IMPACT OF CAGE-FREE LAYING HEN MANAGEMENT AND NUTRITION ON PHYSICAL EGG QUALITY

by

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ABSTRACT

Egg safety and quality are a priority for United States egg producers. With the increase in food manufacturers and retailers pledging to use cage-free eggs by 2025, more egg producers are shifting from conventional cage systems to cage-free housing systems for layers. This change results in a need to evaluate the impact of diet and nutrition and the role of bird management on egg quality. In two studies, the effect of northern fowl mite (NFM) infestation of birds on the quality of cage-free eggs, as well as the effect of dietary supplementation of different omega fatty acids and vitamin D on egg quality were evaluated. NFM infestation reduced volume of the shell, Haugh unit score, shell surface area, shell length, and shell width ($P < 0.05$). Fish oil supplementation negatively impacted physical egg quality ($P < 0.05$) while vitamin D supplementation improved egg quality.

INDEX WORDS: egg safety; cage-free; hen nutrition; egg quality; northern fowl mite

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INTRODUCTION

Purpose of the Studies

The United States (US) egg industry produces approximately 100 billion table eggs per year (American Egg Board, 2019). Advancements in genetics, nutrition, and management contributed to an increase in egg production. In addition to recent advancements, assuring the safety and quality of the eggs is a priority for egg producers. Shell egg producers are transitioning from conventional to cage-free housing systems due to increasing concerns about animal welfare in the US. The adoption of cage-free housing for laying hens requires additional research on hen nutrition and management practices for cage-free systems. Published literature has increased the understanding of different housing systems and the importance of hen nutrition. However, the requirements and practices in housing systems have been evolving in the past decade. With the changes, there is a need to evaluate their impact on hen housing and nutrition.

The objectives of this research were to determine the impact of northern fowl mite (NFM) infestation on cage-free laying hens physical egg quality and to determine the impact of omega fatty acids and vitamin D supplements in the diet on physical egg quality. The results of these studies will provide insight into the impact of NFM on physical egg quality and identify the impacts of hen dietary fat on egg quality in cage-free housing systems.

CHAPTER 2

COMPARISON OF LAYING HEN HOUSING TYPES FROM VARIOUS COUNTRIES AND THE IMPACT OF HOUSING ON EGG QUALITY[1]

[2]Anna M. Hull, Deana R. Jones, Darrin M. Karcher, Manpreet Singh, and Harshavardhan Thippareddi. To be submitted to *World's Poultry Science Journal*.

SUMMARY

Consumers today are concerned about animal welfare, particularly in the food production sector. There has been a push in the European Union (EU), Australia, Canada, and the United States (US) to transition from conventional to cage-free housing systems for laying hens. This transition brings with it an abundance of issues related to the quality of cage-free eggs. The US and EU have different regulations for laying hen housing systems and the production and sale of shell eggs and egg products. Hence, outcomes of research on impact of housing systems on egg quality vary between the two continents. This review will focus on the housing systems and management practices in the layer industry in the US and other countries and how those practices can impact egg quality.

Key words: egg; conventional; cage-free; quality

INTRODUCTION

The EU banned the use of conventional cages for layers in 2012, although enriched cages (large cage enclosures with more room than conventional cages that have perches, nest boxes, and scratch pads) are still being used (Directive, EU, 1999). Many US retailers and food manufacturers have pledged use of cage-free housing for egg production by 2025, thus increasing the need for producers to switch to cage-free housing systems. Although this transition is one that consumers desire, many are unwilling to pay the additional price for cage-free eggs since improvements in egg quality are not evident (Hidalgo *et al.*, 2008).

Published literature on the impact of housing systems on egg quality is increasing. However, differences in laws and regulations within the same production system such as 'cage-free' vary significantly between regions of the world, making it harder to compare literature and draw conclusions. In addition to the housing systems, flock management, hen nutrition, and hen

strain also play a role on egg quality. These additional factors make it harder to definitively attribute housing system impact on egg quality parameters.

It is important to recognize key features (US standards) regarding different housing systems for laying hens. In conventional housing (min 432 cm^2 space/bird), hens are held in cages with *ad libitum* access to food and water, but do not have access to nests, perches, or litter areas. In enriched systems (min 748 cm^2 space/bird), hens are grouped in enclosures, but have additional space compared to the conventional cages to allow for exhibiting natural behaviors. In cage-free systems (min 929 cm^2 space/bird), hens are provided nest boxes, perches, and litter which allow the hens to exhibit natural behaviors (United Egg Producers, 2019). By EU standards, enriched housing systems (min 750 cm^2 space/bird) must include nests, litter, scratch pads, and perches although hens are still caged in groups. Cage-free systems (min 1,111 cm^2 space/bird) must provide nests, litter, and perch space for then hens (Directive, EU, 1999).

EGG PRODUCTS AND PROCESSING LEGISLATION IN THE UNITED STATES

The US Congress passed the Egg Products Inspection Act (EPIA) in 1970, the primary legislation controlling the production and processing of eggs and egg products. The US Department of Agriculture Food Safety and Inspection Service (USDA FSIS) has oversight of shell eggs and the processors must follow the regulations of shell egg production and ensure the safety of the products. The EPIA requires that all egg products distributed for consumption must be pasteurized. Egg products include whole eggs, albumen, and yolks in various forms (frozen, liquid, powder) (American Egg Board [AEB], 2019). An official plant number is assigned which will then be used to label all containers and packages of products from that plant. It is also required that any restricted eggs like checks and dirties must be pasteurized before being sold for human consumption, while any eggs deemed inedible cannot be marketed. The EPIA prescribes

strict temperature requirements, such that shell eggs must be stored at a temperature no greater than 7.2°C. to reduce the risk of pathogen growth in the egg. Thus, egg cartons must be labeled stating that refrigeration is required (USDA, 2005).

The United States Food and Drug Administration (FDA) implemented the Final Rule: Prevention of *Salmonella* Enteritidis in Shell Eggs During Production, Storage, and Transportation (Egg Rule) to mitigate the risk of *Salmonella* Enteritidis (SE) in shell eggs during production, storage, and transportation (FDA, 2009). SE, a common cause of foodborne illness in the United States (Centers for Disease Control and Prevention, 2019), is mitigated by the Egg Rule which was enacted to reduce the risk of SE in shell eggs. Producers are required to implement practices to decrease SE prevalence during production. All egg producers with greater than 3,000 hens are required to register with the FDA and are subject to random inspections to ensure that they follow all aspects of the Egg Rule (FDA, 2009). The USDA Agricultural Marketing Service (AMS) provides continuous on-site grading of shell eggs. They ensure that all eggs being packaged meet the quality and size standards of the grade (USDA, 2012).

Some eggs are produced organically under the voluntary guidelines of the National Organic Program (NOP) operated by USDA-AMS (USDA, 2011). Egg producers can become certified for organic production through the NOP and are permitted to use the USDA organic seal on the cartons and products. In the US, 48 certifying agents (including the NOP) are recognized by the USDA-AMS and can issue organic certificates to producers and farms that abide by USDA organic standards (USDA, 2011). To obtain USDA organic certification, the product must be free of antibiotics, growth hormones, artificial colors, flavors, and preservatives. All hens raised organically must be housed in free-range systems with outdoor access.

EGG PRODUCTS AND PROCESSING LEGISLATION IN THE EUROPEAN UNION

In 2008, The European Union established regulations for the marketing and sale of eggs and egg products in Europe. The legislation outlined the characteristics of a Class A and B egg, whereas in the US, eggs are either Grade AA, A, or B. The EU regulations specify that Class A eggs are not to be washed or cleaned before marketing (Regulation, EU, 2008). To be marketed, shell eggs must be packed and labeled within 10 days of being laid by the hen. Only egg packing centers have the authority to grade, pack, and label their eggs. Egg shipping containers must be labeled with the producer's name and address, the producer code, the number of eggs and their case weight, the date that the eggs were laid, and the date of dispatch of the packaged eggs. This allows for easy traceback of the eggs should a health concern or quality issue arise.

The EU egg legislation also outlines the type of housing and acceptable conditions for the laying hens. The European Union outlawed conventional cage hen housing in 2012, hence the need for hens to be housed in either cage-free, enriched, or free range systems (hens must have access to open-air runs that are covered in vegetation for free range systems). Runs, enclosed outdoor spaces for hens, must not extend further than 150 meters from the building and should be limited to 2,500 hens per 10,000 square meters (Directive, EU, 1999). After the regulations went into effect, extensive research has been conducted in Europe to evaluate the impact of various hen housing systems on egg quality.

EXTERIOR EGG QUALITY

Exterior egg quality focuses on assessments such as egg size, shell cleanliness, shell strength and elasticity, and color. Such characteristics determine egg class, grade, and consumer preference in many countries. With the rise in extensive housing systems around the world,

impact of extensive housing systems on interior and exterior quality of eggs are being actively evaluated.

Eggs from extensive hen housing systems were reported to have higher incidences of cracked and broken eggs compared to those from conventional cage systems. Guesdon *et al.* (2006) reported that eggs laid in enriched systems had a higher percentage of broken and cracked eggs (15.4 and 19.6%, respectively) compared to the eggs produced from conventional systems (8.1 and 12.2% respectively). Similarly, Abrahamsson *et al.* (1995) and Guesdon and Faure (2004) reported lower rate of cracks in eggs produced in conventional systems compared to extensive systems. Accumulation of eggs in the nest boxes in the enriched cages resulted in higher incidence of cracked eggs. Eggs cracked under the weight of one another, a result of infrequent egg collection. This incidence could be remedied through more frequent egg collection or the use of angled floors to facilitate the rolling of eggs onto a conveyor belt for collection once laid. This also minimizes contact time with the hen and other eggs.

Egg shell strength and elasticity vary between conventional and extensive (systems that allow for hens to exhibit natural behaviors) housing systems. Conventionally produced eggs were reported to have a greater shell strength than eggs from cage-free or enriched systems (Valkonen *et al.*, 2006; 2008; Hidalgo *et al.* 2008; Englmaierová *et al.* 2014). However, Guesdon and Faure (2004) did not find conventional eggs to have superior shell strength over extensive systems. Conflicting results are not uncommon when comparing housing systems from different regions, as environmental conditions and regulatory requirements for housing systems vary. However, contradictory results between studies could also be due to improved experimental design and methods of detection over time. All studies that observed conventionally produced eggs to have greater shell strength were published 2-10 years after the Guesdon and Faure (2004)

study. Development and use of automated devices for assessing egg quality probably contributed to improvements in data quality and consistency. For example, Guesdon and Faure (2004) conducted all analyses with previously formulated equations from literature published in the 1980s and 1990s, which potentially allowed for rounding errors or incorrect calculations. Subsequent studies used computerized/automated devices to measure the egg physical quality, thus minimizing errors in data collection (Valkonen *et al.*, 2006; 2008; Hidalgo *et al.* 2008; Englmaierová *et al.* 2014). In China, researchers found that shell strength increased with hen age through 40 weeks of age for both outdoor free-range hens and indoor cage-free hens, and then decreased subsequently. The gradual increase and subsequent decrease in eggshell strength followed the egg production trend and was not affected by housing system (Wang *et al.*, 2009). Hen productivity impacted shell strength more than the housing system design, although the cause is not evident.

In the US, egg safety regulations state that only intact eggs can be marketed to consumers, while the cracked eggs with their contents still internal to the shell can be further processed (USDA, 2005). Leaker eggs must be destroyed and cannot be used for further processing, resulting in an economic loss for producers. Downgraded eggs resulting loss of product are risks to consider when transitioning to extensive housing systems, as the incidence of broken and cracked eggs is much higher in extensive systems than the eggs from conventional housing systems. Unfortunately, literature elucidating the impact of housing system on shell quality is not consistent, making it difficult for producers to adopt systems that assure good shell quality.

Cage-free aviary eggs are normally dirtier than conventional eggs (Abrahamsson and Tauson, 1998; Tauson *et al.*, 1999). Eggs from enriched systems have been reported to be

significantly dirtier than the conventionally produced eggs as the hens lay eggs outside of the available nest boxes (Guesdon and Faure, 2004). Hens will lay outside of the nest boxes in the instances of the boxes being contaminated or dirty. Thick floor litter also promotes laying in litter instead of the available nest boxes. When moving to extensive housing systems, producers need to maintain nest box cleanliness and attractiveness for hens to encourage laying in nest boxes instead of on the floor.

Several countries have egg size or weight requirements for shell egg grading. Some reports highlight that eggs from free range housing systems weighed more than eggs produced from conventional systems (Hughes *et al.*, 1985; Hidalgo *et al.*, 2008), although other studies from around the world disagree (Mostert *et al.*, 1995; Van Den Brand *et al.*, 2004). Others reported that the heaviest eggs came from aviary and enriched systems (Englmaierová *et al.* 2014; Jones *et al.*, 2014), whereas the lightest eggs came from cage-free floor hens (Englmaierová *et al.* 2014). Variation in housing system design on egg weight implies that housing system alone does not impact egg weight but may interact with other factors like hen age or hen strain resulting in heavier or lighter eggs. Hen strain and age are known to impact egg weight and size, highlighting the need to consider all contributing factors when evaluating a housing system's impact on egg quality. Van Den Brand *et al.* (2004) reported that environment alone can affect egg quality, thus maintaining a constant internal and external egg quality is more difficult across extensive housing systems than in conventional systems. Producers should consider hen age, hen strain, and environment as factors for selecting the housing system for a flock.

INTERIOR EGG QUALITY

Interior quality greatly impacts consumer appeal and acceptance. Because of this, many researchers have monitored internal egg quality for various housing systems. Yolk color, vitelline membrane strength and elasticity, and albumen quality comprise the main components of interior egg quality.

