# Feeding brown algae and brown algae extracts from *Saccharina Latissima* to Broiler breeders

– effects on transfer of antibodies and nutrients from hen to egg

*Brunalger och brunalgsextrakt från Sacharina Latissima i foder till slaktkycklingföräldrar*
*- effekter på överföring av antikroppar och näringsämnen från höna till ägg*

Stina Kling

Independent project • 30 hp
Swedish University of Agricultural Sciences, SLU
Department of Animal Nutrition and Management, HUV
Agricultural programme – Animal Science
Uppsala, 2021

# Feeding brown algae and brown algae extracts from Saccharina Latissima to Broiler breeders – Effects on transfer of antibodies and nutrients from hen to egg

*Brunalger och brunalgsextrakt från Sacharina Latissima i foder till slaktkycklingföräldrar - Effekter på överföring av antikroppar och näringsämnen från höna till ägg*

Stina Kling

| | |
|---|---|
| **Supervisor:** | **Emma Ivarsson, SLU, HUV** |
| **Assistant supervisor:** | Malin Boyner, SLU, HUV |
| **Examiner:** | Helena Wall, SLU, HUV |


| | |
|---|---|
| **Credits:** | 30 credits |
| **Level:** | A2E |
| **Course title:** | Independent project in Animal Science, A2E - Agriculture Programme - Animal Science |
| **Course code:** | EX0872 |
| **Programme/education:** | Agriculture programme - Animal Science |
| **Course coordinating dept:** | Department of Animal Breeding and Genetics |
| **Place of publication:** | Uppsala, Sweden |
| **Year of publication:** | 2021 |
| **Cover picture:** | Stina Kling |
| **Keywords:** | broiler, broiler breeder, brown algae, Saccharina Latissima, laminarin, prebiotic, transfer, antibodies, nutrients, iodine, chicken, egg quality |


**Swedish University of Agricultural Sciences**
Faculty of Veterinary Medicine and Animal Science
Department of Animal Nutrition and Management

## Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visiable and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file.
If you are more than one author you all need to agree on a decision. You can find more information about publishing and archiving here: https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.

# Abstract

Brown algae and extracts of algae have been proved to have prebiotic effects. Seaweeds grow very fast and underwater. This is good from a sustainable point of view since algae do not need fertilizers, freshwater or agricultural land for growth. Brown algae is rich in nutrients, vitamins and minerals, and has an especially high content of iodine. This experiment included 45 hens and 9 roosters of the parent line of the fast-growing broiler ROSS 308. The hens were 28 weeks old at arrival and were housed in individual modules. There were three different experimental diets; algae meal (algae), algae extract and control. A boost vaccination was given 21 days after the introduction of the experimental diets. The aim was to analyse egg quality and transfer of antibodies and nutrients from hen to egg in order to determine if feeding broiler breeders brown algae and brown algae extracts could improve egg quality and transfer of antibodies and nutrients, and thereby, improve quality of newly hatched chicks. Maternal antibodies transferred from hen to chick during incubation are highly important since they protect the chick against pathogens during its first weeks in life. Healthy and vital chicks are the keys to good welfare and economic profit in poultry production.

The results showed that egg quality and transfer of antibodies were not improved in eggs laid by hens fed algae or algae extract. No chick parameters were shown to be affected by the hen treatment which indicates that the quality in the newly hatched chicks was not improved. There was, however, a significantly higher concentration of iodine in eggs laid by hens fed algae treatment. A significantly strong positive correlation at 0.69 was found between the egg weight and the chicken weight hatched from that egg, indicating that a small egg gives a small chicken, and a large egg gives a large chicken. Also, the percentage of fat pads of total body weight did differ between treatments and was higher for hens fed algae (2.06%) and algae extract (1.98%) compared to hens fed the control (1.67%).



*Keywords:* broiler, brown algae, Saccharina Latissima, laminarin, prebiotic, transfer, antibodies, nutrients, chicken, egg quality

# Table of contents

# List of tables

# List of figures

# Introduction

The global population is constantly growing and there may be over 9.1 billion people on the planet by the year 2050 (FAO 2009). This means that the food production will need to increase by 70 % in order to feed the world's population (FAO 2009). MacLeod et al. (2013) is forecasting that the demand for chicken meat will increase by 61 % and eggs by 39 % between the years 2005 and 2050. If food production increases, so will the environmental impact. It is therefore very important that the animal production minimizes its environmental impact for sustainability (MacLeod et al. 2013). Algae may contribute to healthier chicks and a more sustainable production (Lawrence et al. 1981). Producing healthy and viable chicks are essential for retaining good welfare and economic stability. Maintaining good chick quality is beneficial for both hatcheries and farmers since reducing the cost for incubation at hatcheries and decreasing mortality gives a higher profit. The maternal antibodies transferred from hen to chick during incubation are highly important due to the fact that they protect the chick against pathogens during its first weeks in life (Lawrence et al. 1981).

Prebiotics are commonly used as a feed supplement to develop better growth and stimulate healthy microflora in chickens (Bednarczyk et al. 2016). When the use of antibiotics as growth promoter in the poultry production was banned in Europe, the prevalence of enteric diseases increased and lead to large economic losses for the farmers (Bednarczyk et al. 2016). Prebiotics have the possibility to function as a solution to this problem, Bednarczyk et al. (2016) showed that injections of prebiotics in ovo resulted in increased lactobacilli and bifidobacteria in chicken feces. It also increased the body weight gain during the first 21 days after hatch. Another study of Canedo-Castro et al. (2019) resulted in increased length of the intestinal villi, and reduced serum cholesterol and triglyceride when broilers diet were supplemented with 2 % green algae (*Ulva rigida*). The increased length of villi produced a larger intestinal surface area and increased the activity of brush border enzymes which led to a larger surface area for absorption and higher capacity to digest. This indicates that algae can improve the microflora and increase the growth rate.

Prebiotics are formed out of three or more monosaccharides and linked by glycosidic bonds (Cheeke & Dierenfeld 2010). Polysaccharides can be branched or linear and are broken down into monosaccharides and oligosaccharides (Zaporozhets et al. 2014). The mucosa in the intestine has binding sites for carbohydrate residues to which also pathogenic bacteria can bind in order to create colonizes and cause diseases (Cheeke & Dierenfeld 2010). Oligosaccharides will bind to these sites instead of the bacteria and result in the bacteria being flushed out

(Cheeke & Dierenfeld 2010). The criteria for a compound being classified as a prebiotic is the following; resistant to acidic pH of the stomach, cannot be hydrolysed by mammalian enzymes or be absorbed in the gastrointestinal tract, can be fermented by intestinal microbiota, and the activity of the intestinal bacteria can be stimulated selectively by the prebiotic which will result in improved health (Gibson et al. 2010). Polysaccharides such as laminarin and fucoidan from brown algae have been proved to have prebiotic effects, both in animals and humans (Zaporozhets et al. 2014). Brown algae grow in coastal regions all over the world and can be used as a feed supplement for production animals (Cock, Peters & Coelho 2011). Algae grows very fast and produces large amounts of nutritive food without much effort (Kadam et al. 2015). Brown algae does for example does not need fresh water, chemical fertilizers, or large areas of agricultural land for good cultivation in comparison to land-based crops (Kadam et al. 2015).

The aim of this project is to analyse egg quality and transfer of antibodies and nutrients from hen to egg in order to determine if feeding broiler breeders brown algae and brown algae extracts can improve egg quality and transfer of antibodies and nutrients and thereby, improve quality of newly hatched chicks.

# 1. Literature Review

## 1.1. Chick quality

There are two different scoring systems developed for determining chick quality on day-old chicks; the Pasgar score and the Tona score. The reason for scoring chick quality is to predict survival and performance (Decuypere & Bruggeman 2007). The Pasgar score was first to be developed and measures reflexes, and appearance of beak, navel, legs and yolk sac. The score is calculated from a score of ten and each abnormality subtracts one score, and ten is considered good chick quality (Boerjan 2006). Tona scoring is most recently developed and the parameters included are; activity, down and appearance, retracted yolk, eyes, the conformation of legs, navel area, remaining membrane, and remaining yolk (Tona et al. 2003). The parameters are weighted differently depending on how important each parameter is for survival. Legs, eyes, and remaining yolk are the parameters that have the strongest impact on the total score (Tona et al. 2003). Activity is measured by laying the chick on its back and record how quickly it returns to its feet. For a high scoring; eyes should be bright and clear, and the navel completely closed. The remaining membrane and yolk should be small, legs should be steady and free from inflammation, the toes should not be crooked, and the chick should be dry and clean in general (Tona et al. 2003).

## 1.2. Egg quality

The average egg from a broiler hen consists of 37.3 g albumen, 18.7 g yolk and 5.39 g shell (Joseph et al. 2002), while the average egg from a laying hen consists of 40 g albumen, 17 g yolk and 6.4 g shell (Nangsuay et al. 2015). An egg's composition is suggested to be affected by the heritage, diet and age of the hen (Rose 1997). All essential nutrients such as lipids, amino acids, and vitamins needed for the embryo to develop are included in the egg from the beginning (Farrell 1997). Variation in the composition of eggs has been found in eggs laid by the same hen (Narushin & Romanov 2002), in eggs laid by different hens and in eggs with different weights (Stadelman 2003). There is also a known variation in the proportion of yolk and albumen between eggs from different hens and the percentage of albumen seems to be increasing with age (Stadelman 2003). Most nutrients may be altered by diet or genetics (Stadelman 2003). When analysing egg quality, there are both external and internal parameters.

### 1.2.1. External parameters

*Egg weight*

Egg weights can vary greatly. A study by Ulmer-Franco et al. (2010) showed a variation between 52 and 70 g. In the same study, a mean value for average, eggs of lower weight and eggs of higher weight were determined as 62.6 g, 58.3 g and 66.8 g, respectively. The same study showed that chicks from younger breeders had equal quality at hatch, but generally a lower final body weight at slaughter than chicks from older breeders. It also showed that eggs of lower weight resulted in small chickens, and heavy eggs resulted in larger chickens at hatch. Other studies have also found a strong positive correlation between egg weight and chick weight (McNaughton et al. 1978; Tona et al. 2003). Apart from age, genotype is a factor that gives variation in egg weight (Scheideler et al. 1998).

*Eggshell and shell colour*

The eggshell protects the egg from physical and pathogenic damage, which the egg can be exposed to in the external environment (Hunton 2005). The shell consists of approximately 94 % calcium carbonate and provides the embryo with calcium (Hunton 2005). It consists of 7 000 to 17 000 pores and each pore has a diameter of around 15-65 μm (Hamilton 1986). The main function of pores is to regulate the passage of water and air (Hunton 2005). A new method for measuring the thickness of the eggshell was constructed by Sun et al. (2012). The parameter is called "uniformity of eggshell thickness" and measures thickness on multiple positions. The authors found a positive correlation between breaking strength and uniformity shell thickness; where breaking strength is measured in order to see how well the eggshell can resist breakage (Sun et al. 2012). A thin eggshell is more likely to get cracks where pathogens can enter (Moyle et al. 2008). Eggs that have intact but thin shells may be dehydrated and result in hatching weak chickens that may perform poorly later on (Moyle et al. 2008). Eggs with thicker shells are to be preferred since the thickness keeps the nutrients in and is good for the development of the embryo (Narushin & Romanov 2002). Even so, too thick shells create a strong barrier and can make it difficult for the chick too hatch, so a balance in thickness must be kept (Narushin & Romanov 2002).

Factors affecting eggshell quality defined as thickness and colour are diets, genetics, housing, and age (Moyle et al. 2008). Shell colour can vary from white to brown in broilers, but the reason behind the variation within strains is not well-known (Moyle et al. 2008). Correlations between shell colour and hatchability in broilers were studied by Moyle et al. (2008), 1 944 eggs where collected from broiler breeders in ages between 33 and 45 weeks. The hatchability of eggs that were extremely pale were lower than in eggs with darker shells. Pale eggs are suggested to be prematurely laid eggs and may not be fully developed.

### 1.2.2. Internal parameters

*Yolk*

The yolk makes up approximately 30 % of the total egg weight and it is a very concentrated biological material which only consists of 48 % water (Stadelman 2003). Stadelman (2003) also stated that the rest of the yolk consists of 16 % protein, 33-35 % lipids, and small parts of minerals and carbohydrates. Two-thirds of the solid parts of the yolk are made up of lipids, mostly present as lipoproteins (Stadelman 2003). Lipids are the main energy source for the developing embryo (Nobel & Cocchi 1990), and they account for 90 % of the total required energy and is derived from the oxidation of fatty acids (Nobel & Cocchi 1990). A reduction of yolk size can thereby have negative consequences for the developing chicken (Ulmer-Franco et al. 2010).

Carotenoids are a group of plant pigments ranging from yellow to red and determine the pigment of the yolk (Cheeke & Dierenfeld 2010). It is important due to different consumer preferences and due to the fact that it has antioxidant activity (Cheeke & Dierenfeld 2010). Carotenoids are essential for birds and needs to be provided through the diet because of the bird's disability to synthesize it (PoultryWourld 2012). Carotenoids are transferred from the yolk to the liver in the developing embryo and decreases after hatch, this indicates that carotenoids help during the stress of hatching (Surai et al. 2016). Paler yolks contain fewer carotenoids than darker yolks.

*Albumen*

The albumen consists of 10.5 % protein and 88.5 % water and is the main source of water and protein for the developing embryo (Willems et al. 2014). The protein part is built up by 75% ovalbumin, 13% ovomucoid, 7 % ovomucin, 3% ovotransferrin, and 2% ovoglobulin. One important aspect of the albumen is to work as a defence against microbial invasion by killing bacteria and making the environment unfavourable for bacteria to grow in (Willems et al. 2014; Stadelman 2003). The viscosity holds the yolk in the right position and reduces the risk of bacteria penetrating and reaching the embryo (Willems et al. 2014). The thick albumen height function as an indicator of freshness in eggs (Scott & Silversides 2002). Long storage time results in lower albumen weight, lower albumen height, and higher albumen pH (Scott & Silversides 2002).

