A COMPARATIVE STUDY OF THE EFFECTS OF MICROALGAE ON POULTRY PRODUCTION

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Taylor Kaitlyn O'Lear Reid

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COMMITTEE MEMBERSHIP

TITLE: A Comparative Study of the Effects of Microalgae on Poultry Production

AUTHOR: Taylor Kaitlyn O'Lear Reid

DATE SUBMITTED: June 2022

COMMITTEE CHAIR: Darin C. Bennett, Ph.D.
Associate Professor, Poultry Science

COMMITTEE MEMBER: Zach McFarlane, Ph.D.
Assistant Professor, Beef Cattle Specialist

COMMITTEE MEMBER: Siroj Pokharel, Ph.D.
Assistant Professor, Meat Food Safety

ABSTRACT

A Comparative Study of the Effects of Microalgae on Poultry Production

Taylor Kaitlyn O'Lear Reid

Research has shown Spirulina to be a viable feedstuff in poultry production. Other species of microalgae are being studied to determine their effects on poultry production. This project compared four microalgaes: Hydrodictyon, Vaucheria, Uronema, and Spirulina and was broken down into three trials. The first experiment investigated the digestibility of an algae species, Spirulina, in broiler chicken diets. Eighty, 25-day-old Ross 708 broiler chicks were sorted into 20 metabolism cages and received one of two diets: Control Diet or Test Diet (75% basal diet + 25% Spirulina). The apparent ileal amino acid digestibility (AIAAD) of lysine, methionine, cysteine, threonine, isoleucine, valine and arginine in Spirulina was 94.5%, 91.3%, 56.1%, 71.4%, 76.8%, 69.8% and 90.4% respectively. Apparent metabolizable energy (AME) of Spirulina was 2279 kcal/kg. The second experiment focused on digestibility of Spirulina and novel algae species in laying hens. Sixty, 80-week-old Lohman LSL-Lite laying hens were randomly assigned to Control Diet, Hydrodictyon Test Diet (87.5% basal diet + 12.5% hydrodictyon), 12.5% or one of three other test diets (75% basal diet + 25% Spirulina, Uronema, and Vaucheria). Spirulina diets contained the highest crude protein (25.5%) and methionine levels (0.68%). Uronema had the highest gross energy content of 3880.5 kcal/kg and lysine levels (1.12%). Hydrodictyon diet was the most nutritionally similar to the control. Crude protein was at 15.3% and 16.6% for control and Hydrodictyon respectively. Gross energy was 3429.6 kcal/g in control versus 3316.1 kcal/kg in Hydrodictyon. Lastly, crude fat was 2.7% compared to Hydrodictyon at 2.4%.

The last trial was to determine the effects of the addition of microalgae in the diet on laying hen production. Seventy-eight, 23-month-old laying hens were assigned to 1 of 3 of the following experimental diets: control, control + 1% microalgae, or control + 2% microalgae. Microalgae, regardless of inclusion level, had no significant effect on egg weight in this study. Increasing level of inclusion of Spirulina, Hydrodictyon, and Uronema from 0 to 2 % significantly increased yolk color. Vaucheria influenced yolk color at the 2% level. No significance differences shown from any of the algae species regardless of inclusion level for egg weight, egg mass, shell thickness, shell strength, shell weight, albumen weight, yolk weight, and albumen height. In addition, there was no significant difference in feed conversion ratio, feed intake, body weight, or tibial strength between the treatments. However, as these are novel algaes, further studies are required to determine the true usability in poultry production.

Keywords: microalgae, broiler, layer, production, digestibility, egg quality

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Chapter 1

INTRODUCTION

Organizations in human food production are researching ways to increase the efficiency and sustainability of their respective products. The poultry industry is one of the top sources of animal protein, as both the resources and time required to bring a bird to full production are lower than other animal agriculture systems. Broilers, swine, and beef production take 7 weeks, 26-28 weeks, and 1 year respectively (Stringham et al., 2022; Dewitt and Droppo, 2021; Beck and Lalman, 2021). One of the biggest problems facing poultry producers today is feed cost. This can be attributed in part to competition over feedstuffs with other industries, environmental factors, cost of the ingredient, and geographic accessibility to feedstuffs. One way to mitigate this cost is to discover alternative feed ingredients that either (1) reduce feed cost or (2) increase final product value. Microalgae is one such ingredient due to its high protein content and presence of natural pigments such as carotenoids and xanthophyl. High protein feedstuffs are important for poultry production, as broilers require 10-23% crude protein in the diet and layers require 15,000mg (or 12.5%) per grams fed per day (Applegate and Angel, 2008). Whereas the pigmentation affects the yolk Roche color score, a system that ranges from 1 being pale to 15 being a dark orange color (Vuilleumier, 1969). The natural pigments not only act as a natural colorant that can affect yolk color, but they also provide health benefits as well. Studies have shown that inclusion of carotenoids in poultry diets can improve production performance, health, and egg and meat quality (Nabi et al., 2020). In addition, carotenoids have anti-inflammatory, antibacterial, and antidiabetic effects (Nabi et al., 2020). Spirulina, a microalgae/bacterium, has already been utilized as an ingredient in human food production; however, few studies have been conducted on these organisms for use in agriculture. For this project, the following novel microalgae species were utilized: Spirulina, Uronema, Vaucheria, and Hydrodictyon. A comparative experiment was completed to understand the microalgae species' respective nutrient digestibility and the effects on egg layer production when compared with the standard US diet. First, we determined the apparent digestibility of the algae. This determined how much of the nutrients present in the algae is being utilized by the hens versus waste excretion. Then, with the proper level of incorporation, laying hens were provided experimental diets to determine the effects on egg production and quality. The goal of this research is to determine whether novel microalgae species are suitable feed ingredients that will provide essential nutrients to layer and broiler production. Spirulina, a commonly studied microalgae, will be included as a foundation for comparison.

Chapter 2
LITERATURE REVIEW

2.1 The Poultry Industry

The world's population is increasing every day, with the estimated population to reach 9.8 billion by 2050 (Department of Economic and Social Affairs, 2017). Food shortages are already prevalent worldwide, causing producers to search for more efficient animal-based protein to meet this ever-increasing demand. The poultry industry is the most produced meat product worldwide at 127 million tons per year, with the United States being the largest supplier (Ritchie and Roser, 2019). The main factors that make poultry an ideal animal product is the fast turnover rate, reduced land usage, lower environmental impact, and feed efficiency.

According to a 2012 USDA report, the average daily rate of lay was 78.5 eggs per 100 layers (National Agricultural Statistics Service, 2012). The United States had a total of 386 million layers alone in 2012 and produced a total of 7.93 billion table eggs (National Agricultural Statistics Service, 2012). Today's broiler chicken can achieve a 5-pound market weight in five weeks compared with forty years ago, where a broiler took 10 weeks to achieve a similar weight (Clauer, 2012). This high rate of production in both segments of the poultry industry, make the poultry industry a prime candidate for helping with world food shortages. In addition, the poultry industry requires less land coupled with a lower environmental footprint when compared with other animal protein industries. Land use in meters squared per kilogram of food product produced is lowest in broiler meat at 12.22 m^2 and egg production at 6.27 m^2 compared with beef production at an average of 326.21 m^2 (Poore and Nemecek, 2018; Ritchie and Roser, 2021). As for land use per 100 grams of animal protein product, beef cattle are highest at 163.6 m^2, followed by swine at 10.7 m^2, broilers at 7.1 m^2, and layers at 5.7 m^2. (Poore and Nemecek, 2018; Ritchie and Roser, 2021). The reduced requirement for land is important not only for poultry producers, but for the planet as well. Poultry production has a lower impact on greenhouse gas emission compared with other animal-based protein sources. This reduction is attributed to the ratio between Greenhouse gas emission to the feed and nutrient utilization efficiency of poultry. Overall, swine and poultry have similar rates, with 12 $kgCO_2eq$ (emission in kg of CO_2 equivalents per kg of food) for chicken and 14 $kgCO_2eq$ for pork (Poore and Nemecek, 2019; Ritchie and Roser, 2021). Research has shown that beef production is highest at 59.6 $kgCO_2eq$, with poultry meat and egg production at 6.1 and 4.5, respectively (Poore and Nemecek, 2018). Compared to other agricultural industries, poultry production does not only utilize less land space, but also the quantity of feed consumed. This can be seen when comparing the feed conversion ratio (FCR) of the various animals.

Feed conversion ratio (FCR) is the amount of feed required to produce 1 kilogram of animal product (Alexander et al., 2016; Ritchie and Roser, 2019). Studies comparing the FCR of beef, swine, broilers, and laying hens states that the feed consumed was 25 kg, 6.4 kg, 3.3 kg, and 2.3 kg, respectively (Alexander et al., 2016; Ritchie and Roser, 2019). In addition, poultry have both a higher level of protein and energy efficiency. This means an increase in output, such as body weight gain, with a lower input of nutrients such as amino acids and carbohydrates. For protein efficiency, egg production is highest at 25 %, followed by poultry for meat at 19.6 %, pork at 8.5 %, and beef cattle at 3.8 % (Alexander et al., 2016; Ritchie and Roser, 2019). Energy efficiency (caloric inputs vs outputs) follows the same pattern with eggs at 19 %, poultry meat at 13 %, pork at 8.6 %, and beef at 1.9 % (Alexander et al., 2016; Ritchie and Roser, 2017). With the ever-increasing need for food with a dwindling amount of available crop land, poultry production is an avenue to provide animal-based protein in an efficient manner. However, there is one major problem affecting the poultry industry as a whole: feed cost.

2.2 Alternative Feedstuffs

The poultry industry is trying to meet this demand but are hindered by the ever-changing cost of feed, as total feed cost constitutes 60-70 % of the final production cost (Thirumalisamy et al., 2016). Feed cost has been steadily increasing in response to crop availability which is due to a variety of factors including seasonal changes, competition over feed stuffs, and geographic location. Available farmland is decreasing on a global scale creating further competition with other food-producing animals as they rely heavily on corn and soybean meal (Austic et al., 2013). Meanwhile, food-producing animals rely heavily on soybean meal (SBM) and corn to meet their protein and energy requirements, creating a direct competition of these two commodities for human consumption (Austic et al., 2013). To combat this, producers are looking at alternative feedstuffs that will provide similar energy and nutritional benefits of the ingredient they are replacing, while also focusing on reducing costs and increasing final product value. One such alternative is microalgae, with Spirulina already utilized as a natural protein supplement in human food production.

2.3 Spirulina

Spirulina is a filamentous, blue-green algae that has a fast growth rate and relative ease of cultivation. The nutritional composition consists of lipids such as omega-3-fatty acids, crude proteins and amino acids, and carotenoids which can act as both a natural pigment and antioxidant (Altmann et al., 2018). However, Spirulina has displayed varying nutritional values across studies found in the literature, suggesting the inconsistent value as an alternative feedstuff. One consistent factor in the literature is that studies have shown Spirulina can substitute the use of soybeans due to the higher crude protein content present.

As stated previously, the average broiler and layer chicken require 10-23 % crude and 12.5 % respectively (Applegate and Angel, 2008). Spirulina contains 58.8 % crude protein, but the lipid content was 4.3% (Altmann et al., 2018). In some cases, the crude protein content of Spirulina was higher, to be between 65 %-70 % (Contreras et al., 1979; Kay and Barton, 1991). However, the crude protein has been described to be particularly low at 26.56 % (Alvarenga et al., 2011). The values change based on the developmental stage of the bird; however Spirulina has also presented higher levels of essential amino acids such as methionine, lysine, and threonine (Alvarenga et al., 2011). In addition, the gross energy content, and apparent metabolizable energy content of Spirulina was higher by 9.6% and 9.8% respectively. (Alvarenge et al., 2011) Due to the high nutritive value of Spirulina, studies have shown that it can be used to replace some of the vitamin and mineral premixes currently present in the poultry industry (Selim et al., 2018). This can be seen as another benefit of Spirulina, as consumers are moving away from synthetic products in favor of natural additives.

2.4 Broiler Production

Due to the high concentration of crude protein present in Spirulina (50-70 %, Altmannn et al., 2018; Contreras et al., 1979; Kay and Barton, 1991), it is seen as a strong candidate for broiler birds as they require a diet with 10-23 % crude protein to sustain their growth potential (Applegate and Angel, 2008). Cobb 500 broilers fed diets containing 2, 4, and 8 g/kg Spirulina had both a significant increase in body weight ($P<0.05$) and significant decrease in FCR ($P<0.05$) when compared to control at 0 g/kg (Jamil et al., 2015). An increase in average daily weight gain has been observed over the course of 42 days for Hubbard broilers supplemented Spirulina (51.42 ± 0.49) compared with control birds (49.32 ± 0.52) (Kaoud, 2012). In addition, FCR was lowered in groups fed Spirulina (1.78) than the control broilers (1.88) (Kaoud, 2012). However, a separate study stated that birds fed Spirulina at 2 g/kg had the highest BW, improved FCR, and final carcass yields (total carcass 4.9 %, front 6.4 %, edible parts 4.4 %) (Abou-Zeid et al., 2015). On the other hand, Spirulina in some trials has shown little to no effect on broiler performance. The effects of Spirulina in broiler chickens fed 40g or 80g showed that body weight did not very between the Spirulina groups and the control diet (Toyomizu et al., 2001). Another study determined that with a substitution of 50% soybean with Spirulina, there were minor differences in carcass indicators such as carcass weight, breast yield, and percent protein of the breast fillet between the groups and little to no changes in meat quality (Altmann et al., 2018). However, when replaced with 7.5 % & 10 % of the control diet with Spirulina, an initial adverse effect on feed intake and body weight gain (Austic et al., 2013) indicating a possible max inclusion level of Spirulina (discussed further on).

