



Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and
Animal Sciences

Identifying factors of importance for chick quality and traits that may predict chick quality

Identifiering av viktiga faktorer för kycklingkvalité och egenskaper som kan förutsäga kycklingkvalité

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Abstract

In the hatcheries today, the eggs that have cracks are sorted out before incubation. When hatched the chicks are graded as saleable or unsaleable, and saleable chicks are transported to the farm. First week mortality (FWM) is a common way to assess chick quality and performance at the farm and it varies among flocks. One disadvantage with FWM is that it gives delayed information regarding chick quality and factors at the farm can influence the FWM. If FWM could be correlated to egg quality traits, chicks of poor quality could be sorted out already before incubation of the egg, which would be a benefit regarding animal welfare and the economy for hatcheries and farmers. Of interest is also to investigate if the immunological function in breeder hens affects the FWM of their progeny. In the present study possible links between FWM and hatching egg quality parameters were investigated. Furthermore, IgY concentration of the serum of the breeder hens and in the yolks of their eggs were analyzed. Four flocks of Ross 308 grandparents, aged between 33-51 weeks, were selected based on the FWM of their offspring. The serum of the grandparents and the yolks of their eggs were analyzed for IgY concentration and eggs were also analyzed for egg quality parameters. In the egg quality analysis there were some storage effects on pH and Haugh units, but no consistent difference between the flocks that could be linked to progeny FWM. The results showed no significant differences between the flocks in IgY concentration of the yolks or in serum. However, there was a large variation in IgY serum levels between individual breeder hens and also in the IgY concentrations in egg yolks within flocks. The reason for this variation is not known. In this study it was not possible to identify which hen that laid a specific egg and it was therefore not possible to link IgY concentrations in egg yolks with IgY in breeder hen serum on individual level. In future studies with a similar approach, being able to collect eggs with known maternal identity is recommended, to be able to follow the transfer of IgY from mother to egg on individual basis, and to link egg quality to specific hens

Sammanfattning

På kläckerierna sorteras ägg med sprickor bort innan äggen placeras i ruvaren. När äggen är kläckta graderas kycklingarna som säljbara eller inte. De kycklingar som kan säljas transporteras till gården för vidare uppfödning medan de andra avlivas. Första veckans dödlighet (FVD) är ett vanligt sätt att utvärdera kycklingarnas prestation och kan variera mellan flockarna på gårdarna. En negativ aspekt med att använda FVD som mått på nykläckta kycklingars kvalitet är att informationen inte är tillgänglig förrän tidigast en vecka efter kläckning och faktorer på gården kan också påverka FVD. Om FVD skulle kunna korreleras till egenskaper hos äggen skulle ägg som ger kycklingar av dålig kvalitet kunna sorteras ut i ett tidigt stadium, till fördel för både djurvälstånd och ekonomi hos kläckerier och lantbrukare. Det är möjligt att avelshönornas immunologiska status har inverkan på deras avkommors FVD. I denna studie undersöktes möjliga kopplingar mellan FVD och olika äggkvalitetsparametrar hos befruktade ägg från avelshöner. Även koncentrationen av IgY i serum hos hönorna och gulorna hos deras ägg analyseras. Fyra flockar av Ross 308 i morföräldrargenerationen valdes ut baserat på deras avkommors FVD. Flockarna var mellan 33–51 veckor gamla. Serum hos höner i de utvalda flockarna och gulorna hos deras ägg analyserades för IgY koncentration, och ägg

analyserades för olika kvalitetsparametrar. Äggvitans pH och Haugh units vid äggkvalitetsundersökningen påverkades av äggens lagringstid innan analys, men inga statistiskt säkerställda skillnader mellan äggens kvalitet i flockarna kunde kopplas till avkommornas FVD. Inga signifikanta skillnader mellan flockarnas IgY koncentration i äggens gula eller hörnas serum framkom. Det var dock en stor variation i IgY koncentration i serum och gulor mellan individuella hönor och ägg inom flock. Orsaken till denna variation är inte känd. I denna studie var det inte möjligt att välja ägg från de individer som valdes ut för analys av serum IgY. I framtida studier rekommenderas att ägg samlas in på ett sätt som gör det möjligt att veta vilken höna som lagt ett ägg, för att följa överförandet av IgY från höna till ägg på individuell nivå och kunna koppla äggkvalitén till specifika hönor.

Table of content

Introduction.....	1
Literature review	3
Chick quality scoring methods.....	3
Qualitative scoring methods	3
Quantitative scoring methods	4
The formation of an egg.....	6
Egg quality	6
External parameters	6
Internal parameters.....	9
Antibodies	10
Material and methods.....	12
Flocks.....	12
Collection of eggs and blood samples for analyses	12
Preparing the samples for analysis of antibodies	12
Blood samples.....	12
Yolk samples.....	12
Egg parameters.....	13
Analysis of antibodies.....	13
Statistical analysis	14
Result	15
Egg quality	15
Effect of day.....	15
Differences between flocks.....	15
Bird weights.....	17
IgY serum concentration.....	18
IgY yolk concentration	18
Discussion.....	20
Conclusion	23
Acknowledgements.....	24
References.....	25
Appendix 1.....	32
Appendix 2.....	35

Appendix 3.....	36
Appendix 4.....	37

Introduction

A healthy and viable chick is not only an important welfare implication but also of economic importance for both hatcheries and chicken farmers. For hatcheries, the chick is the final product of their production chain and for the farmer the newly hatched chick is the start of a new rearing period. The importance of a good quality chick is therefore of concern for both hatcheries and farmers. Today, a common parameter for chick quality is first week mortality (FWM) rates of the flocks, which is delayed information for assessing the chick quality and factors at the farm can influence the FWM. The chick has then already been selected as an egg to be incubated for hatch and at hatch graded as a saleable chick. The assessment of marketability of the chick is made by hatchery staff and saleable chicks are transported to the rearing facilities while the unsaleable chicks are euthanized. The assessments are often made depending on some subjective parameters such as color of the chick, development of the body, navel appearance and vitality of the chick (Ipek & Sözcü, 2013). Attempts have been made to convert these subjective scoring parameters into objective ones (Tona *et al.* 2003; Boerjan, 2006) and to find a link between them and chick performance. Other more objective parameters such as chick length, chick weight and yolk free body mass have also been studied to find possible links to chick performance and thereby be able to make a more reliable assessment of chick quality at the hatcheries (Ipek & Sözcü, 2013). In finding a link between a scoring method and chick performance there is also a possibility to find a link between the scoring methods and egg parameters. At the hatchery the eggs are assessed at arrival if they are cracked or not. Cracked eggs are sorted out while the others are incubated for hatching. The eggs are though different in size and composition regarding e.g., percentages of shell, yolk and albumen, but also nutrient content. These are parameters which could affect the growing embryo during incubation and affect the quality of the chick at hatch. If chick quality could be predicted before incubation by assessments on the egg it could mean a reduction in bad chicks being hatched. It could mean less chicks culled or dying the first week, which is a huge welfare aspect. It would also be of economic benefit for hatcheries by reduced costs for incubation and for the farmers who would be able to gain profit from a higher number of chickens being healthy until slaughter.

The maternal antibodies transferred from the mother hen to the chick during incubation are of importance because they protect the chick against pathogens during its first weeks of life. After that the chick has started producing its own antibodies (Lawrence *et al.*, 1981). The antibodies from the hen are transferred to the egg and then from the egg to the embryo. In contrast to mammals, where antibodies are derived directly from the milk of the mother, the poultry antibody transfer is a twostep process (Patterson *et al.*, 1962). The level of antibodies in the hen's serum and in the egg, could indicate how well the chick survives the first week of life.

The aim of this project is to investigate if there are possible links between selected egg quality traits, mother hen IgY concentration in serum, IgY concentration in yolk and progeny performance in terms of first week mortality. The investigation will be conducted on broiler breeder mothers in the grandparent generation and their progeny in the parent generation.

Literature review

Chick quality scoring methods

Qualitative scoring methods

The qualitative scoring methods assess the general visual appearance of chicks. Qualitative scores are to a higher extent subjective in contrast to the quantitative ones. The color, development, navel quality and vitality of the day old chick are qualitative methods for scoring chick quality (Ipek & Sözcü, 2013).

Color

Good quality chicks are thought to have a deep yellow color, while those of lower quality are pale yellow to white (Meijerhof, 2005a). The yolk is the source of the pigment influencing the color of the down of newly hatched chicks and because the yolk is the source of energy and nutrients for the embryo, it affects the development of the chick. A deep yellow colored chick is therefore thought to be an indication of good yolk uptake and in turn a better developed chick (Petek *et al.*, 2010). However, Petek *et al.* (2010) did not find any significant difference in body weight between newly hatched chicks with deep or pale yellow color of the down. It was suggested the lack of difference could be due to the use of formaldehyde in the hatchery, because formaldehyde colors the down of the chicks. Formaldehyde is usually used as a disinfectant at the hatchery due to its low cost and efficiency to kill bacteria and fungi (Cadirci, 2009). If formaldehyde is used in the hatcher it gives the chicks a deep yellow color (Ceva Santé, 2006).

Development and vitality

Good quality chicks should have a good confirmation of the body, legs, beak and toes. The legs should be straight and there should be no swelling or lesion of the hock and skin. The beak should be well formed and the toes firm and straight (Ipek & Sözcü, 2013). The chick should be alert and investigate its environment, indicating a healthy chick that can find feed and water quickly, which is essential for survival and growth (Meijerhof, 2005a). The chick should also be free of signs of respiratory distress and the body should feel firm at touch (Decuypere *et al.*, 2007).

