Effects of agricultural systems on egg nutritional quality

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ABSTRACT

Eggs are a rich source of nutrients beneficial to human health. Market analysis shows increasing European egg production and consumption. Organic egg production differs from conventional systems (free-range and indoor) in the exclusive use of organically grown feed. Organic and conventional free-range systems share the free access to open-air runs but organic flocks have greater management restrictions, which could potentially differentiate egg composition. In order to investigate the effect of management on egg nutritional quality, three major egg production systems were explored; caged, conventional free-range and organic. The hypothesis developed is based on benefits of increasing pasture and free-range behaviour. To fully investigate the effect, these systems were considered within a framework of a meta-analysis (Chapter Two), a farm survey (Chapter Three) and a retail survey (Chapter Four). In all chapters it was found that management does affect the overall egg nutritional quality, with organic having higher omega-3 (n-3) fatty acids than free-range systems ($P<0.05$ (Chapter Two), $P<0.01$ (Chapter Three) and $P<0.001$ (Chapter Four)) and higher carotenoids than all conventional systems ($P<0.001$ in Chapter Four). At the same time Chapter Four detected seasonal effect for vitamin D_3 ($P<0.001$) and B_2 ($P<0.01$) and interactions with management (B_9, D_3). In Chapter Five, the effect of a sustainable and alternative protein source on egg fatty acid profile was investigated to account for the impact of replacing soybean with insect meal on egg fatty acid within an experimental trial framework. The partial replacement of soybean with insect meal had both positive and negative effects on egg fatty acid composition, flagging promising the replacement of soybean in terms of overall egg nutritional quality. Implications of choosing organic over conventional eggs to human health are focused on higher intakes of long chain n-3 fatty acids, carotenoids and potentially of vitamin A and E. Standardizing egg concentration in vitamin D_3, B_2 and B_9 between summer and winter is also suggested.

DECLARATION

I, Eleni Chatzidimitriou hereby declare that this is my own work and all research carried out within it is my own work unless otherwise stated. The views expressed are of my own.

PUBLICATIONS

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LIST OF ABBREVIATIONS

Abbreviation	Explanation
AA	arachidonic acid
AI	adequate intake
ALA	α-linolenic acid
BS	basket studies
CA	caged
CHD	coronary heart disease
CVD	cardiovascular disease
D6/3	the dietary omega 6 to omega 3 ratio
DHA	docosahexaenoic acid
DMF	dietary monounsaturated fatty acid
Dn3	dietary omega 3 fatty acid
Dn6	dietary omega 6 fatty acid
DPA	omega-3 docosapentanoic acid
DSF	dietary saturated fatty acid
EFSA	European Food Safety Authority
EO	extensive organic
EPA	eicosapentanoic acid
ET	experimental trials
FA	fatty acid
FD	fluorescence detector
FiBL	research institute of organic agriculture, Frick Switzerland
FR	free-range
FS	farm surveys
GC	gas chromatography
HPLC	high pressure liquid chromatography
HU	haugh units
LA	linoleic acid
LIP	lipid content
LOD	limit of detection
LOQ	limit of quantification
lut/zea	sum of lutein and zeaxanthin
MD	mean difference
MUFA	monounsaturated fatty acid
n-3 FA	omega-3 fatty acid
n-3>18C	omega-3 fatty acids with higher than 18 carbons
n-6 FA	omega 6 fatty acid
OA	oleic acid
OR	organic
PRI	population reference intake
PUFA	polyunsaturated fatty acid
RDA	redundancy analysis
SFA	saturated fatty acid
SMD	standardized mean difference
TDI	tolerable daily intake
UK	United Kingdom
WHO	World Health Organization

CHAPTER 1. GENERAL INTRODUCTION

Egg is a rich source of nutrients which are beneficial to human health; such as high quality protein, with an ideal amino acid profile, essential fatty acids, carotenoids and vitamins. Because of the very high content of cholesterol in egg yolk, for many decades it has been perceived that egg consumption may lead to problems that are connected to high blood cholesterol consumption including cardiovascular disease (CVD) (Schaefer, 1997). However, more recent studies have shown that the negative health effects of dietary intake of cholesterol are not relevant for the healthy population (Guo et al., 2017a, Zazpe et al., 2011), yet there is inconsistency through the literature with some studies still suggesting otherwise (Weggemans et al., 2001) or still unable to draw firm conclusions (Berger et al., 2015). Over the last five years, market analysis shows that European egg production and consumption are increasing (FAO, 2017, European Commission, 2018a). In the United Kingdom (UK), according to UK industry data, annual egg consumption has been growing continuously for over a decade reaching 196 eggs per capita in 2017 (UK Industry data, 2018). Compared with other species, poultry farming covers a more diverse classification of production systems. The main egg systems in Europe are enriched cages (from here onwards simplified to "caged", CA), barn, free-range (FR) and organic (OR) systems, accounting for 50%, 29%, 16% and 5% of the total egg production, respectively (European Commission, 2018a). In the UK retail market the relative share is slightly different with the FR systems comprising more than 50% of the total, while barn and OR only 2% each (European Commission, 2018a). The market share of alternative to CA production systems is increasing yearly due to the consumer perception that the management system affects the hen welfare and the overall nutritional quality of the egg (Lee and Yun, 2015, Pettersson et al., 2016).

1.1 EGG NUTRITIONAL COMPOSITION AND ITS CONTRIBUTION TO HUMAN HEALTH

Dietary factors are estimated to be associated with a substantial proportion of deaths from heart disease and other type of diseases (Micha et al., 2017). Egg is a food that is widely consumed throughout the history of humankind. Its richness in nutrients brings it on top of the list of the food commodities that can boost our nutrient intake. It is mainly consisting of protein and fat, being 13% and 9% respectively of a whole egg. Eggs are rich in high quality protein (Andersen, 2015). It is noteworthy that the protein is not only found in the albumen but also in the yolk accounting for 16% of its composition (Public Health England, 2015). Many studies have demonstrated the high quality of egg amino acid composition, yet neither the hens' diet nor genotype has shown to modify it (Lunven et al., 1973). It is not in this study's objective to investigate further egg protein quality and the amino acid profile. On the other hand, egg fat

content, which includes plenty of fat soluble nutrients like carotenoids, vitamin D, A and E and is mainly consisted of fatty acids (FA), is exclusively found in the yolk. At the same time these compounds have been reported to vary according to production parameters including diet, genetics and management practices (Oliveira et al., 2010, Lordelo et al., 2017).

Fatty acids

Fatty acids are the constituents of triacylglycerols, the building blocks of fat. There are three different types of FA, classified as saturated FA (SFA) with no unsaturated chemical bonds between carbons, monounsaturated FA (MUFA) with one unsaturated chemical bond between carbons and polyunsaturated FA (PUFA) with multiple unsaturated chemical bonds between carbons. Among PUFA, there are further structural differences which determine whether the fatty acid is an omega 6 (n-6) or omega 3 (n-3) FA depending on the location of the double bond which is 6 and 3 positions from the carboxylic end of the FA respectively.

The role of FA primarily lies in energy metabolism and their presence as constituents of biological membranes. Fatty acids linoleic FA (18:2n-6, LA) and alpha-linolenic acid (18:3n-3, ALA) are essential to humans, other animals and birds as many organism cannot synthesize them. These FA need to be taken through the diet. PUFA show metabolic regulatory functions through the synthesis of different biologically active compounds as the longer chain FA, either n-6 or n-3 (Burdge and Calder, 2005). Specifically, LA and ALA act as substrates for further elongating and desaturating activities by specific enzymes leading to n-6 and n-3 FA respectively, such as the arachidonic acid (C20:4n-6) and the docosahexaenoic acid (DHA). Figure 1.1 shows the two relative pathways as suggested by Cunnane et al. (1994), Moore et al. (1995) and Schmitz and Ecker (2008).

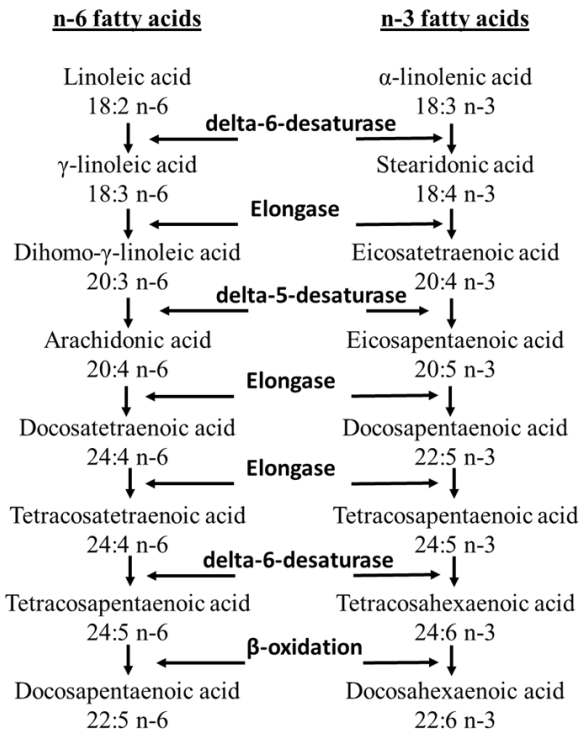

Figure 1. 1. Elongation and desaturation of linoleic acid and α-linolenic acid to longer chain n-6 and n-3 fatty acids adapted from Schmitz and Ecker (2008)

Figure 1.1 also shows competition for common enzymes between n-6 and n-3 FA in their elongation and denaturisation process, with an excess of one causing a significant decrease in the metabolism of the other (Schmitz and Ecker, 2008).

Many studies have already investigated the benefits of n-3 FA consumption to human health, with Cardoso et al. (2016) showing n-3 FA benefiting cognitive and mental health and Lai et al. (2018) reporting long chain n-3 docosapentaenoic (DPA) and eicosapentaenoic acid (EPA) intake from fish to be associated with a higher likelihood of healthy ageing. In general, dietary recommendations to decrease the risk of cardiovascular disease (CVD) have focused on reducing intake of saturated fatty acids for more than 50 years (FAO, 2010, EFSA, 2010b). However, a recent meta-analysis shows unclear evidence in support of cardiovascular guidelines that encourage high consumption of PUFA and low consumption of SFA (Chowdhury et al., 2014). Nevertheless, there is still evidence suggesting that consumption of both LA and n-3 FA, are associated with lowest risk of coronary heart disease (CHD) (Wu et al., 2014, Mozaffarian et al., 2013). On another note, because of the competition for the enzymatic elongation and desaturation of n-6 and n-3 FA, the dietary intake ratio of n-6/n-3 FA is an important indication and should be as low as possible to fully benefit from the n-3 FA potential and exhibit positive health outcomes (Astarita et al., 2014, Gómez Candela et al., 2011, Loef and Walach, 2013, Simopoulos, 2008).

Egg yolk from commercial hens consists of around 30% fat of which 33% is SFA, 42% MUFA and 25% PUFA. PUFA is mainly LA, while n-3 FA accounts for 1-2% of total FA in yolk (Public Health England, 2015). Almost half of the n-3 FA is ALA and the other half is DHA. According to European Food Safety Authority, (EFSA) dietary recommendations for n-3 intake are only relevant for long chain n-3 FA, meaning DHA and EPA and according to the most recent report (EFSA, 2010b), diets including 250 mg of DHA+EPA per day are sufficient to provide any known or assumed health benefits. It is important to mention, that no matter the high fat content of eggs, moderate egg intake has been recently shown to have no overall association with type 2 diabetes, yet with further investigations needed for high egg intake (Drouin-Chartier et al., 2020, Sabaté et al., 2018).

In egg production, it has been shown that hen's dietary FA intake can indeed influence the relative FA content in egg yolk. Extensive research has shown that feed components within different diets can modify the overall egg FA composition (Givens, 2015, Fraeye et al., 2012, Oliveira et al., 2010); a diet rich in linseed will increase the n-3 FA content of the egg yolk, whereas a diet rich in soybean can increase the n-6 FA egg yolk content. Although many studies have investigated the direct relationship of enhancing the yolk's FA content through dietary supplementation, scientific opinions on whether the overall management practices can change the nutritional value of egg vary. Fatty acids, amongst other compounds are naturally found in pasture and specifically in plant based material and insects (other invertebrates included), both known to be rich in n-3 FA (Mugnai et al., 2014, van Huis, 2013). Therefore, management practices that allow access to pasture and hence intake of forage and insects, amongst other parameters, should bear an effect on the final egg FA composition and therefore its contribution to human health.

According to Public Health England (2015) on egg yolk FA composition, an egg (50g) could contribute as much as 13 % of the total daily dietary recommended n-3 FA intake as suggested by EFSA (EFSA, 2010b). When taking into account the actual egg consumption, Givens and Gibbs (2006) suggest that egg intake through '*enriched'* eggs could contribute up to 22% of the daily recommended intake. As previously discussed, the potential for enhancing the yolk total n- FA content through management practices including OR or conventional husbandry systems, genotype selection or seasonal variation, is indeed relevant, as also shown for other animal products including milk and meat (Kamihiro et al., 2015, Srednicka-Tober et al., 2016b). Therefore, FA composition of the eggs is not only relevant for human health, but can be indeed modified and tailored to better meet the nutritional needs of our society. The extent that this can be achieved though management practices as well as the understanding of the natural variation will be discussed below.

Carotenoids

Carotenoids are fat soluble pigments generally with 40 carbons in their chain and are synthesized by plants and microorganisms but not animals (Rao and Rao, 2007). They are grouped into two classes: carotenes (unsaturated hydrocarbons) and xanthophyll carotenoids (oxygenated carotenoids with oxygen atoms). Important members of xanthophyll carotenoids are lutein, zeaxanthin, β-cryptoxanthin, capsanthin and astaxanthin amongst others (Abdel-Aal et al., 2013).

Lutein and zeaxanthin are considered to be the most common in green leafy vegetables (e.g. spinach,) and egg yolks and constitute the majority of egg carotenoids. They are also found at relatively high levels in various varieties wheat and maize (Adom et al., 2003, Andersen, 2015). Lutein and zeaxanthin intake, has been associated with the delay of cataract and age-related macular degeneration (Wang et al., 2007, Karppi et al., 2012). Only those carotenoids are accumulated in the human eye retina from blood plasma (Abdel-Aal et al., 2013).

Lutein and zeaxanthin from egg yolk show higher bioavailability compared to those deriving from fruits, vegetables or supplements because of the high fat content in eggs (Chung et al., 2004, Maiani et al., 2009). Egg consumption has shown to increase human plasma concentrations of lutein and zeaxanthin (Handelman et al., 1999, Kelly et al., 2014).

Levels of carotenoids in egg can be modified by supplementing the diet of hens either with extracts of carotenoids (Grčević et al., 2019) or directly with dietary sources of carotenoids. In this respect, Hammershøj et al. (2010) demonstrated higher carotenoids in eggs from carrot fed hens than control hens fed a compound feed diet. As carotenoids are pigments naturally found in leafy plants, pasture raised hens have also been shown to incorporate higher amounts of carotenoids in their yolks (Mugnai et al., 2014).

As there are no dietary guidelines or recommendations for lutein, zeaxanthin or any carotenoid, the contribution of egg consumption on our total intake can only be considered in relative terms. Overall, egg carotenoids and most importantly lutein and zeaxanthin can indeed be modified and it is worth investigating further as researchers have identified lack of lutein and zeaxanthin primarily as dietary causes in impaired age-related retina functions (Abdel-Aal et al., 2013).

Vitamins A, E and D

As mentioned, egg yolk is rich in fat-soluble vitamins including vitamin A, E and D. Vitamin A refers to a group of retinoids, including retinol, retinal and retinyl esters (Debier and Larondelle, 2005). Some carotenoids, notably β-carotene, also have provitamin-A activity, but these are not found in eggs in significant amounts (Public Health England, 2015). Vitamin E

includes compounds that exhibit the biological activity of a-tocopherol, notably natural stereoisomer RRR-α-tocopherol that accounts for the majority of vitamin E in animal tissues. Other compounds having vitamin E activity are α-, β-, γ-, δ-tocopherols and their respective tocotrienols. Vitamins A and E are essential nutrients with multiple roles, involved in protection against tissue damage, maintenance of the heart, lungs, kidneys and other organs, as well as immune, reproductive, growth and development functions (Debier and Larondelle, 2005, Biesalski and Nohr, 2003).

Vitamin D is a group of calciferols molecules. The main forms found in foods are cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2). The active form 25-hydroxycholecalciferol (25OH-D_3) is naturally found in animal products as it is a metabolite of vitamin D_3 (Borradale and Kimlin, 2009, Ovesen et al., 2003). Vitamin D_3 is strictly speaking, not entirely a vitamin as it can be synthesized in our skin through exposure to ultraviolet light (UVB). However, under conditions of insufficient sun exposure, dietary vitamin D is the most important source of supply. Mammals and birds require vitamin D for embryonic development (Liu et al., 2014). It is typically associated with calcium metabolism and in recent years it has been discovered, to have functions in many body tissues. Vitamin D has also been associated with improved muscle strength, decreased risk of bone fractures and lower incidences of heart failure, type 2 diabetes, colorectal cancer, and coronary artery disease (Holick, 2007, Michos and Melamed, 2008). Dietary intake of all three vitamins (A, E and D) has been associated with reduced risk of CVD and development of some types of cancer (Mamede et al., 2011, Pittas et al., 2010, Weber et al., 1997, Hines et al., 2010). Yet, some studies report this relationship still uncertain (Pittas et al., 2010, Sesso et al., 2005).

According to Public Health England (2015), one egg (50g) will supply 63 µg vitamin A and 0.65 mg of vitamin E. For vitamin D, according to a study by Guo et al. (2017b), one egg yolk from the UK retail market, contains a total of 2 µg of vitamin D, while a 50g egg according to the report from Public Health England (2015), contains around 1.6 µg of vitamin D.

Nutritional reference values for both vitamin A and vitamin E have been recommended for adults (EFSA Panel on Dietetic Products and Allergies, 2015f, EFSA Panel on Dietetic Products and Allergies, 2015e). Thus, concerning vitamin A, one egg per day could potentially contribute to 8% of the population reference intake (PRI – the daily intake of a nutrient that is likely to meet the needs of almost all healthy people in a population) for adults and to a range of 5-25% for all other population groups (infants to lactating women). Concerning vitamin E, one egg per day could potentially contribute 5-6% to the adequate intake (AI, it is the value set when there is not enough data to calculate an average requirement for a nutrient. An AI is the average level of intake of a nutrient, based on observations or experiments that is assumed to be adequate)

for adults recommended by EFSA, whereas 5-13% to the AI for all other population groups. Similarly, for adults a daily intake of 15 μg of vitamin D has been recommended by EFSA Panel on Dietetic Products and Allergies (2016) as an AI for most of the population groups (except infants). Therefore, one egg per day (50g), would contribute about 11% of the AI of vitamin D.

Vitamin A and E are naturally found in cereals like wheat and sunflower, nuts but also some green vegetables (Reboul et al., 2006, Skrivan and Englmaierova, 2014), ingredients which constitute hen diets. At the same time, the total egg content can be increased with manipulation of the dietary intake by hens (Mendonça Jr et al., 2002, Franchini et al., 2002).

The addition of both vitamins to hen diets can increase the egg's content in a dose-dependent manner (Leeson and Caston, 2003). Similarly, dietary vitamin D supplementation in hens can also increase the egg relative content, either in D_3 or the more potent form 25OH-D_3 (Mattila et al., 2004, Mattila et al., 2011, Duffy et al., 2017)

Vitamins B complex and minerals

Riboflavin is also widely known as vitamin B_2 and folate as B_9. Flavins is the name of a group of water-soluble yellow pigments to which B_2, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD) belong. Folate is a generic term that refers to a family of water-soluble B-vitamins that are found in food.

Riboflavin is an important respiratory enzyme constituent and electron donor, essential for the metabolism of carbohydrate, fat and amino-acids (Foy and Mbaya, 1977). It has also been suggested that riboflavin may exert neuroprotective effects in some neurological disorders and that its deficiency have been linked to increased risk for the development of certain types of cancer (Saedisomeolia and Ashoori, 2018). Folate functions as enzyme cofactors by carrying and chemically activating single carbons for biosynthetic reactions. Folate is essential for DNA synthesis, replication and repair and also important for protein biosynthesis (Meshkin and Blum, 2007).

Riboflavin is water-soluble. In the diet, it is naturally present as free riboflavin and as the biologically active derivatives FMN and FAD (Powers, 2003). Dietary sources of vitamin B_2 include liver, fish, milk, eggs, green vegetables, wheat germ and yeast, and is also produced by micro-organisms of the alimentary tract (Foy and Mbaya, 1977). Although grain products contain low natural amounts of riboflavin, fortification practices have increased the content in breads and breakfast cereals (Powers, 2003). Normal chicken eggs contain substantial amounts of riboflavin, all of which is bound to a specific, high-affinity, riboflavin-binding protein (EFSA et al., 2017). Folate occurs naturally in food and folic acid is the synthetic form of this vitamin

that is found in supplements and fortified foods (Meshkin and Blum, 2007). Natural sources of folate include apart from liver and egg, green leafy vegetables and legumes (Scott et al., 2000), which also could comprise part of the hens diet. However, excessive folic acid can mask the symptoms of vitamin B12 deficiency (Cuskelly et al., 2007). Hence a way to avoid excessive folate intake would be ensuring that the diet is adequate in folate so further food fortification of it is avoided. Therefore, novel methods to increase concentrations of folate in foods are increasingly more interesting to researchers.

Nutritional reference values for both B_2 and B_9 have been recommended for adults by (EFSA Panel on Dietetic Products and Allergies, 2014, EFSA, 2017). According to Public Health England (2015) an egg (50g) contains 0.25 mg B_2 and 24 μg B_9 which correspond to 16% and 7% respectively of the PRI of adult population. Regarding the rest of population groups, an egg could contribute a range of 13-42% for B_2 and a range of 5-20% of the PRI for B_9 respectively. Studies on the egg B_2 and B_9 bioavailability and absorption are scarce.

Riboflavin is essential for the health of hens (Squires and Naber, 1993) and since they cannot synthesize it, their intake relies on dietary sources, similarly to folate. Studies have shown that contents of B_2 and B_9 in eggs can indeed be increased by dietary supplementation of hen's diets (Jackson et al., 1946, Brown, 1952, Bunchasak and Kachana, 2009, Hebert et al., 2005, Hoey et al., 2009, House et al., 2002).

Minerals

The egg is also a rich source of minerals important to human health, as previously mentioned. Calcium (Ca), being a vital component of the skeleton, has a structural role while it is needed for skeletal, tissue strength and elasticity. It is stored in bones and also participates in mineral homeostasis. It is interesting to note that an interaction between vitamin D and calcium has been reported with high calcium intake increasing the half-life of 25OHD (Lips, 2012). Iron (Fe), plays a major role in (a) oxygen transport (haemoglobin) and short-term oxygen storage (myoglobin), (b) haem enzymes involved in electron transfer and oxidase activities and (c) energy transduction and oxido-reductase activities. Iron is found in two forms, haem and non-haem iron (EFSA Panel on Dietetic Products and Allergies, 2015b). It is interesting to mention that calcium has been reported to decrease haem and non-haem iron absorption while ascorbic acid (vitamin C) to increase non-haem iron absorption. According to an in-vitro study, simulating a human gastrointestinal digestion, iron in eggs will be more bioavailable if eaten with foods high in ascorbic acid (Thompson et al., 2010). Magnesium (Mg) is a cofactor for more than 300 enzymatic reactions while manganese (Mn) is part of metalloenzymes involved in amino acid, lipid and carbohydrate metabolism (EFSA Panel on Dietetic Products and

Allergies, 2013). Phosphorus (P), is an essential mineral involved in many physiological processes, such as in the cell's energy cycle, cell structure, cell regulation and signalling and in the mineralisation of bones and teeth amongst others (EFSA Panel on Dietetic Products and Allergies, 2015d). Finally, zinc (Zn) has several vital physiological functions. It is most importantly connected with regulating gene expression and intracellular signalling (EFSA Panel on Dietetic Products and Allergies, 2014b).

Nutritional reference values for Ca, Fe, Mg, Mn, P and Zn have been recommended by EFSA (2013, 2014b, 2015a, 2015c, 2015b, 2015d). According to Public Health England (2015) an egg (50g) contains 23mg Ca, 0.86mg Fe, 6.5mg Mg, 0.015mg Mn, 89.5mg P and 0.56mg Zn which correspond to 2%, 8%, 2%, 1%, 16% and 5% respectively of the PRI or AI (for Mg, Mn, P) of adult population. Regarding the rest of population groups, an egg could contribute between 2-56% of the references values for the minerals mentioned above, with the highest contribution percentages being relevant to infants and babies.

All these minerals are also found in cereals, grains and grains products or in dark green vegetables, legumes, nuts. Therefore, they are naturally included in feeding regimes of hens, either in compound standard feed and/or from forage material in FR systems. It is therefore interesting to investigate how management practices can affect the overall mineral content of eggs.

1.2 THE EFFECT OF MANAGEMENT

Three main systems for egg production represent the majority of management practices present in Europe and in the UK; 1) CA or barn systems representing the indoor conventional systems, 2) conventional FR and 3) OR systems. The differences between these systems can be described at two levels a) the use of OR standards covering many aspects of management including feeding, flock sizes, stocking rates and attitudes to maintaining health, comparing OR with conventional systems (either FR or indoor) and b) FR access, comparing FR (either OR or conventional FR) to indoor systems (either CA or barn).

According to Hammershøj et al. (2010) hens with access to pasture can consume up to 120 g of forage material daily and hence FR systems can have an extra source of nutrients through forage material consumption. Since we know that for laying hens, the nutrients we are interested in (e.g. fatty acids, carotenoids, vitamins), are all found in pasture (Dewhurst et al., 2009, Hammershøj and Johansen, 2016, Mugnai et al., 2014, Skrivan and Englmaierova, 2014) and for many, a type of dose-response relationship exists, can we assume that FR eggs will be richer in these compounds than eggs coming from indoor systems?

To put the differences between systems in perspective, OR systems are only allowed to give organically grown feed with no genetically modified or solvent extracted ingredients, no free amino-acids and nothing grown with synthetic fertilizers or pesticides (European Commission, 2008b). With respect to OR feed, the nutritional composition of OR crops have been shown to differ compared with conventionally grown crops (Baranski et al., 2014). Furthermore, the usable area per hen inside the house is larger in OR systems, whereas the respective outdoor useable area when comparing OR to conventional FR is equally at both systems a minimum of $4m^2$ per hen. Nevertheless, the OR standards have restrictions on the maximum number of birds in a flock (3000 hens) whereas FR or CA systems virtually have no maximum flock size under the current legislation. With regards to the indoor stocking density, having fewer birds has been shown to encourage higher outdoor range use (Gilani et al., 2014), which suggests higher forage intake including insects/worms that in turn might enhance the egg nutritional composition. Additionally, stocking rates can influence levels of aggression and injurious behaviour (Kang et al., 2016). Aggressive behaviour such as injurious feather pecking at higher stocking densities, confined housing or even threat from predators found in the FR area can lead to oxidative stress in birds. Oxidative stress is also induced by higher pathogen load in poultry which, again, is linked to higher stocking densities (Gast et al., 2016). As far as OR standards are concerned, they promote animal welfare and husbandry practices such as enriching the outdoor environment to make it safer and more attractive to hens. One can hypothesize that these practices could lead to lower oxidative stress. Finally, when OR are compared to conventional FR systems, the range itself must be rested for at least 9 months between flocks to allow vegetation to grow back, while in FR systems the area only needs to be rested for 2 months (Leenstra et al., 2014). Therefore, it can be assumed that OR hens will have a greater chance to range on a richer pasture than conventional FR hens. It is important to bear in mind that a high content of PUFA makes the feed more susceptible to oxidation and feeds containing oxidised lipids are harmful to the animals, potentially increasing the animals' vitamin E requirements (Lauridsen, 2019).

Many studies report that products of animal origin, such as milk and meat, coming from OR or extensive management systems to be higher in polyunsaturated fatty-acids than those coming from intensive management systems (Srednicka-Tober et al., 2016a, Srednicka-Tober et al., 2016c). Butler et al. (2011) and Stergiadis et al. (2015) reported higher polyunsaturated FA content in milk from OR and high grazing systems compared with milk of conventional systems, while Kamihiro et al. (2015) reported higher polyunsaturated FA for OR meat although this depends on season. Furthermore, retinol and α-tocopherol content in lamb were significantly higher in animals raised on an extensive system than those raised on an intensive

one (Álvarez et al. 2014). On the other hand, Givens et al. (2011) showed that OR poultry meat is not only similar to conventional but sometimes even lower in PUFA and n-3 FA content.

Results of studies are contradictory on whether practices between OR, conventional FR and even indoor systems impact on the egg nutritional profile of fatty acids, carotenoids, vitamins or minerals. Furthermore, results of previous studies evaluating the overall effect of management practices (Filipiak–Florkiewicz et al., 2017, Hidalgo et al., 2008, Lordelo et al., 2017, Poupoulis et al., 2009, Samman et al., 2009), are potentially confounded by factors such as season or genotype, which have not been factored in. These will be discussed in detail in the subsequent Chapters of this thesis.

It is important to mention and consider that industrial development and activity, can increase the residual amounts of contaminants, such as heavy metals (Cd) or Mn and Pb discharged into the air falling on the ground. Consequently, FR hens can potentially have access to such compounds. However, this aspect of egg's profile with potentially negative impact on human health is not the primary objective of this PhD study.

1.3 THE EFFECT OF SEASON

In temperate climates, seasonal variation within animal husbandry is bound to the successive change of seasons and the associated variation in climatic dynamics such as temperature and rainfall, light hours, plant growth and availability and animal behaviour. All these parameters should principally have an impact on outdoor FR egg production, where hens have the freedom to range and feed on pasture plants and invertebrates. In CA and indoor commercial laying husbandry systems, feed regimes are mainly composed of grains and seeds in the form of compound feed. Since grains are grown as annual crops, seasonal variation is less likely to affect these systems and the present thesis will focus on the effect of season within outdoor FR systems.