Abrahamsson and Tauson (1998) reported that yolk color became lighter for multiple hen strains throughout five production cycles in a cage-free aviary system. The egg yolks produced in the first cycle averaged 9.6 points on the color fan and egg yolks in the fifth cycle averaged 6.0 points. Yolk color can negatively impact consumer appeal of the egg compared to other interior quality factors. Van Den Brand *et al.* (2004) also reported that yolk color was impacted with housing system and concluded that eggs from free range hens have darker yolk color than eggs from conventionally caged hens. These results are expected as hens with access to other feed ingredients found in the outdoors like grass and insects consume a greater amount of xanthophylls (the primary determinant of yolk color intensity). Yolk color is greatly dependent on hen diet, a factor that should be considered when selecting a housing system for layers.

Thick albumen height decreases with hen age (Williams, 1992; Silversides and Scott, 2001). However, the impact of hen housing system on albumen height was not definitive (Pavlovski *et al.*, 1981; Mostert *et al.*, 1995). This led researchers to believe that housing systems had little to no impact on albumen quality and that hen age or egg age was the determining factor of internal egg quality. However, Englmaierová *et al.* (2014) reported that conventionally caged hens produced eggs with thicker albumen height and greater Haugh unit scores compared to enriched cage and aviary hens, indicating that hens in conventional housing systems produced greater interior egg quality. In addition, eggs from hens housed in the enriched cages and aviary

had higher yolk index scores, indicating overall taller yolks than yolks from conventionally produced eggs (Singh *et al.* 2009; Englmaierová *et al.*, 2014). These results indicate that each type of housing system is beneficial to internal egg quality, but in different ways. Conventional housing resulted in eggs with taller thick albumen and greater Haugh unit scores whereas the enriched cage and aviaries resulted in eggs with taller yolks. Thus, one system cannot be deemed better than another in relation to internal egg quality, as each system impacted different areas of internal egg quality.

In the US, Jones *et al.* (2014) measured egg quality parameters from conventional, enriched, and aviary systems and found that the internal egg quality characteristics of vitelline membrane strength, vitelline membrane elasticity, and whole egg total solids were not significantly different between housing systems. However, prior research (Jones *et al.* 2002, 2005, 2010) reported lower albumen height and Haugh unit scores with storage time, which is a point to consider when marketing retail shell eggs (regardless of housing system). This is because although housing system has been found to impact egg quality, the egg quality begins to deteriorate as soon as an egg is laid, and the rate of this deterioration can be slowed or promoted by various factors. As outlined, housing system alone is not the only factor that impacts egg quality, although it is a major factor.

CONCLUSIONS

Hen housing system design and hen management impact internal and external egg quality but their effects are still being actively researched. Regardless of the country where eggs are produced, quality of the eggs will be a priority for producers and the housing systems should be designed to optimize bird productivity and egg quality.

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CHAPTER 3

A REVIEW OF LAYING HEN DIET AND NUTRITION AND THE IMPACT ON PHYSICAL EGG QUALITY[2]

[2]Anna M. Hull, Deana R. Jones, Darrin M. Karcher, Manpreet Singh, and Harshavardhan Thippareddi. To be submitted to *World's Poultry Science Journal*.

SUMMARY

Nutrition plays an important role in the physical quality of chicken eggs, such as egg size, shell strength, albumen quality, vitelline membrane strength, and yolk color. Egg quality factors are important, as they impact a consumer's acceptability and preferences of an egg. Understanding how hen diet can be modified to produce eggs that meet desirable egg quality for consumers also provides benefits to the egg producers.

INTRODUCTION

Providing the appropriate concentrations of vitamins, minerals, and fatty acids into poultry diets is critical, as it allows the birds to perform at their full genetic potential (Adhikari *et al.*, 2020). Traditionally, hen diets are formulated based on the minimum dietary requirements published by the National Research Council (NRC, 1994). However, they may need to be refined based on the improved hen genetics, strain, and age of the hen. Also, egg producers may incorporate other ingredients or nutrients to produce eggs with specific quality characteristics such as darker yolk color or stronger vitelline membrane strength, for example. This review will focus on hen diet supplements and their impact on physical egg quality.

NUTRITIONAL IMPACTS ON EGG QUALITY

Egg Weight

Egg size and weight is a factor that influences both egg quality and grade of the eggs. Eggs naturally vary in size depending on genetic strain and hen age (Joyner *et al.*, 1987; Silversides and Scott, 2001; Tůmová and Gous, 2012). Egg weight is comprised of the albumen, yolk, and shell structures. In the United States (US), eggs are classified based on egg weights established by the United States Department of Agriculture (USDA). There are six egg weight classes recognized in the US: jumbo (68.5 g min/egg), extra-large (61.4 g min/egg), large (54.3 g

min/egg), medium (47.2 g min/egg), small (40.2 g min/egg), and peewee (35.4 g min/egg). Extra-large, large, and medium are the most common sizes marketed, with large size eggs making up the greatest percentage of eggs in the market (USDA, 2002).

Modifications to the hen's diet can impact egg weight. Supplementation of a hen's diet with fish oil, a source of omega-3 fatty acids, significantly reduced egg size and weight (Whitehead *et al.*, 1993; Van Elswyk *et al.*, 1994; Gonzalez-Esquerra and Leeson, 2000; Dong, *et al.*, 2018). Whitehead *et al.* (1993) reported that fish oil supplemented into the hen diets caused lower yolk weights compared to the control diet, thus resulting in an overall lighter egg. Gonzalez-Esquerra and Leeson (2000) reported that egg weight decreased linearly by 0.36 g with each 1% increase of fish oil in the diet, resulting in significantly smaller eggs when higher percentages of fish oil were supplemented in the diet. The results of these studies show that laying hen performance (egg size) is negatively affected when their diets are supplemented with fish oil, potentially due to the high levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the oil. EPA and DHA are known to positively impact neural development (Dyall and Michael-Titus, 2008) and to prevent cardiovascular disease risk in humans (Von Schacky and Harris, 2007), which makes fish oil supplementation an appealing option in value-added egg production. Flaxseed oil, another healthy source of omega-3 fatty acids for humans, was found to have no impact on egg size and weight (Ayerza and Coates, 2001; Bean and Leeson, 2003) and it was also shown to increase the omega-3 fatty acid content in the yolk which would benefit human health (Ayerza and Coates, 2001). The effects of these supplementations on egg quality should be considered when enriching the eggs for an increased omega-3 fatty acid content.

Shell Strength

Chickens, especially laying hens, require calcium in their diets to support their skeletal system as well as to create thick, hard eggshells. An eggshell is made up of a 1:50 ratio of protein fibers to calcium carbonate, meaning the eggshell is almost 98% calcium and about 2% proteins and other minerals (Romanoff and Romanoff, 1949). The amount of calcium that is deposited on the eggshell while the egg is developing in the oviduct is dependent on the strain, health status, and nutrition of the hen. On average, hens will consistently deposit about 2 g of calcium onto each eggshell (Gilbert, 1983). As the hen ages, the size of the egg increases and the calcium deposit (2 g) is spread out over a larger shell surface area, resulting in thinner eggshells.

To maintain a consistent calcium deposit each day, a hen must have an adequate amount of calcium in the diet. In hen diets, calcium is typically supplemented through the addition of limestone, a type of sedimentary rock that is composed primarily of calcium carbonate. Multiple studies found that increased milled limestone concentration in the hen diet improved shell breaking strength (the minimum force required to cause failure of the eggshell), thus improving the overall egg quality (Koreleski and Świątkiewicz, 2004; Guo and Kim, 2012). Although improved egg quality is the desired goal, Roberts (2004) reported that calcium availability and deposition are not the only factors that can affect shell strength and quality, as the structure of the shell and the uniformity of the calcium deposition is just as important. Independent to calcium levels in the body, a shell that is poorly constructed in the oviduct will have a relatively low breaking strength, regardless of the other quality factors. This is an important point to consider should issues regarding shell strength or thickness arise in a flock.

Vitamin D is a fat-soluble vitamin that is needed for the proper metabolism of calcium and phosphorus and it plays a role in maintaining skeletal integrity (Adhikari *et al.*, 2020).

Vitamin D is typically obtained through exposure to ultraviolet radiation from the sun, however, cage-free hens do not go outdoors. Therefore, their diets must be supplemented with vitamin D. Calcium will not be absorbed if there is a vitamin D deficiency, regardless of how much calcium is present in the diet. This means calcium will be of little benefit to the body until the vitamin D levels are restored. If a laying hen were to experience a vitamin D or calcium deficiency, the hen would uptake calcium from the bones, leading to loss of bone integrity (Adhikari *et al.*, 2020). Hens require vitamin D in the form of 1,25-hydroxy-vitamin D_3, formally known as calcifediol. Vitamin D is typically added into the diet as D_3 (cholecalciferol) which is metabolized by the hen to calcifediol. In laying hens that are fed a diet that is deficient in vitamin D, the first sign of this deficiency will be the thinning of eggshells leading to reduced shell thickness (Roberts, 2004). In a diet that is completely lacking vitamin D, the egg-laying rates will significantly decrease, along with an increase in eggs with very thin or no shells (Tsang *et al.*, 1990). Although this would be a rare instance in commercial laying operations, identifying the signs of this deficiency is critical to modifying the hen diet to alleviate the deficiency. Reports to the contrary exist in literature. Multiple studies reported that increasing vitamin D supplementation in hen diets did not have an impact on egg breaking strength and quality (Keshavarz, 2003; Mattila *et al.*, 2003). Soares Jr. *et al.* (1988) reported that vitamin D enrichment increased shell quality, but not the breaking strength. Regardless of its potential impacts on exterior egg quality, a minimum dietary vitamin D level of 150 IU/kg from 21-34 weeks of age, and at 250 IU/kg from 30-45 weeks of age (NRC, 1994) is required in the hen's diet to aid in calcium absorption and to improve bone density and eggshell quality.

Haugh Unit and Albumen Quality

The Haugh unit (Haugh, 1937) provides an index of egg quality and is calculated based on the ratio of egg weight (g) to the thick albumen height (mm). There has been a debate on the validity of the Haugh unit score for different egg sizes. One study stated that the weight adjustment for the Haugh unit score was inadequate and suggested that simply measuring the height of the thick albumen was sufficient when comparing eggs from the same flock (Silversides *et al.*, 1993). Another study by Eisen *et al.* (1962) concluded that the Haugh unit, because it is adjusted for a 56.7 g large size egg, resulted in a regression of .05 mm in albumen height per gram of egg weight increase, thus resulting in skewed interior quality measurements. Kidwell *et al.* (1964) stated that the Haugh unit was appropriate for fresh eggs and should not be used as a measure for stored eggs. Although differing opinions exist, the Haugh unit is still considered the "gold standard" for expressing interior egg quality. Several countries, including the US, use Haugh unit scores as a means for egg grade standards. A score of $72\leq$ is grade AA, a score of $60 \leq x < 72$ is a score of A, and a Haugh unit score of <60 is a B grade egg (USDA, 2000). Grade AA eggs have round, firm yolks where the outline of the yolk is indistinct and blends with the surrounding firm, thick albumen. Grade A eggs have yolks with a discernible outline against the surrounding thick albumen and Grade B eggs have thin to flat yolks with an obvious outline surrounded by a watery albumen (USDA, 2002). Regarding Haugh unit, a recent study supplemented hen diets with soybean oil (unsaturated fatty acid), coconut oil (saturated fatty acid), and fish oil (unsaturated fatty acid) to determine how each supplement would impact egg quality (Dong *et al.*, 2018). The primary difference between these diets is that fish oil contains high levels of EPA and DHA whereas soybean and coconut oils do not. No differences were detected between treatments in albumen heights or Haugh unit scores during the first few weeks of experimentation. However, soybean oil supplementation resulted in significantly lower

albumen heights and Haugh unit scores by the end of the experimentation period than the coconut oil and fish oil treatments. Overall, the fish oil treatment resulted in the highest overall albumen heights and Haugh unit scores, but it significantly reduced egg size, yolk size, and weight.

The influence of vitamin D supplementation has also been examined. Park *et al.* (2005) reported that supplementation of a hen's diet with vitamin D resulted in lower Haugh unit scores with increasing cholecalciferol concentrations up to 20,000 IU/kg feed. However, Persia *et al.* (2013) reported minimal impacts on Haugh unit scores of eggs when the hens were fed cholecalciferol concentrations of 2,200 IU to 102,200 IU/kg feed. These contrasting results indicate that vitamin D has little to no relation to Haugh unit and albumen quality in the egg.

Vitelline Membrane Strength

Vitelline (yolk) membrane strength is an important quality characteristic due to its impact in the further processing sector of the egg industry (Kirunda and McKee, 2000). Vitelline membrane strength is also crucial to food safety, as weak vitelline membranes allow penetration of organisms into the yolk. The surge in consumers desiring more convenient cooking options using liquid egg products (whole egg, yolk, or albumen) has increased. However, weak yolk vitelline membrane can rupture upon egg-breaking which may result in higher egg yolk content in the albumen lowering its value and affecting its functional properties. While the vitelline membrane strength may be less of an issue, it can cause major problems in the manufacture of albumen. Therefore, the vitelline membrane must be strong enough to withstand the egg-breaking process (Kirunda and McKee, 2000).

The vitelline membrane is made up of two layers: an inner layer that is formed in the ovary, and an outer layer that is deposited in the oviduct, which are both separated by a thin,

continuous membrane (Bellairs *et al.*,1963). Vitelline membrane strength (VMS) decreases as the hen ages, making it an important quality factor (Fromm and Martone, 1962; Fromm 1964).

