Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences

Analysis of Fatty Acids in Egg Yolks of Various Production Systems

Analys av fettsyror i äggulor från olika produktionssystem

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Department of Molecular Sciences
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Abstract

OBJECTIVES: The objective of this study was to compare fatty acid composition of different eggs. Seven kinds of eggs from three companies were taken, including organic, free range, enriched and "farmer" eggs.

RESULTS: In total 15 fatty acids were identified. The fatty acids with highest content found in the egg yolks were palmitic acid, steric acid, oleic acid and linoleic acid. The saturated fatty acid content was similar in all samples. Different amount of n-3 fatty acids were observed in the eggs. Uggelsta organic had highest docosahexaenoic acid content and Kronägg Guld Gula free range with added algae had low polyunsaturated fatty acids and low docosahexaenoic acid content.

CONCLUSION: Egg yolk from organic hens with access to outdoor exercise yards appears to have a higher DHA content but this result need to be confirmed by further research.

Keywords: egg, fatty acid, omega-3, egg enrichment, docosahexaenoic acid, omega-6/omega-3 ratio, organic, free range, DHA

Sammanfattning

MÅL: Målen med denna studie var att jämföra fettsyrasammansättningen av olika ägg. Sju typer av ägg från tre olika tillverkare valdes för studien, däribland ekologiska-, frigående-, berikade- och bondägg.
RESULTAT: Totalt identifierades 15 fettsyror. De högsta halterna av fettsyror som hittades i äggulorna var palmitinsyra, stearinsyra, oljesyra och linolsyra. Andelen mättade fettsyror i innehållet var likartat i alla prover. Olika mängder fettsyror observerades i de olika äggen. Uggelsta ekologiska hade den högsta dokosahexaensyra -halten och Kronägg Guld Gula frigående berikade med alger hade låg fleromättade fettsyror- och dokosahexaensyra halt.
SLUTSATS: Äggula från ekologiska ägg från höns med tillgång till utanhusrastgårdar ser ut att vara ha en högre DHA halt men detta resultat måste bekräftas av fortsatt forskning.

Nyckelord: ägg, fettsyra, omega-3, äggberikning, docosahexaenoic acid, omega-6/omega-3 kvot, ekologisk, frigående, DHA

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Abbreviations

ALA	α -linolenic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
FADS	Fatty acid desaturase
LA	Linoleic acid
LC	Long-chain
MUFA	Monounsaturated fatty acid
n-3	Omega-3
n-6	Omega-6
PUFA	Polyunsaturated fatty acid
R/C	Rapeseed and corn oil (3:7)
SFA	Saturated fatty acid

1 Introduction

Hen eggs are eaten in most areas of the world (Fraeye *et al.*, 2012; Fredriksson *et al.*, 2006). Eggs supply essential nutrients and defense factors for the developing embryo. The nutrients are minerals, fatty acids, vitamins and essential amino acids (Hansen *et al.*, 1998). The lipid content of the whole egg yolk is about 30% (Nielsen, 1998). The shell and egg white contribute to physical and biological defense mechanisms such as viscosity, pH and antimicrobial properties (Nimalaratne & Wu, 2015). The yolk has a pH of 6.0 and its color is dependent on carotenoids in the feed, e.g. xanthopylls, lutein, luteinmono- and diester, 3´-oxolutein and zeaxanthin. Eggs are better preserved in cold storage because it lowers the losses of carbon dioxide and water (Belitz *et. al*, 2009).

This study was done because we wanted to see if there is in various egg yolks difference in fatty acid composition between conventional and organic egg yolks. The fatty acid compositions were analyzed and the results were compared with other studies of hen systems. A literature review was done to try to find out what kind of diet the laying hens have had from the fatty acid compositions in the eggs. Comparisons between feeds such as fish oil, microalgae, flaxseed and between conventional and certified eggs such as organic and omega-3 (n-3) long-chain polyunsaturated acid (LCPUFA) enriched eggs were made. For comparison, quail egg, being more occasionally used as a gourmet ingredient, was also analyzed (Montagne, 2001). Health aspects have been pointed out of consumptions of n-3 LCPUFA and the importance of the n-6:n-3 PUFA ratio. The focus of this study has mainly been on the n-3 and n-6 PUFAs, such as α-linolenic acid (ALA), linolenic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and on the n-6:n-3 ratio.

2 Background

2.1 Structure, metabolism and biological effects of omega-3 and omega-6 fatty acids

PUFAs have more than two double bonds and when they contain 18 or more carbon atoms they are called LCPUFAs (Beermann *et al.*, 2003; Trautwein, 2001). PUFAs that have 18 carbon atoms are essential to all higher animals and are provided by plants in the food chain (Trautwein, 2001).

Table 1. *List of fatty acids.*

Common name	Chemical name	C:D
Myristic acid	tetradecanoic acid	C14:0
Palmitic acid	hexadecanoic acid	C16:0
Stearic acid	octadecanoic acid	C18:0
Palmitooleic acid	cis-9-hexadecenoic acid	C16:1n-9
Oleic acid	cis-9-octadecenoic acid	C18:1n-9
Eicosenoic acid	cis-11-eicosenoic acid	C20:1n-9
Palmitoleic acid	cis-9-hexadecenoic acid	C16:1n-7
Vaccenic acid	trans-11-octadecenoic acid	C18:1n-7
Linoleic acid	all-cis-9,12-octadecadienoic acid	C18:2n-6
Eicosadienoic acid	all-cis-11,14-eicosadienoic acid	C20:2n-6
Arachidonic acid	all-cis-5,8,11,14-eicosatetraenoic acid	C20:4n-6
α-Linolenic acid	all-cis-9,12,15-octadecatrienoic acid	C18:3n-3
Eicosapentaenoic acid	all-cis-5,8,11,14,17-eicosapentaenoic acid	C20:5n-3
Docosapentaenoic acid	all-cis-7,10,13,16,19-docosapentaenoic acid	C22:5n-3
Docosahexaenoic acid	all-cis-4,7,10,13,16,19-docosahexaenoic acid	C22:6n-3

In the C:D column, C stands for cabons and D for double bonds. The n number gives the carbon from the methyl terminal where the 1st double bond is placed.

Fatty acids contain carbon, oxygen and hydrogen and can be saturated, monounsaturated or polyunsaturated (Table 1) (Omidi et al., 2015). The position of the double bond, the number of double bonds and the total number of carbon atoms give fatty acids their different physiological properties (Trautwein, 2001). PUFAs of n-3 and n-6 types are named based on where the first double bond is placed from the methyl terminal carbon atom of the fatty acid molecule; n-3 fatty acids have the first double bond on the third carbon while n-6 fatty acids have the first double bond on the sixth carbon. Some examples of n-3 are ALA, EPA and DHA and some examples of n-6 are LA and AA.

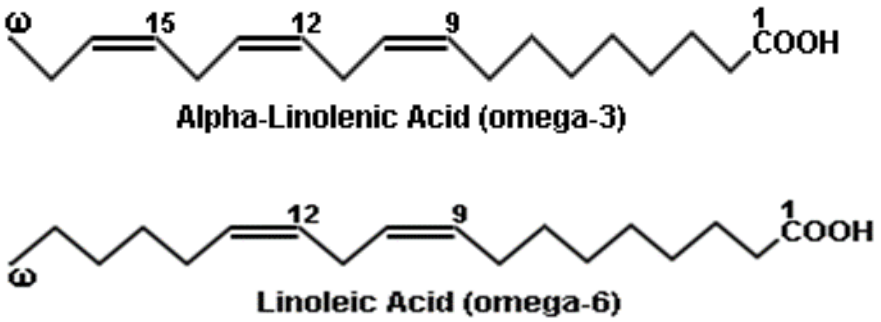

Figure 1. *Chemical structure of the essential fatty acids: alpha-linolenic acid (ALA, 18:3n-3) (above) and linoleic acid (LA, 18:2n-6) (below).*

The land based food chain is rich in α-linolenic acid (ALA) and linoleic acid (LA) (seen in Figure 1) while the LCPUFAs are mostly produced in the aquatic system plants (Lemahieu *et al.*, 2013). ALA is mainly found in triglycerides and cholesterol esters while EPA and DHA are mainly present in phospholipids. In the body, DHA is most abundant in the retina, cerebral cortex, testis and spermatozoa. LCPUFAs are precursor of prostaglandins, tromboxanes and leukotrienes and are very important mediators in normal physiological, inflammatory and immunological processes such as blood clotting and smooth muscle contraction controlling constriction and dilation of blood vessels (Trautwein, 2001). n-3 and n-6 are essential fatty acids (EFA) for humans (Simopoulos, 2002).