Haugh unit is a commonly used parameter when analysing egg quality and it is a measurement where the height of albumen is corrected for egg mass (Haugh 1939). The calculation includes a logarithmic scale which makes it possible to compare the height of the albumen in eggs with different sizes (Haugh 1939). The United States Department of Agriculture (2001) has a ranking of Haugh Unit (HU) value ranging between 0 and 130; AA: 100-72, A: 71-60, B: 59-30, and C: below 29. The letters function as a classification of egg quality where AA indicates the highest quality and B the lowest. For being classified as an AA-egg there are other criteria's that must be fulfilled as well. The eggs must for example be clean, have unbroken

shells, clear albumen, and be free from defects in the yolk. Eggs with scores lower than 60 are considered not to be fresh.

*Vitamins and minerals*

There are 13 commonly accepted vitamins required by humans and eggs contain all of these with the exception of vitamin C (DSM 2018). Leeson & Caston (2003) made a dietary experiment with 60 white leghorn layers that were fed either regular, three or ten times more supplemented vitamins. There was an increase from 59 to 75 µg of vitamin A in 60 g eggs when the supplement was increased from 10,000 IU/kg to 40,000 IU/kg vitamin A. The transfer of vitamins from diet to egg varied among different vitamins. Some vitamins had better transfer than others and are therefore more beneficial to use as enrichment in eggs. Two conventional eggs of large size will meet 30 % of the Recommended Daily Allowance (RDA) in humans for riboflavin, 60 % of vitamin K and around 15 % of vitamin A and D, folate, and vitamin B12 (DSM 2018). The most important factor for altering the vitamin content in eggs is the hen diet and this is especially true for fat-soluble vitamins (vitamin A, D, E, and K) which only are present in the yolk (Schiavone & Barroeta 2011).

Vitamins has several important functions for both the embryo, chicken and hen. For example, vitamin A is vital for normal growth, maintenance of epithelial cells and reproduction (thepoultrysite, 2015). Vitamin D is needed for good absorption and utilization of phosphorus and calcium which is required for eggshell formation, normal growth and bone development. Deficiency of vitamin D can lead to ricket. This can lead to thin-shelled eggs with low hatchability and leg weakness (thepoultrysite, 2015).

Trace minerals can also be transferred from the diet to the egg and are generally most deposited in the yolk (Schiavone & Barroeta 2011). The average hen egg contains between 4 to 10 mg of iodine. The effects on performance and egg traits when supplementing iodine to brown layers were studied by Yalçin et al. (2004). Hen diets were supplemented with 0, 3, 6, 12, and 24 mg iodine per kg feed. The concentration of iodine in eggs was increased by all treatments, but 3 and 6 mg iodine per kg feed was recommended for enriched eggs. Higher levels affected egg traits negatively by reduced egg weight, egg albumen index, and egg Haugh units. Iodine and selenium are involved in the production of thyroid hormones where T4 is iodine depended (Arzour-Lakehal et al. 2013) and T3 is activated by an enzyme that is selenium depended (Arthur & Nicol 1992).

It has been shown that hens fed 0.5 mg/kg selenium transfer increased amounts of selenium to the egg. These chicks have higher tissue concentration selenium 14 days post-hatch than chicks hatched from hens fed less than 0.1 mg/kg selenium (Pappas et al. 2006). Higher selenium reserves in chicks' tissue in brain and liver had a significant linear relation to hatch muscle selenium and improvement of FCR (feed conversion ratio) 0-12 days and 0-21 days after hatch (Couloigner et al. 2015). The authors suggests that this improvement is due to selenium reserves being used to facilitate transition of antioxidant system to GPx (glutathione peroxidase activity) and vitamin E in the liver.

## 1.3. Antibodies

The main function of the immune system is to recognize substances and organisms that are considered antigens (non-self) when entering the body (Poultry hub 2020). The immune system needs to initiate and manage the correct physiological responses in order to neutralize or eliminate the antigen (Poultry hub 2020). There are two types of immune systems; the innate immune system and the adaptive immune system. The innate immune system is the first line of defence against microorganisms, it is non-specific and refers to the natural ability to resist diseases (Cheeke & Dierenfeld 2010). For example, the skin and mucus function as a barrier and make it difficult for microorganisms to enter the body (Poultry hub 2020). A normal microflora with a stable microbial population prevents antigens to get a foothold in the body (Cheeke & Dierenfeld 2010). An increase in body temperature, fever, is a defence mechanism that creates a habitat where some disease-causing bacteria cannot survive

In contrast to the innate immune system, the adaptive immune system is more complex and specific. When the immune system recognizes a foreign molecule in the body, B-cells will start to produce specific antibodies as a response. The antibodies (or immunoglobulins) are glycoproteins that bind to the antigen and marks them for destruction or inactivation (Willey et al. 2012). There are three different classes of immunoglobulins in chickens and these are called IgA, IgY, and IgM (Leslie & Clem 1969). Before the newly hatched chick can develop antibodies itself, it strongly relies on the antibodies transferred from the mother hen (Ulmer-Franco et al. 2012).

The chicken IgA and IgM are only found in the albumen and they have similar properties as IgA and IgM in mammals (Carlander et al. 1999). A study of Kaspers et al. (1996) showed that IgA was transferred from the albumen to the yolk sac. Increased concentrations of IgA were first detected at day 14 of embryonic development and the transfer of IgA from albumen to the yolk sac had had a transfer rate at 44 % on day 21. The albumen is completely absorbed at hatch and the yolk sac is the sole source of IgA. Instead of being transferred into the fetal circulation as most other antibodies, IgA is most likely transferred to the embryonic gut across the yolk sac membrane. Another study of Bar-Shira et al. (2014) agrees with this and has additionally shown that maternal IgA persists in the chick's gut and respiratory system. It seems that IgA is depleted after 7-10 days post-hatch in the domesticated fowl.

Chicken IgY is the main immunoglobulin in serum and is also found in high concentrations in the yolk, the mother hen transfer IgY to the embryo via the yolk (Carlander et al. 1999). The concentration of chicken IgY in the yolk can vary between 1.15-20 mg/ml (Baylan et al. 2017) and between 3.26-6.02 mg/ml in serum (Hamal et al. 2006). Sun et al. (2013) found a positive correlation between the concentration of IgY in serum from hens and in the yolk from eggs laid by the same hen in a study on hens of White Leghorn, Silkie and Dongxiang blue (White Leghorn: $r = 0.404$, $P < 0.001$, $n = 100$; Silkie: $r = 0.561$, $P < 0.001$, $n = 70$; Dongxiang blue-shell: $r = 0.619$, $P < 0.001$, $n = 30$). This indicates that hens with

high concentrations of IgY in their serum may transfer more IgY to their offspring, which is beneficial for the newly hatched chicks.

## 1.4. Brown algae

Brown algae are organisms that grow in coastal regions all over the world, creating undersea forests and has a high level of biodiversity (Cock et al. 2011). It is part of a group of algae called stramenopiles that have evolved independently over a billion years and have resulted in unique features such as multicellularity (Cock et al. 2011). Seaweed is used by several sectors for biofuel, cosmetics, food for humans and farm animals, feed additives, herbal medicine, etc (Tiwari & Troy 2015). Due to its very fast growth rate and the fact that it does not compete with crops at agricultural land, the interest for the use of algae is growing (Cock et al. 2011). Alginates are polysaccharides extracted from the cell walls of brown algae and are used for gelling, viscosifying, and stabilization for example cosmetics or agri-foods (Draget et al. 2006). Brown algae have phenolic compounds (also known as phlorotannins) and these has been shown to have many health benefits for humans due to antioxidant, antiproliferative, antibiotic, anti-allergic, and anti-inflammatory properties (Li et al. 2011).

*Saccharina Latissima* has a high content of minerals (Tiwari & Troy 2015) and especially of iodine that can vary between 1556-7208 $\mu g\, g^{-1}$ with a mean value at 4652 ± 311 μg g−1 (Roleda et al. 2018). The maximum allowance of iodine for broiler feed is 10 mg/kg and this may seem quite much considering that they have a requirement of 0.35 mg/kg iodine in the feed (NRC 1994). Algae is generally rich in fiber (Zaporozhets et al. 2014). Fucoxanthin is a carotenoid found in brown algae and Gumus et al. (2018) made an experiment in order to see if it had any impact on growth performance, antioxidant metabolism, and meat quality of broilers. There was no difference in growth performance or meat quality, but it did increase the antioxidant metabolism and showed a small effect on the microbial quality of meat.

### 1.4.1. Laminarin and Fucoidan

Laminarins are polysaccharides found in the fronds of brown algae composed D-glucopyranose connected through b-1:3-bonds into linear chains and can be slightly branched (Rioux et al. 2010). The level of branching determines water solubility; the high content of branching gives high water solubility in cold water (Kadam et al. 2015). The extraction of laminarin can be done in different ways. Common approaches involve grinding, extraction with an acid (hydrochloric and sulfuric acid are two examples), separation with precipitation in ethanol and dialysis, a separation based on molecular weight, and ultrafiltration to remove other sugar molecules (Yvin et al. 1999; Kadam et al. 2015). The content of laminarin in brown algae varies between 10-35 % (Jiao et al. 2011). An experiment showed that laminarins cannot be decomposed by salivary, gastric, pancreatic, or intestinal enzymes (Deville et al. 2004).

Fucoidan is a series of complexed water-soluble sulfated polysaccharides found in the cell wall of brown algae (Vo & Kim 2012). It is mainly composed of L-fucose and sulfate, but it also contains other monosaccharides (mannose, galactose, and glucose), uranic acids and acetyl groups (Bo et al. 2008). Brown algae contain between 5-20 % fucoidan (Chapman 2012).

In 2017, an experiment was conducted by Sweeney et al. in order to investigate the effects of supplementing diets with laminarin and fucoidan. There were 135 day-old broiler chicks in the experiment, divided into three different treatments: basal diet, basal diet + laminarin, basal diet + laminarin and fucoidan. Chicks fed laminarin and laminarin + fucoidan had a higher total weight gain of 262 g and 254 g respectively in the post hatch period (day 0–13), compared to the chicks fed the basal diet with a gain of 243 g. These chicks also had a higher final body weight (13 days old) at 311 g and 302 g respectively, compared to the chicks fed basal diet with a total final body weight at 290 g. Chicks fed laminarin or laminarin + fucoidan also had an superior feed conversion ratio and ileal villus width.

## 1.5. Fat pads in broiler breeders

The selection of broiler strains has resulted in increased breast muscle and decreased fat pad deposition (Havenstein et al. 2003). Continuously, a 2001 broiler strain had a percentage of carcass at 13.7 % and abdominal fat at 1.4 % at an age of 43 days compared to a 1957 broiler strain at the same weight which at an age of 85 days had 17.9 % carcass fat and 2.0 % abdominal fat. Breast meat was 20 % as a percentage of the total body weight and 12.2 % for the 2001 strain and 1957 strain, respectively. Both strains where fed a 2001 strain diet. These changes are seen as improvements in the production of broiler chickens, but they may have negative effects for the broiler breeders. The abdominal fat pads have declined significantly between 1989 and 2010, from 3 % to 0.5 %. of the total body weight (Emous 2015). Sun & Coon (2005) compared broiler breeders with a diet with a higher inclusion of fat with breeders fed a standard diet during the laying period. Both diets had the same energy content. The result showed increased body weight gain and carcass fat, and larger eggs in breeders with the high fat diet. The study suggests that a certain percentage of body fat is necessary for optimal reproductive performance.

A comparison between ad libitum and restrictively fed broiler breeders were performed by Heck et al. (2004). The result showed that mortality in the ad libitum fed hens where at 40.4 % and 5.6 % for hens fed restrictively. A higher proportion of eggs with soft or broken shells and double yolks were laid by hens fed ad libitum. The hens can develop frustrated behaviour as a consequence of dietary restriction leading to chronical hunger (Sandilands et al. 2006). Symptoms of this can be pecking at non-food objects, excessive drinking, feather picking and aggression (Sandilands et al. 2006). The same study showed that behaviours like this can be reduced by supplementing the diet with oat hulls and CaP.

# 2. Material and method

## 2.1. Animals and housing

### 2.1.1. Parent line

An animal experiment was conducted at the Swedish Livestock Research Centre between November 18th, 2019, and March 23rd, 2020. The experiment was approved by the ethical committee of Uppsala region, approval number Dnr. 5.8.18-10572/2019. Forty-five hens and nine roosters of the parent line of the fast-growing broiler Ross 308 were included in the experiment. The hens were 28 weeks old when they arrived at the research center from a conventional broiler parent farm. Hens were kept in pairs for twelve days and then housed in individual modules. Two days after arrival to the research center a blood sample was taken and infectious bronchitis virus (IBV) titers in serum of the hens were determined. The initial average IBV titer was 5894±3087. Due to a high individual variation in initial IBV titer, the hens were divided in three groups, high, medium and low titer level and the experimental diets were randomly distributed within each titer group. This was done to ensure similar starting level of IBV for all dietary treatments. The change to the respective experimental diets occurred 19 days after arrival and the hens weighed on average 3711 g±397 g at the start of the experiment, there were no differences in weight between treatments. A boost vaccination, IBV (Nobilis®, IB multi, Massachusettes D207/D274 serotypes) was given in the breast muscle 21 days after the introduction of the experimental diets. Each module was provided with a laying nest, raised perch, nipple drinker, and wood shavings. The light was turned on at 8:00 in the morning, just prior to feeding and kept on for 13 h per day, the light intensity was 12 lux at hen height (Mavolux electronic, Gossen). Hens were fed restrictively, 175 g pelleted feed, once a day, and had free access to water. Roosters were kept in two groups of four and five individuals and fed restrictively adjusted according to body condition once a day with a commercial diet for parent line roosters (Lantmännen, Sweden). Hens and roosters were killed on January 27th, 2020 by an overdose of pentobarbital. Hens were weighed and body condition score according to Ross parent line was registered before they were put down. Dissections were conducted, and abdominal fat pads were weighed.

### 2.1.2. Chicks

During experimental day 32-42 hens were naturally mated every 5th day with randomly selected roosters. Eggs were collected and labelled with mother hen identity between experimental days 34-44 for the purpose of hatching chickens. A total of 390 eggs were put in an incubator (J.Hernel Brutgerate GmbH & Co[1]) on experimental day 45. The temperature in the incubator was maintained at 37.5 ± 0.5º C and the humidity kept at 55% ±5%. The eggs were candled on incubation day 5, and 82 % of the eggs were fertile. On incubation day 18, 301 eggs were moved to the rearing facility and placed in individual hatching boxes to maintain information of mother hen identity of every hatched chick. The on-farm hatching occurred between incubation days 20-22, and 85 % of the placed eggs were hatched within this time span. Of the 46 unhatched chicks, 7 was from hens fed the control, 19 from hens fed algae extract and 20 from hens fed algae.