Climatic conditions, such as drought or snow and ice coverage throughout seasons, can decrease or even eliminate the pasture availability to hens. However, in the temperate zone, pasture is available at least at some point throughout the year and the easier the access to it, hens will benefit accordingly from the nutrients present in the diverse pasture components (e.g. grass, insects). In winter, in the majority of the regions within the temperate zones like UK, pasture plants will be scarcer, although, this too is highly dependent on the region, as the contrary was shown for a study in Italy (Mugnai et al., 2014).

With regards to FA profile of forage plant material found in pasture, LA and ALA content, is shown to be different between plant species (Dewhurst et al., 2009, Gilliland et al., 2003).

Similarly, tocopherol and carotene content may vary (Larsen et al., 2012). Season, can affect not only the overall availability but also the plant species available at a given point, as some types of pasture plants systematically precede others according to seasons. The type of pasture has also been reported to affect the pecking behaviour of hens (Breitsameter et al., 2014). Therefore, sward composition affected by seasonal variation could eventually impact the egg composition. However, Gilliland et al. (2003), found that the overall nutritional composition of plants found in pasture, in FA is affected more by climatic conditions than the variety of plants. The same authors also reported that the plant FA content, within the same species, was influenced by seasonal variation by interlinking the developmental and maturation stage of the plant to different FA contents. Lower FA content were noticed during the reproductive growth of the plant, as they mature and produce seed heads (Gilliland et al., 2003). Similarly, Larsen et al. (2012) reported that the concentrations of tocopherols and carotenes in various grasses and legumes although varied with the plant species, variation was much greater according to the plant's maturation stage. Accordingly, Prache et al. (2003), reported a seasonal effect on the lutein and zeaxanthin content of pasture plants. Contrary to this, no effect of season on pasture plants carotenoid content was reported by other studies (Skrivan and Englmaierova, 2014). Considering the intake of forage material and their respective nutrients and how this could be affected from varying climatic conditions, studies suggest that foraging behaviour in hens, especially beyond the immediate zone around the shed house, where higher pasture density is usually noticed, is inversely related to humid and cold weather conditions (Chielo et al., 2016, Gilani et al., 2014). Gilani et al. (2014) however, also noticed the lowest ranging behaviour at May and the highest in mid-winter for farms located in the UK, which has been suggested to be the result of the habituation to the "new", conditions of ranging outside every year (personal communication).

Furthermore, as far as egg vitamin D is concerned, Kühn et al. (2014) concluded that FR systems could increase the respective egg vitamin D content by exposure to sunlight and UVB radiation, provided that the farming and climatic conditions are attractive for hens to range outdoor. Therefore, the effect of season on egg vitamin D content is directly linked to the effect of seasonal variation affecting the amount of hours of available UVB radiation but also indirectly to the ranging behaviour of hens.

In animal products such as milk and beef, there are several studies showing effect of seasonal variation on the final nutritional composition of the product, reflecting pasture growth and composition (Butler et al., 2011, Stergiadis et al., 2019, Kliem et al., 2013). Kamihiro et al. (2015) showed interactions of management systems and season with higher nutritional content in beef during summer, while strong interactions between season and genotype for milk FA

content have also been reported (Stergiadis et al., 2013, Stergiadis et al., 2018). In eggs, very few studies investigated the effect of seasonal variation and as such it has been shown to indeed have an impact under FR management for FA, carotenoids (Mugnai et al., 2014) and 25(OH)D_3, while interactions with D_3 have also been recently reported (Guo et al., 2017b).

Although seasonal variation has been sporadically investigated, not much research has been carried to elucidate interactions between varying seasons and management systems and therefore this is one of the main objective of this thesis.

1.4 THE EFFECT OF GENOTYPE

Hen genotypes currently used in egg production are hybrids of main lines selected for decades for high egg production (Leenstra et al., 2014). Both in conventional and FR systems these highly productive commercial lines are mainly used; even in OR systems (Hammershøj and Steenfeldt, 2015).

According to Bunea et al. (2017), chemical composition of egg yolks, including carotenoids and FA profiles, varies greatly among strains of hen. Similarly, Hammershøj and Steenfeldt (2015), looking into a fast-growing commercial and a slow-growing dual purpose hen genotype under OR management, found that the slow-growing birds were the ones with higher carotenoids yolk content. However, Pintea et al. (2012) did not find differences when similar to the previous study, different genotypes were compared. When white and brown eggs from commercial Lohman lines were compared, no effect of genotype on FA composition was noticed (Johansson, 2010). On the contrary, when two commercial lines, producing white or brown eggs were compared, FA and vitamin profile of eggs did vary between genotypes (Anderson, 2013). It has been noticed that brown hybrids are more sensitive to environmental challenges than white hybrids (de Haas et al., 2014). Genotype can in parallel influence the use of the outdoor area and hence the forage material intake (Elwinger et al., 2008). Therefore, attention should be made while looking into genetic effects, as there could be interactions between system and genotypes. Research has previously shown interactions between genetics and diet exhibiting significant effects in various animal production systems. Stergiadis et al. (2015) showed that interactions between cows' genetics and grazing intake, in low input dairy systems, could drive changes in milk FA profile. Similar interactions were shown for cows' genetics and seasonal variation between winter and summer (Stergiadis et al., 2018). Concerning egg production, studies investigating interactions between genotypes and management or season have not been systematically investigated. The incorporation of n-3 FA acids into the yolk between laying hens has previously been shown to exhibit a genetic × diet interaction (Scheideler et al., 1998, Rizzi and Chiericato, 2010). Ayerza and Coates (2000)

showed a slight and periodical higher incorporation of n-3 FA in yolk from white compared with brown hens, when they were fed chia seeds (high in n-3 FA). On the contrary, Bean and Leeson (2003) found no interactions between genotype and diet when brown and white hens were fed flax seed, a feed ingredient also high in n-3.

Given the variation in results, it is important to further investigate the effect of genotype, accounting in parallel for interactions between management and season, under commercial conditions that represent the majority of consumers' choices on eggs.

1.5 THE EFFECT OF INSECT MEAL AS AN ALTERNATIVE SOURCE OF PROTEIN ON EGG FATTY COMPOSITION

In monogastric animal nutrition, specifically in poultry, the much-needed balance of essential amino acids often drives the composition of feeding regimes. Therefore, to cover amino acid deficiencies in parallel to a rapid production, a major protein source in these feeding regimes is soybean meal (Khan, 2018) because of their amino acid profile and good digestibility (Ravindran et al., 2014). However, relying on soybeans as such an indispensable feed ingredient has been criticized to lead to deforestation, eutrophication, soil erosion, loss of biodiversity, intensive use of pesticides (due to genetic modification) and a high carbon footprint (van Huis, 2015, Semino et al., 2009). Additionally, since the majority of soybean in Europe is imported, naturally it is becoming a highly costly ingredient (Gale and Arnade, 2015). For these reasons there is an imminent need to turn to alternative, sustainable protein sources (Zander et al., 2016), for which, a candidate high on the list are insects.

Insects have been part of the human diet worldwide throughout history (Jongema, 2015). At the same time, insects are a natural integral part of most birds' diet. The recent interest on the further and systematic potential of the use of insects as food and feed has produced quite a lot of interest in the research community (EFSA, 2015, Khan, 2018, Makkar et al., 2014, van Huis, 2015, van Huis, 2013). Production of insect meal to replace costly and unsustainable ingredients in animal diets is being more and more investigated (Wang and Shelomi, 2017, Zhou et al., 2018, DiGiacomo and Leury, 2019)

Insects can be a rich source of nutrients including protein, essential FA and vitamins, potentially covering a large part of human and animal nutritional requirements (Mitsuhashi, 2010). Their potential as feed ingredient to decrease land use and the relative environmental impact (Salomone et al., 2017), make them a promising feed component in line with the environmentally friendly agenda pursued in Europe. Furthermore, limited research has already investigated the use of industrial food waste as the insect growing media (Meneguz et al., 2018, St-Hilaire et al., 2007a) hence recycling nutrients. Another advantage to using insects as feed

ingredient is their nutritional composition is highly depend on the substrate they grown on (Meneguz et al., 2018, Spranghers et al., 2017). For example, unused tissue from animals fed on a high n-3 FA diet will result in insect offal rich in n-3 FA as well (St-Hilaire et al., 2007a). On the other hand the quality of the substrate can negatively impact the nutritional composition of the product by incorporating contaminant such as heavy metals (Purschke et al., 2017). Furthermore, the abundance of edible insects (Jongema, 2015) and the fact that the overall chemical composition of insects can substantially vary between species (Makkar et al., 2014, Guil-Guerrero et al., 2018) assumes several suitable candidates for inclusion in the animal feeding chain, which makes the principle of replacing soybean with insect meal worth investigating.

The interest in insect farming is without a doubt growing. In parallel to fulfilling the nutritional needs of the animals and keeping the production at sustainable levels, insects' nutritional composition can in turn modify the egg nutritional composition. Therefore, replacing basic ingredients in the conventional poultry diets should also be looked in parallel from an egg nutritional quality perspective. Secci et al. (2018) showed higher carotenoids content in yolks from insect fed hens and a slightly modified FA profile. So far, only very limited research on feeding animals and specifically layers with insect meal have investigated the resulting animal product and its nutritional composition and it will further be discussed in the relevant Chapters.

1.6 PHD OBJECTIVES AND HYPOTHESIS

The overall objective of this PhD is to investigate the effect of management practices on the egg nutritional content taking into consideration all available literature (Chapter Two), through a farm survey (Chapter Three) and a supermarket survey (Chapter Four). Additionally, to the main objective, it is planned to also look into the effect of alternative insect fed hens on the egg fatty acid profile.

In the subsequent Chapters, the overall effect of OR FR compared with conventional FR and conventional indoor production systems on egg's nutritional content, mainly on the fatty acid profile (Chapter Two, Three, Four), fat soluble vitamins A, D, E and carotenoids (Chapter Two, Four) was assessed. This was achieved initially by a systematic review followed by a meta-analysis of all the available studies reporting such data (Chapter Two). In the next Chapter, the effect of OR and conventional FR practices were assessed on farm level (Chapter Three). This was achieved by investigating the effect taking into consideration 1) genotypic variation, comparing one brown and one white genotype, and 2) seasonal variation comparing contrasting seasons. In Chapter Four the differences in nutritional content of OR and conventional eggs, were considered at retail level in the UK. This was achieved by sampling eggs from all standard

types (OR, FR and CA), across the biggest supermarkets in the UK four times across a year. In the last Chapter (Chapter Five), an experimental trial was evaluated to investigate the effect of alternative systems and in particular replacing soybean meal with insect feed on the egg fatty-acid profile.

The specific objectives of this PhD project were:

1) To assess the effect OR and conventional management on egg FA profile, egg soluble vitamins A, E and carotenoids, using all the available literature (Chapter Two).

2) To assess the impact of management, season and genotype on egg FA profile (Chapter Three)

3) To assess the egg nutritional profile in the UK retail sector (Chapter Four).

4) To evaluate the impact of replacing soybean meal of hens with insect meal on egg FA profile (Chapter Five).

The hypotheses of the above objectives are as follows:

1) Hypothesis 1: Increasing intensity of forage material consumption found on pasture in hen management will result in enhanced egg nutritional composition. As pasture is a natural source of FA, carotenoids and vitamins, more of these compounds will be available for the hens in FR systems to uptake and deposit in their eggs.

2) Hypothesis 2: Management practices between OR and conventional FR differ not only in feed origin but can also differ in the intensity of free-ranging behaviour and pasture availability. These differences can therefore impact the final nutritional composition of the egg.

3) Seasonal variation can impact the availability and nutritional content of forage material found on pasture. At the same time seasonal variation can interact with management. It is expected that for systems where birds have access to pasture at times with higher pasture availability the egg nutritional profile would be more enriched. Additionally, seasonal variation will interact with management more within systems with intense free-ranging behaviour.

4) The colour of the eggshells is assumed to be controlled by several genes and the brown or white pigment is breed specific (Samiullah et al., 2015). Therefore different genotypes of brown and white egg laying hens can result in differing egg nutritional profiles either because of different behaviours within management systems or because of genetic differences in deposition of nutrient in eggs.

5) The insects used to make the insect meal for this study were fed wheat based diets, a poor source of n-6 FA, compared with soya beans and it is known that insects' FA profile is

dependent on their feed. Therefore, replacing soybean meal with this insect meal should lower dietary n-6 FA content and as a result, the n-6 FA of the egg.

Each of the following Chapters deal with a mix of objectives and is presented in the form of a self-contained paper including an abstract, a short introduction the results and the relevant discussion. The Chapters are then summarized in the Chapter Six of general discussion where the results and findings are linked to the overall objective of this PhD project. The final Chapter will critically evaluate the present project and suggest future research directions.

CHAPTER 2. EGG NUTRITIONAL COMPOSITION BETWEEN ORGANIC AND CONVENTIONAL SYSTEMS: A SYSTEMATIC REVIEW AND META-ANALYSIS

2.1 SUMMARY

Organic egg consumption has increased steadily over the recent years, partly because of perception of consumers that organic food is healthier than non-organic food. Organic egg production differs from conventional systems (free-range and indoor) in the exclusive use of organically grown feed. Organic and conventional free-range systems share the free access to open-air runs although organic flocks have more restrictions into management practices that could potentially drive differences in egg composition. However, there have been no systematic reviews comparing the nutritional composition of organic and conventional eggs, either free-range or indoor. This study reports a meta-analysis comparing the nutritional composition of (a) organic and conventional free-range eggs and (b) organic and conventional indoor eggs. Compositional parameters compared in the study were docosahexaenoic acid (DHA), total omega-3 fatty acids (n-3 FA), carotenoids (as sum and individual compounds), vitamin A, vitamin E and cadmium. Synthesis and statistical analyses of data collected included weighted meta-analysis followed by sensitivity analysis protocols, expressing differences as 'means of estimated effect size of standardized mean difference (SMD)'. The evidence base was generally weak with not many studies retrieved for all outcomes. However, significant differences were determined for fatty acids and carotenoids. Both DHA (SMD=0.71; 95% confidence intervals (CI) 0.05, 1.37) and n-3 FA (SMD=0.58; CI 0.06, 1.10) were higher in organic compared with free-range eggs but not when compared with indoor eggs. Lutein and zeaxanthin were higher in organic eggs compared with either free-range (SMD=2.68; CI 1.78, 3.57) or indoor eggs (SMD=2.13; CI 1.14, 3.11). No differences were detected for cadmium content of eggs. For all parameters included, heterogeneity (I^2) was high. Meta-analysis using moderators such as season to explain heterogeneity were similar to the original meta-analysis results but with lower heterogeneity. However, more studies are required to draw stronger conclusions and improve precision of the results. Potential impacts of the differences in nutritional composition of eggs from difference management systems and their relevance to human health are discussed.

Keywords: meta-analysis, egg composition, organic, free-range, indoor, fatty acids, carotenoids, cadmium

2.2 INTRODUCTION

Organic egg consumption has increased steadily over the recent years with a striking 15% increase at the UK market between 2015 and 2016 alone (Kantar Worldpanel, 2016, Soil Association, 2016). The increase in sales is driven by the perception of consumers that OR food is healthier than that of conventional and that OR production follows higher ethical standards (Hemmerling et al., 2015, Zander and Hamm, 2010). Since the out-dated association between eggs, dietary cholesterol and cardiovascular disease has been reconsidered and their nutrition value and food safety have been restored (Virtanen et al., 2016, Harman et al., 2008, Gray and Griffin, 2009, Group, 2016) egg consumption in general is on the rise. Egg yolk is a dense source of nutrients, including n-3 FA, carotenoids, vitamins A, E and D found in egg lipoprotein composition (Surai et al., 2000, Handelman et al., 1999, Chung et al., 2004, Fraeye et al., 2012). Hence, it has a high potential to contribute to human health and well-being. However, eggs can also be a source of toxic metals as heavy metals like cadmium bio-accumulate from the layer's feed and environment. For instance, egg content of cadmium can contribute from <1% to up to 93% (Malmauret et al., 2002) of the reference tolerable weekly intake of 5 μg/kg of body weight (EFSA, 2009a) for adults. In eggs coming from outside Europe the contribution is often between 4-21%, yet in eggs coming from Europe it is rare that the contribution is >1%. Therefore, it is important to differentiate the nutritional value of OR and conventional eggs, exploring patterns and practices that might drive changes in egg composition. Organic egg production differs from conventional systems (FR, barn and CA) primarily in the restricted and controlled use of genetically modified-free feed, pesticides and veterinary drugs and also the exclusive use of organically grown feeds. Organic and conventional FR share free access to open-air runs but OR flocks have lower stocking density and larger outdoor areas compared with FR, while the range area in OR systems also provides additional protective facilities (European Council 1999/74). Barn and CA systems do not provide access to an outside area although barn systems share all other requirements of conventional FR systems and cage system including cage-constructions for layers within a larger house.

Egg content of FA, vitamins and antioxidants has been shown to vary across different management practices. Dietary supplementation of nutritional compounds tends to show a dose dependent relationship, with varying transfer efficiencies into the egg yolk (Fraeye et al., 2012, Galobart et al., 2001, Mattila et al., 1999a), making diet composition one of the most significant factors affecting egg quality. However, feed components, composition and quality vary according to management practices. Organic cereals, a major component of poultry diets, have been shown to have lower cadmium concentrations (Baranski et al., 2014) and therefore eggs from OR systems are likely to follow this pattern.

Access to nutrient dense pasture also varies according to management systems; CA and barn systems not having access to pasture are more likely to have lower egg carotenoid, vitamin and n-3 FA content than FR systems, either conventional or OR (Mugnai et al., 2014, Krawczyk et al., 2011, Skrivan and Englmaierova, 2014). However, grazing behaviour and pasture access in FR systems varies, therefore management practices that encourage grazing are likely to increase egg nutritional concentration. Some factors that affect range utilisation and grazing by birds are flock size, trees and shade structures, vegetation, climatic condition, time of day and day length (Singh and Cowieson, 2013). Organic systems, in contrast to conventional ones, have restricted flock size and additional protective structures must be provided in the range area, hence OR flocks are likely to use the range area more frequently and therefore increase forage and invertebrate consumption and ultimately the concentration of some egg nutrients. Seasonal variations of the pasture composition and range area, in terms of plant/insect/worm availability, have to been taken into account as well (Boufaied et al., 2003, Mugnai et al., 2014). Interactions between other practices, like genotype selection, and management have also been reported on egg nutritional quality (Krawczyk et al., 2011).

Recent meta-analyses on animal products, showed that OR milk was higher in n-3 FA and vitamin E and OR poultry meat was higher in n-3 FA (Srednicka-Tober et al., 2016a, Srednicka-Tober et al., 2016b) than products from conventional systems, although other, previous reviews had reported otherwise (Dangour et al., 2009). Management practices in egg production are also likely to have an impact on egg nutritional composition and OR management could potentially act as a natural way to enhance the nutritional quality of eggs.

Whereas there have been studies comparing the effect of diets in CA, barn and FR egg production systems, there is limited information on the impact of OR production on nutritionally relevant compounds in eggs and findings are inconsistent. Therefore, the influence of management practices on egg nutritional quality needs further investigation, especially in FR conditions, either OR or conventional, where nutritional relevant compounds could be enhanced by forage material consumption and use of the range area. Methodological approaches can also vary between studies thus leading to inconsistent results. A systematic review and critical appraisal of the quality of each study will take into account those methodological inconsistencies. To my knowledge this is the first meta-analysis assessing the impact of management on egg nutritional quality, hence the need for a systematic review and meta-analysis that will elucidate any nutritional differences between OR and conventional eggs.

The main objective of this review is to systematically assess and synthesize all available research looking into the effect of OR management of layers on egg nutritional quality. This review is designed to answer the following research questions:

1. Is the nutritional profile of OR eggs different from conventional ones?

2. What is the strength of association between OR management and (a) conventional FR-management or (b) indoors management?

3. What is the magnitude of such a difference?

4. How do the effects of management practices vary in different:

(a) Type of studies,

(b) Seasons,

(c) Genotypes.

2.3 MATERIAL AND METHODS

The current section is presented in a form of a systematic review and meta-analysis protocol according to Cochrane Collaboration recommendations (Higgins and Green, 2011). It has to be noted that the protocol has not been bublished or peer reviewed in advance of the data collection as recommended by the Cochrane Collaboration recommendations.

2.3.1. Inclusion and exclusion criteria

Species of interest

(i) Only research looking into the egg composition of the species *Gallus gallus* were included.

Rationale: This review is only interested in the composition of eggs coming from domestic hens since it is the most dominant and commercially available source of eggs in the world. Every breed of this genus was considered.

(ii) Studies looking into egg products (e.g. pasteurised liquid egg yolks) were excluded.

Rationale: The objective of this study is to examine the egg nutritional composition, assuming the consumption of it as a whole. The processing itself and other ingredients added to the egg product might alter the nutritional composition, which is not the objective of this study.

Types of studies

(iii) This review includes data from basket studies (BS), farm surveys (FS) and experimental trials (ET) from which both OR and conventional eggs have been sampled. Basket studies are those using supermarkets and other retail shops as a sampling source, farm surveys use farms as a sampling source and experimental trials have been carried out under controlled conditions.

Rationale: These three types of studies are the most common among comparative studies, covering the range of research available.

Types of interventions and controls

(iv) Only studies including both OR and conventional (FR and cage/barn conventional management systems in the design were included.

Rationale: The effect size metric chosen for the statistical analysis of this study (standardised mean difference) requires means from direct comparison of management systems for the calculations.

(v) Studies were only included when it was stated that OR practices corresponded to certified OR practices. For basket studies, retail OR samples were assumed to be certified produce. For the farm surveys, participating OR farms were assumed to follow OR certification schemes.

For the experimental trials, OR certification of the land and animal husbandry practices had to be mentioned or clearly stated that OR practices had been used. Any other system including CA, FR, barn and backyard, was considered conventional. Conventional systems with special dietary supplementations (e.g. omega-3 enriched) were excluded.

Rationale: Adherence to OR standards has to be confirmed to ensure all OR samples were produced under common management practices.

Types of outcomes

(vi) Only studies looking into fatty acids, carotenoids, tocopherols, vitamins (A and E) and cadmium content were considered. As the pre-defined outcomes the DHA, n-3 FA, lutein, zeaxanthin and any other carotenoids, total carotenoids, vitamin A and E (see 2.3.4 2.3.4. Synthesis procedures and statistical analysis for more details) and cadmium were considered. Eligible studies should report at least one of these outcomes and meta-analysis was performed when at least 3 studies reported data on the same compositional parameter.

Rationale: A scoping search indicated these compounds were consistently amongst those investigated and reported in the literature. Other than protein content, the above egg compositional parameters are nutritionally relevant for human health.

(vii) For each relevant nutritional compound, only studies with at least three replications, within each study, were included (see 2.3.3. Study information and data extraction procedures for more details).

Rationale: As a standard deviation is required for the calculation of the effect size of each study, at least two replications would be needed. However two replications would give a very weak measure of variance. Therefore, a minimum number of three replications were decided as the minimum number needed so as to be included in each basic study.

Search criteria

(viii) Both published and unpublished research post 1992 were included in the study.

Rationale: To avoid publication bias both published and unpublished studies were included (see 2.3.2. Search strategy for finding eligible studies and data management, for more details).

(ix) Abstracts and studies written in languages other than English were included.

Rationale: To construct a more comprehensive review and reduce publication bias. The data and information were extracted from eligible publications written in non-English language under close supervision of native speakers.

2.3.2. Search strategy for finding eligible studies and data management

The following electronic databases were selected to ensure most published and unpublished literature was retrieved: Web of Science, Scopus, Ovid and Elton B Stephens Company (EBSCO-GreenFILE), System for Information on Grey Literature in Europe (SIGLE).

An initial scoping search using Web of Science pinpointed key terms to be included. Relevant publications were identified using the following search phrase, which included relevant search terms for these topics:

(organic OR biodynamic* OR ecologic*) AND ("free-range" OR barn* OR cage* OR backyard* OR "low-input" OR indoor* OR outdoor* OR conventional* OR integrated) AND (egg* OR yolk*)*

Papers in all languages, published or not-published, in peer-reviewed and non-peer-reviewed journals, reporting data on selected compositional parameters were considered relevant for inclusion in the meta-analysis. The search was restricted to the period between 1992 (the year when legally binding OR farming regulations were first introduced in the European Union) and the end of the project in September 2017.

Duplicated articles were deleted and studies relevant for the meta-analysis were identified in the three selection steps:

1. title readout
2. abstract readout
3. full text readout

When the abstract or full text were not available from the database, authors were contacted twice, to obtain missing results. Once initially relevant studies had been identified, their references were also checked for more relevant studies. References from relevant review articles were checked for additional studies on the topic. Study selection for all papers returned from the search, was conducted by two reviewers independently (Eleni Chatzidimitriou and Dr Gultekin Hasanaliyeva) to reduce bias and increase precision (Edwards et al., 2002).

The number of studies found from the initial search and those meeting all selection criteria are provided as a flow chart (Figure 2. 1).

2.3.3. Study information and data extraction procedures

A coding form, extracting information (Appendix B.1.1 Study information form) was completed for all relevant studies. This form was designed to collect data on both the profile of research (e.g. type of study, management practices, year and outcomes) and key data to be used

for analysis (e.g. moderators, sample sizes and variations). After each form was completed, extracted data were copied into electronic spreadsheets.

Sample size extracted for all three types of studies was considered as (1) number of eggs sampled from stores for basket studies (2) number of eggs sampled between all farms for farm surveys (3) egg replicates for experimental trials. Where individual studies reported multiple outcome measures (e.g. multiple breeds, multiple time points and multiple systems), each of these were extracted separately and averaged, if appropriate at a later stage (see 2.3.4. Synthesis procedures and statistical analysis for more details).

A quality appraisal form was completed for each relevant study (Appendix B.1.2 Quality assessment form). Results of quality assessments were used in additional sensitivity analyses in which the effect of exclusion of low-quality studies on the magnitude and direction of the overall effect size was explored.

2.3.4. Synthesis procedures and statistical analysis

Synthesis and statistical analyses of collected data included weighted meta-analysis followed by sensitivity analysis protocols.

Data management

The mean concentrations of lutein, zeaxanthin and individual carotenoids content were extracted as separate values, only if 3 or more studies were found containing these data. Since analytical separation of lutein and zeaxanthin is difficult and they are commonly reported as a sum, hence in this review the sum of lutein and zeaxanthin was also considered as a singular outcome. With regards to n-3 FA, if in the basic study FA analysis had been carried out but the n-3 FA not reported, authors were contacted and/or calculations were employed to sum mean values for n-3 FA content. In case of summing the means of every relevant compound for eithr carotenoids or n-3 FA, the standard deviation was calculated using the formula in the Appendix B.2.

Cadmium and some minor carotenoids were often reported to be below the analytical limits of detection (LOD) or quantification (LOQ). In these cases half of the reported lowest limit of detection were taken as a value for meta-analytical evaluation, together with the respective standard deviations. When content was reported as “trace” values of LOQ instead of LOD were used.

When multiple OR systems were compared within the same study (e.g. biodynamic) only the OR system identified by the authors as the closest to *contemporary* system was used in the meta-analysis, as recommended by Brandt et al. (2011). If authors did not identify any OR

system as contemporary, the study was excluded as not meeting inclusion criteria. For the standard meta-analysis, when multiple contemporary conventional systems were available within the same study (e.g. barn and CA systems or FR and backyard) outcomes from contemporary CA and FR were used over the rest. When authors could not identify a conventional system close to the contemporary one, the study was excluded. In cases where the conventional system was not specified in details, the study was used as both FR and indoor conventional, but excluded from the sensitivity analyses. If outcomes in the same study were reported for different countries or study types, they were treated as independent events. If data in the same study were reported for different regions within the same country or different time points, these were averaged.

Data synthesis

Weighted meta-analysis was carried out using the R statistical environment (https://www.r-project.org/) and the 'metafor' statistical package (Viechtbauer, 2010, Lipsey and Wilson, 2001). Hedges' *d* (standardised mean difference; SMD) (Hedges and Olkin, 1985), being one of the most common metrics, which includes a correction factor for small sample sizes, was used as the measure of effect size, as it is recommended for meta-analyses when there are only few number of studies available (Koricheva et al., 2013).

In case of key data missing (mean value, measure of variability, sample size) even after contacting the authors, multiple imputation methods (Lajeunesse, 2013) were used for aiding these dataset gaps. If retrieving data needed for the calculations was not possible, then the study was excluded.

Two standard analyses were carried out (a) OR eggs vs. conventional FR eggs and (b) OR eggs vs conventional indoor eggs (Table 2.1., Analysis code: 1, 2). The CA systems were primarily used as the main indoor system available, and if not available results from barn systems were used.

Random-effect hierarchical models, weighted by inverse variance (Baranski et al., 2014, Viechtbauer, 2010, Sánchez-Meca and Marín-Martínez, 2010, Lipsey and Wilson, 2001) were used. Statistical significances of a reported effect size and 95% confidence intervals (CI) were calculated according to standard methods (Hedges et al., 1999) inside the 'metafor' package as described by Viechtbauer (Viechtbauer, 2010). Heterogeneity assessments are described below (2.3.5. Quality of evidence assessments).