Navel quality

The navel should be clean and completely sealed with no yolk sac or dried membrane protruding from the navel (Decuypere *et al.*, 2007). It is important for the navel to be closed to minimize the risk of infections (Meijerhof, 2005a), because when the yolk sac is infected the quality of the yolk declines and the chick does not gain the essential nutrients (Rai *et al.*, 2005). A navel that is not closed often leads to high mortality rates (5-10%) during the first few days (peak at 5-7 days) after hatching. The bacteria responsible for the infection are several; coliforms, *Staphylococcus*, *Streptococcus* and *Proteus* (de Lange, n.d.). When the yolk sac was inoculated with *Enterococcus faecalis* no signs of a deviation of body weight, appearance or feed consumption were found (Sander *et al.*, 1998). An increased weight of the yolk sacs was observed when inoculated with *Escherichia coli* and *Staphylococcus aureus* (Khan *et al.*, 2002; Rai *et al.*, 2003). A study on healthy birds showed that there is a natural occurrence of bacteria

(*Pseudomonas* sp., *E. coli.*, enterococcus, *Staphylococcus albus*, *Bacillus* sp) in the yolk sac, indicating that the occurrence of bacteria in the yolk sac does not necessarily imply adverse effects on the survival of the chick (Deeming, 2005).

Quantitative scoring methods

There are four major different quantitative methods for scoring chick quality that are discussed in the literature. These are the Tona score or Pasgar score, chick weight at one day old, yolk free body mass and chick length (Ipek & Sözcü, 2013).

Tona or Pasgar score

Two different scoring systems have been developed in attempts to convert subjective qualitative measures into an objective quantitative score. The scoring systems aims at predicting the survival and performance of the chick (Decuypere & Bruggeman, 2007). The first to be developed was the Pasgar score, which judges reflexes, and appearance of navels, beaks, legs and yolk sac. The chick is given a score between zero and ten based on these parameters, where ten is a good quality chick. The score is calculated with the start at ten and each abnormality in the parameters accounts for subtraction by one (Boerjan, 2006). The Tona score is another scoring system which gives the chick a score between zero and a 100, where 100 is a good quality chick. The Tona scoring system looks at the parameters; activity, down and appearance, retracted yolk, eyes, legs, navel area, remaining membrane in the navel region and remaining yolk. The parameters are weighted depending on their importance, where eyes, legs and remaining yolk have the greatest impact on the total score. The eyes should be open and bright, the chick should easily stand upright on its legs with no inflammation or redness of the hook and there should be no remaining yolk (Tona *et al.*, 2003). The abnormalities in the navel region are suggested to have the highest impact on the survival and growth of the chick. However, the parameters and their impact on the score are suggested to be revised, because some parameters are suspected to always be associated with each other (Tona *et al.*, 2005). The Pasgar and Tona scoring systems are though time consuming since a minimum of 30 and 44 chicks respectively must be evaluated for a representative value (Boerjan, 2006; Molenaar, n.d.). The relevance of the Pasgar score in first grade chicks was questioned by van de Ven *et al.* (2012) who found no correlation between the score and growth after hatch or mortality for first grade chicks. The navel condition was the only criteria of the Pasgar score that affected the weight of the seven day old chicks in that study (van de Ven *et al.*, 2012). Willemsen *et al.* (2008) found no correlation between the Tona score and post hatch performance, which according to the authors could be due to that only first grade chicks were used in their study. The chicks had already been sorted at the hatchery for first and second grade chicks, which could be the reason for lack of anomalies and therefore no great differences in the Tona score was observed (Willemsen *et al.*, 2008).

Chick weight

The weight of the day old chick has been suggested to reflect the quality of the chick and it is claimed that day old weight is reflected in the slaughter weight of the broiler. This correlation is however not fully understood since some have found a relationship between the two traits and some have not (Willemsen *et al.*, 2008). It is argued that the weight of the day old chick is

related to egg weight and not the development of the chick. The weight of the day old chick includes the weight of the residual yolk. A higher amount of residual yolk could be a sign of a less developed chick, since it has not used its energy resources for growth. The method of weighing the day old chick might therefore not be a good predictor of the development of the chick (du Preez, 2007). Those favoring body weight argue that body weight and chick length are highly correlated and weight is much easier to measure than chick length. Then again, the variation of the residual yolk is questioned, some chicks have as little as 2 grams of residual yolk while others have up to 8 grams at hatch (Mauldin *et al.*, 2007).

Yolk free body mass

The yolk free body mass (YFBM) is a third way of assessing the quality of the chick, which is the body weight without residual yolk. It is thought to be a better assessment of chick quality than body weight since it shows how much of the egg weight that has been converted into an embryo and is therefore a better indicator of the development of the chick (du Preez, 2007). Willemsen *et al.* (2008) investigated different chick quality methods for predicting the growth potential of broilers in pure Ross and Cobb lines with parents at different ages. The researchers found a significant correlation of 0.34 between YFBW and day old chick length in Ross lines of different ages, implying the chicks with larger YFBW are longer. However, in the Cobb line, no significant correlation between YFBM and chick length was found. The results from Willemsen *et al.* (2008) were in line with the results from Wolanski *et al.* (2004) who studied different Hybro lines. To calculate YFBM the chick however must be killed (du Preez, 2007), making the method time consuming and costly (Meijerhof, 2005a).

Chick length

Chick length is another predictor of chick quality. The length can be determined by measuring the length of the spinal cord, shank length or the length from the beak to the middle toe (Meijerhof, 2005b). Some studies have shown a correlation between day old chick length and body weight at 6 weeks of age (Wolanski *et al.*, 2004; Molenaar *et al.*, 2008). Molenaar *et al.* (2008) also found an interaction with sex, meaning that length could be a good predictor of chick quality for males but not for females. Another study has shown a significant positive correlation between chick weight and chick length at hatch in progeny from a Ross line at 53 weeks of age but neither in a Cobb line at 42 weeks of age, nor in a 39-week-old Ross line (Willemsen *et al.*, 2008). It is argued that chick length is a better predictor of broiler performance than survival of the first week, due to that length has more to do with the development of the chick than survival (Meijerhof, 2005b). In agreement, Petek *et al.* (2010) did not find a correlation between chick length and chick survival, but suggested that a longer chick has better developed organs and better growth performance. Molenaar & Reijrink (2006) found that longer chicks had heavier hearts, livers and spleens. These are organs important for transportation of oxygen and nutrients to other organs, maintaining the homeostasis in the body and contribute to the function of the immune system. A long chick could therefore have a better potential for optimal growth and development (Molenaar & Reijrink, 2006). Those favoring body weight of the day old chick instead of length argue that measuring length is not an effective method and there is a variation between different observers. Mauldin *et al.* (2007) challenged the theory of variation in measuring length by using two observers, which resulted

in a small but insignificant difference between lengths measured by different observers.

The formation of an egg

The embryo destined to develop and grow into a chick starts its journey in the reproductive tract of the hen, where the egg is supplied with the nutrients needed for embryonic development. The hens' left ovary contains follicles that develop with a time interval of approximately 24 hours. During the growth of the follicle, water, protein, lipids, minerals and vitamins are deposited into the yolk. When ovulated from the ovary the follicle enters the oviduct of the hen, which consists of five different parts where the rest of the components of the egg is developed. The first part is the infundibulum, where the fertilization takes place. The egg then enters the magnum, which is the part where the albumen is formed. The proteins in the albumen are synthesized in the gland of the magnum or formed in the liver and then transported to the magnum. The albumen has different layers with different consistencies due to the proteins being deposited around the oocyte in concentric layers. The egg then moves on to the isthmus where the inner and outer shell membrane is formed around the albumen. The shell membrane is formed by keratin filaments secreted by the glands in the isthmus. On the surface of the outer shell membrane minerals are deposited, these minerals being the minerals that the egg shell is composed of. The next part is the uterus, also known as the shell gland, where calcium carbonate is formed around the egg and a thin membrane that is composed of proteins are thereafter deposited on the outside of the shell. This thin membrane is called cuticle. The egg is then transported through the vagina and out of the cloaca. It is the formation of the shell in the uterus that is the most time-consuming part of the egg formation (Sjaastad, Sand & Hove, 2010).

The embryo is surrounded by an eggshell to keep the important nutrients in place because the embryo continues its development outside the hen. During the incubation period, when the embryo develops into a chick, only oxygen, carbon dioxide, and water diffuse across the eggshell. The embryo is therefore dependent on the nutrients supplied within the shell for development (Sjaastad, Sand & Hove, 2010).

Egg quality

Although the egg laid has been supplied with the nutrients needed for embryonic growth (Sjaastad, Sand & Hove, 2010), there is a variation between eggs laid by the same hen and between eggs laid by different hens, and some eggs are more prone to hatch than others (Narushin & Romanov, 2002). The quality of the egg can be divided into external and internal quality parameters (De Ketelaere *et al.*, 2004). The external quality of the egg is proposed to be determined by egg size, shape, shell structure and thickness and strength of the shell. The internal parameters are e.g. the quality of the albumen, the proportion of the different components in the egg and the integrity of the shell membrane (Wolc & Olori, 2009).

External parameters

Egg weight

The weight of an egg varies greatly between 50-70 g (Ulmer-Franco, Fassenko & O'Dea Christopher, 2010) and at the day of hatch the weight of the chick comprises 62 - 78 % of the

total egg weight (Wilson, 1991). A positive correlation between egg weight and weight of the day old chick was shown by Tona *et al.* (2003).

The weight of the egg is affected by the age of the hen (Ulmer-Franco, Fasenko & O'Dea Christopher, 2010), but also by genotype (Wolanski *et al.*, 2007). The results by Wolanski *et al.* (2007) suggested strain to have a greater impact on the egg weight than the age of the hen when comparing ten different broiler breeder strains. The weight of the egg influences the proportions of different components in the egg. Within flock and age, heavy eggs have a lower weight percentage of yolk compared to eggs of lower weights (Ulmer-Franco, Fasenko & O'Dea Christopher, 2010; Lourens *et al.*, 2006). A lower proportion of yolk could be a disadvantage because the energy required for the embryo is mostly supplied by the lipids in the yolk (Ulmer-Franco, Fasenko & O'Dea Christopher, 2010). However, the results from Ulmer-Franco, Fasenko & O'Dea Christopher (2010) showed no difference between the dry weight of yolk from eggs of different weights, suggesting that the differences in yolk proportion were due to differences in water content and the nutrient content of the yolk was therefore not affected by egg size. In support of this, Lourens *et al.* (2006) showed no difference in relative energy content (megajoule per kilogram dry matter) of the yolk between heavy and light eggs. The researchers also found that lighter eggs resulted in chicks of shorter length at hatch in comparison to the heavier ones.