Chicken quality parameters were measured (Tona et al. 2003) on day one. The following parameters where included: length, weight, activity, down and appearance, retracted yolk, eyes, legs, navel, remaining membrane, and remaining yolk. The length was measured by laying the chick over a ruler and measuring from the tip of the beak to the longest toe. The activity was measured by placing the chick on its back and observe how quickly it returned to its feet. An immediate return to its feet were considered strong activity, while a delayed return on its feet considered a weak chicken. Regarding down and appearance, the chick should be dry and clean. The chick was placed on its back on the hand palm when the retracted yolk was measured. Height of its abdomen and the consistency of the abdomen was estimated through palpation. A chick that had an abdomen containing a large yolk that was hard to the touch was considered of poor quality. The navel was also measured while the chick was placed on its back in the hand palm, and it should be completely closed and have the same colour as its skin around the navel for good quality. Eyes were observed and should be open, alert and bright for a high scoring. Legs were assessed by putting the chick on its feet and observing whether it could stand easily or not. Toes should not be crooked. A quality score of each chick was calculated based on scored physical appearance in accordance with Tona et al. (2003) with the maximum score of 100. Chickens were individually marked with a distinct colour and body placement to keep the individual identify of each chicken. On day seven, these identifications were changed to 1cm x 1cm laminated number tags attached in the neck subcutaneously with plastic strap. After scoring, chickens were housed in groups of 12-13 individuals.

## 2.2. Experimental diets

Hens were divided into three different dietary treatments, resulting in 15 hens per treatment and were fed the experimental diets for 7 weeks. The experimental diets were a control diet formulated according to **ROSS 308** PARENT STOCK, one diet with the inclusion of 0.6 % brown algae meal (Algae), and one diet with the

[1] KG Am Buschbaan 20, 33415 Verl

inclusion of 0.08 % of brown algae extract (Algae extract; Table 1 and 2). The brown algae meal and the extract were from *Saccharina latissima* cultivated at the Sven Lovén Centre for Marine Science at the Swedish West Coast. The algae meal was dried and milled before mixed in the diet. Dried algae were also used as a substrate for the extract. The algae extract was generated by washing with 0.3 M HCl, followed by ultrasonic treatment and precipitation with EtOH. The dried algae meal contained 5.1 % laminarin and the extract contained 40 % laminarin, determined enzymatically by measuring of the β 1,3/1,6-glucan content (K-YBGL 12/16, Megazyme). The algae meal and the algae extract diets were thereby formulated to contain 300 ppm laminarin. The iodine content of the dried algae meal was analysed (EN 15111m:2007) prior to feeding formulation and was 2300 mg/kg. The premix for the algae meal diet was therefore adjusted and did not contain any added iodine.

***Table 1.*** *Ingredient composition for the experimental diets, per kg diet[4] (%)*

| **Component** | **Control** | **Algae** | **Algae extract** |
|---|---|---|---|
| Wheat | 60.58 | 60.58 | 60.58 |
| Soybean meal | 14.38 | 14.38 | 14.38 |
| Oat | 10.00 | 10.00 | 10.00 |
| Calcium | 7.80 | 7.80 | 7.80 |
| Soy oil | 2.43 | 2.43 | 2.43 |
| Barley | 2.00 | 2.00 | 2.00 |
| $CaH_4P_2O_8$ | 1.00 | 1.00 | 1.00 |
| NaCl | 0.27 | 0.27 | 0.27 |
| Premix 1[2] | 0.45 | | 0.45 |
| Premix 2[3] | | 0.45 | |
| Methionine | 0.17 | 0.17 | 0.17 |
| $Na_2CO_3$ | 0.16 | 0.16 | 0.16 |
| Threonine | 0.06 | 0.06 | 0.06 |
| Algae | | 0.60 | |
| Algae extract | | | 0.08 |
| Lysine sulphate | 0.03 | 0.03 | 0.03 |

[2] The premix provided per kg diet: 500 FTU phytase, 0,4 mg biotin, 2 mg folin, 20 mg pantothenic acid, 3.5 mg vitamin B, 0.03 mg vitamin B12, 12 mg vitamin B2 (5-phosphate), 60 mg vitamin B3 Niacin, 6 mg vitamin B6, 3200 IE vitamin D3, 100 mg vitamin E premix, 4 mg vitamin K3, 10 000 IE vitamin A premix, 10 mg Cu-kelat, 5 mg $CuSO^4$, 60 mg $FeSO^4$, 2 mg $Ca(IO^3)^2$, 45 mg Mn-kelat, 100 mg $MnSO^4H^2O$, 0,2 $Na^2SeO^3$, 0.15 mg org selen, 45 mg Zn-kelat, 50 mg $ZnSO^4$, 5.5 mg Canthaxanthin, 2.5 mg Apoester, 0.2 mg cholinchloride

[3] The premix provided per kg diet: 500 FTU phytase, 0,4 mg biotin, 2 mg folin, 20 mg pantothenic acid, 3.5 mg vitamin B, 0.03 mg vitamin B12, 12 mg vitamin B2 (5-phosphate), 60 mg vitamin B3 Niacin, 6 mg vitamin B6, 3200 IE vitamin D3, 100 mg vitamin E premix, 4 mg vitamin K3, 10 000 IE vitamin A premix, 10 mg Cu-kelat, 5 mg $CuSO^4$, 60 mg $FeSO^4$, 45 mg Mn-kelat, 100 mg $MnSO^4H^2O$, 0,2 $Na^2SeO^3$, 0.15 mg org selen, 45 mg Zn-kelat, 50 mg $ZnSO^4$, 5.5 mg Canthaxanthin, 2.5 mg Apoester, 0.2 mg cholinchloride

## 2.3. Egg and feed nutrient analysis

Eggs were collected for nutrient analysis during experimental day 26-31. Eggs were stored for four weeks in 4° C before ten hens per treatment were randomly selected among the hens with confirmed fertile eggs, five fresh eggs were collected from each selected hen. The eggs were sent to Eurofins laboratory for analysis of vitamin A (retinol; EN 12823-1 2014), vitamin $D_3$ (EN 12821: 2009-08), iodine (EN 15111m:2007) and selenium (EN 13805m:2014, EN ISO 17294m:2016). The corresponding analysis was also performed on the experimental diets. The experimental diets were also analysed for dry matter by drying at 103°C for 16 h and ash of feed samples was determined after ignition at 600°C for 3 h (Jennische & Larsson, 1990). The content of crude protein (N × 6.25) was determined by the Kjeldahl method (NMKL, 2003) and the ether extract was determined according to European Communities (1998). In addition, amino acid composition of the experimental diets was analysed according to ISO (2005).

***Table 2.*** *Chemical composition of the diets with different treatments; control, algae and algae extract*

| **Nutrient** | **Unit** | **Control** | **Algae** | **Algae extract** |
|---|---|---|---|---|
| Analysed values | | | | |
| Dry matter | % | 92.8 | 92.9 | 93.2 |
| Crude Protein | g/kg DM | 179 | 176.5 | 172.3 |
| Ether extract | g/kg DM | 46 | 46 | 47.6 |
| Ash | g/kg DM | 128.5 | 123 | 117 |
| Lysine | g/100 g | 0.88 | 0.76 | 0.77 |
| Methionine | g/100 g | 0.41 | 0.37 | 0.41 |
| Threonine | g/100 g | 0.61 | 0.60 | 0.58 |
| Iodine | mg/kg | 1.3 | 12 | 1.7 |
| Vitamin A | µg/100 g | 230 | 201 | 211 |
| Vitamin D3 | µg/100 g | 8.42 | 6.83 | 6.83 |
| Selenium | mg/kg | 0.13 | 0.32 | 0.21 |
| | | | | |
| Calculated values | | | | |
| Metabolizable Energy | MJ | 11.4 | 11.4 | 11.4 |
| Phosphorus | g | 5.4 | 5.4 | 5.4 |
| Magnesium | g | 1.7 | 1.7 | 1.7 |
| Potassium | g | 6.1 | 6.1 | 6.1 |
| Sodium | g | 1.7 | 1.7 | 1.7 |
| Chloride | g | 2.0 | 2.0 | 2.0 |
| C18:2 | g | 19.5 | 19.5 | 19.5 |
| | | | | |
| Available P | g | 3.5 | 3.5 | 3.5 |
| Digestible Lysine | g | 6.0 | 6.0 | 6.0 |
| Digestible Metionine | g | 3.7 | 3.7 | 3.7 |

## 2.4. Scheme for the experiment

| Parameter/ event | Which animals | When | Experimental day |
|---|---|---|---|
| Initial IBV titers in serum | Breeder hens | November 19th | Pre-experiment |
| Start experimental feed | Breeder hens | December 9th | Day 1 |
| Body weight | Breeder hens | December 9th and January 27th | Days 1 and 49 |
| Egg production | Breeder hens | Daily | Days 1–49 |
| Boost vaccination against IBV | Breeder hens | December 30th | Day 21 |
| IBV and IgY level in serum | Breeder hens | January 9th and 21st | Days 31 and 43 |
| Vitamin and mineral content in eggs | Breeder hens | January 3rd to 8th | Days 26-31 |
| Egg quality | Breeder hens | January 9th to 10th; 23-24th | Days 31–32 and 45–46 |
| IgY level in yolk | Breeder hens | January 9th, 10th and 23rd | Days 31, 32 and 45 |
| IgA level in albumen | Breeder hens | January 9th, 10th and 23rd | Days 31, 32 and 45 |
| Collection- hatching eggs | Breeder hens | January 12th to 22th | Days 34–44 |
| Abdominal fat pad and body condition | Breeder hens | January 27th | Day 49 |
| Chick quality | All hatched chicks | February 13-15th | Incubation day 20–22 |
| Chick serum IgY and IBV | 2 focal chicks per module | February 17, 21, 26th | Chick experimental day 3, 7 and 12 |

### 2.4.1. Egg samples

Analysis of IgA and IgY were made on eggs collected at day 31, 32, and 45. Eggs were prepared for analysis at storage day 4 ± 1. Eggs were then cracked open, the yolk was separated from the albumen, and 2 ml of yolk was transferred into Eppendorf tubes. The albumen was homogenized, and 2 ml was transferred into Eppendorf tubes. The samples were centrifuged at 21 000xg for 20 minutes and the water phase was then transferred to Eppendorf tubes by using a 10-100 µl pipette. The samples were stored in + 4° C until analysis.

## 2.5. Egg quality

Eggs were analysed for external and internal egg quality parameters. Exterior parameters included were egg weight, colour of the shell, breaking strength, shell thickness and dry shell weight. Internal parameters included were weight of the albumen, albumen height, Haugh units, weight of the yolk and yolk colour.

### 2.5.1. Collection of eggs

Eggs were collected from each hen in the purpose of analysing external and internal egg quality. This was conducted on experimental day 31, 32, 45, and 46 and approximately 45 eggs per day and 180 eggs in total where collected, depending on the number of eggs laid. Eggs were also collected at day 30 and 44 in case of eggs missing or breakage. The selected eggs should not have visible cracks or double yolks and should be free from dirt. During collection, the eggs were labelled with the date and module number and then packaged with the tip down. The eggs were transported in room temperature in the laboratory at SLU and stored at 4° C until analysis.

### 2.5.2. Procedure for measuring egg quality

1. Shell colour was recorded using an eggshell colour guide from Zinpro[5], ranging from 1 to 10, where 1 was white and 10 dark brown.
2. The egg was weighed.
3. Breaking strength was measured by using an Egg force reader[6].
4. The egg was cracked open onto a glass plate.
5. The colour of the yolk was recorded by using a Yolk colour fan from Roche (1984).
6. Albumen height was measured 0.5 cm from the yolk by using a micrometer.
7. Yolk and albumen were separated using a separator.
8. Albumen and yolk were weighted.
9. Shell thickness was measured on three different spots around the equator of the egg using a micrometre. A mean value was calculated.
10. The weight of the shell, with shell membranes, was recorded after being dried at 103° C overnight and put in a desiccator for 1 hour to adapt to room temperature.

[5] Zinpro corporation, 10400 Viking Drive, Suite 240 Eden Praire, Minnesota, 55344 USA.

[6] Orka Food Technology, 875 Lakeview DR, Bountiful, UT, 84010, USA.

## 2.6. Analysis of antibodies

All analyses were conducted by using Chicken IgY ELISA kit from Immunology Consultants Laboratory[7]. The following analysis were made on hens and their eggs; IgY in yolk, IgY in blood serum, IgA in albumen and IBV in blood serum. Analysis was also made on blood serum from chickens, IBV and IgY.

### 2.6.1. Preparation of reagents

- Diluent Concentrate (5X) was diluted with 1/5 distilled water.
- Wash Solution Concentrate (20X) was diluted with 1/20 with distilled water.
- Enzyme-Antibody Conjugate was prepared by adding 10 µL to 990 µL 1X Diluent for each strip used for the analysis, 120 µL Enzyme-Antibody Conjugate to 12 ml was prepared for a full plate.
- Chicken IgY calibrator was diluted by adding 1 ml distilled water, the calibrator is then at a concentration of 380 µg/ml. The standard was prepared directly prior to use.

### 2.6.2. Preparation of samples

- By adding 5 µL sample to 495 µL 1X diluent, a 1/100 dilution was given.
- Then, 2 µL of the 1/100 dilution was transferred to 998 µL 1X diluent and a 1/50 000 dilution was given.

### 2.6.3. Procedure for analysing antibodies

Standards, one control, and one blank were pipetted onto the plate in duplicates, 100 µL in each well. Samples were pipetted onto the plate in duplicates, 100 µL in each well. The plate was then incubated for 30 ± 2 min at room temperature and washed 4 times. Enzyme-antibody conjugate dilution was pipetted into each well by using a multipette, 100 µL in each well. The plate was incubated for 30 ± 2 min at room temperature and washed 4 times. TMB Substrate Solution was pipetted into each well, 100 µL in each well. The plate was incubated for exactly 10 minutes at room temperature. The stop solution was pipetted into each well, 100 µL in each well and the absorbance was measured at 450 nm. Albumen was analysed for IgA. Sets of Chicken IgA ELISA used for the analysis were also from the organization "Immunology Consultants Laboratory". The same reagents were used, and the procedure was the same, except for shorter incubation times (20 ± 2 min instead of 30 ± 2 min). The samples were 1/50 dilutions.