Dunn-Horrocks *et al.* (2011) researched enriching hen diets with omega-3 fatty acids (fish oil and flaxseed oil), as well as the inclusion of added vitamin B6 to determine how vitelline membrane strength (VMS) was impacted. Vitamin B6 is a cofactor for amino acid metabolism, which allows for egg protein synthesis. Proteins in the VM are directly related to its function and strength (Kelley, 2003). Fish oil and flaxseed oil are known for their high levels of omega-3 fatty acids which have positive health benefits for humans. It was reported that the omega-3 ration with 8-10% flaxseed and 1-2% fish oil significantly reduced the VMS as well as egg weight, although egg weight was not reduced when only flaxseed was added into the diet. It was also reported that the addition of vitamin B6 into the diets increased VMS, as well as egg weight due to the increased amino acid metabolism and protein synthesis (Dunn-Horrocks *et al.*, 2011). These reports indicate the addition of fish oil, even in low amounts in combination with other beneficial oils, negatively impact egg weight. It would be more beneficial to supplement the diets with a combination of increased vitamin B6 and flaxseed oil, without the inclusion of fish oil to avoid the negative impacts on egg quality.

Yolk Color

Yolk color is an egg quality factor that is solely dependent upon the hen's diet, as the hens cannot synthesize color pigments (Bartov and Bornsteins, 1980). The deep yellow-orange color of a yolk is due to carotenoids, particularly hydroxy compounds, xanthophylls (Smith and Perdue, 1966). The xanthophyll contents in the yolk are impacted by the amount of yellow or orange pigmentation in the feed. Lutein is the predominant xanthophyll in yellow corn meal as well as the main carotenoid in eggs from hens fed yellow corn meal (Smith and Perdue, 1966).

Yolk color can be measured using a color fan (subjective method) or electronically with a colorimeter (objective method). A color fan, the most common being the DSM YolkFan™, is a measurement tool that is used to score yolk color. For accurate readings, the color scoring should be conducted when the yolk is against a white background to avoid the influence of contrasting colors and the blades should be spread out right above the yolk. The fan colors range from 1-16, with 16 being the darkest yolk color. The yolk is given a number that correlates to its color, which allows for the yolk colors to be easily compared across a sample of eggs. A past project reported the effects of adding 5%, 10%, and 15% distiller's dried grains with solubles (DDGS) to the hen diet and observing the impact on yolk color. The addition of 5% DDGS into the hen diet resulted in significantly darker egg yolks over a period of 8 weeks (Roberson *et al.*, 2005). The authors also used a colorimeter to determine how redness (a*) and lightness (L*) values were impacted. The addition of 5% DDGS increased the redness in the yolks, and the yolks were all significantly lighter by with each week, regardless of the percentage of DDGS added.

Incorporation of marigold (yellow pigment source), safflower (orange pigment source) and red pepper (red pigment source) improved egg yolk color but did not affect egg production or quality (Rowghani *et al.*, 2006). Safflower petals resulted in the least amount of added color pigmentation because it had the lowest amount of xanthophylls. The marigold flower petals resulted in color fan values close to 10.0, which attests to marigold's benefit in producing a darker yolk color. Most commercial hen diets today include corn meal, but in areas of the world where darker orange yolks are desired, corn meal alone will not produce the desired darker yolks. Natural color pigment sources will be needed to enhance egg yolk color (Gurbuz *et al.*, 2003; Rowghani *et al.*, 2006). Rowghani *et al.* (2006) reported that the addition of 0.5-1% red pepper to the basal corn diet significantly darkened egg yolk color to that which would be

regionally acceptable. These results were confirmed by Gurbuz *et al.* (2003), who conducted a similar study with red pepper pigments.

CONCLUSIONS

It is apparent that hen diet and nutrition play key roles in the quality of eggs that are produced. Although some literature on hen nutrition and egg quality have been contradictory, it is critical to view the results in contrast to the study design. Although inclusion of dietary supplements can negatively impact egg quality, it is equally important to understand the role of these supplements on the eggs and the desirable qualities (e.g. yolk color).

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CHAPTER 4

IMPACT OF NORTHERN FOWL MITE INFESTATION ON THE PHYSICAL QUALITY OF CAGE-FREE EGGS[3]

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ABSTRACT

Ectoparasites, organisms that live externally on a host and derive nutrients at the host's expense, have returned in recent years despite efforts taken by egg producers to rid their farms of ectoparasites. The northern fowl mite **(NFM),** *Ornithonyssus sylviarum,* is a common ectoparasite that affects laying hens. Although there is information available on reducing NFM populations, there is little information on how NFM impact physical egg quality in cage-free housing systems. This study, conducted in two replicates, evaluated the effect of infesting a cage-free laying hen population with NFM and measuring the resulting egg quality characteristics. Both replicates included four rooms, with two rooms being infected by NFM and two rooms serving as the control groups. Replicate 1 hens had intact beaks, while replicate 2 hens were beak-augmented. Eggs were collected from each room at three time periods from 23-47 weeks of age. Eggs were evaluated for physical characteristics in egg shape and volume, static compression shell strength **(SS)**, shell elasticity **(SE)**, shell thickness, Haugh Unit **(HU)**, specific density, vitelline membrane strength **(VMS)**, vitelline membrane elasticity **(VME)**, and whole egg total solids. Three-way interactions between hen age, replicate, and treatment group were found for HU, specific density, shell surface area, shell width, and volume of the shell. The NFM treatment group had a lower average volume of the shell (55.1 mL) than the control group (56.2 mL). The treatment did not impact egg weight. When observing differences for total solids, it was found that replicate 1 eggs had a significantly higher total solids content than replicate 2 eggs (23.08% vs 22.63%, respectively; $P < 0.05$). These results suggest that NFM negatively impact physical egg quality if not controlled.

Key words: ectoparasite, quality, egg, northern fowl mite, cage-free

INTRODUCTION

Northern fowl mites (NFM) are one of the most prominent ectoparasites in the poultry industry. It has been reported that NFM cause decreased egg production, anemia, and weight loss among infected birds. They are also an irritant to egg and bird handlers (DeVaney, 1978). Ectoparasites are also well-known vectors of diseases (Proctor and Owens, 2000). NFM particularly target the vent area of hens, which causes great distress to the birds. NFM are known to decrease egg production and egg weight which indicates that other factors of egg quality can also be affected. Minimal research has been conducted on how NFM impact cage-free housing systems. Vezzoli et al. (2016) found that NFM in conventional cage systems caused a reduced average Haugh Unit (HU) score (91.6 control vs 87.2 NFM), as well as thinner albumen and a lesser egg weight. Devaney (1978) found that NFM resulted in thinner eggshells and a reduced laying rate among the hens in a conventional cage system, however their results were inconclusive as to whether NFM truly impacted the egg quality.

Although literature has been published on NFM impact in conventional flocks, little is known about how NFM can impact cage-free laying hens and cage-free egg quality. The objective of this study is to determine how the infestation of NFM into a cage-free housing system could impact the resulting physical egg quality. The current study monitored differences in egg weight (EW), egg shape, volume of the shell, Haugh unit (HU), shell thickness, specific density, shell strength (SS) and shell elasticity (SE), vitelline membrane strength (VMS) and elasticity (VME), and whole egg total solids with the main effects of hen age, replicate, and treatment.

MATERIALS AND METHODS

Pullet Management. All procedures were approved by Purdue University Institutional Animal Care and Use Committee (IACUC Approval #1706001582). Tetra Brown female chicks were housed in a three-room environmentally controlled windowless building with 400 chicks/room. Water was supplied *ad libitum* and feed was supplied at the rates specified in the management guide for Tetra Brown hens. Pullets in Rep 1 had intact beaks and were vaccinated throughout the rearing period. Pullets in Rep 2 were beak-augmented and were also vaccinated throughout the rearing period. Rearing diets consisting of a starter, grower, and developer diet were formulated to meet or exceed the nutrient requirements of poultry (National Research Council, 1994).

Table 4.1: Vaccination schedule for pullets being used for the mite study at Purdue University.

Age	*Product*	*Protection*	*Administration*
1 Day	Vectormune HVT-IBD + Rispens	Marek's/IBD	Hatchery
	LAH Meganvac 1	SE	Hatchery
16 Days	Intervet Triplevac	NC/B	Coarse Spray
17 Days	LAH Megan-Egg	ST	Coarse Spray
5 Weeks	Intervet Combovac 30	NC/B	Coarse Spray
	LAH Megan-Egg	ST	Coarse Spray
7 Weeks	Biomune Vectormune FP-LT+AE	Fowl Pox, LT, AE	Wing Web
8 Weeks	Intervet Combovac 30	NC/B	Coarse Spray
12 to 13 Weeks	Biomune Layermune 3	NC/B & SE	Breast Injection

Hen Management. This project was conducted in 4 rooms (27.8 m^2/room) of a Big Dutchman Colony 2+ cage-free housing system. A total of 200 hens/room were housed in this facility with 1,393 cm^2 space/bird. There were a total of 6 feed pans (3.8 cm^2/bird), 2 bell drinkers (1.0 cm^2/bird), 10 colony nests (278.7 cm^2/bird), and 10 perches per room (15.2 cm^2/bird). Hens were

assessed every 28 days for mite infestation. Houses were kept in the Thermal Neutral Zone (TNZ) of 21 to 26.6°C to within +/- 3°C.

Treatment Groups. Tetra Brown cage-free laying hens (17 weeks of age) were randomly assigned to the NFM treatment group (Rooms 1-2) and the control group (Rooms 3-4).

Egg Collection. Cage-free nest box eggs (n = 108/treatment collected) were obtained at 23, 35, and 47 weeks of age and placed in foam egg cartons. The eggs were packed into insulated shipping containers and shipped to the Egg Safety and Quality Research Unit (Athens, GA).

Egg Handling. Upon receipt, eggs were inspected and candled for cracks and other shell deformities and then placed in 4°C storage until testing the next day. Only intact eggs were used in the experiment. Eggs were divided into groups for physical quality testing (n = 24 eggs/treatment/collection period).

Physical Egg Quality. Physical egg quality tests included egg weight, volume of the shell, egg length and width, shell surface area, specific density, shell strength and elasticity, Haugh Unit, vitelline membrane strength and elasticity, total egg solids, and shell thickness following methods as outlined by Jones et al. (2018). The yolk and albumen were combined to form pools of 6 eggs (n = 4 pools per treatment at each egg collection period) to conduct whole egg total solids assessments in triplicate according to official methods (AOAC Official Method No. 925.30, 1990).

Statistical Analysis. Differences in egg quality were analyzed by two-way ANOVA using JMP 13 software (SAS Institute, 2017). The model included treatment, hen age, and replicate as the main effects. Up to n = 144 intact eggs were analyzed for each treatment throughout the study. The level of significance used for statistical analysis was $P < 0.05$.

RESULTS AND DISCUSSION

Egg Weight, Volume of the Shell, and Shell Surface Area

The interaction of hen age, replicate, and treatment ($P < 0.05$) on egg weight, volume of the shell, and shell surface area is shown in Figure 4.1. By monitoring all three parameters instead of just surface area alone, a clearer understanding of how egg size was impacted by treatment was obtained. The control replicate 1 eggs had the greatest increase of egg weight, volume of the shell, and shell surface area, while the NFM replicate 2 eggs had the slowest increase in those factors. In replicate 1, there were instances of bird aggression, high mortality, and decreased stocking density due to the hens having intact beaks. By 47 weeks of age, hens from replicate 1 were laying slightly heavier eggs than hens from replicate 2. Although an exact cause of this is unknown, it is hypothesized that the decreased stocking density in replicate 1 allowed the hens to eat more than their recommended daily feed intake, thus gaining more weight and laying heavier eggs than the replicate 2 hens. However, eggs from both replicates increased in weight and volume of the shell as hens aged, which is supported by previous studies (Whitehead et al., 1991; Akyurek and Okur, 2009; Zita et al., 2009). Hens naturally lay larger eggs as they age due to a proportional change in egg components. At the beginning of lay, egg yolks will be relatively small. As the hens age, the yolk increases in size, resulting in a decrease in the proportion of yolk to albumen over time and an overall increase in egg weight (Romanoff and Romanoff, 1949). It should be noted that volume of the shell represents the total volume capacity inside the shell, but it does not consider the size or volume of the air cell. Therefore, the actual volume of egg contents inside the shell is not detected. The NFM treatment resulted in overall smaller eggs than the control treatment (7134.3 mm^2 vs 7226.6 mm^2, respectively; Table 4.2).