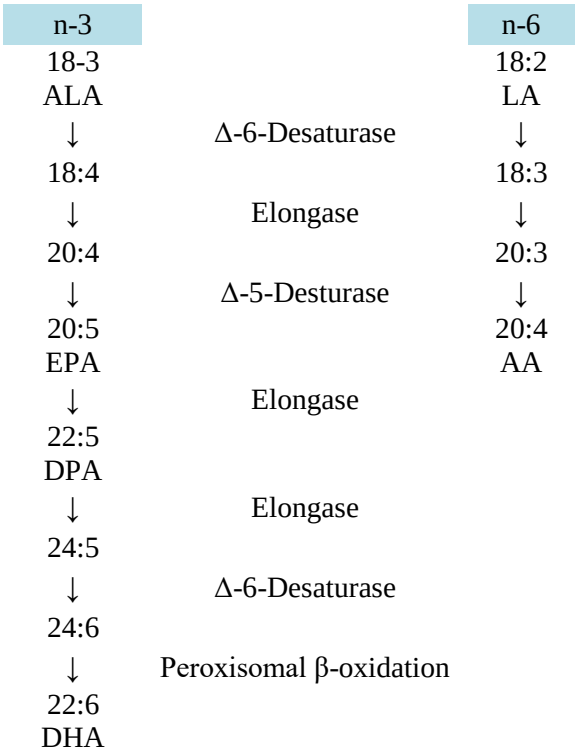

Figure 2. *n-3 and n-6 Fatty acids elongation and desaturation by enzymes. The precursors are α-linolenic acid (C18:3n-3, ALA) and linoleic acid (C18:2n-6, LA). The products are eicosapentaenoic acid (C20:5n-3, EPA), docosapentaenoic acid (C22:5n-3, DPA), docosahexaenoic acid (C22:6n-3, DHA) and arachidonic acid (C20:4n-6, AA).*

Figure 2 illustrates the fatty acid elongation process, i.e. lengthening of the FA chain and desaturation, the process of creating a carbon double bond by removing two hydrogen atoms. LA, in Figure 1, is the major n-6 fatty acid metabolized to AA while ALA is the major n-3 fatty acid metabolized to EPA and DHA (Simopoulos, 2000). Interconvertion of n-3 and n-6 fatty acids is impossible for mammals, because of the lack of Δ-12 and Δ-15 desaturases which are present in lower eukaryotes (Oura & Kajiwara, 2004; Sakuradani *et al.*, 1999), plants (Okuley *et al.*, 1994; Vrinten *et al.*, 2005) and other animals (Ce *et al.*, 1990; Goldberg *et al.*, 2013; Lee *et al.*, 2016).

n-3 and n-6 PUFAs share the same metabolic pathway and hence compete for the same elongase and desaturase enzymes (Trautwein, 2001). The fatty acid desaturase (FADS) genes codes for enzymes critical for the synthesis and regulation of PUFAs. Humans have three FADS including FADS1, FADS2 and FADS3 (Nakamura & Nara, 2004). FADS1 and FADS2 are generally known as Δ-5 desaturase and Δ-6 desaturase respectively. The function of FADS3 in omega FA synthesis and regulation has not been reported (Lee *et al.*, 2016). As can be seen in

Figure 2, Δ-5 desaturase and Δ-6 Desaturase are the critical enzymes which ALA and LA compete with each other for (Neuringer *et al.*, 1984; Neuringer *et al.*, 1986). Δ-6 desaturase has appeared to have the greatest affinity for the greatest number of double bonds in the C18 substrate, i.e. ALA which is later metabolized to DHA (Mennicken *et al.*, 2005; Sardesai, 1992). The LA:ALA ratio also has a role in the conversions of ALA into LCPUFAs. A reduced ratio will encourage more LCPUFA synthesis (Goldberg *et al.*, 2013). The diverse roles FADS on health has been evaluated using genetic and genomics approaches. Activation of fatty acid desaturation leads to pro-inflammatory conditions such as coronary artery diseases in populations that eat excessive meat and not many vegetables (Martinelli *et al.*, 2008; Lee *et al.*, 2016). FADS have also been associated with higher IQ in children (Caspi *et al.*, 2007).

It is known that traditional Inuits don't get diseases such as coronary heart disease that have been traditionally associated with saturated fat and cholesterol, despite their high intake of both saturated fat and cholesterol in their diet. It is believed that this is because their diet also contains high amounts of n-3 LCPUFAs. This has stimulated to more research on n-3 polyunsaturated fatty acids (PUFAs) (Trautwein, 2001). The research has shown that n-3 fatty acids such as α -linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) have health benefits that are well established (Yashodhara *et al.*, 2009) and are needed for normal growth and development (Carrillo *et al.*, 2008), and are important in pre-empt treatments mostly related to cardiovascular diseases, mental health diseases, inflammation, immune functions and cancers (Crespo & Esteve-Garcia, 2001; Fraeye *et al.*, 2012; Gogus & Smith, 2010; Kris-Etherton *et al.*, 2002; Ryan *et al.*, 2013; Simopoulos, 2000; Trautwein, 2001; Yashodhara *et al.*, 2009). Large-scale epidemiological studies have demonstrated evidence that there is a inversibel connection between intake of n-3 PUFAs and coronary heart disease mortality. The protective effect of n-3 PUFAs are especially potent for people who are hypercholesterolemic or have previously had myocardial infarction (Albert *et al.*, 1998; Daviglus *et al.*, 1997; Hu *et al.*, 2002).

Although both DHA and EPA have a protective effect, DHA seems to be more important towards some specific cardiovascular risks but more research is needed to make a conclusion (Cottin *et al.*, 2011). DHA serves as a source for EPA because human tissue can reconvert DHA to EPA through a β-oxidation reaction (Fischer *et al.*, 1987). This happens normally at a rate around 1.4% but may happen at rates up to 12% with high chronic DHA consumption (Arterburn *et al.*, 2006; Brossard *et al.*, 1996; Stark & Holub, 2004; Conquer & Holub, 1996; Conquer & Holub, 1997). Metabolic studies have shown that ALA is converted more efficiently to EPA in non-pregnant women than in men, and it is believed that recommended amounts of ALA will provide sufficient amounts of EPA needed

during pregnancy, lactation and infancy (Jensen *et al.*, 1992). DHA is specially known to have a substantial role in brain-, retinal- and neural tissues in development of fetuses and children. Therefore it is important for pregnant and nursing women to get it in the diet, as it is critical for preterm infants with DHA deficiency (Jordan, 2010; Livsmedelsverket, 2016; Lothaller & Widhalm, 1991). Harris *et al.* (1984) showed that DHA in the diet of the mother increases the DHA content of her milk. The recommended average daily intake of DHA for pregnant and lactating women is at least 200 mg (Kris-Etherton *et al.*, 2009). The recommended daily intake of n-3 PUFAs by several governments and health organizations ranges from 140 to 667 mg/day (Kris-Etherton *et al.*, 2009; Molendi-Coste *et al.*, 2010). Currently the Swedish Food Agency recommends that 5 to 10 percent of the energy we get from food should be PUFAs whereof 1% should be in the form of n-3 LCPUFAs. This would make approximately 2.5 to 3 grams of n-3 PUFAs daily, as much as in a portion of salmon or 1-2 tablespoons of canola oil (Livsmedelsverket, 2016). An intake of 250 mg of n-3 LCPUFAs per day would provide a primary protection against cardiovascular diseases, a limit that are only reached by a few countries of the world today, which are Sweden, Japan, Korea, the Philippines, Finland, Iceland and Norway (Kris-Etherton *et al.*, 2002; Kris-Etherton *et al.*, 2009; Sioen *et al.*, 2009). WHO recommends 300 to 500 mg daily intake of EPA and DHA (Millet *et al.*, 2006).

2.1.1 The n-3 and n-6 ratio

Both n-3 and n-6 PUFAs have many important functions in the human body, e.g. building and repairing cells and regulating blood pressure, functioning kidneys and our immune system (Livsmedelsverket, 2016). The ratio between n-6 and n-3 fatty acids have also become a factor for preventing many chronic diseases (Omidi *et al.*, 2015). High n-6:n-3 fatty acid ratio have shown to be related to many chronic diseases like cardiovascular disease, cancers, inflammatory diseases, autoimmune diseases and many physiological disturbances (Baucells *et al.*, 2000; Kris-Etherton *et al.*, 2002; Livsmedelsverket, 2016; Omidi *et al.*, 2015). n-3 PUFAs lowers the n-6:n-3 fatty acid ratio. During the long evolutionary history of *Homo sapiens*, an n-6:n-3 fatty acid ratio balance has existed, and it is believed that many genetic changes happened partly because of it. The biggest change of n-6:n-3 fatty acid ratio in the food supply of western societies happened during the last hundred years (Simopoulos, 2000), which have shifted the ratio to between 10:1 and 25:1 (Mennicken *et al.* 2005; Simopoulos, 2000), instead of 1:1, which is the case in wild animals and presumably have been the case for human beings during the evolution (Simopoulos, 1991; Simopoulos, 2003; Simopoulos, 2011). Crawford

(1968) showed that animals in the wild have more n-3 fatty acids in their carcass than domesticated animals. n-6 Fatty acids are common in different vegetable oils such as corn oil, sunflower oil, soya oil, rapeseed oil, sesame seed oil and sesame oil, which the population of the western world have increased their intake of during the last century (Livsmedelsverket, 2016). A study by Mennicken *et al.* (2005) with quail showed that breeding for a lower n-6:n-3 PUFA ratio is promising.