One factor that showed the most significant change across the literature was Spirulina’s natural colorant. Like yolk color, the color of raw meat is a factor consumers consider when selecting products with natural colorants becoming more popular due to the growing negative perception to synthetic goods (Toyomizu et al., 2001). Broilers fed Spirulina had flesh that was “orange-yellow” in color versus the “pale yellow” flesh of the wheat-based control diet (Toyomizu et al., 2001). In addition, the color change was present in the breast and thigh muscles (Toyomizu et al., 2001) and in some cases had a darker red tone when compared to control birds (Altmann et al., 2018). Spirulina has shown levels of success in broiler production; however, further research is needed to determine the circumstances in which it would be beneficial to broiler producers.

2.5 Layer Production

Similar to the broiler studies, the use of Spirulina has had a variety of results on laying hen production. Spirulina in some cases has been shown to positively affect the food conversion ratio and body weight gain in laying hens (Bonos et al., 2016). Including Spirulina at as low as 0.3 % concentration improved overall body weight, feed intake, egg mass, and egg production (Selim et al., 2018). In addition, it has been determined that as levels of Spirulina increased, it caused a linear effect on body weight (Selim et al., 2018). However, Spirulina had no significant effect on FCR, feed intake, or egg production when fed at 0.5 %, 1 %, and 2 % concentration (Dogan et al., 2016). Further studies have shown that Spirulina has had a mixed effect on egg quality characteristics. For example, two separate studies by Zahroojian at al. (2011; 2013) stated that the addition of Spirulina, when included at 1.5 %, 2 %, and 2.5 %, had no effect on egg quality. These results fall in line with a similar where Spirulina had no significant effect on yolk weight, albumen weight (egg white), shell weight or Haugh unit score (ratio between the height of the albumen compared to the weight of the egg) (Selim et al., 2018). However, hens fed *Spirulina platensis* had a higher shell thickness, which illustrated linear effect as Spirulina concentration increased (Selim et al., 2018). A separate trial saw similar results with shell thickness, however there was an increase in egg and albumen weight, and albumen height with the inclusion of Spirulina (Omri et al., 2019). One study determined that Spirulina had significant effects on egg albumen index, egg yolk index, eggshell thickness, and eggshell weight (Dogan et al., 2016). No significant differences on shell strength, shell weight, shell thickness, shape index, and albumen index were demonstrated when incorporated at 1 % or 2 % concentration (Curabay et al., 2021).

One aspect of egg quality that previous studies on Spirulina showed the most effect on was yolk color. Desired yolk color can vary based on regional preferences. The United States standard color is around an 8.5 while Europe and Asia prefer a score of 12 (Zahroojian et al., 2011). Yolk color is based on the amount of xanthophyll and carotenoid pigments in the feedstuffs used to formulate the diet. Countries with a corn-based diet will have a higher yolk score compared with wheat-based diets. Marigold, safflower, and synthetic pigments are utilized by the industry to increase the yolk score (Rowghani et al., 2006; Zahroojian et al., 2011). One study compared BASF Lucantin®, a common synthetic pigment, to Spirulina in wheat-fed chickens (Zahroojian et al., 2011). There was a significant increase in yolk color score from groups that were fed Spirulina and the synthetic pigment compared with the control group (Zahroojian et al., 2011). However, there was no significant difference between Spirulina at 2.5 % and the synthetic pigment, which indicated Spirulina could be a replacement to said pigment (Zahroojian et al., 2011). Studies have shown a strong positive relationship between the concentration of Spirulina added and the resulting yolk color, increasing with every level of inclusion (Selim et al., 2018; Anderson et al., 1991; Curabay et al., 2021; Omri et al., 2019). Another study involving Japanese quail illustrated that inclusion level of 1.0% achieved optimal yolk color compared to groups with 0.25 %, 0.5 %, 2.0 % and 4.0 % (Anderson et al., 1991). Contradictory data determined that the highest Roche color score was achieved by the inclusion of 2 % Spirulina (Curabay et al., 2021). The xanthophylls, carotinoids, β-carotene, and vitamin E also act as natural antioxidants (Altmann et al., 2018).

The difference in results may be attributed to the differing egg layer breed used between the studies. Selim et al. (2018) used Norfa laying hens which is a cross between White Leghorn × Fayoumi, Omri et al. (2019) used Lohman white hens, while Zahrojian et al. (2013) utilized Hyline W36 hens. Another factor that may have led to the discrepancy in results is the basal diet where the algae was added. The United Stated incorporates corn into most large-scale production facilities, and corn contains high levels of xanthophyll pigments. Studies that utilized a corn diet with the added algae, would have a higher concentration of the pigments, leading to changes in not only color but antioxidants as well (Hajati and Zaghari, 2019; Omri et al., 2019; Selim et al., 2018, Altman et al., 2018; Dogan et al., 2016). In areas of the world where corn is not considered a staple, wheat or barley is used instead (Curabay et al., 2021; Anderson et al., 1991; Zahroojian et al., 2011; Zahroojian et al., 2013). Wheat contains higher proteins and fiber while corn contains a higher energy content (Jacob, 2022). The decision to include corn and/or wheat will alter the final nutritional profile of the diet, which will affect layer performance. Lastly, the subspecies of Spirulina that was utilized could lead to the discrepancies in the results. *Spirulina platensis* is the commercially utilized form of the algae which was the form predominantly utilized in these studies (Zahroojian et al., 2011; Zahroojian et al., 2013; Curabay et al., 2021; Anderson et al., 1991; Selim et al., 2018; Hajati and Zaghari, 2019). In addition to eqq quality characteristics, bone strength (N) is studied in laying hens. Spirulina at 300 mg/kg has shown to significantly increase the breaking strength ($p<0.001$) of tibias (Ekeuku et al., 2021). Inclusion levels up to 1000 mg/kg of Spirulina significantly increased in bone length, and breaking force (Suzer et al., 2020). The calcium content of Spirulina had a positive effect on bone calcification due to the mineral absorption in the intestinal microflora. (Suzer et al., 2020; Craig and Mangels, 2009). Spirulina has been shown to contain 26 times more calcium than milk (Moorhead et al., 2012).

2.6 Maximum Inclusion

Although levels of incorporation of Spirulina have varied between studies, research has shown that there is a maximum level of inclusion before it becomes detrimental to poultry performance. Spirulina as a protein replacement can reach up to 10 % without having any negative effects on production (Selim et al., 2018). In broiler trials where soybean meal was replaced with Spirulina, inclusion rates up 12 % were viable before negative effects in growth were demonstrated (Alvaranga et al., 2011; Ross and Dominy, 1990). Austic et al. (2013) stated that high levels of inclusion (20 %) let to negative performance in poultry and attributed that to the sulfur containing amino acids and/or digestibility of the protein. Further research is needed to determine the true levels of incorporation for use in the various fields of the poultry industry.

2.7 Sustainability

In addition to the nutritive properties of Spirulina and microalgae, there are sustainability factors. As stated previously, corn and soybean meal are the two main ingredients utilized in poultry diets. However, other agricultural industries utilize these ingredients, leading to a concern over market availability and sustainability soybean cultivation (Altmann et al., 2018). Microalgae can be grown in any environmental condition due the organism's efficient use of solar energy, and it does not require fertile soil compared with other feedstuffs (Hajati and Zaghari, 2019). Algae farms can be created indoors, removing the external factors altogether, or in photobioreactors race-way ponds (Altmann et al., 2018). Microalgae can produce higher levels of crop per unit when compared with traditional feedstuffs (Altomente et al., 2018).

2.8 Discrepancies

Spirulina's definitive use in the poultry industry requires further examination. Through examining previous studies on Spirulina, a discrepancy appeared in the results which may be due to the basal diet, bird breeds, concentration of Spirulina included, and where the algae were acquired from. Some examples for broilers, Bonos et al. (2016) utilized a maize and soybean commercial diet that is common in Greece and added 5 or 10 g/kg of powdered Spirulina to the control diet. The Spirulina was from Serres, Greece but did not say how the algae was dried or stored (Bonos et al., 2016). Of the two levels of inclusion, 5 g/kg increased fatty acids in the thigh meat without any negative significant impact on performance. Tavernari et al. (2018) utilized male Cobb 500 broilers fed at 20 % concentration. The Spirulina was from Spirulina Brasil G&F Incorporated and was air dried before being ground into a powder (Tavernari et al., 2018). For layers, both trials by Zahroojian et al. (2011; 2013) utilized 63-week-old Hyline W_{36} hens and had a control diet with wheat and soybean meal. Both trials used dried organic Spirulina powder from Parry Nutraceuticals in India. No information was provided on how the algae was prepared or stored. The first trial compared the pigmentation capability of Spirulina to a commonly used artificial pigment, with 2.5 % concentration creating a Roche color score of 11.6 compared to the synthetic at 11.9 (Zahroojian et al., 2011). A second trial confirmed that Spirulina at 2-2.5 % had a significant positive effect ($p<0.001$) on yolk color, without having the negative effects on other quality parameters (Zahroojian et al., 2013). Omri at al. (2019) utilized Arthrospira Spirulina platensis, which belong to the same class Cyanophyta as Spirulina and is used as a strain to produce *Spirulina platensis* and the names have been used interchangeably (Bernaerts at al., 2018). White Lohman laying hens were fed a corn-based diet with 1.5 % or 2.5 % Spirulina from Tunisia. (Omri et al., 2019). Both inclusions had no significant effect on egg quality characteristics, except for yolk color ($p<0.0001$) which saw a significant increase with each level of inclusion. Selim et al. (2018) used Norfa (White Leghorn × Fayoumi × White Balad) laying hens fed a corn/soybean diet with pure Spirulina platensis powder from Algal Biotechnology Unit, National Research Centre, Dokki, in Giza, Egypt. Inclusion levels consisted of a control group (0%), 0.1 %, 0.2 %, and 0.3 % (1, 2, and 3kg/ton) (Selim et al., 2018). Unlike the previous trial, there was a significant increase in final body weight, weight gain, feed intake, laying performance, and egg quality characteristics at 0.3% compared to control (Selim at al., 2018). Further studies are needed with a variety in parameters, to determine the cause behind the discrepancies in Spirulina inclusion.

2.8 Objective

The goal of this research is to determine whether novel microalgae species are suitable feed ingredients that will provide essential nutrients to layer and broiler production. Spirulina, a commonly studied microalgae, will be included as a foundation ingredient for comparison among novel species.

Chapter 3

ENERGY AND AIAAD OF MICROALGAE IN BROILER AND LAYING HENS

3.1 Introduction

Microalgae is a feedstuff with growing interest in the poultry industry. Spirulina, a commonly used algae in human food consumption, has been shown to be nutritionally advantageous due to its high lipid and crude protein content (Altmann et al., 2018). The apparent metabolizable energy (AME) of content of Spirulina is estimated between 2,560 and 2,864 kcal/kg as fed (Alvarenge et al., 2011; Tavernari et al., 2018), while crude protein content ranges from 50-70 % as fed, depending on species, storage, and form utilized (Altmann et al., 2018; Contreras et al., 1979; Kay and Barton, 1991). However, for producers to determine whether novel species of microalgae can be a viable replacement, it is important to determine first how much of the nutrients are being utilized by the bird. Nutrient waste is not only costly to the producer, but to environmental sustainability. Birds fed too high of a concentration in their diets, will excrete it as waste. With feed cost consisting of 60-70 %, that loss can impact profitability and production (Thirumalisamy et al., 2016). This waste can also cause harm to the environment by means of nitrogenous waste (Food and Agriculture of the United Nations., 2008). To determine the proper level of algae inclusion in poultry diet, understanding the maximum level of inclusion is required. The objective of these trials was to determine the energy and amino acid digestibility and availability of novel microalgae in broiler and layer diets when included at high levels of concentration. Apparent metabolizable energy (AME) is the energy that is available for growth, maintenance, and reproduction after accounting for fecal and urinary loss (Costa, 2009). This can be calculated by subtracting the total energy in the excreta (excrement that is the mixture of feces and uric acid produced in chickens) from the energy consumed in the diet (Ravindran, 2004). Low AME values indicate that the animal is not utilizing the energy provided in the diet, causing a higher output vs input which provides the animal lower amounts of energy to use towards growth and/or reproduction (Costa, 2009).

In addition to AME, amino acids are another component in poultry diets. Amino acids are the building blocks for protein formation, and some of these amino acids are required externally in the diet (Applegate and Angel, 2008). Without the proper ratio of these limiting (or essential amino acids), any excess amino acids are broken down, causing more nitrogenous waste (Applegate and Angel, 2008). Apparent ileal amino acid digestibility (AIAAD) was utilized as microflora in the ceca can modify the amino acid profiles prior to excretion (Ravindran et al., 2005). To properly discern AIAAD from the diet versus intrinsic manipulation, the excess amino acids from the diet are collected before the ceca (Ravindran et al., 2005).