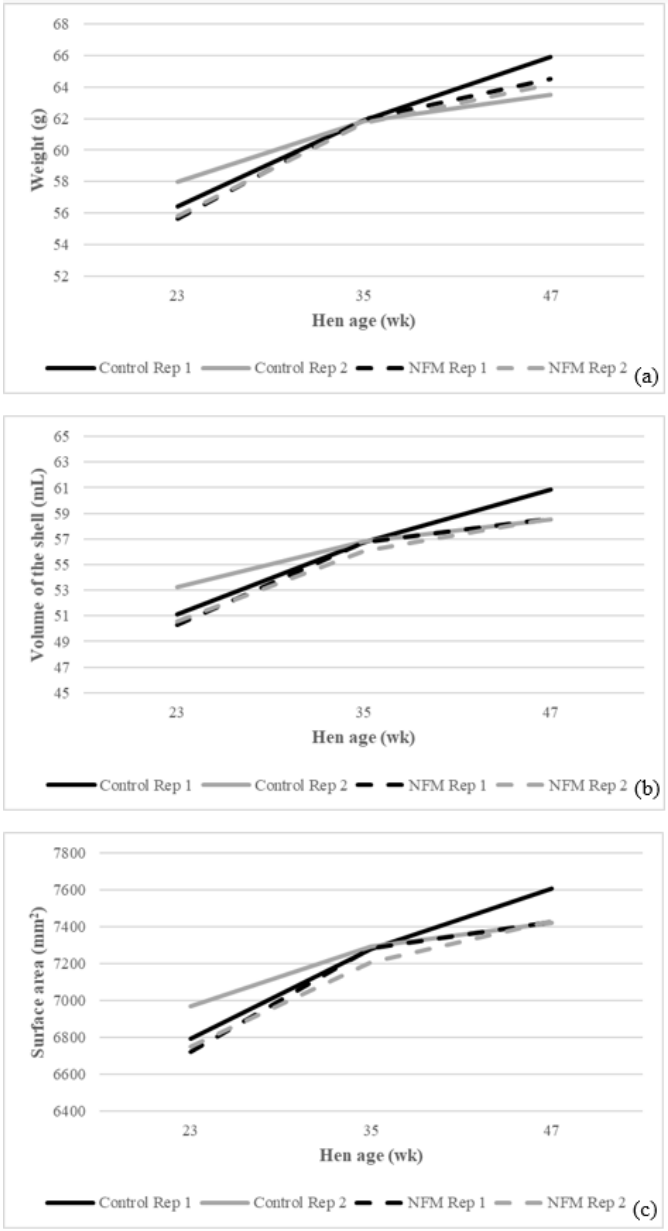

Figure 4.1. Impact of northern fowl mite (NFM), hen age, and replicate interaction ($P < 0.05$) on egg weight (a), volume of the shell (b), and shell surface area (c).

Shell Length and Width

The interaction of replicate, hen age, and treatment ($P < 0.05$) on shell width is presented in Figure 4.2. All treatment and replicate combinations increased in width throughout the production cycle. However, variations in rate of width increase resulted in the three-way

interaction that was found. Both NFM replicates experienced an almost identical rate of increase in shell width, whereas the control replicates were variable in their rates of increase. Eggs in the control replicate 2 group were initially much wider than other eggs and exhibited more consistent egg widths throughout the sampling period. It was also found that eggs from replicate 2 had no significant differences in egg length compared to replicate 1 eggs, indicating that eggs from replicate 2 were getting longer with hen age, but not wider (Table 4.2). Overall, NFM resulted in shorter eggs than the control group (56.1 mm vs 56.7 mm, respectively; $P < 0.001$).

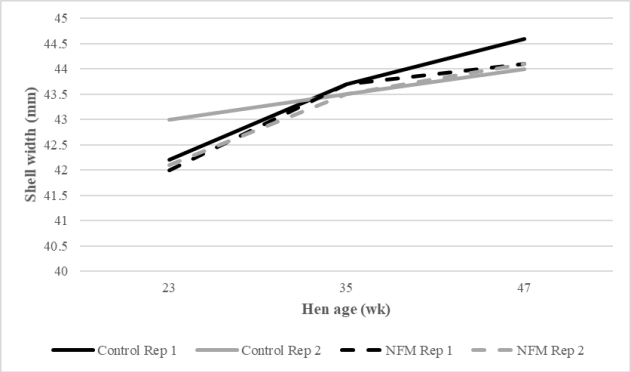

Figure 4.2. Impact of northern fowl mite (NFM), hen age, and replicate interaction ($P < 0.05$) on shell width.

Differences in egg shape index values were found for hen age and treatment (Table 4.2). Shape index is a ratio of shell width to shell length but is not an absolute measure of egg shape. Oval-shaped eggs typically have egg shape indexes of 72-76, whereas rounder eggs have shape indexes above 76 (Altunas and Şekeroğlu, 2008; Duman et al., 2016). For reference, a perfect sphere has a shape index of 100. The NFM eggs reported a significantly greater shape index (77.1) than the control eggs (76.7), although both values indicate a slightly round egg ($P < 0.04$).

The interaction of hen age and replicate ($P < 0.05$) on shell length at maximum width is presented in Figure 4.3. Shell length at maximum width is representative of the overall egg

shape, since egg length and width alone are not indicative of true egg shape. For reference, a perfect sphere has a shell length at maximum width of 50%, indicating that the point of maximum length is also the point of maximum width (the equator of the sphere). Replicate 2 eggs were not found to have differences in shell length at maximum width as hens aged, indicating that eggs were maintaining a consistent shape (despite an increase in size) throughout the laying cycle. However, replicate 1 eggs experienced a consistent decrease in shell length at maximum width, indicating that the point of maximum width was shifting closer to the equator of the egg throughout the laying cycle.

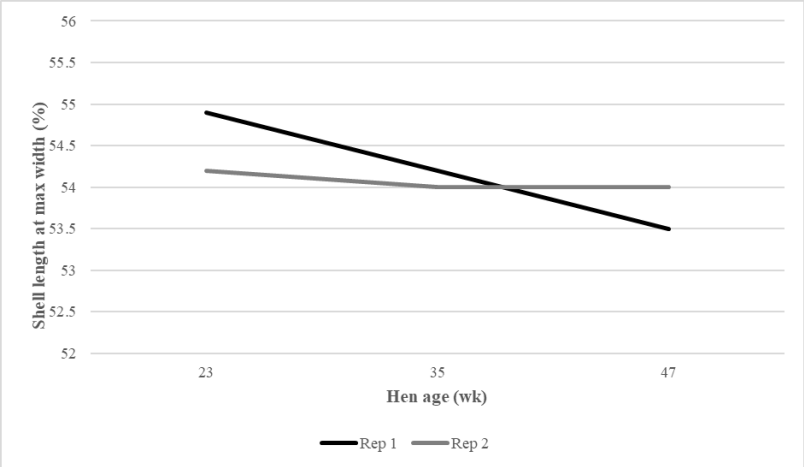

Figure 4.3. Impact of hen age and replicate interaction ($P < 0.05$) from the combined data of the control and northern fowl mite (NFM) treatments on shell length at maximum width.

Although shell length at maximum width is a better indicator of egg shape than shape index, some countries do not possess the technology or testing capabilities to calculate shell length at maximum width. Thus, both shape index and shell length at maximum width data are reported in this study to allow for comparisons to be made based on available technologies.

Specific Density

Specific density was influenced by a three-way hen age, treatment, and replicate interaction ($P < 0.001$; Figure 4.4). Eggs from the replicate 1 control group had masses that were increasing with egg size throughout the production cycle, but the volume of the shell was increasing at a faster rate, decreasing specific density as the hens aged. Hens in the NFM replicate 1 group were initially laying eggs whose volume of the shell was increasing at a higher rate than the mass of the egg, however, egg mass soon started increasing at a higher rate than volume of the shell after 35 weeks, leading to fluctuations in egg specific density. Both the NFM and control group hens from replicate 2 produced eggs whose egg mass and volume increased at the same rate throughout the laying cycle, leading to no change in specific density. Despite variations, both replicates of NFM eggs maintained higher specific densities than the control group replicates.

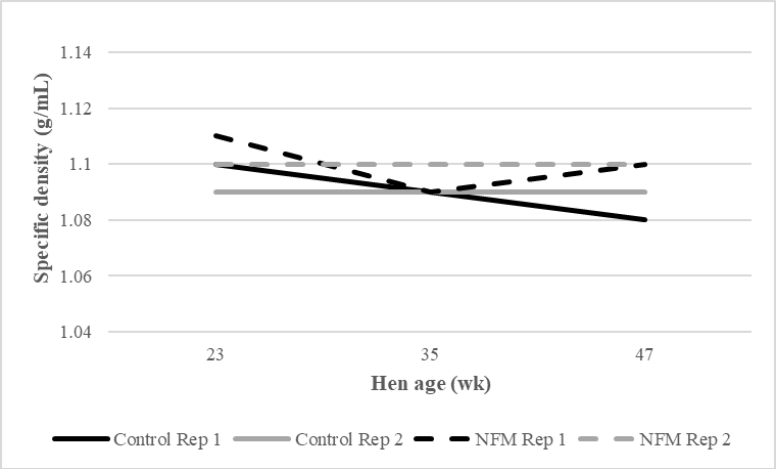

Figure 4.4. Impact of northern fowl mite (NFM), hen age, and replicate interaction ($P < 0.001$) on specific density.

Shell Strength, Elasticity, and Thickness

Shell strength and elasticity were influenced by hen age × replicate interactions ($P < 0.05$), as presented in Figure 4.5. Egg shell strength was variable in replicate 1, however replicate 2 eggs were found to have shell strength values that consistently decreased throughout the production cycle. This decrease aligns with previous work that reported how shell strength decreases with hen age due to the thinning of the shell, which also indicates that shell thickness is correlated with shell strength (Roberts, 2004). As with shell strength, the elasticity of replicate 1 eggshells was not impacted with hen age but was found to decrease in replicate 2 eggs during the first half of the laying cycle. Overall, replicate 1 eggs had less elastic eggshells (0.39 um) than replicate 2 eggs (0.43 um), indicating that replicate 1 eggs were more brittle ($P < 0.001$; Table 4.3).

As hens age, they lay larger eggs, thus spreading out the calcium deposit over a larger shells surface area. Because hens deposit the same amount of calcium onto each eggshell, about 2 g (Roland, 1986), eggshells become thinner and less elastic as larger eggs are laid. However, a decrease in shell elasticity and strength was not observed in eggs from replicate 1. It is hypothesized that due to the hens' potential to eat more food due to the decreased stocking density in replicate 1, they could have obtained an excess calcium intake leading to more calcium to deposit onto the eggshells later in the production cycle.

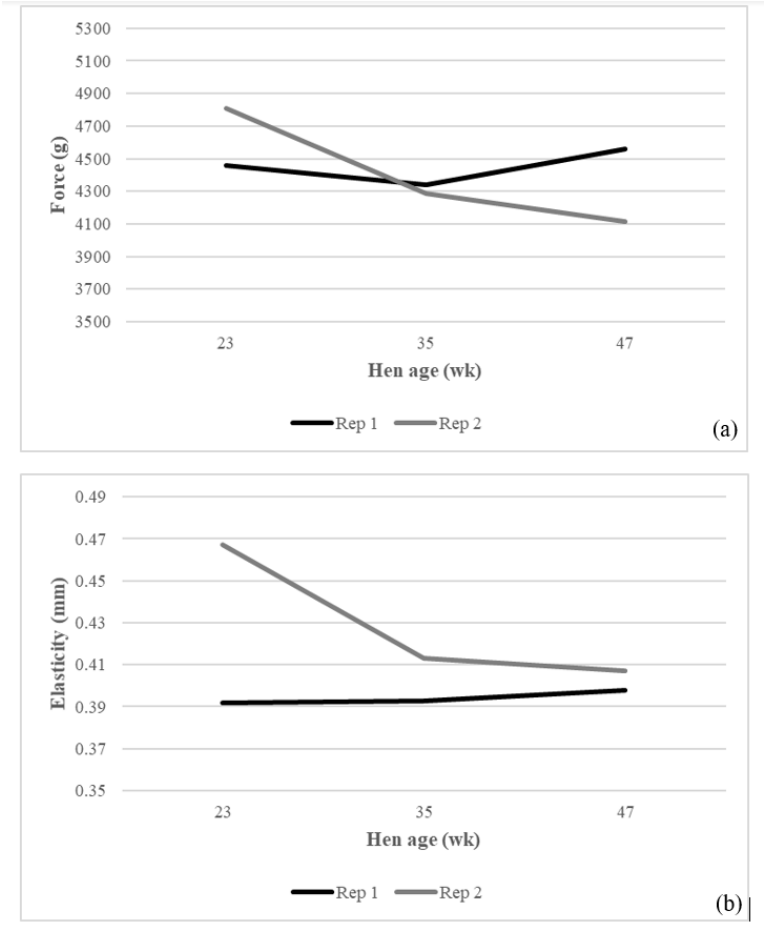

Figure 4.5. Impact of hen age and replicate interaction ($P < 0.05$) from the combined data of the control and northern fowl mite (NFM) treatments on shell strength (a) and shell elasticity (b).

Shell thickness was not impacted by treatment or replicate, but it fluctuated with hen age ($P < 0.05$). This contradicts previous literature that reported a decreased shell thickness in eggs from hens infested with NFM (DeVaney, 1978). Despite significant differences reported, the slight differences in shell thickness between the control and NFM eggs would not be detected by consumers.

Haugh Unit

Haugh unit values were impacted by a three-way hen age, replicate, and treatment interaction ($P < 0.05$), as seen in Figure 4.6. Replicate 2 eggs remained superior in HU scores over replicate 1 eggs throughout the duration of the laying period. Replicate 2 eggs were found

to have decreasing HU scores as hens aged. This finding is supported by previous literature that reported the effects of increasing hen age on HU scores of fresh eggs (Silversides and Scott, 2001; Jones et al., 2002). Although replicate 1 eggs had lower HU values than replicate 2 eggs (82.1 vs 87.1, respectively), their values remained consistent throughout the laying period. Despite the HU values decreasing during the laying period, all eggs had a HU value of 72 or greater, the minimum requirement for US Grade AA (USDA, 2000).

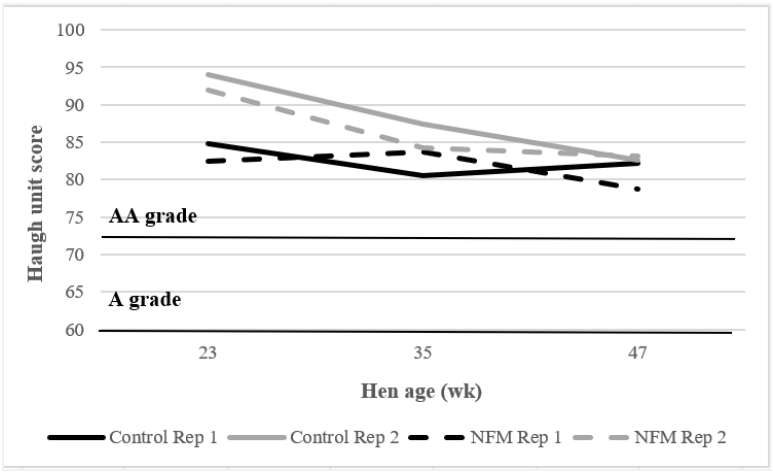

Figure 4.6. Impact of northern fowl mite (NFM) treatment, hen age, and replicate interactions ($P < 0.01$) on Haugh unit score.

