (22.0 lbs./100 birds)	**105 g** (23.2 lbs./100 birds)	**110 g** (24.3 lbs./100 birds)
Protein	%	18.57	17.64	16.80	16.04
Calcium**	%	4.32	4.10	3.90	3.73
Phosphorus***	%	0.66	0.63	0.60	0.57
Av. Phosphorus	%	0.46	0.44	0.42	0.40
Sodium	%	0.19	0.18	0.17	0.16
Chlorine	%	0.19	0.18	0.17	0.16
Lysine	%	0.87	0.82	0.79	0.75
Dig. Lysine	%	0.71	0.68	0.64	0.61
Methionine	%	0.43	0.41	0.39	0.37
Dig. Methionine	%	0.35	0.33	0.32	0.30
Meth./Cyst.	%	0.78	0.74	0.71	0.67
Dig. M/C	%	0.64	0.61	0.58	0.55
Arginine	%	0.89	0.85	0.81	0.77
Dig. Arginine	%	0.73	0.70	0.66	0.63
Valine	%	0.73	0.69	0.66	0.63
Dig. Valine	%	0.62	0.59	0.56	0.53
Tryptophan	%	0.19	0.18	0.17	0.16
Dig. Tryptophan	%	0.15	0.15	0.14	0.13
Threonine	%	0.60	0.57	0.55	0.52
Dig. Threonine	%	0.50	0.47	0.45	0.43
Isoleucine	%	0.69	0.66	0.63	0.60
Dig. Isoleucine	%	0.57	0.54	0.51	0.49
Linoleic Acid	%	2.32	2.20	2.10	2.00

Table A4. Recommended Nutrient Levels for LOHMANN LSL-LITE Layers in Phase 2 for Different Daily Feed Consumptions (41 to 50 weeks ~ up to 59.5 g Egg Mass/Hen/Day) (Source: Lohmann LSL-Lite Management Guide, 2019)

Nutrient		**Daily Feed Consumption /Hen**			
		95 g (20.9 lbs./100 birds)	**100 g*** (22.0 lbs./100 birds)	**105 g** (23.2 lbs./100 birds)	**110 g** (24.3 lbs./100 birds)
Protein	%	18.19	17.28	16.46	15.71
Calcium**	%	4.42	4.20	4.00	3.82
Phosphorus***	%	0.65	0.62	0.59	0.56
Av. Phosphorus	%	0.45	0.43	0.41	0.39
Sodium	%	0.18	0.17	0.16	0.16
Chlorine	%	0.18	0.17	0.16	0.16
Lysine	%	0.85	0.81	0.77	0.73
Dig. Lysine	%	0.70	0.66	0.63	0.60
Methionine	%	0.42	0.40	0.38	0.36
Dig. Methionine	%	0.34	0.33	0.31	0.30
Meth./Cyst.	%	0.76	0.73	0.69	0.66
Dig. M/C	%	0.63	0.60	0.57	0.54
Arginine	%	0.87	0.83	0.79	0.76
Dig. Arginine	%	0.72	0.68	0.65	0.62
Valine	%	0.71	0.68	0.65	0.62
Dig. Valine	%	0.61	0.58	0.55	0.52
Tryptophan	%	0.18	0.18	0.17	0.16
Dig. Tryptophan	%	0.15	0.14	0.14	0.13
Threonine	%	0.59	0.56	0.54	0.51
Dig. Threonine	%	0.49	0.46	0.44	0.42
Isoleucine	%	0.68	0.64	0.61	0.59
Dig. Isoleucine	%	0.56	0.53	0.50	0.48
Linoleic Acid	%	1.68	1.60	1.52	1.45

Table A5. Recommended Nutrient Levels for LOHMANN LSL-LITE Layers in Phase 3 for Different Daily Feed Consumptions (51 to 65 weeks ~ up to 58.9 g Egg Mass/Hen/Day) (Source: Lohmann LSL-Lite Management Guide, 2019)

Nutrient		**Daily Feed Consumption /Hen**			
		95 g (20.9 lbs./100 birds)	**100 g*** (22.0 lbs./100 birds)	**105 g** (23.2 lbs./100 birds)	**110 g** (24.3 lbs./100 birds)
Protein	%	17.62	16.74	15.94	15.22
Calcium**	%	4.53	4.30	4.10	3.91
Phosphorus***	%	0.63	0.60	0.57	0.54
Av. Phosphorus	%	0.44	0.42	0.40	0.38
Sodium	%	0.18	0.17	0.16	0.15
Chlorine	%	0.18	0.17	0.16	0.15
Lysine	%	0.82	0.78	0.75	0.71
Dig. Lysine	%	0.68	0.64	0.61	0.58
Methionine	%	0.41	0.39	0.37	0.35
Dig. Methionine	%	0.33	0.32	0.30	0.29
Meth./Cyst.	%	0.74	0.70	0.67	0.64
Dig. M/C	%	0.61	0.58	0.55	0.52
Arginine	%	0.85	0.81	0.77	0.73
Dig. Arginine	%	0.70	0.66	0.63	0.60
Valine	%	0.69	0.66	0.63	0.60
Dig. Valine	%	0.59	0.56	0.53	0.51
Tryptophan	%	0.18	0.17	0.16	0.15
Dig. Tryptophan	%	0.15	0.14	0.13	0.13
Threonine	%	0.57	0.54	0.52	0.49
Dig. Threonine	%	0.47	0.45	0.43	0.41
Isoleucine	%	0.66	0.62	0.59	0.57
Dig. Isoleucine	%	0.54	0.51	0.49	0.47
Linoleic Acid	%	1.47	1.40	1.33	1.27