2.2 Hen raising systems and fatty acid composition of eggs

Today consumers are becoming more concerned about animal welfare, quality and food safety of conventional foods and have increased the demand for hens raised drug free, and cage-free, and with animal fat-free and hormone-free diet and in the effort to meet the people's demand different alternative production systems have started to become more used and marketed (Cherian *et al.*, 2002; Magkos *et al.*, 2006; Lordelo *et al.*, 2016).

The International Federation of Organic Agriculture Movements' (IFOAM) current definition of organic farming is "Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved" (Paull, 2010). Organically raised hens in Sweden can walk freely in big barns, with maximum of 6 hens per square meter of surface area. A third of the floor has to be covered with litter. They have access to grassed exercise yards where they can scratch, sand bath, peck worms and graze if the weather permits it. At least 95% of their feed has to be organically grown (Sven Secher, 2017). Free range hens that are not organic, can be kept indoors or outdoors. Indoors free range hens can walk freely in big barns and there are maximum of 9 hens per square meter of surface area. Free range outdoor hens have access to four square meters outdoor space, where they can scratch, sand bath, peck worms and graze. Both organically raised and free-range hens can bath and they have access to perches at different heights and nests to lay their eggs in (Sven Secher, 2017).

Hammershøj & Johansen (2016) showed that forage material of grasses and herbs typically affects the egg yolk fatty acid composition to a relatively higher content of PUFAs and especially n-3 fatty acids (Lopez-Bote *et al.*, 1998). Eggs from hens that had access to pasture or forage material could lower the n-6:n-3 fatty acid ratio from between 11 to 19 to around 5 (Hammershøj & Johansen, 2016), because pasture, such as grass, has a relative high proportion of ALA (534

g/kg total fatty acid) (Lopez-Bote *et al.*, 1998). Karsten *et al*. (2010) showed that eggs from pastured hens compared to caged hens had twice as much n-3 LCPUFAs, 2.5 fold more total n-3 fatty acids, and less than half the n-6:n-3 fatty acid ratio. Samman *et al.* (2009) compared fatty acids in eggs from conventional and certified organically raised hens and from enriched n-3 eggs which showed that organic egg yolks contain a higher percentage of palmitic and stearic acids than did conventional yolks and there were no difference in the MUFAs and PUFAs. The n-3 fatty acid enriched eggs contained lower percentage of myristic and palmitic acids and higher n-3 PUFAs. When cage, free-range and barn-laid, eggs were compared, the percentage of stearic acid was much lower for the cage eggs and they also had a very low percentage of AA compared to barn-laid eggs (Samman *et al.*, 2009). Barn-laid hens are similar to free range production systems where hens are the hens are kept indoors (Samiullah *et al.*, 2017). Organic and conventional eggs do not have enough difference in saturated fat that it would have a significant effect on the metabolism of the consumer according to Samman *et al.* (2009). Cherian *et al.* (2002) compared egg components, total fat and fatty acid content in eggs from five different brands. The different eggs in the study were from hens fed a diet free of animal fat, certified organic free-range brown eggs, uncaged unmedicated brown eggs, cage-free vegetarian diet brown eggs, and naturally nested uncaged eggs. Regular white-shelled eggs were used as the control in the study. The total lipid content was lower in the eggs that came from cage-free hens fed vegetarian diet. The content of palmitic, stearic and total saturated fatty acids were lower in eggs from hens fed a diet free of animal fat and no difference were seen in the content of palmitoleic, oleic or total MUFAs. The content of n-3 fatty acids in organic free-range brown eggs, cage-free vegetarian diet eggs and naturally nested uncaged eggs were similar to the control eggs and the content of PUFAs were similar in all analyzed eggs (Cherian *et al.*, 2002).

2.3 Health benefits of eggs

Eggs have traditionally been associated with numerous unfavorable factors for health because of their high content of saturated fatty acids (around 30 g/kg) and cholesterol (around 2 g/kg) (Lamas *et al.*, 2016; Weggemans *et al.*, 2001) but recent research have shown that healthy individuals consuming up to three eggs each day have an overall beneficial effect on biomarkers associated with cardiovascular disease risk (DiMarco *et al.*, 2017). In the study by Oh *et al.* (1991), it was observed that eggs rich in EPA and DHA didn't raise cholesterol and actually lowered blood pressure. Other studies have also affirmed that egg consumption and serum cholesterol are not directly correlated (Chenoweth *et al.*, 1981; Djoussé &

Gaziano, 2008; Ginsberg *et al.*, 1994; Golzar Adabi *et al.*, 2013; Vorster *et al.*, 1992) and that the dietary fat source does of the laying hen diet don't influence cholesterol content in the egg yolk or in the total egg (Millet *et al.*, 2006). SFAs and MUFAs in egg yolk are also hardly affected by the feed in contrast to the PUFAs (Baucells *et al.*, 2000; Dai *et al.*, 2016).

2.4 Enriching eggs with omega-3 fatty acids from various feed sources

As consumers are becoming more aware of the many benefits of n-3 LCPUFAs, a demand for these nutrients elsewhere than marine food have become of interest. Especially poultry eggs and meat have been of interest when it comes to enriching products. It has been reported that consumers are more willing to pay extra for n-3 PUFA enriched eggs (Marshall et al., 1994). Consuming fish several times a day or consuming fish oil capsules are the usual way to obtain n-3 PUFAs (Barclay *et al.*, 1994). Finding new sources of n-3 PUFAs is already considered important for increasing the dietary intake of these beneficial nutrients and to meet the recommended intake. It is most important for people who do not eat seafood for any reason (Palmquist, 2009; Rymer & Givens, 2005). Eggs enriched with DHA can be used in infant formula (Bourre, 2005; Trautwein, 2001).

A large number of studies have concluded that the fatty acid composition of eggs is dependent on the fatty acid composition of the feed of the laying hen, hence enriching of eggs is done by enriching the feed of the laying hens with a source of n-3 PUFAs that will be incorporated into the eggs (Baucells *et al.*, 2000; Dai *et al.*, 2016; Hammershøj & Johansen, 2016). Omidi *et al.* (2015) concluded that the amounts of saturated fatty acids is not affected by dietary manipulation. All poultry diets requires a minimum of 1% added fat to ensure that the hens get adequate levels of LA in the diet and also to increase the absorption of fat soluble vitamins, to enhance egg yield and egg weight, to improve palatability and to reduce dustiness of diets (Omidi *et al.* 2015).

n-3 Fatty acids are mostly found in marine oils but other n-3 PUFAs sources, such as flaxseed and microalgae, can be incorporated into the feed of laying hens (Fraeye *et al.*, 2012; Howe *et al.*, 2002, Palmquist, 2009; Rymer & Givens, 2005). The incorporation of n-3 PUFAs into eggs can result in development of undesirable aroma and flavor attributes in eggs (Goldberg *et al.*, 2013). Hens, humans and other animals are known to have the ability to synthesize SFAs if they need to compensate for a lack of these fatty acids in the diet (Baucells *et al.*, 2000; Millet *et al.*, 2006).

The absorption rate of LA and to a higher extend of ALA increases with the age of the hens and could be due to older hens having larger livers and can thereby more effectively elongate ALA into DHA (Milinsk *et al.*, 2003; Fredriksson *et al.*, 2006). ALA is abundant in the chloroplast and can be added as green leafy vegetables in the hen diet (Livsmedelsverket, 2016; Trautwein, 2001). In a study by Millet *et al.* (2006), where eggs from Aracana and Lohmann hens were compared, it was concluded that the breed as well as the diet influenced the individual fatty acids C18:4n-3 and DPA but no other fatty acids in the yolk. The efficiency of DHA incorporation depends on the concentration of the diet, at low levels efficiencies up to 50% have been seen but efficiency decreases fast at higher concentrations (Fraeye *et al.*, 2012). The EPA is only slightly increased compared to DHA. Herber & Van Elswyk (1996) suggest that it is because DHA is more preferably incorporated into membranes than EPA (Blas *et al.*, 2005; Cheng *et al.*, 2004; González-Esquerra & Leeson, 2001; Herber & Van Elswyk, 1996). Blas *et al.* (2005) compared the enrichment of EPA and DHA in feed of laying hens. Both treatments gave eggs enriched with high amount of DHA compared to EPA. It was shown that the total amount of both EPA and DHA was deposited in the yolk in the form of DHA, which shows that EPA was converted to DHA. The ability of the hen to desaturate EPA to DHA was illustrated by Fredriksson & Pickova (2007) where addition of rapeseed oil in the feed with and without an algae bio mass containing almost 40% of the total fatty acids as EPA. The egg yolk fatty acid composition showed a significant increase in DHA, in spite no DHA was present in the algae species (Fredriksson & Pickova, 2007).

There are also other alternatives to EPA and DHA sources that are currently being explored, such as bacteria, fungi, microalgae and plants; however fungi require an organic carbon source and typically long growth periods, plants need arable land and have no enzymatic activity for producing EPA and DHA (Barclay *et al.*, 1994; Li *et al.*, 2011).