Covariates analyses

In order to explore the impact of covariates on the outcome and to enhance discussions, moderators were included in standard meta-analysis procedures using hierarchical mixed-effect models (Mengersen et al., 2013) and separate subgroup analyses. The following moderators were included one at a time (Table 2.1., Analysis code: 3-8):

1. study type (BS, FS or ET)
2. genotype of a layer; at least three studies had to report data for the same genotype
3. season; depending on the sampling date seasons will be considered winter or summer (between November-March or between April-October respectively for the northern hemisphere and the opposite for the southern hemisphere)

Sensitivity analyses

Four sensitivity analyses for the standard meta-analysis, were carried out to assess robustness with regard to different uncertainties about (Table 2.1., Analysis code: 9-15):

1. effect sizes calculated using imputation methods; if imputation methods were used, studies were excluded
2. egg production systems used; studies using *backyard* instead of FR and barn instead of CA systems were excluded; studies were also excluded if the specific conventional system was not specified
3. Influential effect sizes; studies marked as influential studies by the statistical packages used were excluded
4. risk of bias assessment; studies scored as of potentially lower quality were excluded

Visualisation

Forest plots for random and mixed effect models were used to show pooled SMD and the corresponding 95% CI for all compositional parameters for standard meta-analysis.

Table 2. 1. List of standard, exploratory and sensitivity analyses performed

Code	Type	Comparison	Model
1	**Standard**	**OR FR vs conventional FR**	Random effect model
2	**Standard**	**OR vs indoor system**	Random effect model
3,4	Exploratory	1 and 2 with the study type as a moderator	Mixed effect model
5,6	Exploratory	1 and 2 with genotype as a moderator	Mixed effect model
7,8	Exploratory	1 and 2 with season as a moderator	Mixed effect model
9,10	Sensitivity	1 and 2 with outlier effect sizes studies excluded	Random effect model
11,12	Sensitivity	1 and 2 with effect sizes that were calculated using imputation methods excluded	Random effect model
12,13	Sensitivity	1and 2 with low-quality studies excluded	Random effect model
14, 15	Sensitivity	1and 2 with studies using backyard and/or barn systems excluded	Random effect model

2.3.5. Quality of evidence assessments

Risk of bias

Risk of bias was addressed using the overall score of studies quality assessments (B.1.2 Quality assessment form). Each point is giving a positive overall outcome. A study was considered as "biased" and eventually of lower quality when risk of bias was either positive or unclear for at least one of "baseline confounding", "treatment confounding" and "selective reporting" or negative for "reliable outcome measurements".

Publication bias

Publication bias was addressed using counter enhanced funnel plots to facilitate visual assessment.

Consistency of effect sizes

Heterogeneity (I^2 statistics) was assessed on all summary effect sizes. Homogeneity was indicated if I^2 was <25% and the P value (for the Q statistics) was >0.010. The degree of overlap between study CIs were used to facilitate discussions.

Precision of the estimated total effect size

Precision was based on confidence intervals.

2.3.6. Interpretation of the magnitude of effect size

To avoid hypothesis bias and being data driven to interpret the magnitude of the effect size, a meaningful magnitude, from a human health perspective was decided in advance. These might be open to discussion but the following standards were adopted in this study. With regards to DHA, the European Food and Safety Authority (EFSA) sets the daily recommended value at 250 mg/d (EFSA, 2010b). Any increase in the DHA content of eggs towards this value was considered nutritionally relevant. With regards to total n-3 FAs, since there are no official recommendations for the sum specifically, any statistical difference was considered potentially important and used in combination with the DHA outcome to facilitate discussions. For carotenoids, again there are currently no official dietary recommendations and therefore any positive difference between production systems was considered potentially relevant to human health. With regards to cadmium content, EFSA has set the tolerable weekly intake as 2.5 μg/kg of body weight (EFSA, 2009a) and in the literature egg content of cadmium can contribute from <1% to up to 93% (of the tolerable weekly intake for adults) (Malmauret et al., 2002), yet in Europe it is rare that the contribution is >1%. Considering the accumulative nature of cadmium in liver and its high toxicity, any significant difference between production systems was considered potentially meaningful.

2.3.7. Plans for updating the review

The plan is for Eleni Chatzidimitriou to assume responsibility for bringing up to date the review with new evidence, comments and criticisms and updating the review 5 years after publishing in a peer review journal, assuming funding is available. A network analysis comparing all management systems at the same time will be then considered.

2.4 RESULTS

The magnitude of effect size can be described as positive or negative. A positive effect size means that the respective content is higher in OR eggs, whereas a negative effect size means that the content is lower in OR eggs compared with eggs coming from the respective conventional system.

2.4.1. Selected studies

Figure 2.1. summarizes the eligibility stages performed, the number of studies excluded and finally selected. This meta-analysis was based on data from thirty-seven studies; thirty peer-reviewed papers – including thirty one studies and six non-peer-reviewed papers, including studies reporting basket surveys (n=17), farm surveys (n=12) and experimental trials (n=8). Occasionally papers reported both basket and farm surveys. The majority of the studies were

conducted in Europe (80%), mainly in Italy but also in Germany, Poland, the Netherlands, Greece, Portugal, Ireland, France, Belgium, Switzerland, Austria, Czech Republic, Slovenia, Denmark, Estonia, Latvia, Romania and the UK. Studies from USA, Brazil, Australia, New Zealand, Turkey and Egypt were also present. In total 35% of the studies reported the season when samples were collected. A much lower percentage reported studies on hen genotype or egg colour. The most frequently reported outcomes were DHA and n-3 FA content, and less data were available on carotenoids and cadmium content. Only a few studies reported data on vitamin E (α-tocopherol), vitamin A (retinol) or individual carotenoids contents. It was also found that "poor reporting" was a predominant reason for the probable risk of bias of studies.

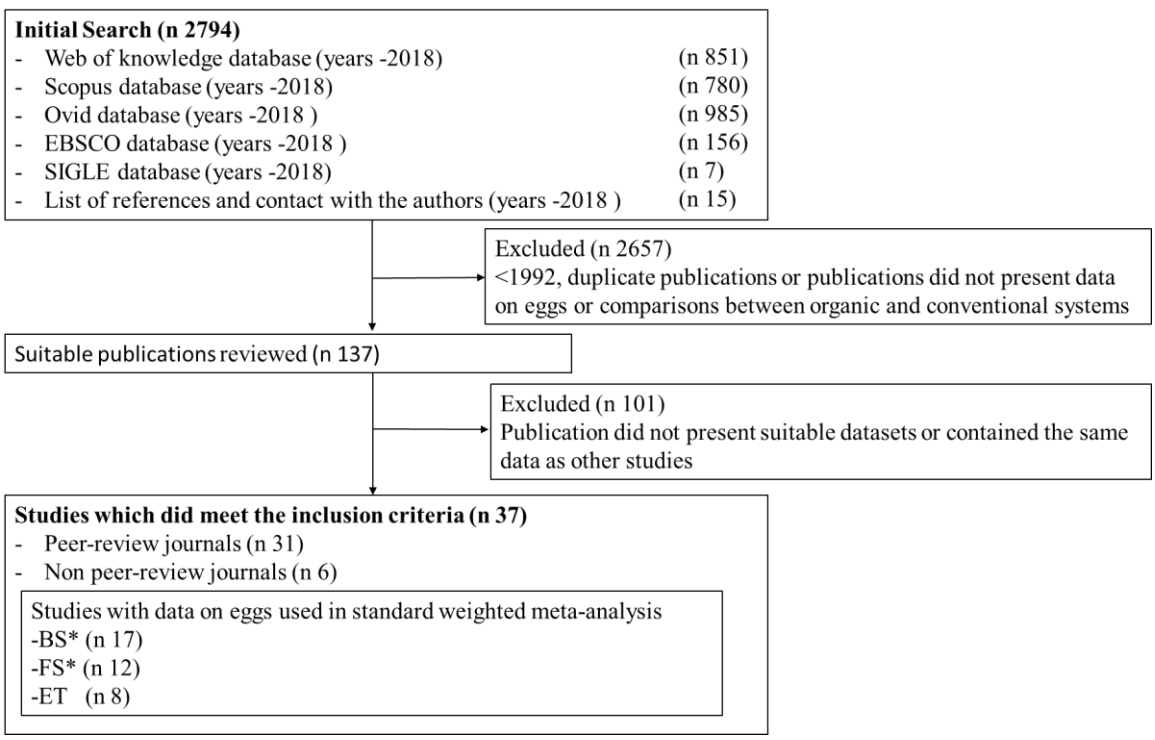

Figure 2. 1 Summary of the search and selection protocols used to identify papers included in the meta-analyses. BS, basket studies; FS, farm survey studies; ET, experimental trials. * One paper had both BS and FS studies

2.4.2. Composition

2.4.2.1. Fatty acids

DHA

Ten data points for the comparison between OR and conventional FR systems (Figure 2. 2) and 14 data points for the comparison between OR and indoor systems (Figure 2. 3) were used to calculate the effect size of management system with regards to DHA content in eggs in the standard weighted meta-analysis. It has to be noted that the Danish National Food Dataset had to be excluded because the value for conventional eggs was reported as "0".

Figure 2.2 shows that OR eggs overall had a significantly higher DHA content than conventional FR eggs, with 4 studies reporting positive effect sizes. Although there was poor consistency between studies, none showed a significant negative direction in DHA content (ie more DHA in eggs from the conventional system). Results are based on all 3 types of studies (BS, FS and ET), with the BS being the most common. Very few studies reported the season when the samples were collected.

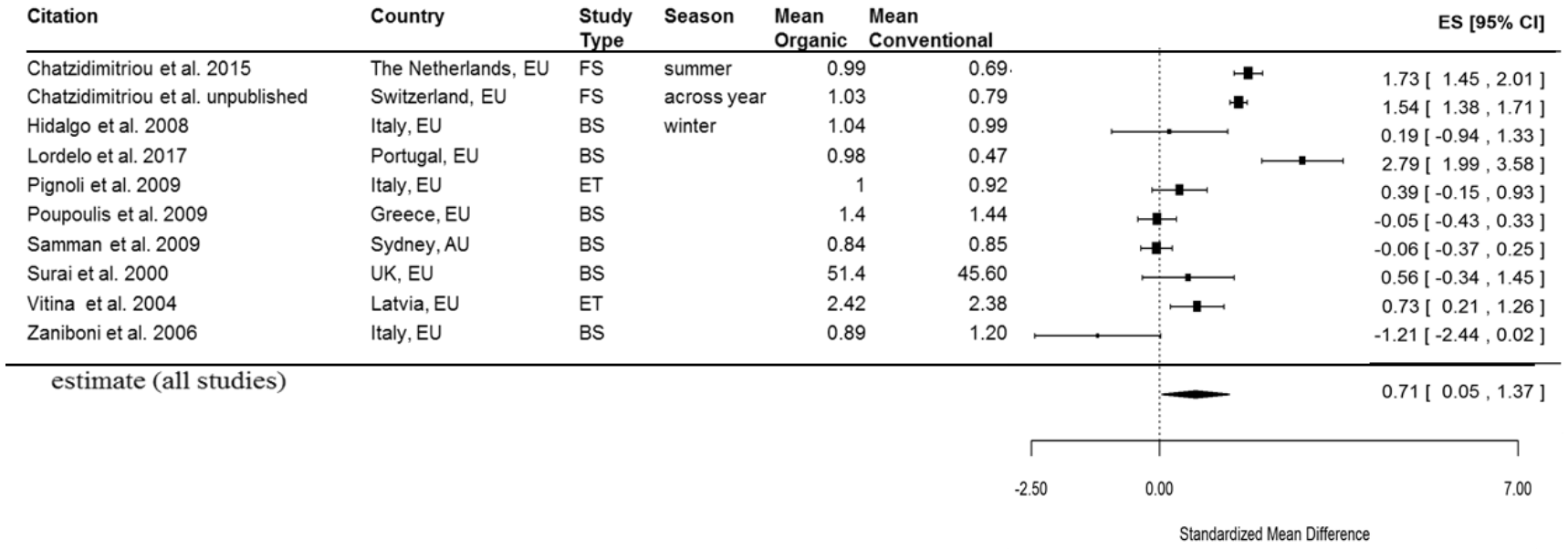

Figure 2. 2. Forrest plot of standardised mean differences (SMD) in docosahexaenoic acid (DHA) content in OR compared with eggs from conventional FR systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial), and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported(% of total FA for all studies except for Surai et al. in which reported in mg/egg. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=96%; P value (Q statistics) <0.001.

Figure 2.3 shows that OR eggs overall had a similar DHA content to indoor eggs, with only 4 studies reporting clear positive effect sizes (more DHA in OR eggs). Moreover, although most studies showed no difference in DHA between management systems, none reported significantly higher content of this fatty acid in conventional FR eggs. The majority of the studies did not report the egg collection season.

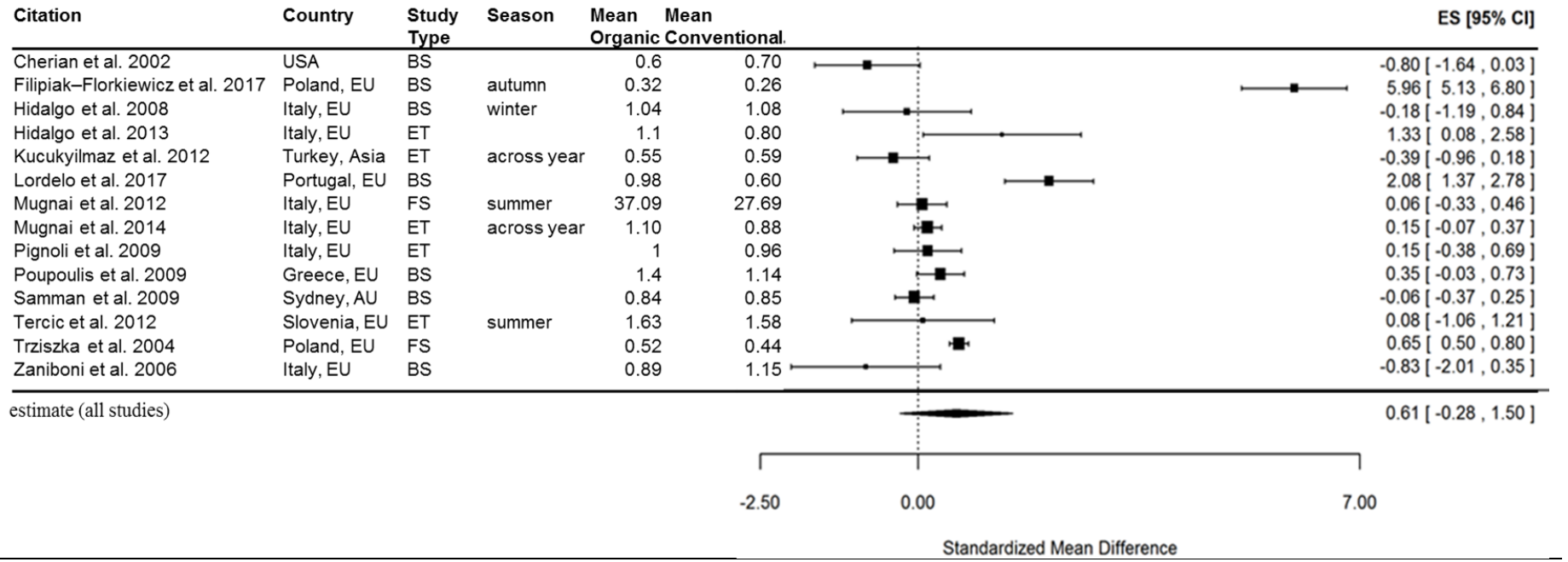

Figure 2. 3. Forrest plot of standardised mean differences (SMD) in docosahexaenoic acid (DHA) content in eggs from OR compared with eggs from indoor systems. Data points are reported as their respective publication reference reporting the the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total FA for all studies except for Mugnai et al. 2012 in which reported in mg/kg yolk). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=96%; P value (Q statistics) <0.001.

Omega-3 FA

The content of total n-3 FA showed a similar pattern to that seen for DHA. Eleven data points for the comparison between OR and conventional FR systems (Figure 2.4) and 17 data points for the comparison between OR and indoor systems (Figure 2.5) were used to calculate the overall effect sizes of management systems effects on the n-3 FA content in eggs. For both comparisons, BS were the most common type of investigation. OR compared with conventional FR eggs (Figure 2.4) had a significantly higher content of n-3 FA, with 3 studies showing distinct positive mean difference (more n-3 FA in OR eggs). The majority of included studies showed positive effect size values. However, with high uncertainty (wide 95% CI) and two publications reported more n-3 FA in eggs from FR conventional management system. Among 3 studies that collected eggs across the whole year, only one had a significant positive effect size and the rest showed only a trend towards higher n-3 FA content in OR eggs.

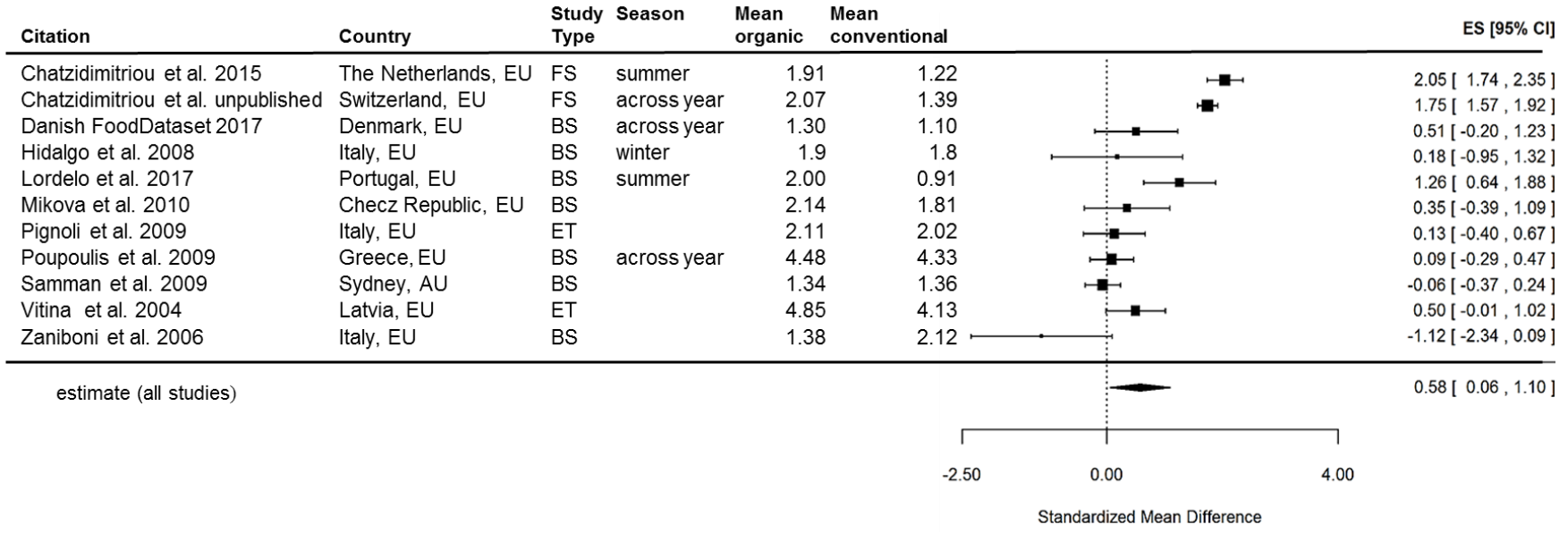

Figure 2. 4. Forrest plot of standardised mean differences in n-3 FA content in eggs from OR compared with eggs from conventional FR systems. Data points are reported as their respective publication reference reporting the country of sampling, study type and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total FA for all studies). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=94%; P value (Q statistics) <0.001.

Figure 2.5 shows that there was no significant effect of management on the n-3 FA content in eggs coming from OR compared with indoor systems. Although almost half of the studies showed a significant positive effect size (more n-3 FA in OR eggs) and only 2 a negative effect size, the overall effect size revealed only a trend towards higher n-3 FA content in OR eggs. One third of the studies showed none or small effect magnitude (from indoor to OR management) and relatively high weight (low variance). Among all studies, only 3 reported eggs collected during summer, showing a consistent positive and significant effect size. Studies collecting eggs throughout the year (5 studies) showed more variable results, with 3 out 5 studies reporting significant positive effect sizes and 1 study reporting a negative effect size.

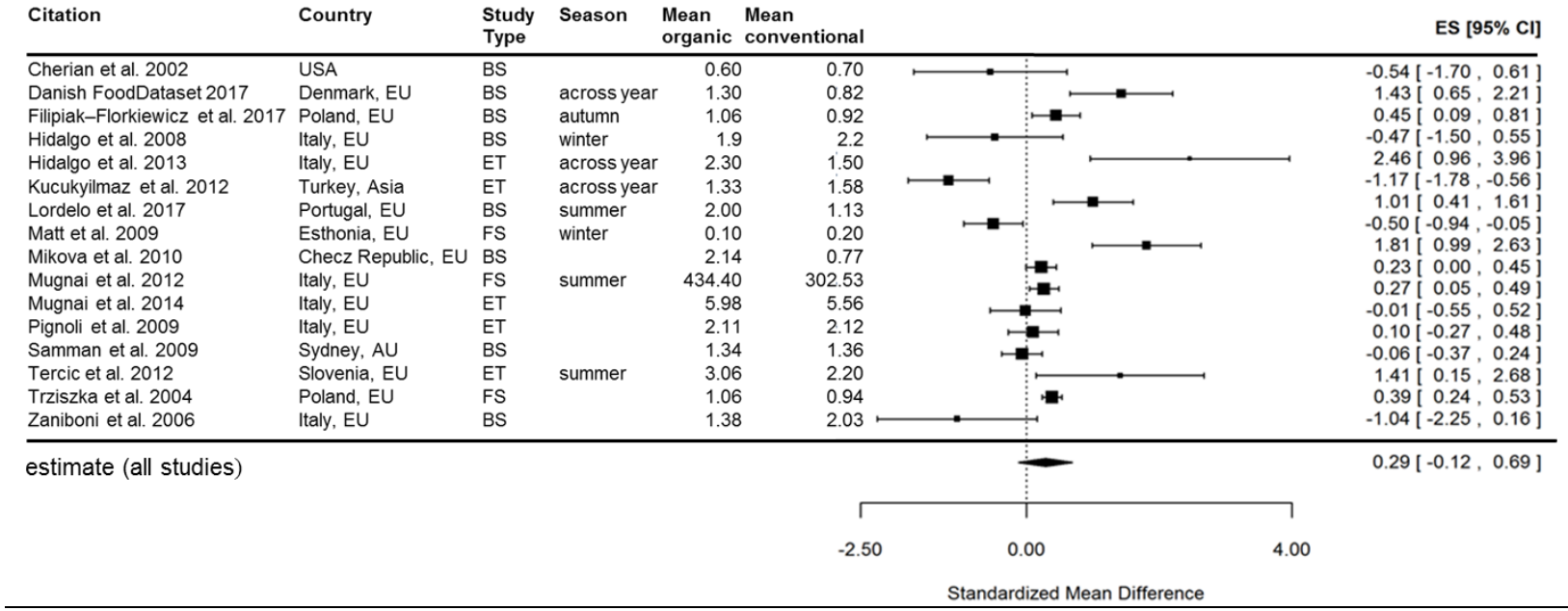

Figure 2. 5. Forrest plot of standardised mean differences in n-3 FA content in eggs from OR compared with eggs from indoor systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total FA for all studies except for Mugnai et al. 2012 in which reported in mg/kg yolk). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant.. I^2=94 %; P value (Q statistics) <0.001.

2.4.2.2. Carotenoids

The sum of lutein and zeaxanthin

Eight data points for the comparison between OR and conventional FR systems (Figure 2.6) and 10 data points for the comparison between OR and indoor systems (Figure 2.7) were used to calculate the effect size of management system with regards to the sum of lutein and zeaxanthin (lut/zea) content in eggs in the standard weighted meta-analysis.

For both comparisons, FS and BS were the main type of investigation, while very few studies reported the sampling season, and those that did were carried out in the summer. There was also high inconsistency in both study types. Nevertheless, OR eggs had a much higher content of lut/zea, regardless of the conventional system they were compared with (FR or indoor).

Only 2 studies show a significantly lower content of lut/zea in OR eggs compared with eggs from indoor system (Figure 2.7). Three studies reporting summer as their sampling season reported a positive effect size whereas one other reported a negative effect size with small confidence intervals, but the difference was not statistically significant.

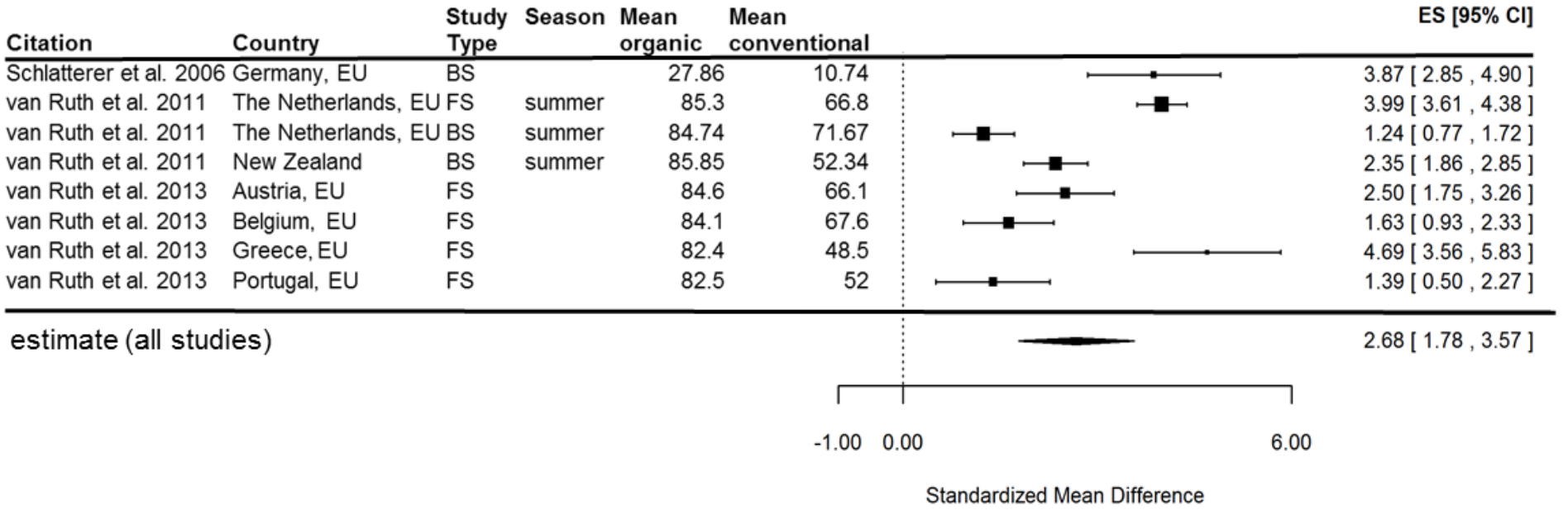

Figure 2. 6. Forrest plot of standardised mean differences (SMD) in sum of lutein and zeaxanthin content in eggs from OR compared with eggs from conventional FR systems. Data points are reported as their respective publication reference reporting the the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total carotenoids for all studies). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=94%; P value (Q statistics) <0.001.

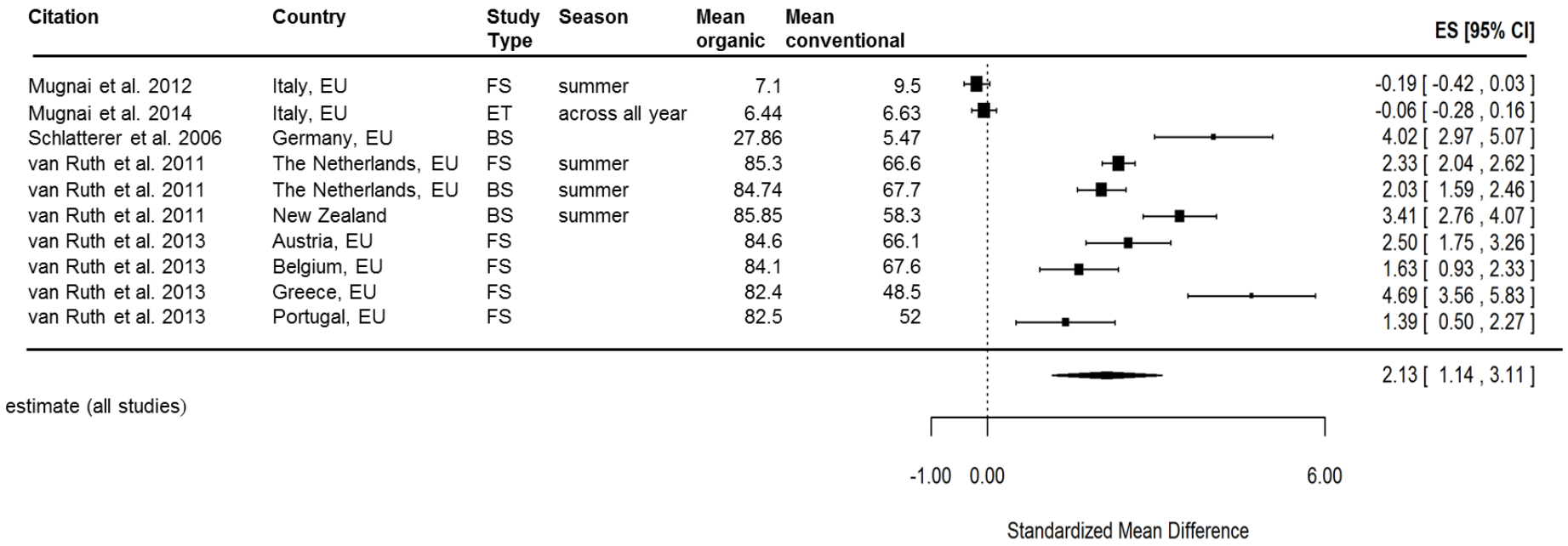

Figure 2. 7. Forrest plot of standardised mean differences (SMD) in sum of lutein and zeaxanthin content in eggs from OR compared with eggs from indoor systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total carotenoids for all studies exepet for Mugnai 2012 and Mugnai 2014 in which values are reported in mg/kg yolk). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=94 %; P value (Q statistics) <0.001.