Egg shell

The eggshell constitutes 10-11% of the total egg weight (Gupta, 2008) and has several roles for the developing embryo. It provides mechanical protection and enables the embryo to develop without outer impact on the content of the egg. The eggshell also functions as a barrier towards microorganisms, a source of calcium for the embryo and enables gas exchange through the membrane without dehydration (Hamilton, 1986).

Thinner eggshells break more easily, resulting in eggs with cracks where pathogens can enter (Moyle, Yoho & Bramwell, 2008). Eggs invaded by pathogens can explode and contaminate the surrounding eggs with pathogens, resulting in bad hatches (Yoho *et al.*, 2008). Eggs with thin, but intact eggshells, are more prone to dehydration and embryonic mortality due to a higher loss of water during incubation. Chicks hatching from eggs with thin shells have reduced livability during the first few days after hatch and thereby also poor performance due to a slow start in life (Moyle, Yoho & Bramwell, 2008). A thicker shell is therefore considered beneficial since it keeps the nutrients in and thereby gives the embryo a possibility to utilize them efficiently (Narushin & Romanov, 2002).

The quality of the egg shell can be determined by measuring the breaking strength of the egg. The breaking strength is the eggshells ability to resist breakage (Peebles & McDaniel, 2013) and is on average >30 Newtons. Other measurements of the egg shell quality are the thickness of the shell, which can be measured in several different ways. When measured with a micrometer the thickness of the shell is approximately 300-350 μm (Gupta, 2008). Another way of measuring the eggshell thickness is by specific gravity, which is one of the easiest and most widely used methods (Moyle, Yoho, Bramwell, 2008). It measures the density of the egg

in relation to water (Gupta, 2008) and can be done by two different methods: the Archimedes method and the salt solution method. The salt solution is the method most commonly used due to being more time efficient. The eggs are put in water tubs that have different concentrations of salt, starting with the lowest concentration and then carrying on to higher. The lowest concentration enabling the egg to float is the measure of specific gravity of the egg (Moyle, Yoho & Bramwell, 2008). The industry standard for hatching eggs is 1.080 for the specific gravity. The results by McDaniel, Brake & Eckman (1981) and Bennett (1992) reported a decrease in the hatchability and an increase in the embryo mortality at specific gravity of below 1.080, which is also supported by Roque & Soares (1994). However, a study by Moyle, Yoho & Bramwell (2008) indicated that the hatchability was not negatively affected until the specific gravity was 1.065 or lower. Ulmer-Franco, Fassenko & O'Dea Christopher (2010) found that the specific gravity varied between 1.066 and 1.075 for a Cobb 500 breed, and therefore never reached the industry standard for broiler eggs. The results of the study showed the hatch of fertile eggs (no of salable chicks hatched/ number of fertile eggs incubated) being 83.1-90.7 %. The researchers suggested the industry standard being outdated and the eggshell of today being different from when the standard was set. Yamak *et al* (2015) measured eggshell thickness of broiler breeder eggs ultrasonically. The results showed no significant difference in hatchability, chick weight or chick length between different eggshell thickness groups. The classification of the different eggshell thickness groups were thin eggs (≤ 0.34 mm), medium (0.35-0.38 mm) and thick (≥ 0.39 mm).

Pores

The shell of the egg consists of 7,000 to 17,000 pores through which the exchange of gases occurs and the blunt side of the egg has the most pores (Hatchability, n.d.). The diameter of the pores is 15-65 μ m (Hamilton, 1986). Shell porosity is a measure used which is based on the concentration of pores and the diameter of the pores. The exchange of oxygen can be obstructed if there are few pores or if the pores are small in diameter or a combination of the two. This could lead to increased embryonic mortality. On the other hand, if the number of pores are high or the diameter of the pores are large, or yet again a combination, it can dehydrate the content of the egg (Narushin & Romanov, 2002).

Color of the shell

There is variation both in color and pigment intensity of the eggshell. The pigmentation of the shell takes place in the last stage of the shell formation in the uterus and it is mainly biliverdin-IX, zinc chelate and protoporphyrin-IX that are responsible for the pigmentation (Butcher & Miles, 1995). It is not clear whether the color of the shell affects chick quality, in the sense of hatchability, or not. It is argued that eggs which are paler in color have been laid prematurely, because the pigment is not applied until just prior to the egg being laid by the hen (Moyle, Yoho & Bramwell, 2008). Possible causes of reduced pigmentation in brown eggs are stress, disease, age of the hen and ingestion of certain drugs (Butcher & Miles, 1995). Moyle, Yoho & Bramwell (2008) studied brown and white eggs and showed that hatchability of broiler breeder eggs that are extremely light in color are lower than those with a dark color. Their study measured the color with a colorimeter, which gave a value of 100 for pure white eggs. The hatchability started to decline for the eggs having a value of 85 or above. When comparing

different intensities of brown eggs from a Hybro breed, Shafey *et al.* (2005) did not find any correlation with eggshell thickness, which is consistent with earlier studies (Shafey *et al.*, 2004). The researchers however found that a combination of shell pigment intensity, age of breeder and light conditions during incubation affected the hatchability (Shafey *et al.*, 2005).

Internal parameters

Albumen

The albumen consists of about 90% water and ten percent protein (Solomon, 2008) and is the major source of water for the embryo (Wolanski *et al.*, 2007). Besides providing the embryo with water and proteins, the albumen protects the embryo from the external environment (Solomon, 2008) by creating an unfavorable environment for growth of bacteria. The viscosity of the albumen holds the yolk in position, away from the shell wall, and makes it difficult for bacteria to penetrate (Willems *et al.*, 2014). It is the protein ovomucin that is largely responsible for the high viscosity of the albumen (Deeming & Ferguson, 1991).

At lay the egg contains high amounts of carbon dioxide, which after lay starts to diffuse through the pores and consequently gives rise to an increased pH in the albumen. An optimal pH is important for the initiation of the embryonic development because the enzymes involved in the process are pH-dependent (Decuypere *et al.*, 2007). At lay the pH of the albumen is 7.4-7.9, which also is close to optimal pH for bacterial growth. During storage the pH can reach up to 9.0-9.6, where most bacteria do not grow (Hatchability, n.d.) and at even higher pH the initiation of embryo growth is affected. If the carbon dioxide does not diffuse properly the pH will instead decrease and a low pH can affect the hatchability negatively (Decuypere *et al.*, 2007). Therefore, pH is often a quality measure of the albumen. Other measurements of the quality of the albumen is height of the albumen and Haugh units (Tona *et al.*, 2002). Haugh units is a measurement based on the height of the albumen corrected for egg mass (De Ketelaere *et al.*, 2004) and decreases as the storage time increases. In a study evaluating eggs from Cobb broiler breeders, at 54 weeks of age, eggs had an average Haugh unit of 80 at day of lay and 75 after eight days of storage in 15-16 °C (Tona *et al.*, 2002). Peebles *et al.* (2000) found that albumen height had no significant effect on weight percentage of yolk, dry matter or lipids. The results though showed that during day two of incubation the concentration of linoleic acid in the yolk was higher in eggs with high albumen height. Increased levels of linoleic acid are indicated to negatively affect the hatchability (Peebles *et al.*, 2000). The weight of the albumen is suggested to be a determinant of the size of the hatching chick (Finkler, Orman & Sotherland, 1998). This could be due to larger eggs having more albumen and chicks hatched from larger eggs had higher body weights at hatch (Wolanski *et al.*, 2007). The proteins in the albumen are either utilized by the embryo during incubation or transferred to the yolk sac, enabling them to be utilized after hatch. It is therefore suggested the quality of the albumen determines the potential performance of the day old chick (Tona *et al.*, 2003).

Yolk

The yolk consists of about 50 % water, 15 % protein, 33 % fat and one percent carbohydrates (Şahan, Ipek & Söczü, 2014). The yolk functions as an energy source and the fat in the yolk is

the main energy source for the developing embryo (Gahri, Najafi & Deldar, 2015) where about 90 % of the energy needed is derived from oxidation of fatty acids during the embryonic development (Noble & Cocchi, 1990). A reduction of the size of the yolk could therefore mean a disadvantage for the developing embryo (Ulmer-Franco, Fasenko & O'Dea Christopher, 2010). The color of the yolk is due to carotenoids, which are an antioxidant responsible for recycling other antioxidants and affecting transcription factors. The concentration of carotenoids in the yolk is affected by the diet and during embryonic development the carotenoids are transferred from the yolk to the embryo and stored mostly in the liver of the chick. After hatch the concentration of carotenoids in the liver of the chick decreases and carotenoids are thought to be important during the stress of hatching (Surai, Fisinin & Karadas, 2016). The yolk constitutes roughly 30 % of the total egg weight (Noble & Cocchi, 1990). The results from Şahan, Ipek & Söczü (2014) showed a significant effect of age on the percentage of yolk in the eggs, where a 52 weeks old Ross breeder flock had a higher percentage of yolk compared to a younger 36-week Ross flock. These results are consistent with the results from Yadgary *et al.* (2010). The size of the yolk can have an effect on the residual yolk sac at hatch. During the first period after hatch the chick is provided with nutrients from the residual yolk sac (Şahan, Ipek & Söczü, 2014). The nutrient content of the yolk sac can therefore affect the performance of the broiler. The chicks are often deprived of food right after hatch and it is suggested they use 60 % of their residual yolk sac the first 48 hours after hatch (Noy & Sklan, 1999). Chicks from eggs of younger breeder hens have shown to have less fat content and less available fat in their residual yolk after hatch compared to chicks from eggs of older hens (Yadgary *et al.*, 2010). Similar results are shown in residual yolk from small and large eggs, where more energy was left in the residual yolk of large eggs than of small eggs at hatch. This implies that chicks from small eggs are as efficient in utilizing the energy in the egg as chicks from large eggs are (Lourens *et al.*, 2006).