2.4.1 Fish oil

The highest amount of LCPUFAs such as DHA and EPA are mostly found in marine oils, like fish oils and the meat of oil-rich fish such as mackerel, herring, salmon and sardines. These fatty acids originates from n-3 PUFA rich marine plankton that was eaten by the fish (Ackman *et al.*, 1964; Fraeye *et al.*, 2012). Carnivorous fish do not synthesize LCPUFAs, rather they obtain them through their diet by eating zooplankton that have fed on algae (Ackman *et al.*, 1964). Fish oil, as a source of LCPUFAs, when added to the diet of laying hens results in egg yolks with high content of DHA (up to 100 mg per egg) and with an increase to a

lesser extent of EPA (Bovet *et al.*, 2007; Cachaldora *et al.*, 2008; Carrillo *et al.*, 2008; Fraeye *et al.*, 2012; Gonzalez-Esquerra & Leeson, 2000; Elswyk *et al.*, 1995; Lemahieu *et al.*, 2015). The content of EPA, DHA and total n-3 fatty acids in the egg yolk are higher in fish oil treatments compared to the feed treatments in other studies (Blas *et al.*, 2005; Bovet *et al.*, 2007; Carrillo *et al.*, 2008; Farrell, 1998; Gonzalez-Esquerra & Leeson, 2000; Herber & Van Elswyk, 1996; Lawlor *et al.*, 2010; Omidi *et al.*, 2015). Supplementing with fish oil can come with problems, for example fish oil can contain heavy metals and PCBs and undesirable aroma and flavor which can be passed over to the eggs (Fraeye *et al.*, 2012; Goldberg *et al.*, 2013). In a study trained panelists compared scrambled eggs from hens fed on diets containing 5, 15, or 30 g menhaden fish oil/kg rich in both EPA and DHA. The eggs that that came from hens fed 30 g menhaden oil/kg received significantly lower positive flavor scores as compared to eggs that came from hens fed 5 and 15 g menhaden oil/kg. This suggest that feeding hens with 15 g menhaden oil/kg is a way to give eggs with both sensory characteristics and nutrition (Elswyk, 1997).

2.4.2 Flaxseed

Flaxseed contains about 34% oil and have a high content of ALA (>50%) (Trautwein, 2001), which makes it a good ingredient for n-3 fatty acid enrichment in egg yolk but, unfortunately flaxseed lacks LCPUFAs like DHA and EPA (González-Esquerra & Leeson, 2001; Cherian & Quezada, 2016; Kim *et al.*, 2016). Although flaxseed lacks LCPUFAs, it has shown to significantly increase both EPA and DHA in the egg yolk (Ao *et al.*, 2015; Kim *et al.*, 2016). This is because hens have a high ability to convert ALA to LCPUFAs (Howe *et al.*, 2002). When overdosing the amount of flaxseed, the content of ALA raised from 13 mg up to 212 mg per egg, but the DHA content also increased substantially, from 28 mg up to 90 mg per egg (Aymond & Van, 1995; Bean & Leeson, 2003; Fraeye *et al.*, 2012; Lemahieu *et al.*, 2015). Lemahieu *et al.* (2015) showed, comparing different sources on n-3 PUFA enrichment in eggs, a slight increase in docosapentaenoic acid (DPA) compared to the control group (5.5 ± 3.0 mg vs 3.0 ± 0.5 mg per egg) when adding flaxseed to the diet of laying hens. In a study by Kim *et al.* (2016), the inclusion of 3 to 5 percent of flaxseed increased the formation of EPA from undetectable levels in the control to 8.3 to 15 mg/g per egg and elevated content of DHA by 2-3 folds. Grounded and not grounded flaxseeds as hen feed were compared which showed that hens that was fed grounded flaxseed resulted in higher levels of EPA and DHA in the yolk (Elswyk, 1997).

2.4.3 Microalgae

Microalgae are the initial EPA and DHA producers in the marine food chain and can naturally grow fast under a variety of autotrophic, mixotrophic and heterotrophic culture conditions with high n-3 LCPUFA production potential (Barclay *et al.*, 1994; Li *et al.*, 2011). They have received much attention as a promising use as animal feed and biofuels (Lum *et al.*, 2013) and have been added to diets of dairy cattle and pigs because of being a rich source of EPA and DHA (Kim *et al.*, 2016). They also function as antioxidants in feed supplementation, good for protection from oxidative deterioration (Herber & Van Elswyk, 1996). Heterotrophic microalgae use organic compounds as a primary source of nutrition, while autotropic microalgae use CO_2 to produce organic compounds with the aid of sunlight (Bruneel *et al.*, 2013). Not all but several microalgae species are rich in EPA and DHA. Examples of algae that have been used to increase the LCPUFAs in eggs are *Phaeodactylum tricornutum*, *Nannochloropsis oculata*, *Isochrysis galbana* and *Chlorella fusca* (Barclay *et al.*, 1994; Lemahieu *et al.*, 2013). There have been fermentation technologies developed to make heterotrophic microalgae with extremely high DHA content (Barclay *et al.*, 1994). Eggs from hens fed with microalgae rich in DHA typically show similar fatty acid composition as eggs from hens that were fed fish oil with high DHA level (Blas *et al.*, 2005; Cheng *et al.*, 2004; Fraeye *et al.*, 2012; Herber & Van Elswyk, 1996; Rizzi *et al.*, 2009; Lemahieu *et al.*, 2016), although microalgae treatment in most studies show a lesser rise in n-3 LCPUFAs (Lemahieu *et al.*, 2016). Herber-McNeill & Van Elswyk (1998) added high amount of heterotrophic microalgae, around 4.8%, to the feed of laying hens and raised the DHA content of eggs to over 200 mg per egg, while still obtaining eggs with an acceptable taste. The redness of the yolk increases when supplementing with microalgae because of the transfer of carotenoids from the microalgae to the egg yolk (Fraeye *et al.*, 2012; Fredriksson *et al.* 2006; Ginzberg *et al.*, 2000; Gouveia *et al.*, 1996; Nitsan *et al.*, 1999; Pickova *et al.*, 1999).

In a study by Bruneel *et al.* (2013), hens were divided into three groups for 28 days, one with standard diet, one that was supplemented with 5% spray dried *Nannochloropsis gaditana* and one that was supplemented with 10% spray dried *N. gaditana*. In the study the efficiency of increasing n-3 LCPUFA levels were low but offer an alternative to other sources for the production of DHA enriched eggs (Bruneel *et al.*, 2013). Fredriksson *et al.* (2006) compared a diet enriched with rapeseed oil to a diet enriched with rapeseed oil and 20% of the microalgae *Nannochloropsis oculata*. *N. oculata* has a unique fatty acid composition high in EPA and ALA, and made it suitable for studying the effect of desaturation of EPA to DHA in the hens. The study showed that by feeding a diet rich in EPA, an increase of DHA was observed in the egg yolk which indicates that elongation of EPA to

DHA had happened. When comparing the rapeseed oil and corn oil (3:7) with the rapeseed oil as feed containing more ALA than corn oil, it showed that hens supplemented with rapeseed oil in their diet resulted in significantly more DHA in the egg yolk, in other words elongation and desaturation of ALA to DHA seems to have happened. Heterotrophic microalgae have shown to produce higher levels of LCPUFAs than autotrophic *N. gaditana* (Barclay *et al.*, 1994).

The use of microalgae instead of fish oil as a way to enrich n-3 LCPUFAs in eggs also have environmental benefits. The number of overfished stocks have increased since the 1950 and the global fish catches have been in decline since the late 1980s, which have impaired the ocean's capacity to provide food, maintain water quality and recover from perturbations (Worm *et al.*, 2006).

3 Aim of the study

The aim of the study was to analyze the variation of fatty acids in egg yolks from different production systems and to see if organic eggs had higher n-3 PUFA content. A literature review was also made to try to determine different sources and factors that could have influenced the fatty acid composition. Egg yolks that were looked at were organic, free range, farm eggs, enriched and quail.

4 Materials and Methods

4.1 Egg collection and sample preparations

Seven different varieties of eggs of three companies were purchased from supermarkets and local farmer in Uppsala (Uggelsta Organic Medium size (Class A) (S1); Uggelsta Free Range Big (Class A) (S2); Kronägg Organic Free Range with access to outdoor exercise yard (Class A) (S3); Kronägg Free Range indoors (S4); Kronägg Guld gula Free Range indoors (S5); Kronägg Gårdsägg (farm eggs) indoors (S6); Tjärdalen Fågel Common quail (S7). Certified eggs were identified according to the type of logo on the package. The eggs were stored in a cold room at 4 °C. Two eggs of each kind were taken for analysis. From each egg variety, two eggs were analyzed and 1 gram from each egg yolk was sampled.

One of the aims of the work was to get the information about laying hen's feed. The egg producers (three companies) were asked if they could provide information about the laying hen's feed but only one of the three answered. Kronägg answered that it was impossible to know because they buy eggs from 25 egg farmers and some of them use their own cereals that they grow at the farm.