Lutein

Studies reporting exclusively lutein content in eggs very were limited. Three data points were found for the comparison between OR and conventional FR systems (Figure 2.8) and 4 for the comparison between OR and indoor systems, respectively (Figure 2.9) for which, all types of studies were present, while only 2 reported the sampling season.

Similarly for both comparisons, eggs from OR, FR or indoor systems did not differ in lutein content. However, the limited evidence and high inconsistency between studies does not allow to draw any evident conclusions on the lutein content in eggs from different management systems.

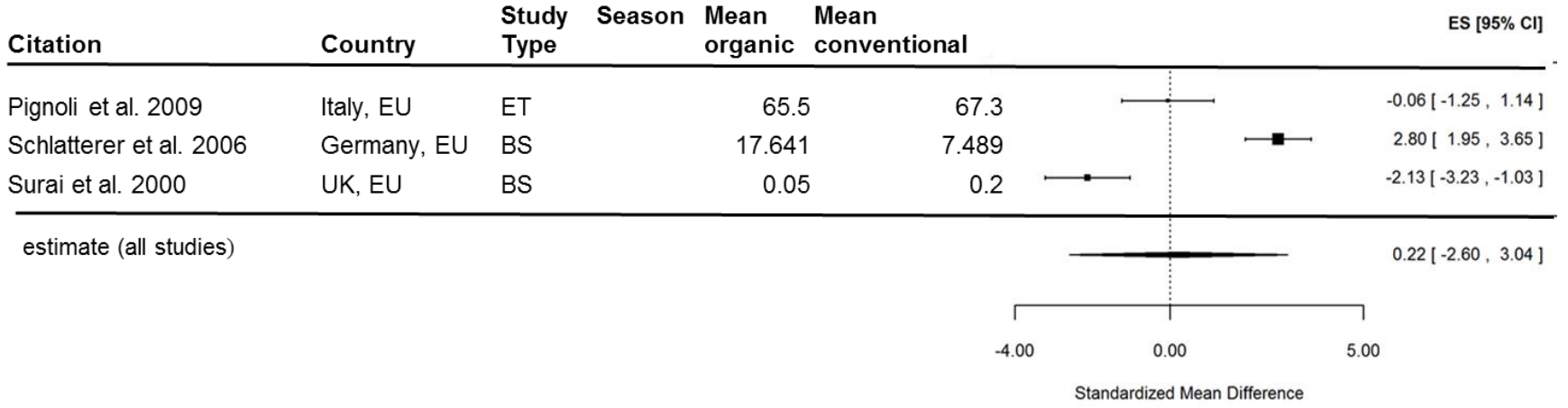

Figure 2. 8. Forrest plot of standardised mean differences (SMD) in lutein content in eggs from OR compared with eggs from FR conventional systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total carotenoids for all studies exepet for Pignoli 2009 (mg/kg lipids) and Surai 2000 (mg/egg)). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=95 %; P value (Q statistics) <0.001.

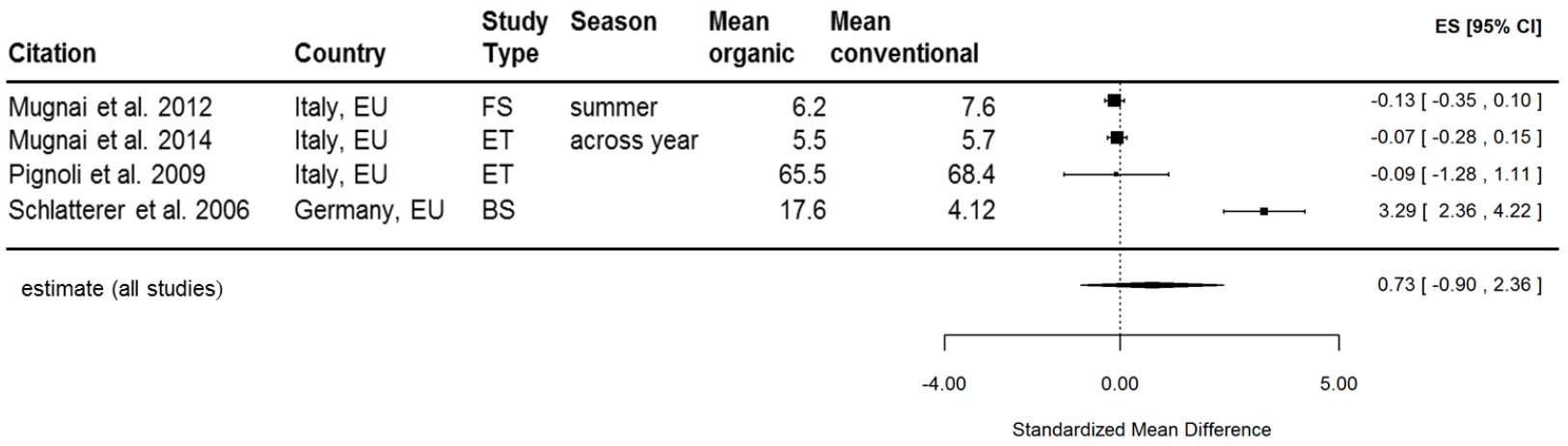

Figure 2. 9. Forrest plot of standardised mean differences (SMD) in lutein content in eggs from OR compared with eggs from indoor systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total carotenoids for all studies exepet for Mugnai 2012 and Mugnai 2014 in which values are reported in mg/kg yolk). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=99 %; P value (Q statistics) <0.001.

Zeaxanthin

Only 3 studies reporting exclusively zeaxanthin content in OR vs indoor system could be found and used in the standard weighted meta-analysis (Figure 2.10). There was no difference in zeaxanthin egg content between OR and indoor conventional eggs. Again, a high inconsistency between studies was observed, with one study reporting no effect, while one reported a negative and one a positive effect size (less and more zeaxanthin in OR eggs, respectively).

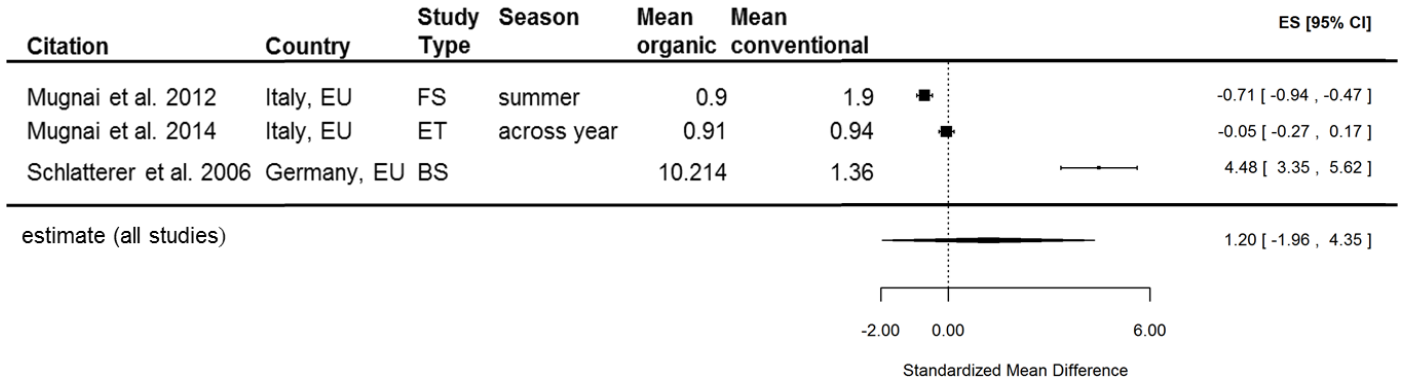

Figure 2. 10. Forrest plot of standardised mean differences (SMD) in zeaxanthin content in eggs from OR compared with eggs from indoor systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total carotenoids for all studies exepet for Mugnai 2012 and Mugnai 2014 in which values are reported in mg/kg yolk). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2>99 %. P value (Q statistics) <0.001.

β-carotene

Six data points for each comparison were used to calculate the effect size of management system with regards to β-carotene content in eggs in the standard weighted meta-analysis. However, all data came from only two publication sources, which could introduce bias in the meta-analysis. Moreover, the same data points from Van Ruth et al. (2013) were used in both comparisons since eggs from both FR and indoor conventional systems were pooled. The data available show β-carotene egg content was similar for both FR conventional and OR systems (Figure 2.11). For four data-points a positive overall effect size was calculated, with one data-point showing no difference and one a strong negative effect size. Only one study (three data-points) reported the egg sampling season.

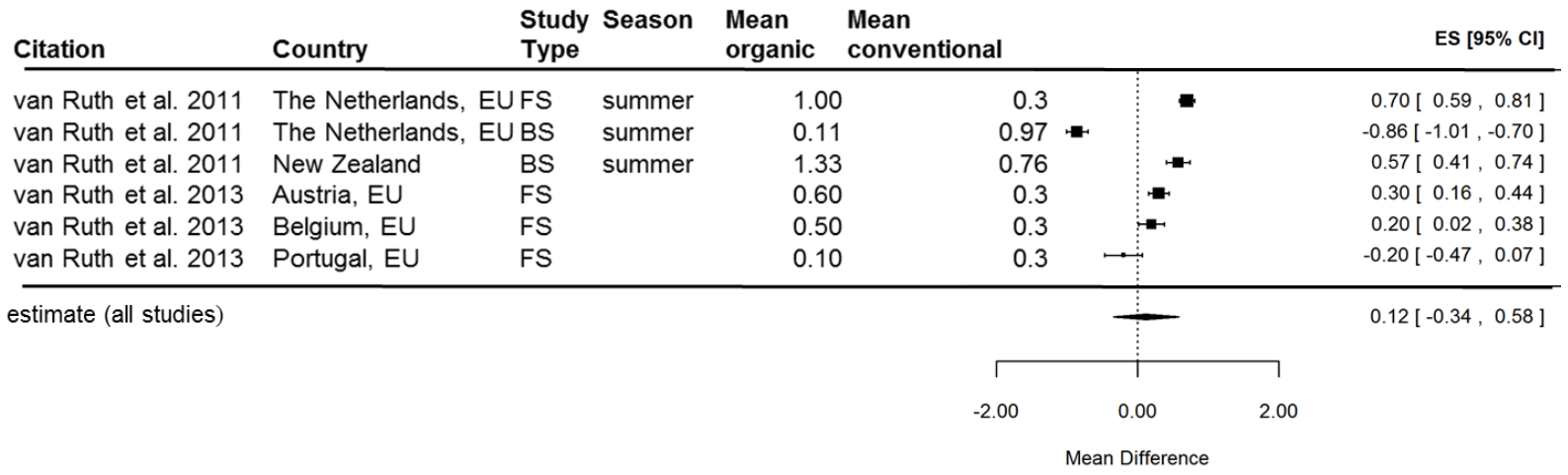

Figure 2. 11. Forrest plot of mean differences (MD) in β-carotene content (%) in eggs from OR compared with eggs from conventional FR systems. . Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total carotenoids for all studies). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=98%; P value (Q statistics) <0.001

Figure 2.12 below shows the comparison between OR and indoor systems. The results were very similar to the previous comparison between FR systems.

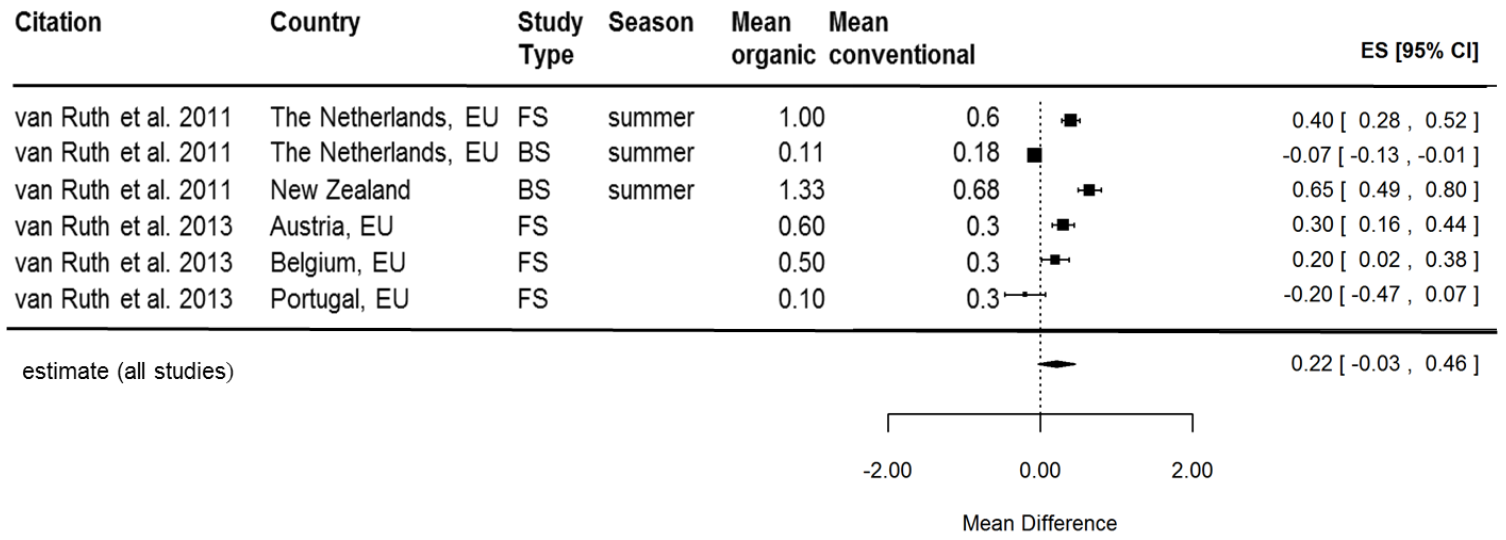

Figure 2. 12. Forrest plot of mean differences (MD) in β-carotene content (%) in eggs from OR compared with eggs from indoor systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total carotenoids for all studies). Values of the effect size (ES) are mean differences, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI are including the value of zero then the P value is > 0.05 and not statistically significantt. I^2=95%; P value (Q statistics) <0.001

The sum of carotenoids

A meta-analysis for the sum of carotenoids was only feasible for the comparison between OR and indoor systems. Figure 2.13 shows no overall effect of the management system on carotenoids content in eggs. The result was based on only four calculated effect sizes of which two showed positive effects and two no effect of OR management. At the same time, two also showed low precision with wide confidence intervals. Three of the four studies reported the respective egg sampling season.

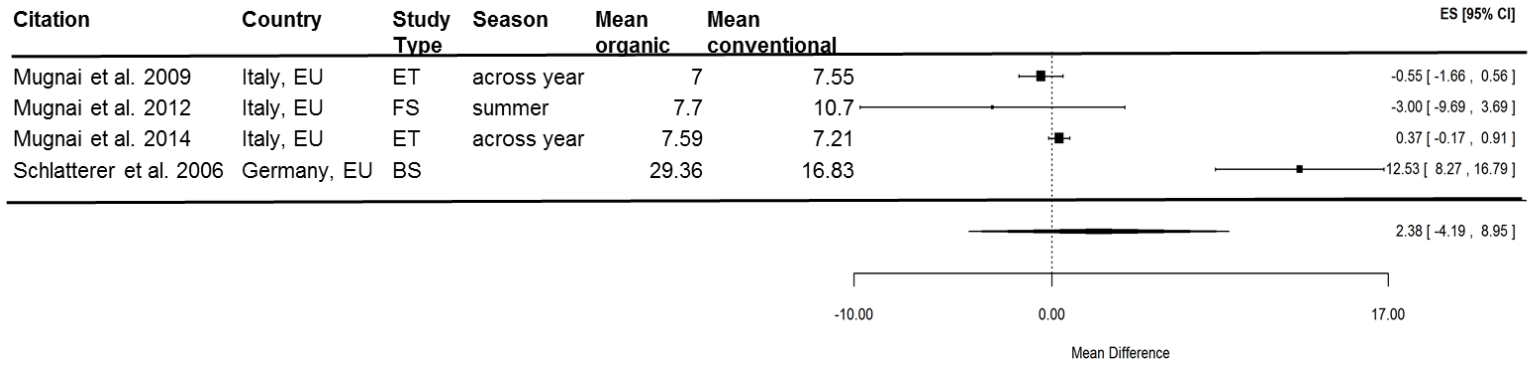

Figure 2. 13. Forrest plot of mean differences (MD) in sum of carotenoids content (%) in eggs from OR compared with eggs from indoor systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (% of total carotenoids for all studies exepet for Mugnai 2009, 2012, 2014 in which values are reported in mg/kg yolk). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant.. I^2=99%; P value (Q statistics) <0.001.

2.4.2.3. Vitamin A

Three data points for the comparison between OR and conventional FR systems (Figure 2.14) and 4 data points for the comparison between OR and indoor systems (Figure 2.15) were used to calculate the effect size of management system with regards to vitamin A content in eggs in the standard weighted meta-analysis. Only one study reported specifically the egg collection season, and one study reported collection of the material throughout the year.

No difference in egg vitamin A content between OR and conventional FR eggs was noted (Figure 2.14). For two studies the calculated effect size was negative while for one the was no effect of management. In consequence there was no significant overall effect of management on vitamin A content was shown.

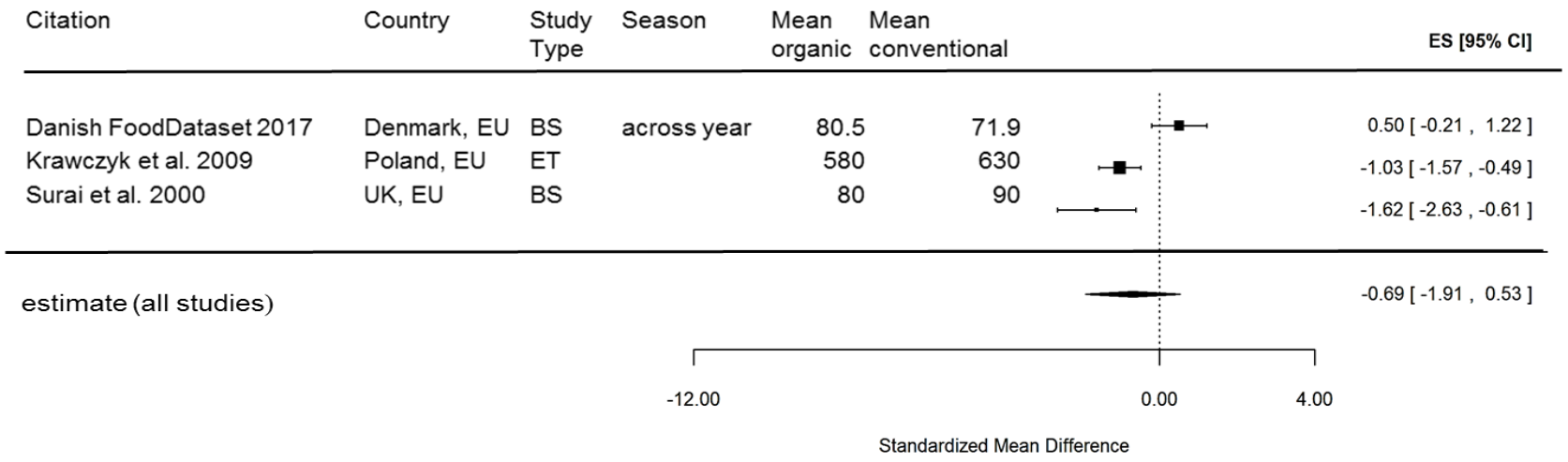

Figure 2. 14. A Forrest plot of standardised mean differences (SMD) in vitamin A content in eggs from OR compared with eggs from conventional FR systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (Danish and Surai (µg/100g egg), Krawczyk (µg/100g yolk)). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant.. I^2=98%; P value (Q statistics) <0.001

Figure 2.15 shows results of the comparison in vitamin A content between indoor and OR outdoor eggs. There was no overall effect of the management system. Among only four datapoints available, for two studies large negative effect sizes were calculated, with relatively large confidence intervals. The other two studies, with a larger weight, reported a positive difference between management systems.

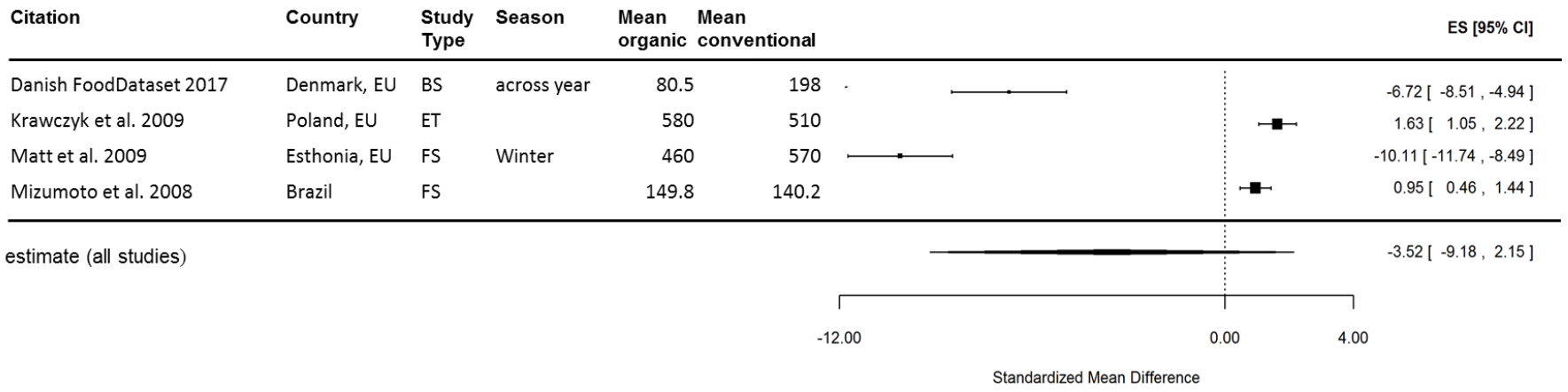

Figure 2. 15. Forrest plot of standardised mean differences (SMD) in vitamin A content in eggs from OR compared with eggs from indoor systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (Danish (µg/100g egg), Krawczyk, Matt and Mizumoto (µg/100g yolk)). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant.. I^2=99%; P value (Q statistics) <0.001.

2.4.2.4. Vitamin E

In the standard weighted meta-analysis only four data points for the comparison between OR and conventional FR systems (Figure 2.16) and 7 data points for the comparison between OR and indoor systems (Figure 2.17) were available to assess the effect size of management system with regards to vitamin E content in eggs. No difference in vitamin E content between eggs from OR and conventional FR system was found (Figure 2.16). A small positive value of the overall effect size was noted in favour of higher vitamin E content in OR eggs but this was not significant and the confidence intervals were large. One study included in analysis showed much larger and significant positive effect of management, while the other three studies reported negative or no effect. This inconsistency among data points led to slightly positive but not significant overall effect of management system on vitamin E content, with low precision exhibited as wide confidence intervals. Studies were from experimental trials and basket surveys, and only one study reported the timing of egg collection.

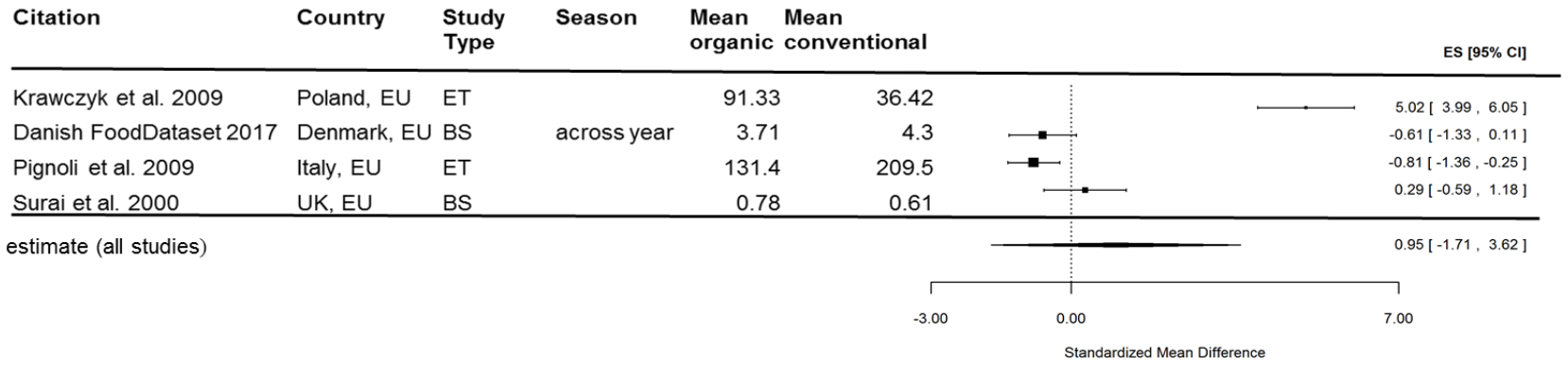

Figure 2. 16. Forrest plot of standardised mean differences (SMD) in vitamin E content in eggs from OR compared with eggs from conventional FR systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (Danish and Surai (µg/100g egg), Krawczyk (µg/100g yolk), Pignoli (mg/kg lipids)). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant.. I^2=98%; P value (Q statistics) <0.001

Similar imprecision was noted in the analysis on differences in vitamin E content between eggs from OR and indoor system (Figure 2.17). While majority of calculated effect sizes showed small effect magnitude around null-effect line, one study with a large negative effect size was found. The overall effect size was therefore imprecise and with a negative value, suggesting lower content of vitamin E in OR eggs compared with eggs from indoor systems. Studies used were from all types of studies, with experimental trials being the majority. Five studies reported the egg collection timing.

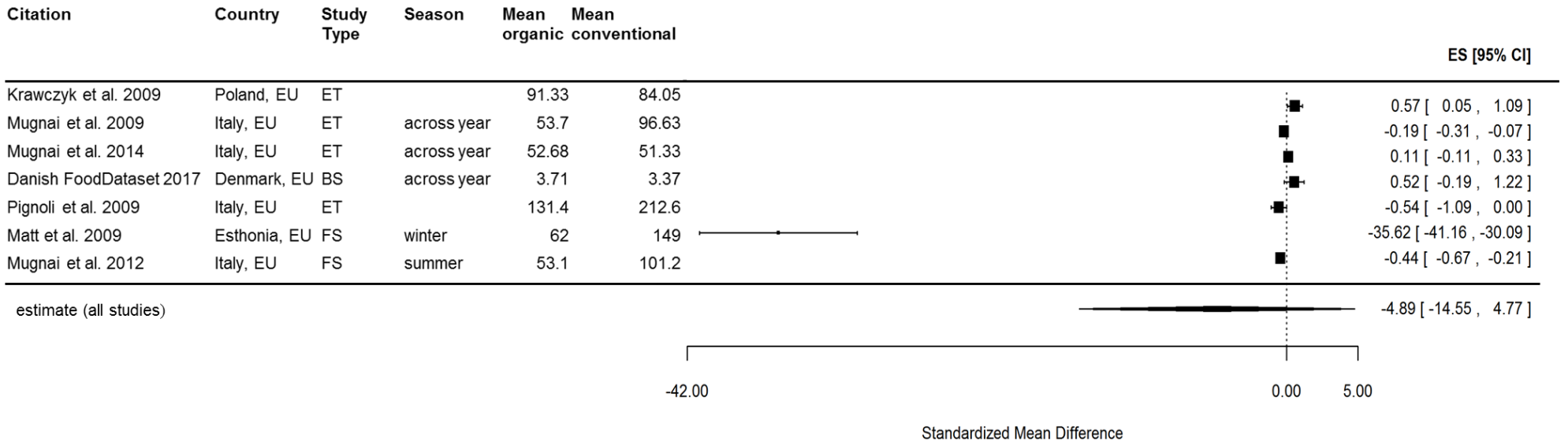

Figure 2. 17. Forrest plot of standardised mean differences (SMD) in vitamin E content in eggs from OR compared with eggs from indoor systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (Danish (µg/100g egg), Krawczyk, Matt (µg/100g yolk), Pignoli (mg/kg lipids), Mugnai (mg/kg yolk)). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2>99%; P value (Q statistics) <0.001.

2.4.2.5. Cadmium

Six data points for the comparison between OR and conventional FR system eggs (Figure 2.18) and 7 data points for the comparison between OR and indoor system eggs (Figure 2.19) were used to calculate difference in cadmium content in the standard weighted meta-analysis. Data points from the Irish report (2004) had to be excluded from both datasets, as a measure of variation could not be calculated. Only two data points for the outdoor comparison and one data point for the comparison between OR and indoor systems reported the sampling season and therefore this is not reported here. For both comparisons, data points were equally from basket and farm surveys. The product type either whole egg or yolk for which the result was associated is also reported. The majority of the results were reported on a whole egg rather than yolk basis.

Figure 2.18 shows that the egg cadmium content was similar in OR and conventional FR eggs, with a slight positive direction and narrow confidence intervals. Half of the studies reported no effect whereas two reported positive effects and one a negative effect meaning higher or lower cadmium content in OR systems respectively. The weights of all data were similar and the precision generally high.

Figure 2.19 shows no overall effect with a negative direction towards OR eggs having lower cadmium content but with wide confidence intervals overall. One study (Kamal et al., 2016), showed a distinct negative effect with relatively low precision. Four studies showed no effect, whereas two showed a negative effect and only one a positive effect of management system. Weights of studies were relatively equal across data points except the mentioned distinct data point.