Antibodies

The immune system is essential for the chick to be able to withstand invading pathogenic microorganisms. There are two different types of immune systems; the innate immune response and the specific immune response. The innate immune system is a nonspecific immune response and the first line of defense against microorganisms. This defense includes structures and functions such as the skin, mucus and antimicrobial mediators (Willey, Sherwood & Woolverton, 2012). In chicks it is the enteric mucosa that is of special interest the first weeks of age, where the defense is both independent and dependent on exposure to feed and bacteria (Bar-Shira & Friedman, 2006). The specific immune system, in contrast to the nonspecific, targets a specific foreign molecule. As the immune system is repeatedly exposed to the foreign molecule the effectiveness of the response increases. Antibodies, or immunoglobulins which they are also called, are a part of the specific immune system. They are produced by the B cells as a response to when foreign molecules are present in the body. These antibodies are glycoproteins and bind to the antigens, which the foreign molecules are called, and marks them for destruction or inactivates them (Willey, Sherwood & Woolverton, 2012). The chick has three different classes of immunoglobulins; IgA, IgM and IgY. The IgA and IgM in chickens have similar molecular properties as those of mammals (Carlander, Ståhlberg & Larsson, 1999) and are only present in the albumen of the egg (Rose, Orlans & Buttress, 1974). The IgY are

similar to the mammalian IgG and IgE, and is thought to combine their properties. IgY has a low molecular weight and is a major defense mechanism against systemic infections; these properties are similar to the properties of IgG. The similarities with the IgE are the ability to mediate anaphylactic reactions (Warr, Magor & Higgins, 1995). The IgY is present in the yolk of the egg (Rose, Orleans & Buttress, 1974). Before the newly hatched chick can derive its own antibodies, it relies on the maternal antibodies transferred from the hen to the chick (Ulmer-Franco *et al.*, 2012). The maternal antibodies are transferred in two steps; first the antibodies are transferred from the blood of the hen to the oocyte. At the second transfer the antibodies are transferred from the yolk to the embryo (Patterson *et al.*, 1962). Lawrence *et al.* (1981) found the IgY-secreting cells not being present in the spleen until six days after hatch in chicks, indicating the transfer of maternal antibodies are essential for immunological protection. The concentration of IgY in the yolk differs between 1.15-20 mg/ml (Baylan *et al.*, 2017). Baylan *et al.* (2017) found eggs with darker egg shells having a higher concentration of IgY in the yolk compared to pale ones. Darker shelled eggs had an average concentration of 6.658 mg IgY/ml, while pale had an average concentration of 5.242mg IgY/ml. When studying the transfer of IgY from the mother hen to the yolk in White Leghorn, Silkie and Dongxiang blue, Sun *et al.* (2013) found a positive correlation between the concentration of IgY in the serum of the hen and concentration in the yolk of the eggs laid by the hens. This indicates that a higher concentration of IgY in the serum of the hen can be beneficial for their offspring. Hamal *et al.* (2006) measured the IgY serum concentration in two meat type hens and found it to range between 3.26-6.02 mg/ml.

Material and methods

Flocks

Four Ross 308 grandparent flocks were selected based on the first week mortality of their progeny. Flock 1 and 2 had a first week mortality of the progeny of 4.64% and 3.68% respectively. In flock 3 and 4 the first week mortality of the progeny was 2.88% and 2.12% respectively. Eggs from these flocks were collected for analyses of egg quality traits and content of maternal antibodies. Egg for analyses of egg quality were sampled in flocks 1, 2, 3 and 4 at 49, 38, 44 and 33 weeks of age respectively. Eggs for analyses of antibodies were collected at 51, 38, 46 and 33 weeks of age in flocks 1, 2, 3 and 4, respectively. Blood samples were collected from the flocks at 49, 38, 44 and 33 weeks of age in flock 1,2,3 and 4 respectively.

Collection of eggs and blood samples for analyses

In each flock 180 eggs were collected, of which 90 were used for analysis of concentration of IgY and 90 for the analysis of external and internal egg quality parameters. The eggs selected fulfilled criteria for incubation implying that they were not dirty, had no visible cracks and were not suspected to have double yolks. The eggs were collected on a second collection of the day to assure that no eggs laid on the day before collection were included. The eggs were collected consecutive on the conveyor belt, packaged with the tip down, labelled with date and time for collection and transported in room temperature to the Swedish University of Agricultural Sciences (SLU) in Uppsala for analysis. The eggs arrived on the day of collection and were stored in + 4°C prior to analyses.

All persons involved in the collection of blood samples had read the ethical permit approved for the study. In each flock 90 blood samples were collected in plastic serum tubes, which had no additives. The hens were chosen at three different spots in the stable (at the door, in the middle and at the back of the stable) and 1 ml of blood was collected from the wing vein of the hen and the hen was also weighed. The blood samples were numbered from one to 90 and the weight of the hen was also noted for each blood sample. The collection of blood samples was conducted at the same day as the collection of the eggs for the egg quality analysis from the flock. After collection, the blood samples were transported in room temperature to SLU.

Preparing the samples for analysis of antibodies

Blood samples

On arrival at SLU the blood samples were stored in room temperature overnight. The day after collection each blood sample was centrifuged in 909xg for 15 minutes. The serum was then poured into an Eppendorf tube and stored in - 20°C until analysis.

Yolk samples

The eggs were cracked open day 5, the yolk was separated from the albumen and 2 ml of yolk was collected into an Eppendorf tube. The samples were then centrifuged in 21'000xg for 20 minutes. The water phase was collected in another Eppendorf tube and stored in + 4°C until analysis.

Egg parameters

Eggs from each flock were analyzed for both external and internal egg quality parameters. Externally the eggs were analyzed for egg weight, shell color, breaking strength, shell thickness and dry shell weight. Internally the eggs were analyzed for albumen height, Haugh units, albumen pH, albumen weight, albumen dry matter, yolk color, yolk weight and yolk dry matter. Eggs were analyzed during several days and were placed in room temperature 30 minutes prior to being analyzed.

Each egg was analyzed accordingly:

1. Color of the shell was recorded using an egg shell color guide from Zinpro¹, which ranged from 1 to 10, where 1 was pure white and 10 dark brown.
2. The egg was weighed.
3. Breaking strength was measured using the Egg force reader².
4. The egg was cracked open onto a glass plate and the yolk color was recorded using a Yolk color fan from Roche (1984).
5. Albumen height was measured 0.5 cm from the yolk by using a micrometer.
6. Yolk and albumen were separated using a separator and the pH of the albumen was measured.
7. Weights of albumen and yolk were recorded.
8. Shell thickness was measured on three different spots around the equator of the egg using a micrometer. A mean value was used for the results.
9. Weight of the shell, with shell membranes, was recorded after being dried in 103°C overnight.
10. Yolk and albumen, respectively, were dried in 60°C overnight and then dried further for at least 48 and 24 hours respectively in 103°C. Before the rise in temperature the yolks were stirred with individual glass rods to facilitate drying.
11. Before weighing the dry yolks and albumens they were put in a desiccator for 1 hour to adapt to room temperature. Based on the dry weight of the albumen and yolk the dry matter content could be calculated.

Analysis of antibodies

Yolk and blood serum were analyzed for the concentration of IgY by using the Chicken IgG ELISA Quantitation set from Bethyl laboratories³. A standard was determined with use of the company set and the dilutions used on the plates for the samples were 1:100 000 and 1:500 000. Duplicates were used for the standard, samples and blanks on each plate, and an average of the duplicates was used when calculating the concentration of IgY. All plates had blanks and the background absorbance was subtracted from the samples and standard in the calculation of concentration. At each washing the plates were washed 6 times. The substrate had a dilution of 1:20 and the stop solution 1.8 M sulphuric acid. The reaction was stopped after five minutes.

¹Zinpro corporation, 10400 Viking Drive, Suite 240
Eden Prairie, Minnesota 55344 USA.

²Orka Food technology, 875 Lakeview DR, Bountiful,
UT, 84010, USA.

³Bethyl Laboratories, Inc, 25043 West FM 1097,
Montgomery, TX 77356 USA.

Statistical analysis

The SAS statistical software⁴ and the MIXED procedure were used for analysis of the fixed effect of day of performing the egg quality analyses for each flock. No random factor was used. Shell color and yolk color was not included in this analysis due to assumption of them not being affected by day. Comparisons with a P-value of <0.05 was considered to indicate a statistically significant difference between days of analyses. Analyses of the differences between egg parameters in the different flocks were described descriptively in graphs, using Microsoft Excel⁵, with a confidence interval of 95% and an assumption of normal distribution due to the high number of samples. If the confidence interval of the different flocks did not overlap they were considered to be significant different from each other. The results of analyzed antibodies in serum samples and egg yolks were described descriptively in graphs. As with the egg parameters a confidence interval was calculated, assuming normal distribution due to the high number of samples, and flocks were considered to be significant different when their confidence intervals did not overlap. In the analysis an outlier was defined as a value outside 1.5 times the interquartile range.

Result

The results from the egg quality analysis, antibody concentration in the yolks and serum for the different flocks are presented below.

Egg quality

Effect of day

There was no parameter with a significant effect of day that was consistent for all flocks. In flock 1 (Appendix 1, table 1) there were significant effects of day of analyses in egg weight and yolk wet and dry weight. The lowest weight for the egg weight, yolk wet and dry weight was day four, while highest means for these parameters were on day six. There was also a tendency ($P < 0.10$) to a difference in percentage of yolk in different days, following a similar pattern over days as the significant parameters. Flock 2 (Appendix 1, table 2) showed a tendency of difference between days in dry matter of the yolk, with a decrease with day. In flock 3 (Appendix 1, table 3) day of analyses had a significant effect on Haugh units and albumen pH, and there was a tendency to significance on height of the albumen. The albumen height and Haugh units were at their highest and lowest levels on days 3 and 4, respectively. In flock 4 (Appendix 1, table 4) there was a significant difference of day on albumen pH and a tendency in albumen height and Haugh unit. The pH increased with day, while the albumen height and Haugh Units were at their lowest on day 3 and highest on day 1.