4.2 Analysis

4.2.1 Lipid extraction

Lipid extraction was performed according to Hara and Radin (1978) with slight modification by Pickova *et al.* (1997). The same method has been used in other studies (Sampels & Pickova, 2011; Fredriksson *et al.*, 2006).

From each egg yolk, one gram was homogenized (3 x 30 s) with a lab homogenizer Ultra-Turrax macerator (Heidolph Diax 600) and extracted with 18 ml HIP

(hexane:isopropanol 3:2, v/v). An amount of 5 ml HIP was used to rinse the homogenizer between each sample, were discarded. Thereafter 14 ml Na_2SO_4 (water free) solution (6.67%) was added to remove non-lipids. The samples were shaken vigorously and centrifuged for 5 minutes at 3,000 rpm and 18°C. The hexane phase was then transferred to new tubes. The samples were stored at -20°C in normal atmosphere for further analysis.

4.2.2 Preparation of Fatty Acid Methyl Esters (FAME)

Preparation of FAME was performed in accordance with Appelqvist (1968). NaOH in dry methanol (0.01 M, 2 ml) was added to each sample. All samples were shaken vigorously and placed in a heating block at 60 °C for 10 min. BF3 (14% boron trifluoride–methanol complex, 3 ml) was added as esterification agent and the vials were reheated at 60 °C for 10 min.

All samples were cooled in cold water and 2 ml H_2O and 3 ml hexane were added. The upper phase containing the FAME was transferred to smaller tubes. The tubes were centrifuged for 3 minutes at 3,000 rpm and 18°C and left to stand for 20 min.

The solvents in the samples were evaporated under nitrogen gas. The lipids were dissolved in 200 µl hexane, vortexed and stored under normal atmosphere at -20°C until further gas chromatographic analysis.

The completions of fatty acid methylation were checked by analytical thin layer chromatography (TLC). Pre-coated glass silica gel TLC plates (20 x 20 cm; Silicagel 60; 0,20 mm layer, Merck, Darmstadt, Germany) were used. A standard were applied as a spot two cm from one side on the plates. The plates were put into a chamber with 100 ml hexane:diethyl ether:acetic acid (85:15:1, v/v/v) used as mobile phase. Plates were air-dried after separation and exposed to iodine vapor.

4.2.3 Gas Chromatography (GC)

The FAME was analyzed with gas chromatograph (CP9001, Chrompack, Middelburg, the Netherlands) equipped with a flame injection detector and split injector using a BPX 70 fused-silica column capillary column (SGE, Austin, Texas), length 50 m, id 0.22 mm, and film thickness 0.25 µm. Samples (1 µl) were injected in split mode with a CP8400 autosampler (Varian). The split ratio was 1:10. Column temperature was programmed to start at 158 °C for 5 min, then increase at 2 min from 158 to 220 °C and remain at 220 °C for 8 min. Injector and detector

temperatures were 230 and 250 °C, respectively. The computer software used was Galaxie.

The peaks were identified by comparing their retention times with those of the standard mixture GLC-68A (Nu-Chek Prep, Elysian, USA) and other authentic standards.

4.3 Statistics and calculations

The results were evaluated in two ways: as percent fatty acid of total identified fatty acids and as percent with the use of an external standard GLC-68A (Nu-Chek Prep, Elysian, USA). Standard deviations were calculated from the GC results. One-way ANOVA and Tukey-Kramer method was used to calculate significance at $P < 0.05$ level.

5 Results

5.1 Fatty acid composition in egg yolk from hens raised in different systems

Table 2 shows the percentage of the fatty acids in each of the egg yolk samples (S1-S7). Among the examined eggs, S1 and S2 from Uggelsta, were organic and free range indoors respectively. S3, S4, S5 and S6 were all from Kronägg but raised in different hen systems; S3 was from organically raised laying hens that had access to outdoors exercise yards, S4 was from laying hens that was raised in a free range system and kept indoors, S5 eggs was from laying hens that was raised in a free range system, kept indoors and given a diet enriched with algae and S6 was from laying hens that was raised in a free range system and kept indoors. S7 was from Tjärdalen Fågel and was from common quail laying hens. The total SFAs ranged from 30.8 to 34.7%, the total MUFAs from 48.4 to 56%, the total PUFAs from 10.5 to 19.4%, the total n-6 PUFAs from 9.53 to 15.9.%, the total n-3 PUFAs from 1.01 to 5.06% and the total n-6:n-3 PUFA ratio from 2.60 to 9.39%. There were no significant difference between the SFAs in all the egg yolk samples. S1, S3 and S4 had a slightly higher MUFA content than S2, S5, S6 and S7. S5 had a lower PUFA content compared to the other eggs, which was largely because of the low n-6 FAs. The content of n-3 FAs and the n-6:n-3 ratios did not differ between the samples. Palmitic acid (C16:0), oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) were the fatty acids with highest content in all egg yolk samples, and contributed in total to 80% of all the FAs. Palmitic and oleic acids did not differ between the samples whereas the content of linoleic acid was almost half in S5 compared to other samples. DPA was found in small amount in all eggs except in S1 and S6 but the differences was not statistically significant.

Table 2. *Fatty acids compositions of egg yolk from seven different samples. Given as mean % ± SD of all analyzed fatty acids.*

Fatty acids	S1 n = 2	S2 n = 2	S3 n = 2	S4 n = 2	S5 n = 2	S6 n = 2	S7 n = 2
C14:0	0.36±0.03	0.30±0.11	0.25±0	0.24±0.02	0.40±0.01	0.27±0.15	0.31±0.07
C16:0	25.6±0.05	24.9±2.56	23.7±0.34	22.6±0.09	24.6±1.00	22.3±0.91	23.8±1.40
C18:0	8.73±0.05	8.74±1.32	8.21±0.48	7.96±0.05	8.44±0.72	8.44±1.73	7.57±0.85
C16:1n-9	0.41±0.58	0.86±0.21	0.80±0.02	0.88±0.02	1.11±0.11	0.85±0.08	0.82±0.03
C18:1n-9	44.4±0.09	45.0±1.93	43.6±0.16	45.0±0.78	48.3±0.11	46.4±2.49	45.2±0.23
C20:1n-9	0.13±0.19	0.27±0.05	0.24±0.01	0.31±0.01	0.09±0.12	0.31±0.01	0.13±0.19
C16:1n-7	2.78^{a}±0.01	2.09^{b}±0	1.83^{b}±0.09	1.58^{c}±0.04	3.83^{d}±0.19	1.32^{c}±0.05	1.91^{b}±0.07
C18:1n-7	0.93±1.32	2.21±0.16	1.91±0.03	2.07±0.05	2.74±0.32	2.09±0.08	2.08±0.06
C18:2n-6	$12.5^{a,b}$±1.13	11.8^{a}±1.3	15.3^{b}±0.49	15.4^{b}±0.37	7.62^{c}±0.61	$14.2^{a,b}$±0.83	11.9^{a}±0.81
C20:2n-6	0.00±0.00	0.09±0.01	0.06±0.09	0.14±0.02	0.02±0.03	0.06±0.09	0.05±0.08
C20:4n-6	1.27±0.30	1.43±0.01	1.48±0.00	1.50±0.03	1.89±0.31	1.28±0.00	1.24±0.26
C18:3n-3	0.62±0.07	1.09±0.29	1.15±0.10	1.12±0.03	0.22±0.03	1.20±0.60	3.90±4.19
C20:5n-3	0.07±0.10	0.01±0.02	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C22:5n-3	0.00±0.00	0.08±0.01	0.04±0.06	0.09±0.01	0.03±0.05	0.00±0.00	0.06±0.09
C22:6n-3	2.15^{a}±0.00	1.16^{b}±0.03	1.42±0.11	1.14±0.04	0.76±0.03	1.33^{b}±0.15	1.10^{b}±0.26
SFA	34.7±0.13	33.9±4.15	32.2±0.82	30.8±0.16	33.4±1.73	31.0±2.07	31.6±2.32
MUFA	48.7^{a}±2.19	50.4^{b}±2.35	48.4^{a}±0.31	49.8^{a}±0.9	56.0^{b}±0.85	51.0^{b}±2.71	50.1^{b}±0.58
PUFA	16.6^{a}±1.33	$15.6^{a,b}$±1.67	19.4^{a}±0.85	19.4^{a}±0.50	10.5^{b}±1.06	18.0^{a}±1.67	18.2^{a}±5.69
n-6 FA	13.8^{a}±1.16	$13.3^{a,b}$±1.32	16.8^{a}±0.58	17.0^{a}±0.42	9.53^{b}±0.95	15.5^{a}±0.92	$13.2^{a,b}$±1.15
n-3 FA	2.84±0.17	2.35±0.35	2.61±0.27	2.35±0.08	1.01±0.11	2.53±0.75	5.06±4.54
n-6:n-3	4.85±0.16	5.67±0.21	6.48±0.30	7.23±0.04	9.39±0.53	6.47±1.62	3.78±2.19

Total SFA (14:0 + 16:0 + 18:0 + 20.0); Total MUFA (16:1n-9 + 18:1n-9 + 20:1n-9 + C16:1n-7 + C18:1n-7); Total n-6 PUFA (18:2n-6 + 20:2n-6 + 20:4n-6 + 22:4n-6 + 22:5n-6); Total n-3 PUFA (18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3).