The object of this study was to determine the AME and ileal amino acid digestibility of microalgae in broiler chickens and laying hens. This data will allow for a more precise level of inclusion in poultry diets.

3.2 Methods and Materials

The study was conducted at the poultry facility of the Animal Science Department, California Polytechnic State University. All experimental procedures were reviewed and approved by the California Polytechnic State University Institutional Animal Care and Use Committee (Protocol #1613, 1908).

3.2.1 Digestibility Trial – Broilers

Eighty, 25-day-old Ross 708 broiler chicks were weighed, sorted, and placed into 20 metabolism cages (2’×2’, 4 birds/cage, 1.0 sq. ft. / bird) with trough feeders and nipple drinkers. Birds received one of two diets (10 cages/diet): Control Diet (Basal corn-soybean meal-based grower diet; Table 3.1) or Test Diet (75 % basal diet + 25 % Spirulina). Titanium dioxide (TiO_2, 5 g/kg) was added to both diets as an indigestible marker in order to calculate the digestibility of the diet. After a 5-day diet adaptation period, excreta were collected from each cage over a 3-day period to determine metabolizable energy. Birds were then euthanized by CO_2 inhalation, and their ileal contents collected and pooled by cage. The ileum was defined as that portion of small intestine extending from Meckel’s diverticulum to a point 40 mm proximal to the ileo-cecal junction. Excreta and ileal samples were oven dried at 50^{o}C and ground to pass through a 0.5-mm sieve. Samples were stored at −20^{o}C for later nutrient analyses.

3.2.2 Digestibility Trial – Layers

Sixty, 80-week-old Lohman LSL-Lite laying hens were randomly assigned into individual metabolism cages (2’ x 2’) with trough feeders and nipple drinkers. Birds received either a control Diet (Basal corn-soybean meal-based layer diet; Table 3.2) or one of four microalgae test diets. Three of the microalgae test diets were 75 % basal diet and 25 % microalgae (Spirulina, Vaucheria, Uronema). The fourth test diet was 87.5 % basal diet and 12.5 % Hydrodictyon. Procedures utilized to estimate digestibility in broilers were repeated for laying hens.

Table 3.1. Composition of the basal grower diet (%, as fed basis) used in the broiler digestibility trial.

Ingredients	
Corn	53.0
Soybean meal	34.2
Corn DDGS	5.0
Vegetable fat	3.3
Limestone	1.2
Monocalcium phosphate and Dicalcium phosphate blend	1.4
Salt	0.32
DL-Methionine	0.35
L-Lysine HCl	0.12
L-Theonine	0.11
Vitamin premix	5.0
Trace mineral premix	3.0
Calculated analysis	
AME (kcal/kg)	3100
Dry matter	88.0
Crude protein	21.5
Crude Fat	6.2
NDF	9.0
Ca	0.87
Available P	0.43
Methionine	0.67
Methionine + Cysteine	0.99
Lysine	1.31
Threonine	0.89

Table 3.2. Composition of the basal layer diet (%, as fed basis) used in the layer digestibility trial.

Ingredients	
Corn	58.3
Soybean meal	11.7
Corn DDGS	10.0
Limestone	8.1
Monocalcium phosphate and Dicalcium phosphate blend	0.90
Salt	0.25
DL-Methionine	0.14
Vitamin premix	0.38
Trace mineral premix	0.11
Calculated analysis	
AME (kcal/kg)	2725
Dry matter	87.9
Crude protein	14.3
Crude Fat	3.5
NDF	13.1
Ca	3.3
Available P	0.35
Methionine	0.37
Methionine + Cysteine	0.55
Lysine	0.69
Threonine	0.51

3.2.3 Nutrient analysis

All nutrient analyses for both digestibility trials (proximate analysis, amino acid content, titanium) were conducted by the University of Missouri Agricultural Experiment Station Chemical Laboratories according to AOAC (2006). Bomb calorimetry for gross energy was conducted at California Polytechnic State University San Luis Obispo. Microalgae were analyzed for dry matter (method 934.01), crude protein (LECO, method 990.03), crude fat (method 920.39), ash (method 942.05), neutral detergent fiber (method 2002.04), acid detergent fiber (method 973.18), amino acid content (method 982.30), and gross energy. Samples of diets and ileal digesta were analyzed for dry matter, titanium (Myers et al., 2004), and amino acid content. Samples of diets and excreta were analyzed for dry matter, titanium, and gross energy.

3.2.4 Calculations

The apparent metabolizable energy (AME) value of each diet (AME_{diet}) was calculated as:

$$AME_{diet} = GE_{diet} - (GE_{excreta} \text{ x } ([Ti]_{diet} / [Ti]_{excreata})).$$

The AME value of the microalgae (Spirulina, Uronema, Vaucheria; $AME_{Microalgae}$) was calculated as follows:

$$AME_{Microalage} = [AME_{Test\ diet} – (AME_{Control\ diet} \text{ x } 0.75)] / 0.25$$

The AME value of the microalgae Hydrodictyon ($AME_{Hydrodictyon}$) was calculated as follows:

$$AME_{Hydrodictyon} = [AME_{Test\ diet} – (AME_{Control\ diet} \text{ x } 0.875)] / 0.125$$

Apparent ileal amino acid digestibility (AIAAD) value of the diets ($AIAAD_{diet}$) was calculated using the following formula:

$$AIAAD_{diet} = \{1- [(AA/Ti)_{ileal\ digesta} / (AA/Ti)_{diet}]\} \times 100$$

where $(AA/Ti)_{ileal\ digesta}$ is the ratio of the amino acid and titanium concentrations in the ileal digesta and $(AA/Ti)_{diet}$ is the ratio of the amino acid and titanium concentrations in the diet. The AIAAD value the microalgae (Spirulina, Uronema, Vaucheria; $AIAAD_{Microalgae}$) was calculated using the following formula:

$$AIAAD_{Microalgae} = [AIAAD_{Test\ diet} – (AIAAD_{Control\ diet} \times 0.75)] / 0.25$$

The AIAAD value of the microalgae Hydrodictyon ($AIAAD_{Hydrodictyon}$) was calculated as follows:

$$AIAAD_{Hydrodictyon} = [AIAAD_{Test\ diet} – (AIAAD_{Control\ diet} \times 0.875)] / 0.125$$

3.2.5 Preliminary evaluation of microalgae on egg yolk color

Over the last three days of the excreta collection in the layer digestibility trial, any eggs laid were collect, weighed, and the yolk color measured. Further analysis into the effects of algae on yolk color is presented in chapter 4.

3.3 Results

3.3.1 Digestibility Trial - Broilers

The energy and nutrient composition of the Spirulina and experimental diets used in the broiler digestibility trial are presented in Table 3.3. The AME and AIAAD of Spirulina is summarized in Table 3.4. Comparison of the total amino acid content (%AF) of Spirulina to soybean meal is presented in Figure 3.1. AME of the Spirulina in this study was 2279 kcal/kg. AIAAD of indispensable amino acids was 81.1 % and dispensable amino acids was 75.5 % (Figure 3.2). Lysine, methionine, and threonine ileal digestibility was 94.5 %, 91.3 %, and 71.4 % respectively (Table 3.4, Figure 3.2). Figures

3.3 and 3.4 compare the AIAAD and AA concentration of Spirulina compared to soybean meal.

3.3.2 Digestibility Trial - Layers

Energy and nutrient composition of the microalgae and diets used in the layer digestibility trial are presented in Tables 3.5 and 3.6, respectively. The AME and AIAAD of the microalgae are summarized in Table 3.7. Note: Except for Spirulina, the values for the microalgae are low. AIAAD of the layer and broiler trial are compare in Figure 3.5. Both indispensable and dispensable AIAAD of Spirulina was spread out between the two groups. Methionine and lysine digestion was higher in broilers at 91.3% and 94.5% versus 79.8% and 58.1%. However, threonine in layers was higher at 86.4% compared to 71.4% in the broiler trial.

3.3.3 Preliminary evaluation of microalgae on egg yolk color

Egg yolk color was significantly increased by inclusion of microalgae in the diets (Figure 3.6, Table 3.8). Control roche yolk color score was an average of 6.3. Hydrodictyon, Spirulina, and Vaucheria was 10.5, 13.2, 9.1.

Table 3.3. Energy and nutrient composition (as fed basis) of the Spirulina and experimental diets used in the broiler digestibility trial.

Composition	Spirulina	Control diet[1]	Test Diet[2]
Dry matter (%)	86.2	88.2	87.6
Gross energy (kcal/kg)	4539	3930	3123
Metabolizable energy (kcal/kg)	2279	2975	2801
Crude protein (%)	61.0	21.3	31.6
Crude fat (%)	3.2	4.1	3.9
Neutral detergent fiber (%)	3.3	9.6	9.4
Acid detergent fiber (%)	1.3	2.0	5.2
Ash (%)	8.1	6.2	6.9
Indispensable amino acids			
Arginine (%)	3.34	1.41	1.97
Histidine (%)	0.85	0.59	0.68
Isoleucine (%)	3.45	0.99	1.48
Leucine (%)	5.14	1.88	2.51
Lysine (%)	2.78	1.32	1.73
Methionine (%)	1.46	0.71	1.16
Phenylalanine (%)	2.72	1.10	1.49
Threonine (%)	2.74	0.95	1.24
Tryptophan (%)	0.62	0.26	0.33
Valine (%)	3.75	1.07	1.59
Dispensable amino acids			
Alanine (%)	4.93	1.10	1.74
Aspartic acid (%)	5.16	2.26	2.99
Cysteine (%)	0.55	0.37	0.42
Glutamic acid (%)	7.31	3.85	4.76
Glycine (%)	3.14	0.91	1.32
Proline (%)	2.05	1.25	1.42
Serine (%)	2.03	0.96	1.21
Tyrosine (%)	2.51	1.75	1.16

[1]Basal diet + Titanium dioxide (5 g/kg)

[2]75 % basal diet + 25 % Spirulina + Titanium dioxide (5 g/kg)

Table 3.4. Apparent metabolizable energy (AME) and coefficient of apparent ileal digestibility of amino acids of the Spirulina fed to broilers (broiler digestibility trial).

	This study	Tavernari et al. (2018)
AME (kcal/kg)	2279	2864
Indispensable amino acids		
Arginine (%)	90.4	74.7
Histidine (%)	76.1	72.0
Isoleucine (%)	76.8	74.7
Leucine (%)	73.9	72.4
Lysine (%)	94.5	76.8
Methionine (%)	91.3	77.2
Phenylalanine (%)	81.3	76.1
Threonine (%)	71.4	72.4
Tryptophan (%)	85.9	-
Valine (%)	69.8	72.5
Dispensable amino acids		
Alanine (%)	62.0	73.6
Aspartic acid (%)	79.1	68.7
Cysteine (%)	56.1	68.7
Glutamic acid (%)	82.6	74.5
Glycine (%)	66.8	66.9
Proline (%)	80.5	74.2
Serine (%)	78.1	68.2
Tyrosine (%)	98.5	70.5

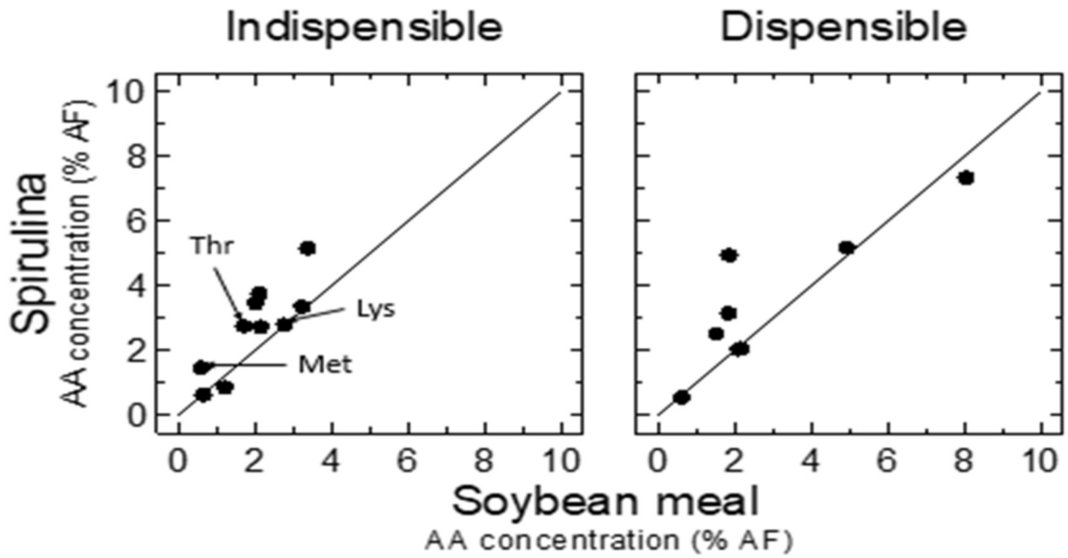

Figure 3.1. Total amino acid content (as fed basis, %AF) of Spirulina (Table 3.3) compared with soybean meal (Bennett et al. unpublished). Line of equality is provided.