[7] ICL, INC, 7150 SW Sandburg Street, Portland, OR, USA 97223

## 2.7. Statistical analysis

The SAS statistical software, ver. 9.4 was used for the analysis of egg quality, chick quality, egg production, level of nutrients, and correlations. For analysis of egg quality parameters, the Mixed procedure was used and treatment, analyze day, and age of hen was used as fixed factors and module as a random factor. Since the residual plots of shell colour and number of eggs with cracked shell did not show a normal distribution they were analysed by the Glimmix procedure in SAS, with treatment as a fixed factor, where a binary logistic model was used to evaluate if shell colour and crack in the shell was affected by the treatment. Prior to the analyses, scoring values of 1 and 2 (pale colour) were converted to binary value 1 and scoring values 4 and 5 (dark colour) to the binary value 0 for shell colour. For number of eggs with cracked shell per week (1 and 2) was converted to binary value 1 and no appearance was converted to 0. The Glimmix procedure was also used for chick quality score with hen treatment as fixed effect, and chicks with the maximum score of 100 were converted to binary value 1 and chicks with less than score 100 to binary score 0.

The Lay percentage, number of eggs laid by a hen per week/7, was analysed with Mixed procedure with treatment and week as fixed factors, the interaction between treatment × week was also included in the model, and the module was included as a random factor. The nutrient content of the eggs was analysed with GLM procedure with treatment as a fixed factor. Pearson correlations (r) were used to correlate the weight of egg to hatching weight of chicken, the antibody level in hen and chick as well as antibody level in serum and yolk. Analysis of antibodies in yolk, serum, and albumen from hens were presented in confidence intervals of 95 % using Microsoft Excel. Antibody levels of different treatments were considered significantly different from each other if the confidence intervals did not overlap.

# 3. Results

On the final day of the experiment the body weight of the hens was on average 4326 g ± 369 g and did not differ between treatments. The percentage of the fat pad of total body weight did differ between treatments (P=0.035) and was higher for hens fed algae (2.06%) and algae extract (1.98%) compared to hens fed the control (1.67%).

The following results are presented below; Analysis of egg quality, transfer of nutrients and egg production, antibody levels in the yolk, albumen and serum for hens, chick quality and correlations between hen and chick in regard to weight and antibody level.

## 3.1. Egg quality

There were no significant differences (>0.05) between treatments with regard to egg quality. There was, however, a tendency (<0.1) to a difference in shell thickness, where hens supplemented with algae had the highest value.

***Table 3.*** *Results from the external and internal egg quality traits. The table shows analysed values for the least squares mean (LSM), standard error (SEM)*

| | Control | Algae | Algae extract | Pooled SEM | P-value |
|---|---|---|---|---|---|
| Egg weight (g) | 64.13 | 63.82 | 64.24 | 0.546 | 0.811 |
| Breaking strength (kgF) | 3.81 | 3.67 | 3.84 | 0.101 | 0.362 |
| Shell thickness (mm) | 0.32 | 0.32 | 0.31 | 0.315 | 0.071 |
| Shell weight (g) | 5.62 | 5.64 | 5.53 | 0.06 | 0.296 |
| Shell percentage (%) | 0.09 | 0.09 | 0.09 | 0.000 | 0.209 |
| Shell colour | 2.92 | 3.00 | 2.70 | 0.535 | 0.554 |
| Yolk weight (g) | 19.72 | 19.6 | 19.79 | 0.273 | 0.810 |
| Yolk colour | 13.71 | 13.81 | 13.85 | 0.105 | 0.501 |
| Yolk percentage % | 0.31 | 0.31 | 0.31 | 0.002 | 0.8396 |
| Albumen height (mm) | 8.01 | 7.79 | 7.9 | 0.127 | 0.3767 |

| | | | | | |
|---|---|---|---|---|---|
| HU | 88.20 | 87.11 | 87.57 | 0.795 | 0.5327 |
| Albumen weight (g) | 35.23 | 34.99 | 35.35 | 0.34 | 0.6712 |
| Albumen percentage (%) | 0.55 | 0.55 | 0.55 | 0.003 | 0.5093 |

### 3.1.1. Egg production

No significant differences were found between treatments with regard to egg production. There were no significant differences in lay percentage between the treatments. The number of eggs with cracked shell for the total period was 9 (1.4% of total eggs) for control and 4 (0.65%) for both algae and algae extract and did not differ between treatments (P=0.305).

***Table 4.*** *Results of the laying of hens. The table includes the lay percentage for the treatments; control, algae and algae extract. Analysed values for the least squares mean (LSM), standard error (SEM)*

| Lay percentage w. | Control | Algae | Algae extract | Pooled SEM |
|---|---|---|---|---|
| 31 | 94 | 98 | 96 | 0.313 |
| 32 | 97 | 96 | 82 | 0.313 |
| 33 | 94 | 90 | 87 | 0.313 |
| 34 | 95 | 96 | 93 | 0.313 |
| 36 | 94 | 90 | 91 | 0.313 |
| 37 | 94 | 95 | 91 | 0.313 |

*P-value Treatment:* 0.127

*P-value treatment*week:* 0.2451

## 3.2. Transfer of vitamins and nutrients

In table 5, significant differences (<0.0001) were found on transfer of iodine from hen to egg in the different treatments. Fresh eggs from hens fed algae had a higher level (4.630 mg/kg) compared to eggs from hens fed control and algae extract, with levels at 0.594 mg/kg and 0.735 mg/kg respectively. A significant difference (<0.05) where found in selenium, where algae eggs had the lowest concentration. Also, a tendency (<0.1) to difference was found in vitamin A where algae extract had the highest concentration.

***Table 5.*** *Results from the analysis of the nutrients iodine, selenium, vitamin A and vitamin D in fresh eggs. The parameters below are; LS means, P-value and SEM are presented*

| | Control | Algae | Algae extract | Pooled SEM | P-value |
|---|---|---|---|---|---|
| Iodine (mg/kg) | 0.594[b] | 4.630[a] | 0.735[b] | 0.157 | <0.0001 |
| Selenium (mg/kg) | 0.392[a] | 0.363[b] | 0.388[a] | 0.008 | 0.0388 |
| Vitamin A (ug/100) | 234 | 218 | 248 | 8.865 | 0.0722 |
| Vitamin D (ug/100) | 1.449 | 1.262 | 1.275 | 0.099 | 0.3441 |

## 3.3. Antibody levels

Antibody levels presented are from hens and their eggs. Analysis on hen's blood serum (IgY and IBV) are presented in figure 1-3. In figure 4-5, antibody levels in eggs are presented (IgA in albumen and IgY in yolk). Antibody levels from each analysing day can be found in appendix, figure 1-8. There were no significant differences between the treatments in any of the analyses below (figure 1-6). However, a tendency (<0.1) to difference was shown in IgA concentration in albumen between algae treatment and control treatment (figure 1). Chicken serum samples were analysed for IgY and IBV but are not presented below and are only presented as correlations to analyses on hens and their eggs in this thesis. No effects on antibody level of hen treatment were shown in the analysis on antibodies performed on chickens.

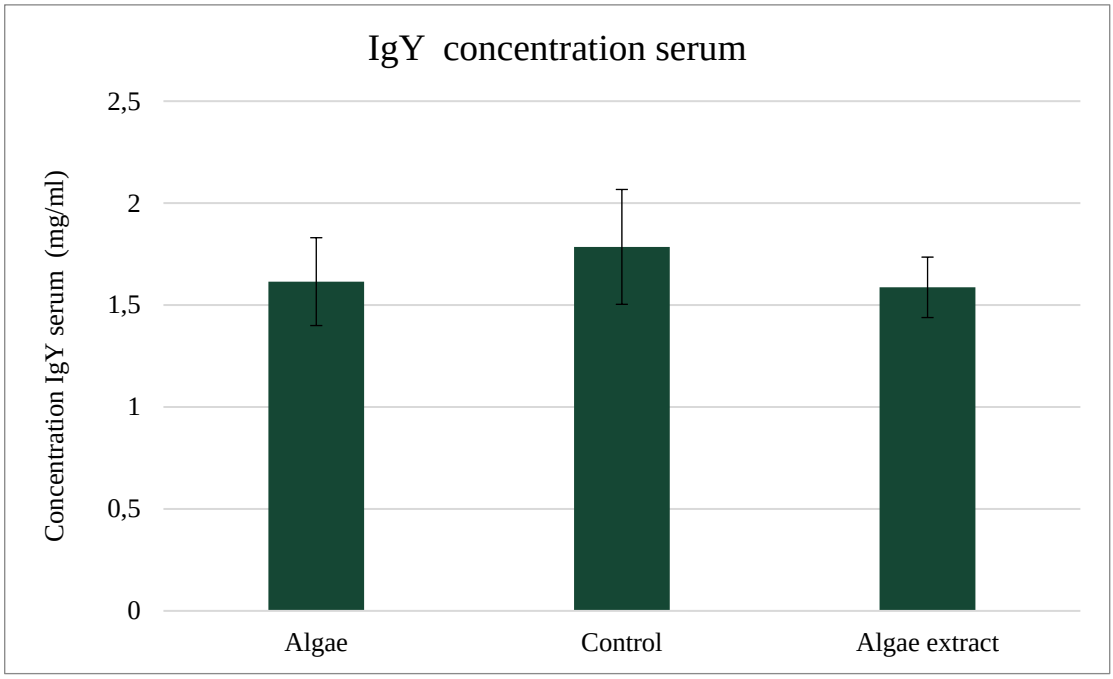


***Figure 1.*** *IgY concentration (mg/ml) in serum from each hen treatment is presented in the bars and the line shows a 95 % confidence interval. The result is an average on all analysing dates. There were no significant differences between the treatments, according to confidence interval.*

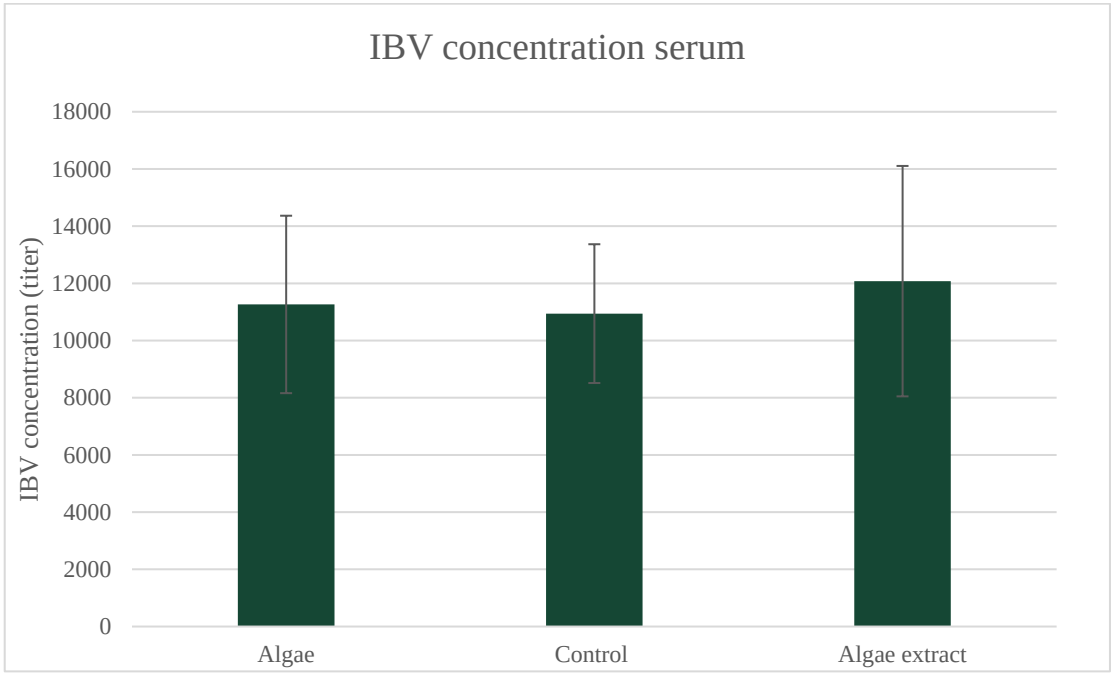


***Figure 2.*** *IBV concentration (titer) in serum from hens 13 days after vaccination (09-01-2020). Each treatment is presented in the bars and the line shows a 95 % confidence interval. There were no significant differences between the treatments, according to confidence intervals*

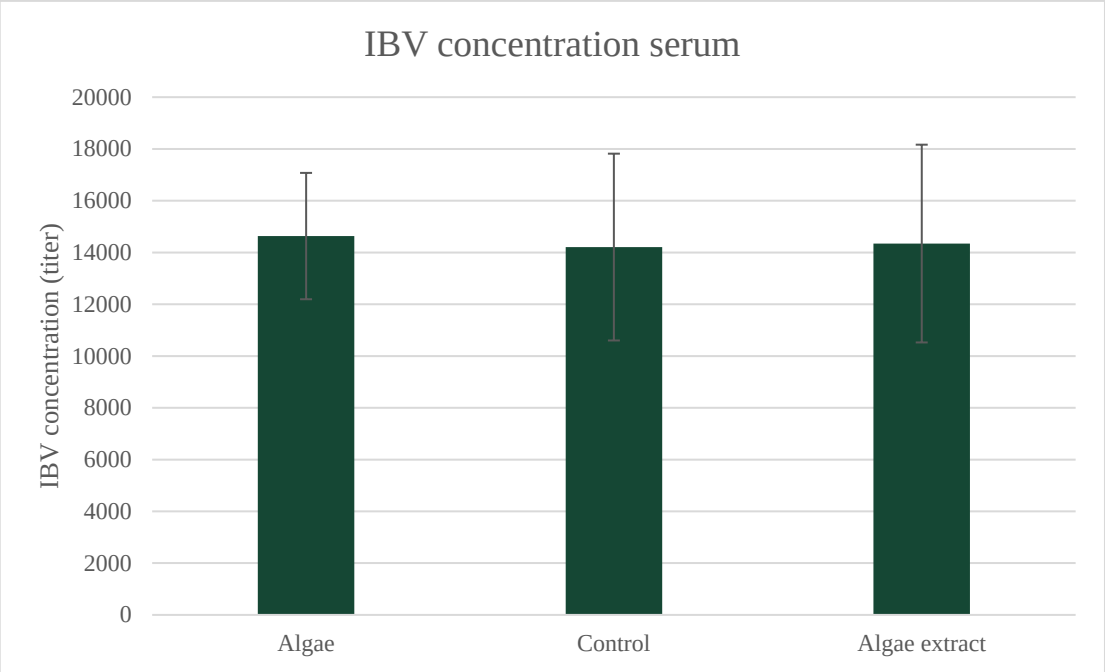


***Figure 3.*** *IBV concentration (titer) in serum from hens at 26 days after vaccination (22-01-2020). Each treatment is presented in the bars and the line shows a 95 % confidence interval. There were no significant differences between the treatments, according to confidence intervals*