Differences between flocks

The egg weight was highest in flock 1 and the lowest in flock 4 (table 1). Based on confidence intervals the egg weights were significantly higher in flocks 1 and 3 compared to flocks 2 and 4. Flock 1 had the highest shell weight and the highest percentage of shell, there was also a significant difference based on confidence intervals between flock 1 and flocks 2, 3 and 4 in shell weight, shell percentage and shell thickness. In addition, flock 4 had a significantly lower shell weight than all other flocks. Albumen height and Haugh units were significantly higher in flocks 2 and 4 compared to flocks 1 and 3. The weight percentage of albumen was significantly higher in flocks 2 and 4 than flocks 1 and 3. The dry matter of the yolk was higher in flock 2 and 4 than in flock 1 and 3, and lower in flock 1 compared to the other flocks. However, the average yolk weights, dry and wet, respectively, were higher in flock 1 and 3 than in flock 2 and 4. The percentage of yolk was higher in flock 1 and 3 than in flock 2 and 4. The highest yolk color grade (not in table) was shown in flock 2 and the lowest in flock 4, and there was a significant difference based on confidence interval between flock 2 and flock 1 and 4 (figure 2). Flock 4 had the highest shell color grade and flock 1 the lowest, but there were no significant differences (figure 1).

Table 1. Results from the analysis of exterior and interior egg quality traits in 4 broiler breeder flocks. The least squares mean (LSM) for each parameter was analyzed and presented below, as well as standard error of mean (SEM) and confidence interval (CI) of 95%.

Parameters	Flock 1				Flock 2				Flock 3				Flock 4			
	LSM	SEM	CI 95% interval		LSM	SEM	CI 95% interval		LSM	SEM	CI 95% interval		LSM	SEM	CI 95% interval	
			min	max			min	max			min	max			min	max
Egg weight (g)	62.88 ^a	0.41	62.36	64.03	59.94 ^b	0.41	59.10	60.70	61.82 ^a	0.45	60.92	62.60	57.55 ^b	0.37	56.81	58.27
Breaking strength (kgF)	3.89	0.079	3.68	3.97	3.67	0.076	3.50	3.80	3.70	0.065	3.59	3.83	3.64	0.055	3.53	3.74
Shell thickness (mm)	0.335 ^a	0.0033	0.329	0.341	0.315 ^b	0.0038	0.308	0.322	0.318 ^b	0.0025	0.313	0.323	0.309 ^b	0.0022	0.305	0.313
Shell weight (g)	5.70 ^a	0.059	5.59	5.81	5.19 ^b	0.055	5.08	5.30	5.37 ^b	0.050	5.28	5.46	5.00 ^c	0.039	4.92	5.08
Shell %	9.07 ^a	0.084	8.91	9.23	8.67 ^b	0.080	8.51	8.83	8.69 ^b	0.056	8.59	8.79	8.70 ^b	0.061	8.58	8.82
Albumen height (mm)	5.7 ^b	0.11	5.5	5.9	6.8 ^a	0.11	6.6	7.01	5.9 ^b	0.10	5.7	6.1	6.7 ^a	0.10	6.49	6.91
HU	72.72 ^b	0.96	70.94	74.50	82.28 ^a	0.68	80.93	83.63	74.97 ^b	0.84	73.18	76.58	82.31 ^a	0.68	80.93	83.69
Albumen pH	8.847 ^a	0.095	8.818	8.880	8.545 ^c	0.035	8.511	8.579	8.899 ^a	0.015	8.864	8.934	8.635 ^b	0.013	8.601	8.669
Albumen weight (g)	34.98 ^a	0.29	34.42	35.54	34.67 ^a	0.33	34.02	35.32	34.48 ^{ab}	0.32	33.89	35.07	33.40 ^b	0.28	32.85	33.95
Albumen dry weight (g)	4.24	0.042	4.16	4.32	4.30	0.051	4.20	4.40	4.21	0.049	4.12	4.3	4.17	0.040	4.09	4.25
Albumen DM (%)	12.12 ^b	0.053	12.02	12.22	12.40 ^{ab}	0.066	12.27	12.53	12.21 ^b	0.068	12.08	12.34	12.49 ^a	0.055	12.38	12.6
Albumen percentage (%)	55.65 ^b	0.31	55.08	56.22	57.75 ^{ab}	0.21	57.33	58.17	55.74 ^b	0.25	55.27	56.21	58.00 ^a	0.19	57.62	58.38
Yolk weight (g)	15.24 ^a	0.18	14.88	15.60	13.25 ^b	0.12	13.02	13.48	14.96 ^a	0.16	14.66	15.26	12.34 ^c	0.14	12.07	12.61
Yolk dry weight (g)	9.60 ^a	0.085	9.43	9.77	8.66 ^b	0.067	8.53	8.79	9.46 ^a	0.083	9.31	9.61	8.18 ^c	0.067	8.05	8.31
Yolk DM (%)	63.19 ^c	0.30	62.62	63.76	65.46 ^a	0.30	64.85	66.07	63.38 ^b	0.241	62.92	63.84	66.52 ^a	0.31	65.9	67.14
Yolk percentage (%)	24.21 ^a	0.20	23.82	24.60	22.13 ^c	0.17	21.79	22.47	24.20 ^b	0.22	23.80	24.60	21.44 ^c	0.20	21.05	21.83

^{a,b,c} Different superscripts indicate a significant difference between flocks based on their confidence interval.

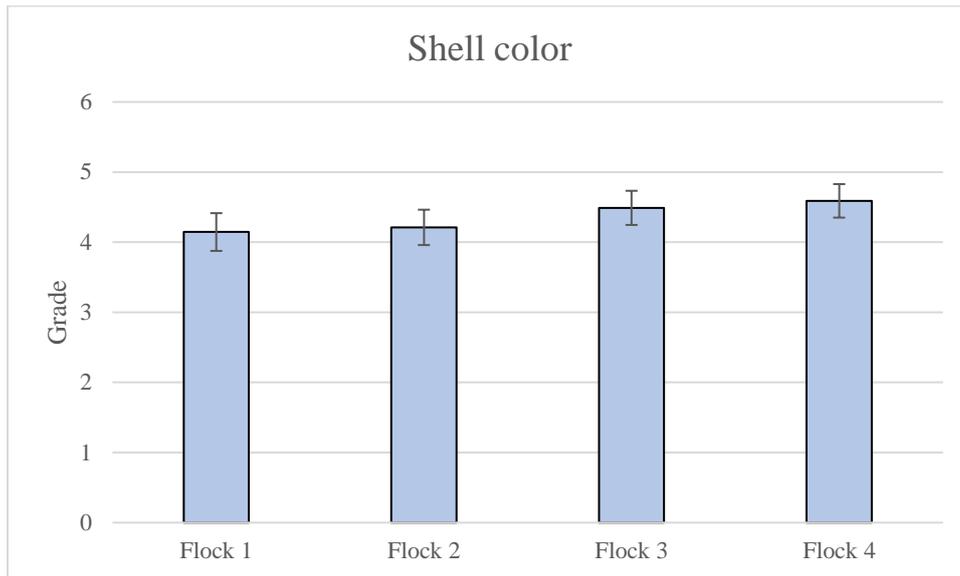


Figure 1. The average grade of the shell color in each flock is displayed in the bar and the line displays 95 % confidence interval for the grade of the different flocks. Significant differences were not detected according to CI.

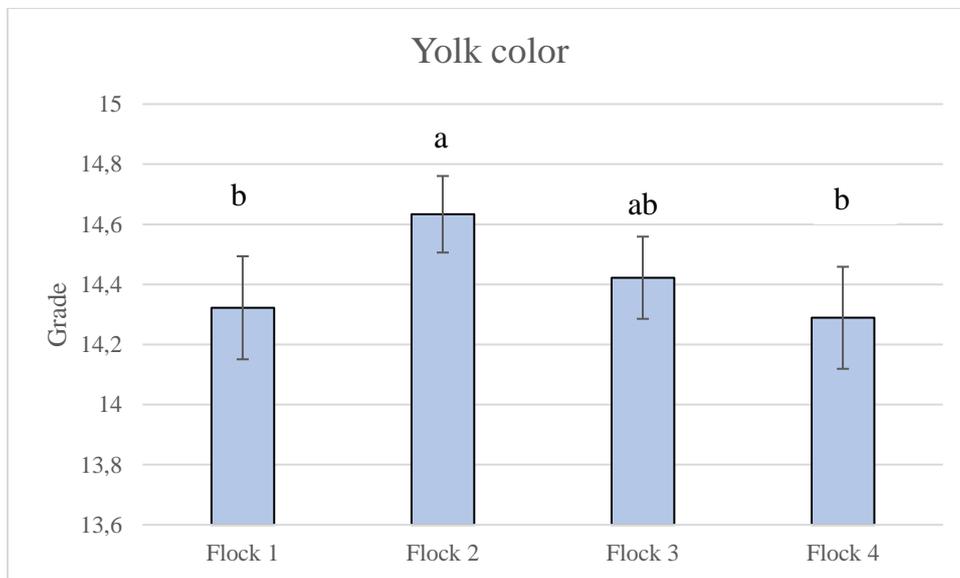


Figure 2. The average grade of the yolk color in each flock is displayed in the bar and the line displays 95 % confidence interval for the grade of the different flocks. Significant differences were detected between flock 2 and flock 1 and 4, according to CI.

Bird weights

There was a significant difference in average weight of birds between flock 1 and flock 4 and between flock 3 and flock 4 (Figure 3). Birds in flock 2 had a lower spread of weights compared to the other flocks, but also more outliers than the others (Appendix 2, Figure 1). In flocks 1 and 3 the average weight was significantly different from the average weight in flock 4 (figure 3).

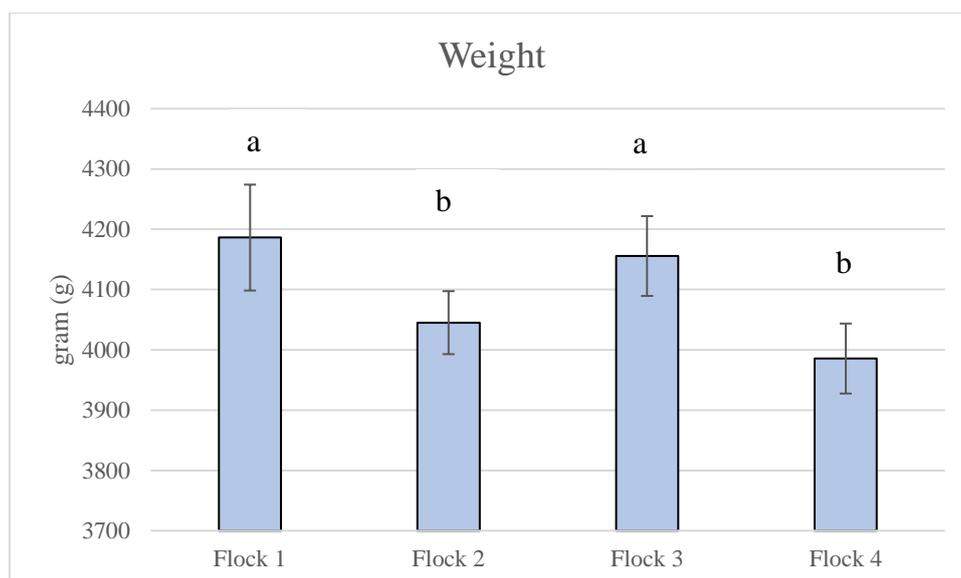


Figure 3. The average hen weight in each flock is displayed in the bar and the line displays 95 % confidence interval for the weight of the different flocks. Significant differences were detected between flock 1 and 4, and between flock 3 and 4 according to CI.