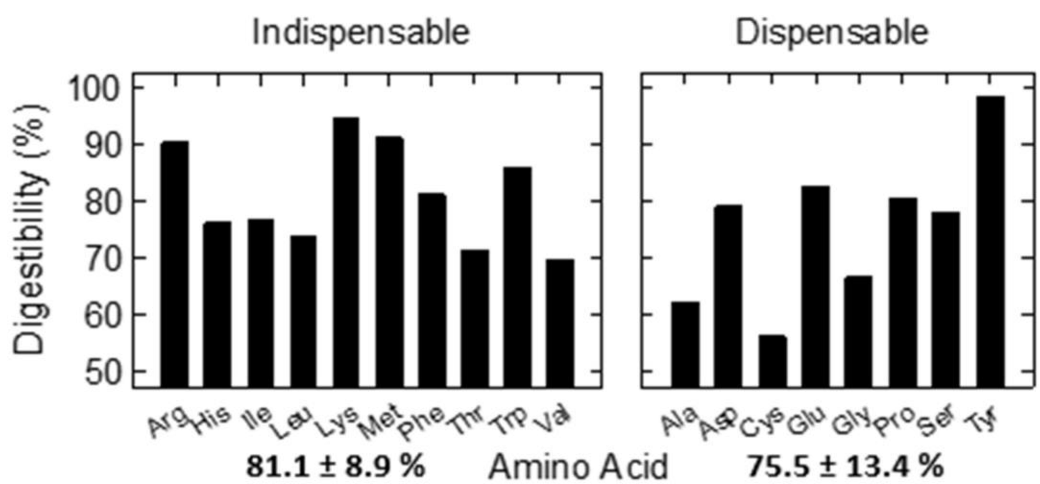

Figure 3.2. Apparent ileal amino acid digestibility (%) of indispensable and dispensable amino acids in Spirulina.

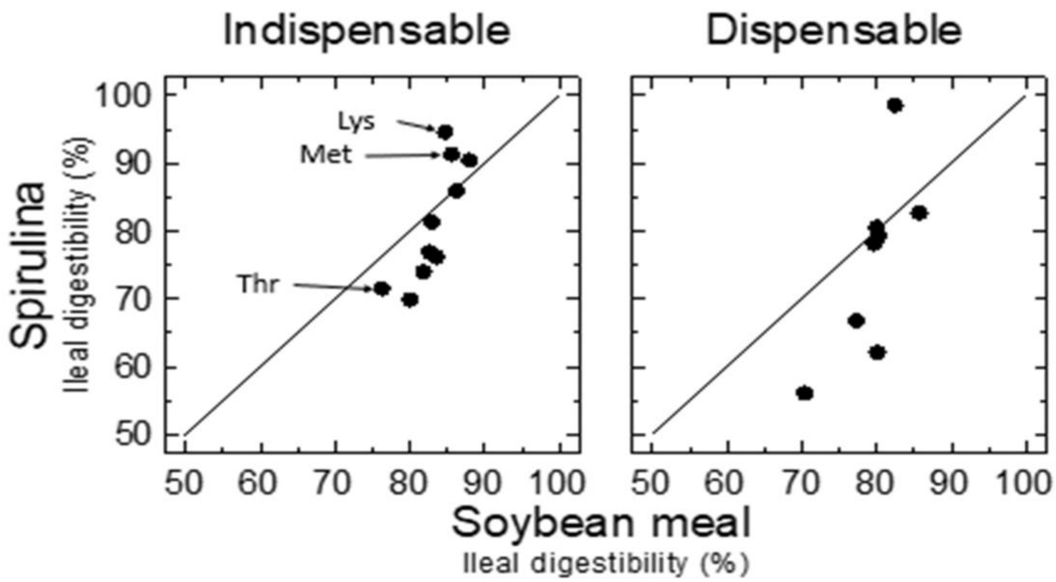

Figure 3.3. Apparent ileal amino acid digestibility (%) of Spirulina (Table 3.4, Figure 3.2) compared with soybean meal (Bennett et al. unpublished). Line of equality is provided.

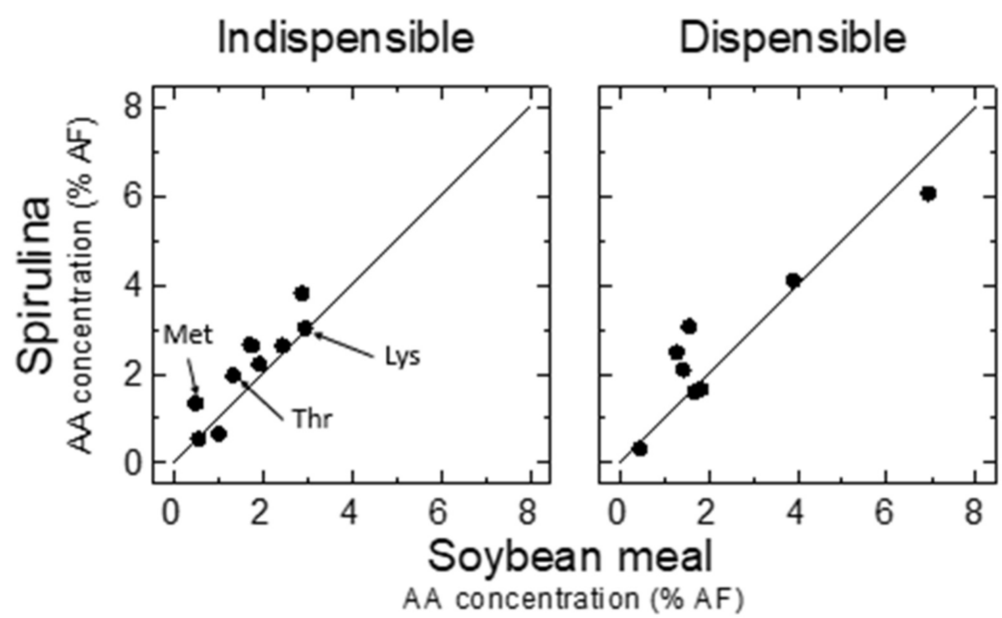

Figure 3.4. Apparent ileal amino acid concentration (as fed basis, %AF) of Spirulina compared with soybean meal (Bennett et al. unpublished). Line of equality is provided.

Table 3.5. Energy and nutrient composition of the microalgae (as fed basis) used in the layer digestibility trial.

Composition	Hydrodictyon	Uronema	Spirulina	Vaucheria
Dry matter (%)	91.5	90.4	87.0	91.4
Gross energy (kcal/kg)	4023.9	4652.2	4569.3	3552.5
Crude protein (%)	35.0	45.4	59.5	36.3
Crude fat (%)	2.7	6.2	2.4	1.7
Neutral detergent fiber (%)	39.3	21.0	3.3	33.7
Acid detergent fiber (%)	20.6	12.6	1.3	24.0
Ash	14.5	9.4	8.2	20.7
Indispensable amino acids				
Arginine (%)	1.39	2.10	3.32	1.21
Histidine (%)	0.44	0.74	0.76	0.39
Isoleucine (%)	1.16	1.82	3.28	1.09
Leucine (%)	2.33	3.44	4.87	2.04
Lysine (%)	1.80	2.47	2.53	2.04
Methionine (%)	0.59	0.84	1.34	0.48
Phenylalanine (%)	1.44	2.21	2.66	1.30
Threonine (%)	1.34	1.89	2.66	1.32
Tryptophan (%)	0.44	0.61	0.69	0.34
Valine (%)	1.65	2.52	3.58	1.66
Dispensable amino acids				
Alanine (%)	2.04	3.09	4.69	2.02
Aspartic acid (%)	2.71	3.58	4.94	2.60
Cysteine (%)	0.49	0.47	0.51	0.72
Glutamic acid (%)	2.93	3.93	7.07	2.85
Glycine (%)	1.65	2.37	3.02	1.64
Proline (%)	1.27	1.82	2.00	1.19
Serine (%)	0.99	1.45	2.11	0.99
Tyrosine (%)	0.93	1.43	2.32	0.91

Table 3.6. Energy and nutrient composition of the microalgae test diets (as fed basis) used in the layer digestibility trial.

Composition	Control	Hydrodictyon	Uronema	Spirulina	Vaucheria
Dry matter (%)	89.5	90.1	90.1	88.7	90.3
Gross energy (kcal/kg)	3429.6	3316.1	3880.5	3785.4	3452.0
Crude protein (%)	15.3	16.6	22.0	25.5	19.1
Crude fat (%)	2.7	2.4	2.7	2.6	2.3
Neutral detergent fiber (%)	13.8	15.5	14.4	12.2	18.1
Acid detergent fiber (%)	5.4	5.7	6.0	3.8	8.7
Ash (%)	13.0	14.9	13.5	10.5	17.3
Indispensable amino acids					
Arginine (%)	0.88	0.83	1.10	1.41	0.88
Histidine (%)	0.41	0.38	0.47	0.48	0.38
Isoleucine (%)	0.62	0.66	0.92	1.27	0.71
Leucine (%)	1.42	1.42	1.89	2.21	1.48
Lysine (%)	0.72	0.79	1.12	1.11	0.94
Methionine (%)	0.45	0.43	0.48	0.68	0.43
Phenylalanine (%)	0.75	0.78	1.09	1.18	0.84
Threonine (%)	0.56	0.59	0.85	1.03	0.68
Tryptophan (%)	0.18	0.16	0.21	0.26	0.16
Valine (%)	0.74	0.80	1.17	1.42	0.90
Dispensable amino acids					
Alanine (%)	0.88	0.93	1.42	1.77	1.05
Aspartic acid (%)	1.27	1.34	1.79	2.11	1.50
Cysteine (%)	0.25	0.26	0.28	0.34	0.33
Glutamic acid (%)	2.66	2.51	2.86	3.56	2.57
Glycine (%)	0.64	0.70	1.06	1.19	0.81
Proline (%)	1.00	0.96	1.17	1.19	0.98
Serine (%)	0.65	0.60	0.77	0.91	0.66
Tyrosine (%)	0.51	0.47	0.66	0.86	0.52

Table 3.7. Apparent metabolizable energy (AME) and coefficient of apparent ileal digestibility of amino acids of the microalgae (as fed basis) fed to layers (layer digestibility trial).

	Hydrodictyon	Uronema	Spirulina	Vaucheria
AME (kcal/kg)	-	-	3347.3	-
Indispensable amino acids				
Arginine (%)	31.4	40.0	80.1	24.8
Histidine (%)	-	4.5	55.1	21.7
Isoleucine (%)	-	-	93.4	24.5
Leucine (%)	16.8	13.8	76.4	35.0
Lysine (%)	-	-	58.1	-
Methionine (%)	27.0	-	79.8	43.0
Phenylalanine (%)	7.5	8.9	77.2	29.7
Threonine (%)	63.3	33.7	86.4	7.4
Tryptophan (%)	53.9	4.2	86.7	43.0
Valine (%)	5.6	2.1	91.0	15.6
Dispensable amino acids				
Alanine (%)	15.8	28.7	103.0	37.4
Aspartic acid (%)	8.0	3.1	80.7	21.8
Cysteine (%)	31.3	-	64.0	-
Glutamic acid (%)	4.4	-	66.7	14.0
Glycine (%)	1.5	16.5	90.7	14.6
Proline (%)	56.4	38.7	70.6	32.3
Serine (%)	52.1	41.6	74.9	7.7
Tyrosine (%)	-	-	83.0	-

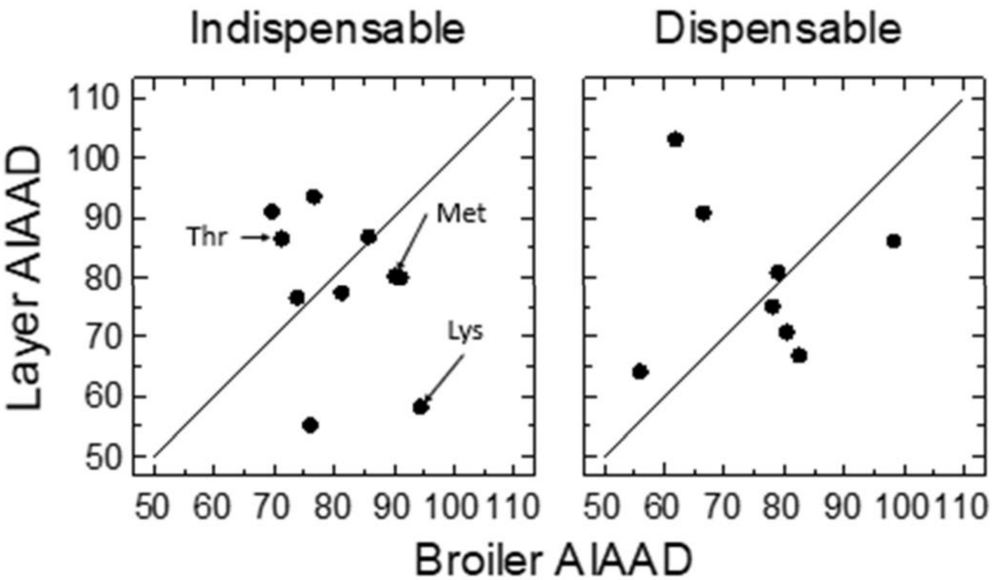

Figure 3.5. Apparent ileal amino acid digestibility (AIAAD, %) of Spirulina measured in the layer trial (Table 3.7) compared with the previous broiler trial (Table 3.4, Figure 3.2). Line of equality is provided.