Vitelline Membrane Strength and Elasticity

VMS decreased with hen age from 213.7 g force to 148.0 g force ($P < 0.05$; Table 4.3), a finding supported by previous literature (Curtis et al., 2005) that reported VMS to be negatively impacted with increasing hen age. Replicate 1 eggs had a higher VMS than replicate 2 eggs (181.8 g force vs 163.6 g force, respectively; $P < 0.003$), potentially due to the higher nutrient intake that the replicate 1 hens had access to. VMS was also negatively impacted by NFM (Table 4.3), indicating that NFM decrease internal egg quality. VME was influenced by a hen age and replicate interaction ($P < 0.05$; Figure 4.7). Hen age had minimal impact on replicate 1 eggs VME but replicate 2 eggs experienced a decrease in VME during the first half of the production

cycle. This indicates that replicate 2 eggs had more brittle and less give in the yolk membranes than replicate 1 eggs.

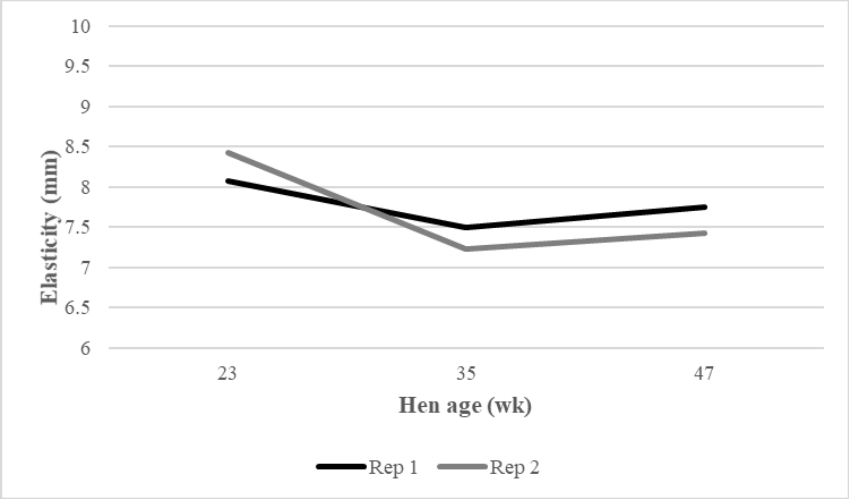

Figure 4.7. Impact of hen age and replicate interaction ($P < 0.01$) from the combined data of the control and northern fowl mite (NFM) treatments on vitelline membrane elasticity.

Whole Egg Total Solids

The interaction of hen age and replicate ($P < 0.05$) on whole egg total solids is seen in Figure 4.8. these interactions indicate differences in egg functional quality as hens aged in each replicate. Previous literature reported that whole egg total solids content increases with hen age (Ahn et al., 1997), a finding that is consistent with those of the current study. Replicate 1 eggs were found to have 1% greater whole egg total solids content than replicate 2 eggs when the hens were 35 weeks of age. Although minute, a 1% difference in whole egg solids content can impact egg functionality. A reason for this significant difference is unknown, but issues with increased hen mortality and a resulting decrease in stocking density in replicate 1 could have been a contributing factor.

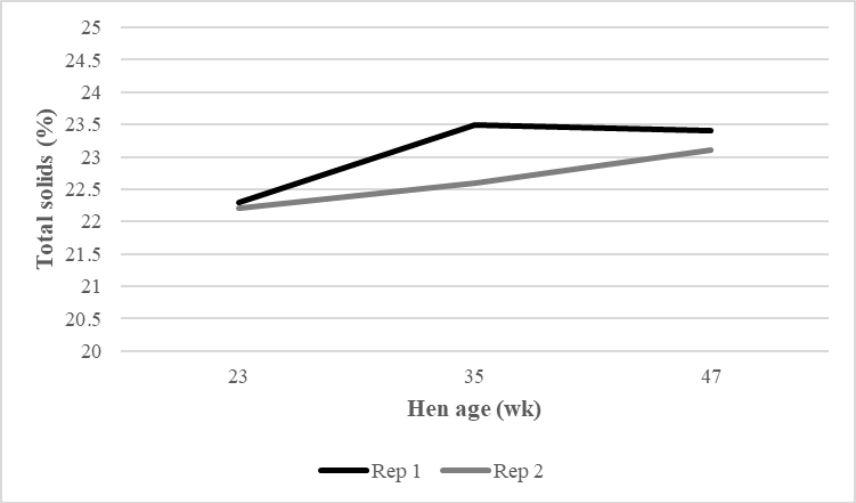

Figure 4.8. Impact of hen age and replicate interaction ($P < 0.05$) from the combined data of the control and northern fowl mite (NFM) treatments on total egg solids.

Conclusions

Overall, this study found that NFM infestation decreased volume of the shell, HU, shell surface area, shell length, and shell width. Hen age had a significant impact on most physical quality parameters. Replicate, where the only difference between replicates was non-beak-augmented vs beak-augmented hens, also had differences. Replicate 1 eggs (from non-beak-augmented hens) had lower HU and shell elasticity values and higher VMS and whole egg total solids content than replicate 2 eggs (from beak-augmented hens). Differences seen between replicates may not only be a result of beak-augmentation, but also a result of issues that were experienced with the flock in replicate 1. Producers should take these results into consideration when deciding whether to beak-augment their hens. Although it is easier to leave the beaks intact, doing so may decrease physical egg quality.

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Table 4.2: Overall effect of treatment group, hen age, and replicate on physical egg quality factors average values across two replicates from 23-47 weeks of age.[1]

	Egg Weight (g) ± SD	Volume of the Shell (mL) ± SD	Shell Surface Area (mm^2) ± SD	Shell Length (mm) ± SD	Shell Width (mm) ± SD	Egg Shape Index ± SD	Shell Length at Maximum Width (%) ± SD	Specific Density (g/mL) ± SD
NFM	60.6 ± 0.3	55.1 ± 0.3	7134.3 ± 444.0	56.1^{b} ± 2.2	43.2 ± 1.4	77.1^{a} ± 0.1	54.1 ± 1.7	1.010 ± 0.01
Control	61.3 ± 0.3	56.2 ± 0.3	7226.6 ± 420.1	56.7^{a} ± 2.1	43.5 ± 1.3	76.7^{b} ± 0.2	54.2 ± 1.7	1.090 ± 0.01
P-Value	NS			0.001		0.04	NS	
23	55.5 ± 0.3	51.3 ± 0.3	6806.5 ± 344.1	54.8^{c} ± 1.7	42.3 ± 1.2	77.3^{a} ± 0.2	54.6 ± 0.1	1.100 ± 0.01
35	61.9 ± 0.3	56.6 ± 0.3	7264.9 ± 312.4	56.8^{b} ± 1.8	43.6 ± 1.0	76.7^{b} ± 0.2	54.1 ± 0.1	1.093 ± 0.01
47	64.5 ± 0.3	59.1 ± 0.3	7471.6 ± 345.2	57.7^{a} ± 1.9	44.2 ± 1.2	76.7^{b} ± 0.2	53.7 ± 0.1	1.091 ± 0.01
P-Value				0.001		0.02		
Rep 1	61.0 ± 0.3	55.7 ± 0.3	7183.0 ± 450.0	56.4 ± 2.2	43.4 ± 1.5	76.9 ± 0.1	54.2 ± 1.9	1.096 ± 0.01
Rep 2	60.8 ± 0.3	55.6 ± 0.3	7178.1 ± 418.4	56.4 ± 2.2	43.4 ± 1.3	76.9 ± 0.2	54.1 ± 1.5	1.094 ± 0.01
P-Value	**	***	***	NS	***	NS	**	***

[a-c] : Means within a column with similar subscripts are not significantly difference ($P < 0.05$).

[1]n = 144/treatment; n=144/rep; n=96/hen age

**: Replicate × hen age interaction; $P < 0.05$.

***: Replicate × hen age × treatment interaction; $P < 0.05$.

NS = Not significant

Table 4.3: Overall effect of treatment group, hen age, and replicate on physical egg quality factors average values across two replicates from 23-47 weeks of age.

	Shell Strength (g)[1] ± SD	Shell Elasticity (mm)[1] ± SD	Shell Thickness (µm)[2] ± SD	Haugh Unit[3] ± SD	Vitelline Membrane Strength (g)[4] ± SD	Vitelline Membrane Elasticity (mm)[4] ± SD	Whole Egg Total Solids (%)[1] ± SD
NFM	4468.1 ± 45.2	0.41 ± 0.01	40.1 ± 2.8	84.1 ± 0.5	169.9[b] ± 4.6	7.68 ± 0.1	22.84 ± 0.1
Control	4387.8 ± 42.7	0.41 ± 0.01	40.1 ± 2.7	85.3 ± 0.6	175.6[a] ± 4.9	7.79 ± 0.1	22.88 ± 0.1
P-Value	NS	NS	NS		0.05	NS	NS
23	4636.1 ± 50.2	0.43 ± 0.01	40.2[a] ± 2.5	88.4 ± 0.7	213.7[a] ± 6.1	8.25 ± 0.1	22.28 ± 0.1
35	4313.4 ± 52.0	0.40 ± 0.01	39.4[b] ± 2.8	84.0 ± 0.6	155.8[b] ± 5.7	7.36 ± 0.1	23.07 ± 0.1
47	4333.7 ± 56.3	0.40 ± 0.01	40.7[a] ± 2.7	81.7 ± 0.6	148.0[b] ± 4.2	7.59 ± 0.1	23.22 ± 0.1
P-Value			0.001		0.001		
Rep 1	4452.3 ± 41.5	0.39 ± 0.01	39.9 ± 2.8	82.1 ± 0.5	181.8[a] ± 5.1	7.78 ± 0.1	23.08 ± 0.1
Rep 2	4403.8 ± 46.3	0.43 ± 0.01	40.2 ± 2.7	87.1 ± 0.6	163.6[b] ± 4.3	7.69 ± 0.1	22.63 ± 0.1
P-Value	**	**	NS	***	0.003	**	**

[a-b:] Means within a column with similar subscripts are not significantly different; $P < 0.05$.

[1]n = 144/treatment; n = 144/rep; n = 96/hen age

[2]n = 143/treatment; n = 143/rep; n = 95/hen age

[3]n = 137/treatment; n = 137/rep; n = 90/hen age

[4]n = 124/treatment; n = 124/rep; n = 83/hen age

**: Replicate × hen age interaction; $P < 0.05$.

***: Replicate × hen age × treatment interaction; $P < 0.05$.

NS = Not significant

CHAPTER 5

IMPACT OF DIETARY OMEGA FATTY-ACIDS AND VITAMIN D ON THE PHYSICAL QUALITY OF CAGE-FREE EGGS[3]

[3]Anna M. Hull, Prafulla Regmi, Darrin M. Karcher, Woo Kim, Deana R. Jones, Cara Robinson, Manpreet Singh, and Harshavardhan Thippareddi. To be submitted to *Poultry Science*.

ABSTRACT

Previous research has found that diet plays an influential role in the quality of chicken eggs. The current study evaluates the effect of adding different omega-fatty acid and vitamin D supplementations to laying hen diets to determine the impact on cage-free egg physical quality. The treatments were flaxseed oil, fish oil, 25-(OH)D_3, and a basal diet (control). Physical quality characteristics were evaluated for differences in egg shape and volume assessment, shell strength and elasticity (**SS, SE**), specific density, shell thickness, Haugh Unit (**HU**) score, yolk index (**YI**), and vitelline membrane strength and elasticity (**VMS, VME**). Hard-cooked eggs were evaluated for differences in yolk texture profile, peak slicing force, and yolk color. Egg weight was greatest for the vitamin D treatment group and lowest for the fish oil group (63.59 g vs. 61.87 g). The vitamin D group also had greater static compression shell strength and elasticity values, and greater vitelline membrane strength and elasticity values than the fish oil group. Fish oil supplementation resulted in the smallest eggs with the weakest eggshells, as well as the lowest average Haugh unit score. Hard-cooked egg texture profiles exhibited the same trend, with the vitamin D treatment group having the greatest overall texture profile and the fish oil group having the lowest (1885.70 g.mm vs. 1680.11 g.mm; $P < 0.05$). The results of this study indicate that fish oil negatively impacts physical egg quality. 25-(OH)D_3 should be considered as a diet supplementation instead due to its positive impacts on egg quality.

Key words: diet, cage-free, quality, egg, vitamin

INTRODUCTION

There are a variety of laying hen housing systems in the United States that are used for 328 million laying hens, with the most popular being conventional cage housing (81.6%) followed by cage-free (13.3%) and organic housing (5.1%) (USDA, 2018). There is an increasing trend of consumers and food manufacturers opting for cage-free eggs instead of conventionally produced eggs due to concerns over animal welfare. The USDA Agricultural Marketing Service has determined that at least 71% of laying hens will need to be in cage-free housing by 2025 to keep up with the expected demand for cage-free eggs (USDA, 2018). Jones et al. (2010) addressed how the US egg industry is now offering more than just conventional egg options in response to the increased consumer awareness of food products and where they come from. With these new options such as free-range and cage-free eggs, further research is needed to determine the impacts of extensive housing systems and nutrition on egg quality. In conventional housing, hens are housed in cages in groups of 6-7 hens per cage to give the minimum allowable space allocation. In cage-free housing, hens may be kept inside the laying facility, but they are no longer restricted to cages. In this study, hens were housed in a multi-tier cage-free aviary which provided them with nest boxes and perches and allowed for vertical movement (AEB, 2019). Regardless of what type of housing system an egg is produced in, physical quality is still of major concern, as any defects can affect consumer appeal and producer profits. As the move from conventional to cage-free housing for laying hens continues to grow, more research is needed to determine how egg quality is impacted.