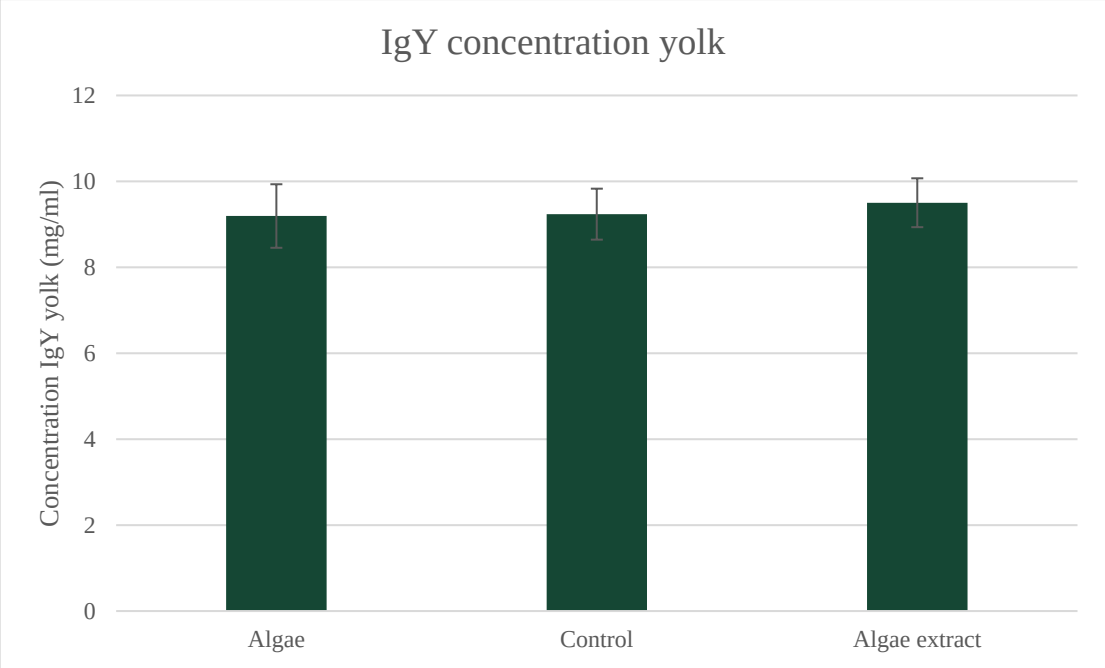


***Figure 4.*** *IgY concentration (mg/ml) in yolk from each treatment is presented in the bars and the line shows a 95 % confidence interval. The result is an average on all analysing dates. There were no significant differences between the treatments, according to confidence intervals.*

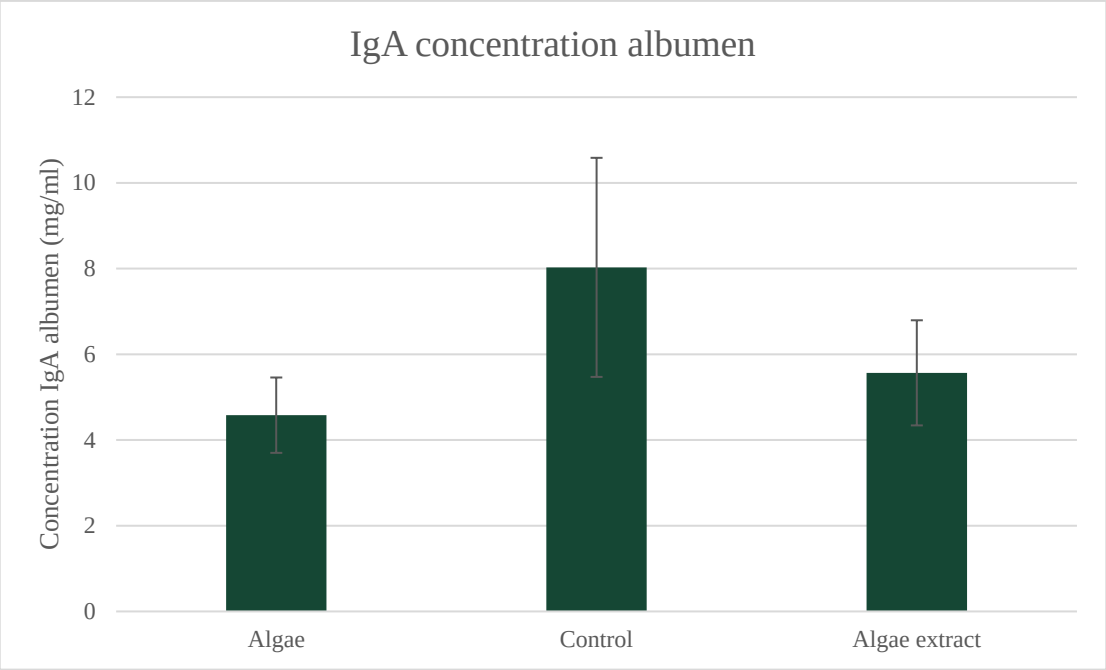


***Figure 5.*** *IgA concentration (mg/ml) in albumen from each treatments presented in the bars and the line shows a 95 % confidence interval. The result is an average of all analysing dates. There were no significant differences between the treatments according to confidence intervals.*

## 3.4. Chick quality

The average hatching weight of the chickens was 46.6±2.38 g and the average length was 17.7 ± 0.51 cm and there was no effect of hen treatment (P>0.05) on these parameters. The chick quality score was on average 97.3 ±4.4 and did not differ between hen treatments (P=0.101). The first week mortality resulted in seven dead chickens out of 255 hatched chickens of which four were from mothers on algae diet, two from mother fed control diet and one from a mother fed laminarin diet.

## 3.5. Correlations

There was a significantly strong correlation at 0.69 with a p-value at <.0001 found between the weight of the egg and the weight of the chicken hatched from the same hen. Mean values for egg weights and chicken weights from each hen were used in the analyse.

A significantly negative correlation at -0.60 with a p-value at <.0001 was found between IgY yolk and IgY serum from the same hen. The first analyse on IgY in serum and the last analyse on IgY in yolk were used in order to be as close as possible in time.

When analysing IgY concentration in serum from chickens and IgY concentration in yolk from their mother hen, a small positive correlation at 0.21 with a p-value at 0.3201 was shown. Mean values for IgY concentration in serum from chickens and IgY concentration in yolk from their mother hen were used in the analyse.

Considering IBV, a mean value from the two analyses of IBV from serum in hens (figure 1 & 2) and a mean value from all analyses on IBV from serum in chickens was used when analysing a correlation. A positive correlation at 0.74 with a p-value at 0.001 was found between IBV from serum in chickens and IBV from serum in the mother hen.

# 4. Discussion

## 4.1. Egg quality

The mean values for all treatments were varying between 35.0-35.35 g in albumen weight, 19.6-19.72 g in yolk weight and 5.53-5.64 g in shell weight (table 3). The literature suggests that an average egg from a broiler hen consists of 37.3 g albumen, 18.7 g yolk and 5.39 g shell (Joseph et al. 2002). These results are very similar to the ones in this study. Total egg weight may vary between 52 and 70 g according to Ulmer-Franco et al. (2010). The variation for all treatments was between 63.82 and 64.24 g (table 3) so the variation was quite small, and the eggs would be considered average or heavy (62.6 g and 66.8 g respectively) according to Ulmer-Franco et al. (2010).

When looking at the ranking for HU developed by The United States Department of Agriculture (2001), the eggs from these hens should be classified as AA eggs (AA: 100-72) since these eggs varied between 87.11 and 88.20 (table 3). Yalcin et al. (2014) stated that levels above 6 mg/kg of iodine in eggs could affect HU. This seems to be correct since the 4 mg/kg of iodine in eggs with algae treatment (table 5) did not significantly affect HU.

There was a tendency to difference considering the shell thickness and the algae treatment had the highest value (table 3). Thicker shells may be positive for the embryo since they keep the nutrients in and are less likely to get cracks (Narushin & Romanov 2002).

Regarding the colour of the eggshell, there were only two different shades (2 and 5) picked out of the eggshell colour guide with ten different shades. The eggs were either very pale or very dark. It could have been interesting to see if the colour of the eggshell had any correlation with hatchability, which Moyle et al. (2008) suggests. Eggs that were extremely pale had lower hatchability and was assumed to be prematurely laid eggs in this study.

Overall, there were no significant differences found between treatments, so it might be assumed that algae and algae extract in the feed did not affect any of the egg quality parameters. Samak & Ibrahem (2012) studied the effects of feeding laying hens with a diet supplemented with Spirulina platensis algae. There where four treatments fed either 0, 0.10, 0.15 or 0.20 % Spirulinapowder between 28 and 52

weeks of age. The result showed that hens fed Spirulina laid significantly heavier eggs and had higher egg yolk percentage and higher yolk colour score. This indicates that algae can improve the egg quality. Brown algae may not be the best option in purpose of improving egg and chicken quality, other types of algae may have better effects. Also, the hens in the study Samak & Ibrahem (2012) were fed the treatments for a longer period of time, 24 weeks compared to seven weeks. Perhaps if the broiler breeders were fed the treatments for a longer period of time, the egg quality would be significantly improved in this study too.

## 4.2. Chicken quality

Regarding the chicken quality, the diets fed to the mother hen did not seem to have any effects on the quality. Chicks from all hen treatments had generally good quality according to Tona et al. (2013), with a mean value at 97.3 ±4.4 on a scale between 0 and 100. This can be due to the fact that the mother hens included in this experiment were in optimal age and therefor produced chicks with good quality. The diets to all hens met the requirements for nutrients, vitamins and minerals. There might have been more obvious effects of the supplemented algae and algae extracts if the basal diet did not meet some of the requirements. Sweeney et al. (2017) supplemented diets for chickens with laminarin and laminarin + fucoidan which improved the growth rate and intestinal structure in the chickens hatched from the hens fed these treatments. Studies where diets supplemented with algae fed to broiler breeders affected chicken quality where not found. However, there is evidence confirming that diets supplemented with vitamin D and E and trace minerals such as selenium, zinc and manganese fed to broiler breeders can improve the quality of chickens (Chang, Halley & Silva 2017).

## 4.3. Vitamins and minerals

Significant differences (<0.0001) were found on the transfer of iodine from hen to the egg in the different treatments (table 5). Eggs laid by hens with algae treatment had a lot higher content of iodine, 4.630 mg/kg, then eggs laid by hens with control and algae extract treatment with concentrations at 0.594 mg/kg and 0.735 mg/kg respectively (table 5). This could be expected with the knowledge of algae having a generally high content of iodine (Roleda et al. 2018). Broiler chickens have a quite low requirement of iodine at 0.35 mg/kg and algae are generally rich in minerals (Tiwari & Troy 2015). Therefore, algae may function as a good feed supplement for minerals in poultry production. It is important to be aware of the mineral's maximum content in the feed. The limit for iodine is at 10 mg/kg feed (NRC, 1994) and there was 12 mg/kg iodine included in the diet with algae treatment (table 2). The inclusion of algae was only 0.6 % of the total diet (table 1) and the possible maximum inclusion of algae meal before maximum iodine level is reached is therefore very limited. There may be negative effects with too much iodine included on for example egg quality (Yalcin et al. 2014). Iodine is consumed by humans through iodine-rich food or salt enriched with iodine. *Saccharina Latissima* is rich

in iodine and 30 mg of this algae would be enough to cover the recommended daily intake (RDI) for humans (Roleda et al. 2018). Eggs enriched with iodine could perhaps be an interesting marketing idea and give an additional cost value. Especially for people suffering from iodine deficiency.

A significant difference (<0.05) where found in selenium in the egg, where algae treatment had the lowest level. Iodine and selenium are involved in the production of thyroid hormones where T4 is iodine depended (Arzour-Lakehal et al. 2013) and T3 is activated by an enzyme that is selenium depended (Arthur & Nicol 1992). So, selenium may have been used for activating the production of thyroid hormones and the transfer of selenium may be lower in the algae treatment due to the high conclusion of iodine. Also, a tendency (<0.1) to difference where found in vitamin A where algae extract had the highest level (table 3). This may be due to fact that algae contain carotenoids (Gumus et al. 2018). Even so, it would have been more logical that algae treatment had the highest level of vitamin A since the green parts and vitamins are washed away when the algae extract is extracted.

Finally, the results indicate that the hens that were fed algae meal treatment had increased concentrations of iodine and algae extract treatment had increased concentrations of vitamin A in their eggs. More research is probably needed, but it might be possible to enrich eggs with vitamins and minerals needed by humans in different regions. This could be very positive for people in developing countries where different deficiencies can be common.

## 4.4. Antibodies

The highest levels of IBV-antibodies were shown in the analyses 26 days after vaccination (figure 3). This is logical due to the fact that it takes some time for the immune system to respond to the vaccination. The treatments algae and algae extract did not seem to have any effect on the levels of IBV-antibodies. No significant differences between hen treatments were determined in any of the analyses made on concentrations of IgA in albumen, IgY in the yolk, IgY in serum, or IBV-antibodies in serum. However, there was a tendency to difference between the treatment algae and control in the analyse on IgA concentration (figure 5). This indicates that the algae treatment might have decreased the level of IgA in the albumen. The IgA-antibodies are transferred from the albumen to the yolk sac and then to the gut prior to hatching according to Kaspers et al. (2016). The literature indicates that IgA-antibodies are important for the gut and respiratory system during the period of hatch and 7-10 days after (Bar-Shira, 2014).