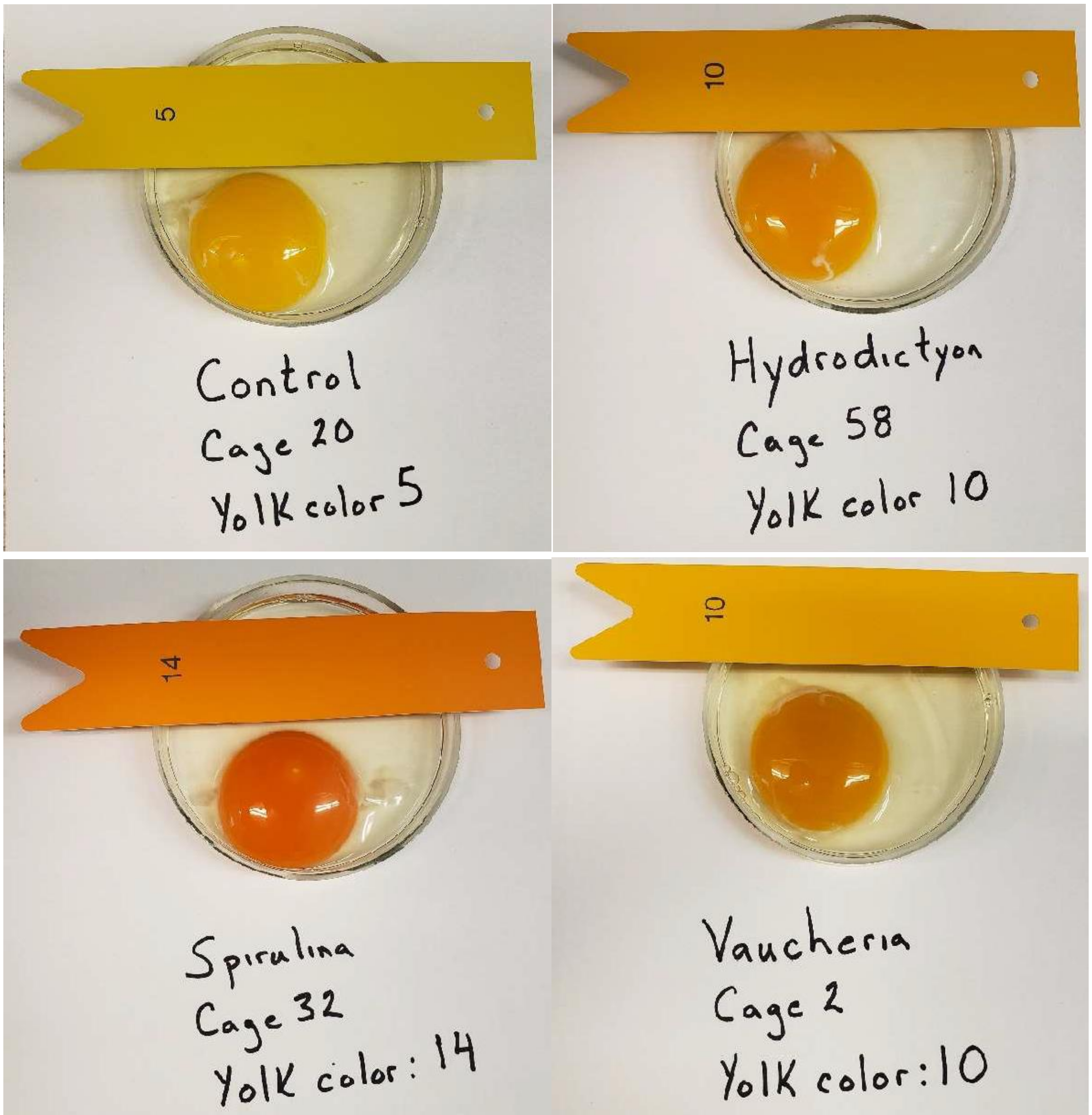

Figure 3.6. Examples of Roche color scores of yolks from eggs collected from hens in the layer digestibility trial. Roche color scores can range from 1 (pale) to 15 (dark orange).

Table 3.8. Roche color scores of yolks from eggs collected from hens fed control and the microalgae diets. See Figure 3.6 for examples.

Diet	Yolk color (mean ± SE)
Control	6.3 ± 0.3
Hydrodictyon	10.5 ± 0.2
Spirulina	13.2 ± 0.2
Vaucheria	9.1 ± 0.8

3.4 Discussion

3.4.1 Broiler Trial

Gross nutrient composition, on an as-is-basis, of the Spirulina was the following: 4569 kcal/kg gross energy, 86.2 % dry matter, 61.0 % crude protein, 3.2 % ether extract, 3.3% neutral detergent fiber, and 8.1% ash. The first two limiting essential amino acids in poultry production are methionine and lysine (Applegate and Angel, 2008; NRC, 1994). These amino acids must be incorporated into the diet as the bird cannot synthesize on its own. In the current study, methionine and lysine, were 1.46% and 2.78% of the diet. Cysteine, threonine, isoleucine, and valine were also present at a higher concentration: 0.55 %, 2.74 %, 3.45 %, and 3.75 %, respectively. Leucine had a concentration of 5.14 % compared to 1.88 % in the control diet. The AIAAD of lysine, methionine, cysteine, threonine, isoleucine, and valine in Spirulina was 94.5 %, 91.3 %, 56.1 %, 71.4 %, 76.8 %, and 69.8 %, respectively. Arginine digestibility was high at 90.4 %, which is a noticeable difference compared to one study at 74.7 % (Tavernari et al., 2018). Diet formulation and experimental period were the same between trials. Sex and strain of broilers may be a factor, Travernari et al. (2018) used Cobb 500 males while Ross 708 broilers of both sexes were used in our study. In addition, our study included Spirulina at 25 % of the diet, whereas the dietary inclusion of Spirulina in Tavernari at al. (2018) was 20 %.

One notable change is the inclusion of Spirulina decreased the gross energy of the diet from 3930 kcal/kg to 3123 kcal/kg. This may be explained due to the removal of corn from the diet with the replacement of Spirulina and corn has an AME value of 3791 kcal/kg on a dry matter basis (Berzegar et al., 2019). In addition, protein has a lower metabolizable energy efficiency compared with other nutrients and Spirulina has a high crude protein content (Tavernari et al., 2018). Previous research determined the gross energy content of Spirulina to be 4,286 kcal/kg while another stated that the AMEn of Spirulina was 3680.6 kcal/kg (Alvarenga et al., 2011; Altmann et al., 2018). Another trial had a similar AME at 3,100-3,200 kcal/kg but Spirulina was incorporated at 0.5 to 3 g/kg (Abou-Zeid et al., 2015). The lowered value of energy in the Spirulina diet consequently led to a lower AME value of 2279 kcal/kg. AME was lower than other studies, where metabolizable energy was 2864 kcal/kg in Spirulina groups (Tavernari et al., 2018). Another possible cause to this variation is the biochemical changes that can occur with the methods the algae was cultivated and growing conditions (Tavernari et al., 2018). Regional sourcing of Spirulina may also change nutritive value, wherein Altmann et al. (2018) utilized Spirulina from Myanmar. Other studies did not state where their algae were derived, which can lead to issues when comparing the algae used across the literature. The level of inclusion would also affect the broilers' ability to digest nutrients and energy present in the diet. Altmann et al. (2018) used Spirulina with a crude protein concentration of 53% and replaced 50% of the diet. As the diet formulated for this trail was a crumble form, there was a possibility that the birds could exhibit sorting behavior. Spirulina has been shown to act as a suitable feedstuff for broiler production; however, future studies need to be conducted to explain the discrepancies in energy content and amino acid digestibility.

3.4.2 Layer Trial

Hydrodictyon, Uronema, and Spirulina all had a high gross energy content of 4023.9 kcal/kg, 4652.2 kcal/kg, and 4569.3 kcal/kg, respectively. However, Uronema and Spirulina had a higher crude protein content of 45.4 % and 59.5 %. The crude protein content of Spirulina in the present study was similar to previous studies that determined Spirulina to consist predominantly of crude protein with a value of 514.7 g/kg (Tavernari et al., 2018). Ross and Dominy (1990) state that the Spirulina in their study had a crude protein content of 60 %. Park et al. (2018) had a crude protein content of 56.7% with freeze-dried Spirulina. One of the Spirulina papers by Zahroojian et al. (2011) stated that the protein content varied from 55 to 69 %. However other studies have had crude protein contents up to 75-90 % (Evans et al., 2015; Alvarenga et al., 2011). The differences in protein level may be attributed to the where the algae originated and how it was prepared, as discussed in chapter 2. Zahroojian et al. (2011;2013) utilized a commercial Spirulina powder from Parry Nutraceuticals in India. Evan et al. (2015) acquired Spirulina from Earthrise Nutritionals in Calipatria, California. Alvarenga at al. (2011) did not state where the algae originated from. All three studies did not specify how the algae was prepared. Vaucheria contained both a lower gross energy at 3552.5 kcal/kg and lower CP content at 36.3%. As stated, Methionine and Lysine are two of the major limiting amino acids in poultry production. Uronema, Spirulina, and Vaucheria all contained high levels of lysine at 2.47 %, 2.53 %, 2.04 % with hydrodictyon with only 1.80%. However, for methionine, Hydrodictyon, Vaucheria, and Uronema were low, at 0.56%, 0.48%, and 0.84%, respectively. Previous studies have stated that Spirulina contained between 1.0-2.0% methionine and 2-5 % lysine (Park et al., Evans et al., 2018; Alvarenga at al., 2011) Uronema had the highest gross energy content of 3880.5 kcal/kg and lysine at 1.12%. Methionine was the second highest, behind Spirulina, at 0.48 %. The Uronema diet had nutritional values similar to the Spirulina diet, while the Hydrodictyon diet was the most nutritionally similar to the control. However, palatability plays a major role in feed consumption as well (Alenier and Combs, 1981). If the birds do not like the taste or fell, the feed will not be consumed, altering the absorption and production results (Alenier and Combs, 1981). Hydrodictyon performed the poorest during the adaptation period, birds were refusing feed, so the algae concentration was reduced from 25 % to 12.5 %.

Further examination is needed on the novel microalgae species to determine the true nutritive value due to the varying levels of proteins, amino acids, and AME. Each algae had strengths and weakness for nutritional uses; thus, future use may be determined by producer needs.

Chapter 4

EFFECTS OF MICROALGAE ON LAYING HEN PRODUCTION

4.1 Introduction

With food shortages occurring around the world, consumers are looking for protein sources that can be produced efficiently. Due to the quick turnover time, poultry production has become the number one meat product worldwide (Ritchie and Roser, 2019). The average laying hen takes 25-35 weeks (depending on breed and/or line) to reach peak production, with average daily rate of lay in 100 birds is 78.5 %, while broiler chickens reach market weight in 5-6 weeks (National Agricultural Statistics Service, 2012: Clauer, 2012; Lera, 2020). However, with feed cost constituting 60-70 % of expenditures, producers are researching alternative feedstuffs, such as microalgae, to provide the same nutrition at a lower cost or higher final product value (Thirumalisamy et al., 2016). Spirulina contains high levels of crude protein, has been shown to improve Roche yolk color scores without negatively impacting production performance (Contreras et al., 1979; Anderson et al., 1991; Zahroojian et al., 2011; Zahroojian et al., 2013; Dogan et al., 2016; Altemann et al., 2018; Selim et al., 2018; Hajati and Zaghari, 2019; Omri et al., 2019; Curabay et al., 2021. With the success Spirulina has demonstrated, can other microalgae species be incorporated as well? The objective of this study was to determine the effects of novel microalgae species on egg quality characteristics, feed consumption, and body weight. Spirulina was used as a foundation microalgae for comparison.

4.2 Methods and Materials

Seventy-eight, 92-week-old Lohman LSL-Lite laying hens were randomly assigned to 9 dietary treatment groups: control diet containing no microalgae (control diet), or diets containing 1 or 2 % of one of four species of microalgae (Hydrodictyon, Spirulina, Uronema, and Vaucheria). The control and 2 % microalgae diets were formulated to meet or exceed the nutrient requirements recommended by the NRC (1994), and the nutritional guidelines for Lohman LSL-Lite laying hens (Lohmann Tierzucht, 2016; Table 4.1). The 1 % microalgae diets were then created by mixing a 50-50 blend of the control and the appropriate 2% microalgae diet. Hens were fed the control diet for one week (Week 0) to make sure the hens laid consistently for the trial and any non-layers were removed. Based on the initial trial period, 2 birds were removed leaving 76 for the trial. Final distribution of birds per treatment are presented in Table 4.2.