^{a,b,c,d} Mean values in the same row with the different letter are significantly different ($P < 0.05$).

Abbreviations=

S1 = Uggelsta Organic (Medium size, Class A)

S2 = Uggelsta Free range indoors (Big size, Class A)

S3 = Kronägg Organic Free range (Class A)

S4 = Kronägg Free range indoors

S5 = Kronägg Guld gula Free range indoors

S6 = Kronägg Gårdsägg (farm eggs) indoors

S7 = Tjärdalen Fågel Common quail

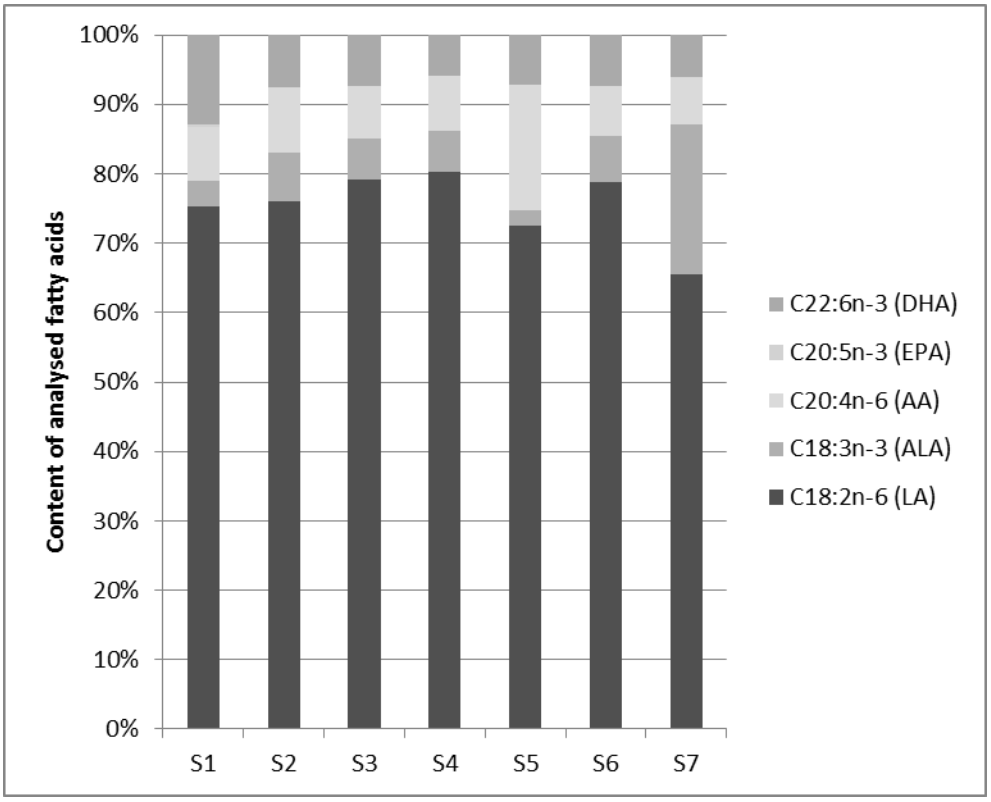

Figure 3. *Content (in percent of the five fatty acids) of C18:2n-6, C18:3n-3, C20:4n-6, C20:5n-3 and C22:6n-3 in seven egg yolk samples.*

Figure 3 shows the results of a few fatty acids, DHA, AA, ALA and LA because they were the FAs most influenced by the diet and EPA to show that it is not stored in the egg yolk. S1, Uggelsta organic egg yolk had highest level of C22:6n-3 (DHA) compared to all the eggs and were 185% of S2, 152% of S3, 188% of S4, 283% of S5, 162% of S6 and 195% of S7. EPA was only found in small amounts in egg yolk samples, because usually EPA is converted to DHA. S5, from Kronägg Guld gula egg yolks from free range laying hens kept indoors had lower C18:2n-6 (LA) compared to the other egg yolk samples and were 61% of S1, 65% of S2, 50% of S3, 50% of S4, 54% of S6 and 64% of S7.

5.2 Fatty acid composition in egg yolk from three different studies

Table 3 and Figure 4, 5 and 6 show results from 3 studies that looked at different kinds of laying hen treatments and the fatty acid composition of the egg yolk.

Table 3. *FA composition of eggs from different studies. Given as mean %.*

	Study 1		Study 2						Study 3	
	Con	FL	Con	FO	OO	GO	CO	SO	Con	A
C14:0			0.45	0.51	0.58	0.45	0.39	0.43	0.26	0.25
C16:0	25.4	24.9	29.3	29.5	29.3	30.2	27.1	26.7	21.65	21.71
C18:0	7.71	7.63	7.86	6.95	7.63	8.16	7.58	8.95	11.75	11.42
C16:1n-9	5.85	5.48	4.47	4.19	3.42	3.13	3.16	2.9	1.28	1.32
C18:1n-9	47.9	46.5	45.7	45.9	47	37.1	47	41.3	38.41	38.61
C20:1n-9									0.26	0.25
C16:1n-7									0.92	0.81
C18:1n-7									1.89	2.03
C20:1n-7	0.31	0.28								
C18:2n-6	9.27	10.4	10.2	8.76	9.79	18.7	12.3	17.3	14.40	14.10
C20:2n-6									0.15	0.15
C20:4n-6	1.69	1.15	1.74	0.59	1.52	1.97	1.74	1.83	3.98	3.35
C18:3n-3	0.42	1.49	0.17	0.28	0.37	0.1	0.81	0.55	1.33	1.47
C20:5n-3			0	0.18	0	0	0	0	0.10	0.30
C22:5n-3	0.06	0.19							0.15	0.30
C22:6n-3	0.66	1.35	0.19	3.21	0.37	0.2	0	0	3.47	3.91
SFA	33.6	33	37.6	36.9	37.5	38.8	35	36.1	33.66	33.38
MUFA	54.1	52.2	50.1	50	50.4	40.2	50.2	44.2	42.75	43.02
PUFA	12.1	14.6	12.3	13	12.1	21	14.8	19.7	23.60	23.59
n-6 FA	11.1	11.7	12	9.35	11.3	20.7	14.03	19.2	18.54	17.61
n-3 FA	1.19	3.09	0.36	3.66	0.74	0.3	0.81	0.55	5.06	5.99
n-6:n-3	5.99	2.8	33.5	2.55	15.3	68.6	17.5	34.9	3.67	2.94

Study 1 by Cherian & Quezada (2016), study 2 by Omidi *et al.* (2015) and study 3 by Fredriksson *et al.* (2006). The treatments were different controls (Con) for each study, flaxseed oil (FL), fish oil (FO), olive oil (OO), grapeseed oil (GO), canola oil (CO), soybean oil (SO) and algae (A). No value means that in that there was no value for that fatty acid from the study.

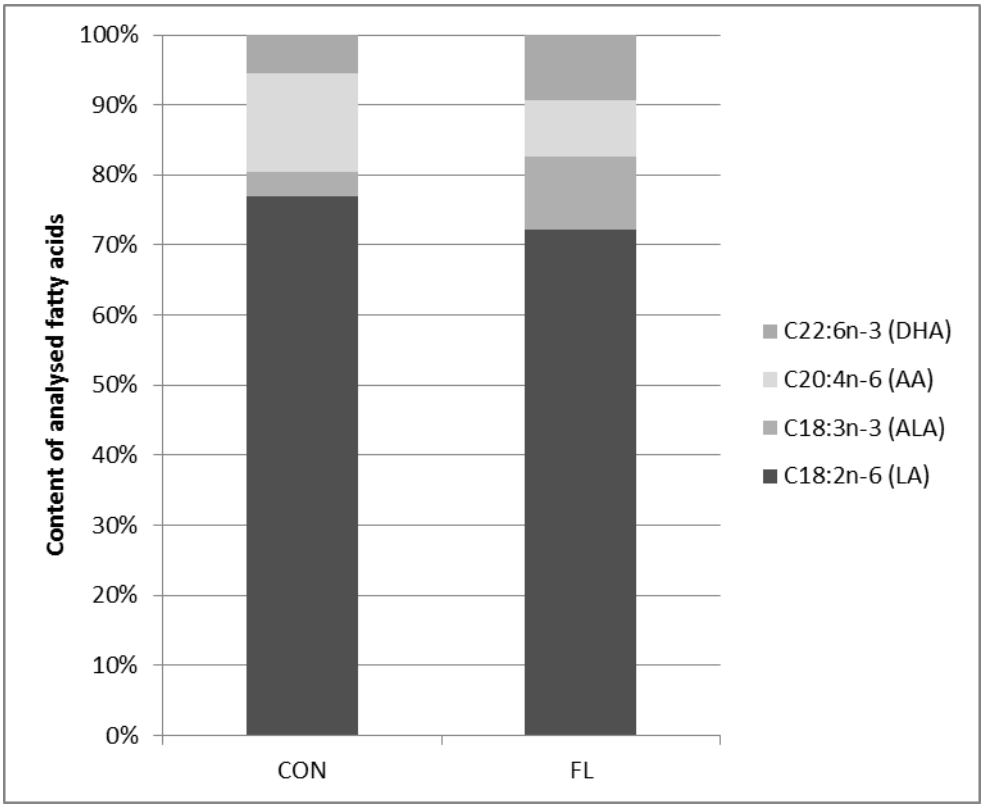

Figure 4. *Study 1 by Cherian & Quezada (2016) shows treatments with (FL) and without flaxseed (CON).*