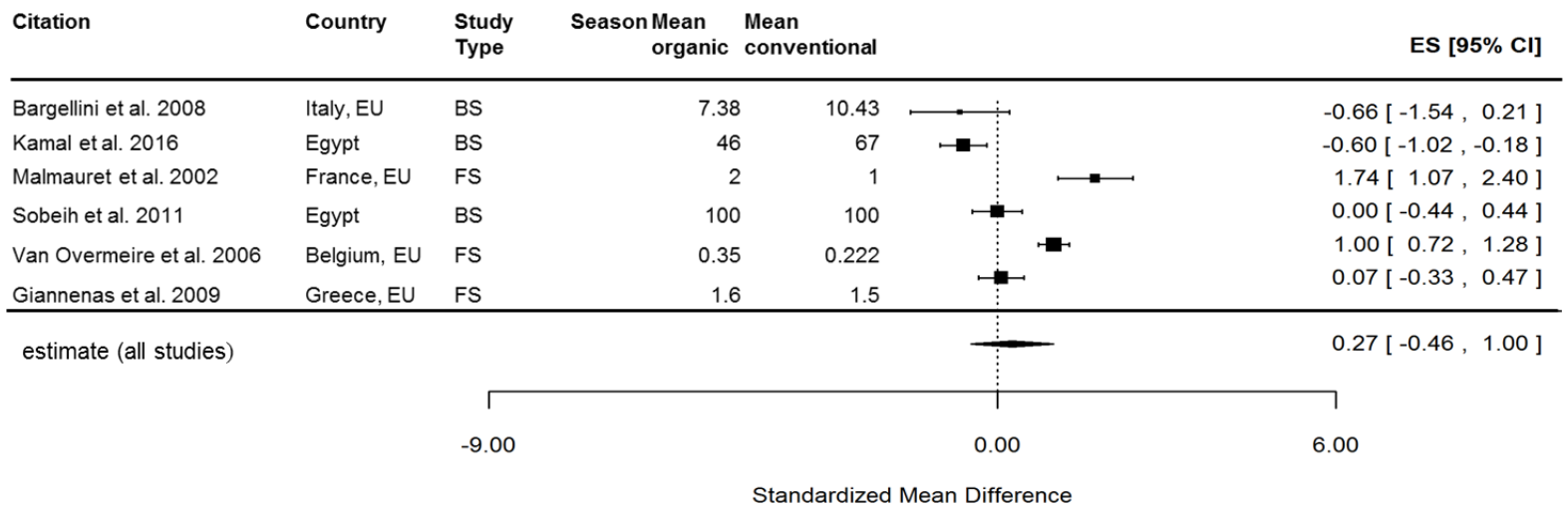

Figure 2. 18. Forrest plot of standardised mean differences (SMD) in cadmium content in eggs from OR compared with eggs from conventional FR systems. . Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (Van Overmeire, Sobeih, Malmauret and Giannenas (ug/kg fresh weight), Kamal and Bargellini (ug/kg dry weight)). Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=94%; P value (Q statistics) <0.001

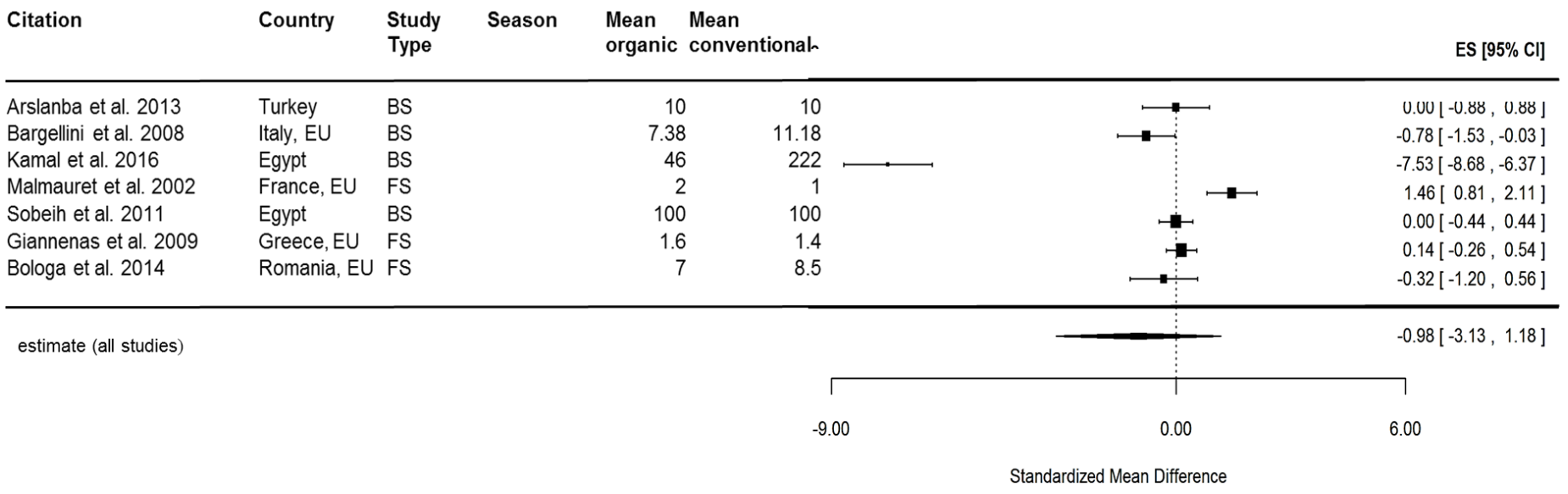

Figure 2. 19. Forrest plot of standardised mean differences (SMD) in cadmium content in eggs from OR compared with eggs from indoor systems. Data points are reported as their respective publication reference reporting the country of sampling, study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Mean values of organic and conventioanl treatments are also reported (Van Overmeire, Sobeih, Malmauret, Giannenas, Arslanba and Bologa (ug/kg fresh weight), Kamal and Bargellini (ug/kg dry weight)).Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=99 %; P value (Q statistics) <0.001.

Table 2. 2. Estimates of Effect size (ES) expressed as standardized mean differences (SMD) unless stated as mean difference (MD), with 95% confidence intervals (CI) and number of data points used in meta-analysis (n) gathers all estimates of effect sizes of the main meta-analysis.

Table 2. 2. Estimates of Effect size (ES) expressed as standardized mean differences (SMD) unless stated as mean difference (MD), with 95% confidence intervals (CI) and number of data points used in meta-analysis (n)

	OR – FR			OR - Indoor		
Outcome	ES	CI	n	ES	CI	n
Docosahexaenoic acid	0.71	(0.05, 1.37)	10	0.61	(-0.28, 1.50)	14
Omega-3 fatty acids	0.58	(0.06, 1.10)	11	0.29	(-0.12, 0.69)	17
lutein and zeaxanthin	2.68	(1.78, 3.57)	8	2.13	(1.14, 3.11)	10
Lutein	0.22	(-2.60, 3.04)	3	0.73	(-0.90, 2.36)	4
Zeaxanthin	x			1.2	(-1.96, 4.35)	3
β-carotene (MD)	0.12	(0.34, 0.58)	6	0.22	(-003, 0.46)	6
Sum of carotenoids (MD)	x			2.38	(-4.9, 8.95)	4
Vitamin A	-0.69	(-1.91, 0.53)	3	-3.52	(-9.18, 2.15)	4
Vitamin E	0.95	(-1.71, 3.62)	4	-4.89	(-14.55, 4.77)	7
Cadmium	0.27	(-0.46, 1.00)	6	-0.98	(-3.13, 1.18)	7

2.4.3. Effects of study type, season and genotype

Information on the study type, such as basket study (BS), farm survey (FS) or experiment trial (ET), was recorded for every publication included in the meta-analysis, allowing the data to be used as a moderator in the regression grouping model. However, because the analysis requires at least two levels of the moderator in one assessment, the analysis including study type as a moderator was only possible for DHA, n-3 FA, lut/zea, β-carotene and cadmium. With regards to fatty acids (both DHA and n-3 FA) and the comparison between eggs from OR and conventional FR system, FS and ET studies were only represented by 2 data points each (Appendix B3, Figure B3.1-2) and only the two FS studies had similar ES to the main meta-analysis. When comparing OR with indoor systems, all study types showed a statistically similar ES (Appendix B3, Figure B3.3-4). The sum of lut/zea and cadmium showed that BS and FS studies had broadly similar results with the main meta-analysis (Appendix B3, Figure B.3.5-7).

Only a few studies reported the egg collection season and therefore the use of season as a moderator was limited. With regards to comparison of OR to conventional FR eggs, for both DHA and n-3 FA there were 3 data points for summer and 2 data points for winter (Appendix B3, Figure B.3.8-9). In both cases when season was used as a moderator the measure of heterogeneity were lower for each subgroup of data points included. The effect size for summer was closer to the overall effect size of the standard meta-analysis and although winter was

represented only by two data points, a trend for a smaller positive effect size in winter was noticed. Similar patterns were observed for egg content in DHA and n-3 FA when OR eggs were compared with those from indoor systems (Appendix B3, Figure B.3.10-11). A comparison between OR and conventional n-3 FA egg content, including both FR and indoor systems, was also explored. Five data points for winter and 7 for summer showed a positive effect size for summer and a null effect for winter (summer CI: 0.44, 1.75; winter CI: -0.50, 1.09).

Lutein and zeaxanthin (lut/zea) egg content in OR eggs compared with indoor systems eggs only in summer, the overall estimate was positive suggesting more of those antioxidants in OR eggs, similarly to the result from standard meta-analysis (Appendix B3, Figure B.3.12). When season was used as a moderator the measure of heterogeneity was lower but with a P value > 0.1. With regards to total carotenoids content, similar to the standard meta-analyses, a null effect was noticed for the indoor comparison but with a negative direction for the effect size, especially for summer eggs analysis (Appendix B3, Figure B.3.13). With regards to the β-carotene of eggs, a meta-analysis was only possible including summer as a moderator and the results were similar to the standard meta-analysis, with a null effect in both types of comparisons and a positive direction (Appendix B3, Figure B.3.14-15). With regards to vitamin E and the comparison between OR and indoor systems, no difference to the main meta-analysis was noticed when season was included as a moderator, with large confidence intervals in both seasons and a stronger negative direction in winter (summer CI: -16.29, 15.88, 4 studies; winter CI: -27.76, 4.62, 4 studies, data not shown).

2.4.4. Effect of other sources of variation

Heterogeneity among studies was high (I^2>75%), for all compositional parameters and the P values for heterogeneity statistics were below alpha 0.05. Sensitivity analyses designed to identify the effect of different inclusion criteria and data handling methods yielded results broadly similar to the standard meta-analyses.

The sensitivity analyses conducted to identify the effect of excluding influential points from the analyses were designed to exclude the most influential study for each parameter and comparison. The results of meta-analysis after exclusion of the study by Filipiak–Florkiewicz et al. (2017), showing large positive effect of the OR management on DHA content in eggs, were similar to the standard meta-analyses (Appendix B3, Figure B.3.16). Exclusion of the study by Schlatterer and Breithaupt (2006) resulted in an overall similar, non-significant effect of management system on lutein content in eggs, although the trend towards positive direction changed to a negative one (Appendix B3, Figure B.3.17). With regards to cadmium, excluding

one study with a very high positive effect for the FR comparison and a study with a very high negative effect size for the indoor system comparisons had no statistical impact on the overall estimations (Appendix B3, Figure B.3.18-19). However, the negative trend, when OR eggs where compared with indoor system eggs, shifted to a slightly positive one.

The sensitivity analyses to identify the effect of excluding studies for which the imputation method was used to calculate missing values resulted in similar results to those from the standard meta-analysis. In the analysis of DHA and n-3 FA content for both comparisons between FR systems and indoor systems, excluding three studies did not change the direction of the overall effect size but did slightly increase the value of estimation (Appendix B3, Figure B.3.20,21). Concerning the n-3 FA, no change to the overall effect size and the relevant direction was noticed. Concerning the lut/zea, lutein and vitamin A content within the OR vs indoor systems comparison, excluding one data point in each analysis by Schlatterer and Breithaupt (2006), Pignoli et al. (2009) and the Danish FOODDATA respectively had no impact on the overall effect size or its direction (Appendix B3, Figure B.3.22-24).

Sensitivity analyses in which alternative (backyard, barn or mixed) management systems were excluded yielded similar results to the standard analysis on DHA and n-3 FA content. Excluding one study point with a rather neutral effect size (Samman et al., 2009) (mixed systems) across both comparisons and for both DHA and n-3 FA did not impact on the magnitude of effect size or its direction. For lut/zea, excluding data points by Van Ruth et al. (2013), included in both comparisons as the eggs were pooled from both outdoor and indoor systems, had no impact on the effect size of the meta-analysis of the OR to FR systems (Appendix B3, Figure B.3.25). However, for the OR vs indoor systems comparison, the meta-analyses of the 3 remaining studies resulted in a not significant difference between systems, with a positive direction of the effect size (Appendix B3, Figure B.3.26). For vitamin A and E content, no significant differences were noted when the study by Krawczyk (2009) (barn system) was excluded from the comparison of OR vs. indoor system. However, the magnitude of the negative effect of management system (for comparison of OR vs. indoor) on vitamin A in eggs slightly increased (Appendix B3, Figure B.3.27). No significant differences of the effect size were noted concerning egg cadmium content when data points were excluded within the outdoor (Kamal et al., 2016, Malmauret et al., 2002) and the indoor (Bargellini et al., 2008, Malmauret et al., 2002) comparison with the not significant effect remaining in both cases (data not shown).

Sensitivity analysis in which studies of lower quality were where excluded, was conducted only for DHA, n-3 FA lut/zea and b-carotene egg content. Concerning DHA egg content in the comparison between OR and FR, excluding 6 out of 10 studies resulted in a non-significant difference between systems in contrast with the main meta-analysis and larger confidence

intervals with a positive direction (Appendix B3, Figure B.3.28). Within the comparison of OR vs indoor systems, excluding 6 out of 14 studies had no significant difference on the effect size (Appendix B3, Figure B.3.29). Similar results were shown for n-3 FA, excluding 7 and 11 studies respectively (Appendix B3, Figure B.3.30, 31). Sensitivity analyses for lut/zea yielded the same results as in the sensitivity analyses for alternative systems when OR systems were compared with conventional FR (Appendix B3, Figure B.3.25) and similar results to the main meta analyses when excluding 7 out of 10 data points (Appendix B3, Figure B.3.32). Similar results to the standard meta-analyses yielded the sensitivity analyses for β-carotene within both comparisons (Appendix B3, Figure B.3.33, 34).

Substantial funnel plot asymmetry (in some cases even non-funnel shaped) was detected for all parameters investigated in the standard meta-analyses (Appendix B3, Figure B.3.35, 36).

2.5 DISCUSSION

At this point in time this is the first meta-analysis focusing on the nutritional differences between OR and non-OR eggs and the number of qualifying papers was disappointing. Results reported in other meta-analyses looking into OR and non-OR food commodities showed higher n-3 FA and lower SFA in OR meat, cow's milk, as well as higher polyphenols and lower cadmium content in OR crops (Baranski et al., 2014, Srednicka-Tober et al., 2016a, Srednicka-Tober et al., 2016b). Similarly, results reported in this study indicated that there are some nutritionally related differences between OR and non-OR eggs. However, variability between available data found in this study indicated the overall weakness of evidence and hence resulted in uncertainty of outcomes.

Results of the present study indicated that OR eggs contained more DHA than conventional FR eggs, and similar amount of DHA when compared with eggs of hens kept in indoor systems. As DHA accounts for 40-50% of the total n-3FA (Table 3.2, Table 4.3), the overall profile of n-3 FA is highly dependent on the DHA content, following the same relationship with DHA. Additionally, the sum of lutein and zeaxanthin content, accounting for more than 80% of the total egg carotenoids was higher in OR eggs than in eggs from FR and indoor systems. However, not all carotenoids showed the same pattern and eggs from OR and indoor management systems did not differ in total carotenoid content. Finally, cadmium content was similar within eggs of all origin.

Since the available data within the literature from controlled studies exploring nutritionally relevant parameters was neither in large quantity nor consistent, the objective of this study evolved to elucidate any consistent differences or patterns in the composition of eggs originating from OR versus both conventional FR and indoor systems. For n-3 FA and DHA

11 and 17 data points were used respectively, whereas for carotenoids this was only between 3 and 10 data points. Finally, cadmium content comparisons were based on 6 and 7 data points for the outdoor and indoor systems respectively. In general, variation across studies was evident. Concerning the seasonal variation and its impact on the effect size when used as a moderator, season might have been greater if more studies reported the season of egg sampling and therefore increase the sample sizes.

The following discussion focuses on the outcomes in relation to (a) the current knowledge about differences in management systems both in egg production but also in feed composition between OR and non-OR ingredients, (b) the strength of evidence for each parameter and the respective heterogeneity, (c) the nutritional relevance of the results and (d) the need for more robust data which could expand current body of evidence.

2.5.1. Links between layer management and egg composition

Previous research has shown feed composition is the most influential factor that affects egg composition. It was also suggested that enrichment of the layers diet in just one compound is a very efficient way to increase the egg's content of that compound, when talking about fatty acids, carotenoids (Hammershøj et al., 2010, Fraeye et al., 2012) or even heavy metals like cadmium (Bokori et al., 1995). At the same time, foraging in FR systems has been shown to increase the nutritional profile of eggs in terms of n-3 FA and carotenoids content (Skrivan and Englmaierova, 2014, Mugnai et al., 2014).

FR egg production standards, either OR or conventional, require that layers have access to outdoors and hence allowing flora and fauna consumption to play a significant role in egg composition. On the contrary, CA hens stay exclusively in furnished cages under controlled environmental conditions (European Council, 1999/74). However, even within FR systems, standards differ between OR and conventional management. Organic standards demand for example, lower indoor and outdoor stocking densities than conventional FR standards (European Commission, 2008b). As such, the outdoor ranging behaviour is not only strongly stimulated but also complemented with a richer outdoor environment in forage and cover protection (Soil Association, 2019). Therefore, differences in forage consumption between OR and conventional systems are likely to drive some systematic differences in egg composition and it will further be discussed as part of the differences in feeding regimes and seasonality.

Feeding regimes

Dietary requirements for layers mean that a high protein diet is needed to maintain constant egg production. Furthermore, the overall dietary and energy requirements for the often-used commercial lines of layers are well established. Therefore, compound layer feed would be fairly

similar in composition, including notably soybean and various cereals (e.g. wheat and oats) and other oilseeds (sunflower) in both systems. On one hand, since the use of solvent extracted feeds are not permitted in OR diets, higher residual oil content in OR compared with conventional feeds is expected (extracted soya =2-3% lipid vs ~5% in expeller soya). Soya beans are very high in linoleic acid (C18:2, LA), the main omega-6 FA in yolk. As a result, it would be expected that OR compound feed, having a higher proportion of this group of FA, potentially could change the n-3 FA proportion in the egg as well. On the other hand, soybean in OR feed is likely to be used less, as OR, non-genetically modified soya is not only scarcer but also considerably more expensive than other protein sources. Unfortunately information on dietary FA composition and feed intake of the studies used in this meta-analysis was generally not available. Data on compound diets' FA profile used in OR and FR systems found in Chapter Three, Table 3.1 indicate indeed that even though OR dietary intake is not significantly higher in LA, it also to be higher in n-3 FA.

Forage consumption itself and overall OR management has been reported to increase n-3 FA content in milk (Srednicka-Tober et al., 2016b). Similarly for egg quality, since pasture plants, insects and other invertebrates are consumed while layers forage outdoors, these can potentially be an important source of supplementary n-3 FA and carotenoids in the diet (Hammershøj and Johansen, 2016, Kouřimská and Adámková, 2016). Systematic differences in egg composition indicated by this meta-analysis could be explained by the highest foraging behaviour in OR system among all the systems considered. Within the FR comparison, meta-analysis results suggest that both DHA and total n-3 FA concentrations were higher in OR eggs. Despite taking into account the substantial heterogeneity between studies and the estimated effect size only marginally reached statistical significance, it can be concluded that there is a trend towards nutritional superiority of the OR eggs in comparison to eggs from FR system in terms of FA profile. Layers in OR systems, not only have a larger and more enriched outdoor ranges but, as mentioned, due to the lower indoor stocking density it is more likely for them to forage outdoors (Gilani et al., 2014). Similarly to results presented here, Srednicka-Tober et al. (2016a) reported higher n-3 FA in OR poultry meat compared with meat from conventional systems but no difference for individual n-3 FA. Since conventional systems have no minimum standards concerning flock size, as long as the stocking density is less than 9 hens per m^2, FR could in theory reach the housing and ranging standards of OR systems, although commercially it is possibly unlikely. Additionally, with the maximum OR colony of 3000 hens and minimum of $4m^2$ range per layer, the flock size is inversely related to egg n-3 FA, as shown on the redundancy plot in Chapter3, Figure 3.7, agreeing with Mugnai et al. (2014a) who report the higher the foraging area the higher the n-3 FA content. Given this, it would be unlikely to expect a lower n-3 FA content in OR compared with FR eggs, and this is confirmed in this meta-

analysis in which almost all studies (except one of low precision) confirm this direction. In the comparison of OR with indoor systems, although there was a similar positive direction when OR was compared with FR systems, there was no significant difference between the systems. Although it might be expected that the difference would be more prominent since there is no forage consumption in indoor systems, around one third of the studies showed a similar n-3 FA content between the systems. Another third showed a statistical positive effect size of OR, whereas the last third of the studies, showed a negative direction for OR eggs which was either statistically significant or with no significant effect. Overall, compared with the results of the meta-analysis of OR vs conventional FR systems, there were more studies included but they were more variable with larger confidence intervals. The comparison of n-3 FA content between OR and indoor systems will further be discussed in relation to season.

The carotenoid content in cereals is reported to be similar for OR and conventional crops (Baranski et al., 2014). Therefore, higher dietary intake through more intensive forage material consumption could explain the results of this meta-analysis since carotenoid supply from cereals in the diets would be expected to be similar. Indeed, most data points from papers on carotenoids content of eggs indicated significantly more lutein and zeaxanthin concentrations in OR eggs in both comparisons, despite heterogeneity among the studies. It has to be noted though that most studies belonged to the same author which potentially could introduce bias. Yet the fact that the data points came from different countries should make them independent measurements, as discussed in the data management earlier in this Chapter. Additionally, since the sum of lutein and zeaxanthin concentrations constitutes the largest fraction of the carotenoid content in eggs (Bunea et al., 2017) this result could be used to represent the trend in the total carotenoid content in eggs from different systems. Lutein content by itself was analysed in too few studies, whereas it was only possible to complete a meta-analysis on zeaxanthin and the sum of these and total carotenoids to compare OR with indoor system eggs. Within the comparison of zeaxanthin, for the two studies reporting either no difference or a negative effect size (Mugnai et al., 2012, Mugnai et al., 2014), the OR systems had a quite intensive approach, potentially explaining the lack of effects of OR management. There is a need for more studies which separate these compounds as some authors suggested that zeaxanthin content is actually much higher in OR compared with conventional FR and CA eggs (Schlatterer and Breithaupt, 2006). Considering β-carotene, all 6 data points came from 2 studies by the same group (van Ruth et al., 2011, Van Ruth et al., 2013), one of which was used in both comparisons (Van Ruth et al., 2013) as eggs from conventional systems were pooled. Therefore, taking into account that β-carotene is found in eggs at relatively low concentrations, differences coming from other carotenoids between management systems should be more influential for the direction of the overall estimated effect. Overall, there is a need for more data to allow conclusions to be made

on β-carotene content of eggs with any confidence. In all cases, given the small number of studies and poor precision the results for the sum of these carotenoids should be interpreted cautiously Similarly, meta-analysis on vitamin A content in eggs, for both comparisons, were carried out with too few studies.

Wider confidence intervals of the effect sizes calculated for the OR compared with indoor system eggs were noticed for two studies reporting much lower vitamin A content in OR eggs (Fooddata DK, Matt et al., 2009). Matt et al. (2009) used eggs coming from one single diet formulation for OR and another diet formulation for conventional systems. The comparison of vitamin E content between OR and FR system eggs showed no difference between these two management systems. Additionally, when sensitivity analysis was conducted after removing the study with the large positive effect size (Krawczyk, 2009), no significant differences to the standard meta-analysis were noted. Finding that there were no significant differences between systems for both vitamin A and E, suggests also either that not all hens from conventional systems received high vitamin supplements or/and that forage or invertebrate consumption in the conventional FR systems is able to compensate for the dietary supplementation in conventional housed systems. On the other hand, current poultry diet formulation is precisely tailored, and the vitamin requirements of the birds are normally addressed in modern systems.

Regarding cadmium content in eggs and feeds, as mentioned this metal is expected to be lower in feed based on OR crops, as reported in previous meta-analysis by Baranski et al. (2014), probably linked to differences in fertilisation between OR and conventional agriculture. However, as shown here, there was no difference in Cd content of eggs between systems. In both comparisons, OR versus conventional FR and OR versus indoor system, cadmium contents in eggs were not different, even when influential studies were excluded at sensitivity analyses. On the other hand, within the OR vs. FR system comparison greater variation of the direction of the effect size was noted, and availability of the information on soil quality could help to elucidate these differences. The large negative effect size in the comparison of OR to indoor systems could also be linked to the feed composition, being an important driver for egg quality. In this meta-analysis half of the studies in each comparison included, reported that Cd was non-detected. While it is common for heavy metals, including cadmium, to be below the limit of detection (natural or industrial contaminants are fortunately in very low quantities under normal conditions), the use of these values in this meta-analysis is discussed below in the strength of evidence section.

Seasonality

Pasture availability and foraging behaviour of laying hens can vary upon season. For example, Chielo et al. (2016) showed that lower temperature and humid weather negatively affect the use of pasture. As a result, as long as the compound feed composition is constant across seasons, one would expect differences in the nutrient content of eggs due to pasture access to be less pronounced in winter than in summer. Moreover, sward composition and invertebrate activity will vary throughout the seasons, which has also been reported to affect foraging behaviour (Breitsameter et al., 2014). Additionally, it is also important to take into account that depending on the growth stage of pasture plants the saturation in carotenoids, FA and other components of pasture plants may vary (Mugnai et al., 2014).

Borenstein et al. (2009) advise to use heterogeneity (I^2 statistic) as a criterion to decide if a moderator analysis should be considered in a meta-analysis. In the case of this study which revealed high I^2 across all analyses, egg collection months were grouped into 2 seasons, winter or summer as described in material and methods (*2.3.4. Synthesis procedures and statistical analysis*). It is also important to keep in mind that depending on the specific climatic zone and microclimatic conditions, it is unlikely that pasture availability according to season is identical at all locations, even within Europe.

For all outcomes, few studies reported the egg collection season. For DHA and n-3 FA for both comparisons, meaningful results were obtained mostly for summer only. In all cases when season was used as a moderator, neither the direction nor the magnitude and the statistical significance of the effect size changed, compared with the standard meta-analysis. Given the fact that there were only 3 and 4 studies respectively for both comparisons (down from 10 and 14 respectively) the confidence intervals when season was included as a moderator, were slightly wider. However, in the case of n-3 FA looking exclusively into summer studies, the heterogeneity decreased from 94 to 71 % in the FR comparison and from 94 to 77 % in the OR vs. indoor system comparison. This might imply that seasonal variation could explain part of the overall variation, but yet more studies are needed to be able to draw definite conclusions. Sensitivity analyses using season as a moderator, did not change the direction or the magnitude of the effect sizes in any for the carotenoid outcomes. Similarly to FA sensitivity analyses, the data points included in analyses of carotenoids were very limited. Sensitivity analysis within the OR to indoor comparison, showed also a stronger trend towards OR eggs having lower content of vitamin E than indoor system eggs in winter than in summer. This pattern could imply interactions between systems and season. Assuming that in winter fresh forage or/and insect consumption is less than in summer, OR crops have a slightly lower content in vitamin E (Baranski et al., 2014) and conventional diets are routinely supplemented, the vitamin E

intake could be more consistent in indoor system eggs where supply of the vitamin is constant all year long. It would be interesting to see if this pattern is similar for the other outcomes, such as n-3 FA and carotenoids that are all found in forage material (although not routinely added to layer diets), but not enough studies report results for winter eggs. It was therefore not possible to conduct meaningful meta-analysis to consider season, except for n-3 FA which showed a small trend of being slightly lower in OR compared with indoor system eggs during winter. In this respect the unexpected overall results of the standard meta-analysis showing no differences in n-3 FA content between OR and indoor system eggs could partially be explained if the studies used in this meta-analyses were mostly conducted during winter months. As suggested for dairy cows and enhanced milk nutritional quality (Stergiadis et al., 2018) it will be important in the future to adapt layer genotypes to local environments for higher forage consumption.

Layer genotype and study type

Although for OR production the use of different genotypes from those used in conventional production is recommended, the commercial poultry management has been using genotype lines either brown or white, that have been extensively developed for intensive egg production (Leenstra et al., 2012, Leenstra et al., 2014). At the same time, studies used for this meta-analysis did not provide sufficient data on the genotypes. Even when trying to group the genotypes into brown and white lines, no sufficient data were available. On top of that, most of the European production uses brown line genotypes and since studies used here were mostly carried out in Europe, no balanced comparison could be expected from farm-based studies. Therefore, it was not possible to analyse and determine the effect of genotype on egg compositional parameters.

Some authors report that genotypes reared and kept organically have similar egg nutritional profile, suggesting it is actually the management that drives the differences not the genetics of the poultry varieties (Pintea et al., 2012). However, there were controlled experiments that showed consistent differences in both FA and carotenoid content of eggs between layer genotypes (Bunea et al., 2017). The genotype effect under OR and conventional systems should be further explored as there could be genotype and environment interactions.

In comparison with the results from the standard meta-analysis, grouping the data according to the type of studies did not seem to have a notable impact on the overall estimates, similar to the other meta-analyses on crops or meat (Baranski et al., 2014, Srednicka-Tober et al., 2016a). However, a trend that FS rather than BS or ET studies showing stronger differences in n-3 FA content in OR than FR system eggs is worth mentioning and it should be explored in relation

to the respective study designs. However, the studies and comparisons available to draw firm conclusions were very limited, so (as with others) this conclusion should be treated with care.