The mean values for IgY in the yolk from hens fed either algae treatment, control treatment or algae extract treatment were 9.2 mg/ml, 9.23 mg/ml and 9.5 ml/mg respectively (figure 4). A study of Baylan et al. (2017) found a variation of chicken IgY in yolk between 1.15-20 mg/ml and this indicates that the levels of IgY in the yolk from this study would be quite average. The mean values for IgY in blood serum from hens fed either algae treatment, control treatment or algae extract treatment where 1.61 mg/ml, 1.79 mg/ml and 1.59 ml/mg respectively (figure 2).

When comparing the results from this study to the ones found in the literature that were between 3.26-6.02 mg/ml in serum (Hamal et al. 2006), the results from this study seems to be at very low concentrations. It is difficult to compare results from ELISA-tests. Different ELISA-kits can vary according to reagents, dilutions and incubation times. Another factor to consider is the person performing the test. All persons pipette differently even if there are guidelines for accuracy and the speed can also vary greatly.

## 4.5. Correlations

A significantly strong positive correlation at 0.69 was found between the egg and the chicken hatched from that particular egg. This states that a small egg gives a small chicken, and a large egg gives a large chicken. Other studies also suggest a strong positive correlation between egg weight and chick weight (McNaughton et al. 1978; Tona et al. 2003). The same result was found in a study by Ulmer-Franco et al. (2010) where eggs of lower weight resulted in small chickens and heavy eggs larger chickens at hatch. Broilers hatched from heavy eggs were still heavier than those chicks hatched from eggs with lower weights after 21 days, but chickens hatched from average eggs had caught up. The same study also showed that chicks hatched from an egg with lower weight hatched earlier than average or heavy eggs.

There was a significantly negative correlation at -0.60 between IgY serum and IgY yolk from the same hen. This indicates that a hen with a low concentration IgY in serum has a high concentration in the yolk. In opposite to this result, Sun et al. (2013) found a positive correlation between the concentration of IgY in serum and in the yolk from the same hen in a study on hens in the breeds White Leghorn, Silkie and Dongxiang blue. The methods differed due to the fact that eggs were collected 7 days after the blood samples in the literature and 16 days after the blood samples in this study. Why the results differ between these two studies is still unclear. Further research could include parameters such as; at which point the antibodies transfer from the blood to the yolk, when the optimal time for measuring the antibody levels for the most accurate result occurs, if the hen can save IgY in the serum, if the transfer itself can be more efficient in some hens and if there are differences between breeds regarding these aspects.

A positive correlation at 0.74 was found between IBV from serum in chickens and IBV from serum in the mother hen. Also, a small positive correlation was found between IgY from serum in chickens and IgY from yolk in the mother hen was found. This suggests that hens with high concentrations of antibodies in their serum can transfer more antibodies to their offspring, which is beneficial for the newly hatched chicks.

## 4.6. Fat pads

The percentage of the fat pad of total body weight did differ between treatments (P=0.035) and was higher for hens fed algae (2.06%) and algae extract (1.98%) compared to hens fed the control (1.67%). These results indicates that hens fed diets with algae or algae extracts can reserve more body fat without increasing the amount of energy in the diet or the amount of fed. A study of Sun & Coon (2005) suggests that a certain percentage of body fat is necessary for optimal reproductive performance. It can be challenging to balance the right proportion of body fat since broiler breeders needs to be fed restrictively in order to avoid mortality and low egg quality (Heck et a. 2004). Nutritional studies have been conducted in order to increase the percentage of body fat without increasing the amount of feed or the content of energy in the feed (Sun & Coon 2005). Studies have also been made on adding feed supplements in purpose of reducing unwanted behaviours related to chronical hunger. Sandiland et al. (2006) showed that unwanted behaviours were reduced by supplementing the diet with oat hulls and CaP. So, it is possible to impact the percentage of body fat and unwanted behaviours as a result of chronical hunger by feed supplements. Algae is generally rich in fibre (Zaporozhets et al. 2014) and minerals (Tiwari & Troy 2015). It would be interesting to investigate if algae could reduce behaviours related to restricted diets and chronical hunger, since oats hulls contains fiber and calcium and phosphor (CaP) are minerals which showed effects in the study of Sandiland et al. (2006). Further research is needed for knowing what caused the higher percentage of fat pads in the treatments with algae and algae extracts.

## 4.7. Sustainability

Brown algae is produced in a sustainable way for many reasons. It does not need agricultural land since it grows in water, and it thereby does not need fertilizers or freshwater for growth (Kadam et al. 2015). It also has a very fast growth rate (Kadam et al. 2015). These arguments point to algae having a small impact on the environment. This is very positive due to the increasing demand for meat and eggs, and the increasing population all over the world (FAO 2006).

Li et al. (2011) stated that brown algae have phenolic compounds and has many health benefits for humans and perhaps these could be health aspects for poultry that researchers are not aware of yet. Laminarin and fucoidan from brown algae have been proved to have prebiotic effects, both in animals and humans (Zaporozhets et al. 2014). Further research on the prebiotic effects from algae extracts on the microflora and disease resistance would be interesting to investigate. Even if the transfer of antibodies or the egg quality did not significantly differ in hens fed with algae or algae extract, there may be several other health aspects such as antioxidant metabolism and meat quality that could be investigated when using algae as a feed supplement. A healthy and viable chick is important for economic stability and minimizing the environmental impact (MacLeod et al. 2013).

# 5. Conclusion

The egg quality and transfer of antibodies were not improved in eggs laid by hens fed algae or algae extract treatment. No chick parameters were shown to be affected by the hen treatment which indicates that the quality of the newly hatched chicks was not improved. There was, however, a significantly higher concentration of iodine in eggs from hens fed algae treatment. Also, a significantly strong positive correlation at 0.69 was found between the egg and the chicken hatched from that egg, confirming that a small egg gives a small chicken, and a large egg gives a large chicken. Also, the percentage of fat pads of total body weight did differ between treatments and was higher for hens fed algae (2.06%) and algae extract (1.98%) compared to hens fed the control (1.67%).

# References


Arthur, J.R & Nicol, F. (1992). The role of selenium in thyroid hormone metabolism and effects of selenium deficiency on thyroid hormone and iodine metabolism. Biological trace element research, vol. 33(3), pp. 27-42.

Arzour-Lakehal, N., Siliart, B. & Benlatreche, C. (2013). Relationship between plasma free thyroxine levels and some biochemical parameters in two strains of broiler chickens. Global Veterinaria, vol. 10(3), pp. 243-249.

Bar-Shira, E., Cohen, I., Elad, O. & Friedman, A. (2014). Role of goblet cells and mucin layer in protecting maternal IgA in precocious birds. *Developmental and comparative immunology*, v. 44, pp. 186-194.

Baylan, M., Celik, L.B., Akpinar, G.C., Alasahan, S., Kucukgul, A. & Dogan, S.D. (2017). Influence of eggshell color on egg yolk antibody level, incubation results, and growth in broiler breeders. *Brazilian journal of animal science*, vol. 46, pp. 105-112.

Bednarczyk, M., K. Stadnicka, K., Kozłowska, I., Abiuso, C., Tavaniello, S., Dankowiakowska, A., Sławińska, A. & Maiorano, G. (2016). Influence of different prebiotics and mode of their administration on broiler chicken performance. *Cambridge University Press*, vol. 10(8), pp. 1271-1279.

Boerjan, M. (2006). Chick vitality and uniformity. *International hatchery practice*, vol. 20, pp. 7-8.

Chapman, V.J. & Chapman, D.J. (2012). *Seaweeds and Their Uses*. London: Springer Science & Business Media.

Cheeke, P.R. & Dierenfeld, E.S. (2010). *Comparative animal nutrition and metabolism.* Wallingford: CAB International.

Cock, J.M., Peters, A.F. & Coelho, S.M. (2011). Brown algae – quick guide. *Current biology*, vol. 21, pp. 573-575.

Decuypere, E. & Bruggeman, V. (2007). The endocrine interface of environmental and egg factors affecting chick quality. *Poultry science*, vol 86, pp. 1037-1042.

Deville, C., Damas, J. & Forget, P. (2004). Laminarin in the dietary fiber concept. *Journal of the science of food and agriculture*, vol. 84, pp. 1030–1038.

Draget, K.I., Moe, S.T., Skjak-Bræk, G. & Smidsrød, O. (2006). Alginates. In: Stephen, A.M., Phillips, G.O. & Wiliams, P.A. *Food Polysaccharides and their Applications.* CRC Press: Boca Raton, vol. 14, pp. 159–1178.

DSM (2018). *Increasing the vitamin content of eggs*. Available: https://www.dsm.com/anh/en/feedtalks/increasing-vitamin-content-eggs.html [2020-06-30]

European Communities (EC), 1998. Commission Directive 98/64/EC of 3 September 1998 establishing community methods of analysis for the determination of amino acids, crude oils and fats, and olaquindox in feeding stuffs and amending directive 71/393/EEC. Official Journal of European Communities, pp. L 257/23.

FAO (2009). *How to Feed the World in 2050.* Available: http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf [2020-03-14]

Gibson, G.R., Scott, K.P., Rastall, R.A., Tuohy, K.M., Hotchkiss, A., Dubert-Ferrandon, A., Gareau, M., Murphy, E.F., Saulnier, D. & Loh G. (2010). Dietary prebiotics: Current status and new definition. *Food Science Technology Bulletin,* vol. 7, pp. 1–19.

Hamilton, R.M.G. (1986). The microstructure of the hen's egg - a short review. *Food microstructure,* vol. 5, pp. 99-110.

Haugh, R.R. (1937) The Haugh unit for measuring egg quality. *United States egg poultry magazine,* vol. 43 pp. 522- 555, 572-573.

Havenstein, G. B., P. R. Ferket, P.R & Qureshi, M.A. (2003). Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poultry Science,* vol. 82, pp. 1500-1508.

Heck, A., Onagbesan, O., Tona, K., Metayer, S., Puterflam, J., Jego, Y., Trevidy, J.J., Decuypere, E., Williams, J., Picard, M. & Bruggeman, V. (2004). Effects of ad libitum feeding on performance of different strains of broiler breeders. *British poultry science,* vol. 45, pp. 695-703.

Hunton, P. (2005). Research on eggshell structure and quality: an historical overview. *Brazilian Journal of Poultry Science,* vol. 7, pp. 67–71.

impact of feeding strategies. PhD thesis: 978-94-6257-238-6. Wageningen University. The Rector Magnificus. https://core.ac.uk/download/pdf/29207174.pdf

ISO. (2005). Animal feeding stuffs — determination of amino acids content ISO 13903:2005 (E). Geneva, Switzerland.

Jennische, P., and K. Larsson. (1990). Traditonella svenska analysmetoder för foder och växtmaterial. National Laboratory of Agricultural Chemistry, Uppsala, Sweden.

Jiao, G., Yu, G., Zhang, J. & Ewart, H.S. (2011). Chemical structures and bioactivities of sulfated polysaccharides from marine algae. *Marine Drugs,* vol. 9(2), pp. 196–233.

Joseph, N.S., Dulaney, A.A.J., Robinson, F.E., Renema, R. & Zuidhof, M. (2002). The effects of age at photostimulation and dietary protein intake on reproductive efficiency in three strains of broiler breeders varying in breast yield. Poultry science, vol. 81(5), pp. 597-607.

Kadam, S.U., Álvarez, C., Brijesh., Colm, K.T. & Mazarrasa, P.O. (2015). *Seaweed sustainability: Food and Non-Food Applications*. Dublin: Academic press.

Kaspers, B., Bondl, H. & Gobel, T.W.F. (1996). Transfer of IgA from Albumen into the Yolk sac during Embryonic Development in the Chicken. *Journal of veterinary medicine,* vol. 43, pp. 225-231.

Lawrence, E.C., Arnaud-Battandier, F., Grayson, J., Koski, I.R., Dooley, N.J., Muchmore, A.V. & Blaese, R.M. (1981). Ontogeny of humoral immune function

in normal chickens: a comparison of immunoglobulin-secreting cells in bone marrow, spleen, lungs and intestine. *Clinical and experimental immunology,* vol. 43, pp. 450-457.

Leeson, S. & Caston, L.J. (2003). Vitamin Enrichment of Eggs. *The journal of applied poultry research*, vol. 12, pp. 24-26.

Leslie, G.A. & Clem, L.W. (1969). Phylogeny of immunoglobulin structure and function: 3. Immunoglobulins of the chicken. *Journal of experimental medicine,* vol. 130, pp. 1337-1352.

Li, Y.X., Wijesekaraa, L., Li, Y. & Kim, S.K. (2011). Phlorotannins as bioactive agents from brown algae. *Process biochemistry*, vol. 46, pp. 2219-2224.

MacLeod, M., Gerber, P., Mottet, A., Tempio, G., Falcucci, A., Opio, C., Vellinga, T., Henderson, B. & Steinfeld, H. (2013). *Greenhouse gasemissions from pig and chicken supply chains – A global life cycle assessment.* Food and agriculture organization of the United Nations. Available: http://www.fao.org/3/i3460e/i3460e.pdf [2020-03-14]

McNaughton, J.L., Deaton, J.W. & F.N. Reece. (1978). Effect of age of parents and hatching egg weight on broiler chick mortality. *Poultry science extension,* vol. 57, pp. 38-44.

Moyle, J., Yoho, D. & Bramwell, K. (2008). Measuring hatching egg shell quality. *Avian advice*, vol. 10, pp. 7-9.

Nangsuay, A., Molenaar, R., Meijerhof, van den Anker, I., Heetkamp, M.J.W., Kemp, B. & van den Brand, H. (2015). Differences in egg nutrient availability, development, and nutrient metabolism of broiler and layer embryos. *Poultry science*, vol. 94(3).

Narushin, V.G. & Romanov, M.N. (2002). Egg physical characteristics and hatchability. *World´s poultry science journal,* vol. 58, pp. 297-303.

NMKL. (2003). Determination in feeds and faeces according to Kjeldahl. Nordic committee on food analysis, Olso, Norway.

NRC. (1994). *Nutrient requirements for poultry, 9th edition*. Washington, D.C; National Academy Press.