IgY serum concentration

The average, median, max and min are presented in table 2. There was no significant difference in IgY serum concentration between the different flocks (figure 4) but there was a considerable variation in IgY concentration in hens' serum in all flocks (Appendix 3, figure 1). Flock 3 had the lowest spread and lowest average concentration of IgY in hens' serum (table 2). The most hemolysis was seen in the blood samples from flock 3. Flock 2 had the highest average IgY serum concentration (table 2). The number of outliers were higher in flock 1, and flock 2 had the largest spread (Appendix 3, figure 1).

IgY yolk concentration

The results from the analysis of IgY concentration in the yolk are presented in table 2. The spread was of similar level in all flocks (Appendix 4, figure 1), somewhat lower in flock 2. Flock 2 had the lowest average concentration of IgY in the yolk and flock 4 the highest (table 2). The IgY yolk concentration did not significantly differ between the flocks (figure 5).

Table 2. The average and median concentration of IgY in mg/ml are represented below for the different flocks. The maximum and minimum values are also presented. The first week mortality (FWM) percentage are shown in the table for the different flocks.

	IgY concentration serum (mg/ml)				IgY concentration yolk (mg/ml)				FWM (%)
	Average	Median	Max	Min	Average	Median	Max	Min	
Flock 1	14.58	7.51	40.58	1.18	8.44	7.83	20.42	3.15	4.55
Flock 2	15.00	14.00	40.08	0.79	7.62	7.36	19.18	3.08	3.66
Flock 3	12.04	9.48	36.85	0.87	7.81	7.60	16.92	1.53	2.94
Flock 4	12.92	12.19	30.64	0.80	8.85	8.46	22.62	3.42	2.12

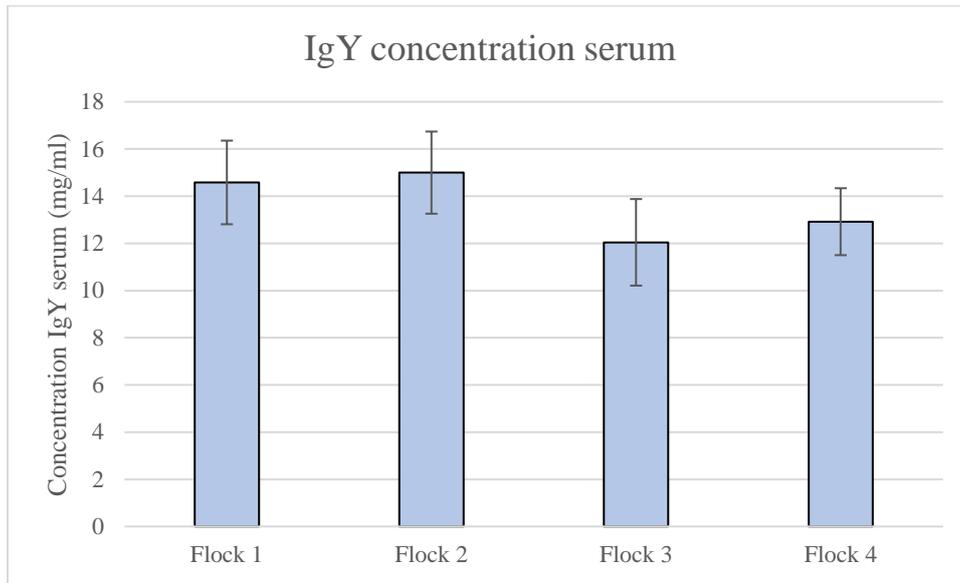


Figure 4. The average IgY serum concentration for each flock is displayed in the bar and the line displays 95 % confidence interval for the IgY serum concentration of the different flocks. No significant differences were detected according to CI.

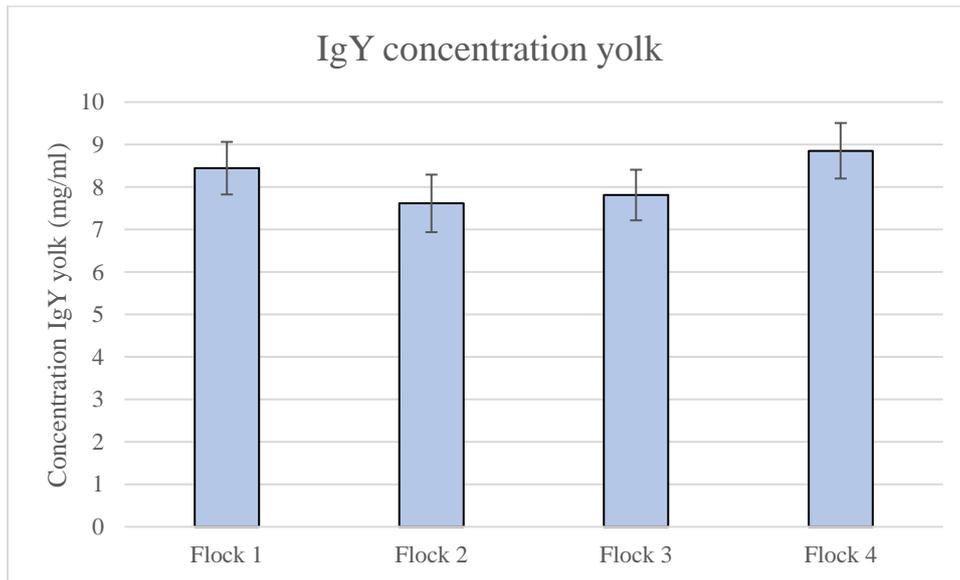


Figure 5. The average IgY yolk concentration in each flock is displayed in the bar and the line displays 95 % confidence interval for the IgY yolk concentration of the different flocks. No significant differences were detected according to CI.

Discussion

In this study four Ross grandparent flocks were selected based on the first week mortality of their progeny. However, the flocks were of different ages which is likely reflected in some of the parameters studied.

The weights of eggs from the breeders in the flocks were within the normal range (Ulmer-Franco, Fasenko & O'Dea Christopher, 2010). There were significant differences in egg weight between flocks related to their age, with the highest weight of eggs in the oldest flock (flock 1, 49 weeks) and lowest in the youngest flock (flock 4, 33 weeks). This is in accordance with older hens having higher egg weights (Ulmer-Franco, Fasenko & O'Dea Christopher, 2010).

The thickness of the shell was within the normal range, but the shell weight percentage was lower, between 8.7-9.0%, than the shell weight percentage stated by Gupta (2008), which were 10-11%. The reason for the difference could be that the shell weight percentage stated by Gupta (2008) was for table eggs, while the present experiment measured broiler breeder eggs. There was a significant difference in egg shell thickness between flock 1 and the other flocks, where flock 1 had the thickest (0.335mm) and flock 4 the thinnest (0.309 mm) shell thickness. The results regarding shell weight and shell percentage point in the similar direction. Flock 1 was the flock with the highest percentage of first week mortality and highest egg weight, where the higher shell weight per se contributed to the higher egg weight. One could speculate in that a thick shell requires more energy of the chick to break through at hatch, resulting in less energy reserves after hatch and thereby a chick more susceptible to pathogens when entering the farm. However, the breaking strength of the eggs in the different flocks were though not significantly different, implying that the egg shells would demand an equal effort by the chickens at hatch. The breaking strength of the different flocks were well above the recommended value of >3.059 for table eggs (Gupta, 2008).

The wet weights of the albumen were highest in flock 1 and lowest in flock 4, while albumen dry matter and albumen percentage were lower in flocks 1 and 3 compared to flocks 2 and 4. In flock 1 and 3 the egg weight was higher, and therefore the weight of the egg components were higher. The egg size could therefore be the reason for the difference in wet albumen weight. The greater albumen weight could be explained by a higher inclusion of water in the albumen in flock 1 (49 weeks old) and 3 (44 weeks old) compared to flock 2 (38 weeks old) and 4 (33 weeks old).

When considering the albumen height there was a tendency to an effect of day of analyses in flock 3, and significant effect in Haugh units. In flock 3, both the albumen height and Haugh units decreased with storage time. Besides a peak day four the same trend could be seen in flock 4. The peak at day four was due to some extreme values creating a higher average. The findings that albumen height and Haugh units decrease with time are in line with the findings by Tona *et al.* (2002). The reason for the decrease with time is due to the degradation of ovomucin, creating a lower viscosity and thereby a lower height of the albumen (Robinson & Monsey, 1972). In eggs incubated after storage there is a risk that the egg yolk is not positioned

in the middle of the egg due to reduced albumen viscosity. A shorter distance from the egg shell to the egg yolk and reduced viscosity facilitates for bacteria to reach the growing embryo (Willems *et al.*, 2014).