2.5.2. Strength of evidence and potential reasons for the heterogeneity of the data

Heterogeneity was high for all compositional parameters explored in this meta-analysis for eggs, in accordance with the meta-analyses on meat (Srednicka-Tober et al., 2016a). According to Ioannidis et al. (2007) heterogeneity (I^2) should not be considered as a measure of real variation of the true effects but rather as a measure of inconsistency across the findings. However, these authors also suggest that the observed variation incorporates both random error and true heterogeneity, highlighting that this measure is not directly affected by the number of studies in the analysis. As discussed, the intensity of layers management systems and foraging behaviour could explain compositional differences. However, the intensity of foraging behaviour could only be explored taking into account the regulatory standards and the seasonal differences within and across the outdoor or indoor system, whenever this information was available. The extensive period that studies were considered (>15 years), variation in agronomic practices along the years and geographic locations could all contribute to the observed heterogeneity.

Additionally, several sensitivity analyses were designed to explore the impact of different inclusion criteria and data handling methods on the magnitude and direction of the calculated effect size. These sensitivity analyses were only conducted if more than 3 studies were available. As results showed a few estimations were sensitive to variable inclusion criteria. The sensitivity analysis with the largest difference in the effect size compared with the main meta-analysis, was excluding the "lower quality" studies for DHA and n-3 FA when OR were compared with conventional FR systems. In these cases, whereas the standard meta-analysis showed a significant positive overall effect size for OR eggs, the statistical significance disappeared. However, more than 60% of the studies were excluded in both cases, leaving just 4 studies for each comparison used at the end. Taking into account that lower quality studies were rated with a broad range of quality criteria, from unpublished to underreported information, the loss of statistical significance in this case should be treated cautiously and the need of more studies with standardized study design and reporting protocol is highlighted. Excluding the most influential data point had an impact on the direction of the effect size in case of lutein and cadmium within the OR vs. indoor system comparison. Whereas the lack of significance remained, the direction changed to negative and positive for lutein and cadmium, respectively. However, the magnitude of the effect size in both cases (-0.10 and 0.09 respectively) was considered to be very small. Furthermore, as expected, excluding only one data point with small effect size and additionally, with eggs pooled from both indoor and

outdoor systems from the standard meta-analyses did not have a significant impact on DHA or n-3 FA results. On the contrary, the higher content of the sum of lutein and zeaxanthin in OR compared with indoor system eggs, lost its statistical significance when alternative systems (3 data points of barn systems and 4 data points of mixed systems) were excluded. Nevertheless, in this case, only 3 studies remained for the meta-analysis and therefore any conclusions drawn should take into account the uncertainty because of the low number of studies. This meta-analysis deals with the question in potential differences between OR and FR or indoor systems separately. Taking into consideration that consumers are exposed to, and asked to make a choice between all systems, it is important to report the compositional differences and conclude individually for each system. With respect to cadmium content, it is interesting to mention that it was not possible to explore the impact of using imputation methods on the overall result because the majority of the studies included not-detectable values and imputation methods were used to estimate the cadmium content. Management and statistical analysis of such *left censored data* (data points below certain value –in this case LOQ- although it is unknown by how much) is a well-known and complex uncertainty in dietary risk assessment. The approach of substituting the values below the LOQ have been previously suggested (EFSA, 2010a), yet with considerable bias. Similar to the treatment of data in this meta-analysis, EFSA (2010a) suggested that heterogeneity could be explored by using random effect modelling further than categorising the effects according to various parameters, such as country of origin. Although in this case there was not enough data to examine season, genotype or country effects, it would be interesting to consider including these explanatory data but only when observations under identical conditions have been made for both OR and conventional systems. This might also increase the sample size of studies included and increase the validity of the reported result.

2.5.3. Potential nutritional impact of composition differences

Omega-3 FA

During the last decades, omega-3 FA intake has been linked to various benefits for human health including protection against CVD and CHD (Adkins and Kelley, 2010, Simopoulos et al., 2004), as well as brain development (Ryan et al., 2010) and cognitive function during ageing (Cardoso et al., 2016). However, a recent meta-analysis concluded that ALA may only slightly reduce risk of CVD events and evidence supporting long chain FA reducing CHD events is low quality (Abdelhamid et al., 2018). Taking into account that egg consumption can be a significant contributor of n-3 FA (Givens, 2009) in parallel with the latest reports that dietary cholesterol through egg intake and its link to CVD is nowadays questioned, it is important to assess the potential impact and differences on the omega-3 dietary intake between consuming OR and conventional eggs.

This meta-analysis showed that the effect of OR, rather than conventional outdoor management not only significantly but also considerably increased DHA content in eggs. According to a generic approach the SMD value of 0.2 is considered to be small, 0.5 medium and 0.8 large (Koricheva et al., 2013). Here a medium effect size was noticed for total amount of n-3 FA in eggs, where ALA and DHA were the biggest contributors in total n-3 FA content. Taking into account that DHA can be generated from dietary ALA since humans are capable of elongating ALA to DHA at low rates (Burdge and Calder, 2005, Childs et al., 2008), the possibility of increased long chain n-3 FA intake through a change to consumption of OR eggs which would contribute towards EFSA's recommended intake of EPA and DHA (EFSA, 2010b) is demonstrated. On the other hand, comparing OR with indoor system eggs, a similar statistical significance favouring OR eggs was not shown. Moreover, even though there was a trend towards higher DHA and n-3 FA in OR eggs, the effect sizes were medium and small respectively. Thus, whether consuming OR eggs could result in health-related changes in n-3 FA intakes is uncertain and requires further investigation.

Carotenoids and vitamins A and E

Lutein and zeaxanthin represent most of the carotenoid content in hen's eggs. It is well established not only that these carotenoids pigments accumulate in the macular region of the retina and are protective towards macular degeneration but that they are also the predominant retinal carotenoids (Zaheer, 2017, Abdel-Aal et al., 2013, Karppi et al., 2012, Sideri et al., 2019, Wang et al., 2007). Taking into consideration that egg consumption has the ability to raise lutein and zeaxanthin concentration in plasma (Chung et al., 2004, Handelman et al., 1999, Kelly et al., 2014), the results of this study could be quite relevant to human health. Large and constant magnitude of effect sizes favouring OR eggs should not be overlooked and should be taken into account when discussing nutritional policy and health, especially for the ageing population. β-carotene was shown to be statistically higher in OR compared with FR system eggs, despite the effect size being rather small (0.12) and the absolute content in eggs is rather limited. Hence there is probably no meaningful impact of these differences in β-carotene content on human health regarding any differences found between eggs. Concerning egg content of vitamin A and E, there were too few data points to draw conclusion on the potential impact of the management system. Although a large negative trend was shown favouring indoor system eggs, the result was determined by a single study and hence no firm conclusions can be drawn either.

Cadmium

Cadmium is well known to be toxic to the kidney and liver, and its cancerogenic potential has also been demonstrated (EFSA, 2009a). Its bio-accumulative nature and the suggested recommendations on lowering intake (EFSA, 2009a, Satarug et al., 2017, EFSA, 2012) have to

be taken into account when assessing the potential nutritional impact of choosing eggs from different management systems. The results showed that although none of the comparisons found statistically significant differences, the meta-analysis did find a higher cadmium content in OR rather than FR system eggs. Even though a low effect size was found, it should be further investigated with more and of higher quality studies. A more pronounced trend favouring a lower cadmium content in OR rather than indoor system eggs was also highlighted. The real impact of these trends should be further explored upon reception of more data and any conclusions cannot currently be drawn firmly. At the same time it is known that hens can accumulate various contaminant from feed and soil intake in eggs (e.g. persistent OR pollutants) (Kan and Meijer, 2007, Domingo, 2014). Ideally, if more and higher quality data are acquired in the future, the comparison should be done on absolute values of cadmium in order to be able to discuss any differences within a perspective of clinical significance.

More data on egg contaminants across management systems should also be generated to be able to conduct meta-analyses.

The current high level of consumption of saturated fatty acids, found in high proportions in eggs, is considered undesirable and linked to cardiovascular and heart disease, while it is commonly suggested that these fats should be replaced with unsaturated FA (Jakobsen et al., 2009, Briggs et al., 2017). However, although it has been suggested that saturated FA content in eggs cannot be markedly manipulated (Kuksis, 1992), slight changes depending on the saturated FA profile of the feed have been reported (Oliveira et al., 2010). Since egg is rich in multiple fat-soluble nutrients, identifying alternative approaches to increase the egg nutritional composition, balancing at the same time the contaminants, with a risk-benefit approach should be considered.

Overall, this study indicates that layer management can potentially change the contents of FA, carotenoid and potentially some other nutrients in eggs. Results from epidemiological studies showed positive associations between OR milk consumption and lower eczema risk in young children (Kummeling et al., 2008). If differences in egg composition are nutritionally relevant it could justify conducting human intervention or cohort studies designed to examine the impact of switching egg consumption to those from OR production.

2.5.4. Deficiencies in the evidence base

Similarly to the limited datasets for meat composition reported by Srednicka-Tober et al. (2016a), the datasets available for egg compositional parameters, except for DHA and n-3 FA, were limited. Furthermore, for the majority of the studies included in this meta-analysis but also for those excluded, deficiencies were identified. In general, bad reporting (mainly measures of

variation and replication) and lack of standardised measurements were identified, potentially leading to bias due to low quality evidence. A lack of detailed information on time of sampling, country and region of origin, genetics of layers (for field surveys and experiment trials) that could further explain the variation was also noticed.

The high funnel plot asymmetry and actually in most of the cases the lack of funnel plot shape for all parameters interconnects with the equally high heterogeneity, although these results should be interpreted with caution as the statistical tests ought to be done with 10 or more studies, and since the weight of the study greatly affects the plot asymmetry (Sterne et al., 2011). Overall it should be noted that the funnels plots conducted within this study suggest that the outcomes of this meta-analysis should be interpreted cautiously as there is a risk that they were calculated in a biased way not completely representing the relative systems.

The low statistical power for most parameters and high heterogeneity did not allow for a good reliability of the studies included. However, for some parameters the overall effect size was large and also accompanied with a high precision hence statistical significance. The addition of data from well-designed studies would potentially allow better understanding and use of further covariates to explain the between study heterogeneity.

At this point, it should be noted that some of the studies used within this meta-analysis made their analyses on pooled egg samples. That means that the derived standard deviation was reported as per pooled sample. At the same time the sample size chosen to calculate the effect size was as described in the material and methods was not the pooled sample but the readjusted individual egg count. Therefore it is clear that the units of the measure of variance and sample size used to calculate the effect size was different. It is possible that the effect of the incoherence between units to calculate the SMD, could change the calculated effect sizes and hence the conclusions for each outcome, which should be highlighted as a weakness of the study. Ideally, analysing individual samples would solve this incoherence, but the resources to do so were limited. Alternatively an advanced mathematical way to adjust the pooled standard deviation to reflect the individual egg count would consist of adjusting the calculated standard deviation of pooled egg analyses according to the number of eggs pooled per replicate (Caudill, S. P., 2010). However, in the framework of this thesis such adjustments were not possible. Future analyses reporting measures of variance on pooled samples should consider to adjust the outcomes taking into account the total number of samples used to make up the pooled sample.

Therefore, not only more studies are required to increase statistical power, but also data from studies conducted according a standardised reporting protocol. Furthermore, for the advancement of the field, future studies should also take into account the aforementioned methodology on adjusting standard deviations when analyses are conducted on pooled samples.

Overall, it is important to develop recommendations for conducting and reporting comparative studies on food compositional parameters in order to minimise and explain heterogeneity.

2.6 CONCLUSION

This is the first meta-analysis investigating the effect of OR compared with conventional management system on the egg nutritional composition including DHA, n-3 FA, lutein, zeaxanthin and the sum carotenoids, vitamin A, E but also on the heavy-metal cadmium. The aim of this meta-analysis was to estimate the effect and the combined effect size and the respective confidence intervals, while trying to explain the reasons of this variance. The evidence base was generally weak with limited studies for the selected outcomes. Significant differences between OR and conventional eggs were determined for fatty acids and carotenoids. Both DHA and n-3 FA were higher in OR compared with FR eggs but not when compared with indoor eggs. Lutein and zeaxanthin were higher in OR eggs compared with either FR or indoor eggs. No differences were detected for vitamin A, E and cadmium content of eggs. For all parameters included, heterogeneity was high. Meta-analysis using moderators such as season to explain heterogeneity were similar to the original meta-analysis results but studies included were very limited. More studies of standard high quality are required to draw stronger conclusions and improve precision of the results.

CHAPTER 3. EFFECTS OF FREE-RANGE MANAGEMENT, SEASON AND GENOTYPE ON EGG FATTY ACID COMPOSITION

3.1 SUMMARY

Demand for free-range eggs has progressively been growing. Organic and conventional free egg production systems are both sharing the mandatory access to outdoor area for hens to roam. Forage intake while ranging, can change the egg fatty acid content found in the yolk and omega-3 fatty acids, that can be found in leafy greens and insects within pasture are beneficial to human health. Hence, an increase in the relative human intake by means of an egg profile higher in the relative fatty acids is worth investigating. The production parameters such as feed composition and outdoor area standards of organic and conventional free-range systems that correlate with higher forage material intake and hence, egg omega-3 fatty acid content can be different between the two systems. Additionally, seasonal variation and hen genotype selection can lead into variations in the final egg fatty acid content. This study is investigating the effects of free-range systems, season and genotype on egg fatty acid composition. Eggs were collected from thirteen flocks of white and brown egg colour hen genotypes of organic and free-range systems in Switzerland. Eggs were collected twice, once in winter and once in summer, where summer was successive to winter as far as flock age is concerned. Five pooled samples were collected through summer and winter, along with one sample of feed of each system and a farmer's questionnaire relative to egg production parameters. Data and eggs were analysed for egg production, feed and egg fatty acid content. Organic eggs were higher in total omega-3 fatty acids (+49%) and long chain fatty acids (+31%). Seasonal variation of omega-3 fatty acids was confirmed but contrary to what expected, higher omega-3 fatty acids were noticed in winter. Eggs from brown genotypes had lower saturated fatty acids. This study provides evidence that in Swiss free-range systems egg fatty composition could be improved by adopting organic practices without compromising egg production.

Keywords: farm survey, egg composition, organic, free-range, season, genotype, fatty acids,

3.2 INTRODUCTION

Consumers' perception of animal welfare, health and environment associated with free-range (FR) eggs and outdoor ranging (Weeks et al., 2016b), in parallel with the EU Directive of European Council (1999/74) to ban cages, has shifted demand towards cage free eggs in several EU members and the UK (Scrinis et al., 2017). Additionally, the perception of organic (OR) food being more nutritious (Lee and Yun, 2015) has also increased demand for specialty eggs within FR systems. Yolk lipid can substantially contribute to human intake of omega-3 fatty acids (n-3) which are important for human health (Givens and Gibbs, 2006) and its fatty acid (FA) composition can easily be modified by manipulating the hens' diet (Fraeye et al., 2012). Besides the contribution of the FA profile of the feed offered to the overall egg FA composition, FR poultry can also take up additional nutrients through consumption of forage material and invertebrates present on the range. Intake of forage material has been well documented not only to increase the n-3 FA in cow's milk (Stergiadis et al., 2012, Butler et al., 2008) but a similar deposition has also been suggested for FR eggs (Anderson, 2011, Lopez-Bote et al., 1998). However, the latter relationship has been reported with some controversy in the literature (Mugnai et al., 2014, Lordelo et al., 2017).

While OR food is regulated under strict standards, FR systems are not extensively regulated and may vary considerably and therefore it is relevant to investigate nutritional differences in egg FA profile between conventional FR and OR FR systems. Any feed offered to OR hens has to be produced to OR standards, prohibiting genetically modified and solvent-extracted ingredients in addition to the use of ingredients produced without the use of pesticides and synthetic fertilisers, and so could substantially differ from that offered to conventional FR flocks. In addition, outdoor and indoor stocking density, flock size and the outdoor environment overall are other management features that can vary considerably according to European standards (European Commission, 2008a, European Commission, 1991) between conventional and OR FR systems. Although both systems allow hens outdoor access, in OR systems maximum stocking densities and flock sizes are lower than conventional FR both systems encourage outdoor ranging and hence forage material consumption (Gilani et al., 2014). Recently, commercial FR poultry have been criticised for allowing only limited ranging behaviour (Chielo et al., 2016). Seasonal variation has also been reported to affect several production factors including the frequency of hens roaming outside (Gilani et al., 2014), pasture availability, the overall qualitative characteristics of available pasture and therefore the subsequent accumulation of n-3 FA in eggs (Mugnai et al., 2014).

Farm level surveys and trials on this aspect have mostly compared FR systems to indoor systems (Anderson, 2011, Krawczyk et al., 2011, Mugnai et al., 2014, Simčič et al., 2011),

while studies investigating differences in egg FA profile specifically between FR and OR systems between seasons are not documented. It is worth mentioning that the few retail studies comparing these systems present contradicting results (Cherian et al., 2002, Filipiak–Florkiewicz et al., 2017, Lordelo et al., 2017). Furthermore, although few studies have examined the impact of strain of hens and egg colour, brown or white, (Anderson, 2013, Rizzi and Marangon, 2012) on egg FA profile, no studies so far have examined this relationship between FR and OR systems specifically. This study was designed as part of a larger farm survey looking into differences in production parameters (Leenstra et al., 2012) between FR and OR systems of Swiss flocks and its aim was to investigate the relationship between different management systems, seasons and genotypes on egg production and quality, specifically lipid and FA composition.

3.3 MATERIAL AND METHODS

3.3.1. Survey design

Thirteen flocks of either white or brown egg colour hen genotypes within OR and FR systems in Switzerland were randomly selected to take part in this survey. Eggs were collected during winter (December 2012-February 2013) and summer (May-August 2013). During winter flocks were 42-51 weeks old and during summer 63-69 weeks old with one flock being 21 weeks old. Egg colour genotypes consisted of the white egg colour genotypes Lohmann LSL-Classic and H&N Super Nick and brown egg colour genotypes Lohmann Brown Classic and H&N Brown Nick. One flock had mixed genotypes of H&N Super Nick and H&N Brown Nick and these were treated as independent genotypes within the same flock, white and brown respectively.

Twenty five eggs were sampled from each genotype within each flock and transported to the Research Institute of OR Agriculture in Switzerland (Research Institute of OR Agriculture, FiBL, Ackerstrasse 113 / Postfach 219, CH-5070 Frick, Switzerland). Yolks were separated from egg white, randomly pooled by 5, generating 5 replicates per genotype and flock and were kept at -20˚C until transported on dry ice to Newcastle University through airmail where they were stored at -20˚C until further analysis.

Flocks were fed compound feed and grains; feed samples from each flock were sampled and delivered along with egg samples at winter sampling. Only one FR and one OR grain sample was sampled. A questionnaire to record the flock's management and feeding practices during the sampling period was completed with farmers (Appendix A.1). Details recorded included egg output, age (in weeks) and size of flock. Feed and grain intakes (grams/hen/day) were calculated at 60 weeks of age.

3.3.2. Egg production

Egg production was calculated as a percentage of eggs laid to actual number of hens during the week of egg sampling. The average of eggs laid during the week was used.

3.3.3. Fatty acid analysis of feed and eggs

3.3.3.1. Chemicals and analytical standards for fatty acid analysis of lipids

Hexane (≥99.9%) and toluene (≥99.5%) were purchased from Sigma–Aldrich (Gillingham, UK). Methanol (≥99.8%), chloroform (≥99.8%), 2,2,4-trimethylpentane (isooctane; ≥99.5%), boron trifluoride 12% in methanol, acetyl chloride (≥98.0%), sodium chloride (≥99.9%), potassium chloride and sodium hydroxide pellets were purchased from Thermo-Fischer Scientific Ltd. (Loughborough, UK). Analytical standard 52 FA methyl esters standard (GLC463) was purchased from Nu-Chek Prep Inc. (Elysian, MN, USA).

3.3.3.2. Feed fatty acid analysis

Lipids were extracted with petroleum ether under controlled conditions using the Soxhlet method (Ministry of Agriculture, 1973) for 6h. Fifty mg of the extracted lipid was transferred in a glass tube and the same procedure was followed as described in Butler et al. (2011). Feed fatty acid methyl esters (FAMEs) were analysed identically to yolk FAMEs with the chromatographic conditions described below.

3.3.3.3. Egg fatty acid analysis

Yolk samples were thawed at room temperature and thoroughly homogenized. Lipids were extracted from yolk according to the method described by Folch et al. (1957) and lipid content as a percent of yolk was determined gravimetrically.

Preparation of FAME was done according to the method of Joseph and Ackman (1992) on the lipids extracted according to the method above. Briefly, 1.5ml of methanolic 0.5N NaOH was added and after vortexing, samples were blanketed with nitrogen, again vortexed and heated in a hot block at 100˚C for 5 min. Samples were left to reach room temperature and 2ml of 12% boron trifluoride (BF_3) in methanol was added. Purging with nitrogen and vortexing before and after the purge was repeated after every solvent addition. Samples were heated in a hot block at 100˚C for 30min and after leaving them to cool for 5min, 1ml of isooctane and 5ml of saturated NaCl were added. Samples were then centrifuged for 5min at 2000rpm and the upper layer was transferred into a new glass tube. This step was repeated and samples were dried down under a stream of nitrogen at room temperature. Tubes were rinsed with 1ml of hexane and 0.4ml was transferred into a sealed amber vial and stored at -20 ˚C until the GC analysis.

Analysis FAMEs was carried out by gas chromatography (GC) (Shimadzu, GC-2014, Kyoto, Japan) fitted with a flame ionization detector (FID) and using an Agilent CP-Sil 88 column (100m x 0.25mmID x 0.20 μm film thickness). Purified helium was used as the carrier gas with a pressure of 210kPa and a column flow of 1ml/min. 1 μl of sample was injected to the column by an auto injector (Shimadzu, AOC-20i) using a split injection mode with a split ratio of 50, an injector temperature of 255˚C with a FID temperature of 260˚C. Sample was injected at an initial column temperature of 70˚C and held at this temperature for 1min. The temperature was then raised to 100˚C at a rate of 5˚C/min, held there for 2min and then raised to 160˚C at a rate of 10˚C/min and held for 71min. Finally, the temperature was increased at 240˚C at a rate of 5˚C/min and then held at this temperature for 29 min leading to a final gradient profile with a 131min total runtime. Identification of peaks was achieved by comparing retention times with those of a 52 FAME standard but also by using already identified peaks by published papers (Stergiadis et al., 2015) using the same GC conditions. Peaks were integrated using the GC Solution Shimadzu software and quantification was based on peak areas of individual FA, expressed as a percentage of the total peak area for known quantified fatty acids.

3.3.3.4. Calculated dietary intakes

Dietary intakes (g/hen/day) of individual FA and FA groups of flocks from different management systems were calculated as follows:

FA intake (g) = total feed intake (g) × [feed lipid content (g/100 g feed)/100] × % of [FA (g/100 g total FA in feed)/100].

It is noted that a correction factor to allow for the fact that the total fat is not only FA has not been used as the diet used in this chapter was a mix of various cereals whose exact concentration and correction factors rest unknown.

3.3.4. Statistical analysis

All analyses of variance (ANOVA), derived from linear mixed-effects models were performed in R statistical environment (R Development Core team, 2009). Management (OR or FR), season (winter or summer) and egg colour genotype (brown or white) were considered as fixed factors, while flock replicate and egg replicates were treated as random factors. The main effects of and interactions between the above factors were assessed. For egg production, mixed flocks (1 in summer and 1 in winter) were excluded from the analyses. Dietary feed FA profile and intake differences were assessed only between management systems. Pairwise comparisons of means ($P < 0.05$) were performed using post-hoc Tukey's honestly significant difference test.

Redundancy analysis was performed to investigate the influence of management practices and dietary FA intakes on yolk lipid content and FA profile, using the CANOCO package (Cajo and Petr, 1998) with automatic forward selection of variables and significances with no interaction terms using Monte Carlo permutation tests. Drivers were size and age of the flock as well as the flock's dietary intakes of FA groups. Management, season and egg colour genotype were included as passive drivers.

3.4 RESULTS

3.4.1. General

All differences discussed are statistically significant ($P < 0.05$) unless stated otherwise. The effect of management on dietary intakes of each flock and dietary FA profile of compound feed are shown in Appendix B.4 table 1. Feed lipid content was similar between feeds in different systems (data not shown). Feed intake was not different between OR and FR flocks, whereas grain intake was higher in OR flocks. However, since grain intake was only a small part of the diet, (<7% in OR and <2% in FR) total feed intake was similar between systems. Diets' FA composition was also similar for both systems with only C16:0 differing; 22 % lower in OR compared with FR feed. Grain FA profile and dietary intakes of g of FA/hen/day were similar between systems, despite some minor differences in feed lipid composition (Figure 3.1, Table 3.1).

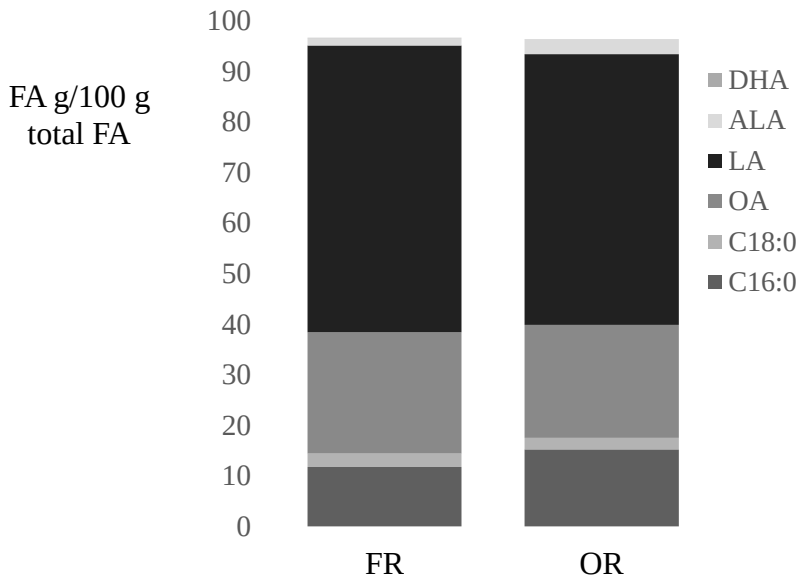

Figure 3.1. Grain fatty acid (FA) profile (g/100g total FA) of grains used in free-range (FR) and organic (OR) flocks in Switzerland; OA= oleic acid; LA = linoleic acid; ALA = α-linolenic acid; DHA = docosahexaenoic acid.

Table 3.1. Dietary and total fatty acid (FA) intakes (g/hen/day) of FR and OR flocks in Switzerland.

	Management		
	FR n=7	OR n=6	ANOVA P-values
FI[1]	119.8±3.7	118.3±6.79	0.846
GI	1.7± 0.89	8.6±2.95	0.037
TFI	121.5±2.97	126.9±7.01	0.472
TFAI[2]			
C14:0	0.04±0.01	0.01±0.00	0.090
C16:0	1.20±0.13	0.87±0.12	0.091
C18:0	0.35±0.06	0.33±0.06	0.817
OA	1.70±0.15	1.63±0.33	0.847
LA	1.79±0.21	2.05±0.34	0.521
ALA	0.09±0.02	0.13±0.02	0.166
DHA	0.001±0.00	0.002±0.00	0.178
TFAI groups			
SFA	1.70±0.17	1.31±0.19	
MUFA	1.81±0.16	1.74±0.35	0.846
PUFA	1.94±0.22	2.24±0.37	0.485
n-3	0.11±0.02	0.15±0.02	0.213
n-6	1.80±0.21	2.06±0.34	0.516
n-6:n-3	17.71±1.4	14.12±0.85	0.059
n-3>18C	0.01±0.00	0.01±0.00	0.273

[1] FI = feed intake g/hen/day; GI = grain intake (g/hen/day); [2]TFI = total feed intake (g/hen/day); TFAI = total FA intake (g FA/hen/day); c OA= oleic acid; LA= linoleic acid; ALA = α-linolenic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-3 = omega-3 FA; n-6 = omega-6 fatty acids;n-6/n-3 = omega-6 to omega-3 ratio; n-3>18C = omega-3 fatty acids with higher than 18 carbons.

3.4.2. Egg production

Neither management nor egg colour genotype influenced egg production (Table 3.2). However, egg output in summer was 8% lower compared with winter. There were no interactions between treatment factors.

3.4.3. Fatty acid composition of yolk

Mean concentrations of yolk lipid and FA are shown in Table 3.2. The most abundant FA in yolk were C16:0 followed by stearic acid (C18:0) for SFA, oleic acid (c9C18:1; OA) for monounsaturated FA (MUFA) and linoleic acid (LA) for polyunsaturated FA (PUFA). The major n-3 FA was docosahexaenoic acid FA (DHA).

3.4.3.1. Main effects

Management affected egg FA profile; OR eggs had less total SFA (-3%) and MUFA (-14%) but higher total PUFA (+34%) than FR eggs. Similarly, concentrations of individual SFA (C14:0 and C16:0) and MUFA (OA, c9C16:1 and c11C18:1) were lower in OR than FR eggs. Total omega-6 (n-6) and the dominant n-6 FA LA were 34% and 38% higher in OR eggs, respectively. Organic eggs were also higher in total (+49%) and individual n-3 FA of α-linolenic acid (9c12c15C18:3, ALA) (+97%), docosapentaenoic acid (c7,c10,c13,c16,c19C22:5, DPA) (+43%) and DHA (+30%), as well as being higher in long chain n-3 (i.e. with more than 18 carbons (n-3>18C) (+31 %). The n-6/n-3 ratio was not affected by management. Production season influenced yolk composition with summer eggs showing lower lipid content (-8%) than winter eggs. Although differences between summer and winter eggs were significant for C16:0, c9C16:1, c11C18:1 and DHA, the differences were relatively small; C16:0 and c9C16:1 were 1% and 6% higher in summer whereas c11C18:1 and DHA were 7%, and 5% respectively higher in winter. Total PUFA, n-3 and n-3>18C were lower in summer than winter by 3%, 7% and 6% respectively. Fewer differences due to egg colour genotype were detected. White eggs had more of both total SFA (+6%) and individual C16:0 (+5%) and C18:0 (+12%) and less DPA (-27%) than brown eggs. White eggs were also higher in arachidonic acid (c5c8c11c14C20:4; AA) but the difference was small (+2%).