Vitamin D, particularly in the form of cholecalciferol, is necessary in hen diets to regulate calcium absorption and metabolism (Hurwitz, 1987). Vitamin D is a cofactor for calcium metabolism and absorption, making it an essential vitamin in the hen diet. Alvarez et al. (2004)

found that fish oil did not impact egg weight, shell thickness, or yolk color in conventional eggs, although other studies (Whitehead et al., 1993; Dong et al., 2018) have found that fish oil resulted in significantly smaller eggs than the control groups. Contrasting studies highlight the need for further research to determine how certain diet supplementations can impact cage-free egg quality. The objective of this study was to determine how different omega fatty-acids and 25-(OH)D_3 supplementation impacted the physical quality of cage-free eggs.

MATERIALS AND METHODS

Pullet Management. All procedures were approved by Michigan State University Institutional Animal Care and Use Committee (IACUC Approval #1802001691). Lohmann Brown-Lite chicks were brooded at the facility in environmentally controlled rooms that contained plastic flooring. Chicks were given floor access at 3 weeks of age. Ramps and slats for roosting were also installed on an as-needed basis and photoperiod was decreased from 24 hours per day to 10 hours per day from Day 1 to Week 16 of rearing. Pullets were beak-augmented and then vaccinated throughout the rearing period.

Table 5.1: Vaccination schedule for pullets used in the dietary fat study at Michigan State University.

Age	*Product*	*Protection*	*Administration*
1 Day	Vectormune HVT-IBD + Rispens	Marek's/IBD	Hatchery
2 Days	LAH Meganvac 1	SE	Hatchery
16 Days	Intervet Triplevac	ND/IB	Coarse Spray
17 Days	LAH Megan-Egg	ST	Coarse Spray
5 Weeks	Intervet Combovac 30	ND/IB	Coarse Spray
	LAH Megan-Egg	ST	Coarse Spray
7 Weeks	Biomune Vectormune FP-LT+AE	Fowl Pox, ILT, AE	Wing Web
8 Weeks	Intervet Combovac 30	ND/IB	Coarse Spray
12 to 13 Weeks	Biomune Layermune 3	NC/B & SE	Breast Injection

Rearing diets consisting of a starter, grower, and developer diet were formulated to meet or exceed the nutrient requirements of poultry (National Research Council, 1994). Calcium was included at a rate of 0.90% and phosphorus at a rate of 0.37%. Chicks were fed the same basal diet through 11 weeks of age. At 12 weeks of age, they were randomly assigned to one of 4

dietary treatment groups that included a basal diet and three diets supplemented with additional fatty acids or 25-(OH)D_3.

Hen Management. This project was conducted in 4 multi-tier Natura-60 Big Dutchman® aviary rooms (929.0 cm^2/bird) and hens were moved into the cage-free aviary housing at 17 weeks of age. Each room had 4 sections and a total of 576 hens/room (144/section). Hens had 5.08 cm^2/bird of feeder space, 40.6 cm^2/bird of perch space, 9 hens per nipple drinker, and 83.8 cm^2/bird of nest box spacing. Houses were kept in the Thermal Neutral Zone (TNZ) of 21to 26.6^{o}C to within +/- 3^{o}C.

Treatment Groups. Lohmann Brown-Lite cage-free laying hens (17 weeks of age) were continued on one of four dietary treatment groups started at 12 weeks of age during the pullet phase: flaxseed oil for linolenic acid, fish oil for EPA and DHA, vitamin D(OH)D_3, and a basal diet (control). Diet formulation is listed in Table 5.2.

Table 5.2: Dietary treatment formulations for each of the treatment groups used in the study.

ITEM (INGREDIENT %)	CONTROL	TREATMENT 1 (FLAXSEED OIL)	TREATMENT 2 (FISH OIL)	TREATMENT 3 (VITAMIN D)
Corn	45.00	45.00	42.00	45.00
Wheat Middlings	18.44	18.44	21.54	18.44
Soybean Meal (48%)	25.00	25.00	25.00	25.00
Flaxseed oil	**0.00**	**4.00**	**0.00**	**0.00**
VPgold oil	**0.00**	**0.00**	**4.00**	**0.00**
Soybean oil	**5.00**	**1.00**	**1.12**	**5.00**
Limestone	1.69	1.69	1.70	1.69
Monocal Phosphate	0.98	0.98	0.94	0.98
Salt	0.21	0.21	0.25	0.21
Vitamin D_3	**2,760 IU/kg**	**2,760 IU/kg**	**2,760 IU/kg**	**0.00**
Vitamin D 25-(OH)D_3	**0.00**	**0.00**	**0.00**	**2,760 IU/kg**
Vitamin Pre-mix	0.05	0.05	0.05	0.05
Mineral Pre-mix	0.08	0.08	0.08	0.08
DL-Methionine	0.05	0.05	0.04	0.05
L-Lysine	0.00	0.00	0.00	0.00
Threonine	0.00	0.00	0.00	0.00
Filler	3.45	3.45	3.22	3.45
Coccidiostat	0.05	0.05	0.05	0.05
Omega (n-6/n-3) ratio	6.750	0.534	0.534	6.750
ME (kcal/kg)	2,910	2,900	2,900	2,910
CP (%)	18.37	18.37	18.64	18.37
Ca (%)	0.90	0.90	0.90	0.90
Available P (%)	0.37	0.37	0.37	0.37
Total Met (%)	0.34	0.34	0.34	0.34
Total Lys (%)	0.98	0.98	1.01	0.98
Total Thr (%)	0.69	0.69	0.71	0.69

Egg Collection. Cage-free nest box eggs (n = 54/treatment) were collected at 25, 29, 32, 36, 41, 44, and 49 weeks of age and placed in foam egg cartons. The eggs were packed into insulated shipping containers and shipped to the Egg Safety and Quality Research Unit (Athens, GA).

Egg Handling. Upon receipt, eggs were inspected and candled for cracks and other shell deformities and then placed in 4°C storage until testing the next day. Only intact eggs were used in the experiment. Eggs were divided into groups for physical quality testing (n = 24 eggs/treatment) and hard-cooking (n = 12 eggs/treatment). Eggs were removed from cold storage promptly before testing began, and the temperatures were monitored throughout the quality tests and ranged consistently between 6-9°C.

Physical Egg Quality. Physical egg quality tests included egg weight, volume of the shell, egg length and width, shell surface area, specific density, shell strength and elasticity, Haugh Unit, yolk index, vitelline membrane strength and elasticity, and shell thickness following methods as outlined by Jones et al. (2018). The eggs for hard-cooking were divided into weight categories (large, extra-large, and jumbo (USDA, 2000)) and hard-cooked in an InstantPot® (Duo 8 Quart InstantPot, Instant Brands Inc., Ottawa, Canada). To hard-cook eggs, 500 mL of water was placed into the stainless-steel pot. The bottom shelf of the egg stand was placed inside the stainless-steel pot, and 6 eggs were loaded into the egg stand air cell end up. The top egg stand with eggs was placed on top of the bottom egg stand, for a total of 12 eggs in the InstantPot® at once. The lid was placed on the InstantPot® and the valve was set to sealing. The "egg" button was pressed to hard-cook the eggs on high pressure. Large eggs were cooked for 5 minutes, extra-large eggs for 6 minutes, and jumbo eggs for 7 minutes. After the InstantPot® finished hard-cooking the eggs, the valve was released to "venting" to allow for immediate pressure release. After 3 minutes of venting, the "cancel" button was pressed and the lid was removed. The hard-cooked eggs were immediately placed in an ice water bath for 20 minutes. They were then removed from the ice bath, surface dried, and refrigerated overnight in sealed bags. The next morning, the shells and albumen were peeled off. A texture analyzer with a wire (TA-026,

Texture Technologies, Hamilton, MA) and a 500 g load cell was used to slice the yolk with a test speed of 0.5 mm/s, a slicing distance of 22 mm, and a trigger force of 5 g. Using a chromameter (CR-400, Konica Minolta, Tokyo, Japan), average L*a*b* color values were determined by taking one reading in the middle of each hard-cooked yolk half.

Statistical Analysis. Differences in egg quality were analyzed by two-way ANOVA using JMP 13 software (SAS Institute, 2017). The model included treatment and hen age as the main effects. Up to n = 168 intact eggs were analyzed for each treatment throughout the study for physical quality tests and up to n = 84 intact eggs were used for hard-cooking. The level of significance used for statistical analysis was $P < 0.05$.

RESULTS AND DISCUSSION

Egg Weight, Volume of the Shell, and Shell Surface Area

The interaction of hen age × treatment ($P < 0.001$) on egg weight, volume of the shell, and shell surface area is displayed in Figure 5.1. There was an initial sharp rate of increase in all three factors from 25-29 weeks of hen age due to the egg yolks getting larger and increasing in size and weight. However, this rate of increase was not observed for fish oil as with the other treatment groups. Egg weights from all treatment groups overall increased with hen age which has been previously reported (Whitehead et al., 1993; Akyurek and Okur, 2009; Zita et al., 2009). After 29 weeks of age, eggs from the flaxseed oil and control groups remained consistent in egg weight, volume of the shell, and shell surface area. However, eggs from the vitamin D treatment group fluctuated but still maintained the highest overall values out of all treatments for these physical quality factors. In a previous study, vitamin D enrichments added at a rate of 2,500 IU/kg resulted in an average egg weight of 63.3 g (Mattila et al., 2004). These findings were supported with the results of the current study where vitamin D added at a rate of 2,760 IU/kg resulted in an average egg weight of 63.5 g. Although these results are almost identical, it should be noted that Mattila et al. used caged laying hens instead of cage-free. Eggs from the fish oil treatment group consistently had the lowest overall values for egg weight, volume of the shell, and shell surface area (Table 5.3). The eggs from the fish oil treatment group were also considerably smaller than eggs from other treatment groups. Hens fed the fish oil treatment did not start laying extra-large sized eggs until 32 weeks of age, whereas hens from the other treatment groups were laying extra-large eggs by 29 weeks of age.

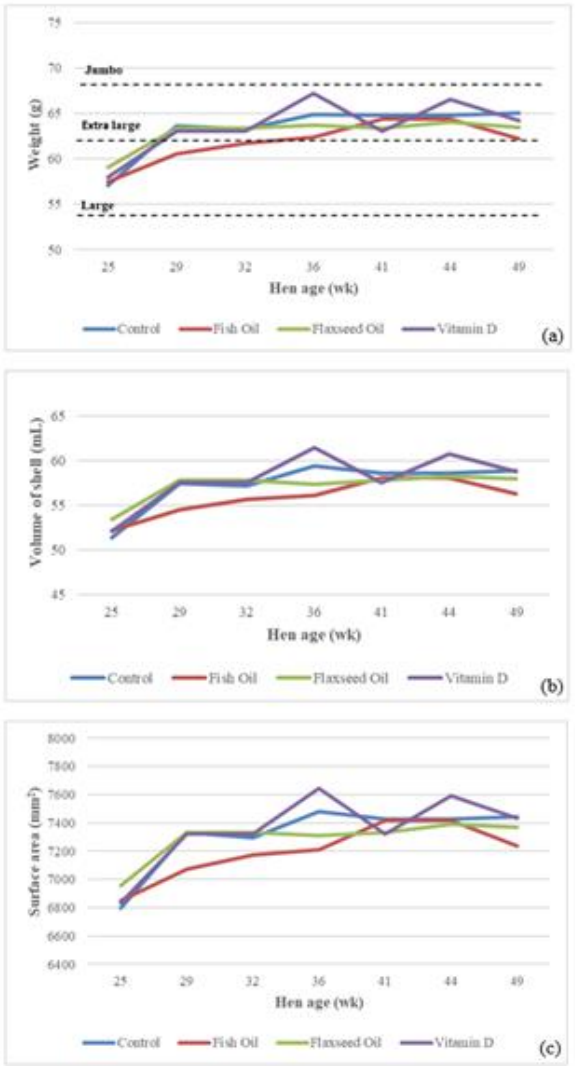

Figure 5.1. Impact of fish oil, vitamin D, and flaxseed oil treatments × hen age interaction ($P < 0.001$) on egg weight (a), volume of the shell (b), and shell surface area (c).

Shell Length and Width

Shell length and shell width were influenced by hen age × treatment interactions ($P < 0.001$; Figure 5.2). Although shell length increased throughout the laying period for all treatments, eggs from the vitamin D treatment fluctuated in shell length at 41 weeks of hen age. Shell width increased for all treatment groups except fish oil at the beginning of the laying cycle and then remained consistent throughout the rest of the laying period. The fish oil treatment resulted in eggs that significantly increased in egg length but remained constant in width as hens

aged. Because of this, egg shape index for eggs from the fish oil treatment consistently decreased throughout the duration of the experiment. Shell length at maximum width, a representation of overall egg shape, was not impacted by any interactions (Table 5.3). However, it was found to be affected individually by each of the effects. At 25 weeks of hen age, shell length at maximum width was greater than at any other point in the study ($P < 0.05$). This is a result of the eggs increasing in size at a faster rate of change that at other hen ages, as discussed previously. After 25 weeks, the shell length at maximum width decreased but then remained consistent throughout the remainder of the experiment, indicating that the point of maximum width shifted closer to the equator of the egg after 25 weeks of hen age.