Pappas, C.A., Surai, P., Acamovic, T. & Mcdevitt, R.M. (2006). Maternal Organo-Selenium Compounds and Polyunsaturated Fatty Acids Affect Progeny Performance and Levels of Selenium and Docosahexaenoic Acid in the Chick Tissues. Poultry science, vol. 85, pp. 1610-1620.

Poultry hub (2020). Immune system. Available: http://www.poultryhub.org/physiology/body-systems/immune-system/ [2020-07-20]

Poultry world (2012). *The effect of carotenoids on yolk and skin pigmentation*. Available: https://www.poultryworld.net/Broilers/Nutrition/2012/8/The-effect-of-carotenoids-on-yolk-and-skin-pigmentation-WP010752W/ [2020-08-14]

Renema, R. A., Moraes, T.G.V. & Zuidhof, M. J. (2013). Effects of broiler breeder nutrition on chick quality and broiler growth. Proc. 2nd Int. Poult. Meat Congress, Antalya, Turkey pp. 212-222.

Rick van Emous. (2015). Body composition and reproduction in broiler breeders:

Rioux, L.E., Turgeon, S.L & Beaulieu, M. (2010). Structural characterization of laminaran and galactofucan extracted from the brown seaweed Saccharina longicruris. Phytochemistry, vol. 71(13), pp. 1586-1595.

Roleda, M.Y., Skjermob, J., Marfainge, H., Jónsdóttird, R., Reboursa, C., Gietlf, A., Stengelf, D.B. & Nitschkef, U. (2018). Iodine content in bulk biomass of wild-harvested and cultivated edibleseaweeds: Inherent variations determine species-specific daily allowable consumption. *Food chemistry*, vol. 254, pp. 333-339.

Rose, S.P. (1997). *Principles of Poultry Science.* Cab International, Wallingford, UK.

Samak, H. & Ibrahem, M.A. (2012). Effect of using Spirulina platensis algae as feed additive for poultry diets: 1-productive performance of local laying hens. Semantic scholar.

Sandilands, V., Tolkamp, B., Savory, C. &Kyriazakis, I. (2006). Behaviour and welfare of broiler breeders fed qualitatively restricted diets during rearing: Are there viable alternatives to quantitative restriction? *Applied animal behaviour science, vol. 96, pp. 53-67.*

Scheideler, S.E., Jaroni, D. & Froning, G. (1998). Strain and age effects on egg composition from hens fed diets rich in n-3 fatty acids. *Poultry Science*, vol. 77, pp. 192-196.

Schiavone, A. & Barroeta, A.C. (2011). Egg enrichment with vitamins and trace minerals. In: Immerseel, F.V., Nys, Y. & Bain, M. *Improving the Safety and Quality of Eggs and Egg Products.* Cambridge: Woodhead Publishing, pp. 289-320.

Scott, T.A. & Silversides, F.G. (2002). The effect of storage and strain of hen on egg quality. *Poultry Science*, vol. 79(12), pp. 1725–1729.

Stadelman, W.J. (2003). Dietary importance. *Encyclopedia of Food Sciences and Nutrition: 2nd Edition*, pp. 2005-2009.

Stadelman, W.J. (2003). EGGS – Structure and composition. *Encyclopedia of Food Sciences and Nutrition: 2nd Edition,* pp. 2005-2009.

Sun, C.J., Chen, S.R., Xu, G.Y., Liu, X.M. & Yang, N. (2012). Global variation and uniformity of eggshell thickness for chicken eggs. *Poultry Science*, vol. 91, pp. 2718–2721.

Sun, C.J., Chen, S.R., Xu, G.Y., Liu, X.M. & Yang, N. Sun, H., Chen, S., Cai, X., Xu, G. & Ou, L. (2013). Correlation analysis of the total IgY level in hen serum, egg yolk and offspring serum. *Journal of animal science and biotechnology*, vol. 4, pp. 1-4.

Sun, J. & Coon, C.N. (2005). The effects of body weight, dietary fat, and feed withdrawal rate on the performance of broiler breeders. *Journal of Applied Poultry Research,* vol. 14, pp. 728-739.

Surai, P.F., Fisinin, V.I. & Karadas, F. (2016). Antioxidant system in chick embryo development. Part 1. Vitamin E, carotenoids and selenium. *Animal nutrition*, vol. 2, pp. 1-11.

Sweeney, T., Meredith, H., Vigors, S., Mcdonnell, M.J., Ryana, M., Thornton, K. & V.O'Dohertyb, J. (2017). Extracts of laminarin and laminarin/fucoidan from the marine macroalgal species Laminaria digitata improved growth rate and

intestinal structure in young chicks but does not influence Campylobacter jejuni colonization. *Animal Feed Science and Technology,* vol. 232, pp. 71-79.

The poultry site. (2015). Importance of Vitamins in Poultry Production. Available: https://www.thepoultrysite.com/articles/importance-of-vitamins-in-poultry-production [2020-12-28]

Tiwari, B.K & Troy, D.J. (2015). *Seaweed sustainability: Food and non applicactions.* Academic press: Cambridge.

Tona, K., Bamelis, F., De Ketelaere, B., Bruggeman, V., Moraes, V.M.B., Buyse, J., Onagbesan, O. & Decuypere, E. (2003). Effects of egg storage time on spread of hatch, chick quality, and chick juvenile growth. *Poultry science,* vol. 82. pp. 736-741.

Ulmer-Franco, A.M., Fasenko, G.M. & O'Dea Christopher E.E. Hatching egg characteristics, chick quality, and broiler performance at 2 breeder flock ages and from 3 egg weights. *Poultry science,* vol. 89. pp. 2735-2742.

United States Department of Agriculture (USDA). (2000). United States Standards, Grades, and Weight Classes for Shell Eggs. Available: https://www.ams.usda.gov/sites/default/files/media/Shell_Egg_Standard%5B1%5D.pdf [2020-04-28]

Vo, T.S. & Kim, S.K. (2012). Fucoidans as a natural bioactive ingredient for functional foods. *Journal of functional foods,* v. 5(1), pp. 16-27.

Willems, E., Decuypere, E., Buyse, J. & Everaert, N. (2014). Importance of albumen during embryonic development in avian species, with emphasis on domestic chicken. *World's poultry science journal,* vol. 70, pp. 503-518.

Willey, J.M., Sherwood, L.M. & Woolverton, C.J. (2012). *Prescott́s principles of microbiology*. New York: McGraw-Hill.

Yalçin, S., Kahraman, Z, Yalçin, S, Yalçin, S.S. & Dedeoğlu, H.E. (2004). Effects of supplementary iodine on the performance and egg traits of laying hens. *Britich poultry science,* vol. 45(4).

Zaporozhets, T.S., Besednova, N.N,. Kuznetsova, T.A., Zvyagintseva, T.N., Makarenkova, I.D., Kryzhanovsky, S.P & Melnikov, V.G. The Prebiotic Potential of Polysaccharides and Extracts of Seaweeds. *Russian Journal of Marine Biology,* vol. 40, pp 1–9.

# Acknowledgements

I would like to express my gratitude to my supervisors Emma Ivarsson and Malin Boyner. They have provided me with all the necessary material and assistant I have been needing during this period. Their patience, shared experiences and friendly approaches has really contributed to me successfully finishing the experiments and the writing of this thesis. I have gained a lot of knowledge and I am very happy to have contributed with a small part to this very interesting and important research, and for that I am very grateful.

# Appendix

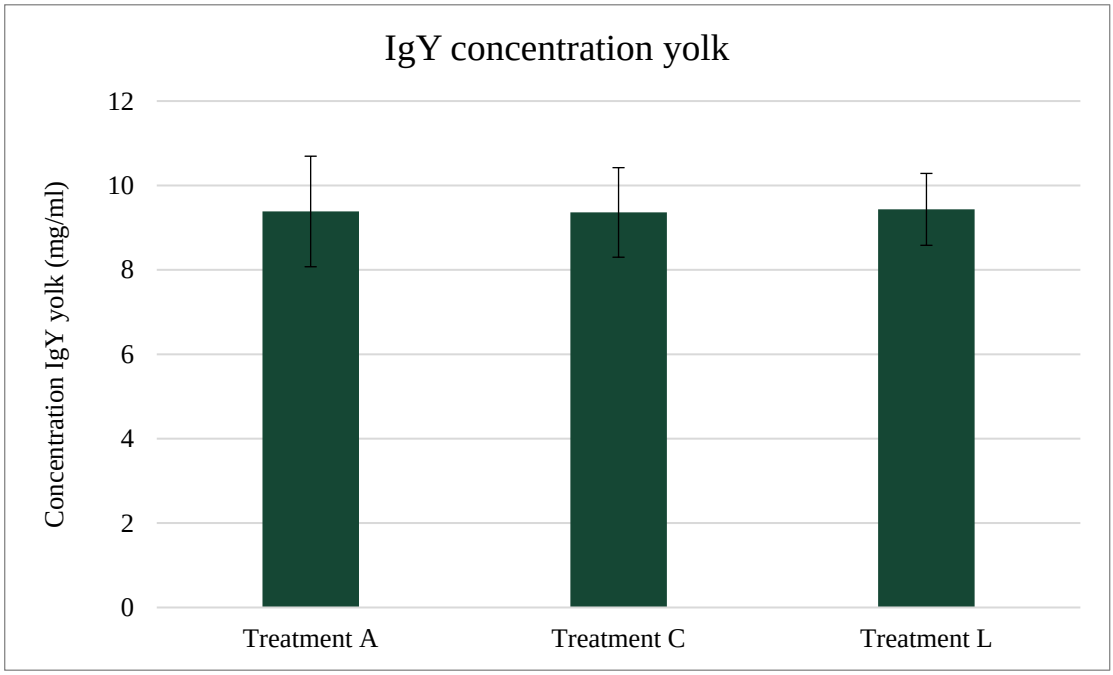


***Figure 1.*** *IgY concentration yolk 35 days after the change to experimental diets from each treatment is presented in the bars and the line shows a 95 % confidence interval. There were no significant differences between the treatments, overlapping confidence interval does not differ*

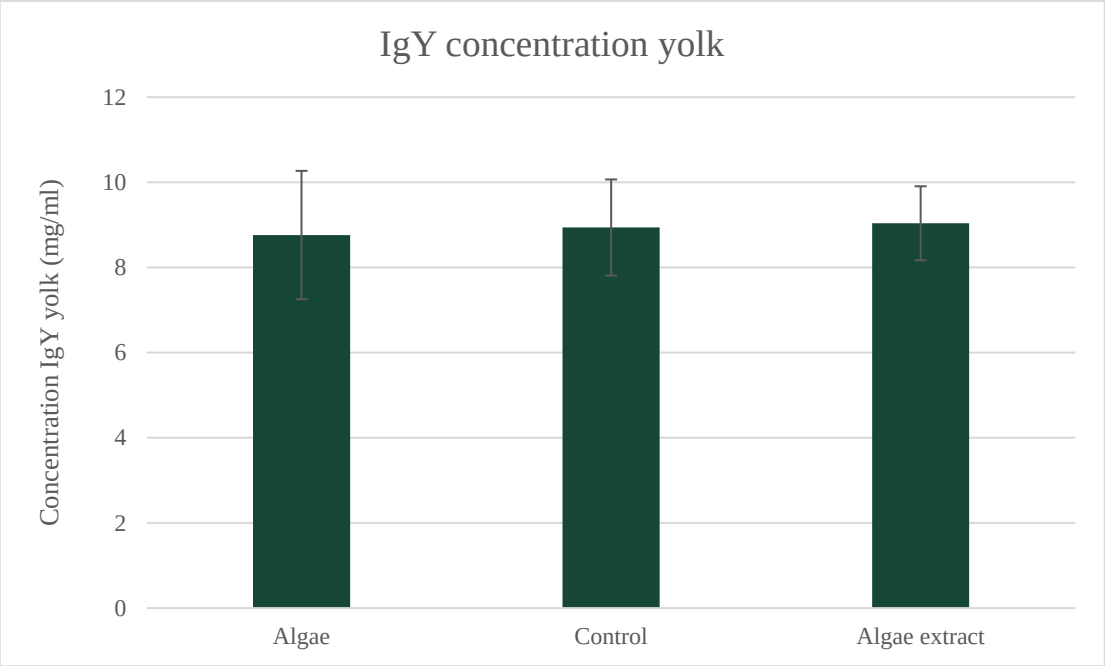


***Figure 2.*** *IgY concentration yolk 36 days after the change to experimental diets from each treatment is presented in the bars and the line shows a 95 % confidence interval. There were no significant differences between the treatments, overlapping confidence interval does not differ*

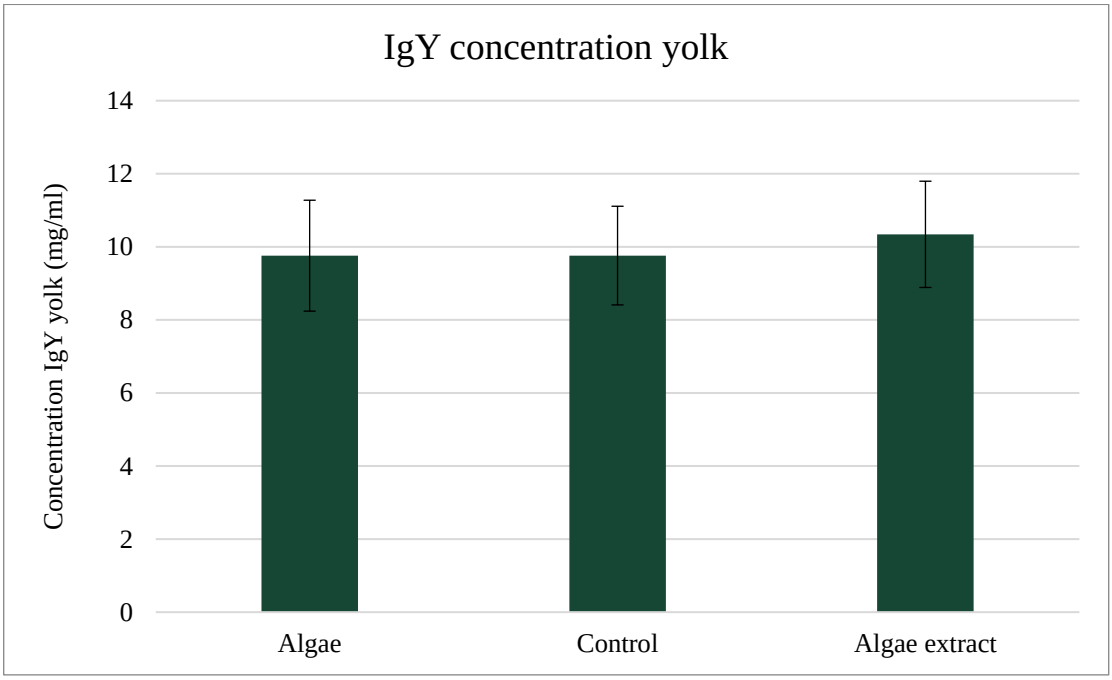


***Figure 3.*** *IgY concentration yolk 49 days after the change to experimental diets from each treatment is presented in the bars and the line shows a 95 % confidence interval. There were no significant differences between the treatments, overlapping confidence interval does not differ*