Table 3.2 Means ± SE and ANOVA P values for the effect of management (free-range (FR), organic (OR)), season and egg colour genotype on flock egg production (%), yolk lipid content (%) and fatty acid (FA) profile (g/100g total FA) of brown and white eggs collected from FR and OR farms in Switzerland during winter and summer.

							ANOVA - P values						
	Management (M)		Season (S)		Egg colour genotype (EC)		Main Effects			Interactions			
	FR n=14	OR n=10	Winter n=12	Summer n=12	Brown n=10	White n=13	M	S	EC	M:S	M:EC	S:EC	M:S:EC
Egg Production	89.2±2.6	91.8±1.1	94.2±0.7	86.4±2.7	91.7±1.3	89.3±2.6	0.457	0.016	0.463	0.353	0.547	0.302	0.505
	FR n=70	OR n=70	Winter n=70	Summer n=70	Brown n=60	White n=80							
Lipid	33.7±0.40	32.7±0.40	34.4±0.45	32.1±0.29	33.5±0.48	33.0±0.34	0.452	<.0001	0.196	0.590	0.653	0.033	0.140
Fatty acids[1]													
SFA													
C14:0	0.30±0.01	0.23±0.00	0.27±0.01	0.26±0.01	0.28±0.01	0.26±0.00	0.021	0.348	0.674	0.003	0.166	0.107	0.008
C16:0	24.88±0.11	23.85±0.10	24.22±0.12	24.51±0.13	23.72±0.13	24.84±0.09	0.007	0.002	<.0001	0.042	0.535	0.397	0.441
C18:0	8.46±0.11	8.44±0.06	8.50±0.09	8.40±0.08	7.91±0.07	8.85±0.06	0.920	0.177	<.0001	0.569	0.054	0.000	0.000
MUFA													
c9C16:1	2.08±0.05	1.62±0.03	1.80±0.05	1.90±0.05	2.00±0.06	1.73±0.04	0.008	0.005	0.311	<.0001	0.112	0.795	0.714
OA	38.99±0.33	33.59±0.20	36.06±0.41	36.53±0.44	36.19±0.51	36.37±0.36	0.001	0.062	0.057	0.513	0.582	0.423	0.963
c11C18:1	2.24±0.04	1.93±0.02	2.15±0.03	2.01±0.04	2.19±0.05	2.01±0.03	0.012	<.0001	0.160	0.291	0.099	0.188	0.948
PUFA													
LA	16.99±0.36	23.53±0.25	20.53±0.49	19.99±0.51	20.97±0.62	19.72±0.40	0.000	0.068	0.618	0.694	0.810	0.135	0.315
ALA	0.39±0.03	0.77±0.03	0.60±0.04	0.56±0.03	0.66±0.05	0.52±0.03	0.007	0.050	0.839	0.716	0.360	0.000	0.184
AA	1.95±0.01	1.98±0.01	1.97±0.01	1.96±0.01	1.95±0.01	1.98±0.01	0.635	0.317	0.001	0.113	0.935	0.084	0.317
DPA	0.07±0.00	0.10±0.00	0.09±0.00	0.09±0.00	0.11±0.00	0.08±0.00	0.016	0.059	<.0001	0.588	0.706	0.001	0.186
DHA	0.79±0.02	1.03±0.01	0.94±0.02	0.89±0.02	0.94±0.03	0.89±0.02	0.010	0.002	0.892	0.566	0.615	0.006	0.024

Continue of Table 3.2

FA groups	FR n=70	OR n=70	Winter n=70	Summer n=70	Brown n=60	White n=80	M	S	EC	M:S	M:EC	S:EC	M:S: EC
SFA	33.94±0.18	32.86±0.15	33.30±0.18	33.50±0.18	32.24±0.14	34.27±0.12	0.019	0.110	<.0001	0.173	0.597	0.008	0.011
MUFA	44.27±0.40	38.00±0.22	40.90±0.47	41.36±0.52	41.41±0.60	40.92±0.41	0.001	0.128	0.055	0.832	0.904	0.609	0.950
PUFA	21.37±0.40	28.67±0.28	25.34±0.55	24.70±0.57	25.87±0.69	24.37±0.44	0.000	0.039	0.689	0.675	0.745	0.101	0.406
n-3	1.39±0.05	2.07±0.04	1.79±0.07	1.67±0.06	1.86±0.08	1.63±0.05	0.006	0.001	0.500	0.653	0.481	0.001	0.642
n-3>18C	0.89±0.03	1.17±0.01	1.06±0.03	1.00±0.03	1.08±0.03	0.99±0.02	0.009	0.001	0.351	0.646	0.661	0.004	0.027
n-6	19.84±0.36	26.49±0.25	23.43±0.49	22.90±0.52	23.88±0.63	22.63±0.41	0.000	0.072	0.712	0.760	0.817	0.177	0.372
n-6/n-3	15.10±0.36	12.99±0.20	13.78±0.35	14.31±0.28	13.47±0.29	14.48±0.32	0.100	0.053	0.421	0.519	0.388	0.008	0.230

1 SFA = saturated FA(C12+C11:1, C14:0+9C13:1, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0); MUFA = monounsaturated FA (c9C14:1, t9C16:1, c9C16:1, c9C17:1, t6+t7+t8C18:1, t9C18:1, t11C18:1, c9C18:1 (OA), c11C18:1, c13C18:1, c5C20:1, c8C20:1, c15C24:1; PUFA = polyunsaturated FA (t10t14C18:2, t8c13C18:2, c9t12C18:2, t9c12C18:2, ctmix10,14+12,16C18:2, c9c12C18:2 (LA), c9c15C18:2, c6c9c12C18:3(GLA),c 9c12c15C18:3 (ALA), conjugated linoleic acid (CLA), t11c13C18:2, unknown CLA(t,t), unknown CLA(t,t), c9c13c15C18:3, c11c14C20:2, c9c11c15C18:3, c8c11c14C20:3, c11c14c17C20:3, c5c8c11c14C20:4 (AA), c13c16C22:2, c5c8c11c14c1720:5(EPA), c7c10c13c16C22:4, c4c7c10c13c16C22:5, c7,c10,c13,c16,c19C22:5(DPA), c4,c7,c10,c13,c16,c19C22:6 (DHA)); n-3 = c9c15C18:2, ALA, c9c13c15C18:3, c9c11c15C18:3, EPA, c11c14c17C20:3, DPA, DHA; n-6 = c9t12C18:2, t9c12C18:2, LA, GLA, c11c14C20:2, c8c11c14C20:3, AA, c13c16C22:2, c7c10c13c16C22:4, C22:5n-6; c = cis; t = trans

3.4.3.2. Interactions

Significant interactions between management and season were detected for concentrations of C14:0, C16:0 and c9C16:1 (Figure 3.2). Differences between management systems were only significant in winter with OR eggs having less C14:0 (-30%), C16:0 (-5%) and c9C16:1 (-29%) than FR eggs. Concentrations of C14:0 in FR eggs were lower in summer (-11%) compared with winter, whereas OR eggs did not differ across seasons. Concentrations of C16:0 and c9C16:1 showed seasonal differences only under OR management with higher concentrations of both FA in summer (+2% and +18% respectively). Although no significant interactions between management and egg colour genotype were detected, several interactions were detected between season and egg colour genotype (Figure 3.3). Yolk lipid content was lower in summer for both genotypes but the drop in lipid concentration in summer was more pronounced in brown (-10%) than in white eggs (-4%). Egg colour genotype only influenced the yolk lipid concentration in winter, being higher in brown eggs. A seasonal effect on concentrations of C18:0 was detected only for white eggs decreasing by 3% in summer, whereas brown eggs had similar concentrations of this FA across seasons. White eggs were higher in C18:0 than brown eggs both in winter and summer. However, the difference in summer was smaller. In contrast, a seasonal effect on concentrations of total SFA was found only for brown eggs, which had slightly (but significantly) more SFA (+2%) in summer. White eggs were consistently higher in SFA across seasons. There was a seasonal change in concentration of total n-3 for brown eggs only, being the highest in winter and decreased in summer by 14%. Even though not significantly different, brown eggs showed a trend of slightly higher n-3 concentrations both in winter and summer than white eggs. However, the difference was less pronounced in summer; 6% vs 18% in winter. Similarly to n-3, n-3>18C content of brown eggs decreased in summer by 10% but white eggs in summer were also significantly lower than brown eggs in winter (-13%). Very similar patterns to total n-3 concentrations were observed for the individual FA ALA, DPA and DHA (Figure3.4). Concentrations of DPA in white eggs though, constant across seasons, were significantly lower than brown eggs both in winter and summer. Seasonal difference in the ratio n-6/n-3 was shown only for brown eggs, which increased by 11% from winter to in summer. Even though not significant, brown eggs showed a trend of slightly lower n-6/n-3 both in winter and summer than white eggs, with a smaller difference in summer. Three-way interactions were found for C14:0, C18:0, DHA, SFA and n-3>18C (Table 3.2).

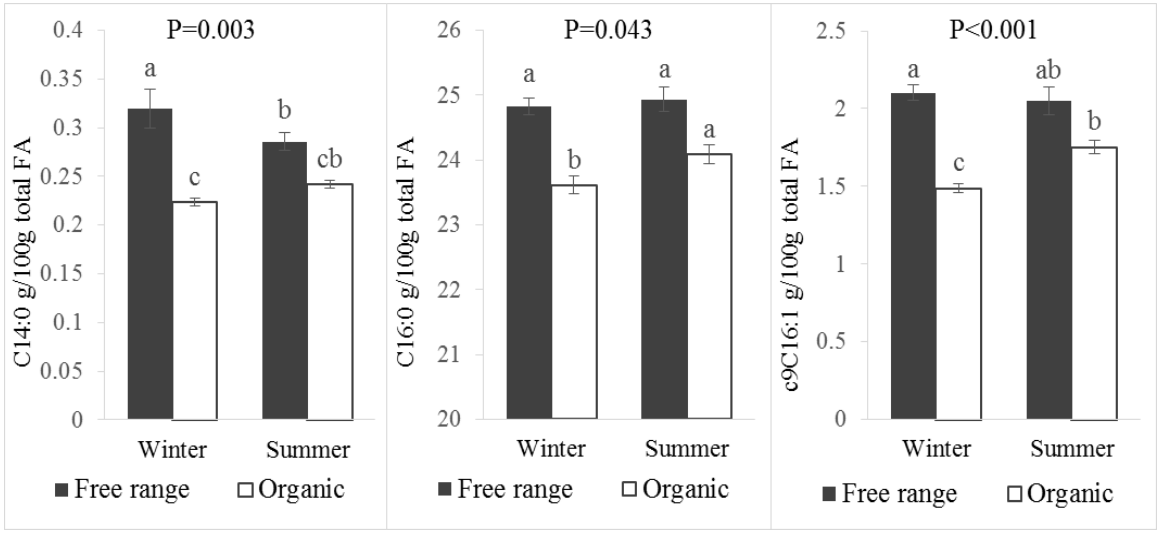

Figure 3. 2. Interaction means ± SE for the effects of management (FR vs OR) and season (winter vs summer) on the concentrations of myristic acid (C14:0), palmitic acid (C16:0) and palmitoleic acid (c916:1) of eggs collected from free-range and organic farms in Switzerland during winter and summer seasons. P represents the ANOVA P-value for the interaction. Bars labelled with different letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$).

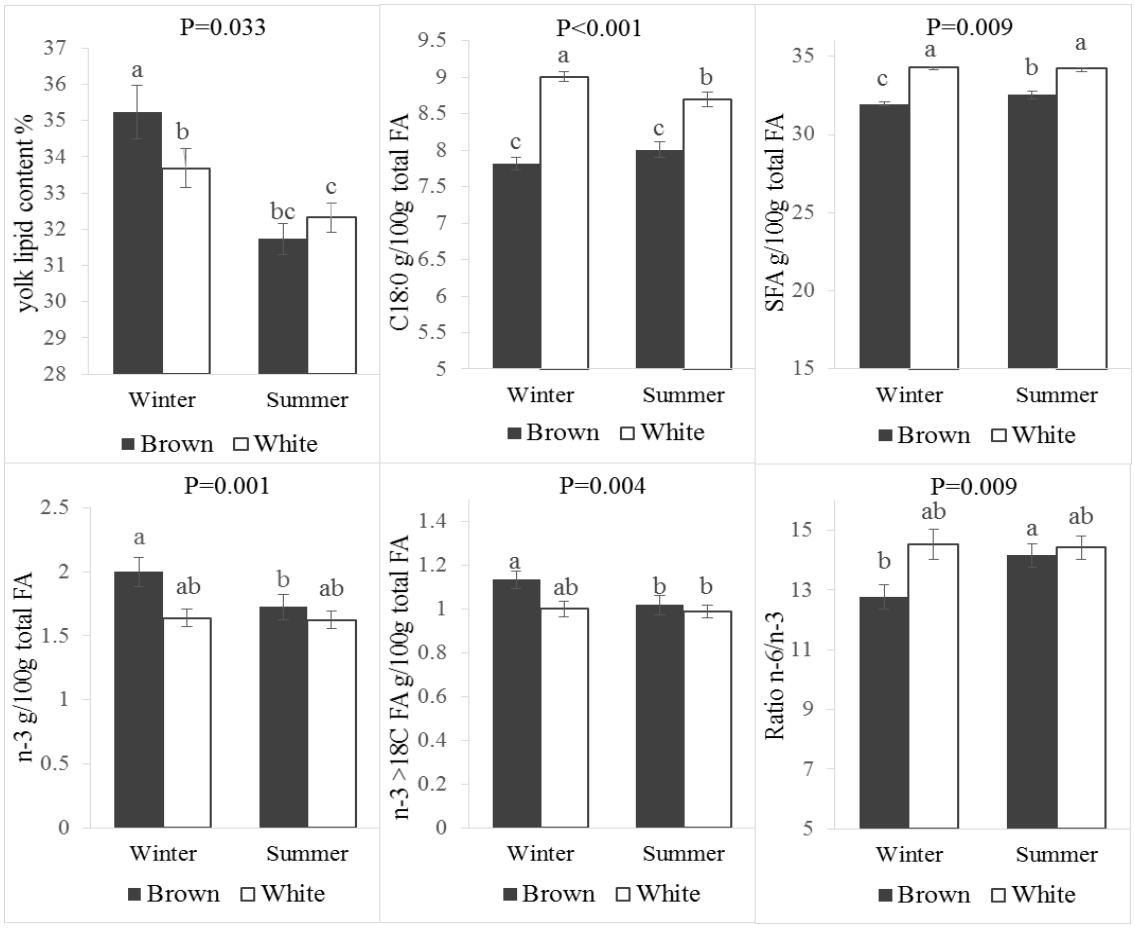

Figure 3.3 Interactions means ± SE for the effects of season (winter vs summer) and egg colour genotype (brown vs white) on the concentrations of yolk lipid content, stearic acid (C18:0), total saturated FA (SFA), omega-3 FA (n-3), long chain n-3 FA (n-3>18C) and omega-6/omega-3 ratio (n-6/n-3) of eggs collected from free-range and organic farms in Switzerland during winter and summer seasons. P represents the ANOVA P-value for the interaction. Bars labelled with different letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$).

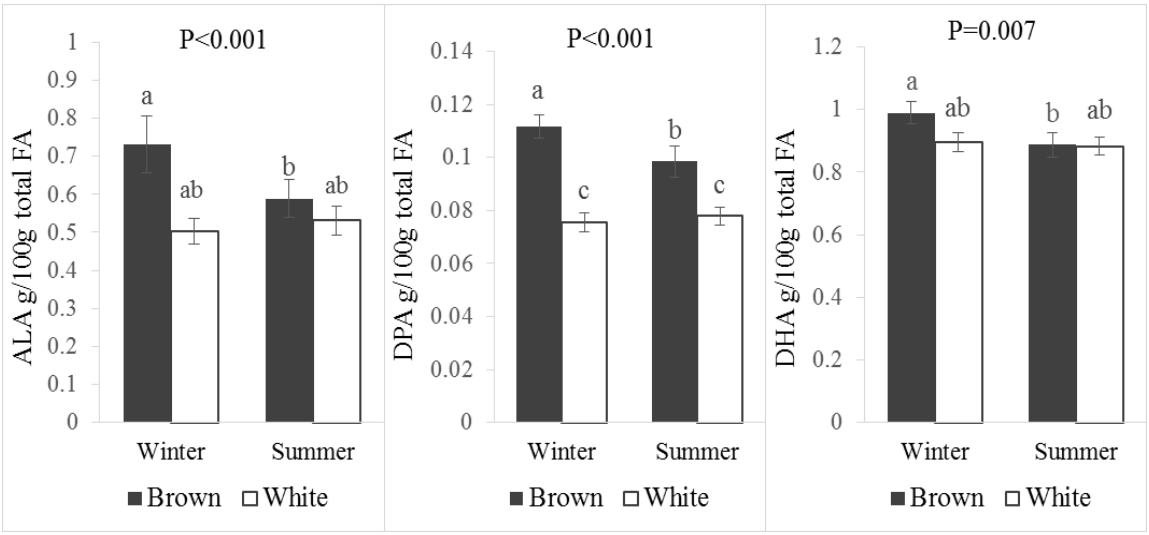

Figure 3.4. Interactions means ± SE for the effects of egg colour genotype (brown vs white) and season (winter vs summer) on the concentrations of α-linoleic acid (ALA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). P represents the ANOVA P-value for the interaction. Bars labelled with different letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$).

To further explore these interactions two-way ANOVAs were used and, where differences were significant, results are shown in Figure 3.5. In winter, OR brown eggs were 40% lower in C14:0 than FR brown eggs whereas in summer this was only 15% and not statistically significant. Additionally, while C14:0 in FR eggs were lower in summer by 21%, OR eggs had 13% more C14:0 in summer, yet not statistically significant between seasons. Although both FR and OR eggs in summer seeed to have less C14:0 compared with the highest observed concentration in FR eggs in winter, OR eggs in summer had statistically similar concentration to the latter. Concentrations of DHA were consistently higher in white eggs from OR than FR, irrespective of season, However, the difference in summer was smaller than in winter (+26 % vs +38%) as DHA concentrations in OR eggs slightly decreased (-5%) whereas FR eggs it slightly increased (+3%) between winter and summer. Whereas Figure 3.3 indicates, on the whole, white eggs had similar SFA concentrations across seasons, with summer only slightly (-1.5%) lower than winter, closer scrutiny shows greater differences between seasons under FR management (Figure 3.5). For white genotypes, OR eggs were lower in SFA than FR eggs during winter (-4.4%) but not in summer. Interactions for C18:0 show (a) OR brown eggs being higher than FR brown in winter, while white eggs being similar across systems (b) an increase in brown eggs and (c) a decrease in white eggs of C18:0 in summer compared with winter eggs under FR system (Figure 3.6)

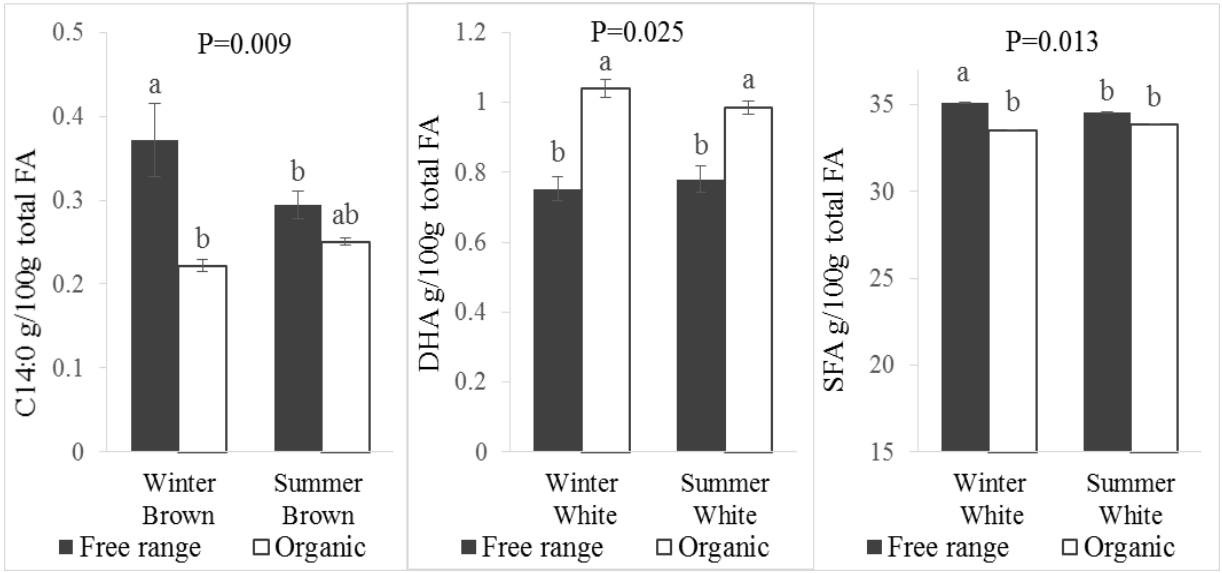

Figure 3.5. Interactions means ± SE for the effects of management and season (free-range vs organic) and season (winter vs summer) on the concentrations of mysristic acid (C14:0), docosahexaenoic acid (DHA), and total saturated FA (SFA) of either brown or white eggs collected from free-range and organic farms in Switzerland during winter and summer seasons. P represents the ANOVA P-value for the three-way interaction. Bars labelled with different letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$).

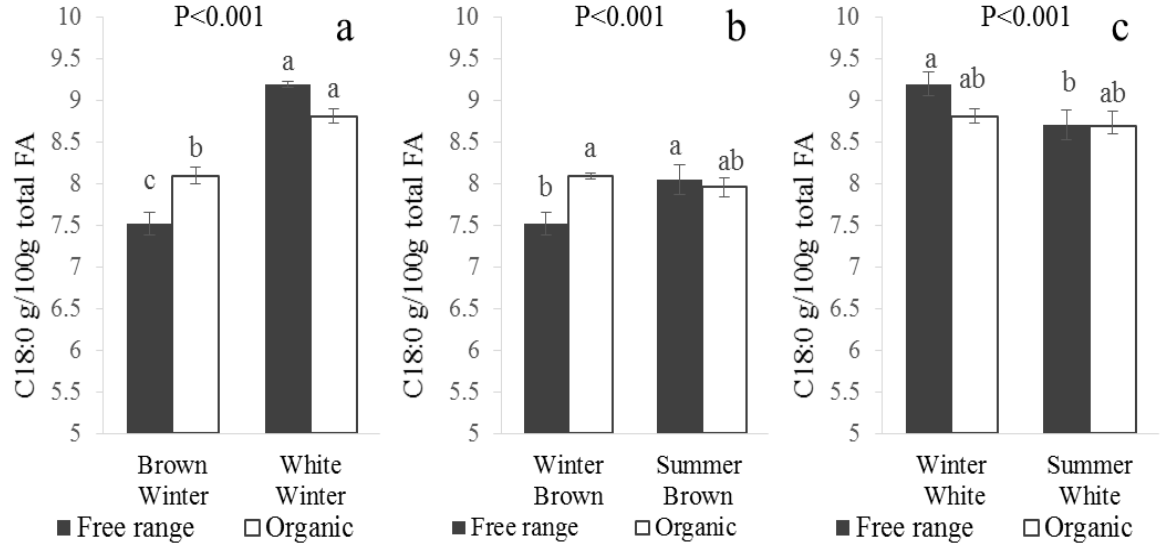

Figure 3.6. Interactions means ± SE for the effects of (a) management (free-range vs organic) and egg colour genotype (brown vs white) in winter and (b) management (free-range vs organic) and season (winter vs summer) in brown and (c) white eggs on the concentrations of stearic acid (C18:0) of eggs collected from free-range and organic farms in Switzerland. P represents the ANOVA P-value for the three-way interaction. Bars labelled with different letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$).

3.4.3.3. Redundancy analysis (RDA)

Results from the RDA (Figure 3.7) showed that management (OR and FR) including flocks' dietary intakes of SFA (DSF), MUFA (DMF), n-3 (Dn3), the n-6/n-3 ratio of their diet (D6/3) and size of the flock were strong ($P < 0.001$) drivers of yolk FA composition. In contrast, season

and egg colour genotype, dietary intakes of PUFA (DPF) and age of hens had weaker influences. Concentrations of yolk PUFA including LA, DHA, ALA, DPA and to a lesser extent eicosapentaenoic acid (c5c8c11c14c1720:5, EPA), were positively associated with Dn3 and OR system, but negatively associated with FR system and higher DSF, DMF, D6/3and flock size. In contrast, concentrations of yolk C16:0, OA, n-6/n-3 and to a lesser extend C14:0 and c9C16:1 (PLO) were positively associated with FR system along axis 1. Yolk lipid content (LIP) along axis 2 was negatively associated with hen age and summer production. Neither C18:0 or AA concentrations were influenced strongly by any of the drivers used in this analysis.

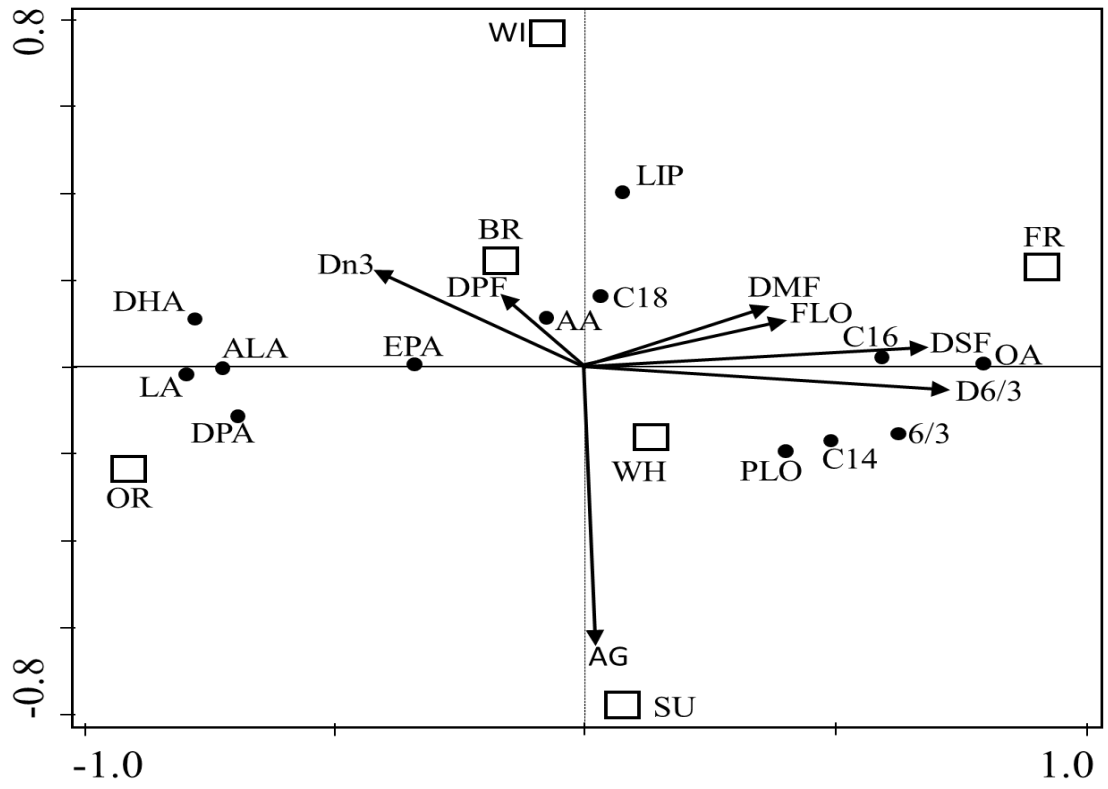

Figure 3.7. Biplot deriving from the redundancy analysis showing the relationship between egg yolk lipid content and fatty acid (FA) profile (shown as dots): LIP= lipid content (%), C14 = mysristic acid, C16 = palmitic acid, C18 = stearic acid, PLO = cis-9 palmitoleic acid, OA = oleic acid, LA = linoleic acid, ALA = α-linolenic acid, AA = arachidonic acid, EPA = eicosapentaenoic acid, DPA = docosapentaenoic acid, DHA = docosahexaenoic acid and 6/3= omega-6/omega-3 ratio and management explanatory continuous variables (shown as arrows): AG = age of layers in weeks (F = 2.6, P = 0.072); DSF = total saturated FA intake (g/hen/day) (F = 41.1, P < 0.001); DMF = total monousaturated FA intake (g/hen/day) (F = 10.7, P < 0.001); DPF = total polyunsaturated FA intake (g/hen/day) (F = 2.8, P = 0.066); Dn3 = total omega-3 FA intake (g/hen/day) (F = 14.1, P < 0.001); D6/3 = omega-6/omega-3 ratio of dietary intake (F = 47.8, P < 0.001); FLO = flock size of layers (F = 12, P < 0.001).Categorical variables (shown as squares) were used as supplementary (passive) data: OR: OR management, FR: FR management, SU: summer (eggs collected between May-August), WI: winter (eggs collected between December-February), WH: white eggs, BR: brown eggs. Axis 1 explained 46.8 % of the variation and axis 2 a further 5.7 %.

3.5 DISCUSSION

3.5.1. General

This study reports for the first time the effects of management, season and brown or white egg colour, hen genotype and their interactions on (i) egg production and (ii) lipid content and FA composition of yolk by comparing FR and OR systems in Switzerland in winter and summer. The study was designed as part of a bigger farm survey on performance on commercial farms in Switzerland and the Netherlands (Leenstra et al., 2012).