The younger flocks (flock 2 and 4) had higher albumen heights and Haugh units than did the older flocks, which is probably due to a storage effect, where the older flocks' eggs were stored for a longer time before being analyzed (Scott & Silversides, 2000). It could also be the effect of age, as the albumen height and Haugh units decreases with age (Tona *et al.*, 2004). When comparing the average Haugh units of the older flocks (44 and 49 weeks old) in our study with a Cobb broiler breeder flock at 54 weeks, the albumens in our study had lower Haugh units, at 72 HU and 75 HU, than the Cobb broiler breeder flock right at lay (80 HU). However, after eight days of storage of eggs from the Cobb breeders the Haugh units was almost the same as in the present study (75 HU) (Tona *et al.*, 2002). Flock 2 and 4, aged 38 and 33 weeks, both had an average of 82 HU and were close to the average Haugh units at lay in the Cobb broiler breeder flock in the study by Tona *et al.* (2002). The difference is probably due to the age, where the albumen height decreases with age (Tona *et al.*, 2004). In this study Ross broiler breeders were used while in the study by Tona *et al.* (2002) Cobb broiler breeders were used, the difference could therefore be linked to the different breeds, where Cobb has a higher viscosity of their albumens. The eggs in the study by Tona *et al.* (2002) were from older hens and if younger hens would have been used it is hypothesized that the Haugh units would have been even higher since albumen height decreases with age. It can also be speculated the higher inclusion of water in the older flocks (flock 1 and 3) could explain the lower albumen height and Haugh units, where a higher inclusion of water could dilute the concentration of ovomucin and thereby affect the viscosity.

The pH of the albumen was expected to be affected by storage, but was only affected by the number of days stored prior to analyses in flock 3 and 4. In flock 4 there was a rise in pH with day in storage, which was in line with the results by (Decuypere *et al.*, 2007), but in flock 3 it peaked in day three and then decreased. The reason for the peak could be that some of the eggs analyzed day three had a higher pH than the others and thereby contributed to a higher average that day. The average pH in each flock was close to nine, which is considered be a normal pH after storage. A pH around nine is not too high to inhibit embryo growth, but high enough to inhibit most bacterial growth (Hatchability, n.d.). Flock 2 and 4 were analyzed quicker after lay compared to flock 1 and 3, which is reflected in a lower pH in flock 2 and 4. This difference was significant and is probably due to the difference in number of days required for completing the egg analyses.

There was a significant effect of day of analyses on the wet and dry weight of the yolk in flock 1, and a tendency of an effect of day on the percentage of yolk. The egg weight was significantly affected by day, which could explain the effect on the yolk as well. Since the weight of the egg correlates with the weight of its components it could be that the smallest eggs by random were analyzed on day 4, inducing the effect of day.

There was a significant difference in the percentage of yolk and wet and dry yolk weight between flock 1 and flocks 2 and 4, as well as between flock 2 and flock 3, where flock 1 and 3 had higher wet and dry weights. It could be the weight of the eggs reflecting the weight of the yolk, where flock 1 and 3 had higher egg weights and therefore higher yolk weights. The higher percentage of yolk in the older flocks (flock 1 and 3) are in accordance with the results by Sahan, Ipek & Sözcü (2014) and Yadgary *et al.* (2010). The dry matter of the yolk was though lower in flock 1 and 3 than in flock 2 and 4, which indicates a higher water content in the yolk of the older flocks. A higher yolk content is therefore not directly related to a greater inclusion of nutrients and thereby possibly a better condition for the developing chick, the dry matter should be considered instead. In comparison with the literature (Noble & Cocchi, 1990) the percentage of yolk is lower in the present study, where the literature states the yolk being around 30% of the total egg weight compared to 21-24% in the present study. Possible factors affecting the percentage of yolk are differences in genetics and condition of the hen.

There was a significant difference in the yolk color between flock 2 and 4, where flock 2 had the darkest colored yolks and flock 4 the lightest. Carotenoids are responsible for the color of the yolk; the darker colored yolks have more carotenoids than the lighter ones. Content of carotenoids in bird diet affects egg yolk color, as shown by Surai, Fisinin & Karadas (2016). The content of carotenoids in the diets provided to the four breeder flocks in the present study is not known, and it cannot be excluded that there were dietary differences. Other reasons could be a difference in the uptake of carotenoids by the hen. Based on the yolk color in the present study, progeny from flock 2 were hatched from eggs with a darker yolk color, implying a higher content of carotenoids. As carotenoids are stated to be of importance to support the chick during the stress of hatching (Surai, Fisinin & Karadas, 2016), chickens from flock 2 would have been expected to be more viable. However, in contradiction, flock 2 had the second highest first week mortality of the four compared flocks, whereas flock 4, with the palest yolks, had the lowest first week mortality.

The average hen weight in the flocks were lower in the younger flocks (flocks 2 and 4), and differences in hen weights were likely related to the age differences. The significant difference between flock 1, 3 and flock 4 can also be explained by the age difference, whereas older hens are heavier. Flock 2 had the lowest spread of individual bird weights and if the sample is to reflect the population, it means that the hens in flock 2 had a lower within flock variation regarding weight compared to the other flocks. However, it cannot be excluded that the staff catching the hens by chance selected more hens of the same size even though the catching procedure was to be standardized.

There was a considerable variation in IgY serum concentration between hens in all flocks, and no significant differences were detected between flocks. This variation could reflect a natural variation in the IgY serum concentration of the hens, in accordance with the variation in the study by Hamal *et al.* (2006) with concentrations between 3.26 and 6.02mg/ml. It is also possible that some hens had an infection or an overactive immune system, resulting in an increased concentration of IgY in their serum. Since there was no significant difference in IgY

between the flocks in the present study, a link between first week mortality and IgY serum concentration could not be proved. However, although not significantly different, the IgY serum concentrations were in absolute numbers higher in flocks with higher first week mortality (flock 1 and 2) compared to flock 3 and 4. Flock 3 had a lower variation and the lowest average IgY serum concentration, and it cannot be excluded that the observed higher degree of hemolysis in the samples affected the result. The samples with severe hemolysis were not included in the results but it cannot be neglected that samples with moderate hemolysis, could have influenced the results of the analyzed concentration of IgY in the serum. Kurian *et al.* (2012) showed that severe and moderate hemolysis did affect the ELISA results when testing for infectious bronchitis virus, avian encephalomyelitis virus and chicken anemia virus. However, the hemolysis in this study probably had no significant effect since no significant differences were evident in the IgY serum analysis. The reason for the higher prevalence of hemolysis in this flock can only be speculated on. It is possible that something differed in the sampling technique when taking the blood samples, something happened during transport or that for some unknown reason the blood from flock 3 was more prone to hemolysis.

There were no significant differences in the IgY yolk concentration between flocks. In absolute numbers flock 2 had the lowest IgY yolk concentration and flock 4 the highest and the variation was of equal size in all flocks. According to the flock means, IgY yolk concentration and IgY serum concentration do not seem to be correlated. Which is not in accordance with the results by Sun *et al.* (2013) who found a positive correlation between the IgY serum concentration and IgY yolk concentration. The reason for the difference could be different breeds or that in the present study, the transfer of IgY from hen to egg on individual basis could not be followed as hens and eggs were randomly chosen. Based on the mean values, there is an indication of that a higher level of serum IgY was transferred to the yolks in flock 4 than in the other flocks.

Conclusion

In the present study no results of the egg quality analysis or the antibody status show any significant differences that can be linked to a difference in the first week mortality of the flocks' offspring's. The results though show an effect on some of the egg quality parameters that could be explained by the age, which are consistent with the findings of other studies. Although there were no significant differences, the IgY serum concentration had a trend of following the first week mortality, where the flock with highest concentration also had the highest first week mortality. The transfer of the IgY though seemed to be most efficient in the flock with lowest IgY serum concentration and the lowest first week mortality. It would be of interest to repeat the study with flocks that were of the same age at egg collection to be able to not consider the age effect and see if there are any difference in the egg quality parameters. The transfer of IgY from the individual hen to her own egg would also be of interest to investigate in order to see if there is a correlation between the transfer of IgY and the first week mortality rates. To investigate the reason to why the first week mortality rates differ between flocks it would be interesting to include several aspects such as differences in nutrition, management, housing conditions and routines at the hatchery and farms for example.

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Appendix 1

Table 1. Results from the analysis of exterior and interior egg quality in flock 1. The least squares mean (LSM) for each parameter and day they were analyzed are presented as well as P-value. The P-value marked are *=P<0.05, **=P<0.01 and ***=P<0.001.

Flock 1						
Parameters	LSM day of analyses					P-value day
	2	3	4	5	6	
Egg weight (g)	62.30	63.96	59.84	63.04	65.27	***
Breaking strength (kgF)	3.917	3.808	3.841	3.773	3.853	0.99
Shell thickness (mm)	0.339	0.331	0.329	0.335	0.340	0.78
Shell weight (g)	5.68	5.76	5.50	5.64	5.91	0.18
Shell %	9.12	9.01	9.23	8.96	9.05	0.85
Albumen height (mm)	5.6	5.5	6.0	5.5	5.9	0.53
HU	72.21	70.69	76.28	71.26	73.14	0.36
Albumen pH	8.911	9.048	8.831	8.895	8.550	0.42
Albumen weight (g)	34.60	35.50	33.94	34.80	36.06	0.13
Albumen dry weight (g)	4.16	4.30	4.08	4.29	4.38	0.16
Albumen DM (%)	12.00	12.11	12.03	12.31	12.14	0.30
Albumen %	55.50	55.49	56.87	55.17	55.21	0.36
Yolk wet weight (g)	14.97	15.62	13.95	15.40	16.28	***
Yolk dry weigh (g)	9.41	9.77	9.01	9.64	10.16	***
Yolk DM (%)	63.04	62.62	64.77	62.87	62.60	0.13
Yolk %	24.06	24.44	23.26	24.40	24.90	0.090

Table 2. Results from the analysis of exterior and interior egg quality in flock 2. The least squares mean (LSM) for each parameter and day they were analyzed are presented as well as P-value. The P-value marked are *=P<0.05, **=P<0.01 and ***=P<0.001.