Egg production (Hen-day production, %) was recorded daily and calculated weekly. Once a week, all eggs laid that day were weighed, and their eggshell quality (weight, thickness, and breaking strength) was assessed. Egg mass (g/d) was then calculated from egg production (Hen-day production, %) and egg weight (g) data. Eggshell thickness (mm) was measured using an ultrasound micrometer (ORKA Technology LLC, UT, USA). Egg shell breaking strength (Newtons (N) of force to break) was measured using an ORKA Egg Force Reader (ORKA Technology LLC, UT, USA). At the end of week 8, birds were euthanized by CO_2 gas inhalation, and their tibia bones harvested, cleaned of adhering tissue and frozen for later analysis.

4.2.1 Statistical Analysis

All data was analyzed using IBM SPSS Statistics Version 28.0.0.0 (190) and are reported as means +/- SE. Body weight, feed intake, FCR, and egg data were analyzed two ways. First, a one-way repeated measures ANOVA was run on each individual species microalgae (Hydrodictyon, Spirulina, Uronema, Vaucheria), with inclusion level (control, 1%, 2%) as the main effect and week as the repeated measure. Second, a two-

Table 4.1 Composition of the layer diets (%, as fed basis) used in the layer production trial.

Ingredients	Control	2 % Microalgae
Corn	58.4	56.7
Soybean meal	11.7	10.8
Corn DDGS	10.0	10.0
Microalgae	0.0	2.0
Limestone	8.1	8.6
Monocalcium phosphate and Dicalcium phosphate blend	0.90	0.97
Salt	0.25	0.25
DL-Methionine	0.14	0.12
Vitamin premix	0.38	0.38
Trace mineral premix	0.11	0.11
Yeast culture (Diamond V XPC)	0.08	0.08
Calculated analysis		
AME (kcal/kg)	2760	2750
Dry matter	87.9	88.0
Crude protein	14.3	14.9
Crude Fat	3.5	3.2
NDF	13.1	13.3
Ca	3.3	3.5
Available P	0.35	0.35
Methionine	0.37	0.37
Methionine + Cysteine	0.62	0.62
Lysine	0.69	0.71

Table 4.2. Distribution of Lohman LSL-Lite laying hens into the control and eight test diets for the production trial.

Dietary Treatment		
Microalgae	Inclusion level	Number of replicate cages (birds)
Control	0	10
Hydrodictyon	1	8
Hydrodictyon	2	8
Spirulina	1	10
Spirulina	2	9
Uronema	1	7
Uronema	2	8
Vaucheria	1	8
Vaucheria	2	8

way repeated measures ANOVA was run with microalgae species (Hydrodictyon, Spirulina, Uronema, Vaucheria) and with inclusion level (1%, 2%) as the main effects and week as the repeated measure. Bone strength was similarly analyzed using both a one-way ANOVA and two-way ANOVA.

4.3 Results & Discussion

The effects of the treatment groups on egg analysis characteristics are shown in Figures 4.1-4.11. Each table displays the average of each week by treatment group and compared the individual treatments to control, to determine if there was any significant change from the US standard poultry diet. Each figure displays the average of each week to determine if there was any significant change from the corn-SBM control diet. Figure 4.12-4.13 describe average daily feed consumption and body weight gain respectively. In both cases, treatments groups had no significant effect compared to control.

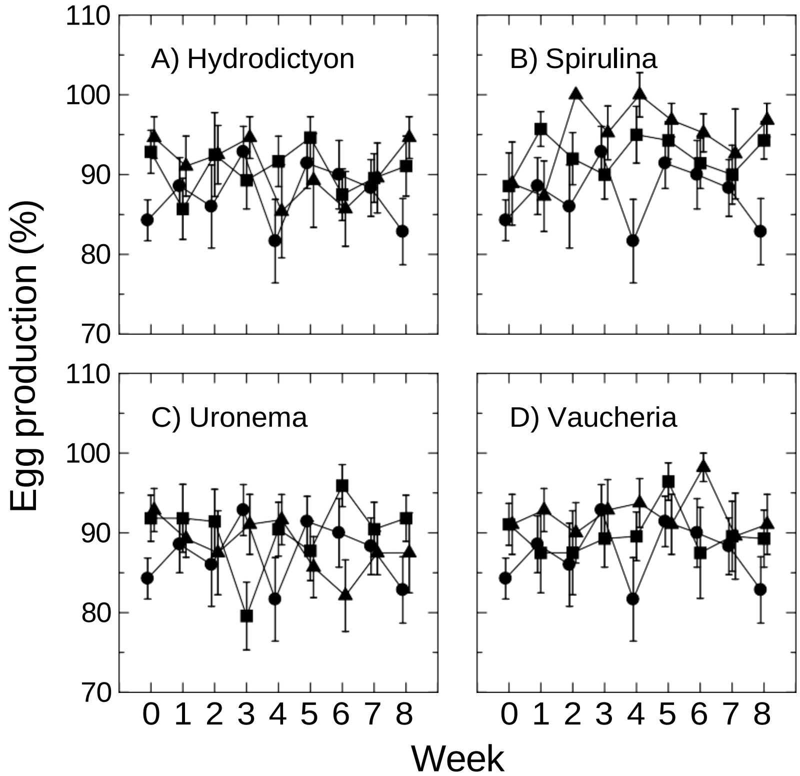

Figure 4.1. Average egg production (%) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Overall mean = 90.7 ± 0.4 %.

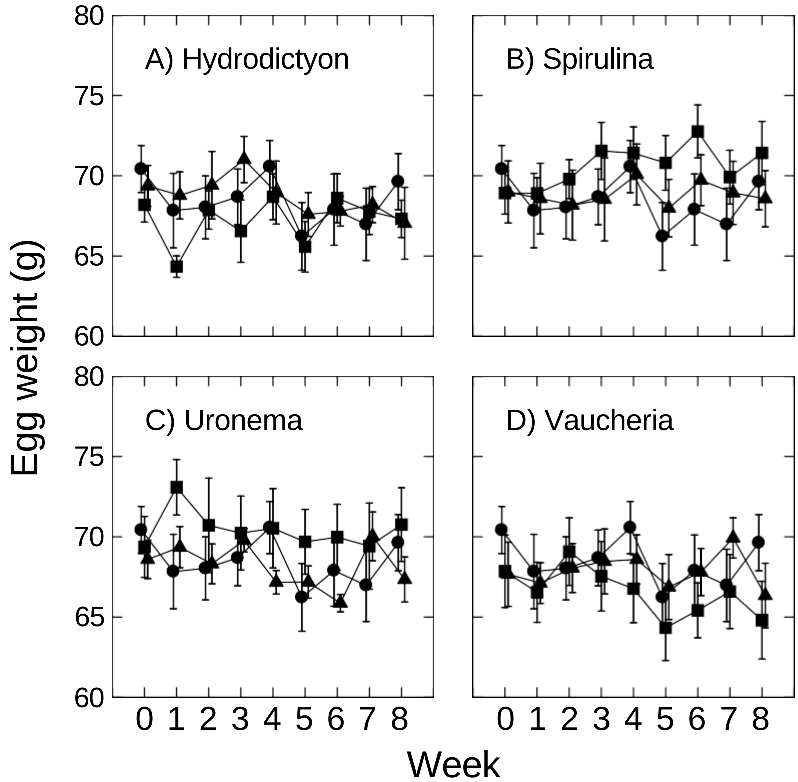

Figure 4.2. Average egg weight (g) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Overall mean = 68.6 ± 0.2 g.

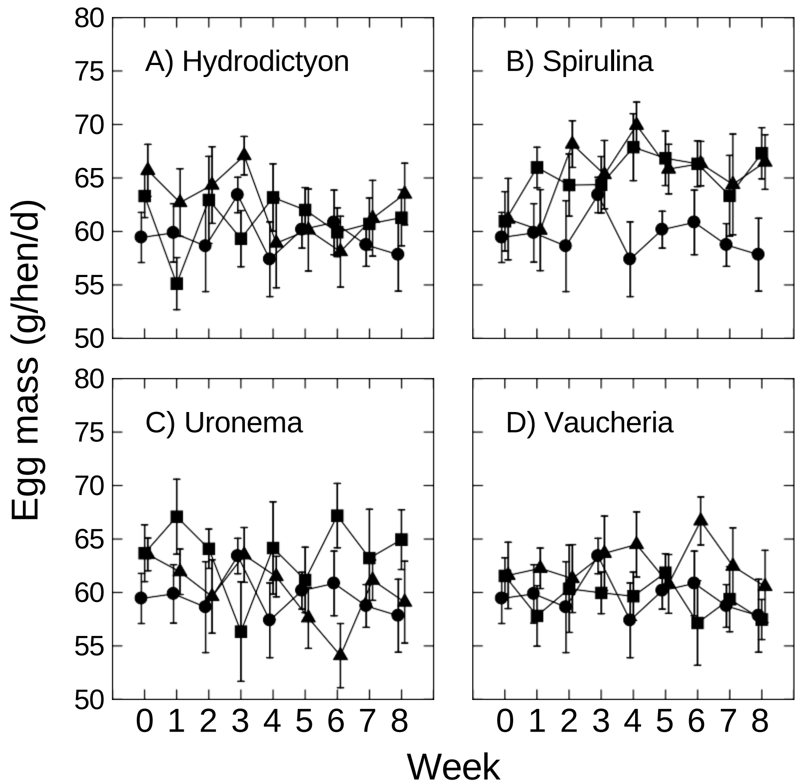

Figure 4.3. Average egg mass (g/d) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Overall mean = 62.2 ± 0.3 g/hen/day.

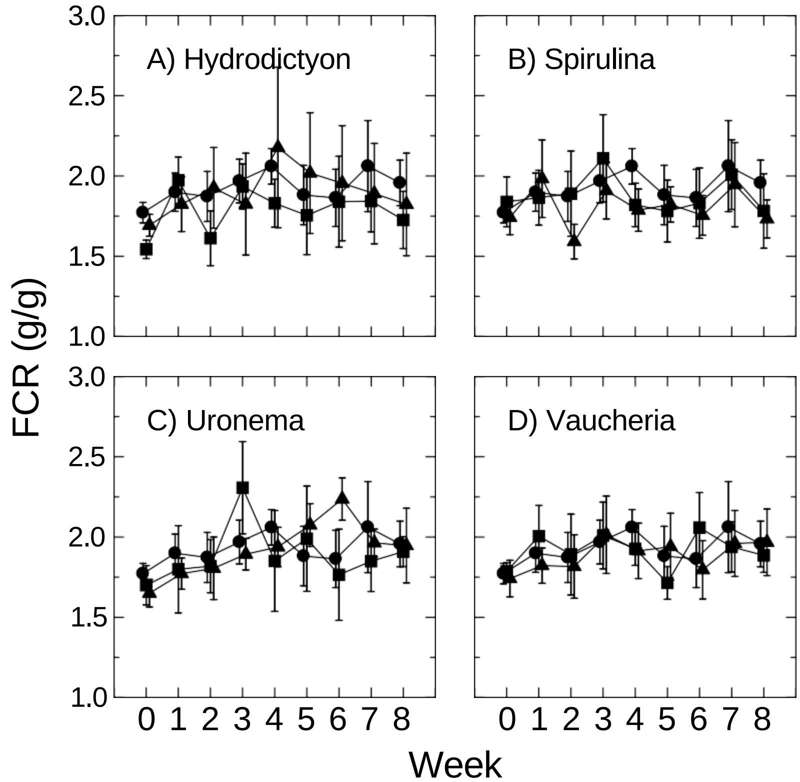

Figure 4.4. Average Feed conversion ratio (FCR, g/g) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Spirulina 1%: Cage 36, Week 8 – Changed FI/FCR from 391.4/6.319 (Spillage) to 117.1/1.890. Overall mean = 1.88 ± 0.02 g/g.

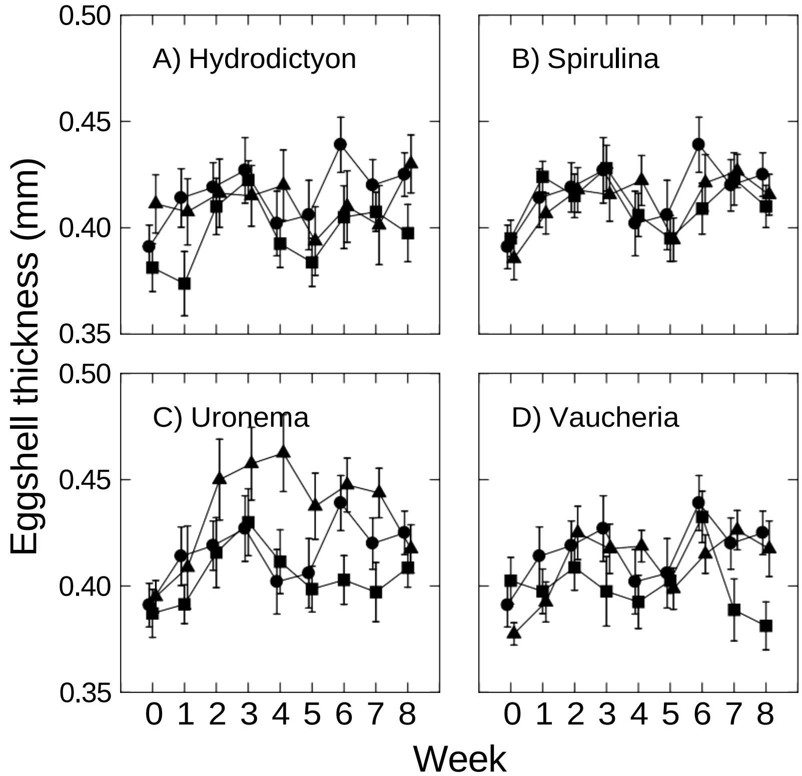

Figure 4.5. Average eggshell thickness (mm) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Overall mean = 0.411 ± 0.001 mm.