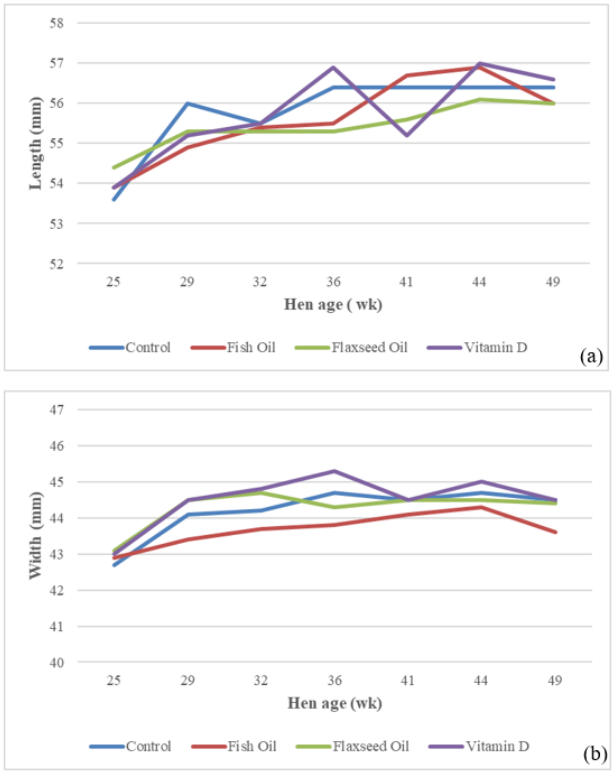

Figure 5.2. Impact of fish oil, vitamin D, and flaxseed oil treatments × hen age interaction ($P < 0.05$) on shell length (a) and shell width (b).

Specific Density

Egg specific density was impacted by hen age × treatment interactions ($P < 0.001$; Figure 5.3). Although significant differences were detected, each treatment group except fish oil experienced vast fluctuations in specific density throughout the duration of the laying cycle, leading to significant treatment by hen age interactions. Eggs from the fish oil treatment group had specific density values that did not change during the extent of the experiment.

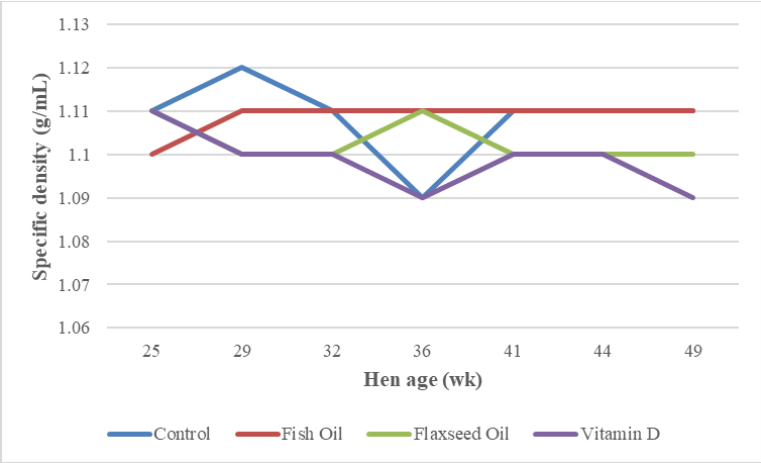

Figure 5.3. Impact of fish oil, vitamin D, and flaxseed oil treatments × hen age interaction ($P < 0.001$) on specific density.

Shell Strength, Elasticity, and Thickness

The interaction of hen age × treatment ($P < 0.05$) on shell strength and shell elasticity is displayed in Figure 5.4. Despite the variability in shell strength values throughout the duration of the laying period, there was an overall decrease in shell strength for all treatments from 5826.0 g force to 5252.8 g force (Table 5.4). Eggs from the fish oil and vitamin D treatment groups had almost identical shell strength values from 36 to 49 weeks. Overall, shell elasticity remained consistent for all treatment groups throughout the duration of the laying period. This indicates that shell elasticity was not impacted by decreasing shell strength. Eggs from the flaxseed oil

treatment had thicker eggshells than eggs from other treatment groups (Table 5.4), although this is not a difference that would be detectable by consumers.

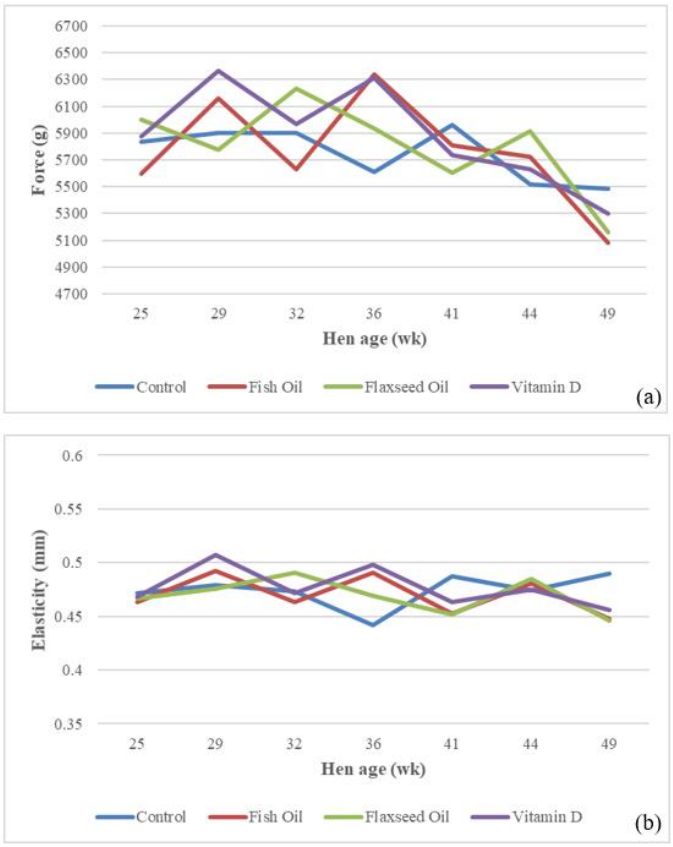

Figure 5.4. Impact of fish oil, vitamin D, and flaxseed oil treatments × hen age interaction ($P < 0.01$) on shell strength (a) and shell elasticity (b).

Haugh Unit and Yolk Index

Haugh unit values were impacted by hen age × treatment interactions ($P < 0.001$; Figure 5.5). There was an overall decrease in HU scores for all treatment groups except for flaxseed oil as hens aged, but no differences in HU scores between treatment groups were found (Table 5.4). Eggs from all treatments remained within the US Grade AA category (USDA, 2000). Yolk index was impacted by hen age and treatment (Table 5.4). Hens at 25 weeks of age were producing eggs with the greatest yolk index values, as they were still coming into production and producing

smaller yolks. Yolk height remained consistent as hens aged, although the yolk width increased, causing a decrease in yolk index by 49 weeks of age (Table 5.4).

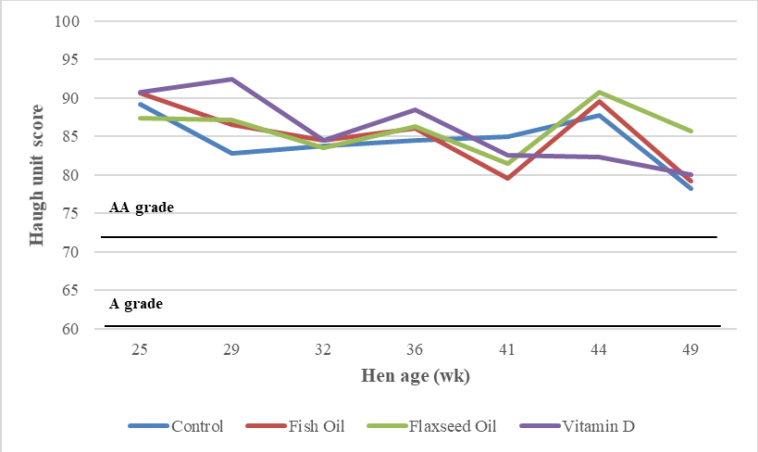

Figure 5.5. Impact of fish oil, vitamin D, and flaxseed oil treatments × hen age interaction ($P < 0.05$) on mean Haugh unit score.

Vitelline Membrane Strength and Elasticity

Vitelline membrane strength and elasticity were both impacted by hen age × treatment interactions ($P < 0.001$; Figure 5.6). The trend for each treatment is similar for both VMS and VME, indicating that VME was impacted by the VMS in this study ($P < 0.001$). The vitamin D treatment resulted in yolks with the greatest VMS and VME, while the flaxseed oil treatment resulted in the weakest yolks and lowest elasticity values overall (Table 5.5). This finding is supported by previous work that found omega-3 enriched eggs to have weaker vitelline membranes than eggs from other diet enrichments (Dunn-Horrocks et al., 2011).

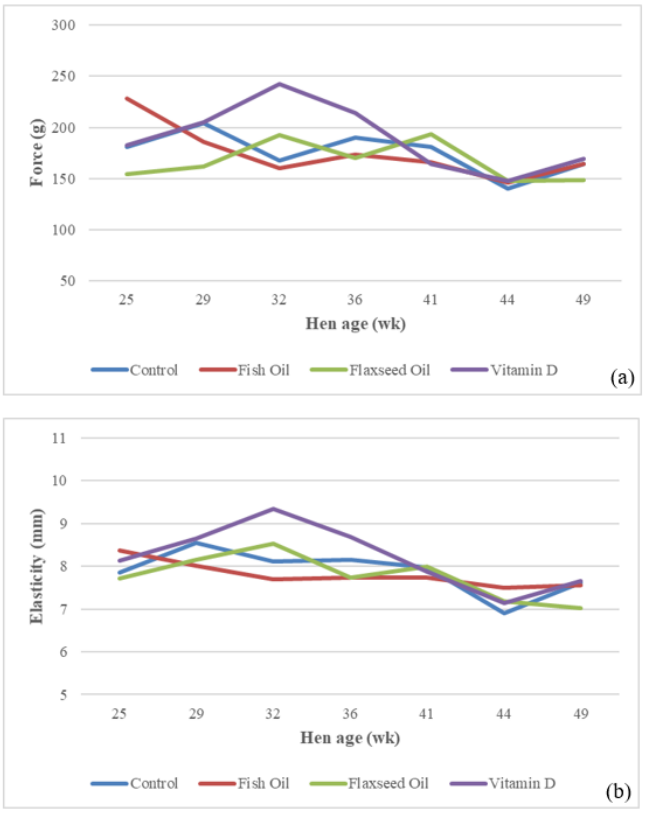

Figure 5.6. Impact of fish oil, vitamin D, and flaxseed oil treatments × hen age interaction ($P < 0.05$) on vitelline membrane strength (a) and vitelline membrane elasticity (b).

Hard-Cooked Yolks

Hard-cooked yolk texture profile, the total amount of work required to slice through the hard-cooked yolk, was found to differ between treatment groups (Table 5.6). The greatest amount of energy was needed to slice through hard-cooked yolks from the flaxseed oil and vitamin D treatments, whereas the least amount of energy was needed to slice hard-cooked yolks from the fish oil treatment group. This indicates that hard-cooked yolks from eggs in the fish oil treatment are softer than those from other treatments. As hens aged, hard-cooked yolks became harder to slice ($P < 0.001$).

Peak force, the point of maximum force required to slice through the hard-cooked yolk, was impacted by hen age × replicate interactions ($P < 0.001$; Figure 5.7). Eggs from the flaxseed

oil treatment maintained similar peak force values as the control group as hens aged. However, hard-cooked eggs from the fish oil treatment had the lowest peak slicing values throughout the duration of the laying period while eggs from the vitamin D treatment group had the greatest (Table 5.6). Peak distance, the point where peak force is detected, was only impacted by hen age ($P < 0.001$; Table 5.6). Despite significant differences, peak distance varied throughout the laying period.

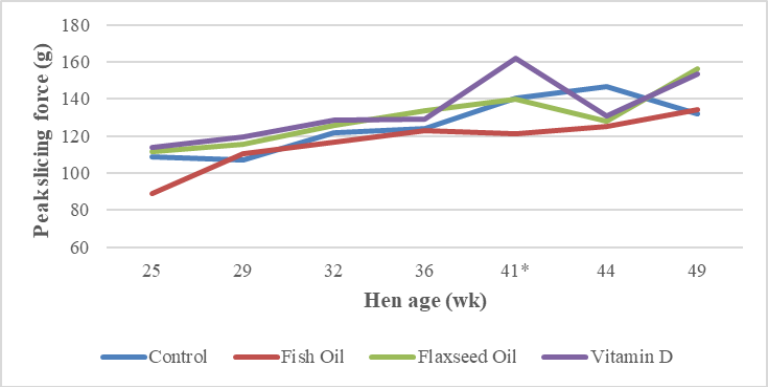

Figure 5.7. Impact of fish oil, vitamin D, and flaxseed oil treatments × hen age interaction ($P < 0.001$) on hard-cooked yolk peak slicing force.

Hard-cooked yolk color L*a*b* values were measured, and differences were detected (Table 5.6). The L* and a* values were only impacted by hen age. Yolks naturally become lighter when cooked due to the denaturation of proteins, but they were observed to also become lighter with hen age, as indicated by the increasing L* values throughout the laying period from 85.98 to 88.34 ($P < 0.001$). Differences in red and green color pigmentation were also detected from the a* value results, however, no obvious trends were detected. The b* value of hard-cooked yolks was influenced by hen age × treatment interactions ($P < 0.001$; Figure 5.8). Hard-cooked yolk b* values from all treatments indicate that yolks were gaining more blue pigmentation and less yellow pigmentation as hens aged, a finding that supports the L* value results. The vitamin D treatment resulted in hard-cooked yolks that were more yellow in color

from 25 to 36 weeks of hen age. The post-peak production yellow coloration then equalized between the treatments.