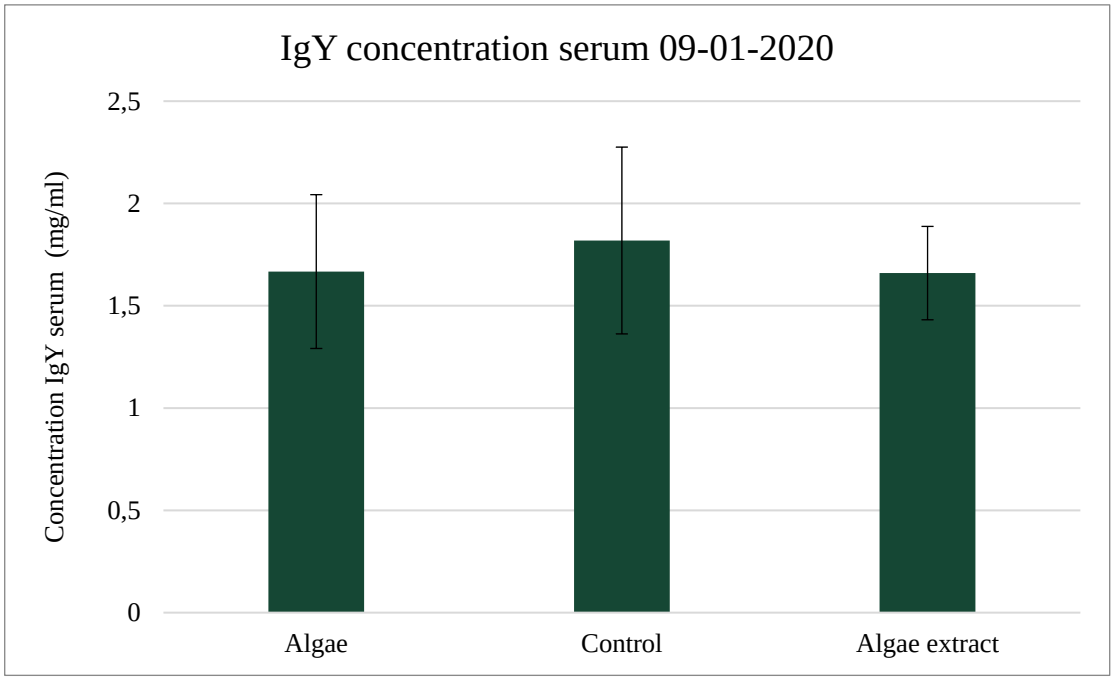


***Figure 4.*** *IgY concentration yolk 35 days after the change to experimental diets from each treatment is presented in the bars and the line shows a 95 % confidence interval. There were no significant differences between the treatments, overlapping confidence interval does not differ*

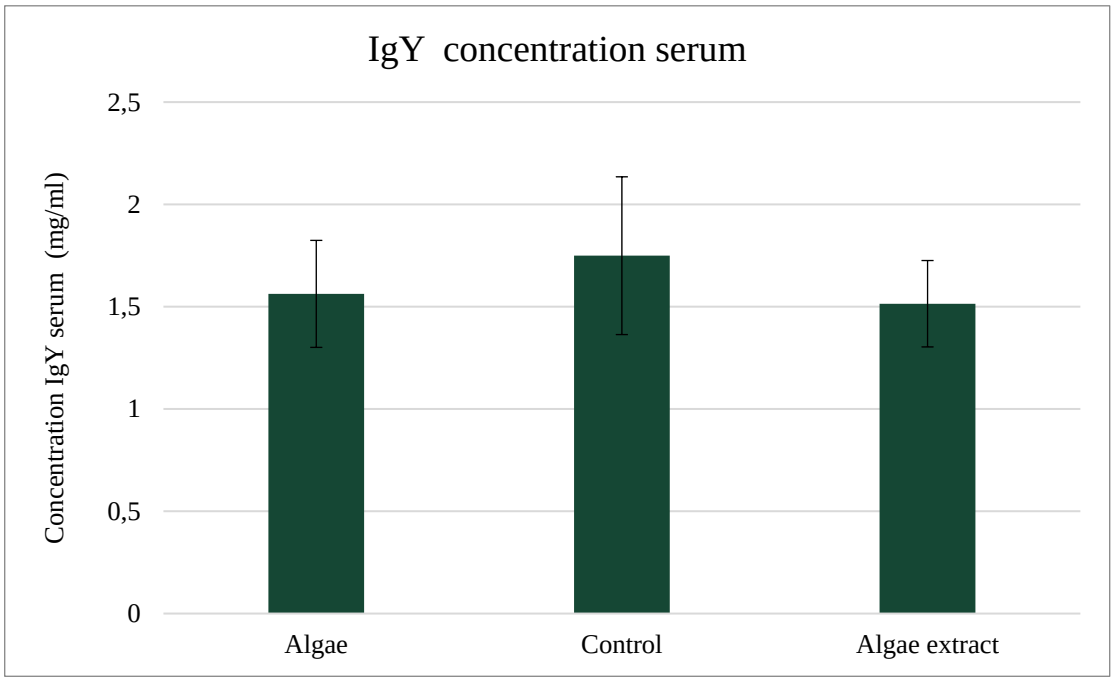


***Figure 5.*** *IgY concentration serum 46 days after the change to experimental diets from each treatment is presented in the bars and the line shows a 95 % confidence interval. There were no significant differences between the treatments, overlapping confidence interval does not differ*

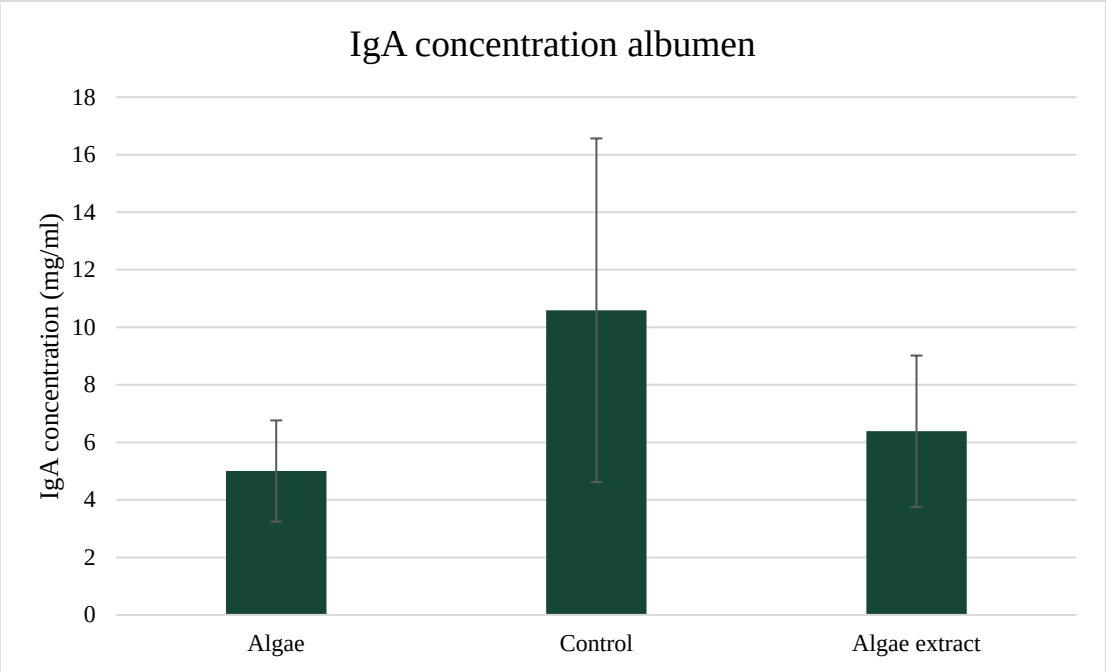


***Figure 6.*** *IgA concentration albumen 35 days after the change to experimental diets from each treatment is presented in the bars and the line shows a 95 % confidence interval. There were no significant differences between the treatments, overlapping confidence interval does not differ*

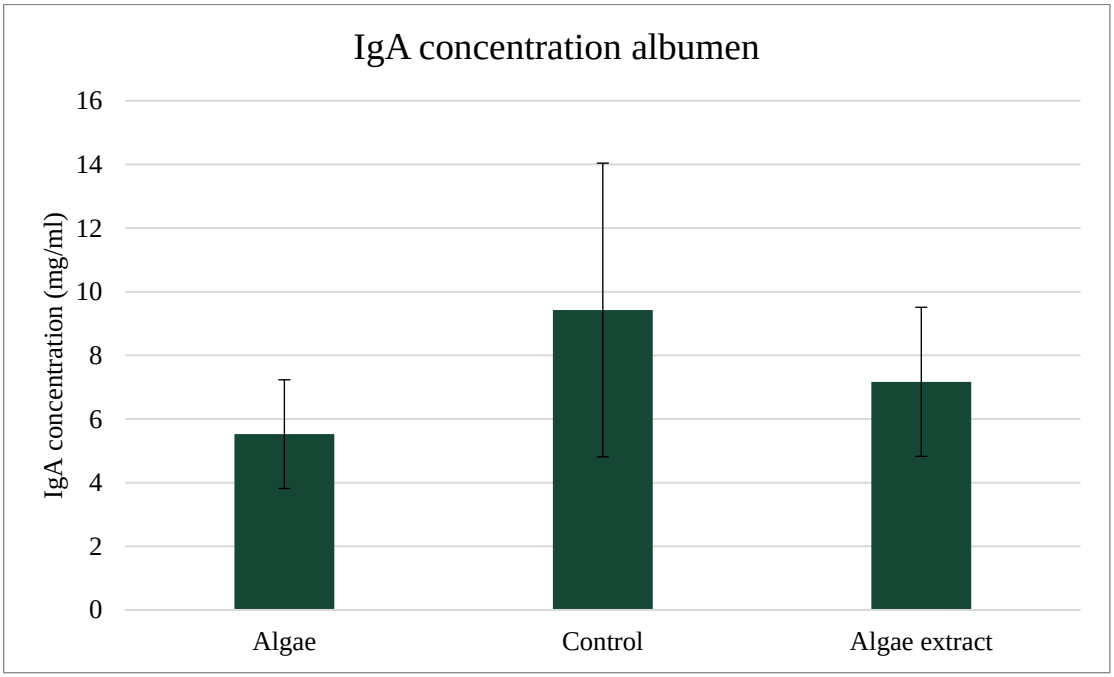


***Figure 7****. IgA concentration albumen 36 days after the change to experimental diets from each treatment is presented in the bars and the line shows a 95 % confidence interval. There were no significant differences between the treatments, overlapping confidence interval does not differ*

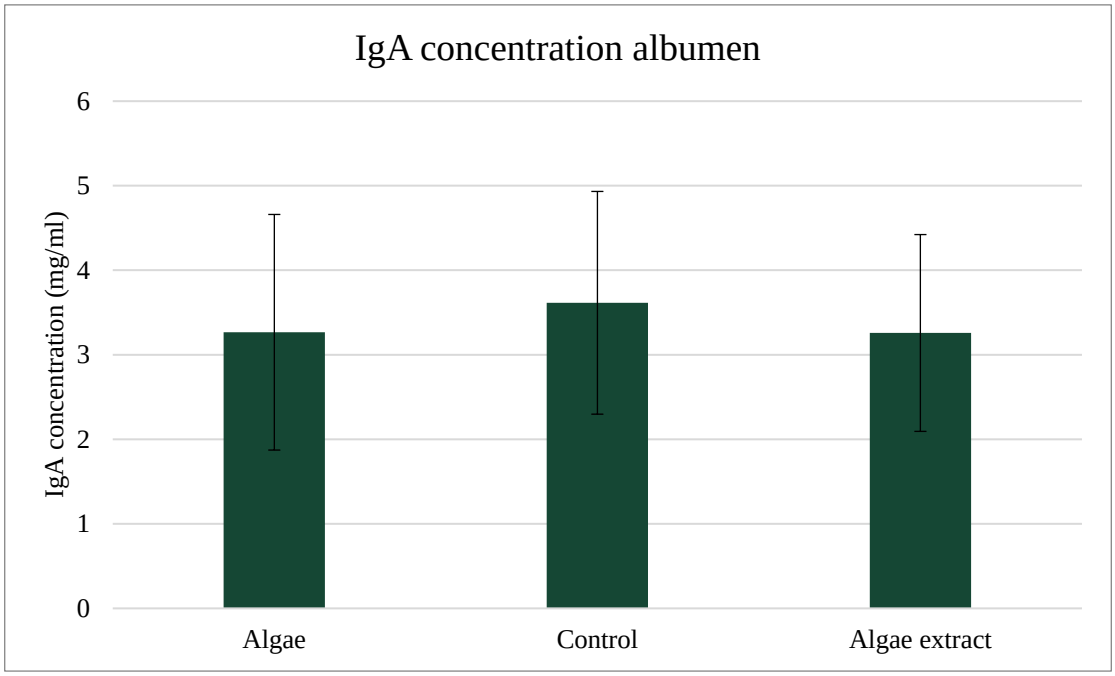


***Figure 8***. *IgA concentration albumen 49 days after the change to experimental diets from each treatment is presented in the bars and the line shows a 95 % confidence interval. There were no significant differences between the treatments, overlapping confidence interval does not differ*