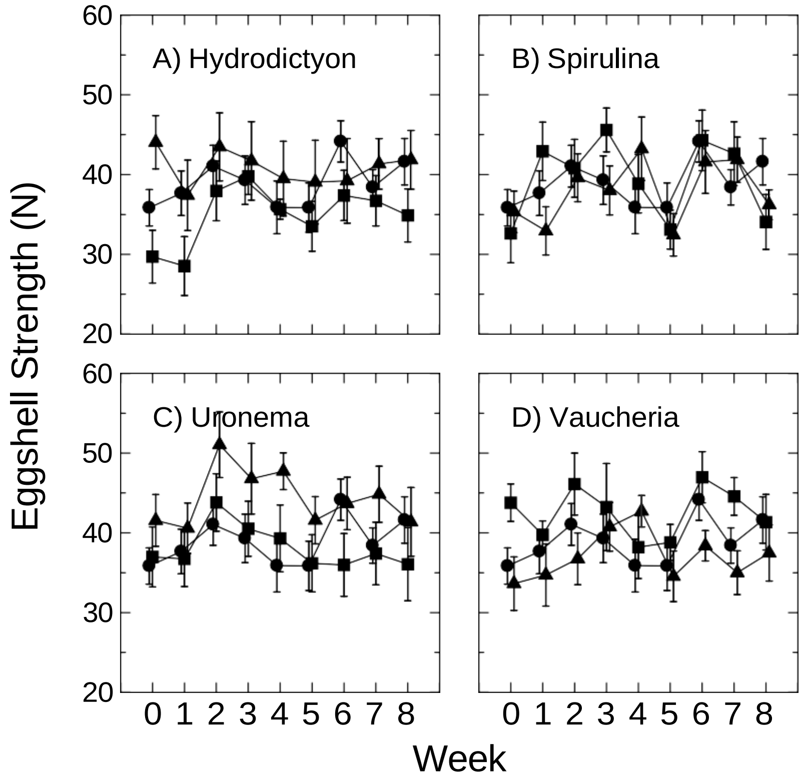

Figure 4.6. Average eggshell strength (N) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Overall mean = 39.3 ± 0.4 N.

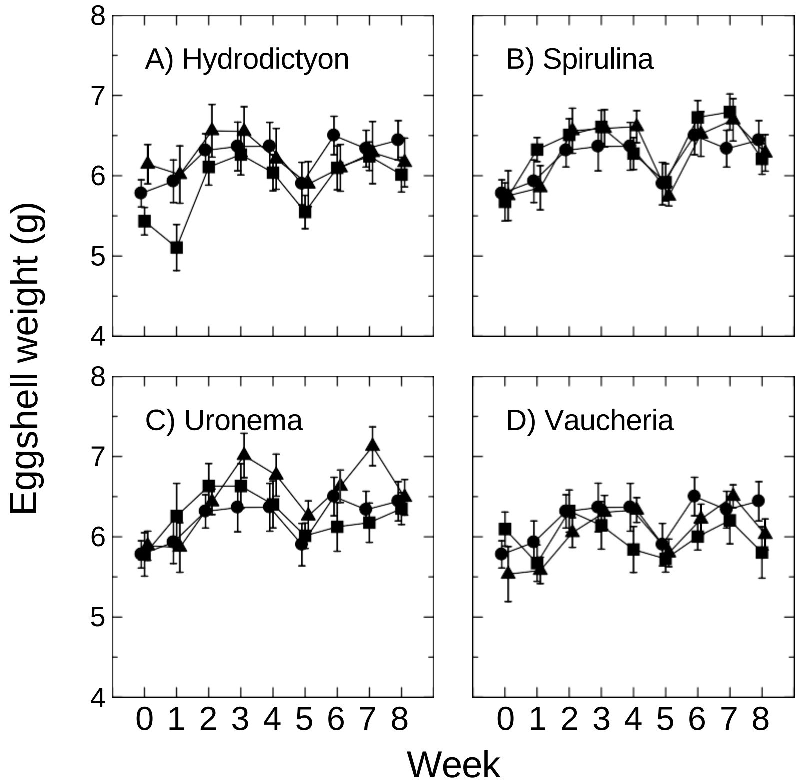

Figure 4.7. Average eggshell weight (g) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Overall mean = 6.19 ± 0.03 g (9.0 ± 0.04 % egg weight).

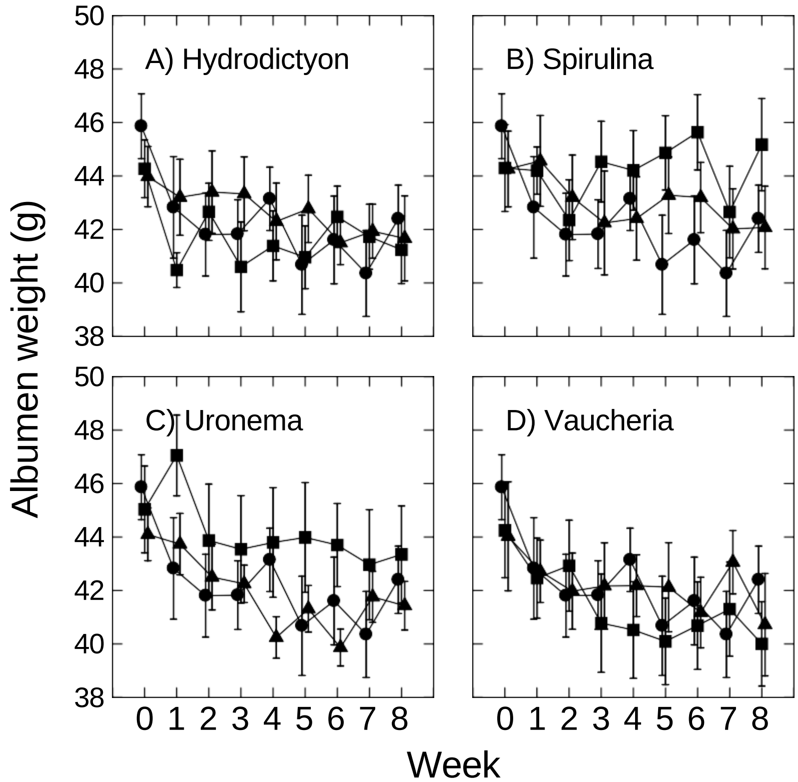

Figure 4.8. Average albumen weight (g) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Overall mean = 42.7 ± 0.2 g (62.1 ± 0.1 % egg weight).

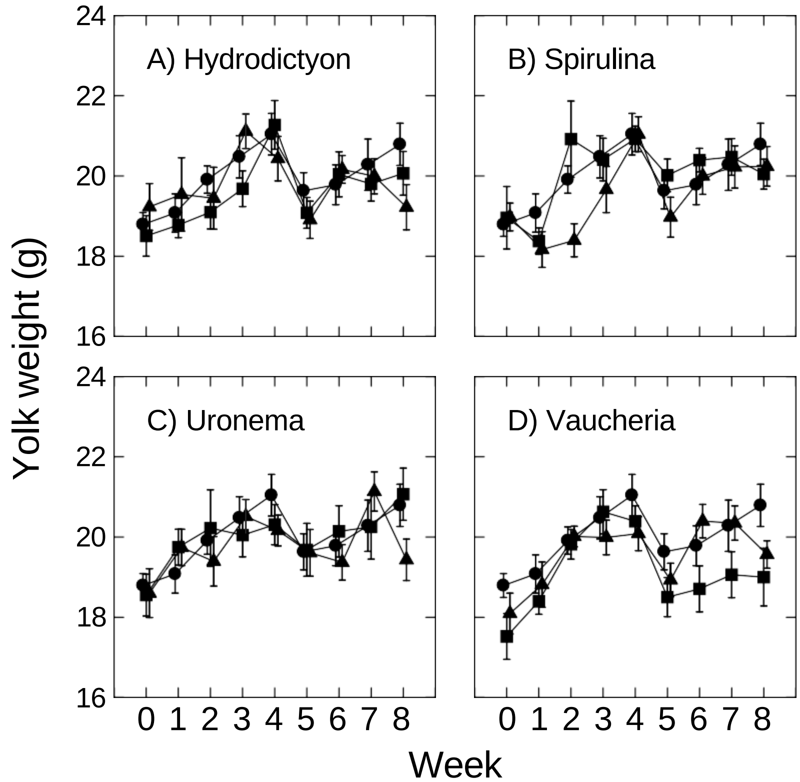

Figure 4.9. Average yolk weight (g) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Overall mean = 19.7 ± 0.1 g (28.8 ± 0.1 % egg weight).

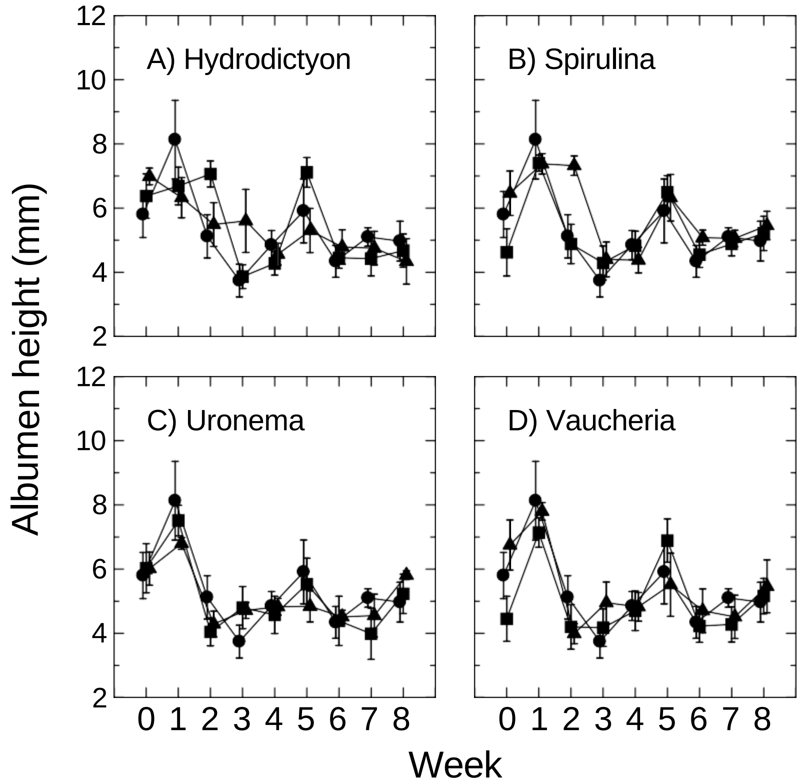

Figure 4.10. Average albumen height (mm) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Overall mean = 5.3 ± 0.1 mm.

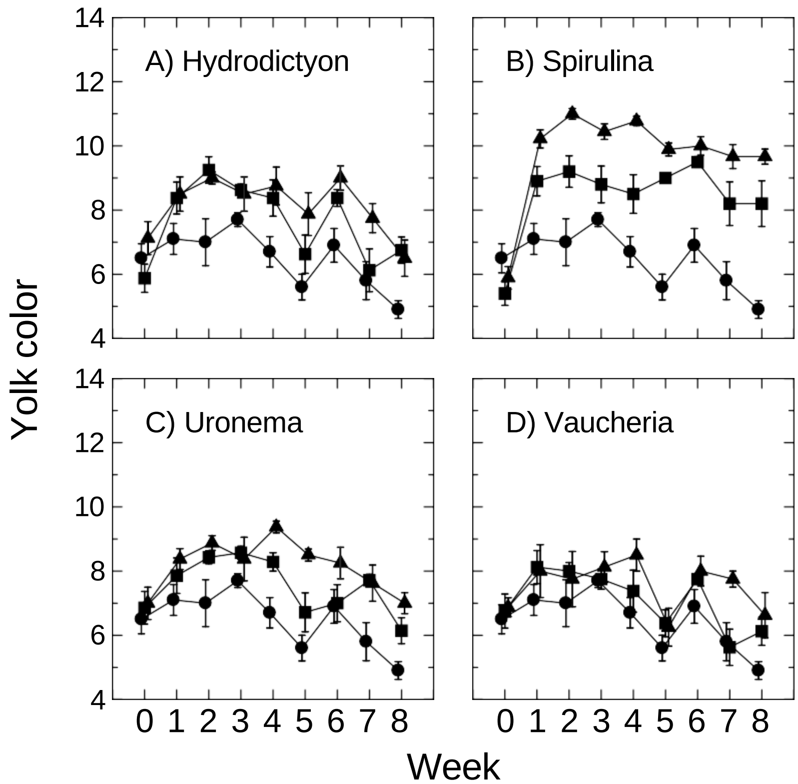

Figure 4.11. Average yolk color (Roche yolk color) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline.