Cherian & Quezada (2016) (study 1) compared eggs from laying hens with and without flaxseed and camelina added to the diet. DHA, AA, ALA and LA in Figure 4 were the FAs most influenced by the diet. In Table 3 and Figure 4 only the flaxseed and the control samples are shown. Both diets contained a corn-soybean meal basal diet. The experiment contained five laying hens per treatment and was ongoing for a period of 16 weeks. The egg production was higher in the eggs of hens fed flaxseed than in the eggs of the control. In the eggs of the flaxseed treatment, there were a significant increase in ALA (18:3n-3), DPA (22:5n-3) and DHA (22:6n-3) (Table 3 & Figure 4). The total n-3 fatty acids constituted 1.19% in control eggs compared to 3.09% in flaxseed eggs (Cherian & Quezada, 2016).

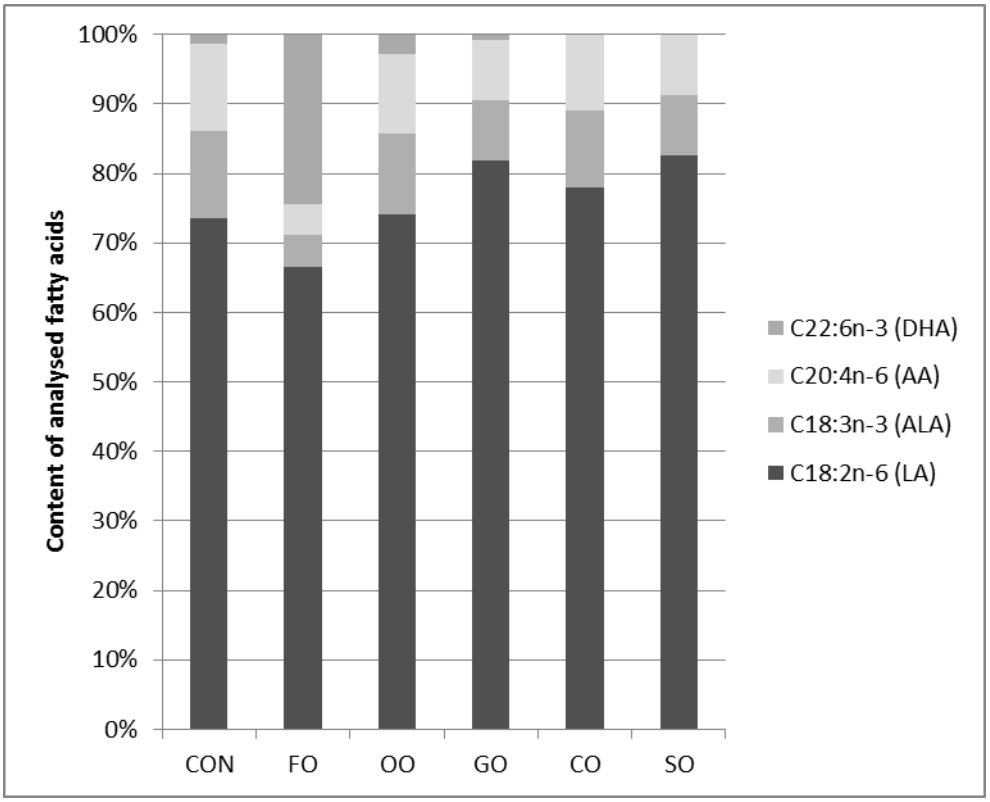

Figure 5. *Study by Omidi et al. (2015). In treatments that were compared were control (no oil)) (CON), 3% fish oil (FO), 3% olive oil (OO), 3% grapeseed oil (GO), 3% canola oil (CO) and 3% soybean oil (SO).*

Omidi *et al.* (2015) (study 2) evaluated the effects of different dietary oil sources supplementation on laying hen's performance and fatty acid profile of egg yolks, the fatty acid profile is shown in Table 3 and Figure 5. It is one of few studies that directly compare the effectiveness of different n-3 PUFA sources for enrichment of egg yolk with n-3 LC-PUFAs. Twenty three week old laying hens were divided into six experimental diets in a completely randomized design for nine weeks. The hens in the experiment where divided into sex groups according to their diet, consisting of control (no oil), 3% fish oil, 3% olive oil, 3% grapeseed oil, 3% canola oil or 3% soybean oil. FA profiles in the eggs were affected by the experimental diets. DHA, AA, ALA and LA shown in Figure 5 were most influenced by the diet. Fish oil significantly reduced n-6 and AA and increased DHA in the egg yolk, seen in Table 3. The n-3 increased and therefore also the n-6:n-3 ratio was reduced (Omidi *et al.*, 2015). High content of LA in egg yolks could be seen in both the grapeseed oil and the soybean oil treatments. Grapeseed oil treated eggs had the highest n-6:n-3 ratio because of the high LA and the absence of LCPUFAs in grapeseed oil.

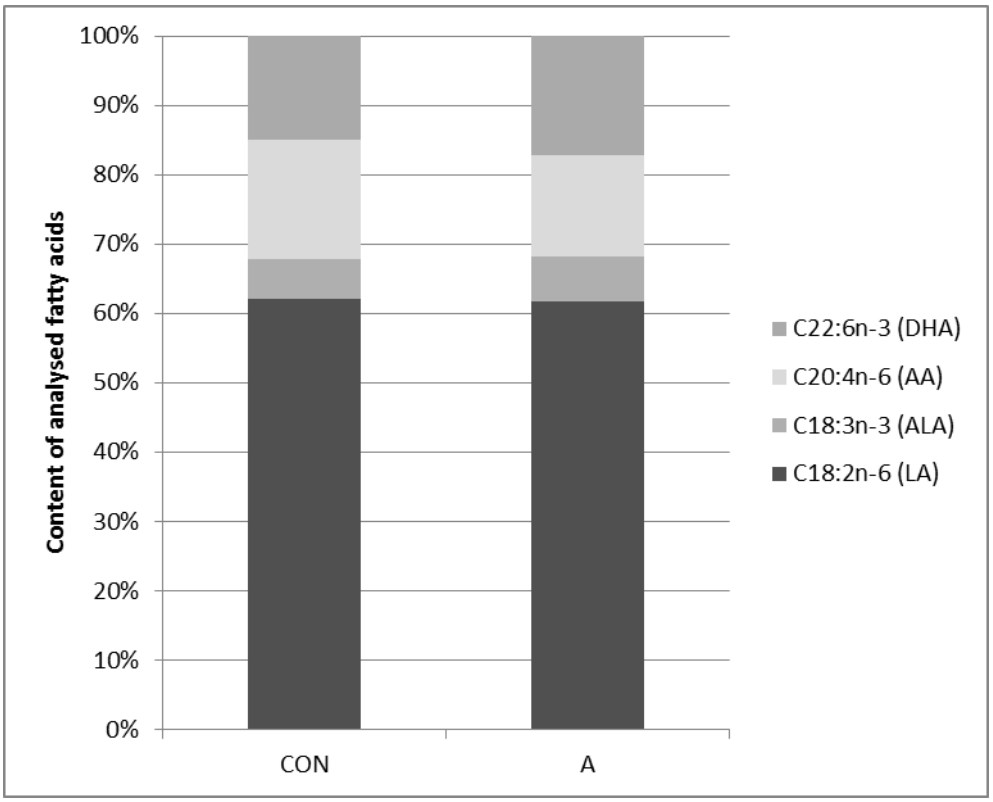

Figure 6. *Study 3 by Fredriksson et al. (2006). Treatment with 20% microalgae (A) added to the hen's diet and a control without added microalgae (CON).*

In Fredriksson *et al.* (2006) (study 3) hens were fed diets based on a mix of rapeseed oil and corn oil with and without the addition of the marine microalgae, *Nannochlorpsis oculata*. DHA, AA, ALA and LA shown in Figure 6 were the most influenced FAs by the diet. A higher amount of EPA and DHA was obtained in the egg yolks from laying hens that were given a diet containing 20% *N. oculata* algae, which can be seen in Table 3. The color a* value and carotenoid content of the egg yolks also increased when *N. oculata* was added to the diet (Fredriksson *et al.*, 2006).