Table A6. Recommended Nutrient Levels for LOHMANN LSL-LITE Layers in Phase 4 for Different Daily Feed Consumptions (after week 65 ~ up to 56.3 g Egg Mass/Hen/Day) (Source: Lohmann LSL-Lite Management Guide, 2019)

Nutrient		**Daily Feed Consumption /Hen**			
		95 g (20.9 lbs./100 birds)	**100 g*** (22.0 lbs./100 birds)	**105 g** (23.2 lbs./100 birds)	**110 g** (24.3 lbs./100 birds)
Protein	%	17.05	16.20	15.43	14.73
Calcium **	%	4.74	4.50	4.29	4.09
Phosphorus ***	%	0.61	0.58	0.55	0.53
Av. Phosphorus	%	0.43	0.41	0.39	0.37
Sodium	%	0.17	0.16	0.15	0.15
Chlorine	%	0.17	0.16	0.15	0.15
Lysine	%	0.80	0.76	0.72	0.69
Dig. Lysine	%	0.65	0.62	0.59	0.56
Methionine	%	0.39	0.37	0.36	0.34
Dig. Methionine	%	0.32	0.31	0.29	0.28
Meth./Cyst.	%	0.72	0.68	0.65	0.62
Dig. M/C	%	0.59	0.56	0.53	0.51
Arginine	%	0.82	0.78	0.74	0.71
Dig. Arginine	%	0.67	0.64	0.61	0.58
Valine	%	0.67	0.64	0.61	0.58
Dig. Valine	%	0.57	0.54	0.51	0.49
Tryptophan	%	0.17	0.16	0.16	0.15
Dig. Tryptophan	%	0.14	0.14	0.13	0.12
Threonine	%	0.55	0.53	0.50	0.48
Dig. Threonine	%	0.45	0.43	0.41	0.39
Isoleucine	%	0.64	0.60	0.57	0.55
Dig. Isoleucine	%	0.52	0.50	0.47	0.45
Linoleic Acid	%	1.26	1.20	1.14	1.09

Table A7. Mineral Composition of Ground and Extruded *Chondrus crispus*, as fed

	Ground *Chondrus crispus*	Extruded *Chondrus crispus*
Dry Matter (%)	85.38	90.105
Crude Protein (%)	17.75	18.06
Calcium (%)	0.375	0.471
Potassium (%)	3.691	3.726
Magnesium (%)	0.663	0.685
Phosphorus (%)	0.260	0.268
Sodium (%)	3.701	0.3984
Copper (ppm)	ND	ND
Manganese (ppm)	85.56	96.52
Zinc (ppm)	16.74	43.74
Crude Fat (%)	ND	ND

* ND indicates the analysis value is below reporting limit, which is the lowest concentration that will be reported for a specific method

Table A8. Amino Acid Composition of Raw Cultivated *Chondrus crispus*

Amino Acid	Percentage (%)
Alanine	0.84
Arginine	0.94
Aspartic Acid	1.44
Cystine	0.28
Glutamic Acid	1.78
Glycine	0.72
Histidine	0.22
Iscoleucine	0.59
Leucine	0.96
Lysine	0.81
Methionine	0.28
Phenylalanine	0.84
Proline	0.70
Serine	0.67
Threonine	0.63
Tryptophan	0.17
Tyrosine	0.36
Valine	0.71
Total Protein	12.94

Table A9. Amino Acid Composition of Sundried, Ground *Ascophyllum nodosum*, Tasco®

Amino Acid	Percentage (%)
Alanine	0.34
Arginine	0.22
Aspartic Acid	0.53
Cystine	0.07
Glutamic Acid	0.71
Glycine	0.30
Histidine	0.07
Iscoleucine	0.26
Leucine	0.38
Lysine	0.30
Methionine	0.11
Phenylalanine	0.24
Proline	0.25
Serine	0.27
Threonine	0.25
Tryptophan	0.06
Tyrosine	0.12
Valine	0.27
Total Protein	4.78

Table A10. Fiber Composition of Raw versus Extruded *Chondrus crispus,* As Fed

Feed Component	Raw CC	Extruded CC
NDF (%)	87.68	91.02
ADF (%)	3.06	2.95
	35.86	14.79