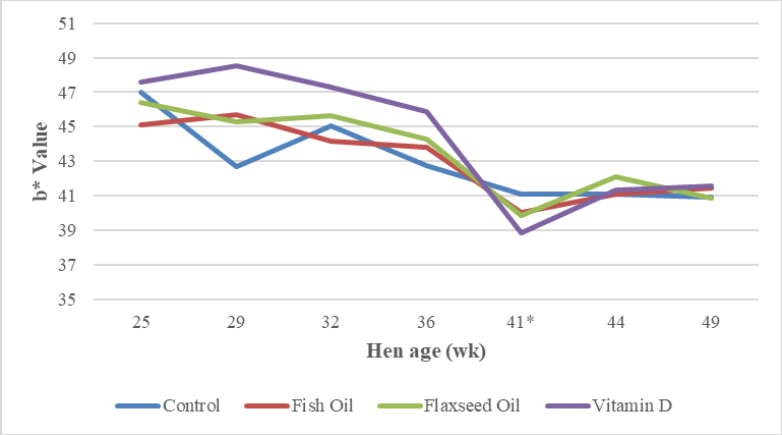

Figure 5.8. Impact of fish oil, vitamin D, and flaxseed oil treatments × hen age interaction ($P < 0.001$) on hard-cooked yolk color (difference in blue and yellow color pigmentation).

Overall, it was found that fish oil negatively impacted egg quality while vitamin D improved it. If producers are looking for ways to enrich eggs with omega fatty acids, flaxseed oil should be used as an alternative to fish oil, as it did not hinder egg quality.

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Table 5.3: Overall effect of treatment and hen age on physical egg quality factors average values from 25-49 weeks of age[1].

	Egg Weight (g) ± SD	Egg Volume (mL) ± SD	Shell Surface Area (mm^2) ± SD	Shell Length (mm) ± SD	Shell Width (mm) ± SD	Egg Shape Index ± SD	Shell Length at Maximum Width (%) ± SD	Specific Density (g/mL) ± SD
Fish Oil	61.9 ± 4.6	55.9 ± 4.1	7197.0 ± 359	55.6 ± 1.9	43.7 ± 1.1	78.6^{c} ± 2.2	53.4^{b} ± 1.5	1.108 ± 0.01
Flaxseed Oil	62.9 ± 3.8	57.2 ± 3.9	7288.6 ± 307	55.5 ± 1.9	44.3 ± 1.0	79.9^{a} ± 2.2	53.9^{a} ± 1.5	1.100 ± 0.01
Vitamin D	63.6 ± 4.0	57.9 ± 3.5	7352.4 ± 340	55.7 ± 1.6	44.5 ± 1.0	79.9^{a} ± 1.9	53.7^{ab} ± 1.5	1.098 ± 0.01
Control	63.3 ± 4.4	57.4 ± 4.2	7314.9 ± 355	55.8 ± 1.7	44.2 ± 1.1	79.2^{b} ± 2.4	53.9^{a} ± 1.4	1.105 ± 0.01
P-Value						0.001	0.01	
25	57.9 ± 3.2	52.3 ± 2.9	6860.3 ± 255	53.9 ± 1.3	42.9 ± 0.8	79.6^{ab} ± 1.9	54.2^{a} ± 1.4	1.108 ± 0.01
29	62.6± 3.5	56.8 ± 3.4	7264.2 ± 292	55.4 ± 1.6	44.1 ± 1.0	79.8^{a} ± 2.3	53.7^{b} ± 1.4	1.103 ± 0.01
32	62.9 ± 3.4	57.0 ± 3.1	7280.3 ± 265	55.4 ± 1.4	44.2 ± 0.9	79.9^{a} ± 2.1	53.6^{b} ± 1.5	1.102 ± 0.01
36	64. ± 4.2	58.0 ± 4.1	7411.7 ± 348	56.1 ± 1.7	44.5 ± 1.1	79.4^{ab} ± 2.1	53.6^{b} ± 1.5	1.102 ± 0.01
41	63.9 ± 3.6	58.0 ± 3.3	7374.1 ± 276	55.9 ± 1.7	44.4 ± 0.9	79.5^{ab} ± 2.4	53.6^{b} ± 1.5	1.102 ± 0.01
44	64.9 ± 3.8	58.9 ± 3.6	7456.4 ± 295	56.6 ± 1.6	44.5 ± 1.0	78.8^{b} ± 2.2	53.8^{b} ± 1.5	1.102 ± 0.01
49	63.8 ± 3.7	58.0 ± 3.5	7370.3 ± 301	56.3 ± 1.7	44.3 ± 1.0	78.7^{b} ± 2.4	53.8^{b} ± 1.5	1.100 ± 0.01
P-Value	**	0.001	**	**	**	0.003	0.04	**

[a-c:] Means within a column with similar subscripts are not significantly different; $P < 0.05$.

[1]n = 168/treatment; n = 96/hen age

**: Hen age × treatment interaction; $P < 0.05$.

Table 5.4: Overall effect of treatment and hen age on physical egg quality factors average values from 25-49 weeks of age.

	Shell Strength (g)[1] ± SD	Shell Elasticity (mm)[1] ± SD	Shell Thickness (um)[2] ± SD	Haugh Unit[3] ± SD	Yolk Index[3] ± SD	Yolk Height (mm)[3] ± SD	Yolk Width (mm)[3] ± SD
Fish Oil	5763.2 ± 805	0.47 ± 0.05	43.2^{ab} ± 2.8	85.2 ± 7.5	0.536^{b} ± 0.04	21.0^{a} ± 1.0	39.3^{b} ± 2.5
Flaxseed Oil	5798.5 ± 943	0.47 ± 0.05	43.9^{a} ± 2.7	86.1 ± 6.1	0.548^{a} ± 0.04	21.3^{ab} ± 1.1	38.9^{bc} ± 1.8
Vitamin D	5887.9 ± 876	0.48 ± 0.05	43.2^{ab} ± 2.1	85.9 ± 7.6	0.541^{ab} ± 0.04	21.5^{c} ± 0.9	39.8^{a} ± 1.7
Control	5745.8 ± 834	0.47 ± 0.06	43.0^{b} ± 2.5	84.3 ± 7.1	0.545^{ab} ± 0.04	21.1^{bc} ± 1.0	38.8^{c} ± 2.4
P-Value			0.01		0.01	0.05	0.05
25	5826.0	0.47 ± 0.05	43.4^{ab} ± 2.7	89.5 ± 5.9	0.566^{a} ± 0.03	21.1^{cd} ± 0.8	37.3^{e} ± 1.7
29	6053.9	0.49 ± 0.05	43.4^{ab} ± 2.5	87.2 ± 6.6	0.555^{a} ± 0.03	21.6^{ab} ± 0.9	39.0^{cd} ± 1.2
32	5929.3	0.48 ± 0.05	43.7^{a} ± 2.0	84.0 ±6.6	0.554^{a} ± 0.03	21.8^{a} ± 0.9	39.3^{bc} ± 1.6
36	6048.7	0.48 ± 0.05	43.5^{ab} ± 2.4	86.3 ± 6.2	0.520^{b} ± 0.03	20.7^{d} ± 0.9	39.9^{ab} ± 1.5
41	5776.8	0.46 ± 0.05	43.0^{bc} ± 2.3	82.0 ± 7.6	0.530^{b} ± 0.04	21.3^{c} ± 1.0	40.1^{ab} ± 3.7
44	5695.7	0.48 ± 0.06	43.7^{a} ± 2.3	87.7 ± 6.6	0.554^{a} ± 0.03	21.3^{c} ± 1.2	38.4^{d} ± 1.4
49	5252.8	0.46 ± 0.06	42.5^{c} ± 2.5	80.7 ± 6.2	0.517^{b} ± 0.03	20.8^{d} ± 1.0	40.2^{a} ± 1.4
P-Value	**	**	0.01	**	0.001	0.05	0.05

[a-d:] Means within a column with similar subscripts are not significantly different; $P < 0.05$.

[1]n = 168/treatment; n = 96/hen age

[2]n = 166/treatment; n = 95/hen age

[3]n = 162/treatment; n = 88/hen age

**: Hen age × treatment interaction; $P < 0.05$.

Table 5.5: Overall effect of treatment and hen age on vitelline membrane strength and elasticity average values from 25-49 weeks of age[1].

	Vitelline Membrane Strength (g) ± SD	Vitelline Membrane Elasticity (mm) ± SD
Fish Oil	175.1 ± 62.3	7.81 ± 1.1
Flaxseed Oil	167.0 ± 65.9	7.78 ± 1.2
Vitamin D	190.9 ± 74.2	8.25 ± 1.3
Control	176.1 ± 65.8	7.90 ± 1.2
25	185.9 ± 71.7	8.02 ± 1.1
29	188.4 ± 69.6	8.33 ± 1.1
32	192.0 ± 78.5	8.45 ± 1.1
36	187.2 ± 55.2	8.08 ± 1.0
41	176.2 ± 58.1	7.90 ± 1.0
44	145.3 ± 62.0	7.19 ± 1.3
49	162.0 ± 65.0	7.47 ± 1.1
P-Value	**	**

[a-e:] Means within a column with similar subscripts are not significantly different; $P < 0.05$.

[1]n = 146/treatment; n = 84/hen age

Table 5.6: Overall effect of treatment and hen age on hard-cooked yolk physical egg quality factors average values from 25-49 weeks of age[1].

	Texture Profile (g.mm) ± SD	Peak Force (g) ± SD	Peak Distance (mm) ± SD	Yolk Color L* Value ± SD	Yolk Color a* Value ± SD	Yolk Color b* Value ± SD
Fish Oil	$1680.1^{b} \pm 316$	117.4 ± 21.7	10.99 ± 1.5	87.26 ± 2.0	-4.991 ± 0.53	43.46 ± 2.6
Flaxseed Oil	$1860.2^{a} \pm 353$	129.9 ± 24.2	11.25 ± 2.1	86.93 ± 2.3	-4.998 ± 0.55	43.47 ± 3.0
Vitamin D	$1885.7^{a} \pm 378$	133.1 ± 25.9	11.38 ± 1.6	86.80 ± 2.4	-4.954 ± 0.53	44.84 ± 3.7
Control	$1791.3^{ab} \pm 379$	125.2 ± 21.7	11.12 ± 2.1	87.17 ± 1.8	-5.106 ± 0.50	43.03 ± 2.9
P-Value	0.001		NS	NS	NS	
25	$1525.4^{e} \pm 88$	106.3 ± 18.7	$10.66^{a} \pm 1.3$	$85.98^{ab} \pm 2.2$	$-4.962^{ab} \pm 0.58$	46.40 ± 2.2
29	$1620.9^{de} \pm 269$	113.0 ± 16.6	$11.08^{a} \pm 1.4$	$85.58^{a} \pm 2.2$	$-5.237^{bc} \pm 0.59$	45.57 ± 3.0
32	$1715.9^{cde} \pm 243$	122.9 ± 14.8	$11.12^{a} \pm 1.8$	$86.56^{ab} \pm 1.6$	$-4.948^{ab} \pm 0.46$	45.50 ± 2.2
36	$1774.1^{cd} \pm 268$	127.3 ± 21.9	$10.63^{a} \pm 1.9$	$88.32^{c} \pm 1.2$	$-4.967^{ab} \pm 0.35$	44.18 ± 2.3
41[#]	$1974.2^{ab} \pm 365$	140.9 ± 24.4	$12.60^{b} \pm 1.8$	$86.73^{abc} \pm 1.0$	$-5.501^{c} \pm 0.29$	39.84 ± 1.5
44	$1879.9^{bc} \pm 333$	132.9 ± 21.8	$10.92^{a} \pm 1.6$	$87.08^{b} \pm 2.8$	$-4.985^{ab} \pm 0.67$	41.36 ± 2.0
49	$2162.2^{a} \pm 361$	144.0 ± 24.0	$11.58^{ab} \pm 2.1$	$88.34^{c} \pm 1.1$	$-4.852^{a} \pm 0.41$	41.20 ± 2.0
P-Value	0.001	**	0.001	0.001	0.001	**

[a-e:] Means within a column with similar subscripts are not significantly different; $P < 0.05$.

[1]n = 84/treatment; 48/hen age, except at 41 weeks

[#]Due to unforeseen circumstances, many of the eggs from the week 41 collection arrived broken. There were only 17 total eggs available for hard-cooked analysis from all treatments instead of 48. Any variation in results at this age for hard-cooked data could possibly be due to this discrepancy.

**: Hen age × treatment interaction; $P < 0.05$.

CHAPTER 6

SUMMARY OF THESIS

Based on the results of this research, NFM does have the potential to significantly reduce egg quality, which aligns with previous studies regarding the negative impact NFM have on hens and egg quality. Adhering to strict biosecurity measures and management practices can reduce the chance of NFM infestation and maintain optimum egg quality that consumers desire.

Other results of this research indicate that fish oil supplementation into a hen's diet did not prove to be the best source of omega fatty acids due to the significantly lower egg quality results than other treatment groups. Other research studies support these findings and suggest other sources of omega fatty acids like flaxseed oil should be used instead. Although maintaining the minimum laying hen dietary requirements provided by the NRC is a must, understanding what additional supplements can further improve egg quality is beneficial to both the producer and the consumer.