Microalgae, regardless of inclusion level, had no significant effect on egg weight in this study (Figure 4.2). Similarly, Ross and Dominy (1990) found that with the inclusion of ?? at 2 %, egg weight was not significantly different, only at 6 % was there a significant difference. Selim et al. (2018) saw egg weight to be significantly higher in birds fed 0.3 % Spirulina. Other studies have shown that Spirulina does not have a significant impact on total egg weight (Zahroojian et al., 2013; Omri at al., 2019; Dogan at al., 2016). Dogan et al. (2016) states that Japanese quail fed Spirulina between 0.5-2.0 % showed no significant difference on eggshell weight. Zahroojian et al. (2013) saw no significant changes in egg weight at 1.5-2.5 % with laying hens, which matched the results from Omri et al. (2019) with Spirulina at 1.5-2.5%. Curabey et al. (2021) received similar results, with Spirulina at 1% and 2% having no significant change in the total egg weight.

Yolk color saw the most significant increase amongst the egg quality characteristics. Increasing level of inclusion of Spirulina, Hydrodictyon, and Uronema from 0 to 2 % significantly increased yolk color as shown in Figure 4.1. In addition, Vaucheria influenced yolk color, but only at the 2% level. The change in yolk color goes along with previous literature, which have shown a linear relationship between concentration and final yolk color (Anderson et al., 1991; Zahroojian et al., 2011, Zahroojian et al., 2013, Curabay et al., 2021). In the Anderson study, 1% inclusion of Spirulina in a barley-based diet brought the roche score to 8.3, the standard color in the corn control diet used in this study (Anderson et al., 1991). Zahroojian et al. (2011;2013) compared the use of Spirulina in a barley-based diets and determined that with the inclusion of only 1.5% brought the yolk color to 10.5. Increasing the rate to 2.5% saw the color to reach 11.6, which matched the synthetic pigment in that trial at 11.9 (Zahroojian et al., 2011; Zahroojian et al., 2013). Omri et al. (2019) saw similar results with inclusion of 1.5 and 2.0% Spirulina in laying hens, with $p<0.0001$ for both treatment groups. Ross and Dominy et al. (1990) saw a significant difference in yolk color with the inclusion of 1.5%, but at 12% the color score tripled in value compared to control. Selim et al. (2018) saw significant changes in yolk color with inclusion levels as low as 0.1%. Variation in yolk color between the algae may be due to different concentrations of xanthophylls and carotenoid pigments. The methods on how the algae was prepared can also alter this value (Anderson et al., 1991). Anderson et al. (1991) stated that algae that was freeze dried had an increase of 5,000 mg/kg xanthophylls and 6,000 mg/kg carotenoids compared to oven-dried Spirulina. No significance differences shown from any of the algae species regardless of inclusion level for egg weight (Figure 4.2), egg mass (Figure 4.3), shell thickness (Figure 4.5), shell strength (Figure 4.6), shell weight (Figure 4.7), albumen weight (Figure 4.8), yolk weight (Figure 4.9), and albumen height (Figure 4.10). Spirulina has not had any significant effects in those areas in prior studies (Curabay et al., 2021; Selim et al., 2018; Zahroojian et al., 2013). Selim et al. (2018) saw no significance in any of the egg quality characteristics, except for egg yolk color. This follows the study by Zahroojian et al. (2013), where yolk color was the only parameter to have a significant effect due to Spirulina. However, Dogan et al. (2016) saw a significant albumen and yolk index in their study on Japanese quail. On the other hand, Hajati and Zaghari (2019) saw a decrease in albumen height in all levels of inclusion of Spirulina.

In addition, there was no significant difference in feed conversion ratio, feed intake, body weight, or tibial strength between the treatments (Figure 4.4, Figure 4.12,

Figure 4.13, Table 4.3). However, Spirulina at 2% saw a significant increase (p=0.008) in egg production. Ross and Dominy (1990) saw growth depression with Spirulina, however, this was with inclusion of 20%. In the second part of their study, Spirulina was included at 12% and it had no effects on growth compared to the control group. Curabay et al. (2021) saw no significant difference in both FCR (p=0.31) and body weight (p=0.59).

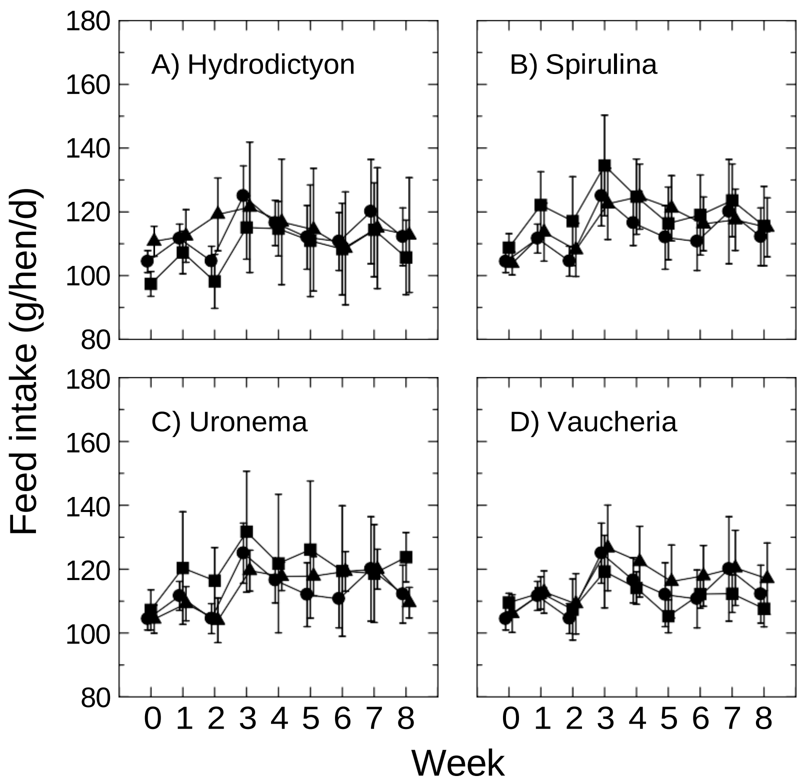

Figure 4.12. Average daily feed intake (g/hen/d) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Spirulina 1%: Cage 36, Week 8 – Changed FI/FCR from 391.4/6.319 (Spillage) to 117.1/1.890. Overall mean = 114.9 ± 1.2 g/hen/d.

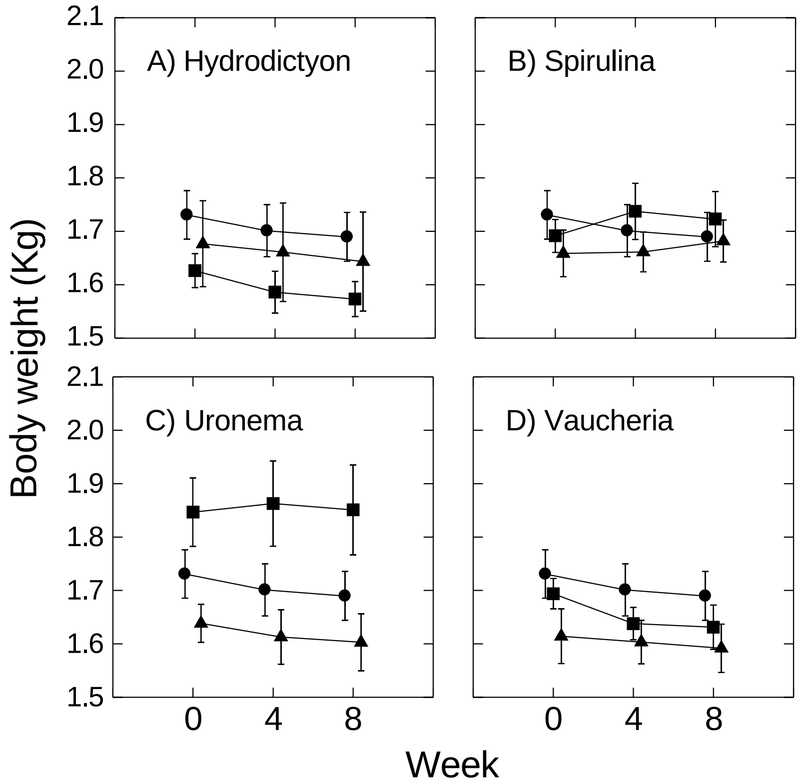

Figure 4.13. Initial (week 0), mid-trial (Week 4), and final (Week 8) body weight (kg) of the hens fed the experimental diets (0% ●, 1% ■, 2% ▲ microalgae). Note: the control group (0% microalgae) was common to all four experimental microalgae groups. Week 0, all birds were on the control diet to determine baseline. Overall mean = 1.67 ± 0.01 kg.

Table 4.3. Effect of dietary microalgae on breaking strength (N) of tibias.

*Significance at $p<0.05$

Dietary Treatment		Breaking strength	
Microalgae	Inclusion level (%)	(N)	p-value
Control	0	154.0	
Hydrodictyon	1	133.6	0.417
Hydrodictyon	2	153.2	0.999
Spirulina	1	137.5	0.635
Spirulina	2	175.9	0.474
Uronema	1	164.4	0.830
Uronema	2	152.8	0.997
Vaucheria	1	130.5	0.366
Vaucheria	2	140.5	0.711

In a study by Zahroojian et al. (2013), Spirulina included at 1.5-2.5% reported no significance difference in feed intake and feed conversion. One study saw a significant difference in body weight with laying hens fed Spirulina at 0.3% of the diet (Selim, 2018). Hajati and Zaghari (2019) saw that with an inclusion level of 20g/kg in quail led to an increase in feed intake.

One of the probable causes behind the variation in the results is the age of the birds utilized in the present study. The birds used in this study were at the end of their usable production life and had never been molted. A hen will lay eggs until about 70 weeks, where it is either molted or removed from production. Continued lay by that bird, leads to a decrease in production as shown in a study by Hendrix Genetics comparing brown egg laying hens from 1980 to 2020 (Figure 4.14) (Lera, 2020). Another cause may be due to the food system in which the birds received the diets. Diets were in a crumble form, which allowed for the possibility for the birds to pick and choose the items they consumed. Birds could exhibit food sorting behavior by removing ingredients out of the trough which would affect consumption values. For further studies in the poultry research barn, one must devise a more cohesive and consistent system to prevent feed loss and mixing.

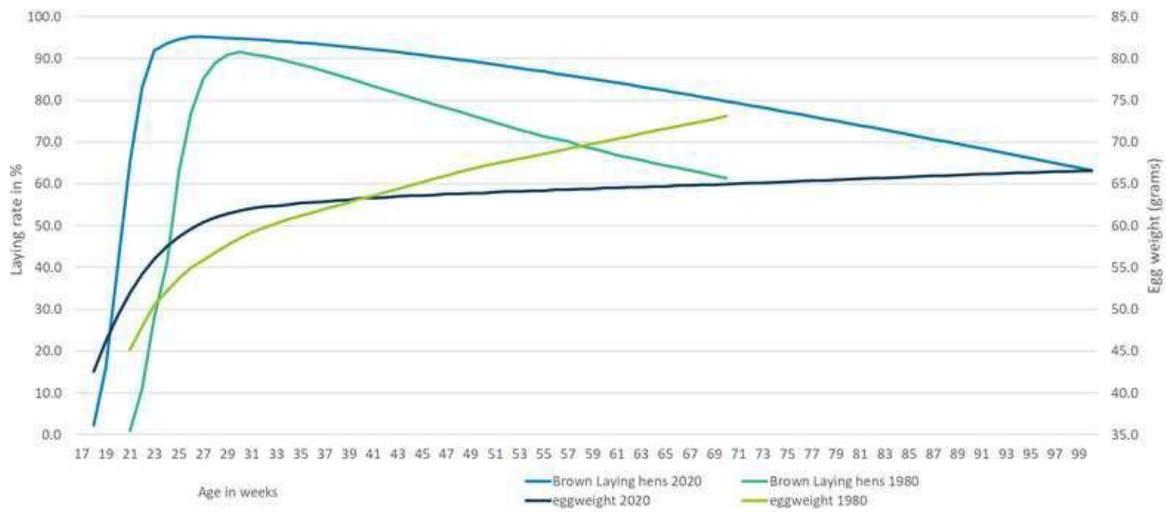

Figure 4.14. The relationship between age of a bird (in weeks) and the effects on laying rate (%) and egg weight (g) (Lera, 2020).

Chapter 5

CONCLUSION

Microalgae is a growing product used in both human and agriculture industries. Spirulina saw the greatest increase in weekly egg production at 2%. All algae groups had no significant effect on egg quality parameters compared to the control. However, at both inclusion levels, except for Vaucheria at 1%, significantly increased yolk color score. These may be viable inclusions into layer diets, as the xanthophylls present can act as a natural antibiotics and yolk pigment enhancer. In addition, with the high levels of crude protein present in the microalgae, they can act as a supplement for vitamin premixes utilized in the poultry industry. Both are beneficial concepts for the producer, as consumers are moving towards more natural products. However, the novel microalgae had varying AME and AIAAD values in the digestibility trials within test groups, which prevented the determination if the change was from the diet or other factors such as the age of the layers. Further study on these novel microalgae is needed to determine usability in broiler and layer production.

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