Flock 2					
Parameters	LSM day of analyses				P-value day
	1	2	3	4	
Egg weight (g)	60.32	59.01	60.14	60.28	0.60
Breaking strength (kgF)	3.63	3.50	3.77	3.73	0.56

Shell thickness (mm)	0.312	0.316	0.318	0.314	0.96
Shell weight (g)	5.18	5.07	5.25	5.27	0.55
Shell %	8.61	8.60	8.73	8.76	0.86
Albumen height (mm)	7.3	6.8	6.7	6.6	0.19
HU	84.70	82.49	81.53	80.39	0.18
Albumen pH	8.453	8.565	8.487	8.674	0.14
Albumen wet weight (g)	35.11	33.96	34.81	34.78	0.63
Albumen dry weight (g)	4.37	4.23	4.26	4.35	0.72
Albumen DM (%)	12.42	12.45	12.22	12.50	0.43
Albumen %	58.09	57.50	57.80	57.61	0.79
Yolk wet weight (g)	13.16	13.11	13.13	13.60	0.43
Yolk dry weight (g)	8.73	8.62	8.58	8.70	0.86
Yolk DM (%)	66.47	65.89	65.40	64.10	0.057
Yolk %	21.86	22.22	21.87	22.58	0.42

Table 3. Results from the analysis of exterior and interior egg quality in flock 3. The least squares mean (LSM) for each parameter and day they were analyzed are presented as well as P-value. The P-value marked are *=P<0.05, **=P<0.01 and ***=P<0.001.

Flock 3						
Parameters	LSM day of analyses					P-value day
	2	3	4	5	6	
Egg weight (g)	61.78	62.00	61.58	62.59	61.17	0.81
Breaking strength (kgF)	3.553	3.831	3.736	3.711	3.679	0.82
Shell thickness (mm)	0.312	0.323	0.315	0.316	0.323	0.52
Shell weight (g)	5.25	5.50	5.32	5.39	5.39	0.68
Shell %	8.50	8.86	8.65	8.62	8.82	0.24
Albumen height (mm)	6.3	6.3	5.6	5.7	5.7	0.066
HU	78.16	78.38	71.39	73.29	73.61	*
Albumen pH	8.982	9.064	8.804	8.784	8.862	***
Albumen weight (g)	34.78	34.41	33.92	34.96	34.32	0.85
Albumen dry weight (g)	4.26	4.16	4.16	4.29	4.18	0.86
Albumen DM (%)	12.26	12.09	12.24	12.27	12.17	0.92

Albumen %	56.30	55.48	55.02	55.81	56.09	0.53
Yolk wet weight (g)	14.98	14.79	14.80	15.18	15.04	0.92
Yolk dry weight (g)	9.35	9.47	9.50	9.55	9.43	0.96
Yolk DM (%)	62.55	64.06	64.32	63.06	62.91	0.11
Yolk %	24.24	23.86	24.02	24.27	24.61	0.77

Table 4. Results from the analysis of exterior and interior egg quality in flock 4. The least squares mean (LSM) for each parameter and day they were analyzed are presented as well as P-value. The P-value marked are *=P<0.05, **=P<0.01 and ***=P<0.001.

Flock 4					
Parameters	LSM day of analyses				P-value day
	1	2	3	4	
Egg weight (g)	58.40	57.34	57.62	56.82	0.54
Breaking strength (kgF)	3.645	3.649	3.620	3.628	0.10
Shell thickness (mm)	0.311	0.307	0.308	0.311	0.90
Shell weight (g)	5.05	4.95	4.99	5.01	0.83
Shell %	8.65	8.65	8.67	8.83	0.71
Albumen height (mm)	7.1	6.6	6.3	6.9	0.05
HU	84.57	81.65	79.64	83.38	0.065
Albumen pH	8.472	8.599	8.706	8.762	***
Albumen wet weight (g)	33.99	33.59	33.48	32.53	0.34
Albumen dry weight (g)	4.29	4.20	4.19	4.01	0.14
Albumen DM (%)	12.62	12.49	12.50	12.33	0.38
Albumen %	58.21	58.50	58.04	57.25	0.14
Yolk wet weight (g)	12.35	12.08	12.46	12.45	0.71
Yolk dry weight (g)	8.27	8.10	8.15	8.18	0.85
Yolk DM (%)	67.22	67.27	65.49	66.11	0.10
Yolk %	21.11	21.09	21.67	21.87	0.39

Appendix 2

Table 1. The average and median weight of 90 birds in each flock are presented in the table. The maximum and minimum weight are also represented for the different flocks.

	Weight (g)			
	Average	Median	Max	Min
Flock 1	4186	4041	5384	3448
Flock 2	4045	4029	4975	3509
Flock 3	4156	4111	4867	3595
Flock 4	3986	3996	4572	3253

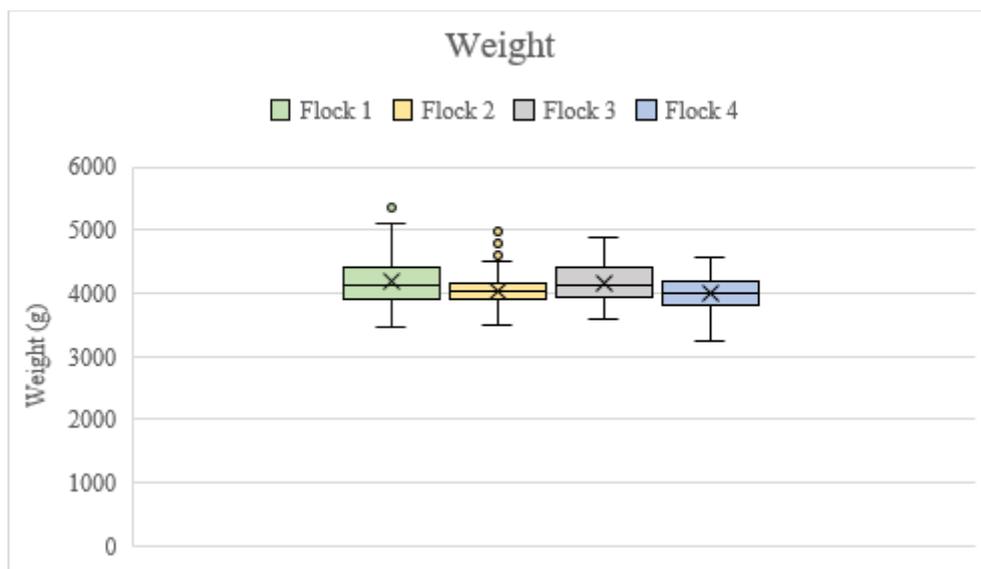


Figure 1. The weight of 90 birds in different flocks are presented in a boxplot. The median value is displayed with a line in the box and the average with an X. The box encloses 50% of the data, the whisker marks the maximum and minimum and circles represent outliers.

Appendix 3

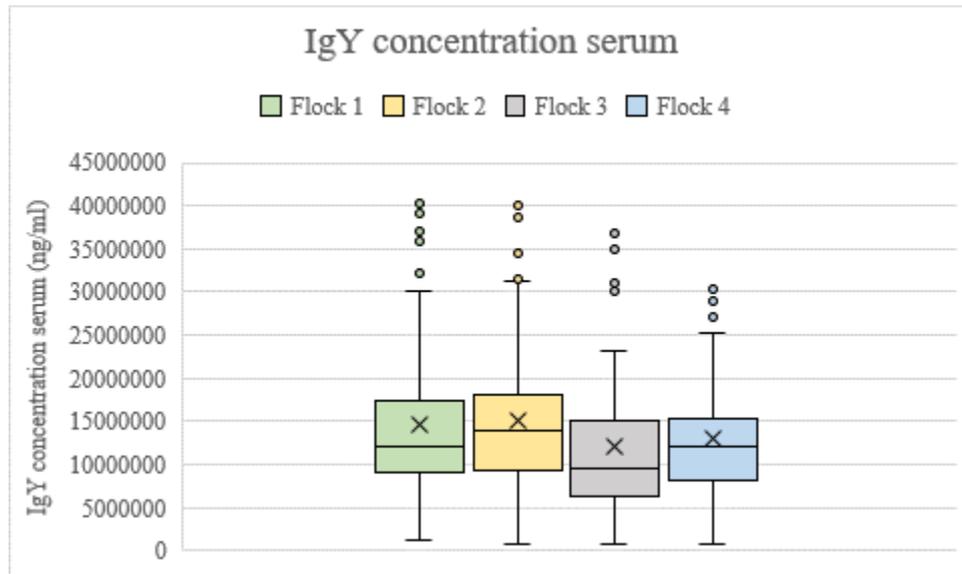


Figure 1. The IgY serum concentration in the different flocks are presented in a boxplot. The median value is displayed with a line in the box and the average with an X. The box encloses 50% of the data, the whisker marks the maximum and minimum and circles represents outliers.

Appendix 4

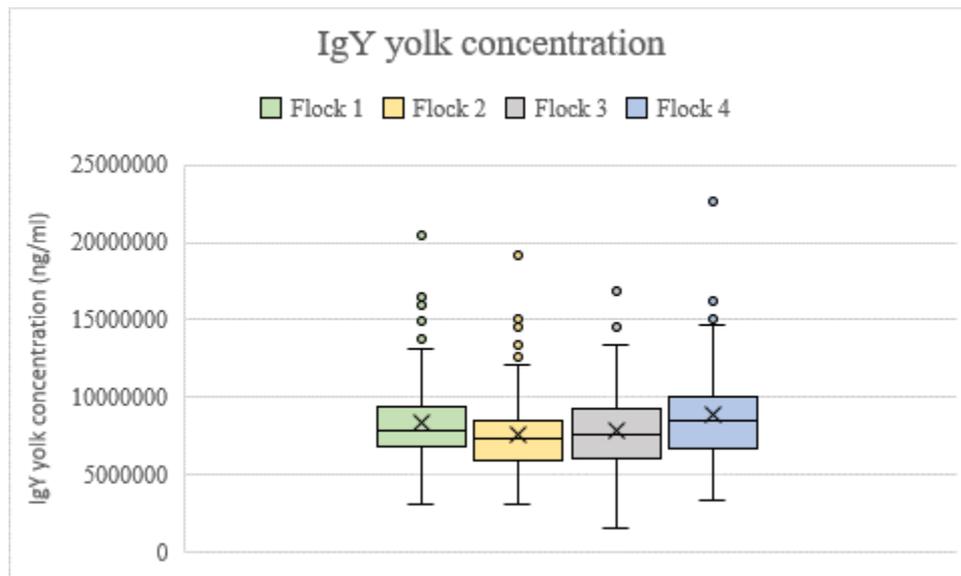


Figure 1. The IgY yolk concentration in the different flocks are presented in a boxplot. The median value is displayed with a line in the box and the average with an X. The box encloses 50% of the data, the whisker marks the maximum and minimum and circles represents outliers.