6 Discussion

Hen eggs are used in all parts of the world and are very nutritional food that provides essential nutrients (Dai *et al.*, 2016; Fraeye *et al.*, 2012; Fredriksson *et al.*, 2006). The nutrients are there to the give the embryo all it needs to grow and also provide defense factors (Hansen et al., 1998; Nimalaratne & Wu, 2015). The SFAs and cholesterol in egg have given it a bad reputation of being a food that increases the risk of developing many diseases. n-3 Fatty acids, especially EPA and DHA are known to prevent or treat many diseases that are believed to be caused by cholesterol and saturated fat and are recommended by food agencies around the world (DiMarco *et al.*, 2017; Lamas *et al.*, 2016; Weggemans *et al.*, 2001; Trautwein, 2001). These LCPUFAs are known to have important roles in the brain, retinal and neural tissues and especially important for pregnant and nursing women (Carrillo *et al.*, 2008; Kris-Etherton *et al.*, 2002; Simopoulos, 2000). The n-6:n-3 ratio was 1:1 when humans developed and is believed to be an important factor for the genetic changes that happened during the human evolution but in the last hundred years there have been a big change in the western diet changing to more n-6 rich foods such as cereals (Simopoulos, 1991; Simopoulos, 2003; Simopoulos, 2011). Fraeye *et al.* (2012), take the view that the total EPA and DHA content in eggs are generally below 100 mg per egg which could make more direct supplementation more interesting and by enriching the eggs with EPA and DHA, LCPUFAs could be increased to 200 mg per egg (Herber-McNeill & Van Elswyk, 1998). Research has shown that age, breed and nutrition of laying hens influence the fatty acid composition in the egg yolk, but the dietary fatty acids are acknowledged to have the biggest influence on fatty acid composition. The most influenced fatty acids by diet are DHA and other PUFAs, while total SFA content is kept almost constant. Though the content of individual SFAs such as C14:0, C16:0 and C18:0 may vary. These conclusions have been made in many reports where hens have been fed different kinds of diets and as a consequence the differences in SFA content of hens raised in different systems are not believed to have any negative health ef-

fects on the consumer (Baucells *et al.*, 2000; Dai *et al.*, 2016). The small changes in SFA content can be due to factors such as age and breed differences of the laying hens.

Palmitic acid (C16:0), oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) were the most abundant fatty acids found in the egg yolks (S1-S7), which have also been shown in other studies (Cherian & Quezada, 2016, Fredriksson *et al.*, 2006, Lordelo *et al.*, 2016, Omidi *et al.*, 2015). The total SFA content in egg yolks of this study ranged from 30.8 to 34% and in the studies that were used for comparison SFA ranged from 33 to 38.8% (Table 3), which shows that SFA is kept in a very small range. It is obvious from all studies, including the present study, that EPA is not stored in the yolk, because all samples had none or very low values (Table 2-3). The reason for this lack of one of the important LCPUFAs is not investigated here but it is noteworthy.

S1 and S2 samples in Table 2 were from the same company, Uggelsta. S1 samples were from organic hens and S2 samples from free range hens kept indoors. Both S1 and S2 samples had similar total content of SFA, MUFA and PUFA but the S1 samples had higher DHA content than the S2 sample. The S1 samples had the highest levels of DHA of all the analyzed egg yolks in this study. It is possible that the diet of the S1 hens contained some food source high in n-3 PUFAs, for example microalgae, fish oil or oily fish like salmon, trout or sardines or a source of ALA which have been converted into DHA. Fish oil treatments in other studies have shown the highest increase of EPA, DHA and total n-3 content in eggs (Bovet et al., 2007; Blas *et al.*, 2005; Carrillo *et al.*, 2008; Farrell, 1998; Gonzalez-Esquerra & Leeson, 2000; Herber & Van Elswyk, 1996; Lawlor *et al.*, 2010; Omidi *et al.*, 2015). Flaxseed treatment by Cherian & Quezada (2016) increased DHA in egg yolk but also gave an increase in ALA content, which was low in S1 eggs. It is important to note that fortification with n-3 supplements is expensive and therefore it is presumably not done often.

The organic and conventional eggs didn't show any difference in MUFA and PUFA content in the study by Samman *et al.* (2009) but it is not clear if the laying hens of the conventional eggs were from indoor or outdoor laying hens. In the present study, it was only the hens with organic treatment that had access to go out on grassed exercise yards. In the study by Hammershøj and Johansen (2016) where eggs from organic egg production was studied and Karsten *et al.* (2010) where the composition of caged and pastured hen eggs was studied, it was shown that egg yolks of hens that had access to a yard had a significantly higher amount of n-3 PUFAs than eggs from hens that were kept indoors. When the hens are outdoors they have access to pasture and forage material of grasses and herbs (Hammershøj & Johansen, 2016). Lopez-Bote *et al.* (1998) showed that grass have relative high proportion of ALA. It is also important to note that S1 eggs was of medi-

um size and S2 eggs of big size, which also could have an impact on fatty acid composition, but that was not investigated in this experiment. Both S1 and S2 samples had low n-6:n-3 ratio compared to S3-S6.

Samples S3, S4, S5 and S6, in Table 2, were all from Kronägg but the laying hens were raised in different hen systems. S3 was from organic hens, S4 from free-range hens raised indoors, S5 from free-range hens that were raised indoors and given a diet enriched with algae and S6 from farm hens that were raised indoors. The organic S3 eggs from hens with access to go outdoors showed little higher DHA content than the other Kronägg eggs but this was not statistically significant ($P < 0.05$). In the study by Samman *et al.* (2009) there was not any difference of PUFAs in egg yolk from organic and conventional hens and in the study by Hammershøj & Johansen (2016) eggs from hens that had the ability to go outside had a higher content of PUFAs, especially n-3 fatty acids. Hence it is more likely that the difference was due to that the S3 eggs came from hens that had access to go outdoors rather than because they were organic. The fatty acid profile of S3 and S4 were very similar and the small changes could be due to other factors such as breed and age of the hen. In comparison, S5 had a very different fatty acid profile from S3 and S4 eggs with low LA, ALA and DHA and high AA content. Some red algae species have high AA, for example *Porphyridium cruentum* has as high as 36% AA of the total lipid content and could possibly have been used as feed for S6 (Ahern *et al.*, 1983). The LA could also have been transformed into AA. When the egg yolks were compared, the S5 had a darker red color than S3 and S4, because of the transfer of carotenoids from the algae to the egg yolk. The low PUFA in S6 could have been because the laying hen diet contained a more vegetarian diet. In the study by Cherian *et al.*, (2002) it was pointed out that eggs from vegetarian laying hens had lower PUFA content. Normally algae treatment increases n-3 PUFAs, for example in the study by Fredriksson *et al.* (2006), but in this study those fatty acids were lower in algae treated eggs compared to the other egg treatments. Using microalgae also have environmental benefits, because it is an alternative to fish oil that would lower overfishing, increase the ocean's capacity to provide food, maintain water quality and recover from perturbations. The high levels of DHA and ALA gave higher n-3 and lower n-6:n-3 ratio. Total n-6, DHA, LA and AA was already high in the basal diet.

Egg yolk samples of Tjärdalen Fågel didn't show any differences compared to the other egg yolks, except that it had the lowest n-6:n-3 ratio and highest ALA content but the duplicated differed a lot and therefore it is not significant. A comparison between hen eggs and quail eggs can be complicated to do because of the differences between species.

In this study, all the eggs that were bought came from laying hens that were kept indoors, except for the organic laying hens which had access to grassed exer-

cise yards where they can scratch, sand bath, peck worms and graze (Sven Secher, 2017).

6.1 Conclusion

The study has shown that sample S1 from Uggelsta organic egg yolk samples had statistically higher DHA content and S3 Kronägg organic egg yolk samples had on average but not statistically ($P < 0.05$) higher DHA content in their egg yolk samples compared to the other Kronägg egg yolk samples. In the organic hen system the laying hens have access to grassed exercise yards which have shown to have a positive impact on n-3 PUFA content in the egg yolk. Sample S5 from Uggelsta Guld Gula egg yolk samples from hens with algae enriched diet had high C16:1n-7, low LA, low DHA and low overall n-6 PUFA content and could have been because a red algae with high AA was used as enrichment.

Observations were that palmitic acid, steric acid, oleic acid and linoleic acid were the fatty acids with highest content in the egg yolks, SFA content was similar for all egg varieties, and EPA was found in some of the egg yolks in small amounts. It is also important to note that eggs were of different size, which also could have an impact on fatty acid composition, but that was not investigated in this experiment.

The companies were asked what the laying hens had got as feed but only one answered that the feed varies between hen ages and it was not possible to know the exact feed because the eggs come from many different farms and the feed varies from one farmer to the next, including some farmers using their own cereals from the farm. More conclusions could have been made if we had received information about the feed of the laying hens from the companies. Further, researchers should buy eggs from the farmer instead of the super market because then it would be easier to get information about the laying hens' feed. It would also have been better to analyze more than two eggs from each kind of egg type. A bigger sample size and replicates would have given a better picture on the variability of the fatty acids in the eggs.

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