3.5.2. Egg production

Egg production is commonly reported to be lower in FR compared with indoor systems (Rakonjac et al., 2014) though a few studies show the opposite (Yilmaz Dikmen et al., 2016). However, although there are not many direct comparisons available between FR and OR systems, research shows comparable production performance (Hammeishøj, 2011, Vitina et al., 2004). In this study, alongside a similar feed intake, our results also show no significant differences in egg production between systems or genotypes, in agreement with the larger dataset of Leenstra et al. (2012) which also reported a significant interaction between system and country. The lack of differences between genotypes can be attributed to the fact that strains forming this study's white or brown groups are all commercial crosses with similar selection pressure for high egg production. The lack of interactions further suggests that both genotypes could similarly adapt in either FR or OR production conditions without compromising egg production. It is important to note that although mortality data, which directly impact production, was not available for this subset, Leenstra et al. (2012) show no statistical difference in mortality in Swiss flocks and further suggested comparable performance of these genotypes under the two systems. Furthermore, a recent meta-analysis on cumulative mortality reportd no differences between FR and OR systems (Weeks et al., 2016a). Even though there has been no research showing clear suitability of genotypes for FR systems, interactions between genotypes and environment were reported (Leenstra et al., 2012), suggesting there is need for further research on selective breeding within FR systems. In this study, summer was successive to winter and as expected, egg production in summer significantly decreased, possibly depicting an aging rather than a seasonal effect, as production naturally decreases as hens age towards the end of the production cycle.

3.5.3. Lipid content and fatty acid composition of yolk

Although the impact of feed composition on egg FA profile has been studied in detail (Fraeye et al., 2012, Poureslami et al., 2012), the impact of FR management practices varies through

the literature. This inconsistency might be explained by the range of factors composing the environment within which the FA profile is formed, as well as the statistical power of each study itself. Results from the RDA in this study confirm the impact of feeding regimes on FA composition and at the same time suggest that management as a whole, explains almost half the variation of the composition of the yolk. However, even though FA composition of FR and OR feed revealed some trends in differences, the dietary FA intake of hens of both systems were similar, suggesting that factors beyond the FA profile of feed offered should be examined in order to further explain differences between FR and OR egg composition. It has to be noted that for the calculation of the diet FA intakes, a correction factor to allow for the fact that the total fat is not only FA has not been used. Undoubtedly, this is a weakness of the study and not ideal as it does not give the exact and real amounts of FA. However, in the framework of this chapter the dietary FA intakes have been discussed relatively between diets, while their transfer to eggs has also been relatively discussed. Concerning the samples analysed, the analysis of pooled egg samples was opted which is a commonly encountered methodology in studies assessing egg nutritional composition (van Ruth et al., 2013, Skrivan and Englmaierova, 2014, Filipiak–Florkiewicz et al., 2017). Retrospectively, a more advanced approach would consist of analysing individual egg samples or even adjusting the calculated standard deviation of pooled egg analyses according to the number of eggs pooled per replicate (Caudill, S. P., 2010). Future studies should take into account the aforementioned methodology for the advancement of the field.

3.5.3.1. Lipid content

Management did not affect total yolk lipid content, in agreement with Hidalgo et al. (2008) and Pignoli et al. (2009), who did not find differences or interactions between FR systems and conventional or OR feed. In our study, lipid content of yolks being higher in winter might be explained by lower bird activity, leading to lower energy use and higher consumption of compound feed rather than forage material during periods when environmental conditions do not encourage ranging outside (Richards et al., 2011). However, a retail study carried out in winter failed to show such a relationship (Hidalgo et al., 2008). Mugnai et al. (2014a) reported lower feed intake in summer compared with winter in OR systems. Feed intake information for each season and interactions with management or/and genotype could be helpful to further explain these results. Aging of hens towards summer might have a confounding effect on fat deposition although evidence is conflicting. While Minelli et al. (2007) showed a 2% higher lipid content in eggs from 47-50 compared with 70-73 weeks old hens, Johansson (2010) reported the opposite with lipid in yolk increasing by 16% with age between age 52 and 72

weeks old hens. However, neither Nielsen (1998) nor Aida et al. (2005) observed any age effects on yolk lipid. Although there was no clear effect of egg colour genotype on lipid content here, there was an interaction between season and genotypes. In this work, findings that lipid content in brown eggs was higher than white in winter, while being similar during summer, are in contrast with Anderson (2013) and Jones et al. (2010) who reported white eggs having higher lipid content than brown eggs by 9% and 12% respectively. While lipid accretion in eggs is supported by hepatic synthesis with a constant supply of lipids to support embryo development (Kuksis, 1992), this study shows some variation which should be further explored along with any relationship between deposition of total lipid into eggs and any preference for specific FA.

3.5.3.2. Effect of management

In contrast to total lipid content, yolk lipid composition was affected by management. The most pronounced impact in FA content was noticed on PUFA and MUFA levels. Concentrations of all PUFA groups and the relevant individual FA, with the exception of AA, were significantly higher in OR eggs, in accordance with a large scale farm survey in the Netherlands (Chatzidimitriou et al., 2015) (Appendix A.2). In contrast, some retail surveys report no differences in PUFA levels or the dominant LA between eggs from differing production systems (Lordelo et al., 2017, Hidalgo et al., 2008). In a Greek retail survey, although LA levels were similar between systems, total PUFA levels were higher in OR eggs, due to changes in the minor n-6 FA (Poupoulis et al., 2009). Whilst PUFA and the leading n-6 FA LA in offered feed was neither statistically different between systems, nor a strong RDA driver for LA content in eggs, OR feed was 18% and 16% higher in PUFA and LA respectively and OR eggs had similarly more PUFA and LA respectively. This pattern could be explained by the higher supplementary grain intake by OR compared with FR hens, as seeds and grains, are relatively high in LA (Woods and Fearon, 2009). It is worth mentioning that the small sample size of feed samples may have not been adequate to show statistical significance. Similarly to our results, Lordelo et al. (2017) reported OR eggs having approximately two-fold levels of the beneficial total n-3 FA, ALA, and DHA. Vitina et al. (2004), also showed a higher level of n-3 FA in OR eggs but not as distinct and only driven by significant differences in ALA rather the long chain FA. Although the diet FA profiles revealed only a trend of OR feed being higher in n-3 FA, the RDA indicates individual n-3 FA intakes were strong drivers of their transfer into egg. However, whereas OR feed was 48% higher in ALA, OR eggs were 97% higher than FR eggs. Recorded dietary intakes of n-3 FA maybe strong drivers but they do not fully explain n-3 FA transfer into eggs, confirming there other factors within each system influencing the final level in eggs. It is interesting to mention Pignoli et al. (2009); although they fed hens OR feed

significantly higher in n-3 FA than the conventional feed, this difference was not seen in the eggs from their FR and OR systems. Outdoor access in FR systems has been reported to increase the n-3 FA content in eggs (Chatzidimitriou et al., 2015, Mugnai et al., 2014) as forages, grass and invertebrates are potentially good sources of n-3 FA (Woods and Fearon, 2009, Guil-Guerrero et al., 2018). Conventional FR systems can have higher outdoor stocking densities than OR, possibly leading to quicker depletion of pasture and associated invertebrate populations. Additionally, flock size tends to be smaller in OR systems and predator protection like trees on the range greater, both influencing ranging behaviour and therefore forage material consumption (Steenfeldt and Nielsen, 2015, Gilani et al., 2014, Leenstra et al., 2012). The RDA also confirm a slight inverse relationship of flock size and n-3 FA in eggs. Since OR management influenced both n-6 and n-3 content equally relative to FR eggs, the n-6:n-3 ratios were the same in both systems, which is in line with the literature (Chatzidimitriou et al., 2015, Hidalgo et al., 2008, Pignoli et al., 2009, Poupoulis et al., 2009).

All individual MUFA reported here, as well as the total MUFA, were lower in OR eggs, in line with some results from the literature (Chatzidimitriou et al., 2015, Lordelo et al., 2017), However, Lordelo et al. (2017) reported OR eggs being higher in the minor c9C16:1. On the contrary, Pignoli et al. (2009) and Hidalgo et al. (2008) found no significant differences in MUFA concentrations between FR and OR eggs, as did Poupoulis et al. (2009), even though OA was lower in OR eggs. The similarity in feed MUFA levels and dietary MUFA intakes in this study did not directly drive the content of the dominant OA in eggs, yet they appeared to be strong drivers of egg FA composition, suggesting that, as with n-3, there are other factors involved in the deposition of MUFA. It is interesting to mention that Kuksis (1992) reported LA to be deposited in egg yolk with simultaneous suppression of OA, consistent with our results for OR eggs having higher LA while lower OA and MUFA.

A significantly higher content of C16:0 in FR feed, a trend for the total SFA and dietary SFA intakes significant driving C16:0 levels in eggs resulted in OR eggs being lower in SFA, C14:0 and C16:0, in line with Chatzidimitriou et al. (2015) reporting C16:0 being lower in OR compared with FR eggs. However, in our results, even though C16:0 in OR feed was markedly lower than in FR feed a proportional difference was not seen in the eggs, although still significant. On the contrary, Hidalgo et al. (2008) reported SFA being higher in OR eggs, whereas neither Poupoulis et al. (2009) nor Pignoli et al. (2009) found any differences, although the latter reported differences in feed SFA. Although RDA shows dietary SFA as a strong driver of egg FA composition this should be cautiously interpreted as it could be driving the deposition of the spectrum of FA, not particularly SFA in egg. The main SFA are reported to successfully

be synthesised *de novo* in the hen's liver (Speake et al., 1998) and therefore not being directly driven by dietary means. According to Jakobsen et al. (2009) who suggested that replacing dietary SFA with PUFA rather than MUFA prevents coronary heart disease in human, this study suggests that eggs managed by OR practices, having less SFA and MUFA and more PUFA could potentially contribute to a healthier dietary intake of fats.

3.5.3.3. Effect of season

Seasonal variation between winter and summer had a less pronounced impact on egg FA composition than management. Although studies reporting egg composition through seasons are very limited (Mugnai et al., 2014, Poupoulis et al., 2009), there is an agreement through the literature that pasture access affects egg FA profile (Karsten et al., 2010, Lopez-Bote et al., 1998, Anderson, 2011, Simčič et al., 2011), mainly by incorporating n-3 FA into the yolk. Interestingly, in our study, eggs were richer in PUFA, n-3FA and n-3>18C in winter rather than in summer, although the differences were small. Unfortunately, this study does not include information of forage intake or grass availability amongst seasons, as the farmers failed to answer such questions systematically. Assuming lower availability of pasture in Switzerland during winter and less ranging activity by hens due to harsher environmental conditions (Richards et al., 2011), it was expected summer feeding conditions would favour higher PUFA and n-3 FA yolk levels. However, findings point towards the opposite direction. Pasture plants' FA composition depends on season and vegetative stage (Morand-Fehr and Tran, 2001, Mugnai et al., 2014, Woods and Fearon, 2009) with younger plants in winter and spring having a higher ALA content than reproductive growth in summer. Therefore, assuming there were available plants in pasture in winter, even with slow growth, it would have kept the high n-3 FA content. Contrary to our results and the reported grass FA composition from the literature, Mugnai et al. (2014a) reported higher DHA within the extensive organic eggs in summer compared with winter, although levels in eggs from a higher density OR system were similar in both seasons. The latter is in agreement with Poupoulis et al. (2009) who found no DHA variation between Greek FR and OR retail eggs from January/February and May/June. Likewise and similar to our results, ALA has been reported to be consistent within systems and across seasons (Mugnai et al., 2014) and between winter and summer months (Poupoulis et al., 2009). However, Poupoulis et al. (2009) reported the highest DHA content in autumn eggs whereas Mugnai et al. (2014a) in spring. This could possibly be explained by the vegetative stage of the plants, which could be actively growing in both seasons or the depletion of pasture plants during summer months either because of the consumption itself or various climatic conditions. Some studies suggest that dried alfalfa or lucerne, commonly used as forage in winter especially in

OR systems, could raise the relative n-3 FA levels in eggs (Hammeishøj, 2011, Karsten et al., 2010). Yet, in our survey, farmers do not report feeding additional forage. We should also not exclude the impact of insect or larvae consumption from the range, which along with the scarce pasture plants, could impact on egg FA profile. Even so, this would have been accompanied by relatively higher SFA or C16:0, which was not shown by our results. On the contrary, slightly higher levels of C16:0 were found in summer eggs in contrast to Poupoulis et al. (2009) who reported higher C16:0 in winter eggs and Mugnai et al. (2014a) who found no differences in C16:0 in eggs, even though grass in winter was higher in SFA. Potential forage material consumption has not been associated with lower SFA content in eggs when FR is compared with CA systems (Simčič et al., 2011, Karsten et al., 2010). No significant differences were noticed for total MUFA between seasons, which is in agreement with Mugnai et al. (2014a) and Poupoulis et al. (2009) who reported though autumn eggs differing compared with winter, summer and spring eggs.

As mentioned, summer in this study, coincides with hens being older and closer to the end of their production cycle when compared with winter results, therefore we cannot exclude a confounding age effect on yolk FA deposition. However, the RDA suggests age of hens is not strongly driving egg FA levels, in agreement with Anderson (2011), who did not find any age effect on yolk FA profile. Additionally, hen ranging behaviour, and consequently potential forage material consumption might change with age, but Richards et al. (2011) reported no effects of age and Gilani et al. (2014) suggested the older hens ranging more, which seems to be the opposite direction to that indicated from our results.

It is possible the seasonal effect on FA profiles is aligned, at least partly, with the higher lipid content in winter eggs, the source of which is still under discussion. Perhaps confirming the strong impact of diets' FA composition on egg FA profile. Since seasonal variation is seen especially in the beneficial n-3 FA content of eggs, it is important for researchers to report and comment on the time of year and range conditions in which studies are carried out. Future monitoring where (a) young hens will be in summer and older hens in winter and (b) more regular seasonal assessment of pasture and egg FA profile are necessary to fully examine the effect of age and season on egg FA profile.

3.5.3.4. Effect of egg colour genotype

This study investigated genotype effects on FA profile by comparing eggs coming from two strains of commercial brown egg birds with two strains of white egg colour birds. It has to be noted that egg colour is controlled by several genes (Samiullah et al., 2015), specific to the

breed. It is also worth noting that both the brown and the white genotypes used in this study are coming from the same breeder company (Hendrix Poutlry) (Silversides, 2010) while the exact pure lines used for this genotypes are unknown. The effect of genotype was looked from an egg colour group perspective as white birds are genetically quite similar within their colour group; brown-egg layers have been derived from several different breeds and white-egg layers from only one, with most commercial white-egg lines probably sharing at least part of their genetic background (Silversides, 2010). Additionally as previously mentioned, genotype can affect birds' behavioural aspects. With this respect, it has been noticed that brown hybrids are more sensitive to environmental challenges than white hybrids (de Haas et al., 2014). Overall, genotype has been shown to influence birds' free-range behaviour and therefore potentially the forage material intake (Elwinger et al., 2008), the respective nutrient uptake and their egg deposition.

With regards to egg FA profile, only a limited genotype effect was noticed, mainly focused on SFA, including C16:0 and C18:0, being lower in brown than white eggs. A similar effect of brown eggs having lower SFA and C16:0 than white was reported by Anderson (2013) and Johansson (2010) respectively. Likewise, Rizzi and Chiericato (2010) and Kucukyilmaz et al. (2012) reported brown having less SFA and C18:0 than white eggs, although failed to show any differences for C16:0, whereas Millet et al. (2006) only showed C18:0 being lower in brown eggs. Another effect was identified for on AA and DPA being lower and higher in brown eggs respectively, although differences were small. These long-chain PUFA are synthesized from LA and their varying content suggests a variation in these substrates. Similar to our results, Rizzi and Chiericato (2010) and Millet et al. (2006) reported higher DPA in brown eggs but did not find any differences in AA content. Bean and Leeson (2003) showed differences in DHA only, with brown having higher content than white eggs. Since, brown hens in this study were heavier, the authors speculated an associated greater liver capacity to elongate ALA increasing DHA in eggs. On the contrary Ayerza and Coates (2000) reported a trend of white egg hens incorporating more PUFA, n-6 FA and n-3 FA into their yolk than brown egg hens, similarly to Anderson (2013) who showed the same trend for PUFA and n-6 FA although found no differences for n-3 FA. Effect of genotype on MUFA has been reported to be either in line with our results with no difference between brown and white eggs (Anderson, 2013, Johansson, 2010) or for brown eggs having higher OA (Ayerza and Coates, 2000, Millet et al., 2006) and consequently higher MUFA levels (Rizzi and Chiericato, 2010) than white. Considering primarily the lower SFA content and secondly the occasionally reported higher DPA in brown rather than white eggs, brown eggs seems to be more promising in following EFSA (2010b)

recommendations on fat intakes. Future studies taking into account the flock individual FA intake can be helpful so as to investigate the transfer of dietary FA and further understand any genetic effects on FA metabolism and storage of fats.

3.5.4. Interactions

Very few interactions between management and season were significant, suggesting that factors within each system affecting egg FA composition were similar throughout winter and summer. Organic eggs were lower in C14:0 and C16:0 than FR during winter (but not summer), when hens possibly rely less on forage consumption, highlighting the impact of feed FA intake. As mentioned, OR feed was significantly lower in C16:0, following a similar trend for C14:0, most possibly due to the fact that OR feeds have less soya which is a rich source of C16:0 (Woods and Fearon, 2009). However, in summer, when outdoor ranging introduces more potential supplementary sources of FA, including high C12:0 content insects, egg C16:0 increases (Chapter Five) and the differences between systems are smaller and not statistically significant. A similar pattern is seen for c9C16:1 possibly coming from desaturation of C16:0. On the contrary, C14:0 although also lower in OR eggs during winter, decreased in FR eggs and reached similar levels to OR eggs during summer. A similar seasonal variation has been reported for low-input milk (Stergiadis et al., 2018) but the reason seems unclear. Further exploring the C14:0 concentrations through the three way interaction, we see that for brown eggs the slight increase in OR eggs in summer, reaches FR levels across seasons, in line with Millet et al. (2006) who reported an interaction between breed and nutrition, yet no clarifying the contribution of different genotypes. Although C14:0 is a minor FA in yolk, it has been negatively linked to human health (EFSA, 2010b) and variation should be further explored. Based on Mugnai et al. (2014a), in systems with a lower outdoor density, DHA concentrations might increase in summer but this was not confirmed by our results. Poupoulis et al. (2009) also showed a clear interaction between management and season for ALA and DHA levels, although details were not reported so it is not clear how specifically FR and OR groups differ over time. The lack of interactions between management and season for PUFA and n-3 FA suggests that, at least in Switzerland, FR management promotes outdoor ranging and grazing equally and irrespective of season, suggesting that any variation in n-3 FA between systems are possibly coming from differences in offered feed. Nonetheless, the loss of grazing in summer due to drought or consumption might mask any potential interactions and an investigation of these interactions during other seasons could be interesting.

The complete lack of interactions between management and egg colour genotype suggests that all genotypes were equally suited to both production systems in this study. A lack of interactions

between genotypes for brown or white egg and dietary FA reported for CA systems (Millet et al., 2006, Bean and Leeson, 2003) also suggest the genetic similarity with respect to FA metabolism in commercial available birds or possibly a similarity in the systems compared. On the other hand Kucukyilmaz et al. (2012) reported interactions between management and brown or white genotypes for SFA and ALA with genotype only having an effect in controlled CA but not in OR systems. Since a genetic effect on yolk FA profile has been reported for less commercial genotypes (Rizzi and Chiericato, 2010) it is worth further investigating if any genotypes interact with management within FR systems.

The interactions noticed between season and genotype focused on SFA and n-3 FA. Apart from the previously mentioned genetic effect on C18:0, seasonal variation was noticed with white egg hens deposing less C18:0 into their yolk in summer compared with winter, resulting in a smaller , yet significant, difference between genetic strains in summer than winter. A more complex relationship is revealed by the three way interaction where a summer reduction for concentration of C18:0 in white eggs and an increase in brown is only significant in FR rather than OR system. A genetic interaction with diet and age affecting the deposition and metabolism of C18:0 has been suggested before (Scheideler et al., 1998) but not fully elucidating a relevant mechanism or pattern. The results here suggest OR eggs are more consistent in C18:0 within the same genotype compared with those under FR management. As C18:0 is neutral with respect to health it is worth understanding and exploiting its mechanism to increase or standardize its content over the more harmful C16:0. Total SFA, possibly driven by differences in C18:0, reveal a very similar pattern, where brown egg hens increase yolk SFA content in summer whereas in white eggs SFA is reduced but only in FR systems. Total n-3 and n-3>18C FA and ALA and DHA follow a similar pattern; levels in brown eggs are lower during summer whereas in white eggs stay constant across seasons. A closer look into the three way interaction for DHA shows a further interaction with management suggesting that white hens deposited the same amount of DHA into eggs irrespectively of system and season unlike those laying brown eggs. This could be either down to the constant supply of slightly more n-3 FA through feed in OR systems or to the overall higher foraging by OR hens compared with FR hens. Alternatively, a possible explanation of greater variation across seasons in brown eggs and the lack of a three way interaction, is as suggested, due to a possible weaker ranging behaviour of brown egg hens during winter and a subsequent higher consumption of offered feed. However, the reduction of lipid content in summer is significant for white as well as brown eggs, although the difference is smaller and therefore possibly not translated into n-3 FA variation during summer. A slightly different pattern was shown for DPA concentrations, an

intermediate n-3 FA between ALA and DHA. The strong genetic effect of lower DPA content in white compared with brown eggs, cannot be fully explained and needs further investigation since both ALA and DHA are within similar levels in both winter and summer.

Overall, during summer the differences in FA composition between systems and genotypes are smaller than in winter, suggesting that forage consumption under the factors investigated can be considered as an explanatory factor with regards to the egg FA variability.

3.6 CONCLUSION

This study provides evidence that in Swiss FR systems egg FA composition could be improved by adopting OR practices without compromising egg production. However, the hypothesis of higher forage intake during summer or any interactions between systems and seasons could not be confirmed. Still, during summer the differences between genotypes and systems were smaller than in winter. Although a seasonal effect was noticed, the results were unexpected having overall higher n-3 FA in winter, possibly highlighting the impact of diets' FA profile. Egg yolks from brown genotypes systematically had lower SFA - better suited to meeting the EFSA guidelines towards a healthier diet.

CHAPTER 4. VARIATION IN NUTRITIONAL AND STANDARD QUALITY IN RETAIL EGGS IN THE UK

4.1 SUMMARY

The nutritional content of eggs including fatty acid (FA) and carotenoid composition, vitamins A, D, E, B_2 and B_9 and mineral composition, was measured over one year to examine the effects of management system (extensive organic (EO), organic (OR) vs free-range (FR) vs caged (CA)) and season on the nutritional content of eggs found in the market of United Kingdom. Results demonstrated a strong management effect on egg FA composition and more specifically a higher omega-3 FA content ($P<0.001$) in EO (2.67g FA/100 g FA) and OR eggs (2.82g FA/100 g FA) compared with FR (2.01g FA/100 g FA) and CA eggs (1.80g FA/100 g FA). Season had no effect in the overall FA composition. A strong management effect was also demonstrated for the egg carotenoid composition. Lutein content was higher ($P<0.001$) in EO eggs (1142µg/100 g yolk) compared with OR (751µg/100 g yolk) and FR and CA eggs (461 and 285 µg/100 g yolk respectively). No season effect was noticed apart from γ- and δ-tocopherol ($p<0.05$ and $p<0.01$ respectively). Vitamins D_3, B_2 and B_9 content did not differ in eggs amongst different systems. On the contrary, a date effect between July and January was noticed for all vitamins above except B_9. Vitamin D_3 and B_2 were higher ($P<0.001$ and $P<0.01$ respectively) in July (14.9 mg vitamin D_3/100g yolk and 0.43 mg vitamin B_2/100g egg) compared with January (11.5 mg vitamin D_3/100g yolk and 0.39 mg vitamin B_2/100g egg). An interaction between management and date was noticed for vitamin D_3 and B_9 showing eggs coming from EO systems being the most affected and with the lowest content of both vitamins in January compared with July. A varying management effect was found for mineral composition of eggs, whereas an effect of date ($P<0.01$) was noticed only for Manganese egg content (0.04 mg/100g egg in July and October vs 0.03 mg/100g egg in January and April). The results of this study indicate that eggs coming from different management systems are different in their nutritional composition and therefore right labelling would be recommended.

Keywords: retail survey, egg composition, extensive organic, organic, free-range, caged, fatty acids, carotenoids, vitamins, minerals

4.2 INTRODUCTION

Eggs are a rich source of several nutrients beneficial to human health; from fat soluble vitamins such as vitamin A, E and D_3 to water soluble vitamins (B_2 and B_9) and chemical elements such as phosphorus and magnesium. Egg consumption in the UK has been steadily increasing for the past decade (UK Industry data, 2018), possibly driven by the relatively low price of eggs and the fact that the possible link between egg intake and adverse health effects such as cardiovascular disease concerning healthy populations, is nowadays not as relevant (Guo et al., 2017a) as in the past.

Options for consumers of egg types within the UK retail system, where the majority of eggs are supplied and purchased (UK Industry data, 2018), are eggs mainly from CA, FR and OR systems. Egg choices are driven not only by price and taste criteria but also by perceptions relating management systems to welfare and nutritional quality (Lee and Yun, 2015, Weeks et al., 2016b). It is well established that egg production systems can largely affect the nutritional composition of eggs through different feeding regimes, including forage material intake that can increase the intake of several nutrients (Mugnai et al., 2014, Zhu et al., 2015, Schlatterer and Breithaupt, 2006). The nutritional composition of OR and conventional feed has already been shown to differ between management systems (Baranski et al., 2014) and therefore this is likely to affect the respective egg quality. Eggs from systems that permit birds to have outdoor access grazing on pasture, are more likely to have a higher content of nutrients found in pasture, such as carotenoids, n-3 FA or vitamin A. This means that FR eggs, either conventional FR or OR, can be richer in pasture-related nutrients. Organic systems having different standards to conventional systems, promoting grazing behaviour can lead to eggs with a more beneficial nutritional profile. However, depending on the intensity and the characteristics of the systems, the differences might be small or even non-existent. It has been shown that within FR systems and even within OR systems, the intensity of foraging activity is driven by several factors such as internal and external stocking densities (Gilani et al., 2014), can actually result in different egg nutritional profiles (Mugnai et al., 2014). Consequently, systems that allow and promote extensive grazing and foraging behaviours could result in further enriched egg nutritional profiles. However, usually eggs from these systems can only be found within small and local supply chains or at farm gates rather than through big retails supermarkets. In addition, seasonal variation has also been connected with variations in grazing behaviour (Gilani et al., 2014) and hence a potential variation in egg composition especially in FR systems, where pasture availability and forage intake can be largely affected across seasons.

Taking into consideration consumers' perceptions on egg types and Additionally, that in the UK, the share of FR egg production is growing and is currently more than 50% of total production (DEFRA, 2018), differences between egg types that are commonly available in big retail supermarkets should be elucidated. Additionally, the nutritional quality of eggs from systems with extensive foraging behaviours should be also be compared to the ones regularly found in the supermarkets. This study was designed to look into differences of egg nutritional differences from conventional (CA and FR), OR and extensive organic (EO) egg production systems that are available to consumers. The main objective of the study was to assess the egg nutritional quality coming from the main management systems within the UK retail sector. In parallel including, eggs coming from farms with extensive foraging practices, will more clearly show any effects of pasture-raised hens to the overall egg nutritional quality. A second objective was to investigate seasonal variations and how this could relate to the respective management systems.

4.3 MATERIAL AND METHODS

4.3.1. Survey design

Eggs from all standard commercial systems available to consumers via supermarkets were included in the experimental design; including eggs from CA, FR and OR systems. Eggs were also collected directly from OR small scale farms, supplying their eggs only locally and using maximum foraging practices representing EO systems. These farms were chosen after inspection based on their OR certification and only farms with outdoor stocking density of at least $10m^2$ per bird were considered. The survey was repeated throughout the year to reflect potential seasonal differences.

4.3.2. Sample Collection

Eggs from CA, FR, OR and EO systems were collected five times every three months starting April 2017 till April 2018, from eight supermarkets in the Newcastle upon Tyne region (CA, FR and OR) and five farms (EO); 4 in Southern England and 1 in Wales. However, during the first date (A, April 2017), all poultry across the country were kept inside to as a precaution against Avian Influenza infection. However, the EO farms had already released their birds upon collection. Therefore, this date could not be considered in the main statistical analysis. On each date, three half dozen, medium sized egg boxes of the supermarket own brand were randomly picked from each supermarket for each production system available; CA (4), FR (8) and OR (8) (numbers in parenthesis represent the number of supermarkets providing eggs). Mixed sized eggs were purchased when medium were not available and all eggs were brown. Table 4. 1 shows the sampling design.

Table 4. 1. Retail points for egg sampling; □available, na: not available

Date		**System (n=#supermarket/farms)**		
	CA[1] (n=4)	**FR[2]** (n=8)	**OR[2]** (n=8)	**EO farms** (n=5)
A – April 2017	n=4	n=8	n=8	n=5
B – July 2017	n=4	n=8	n=8	n=5
C – October 2017	n=4	n=8	n=8	n=5
D – January 2018	n=4	n=8	n=8	n=5
E – April 2018	n=4	n=8	n=8	n=4

1 LIDL, Morrison's, ASDA, Tesco; [2] COOP, Waitrose, M&S, LIDL, Morrison's, ASDA, Sainsbury's, Tesco

Eggs were collected from the EO farms just before supermarket purchases. When farms had multiple flocks of varying ages, twelve eggs were collected from each age group to reduce sampling bias; age groups were considered as young (20-36 weeks), medium (37-55 weeks) and old (> 56 weeks), sampling a total of 36 eggs in this case per farm. Twenty one eggs were

sampled in case of only one age group as for supermarket sampling. Three farms had flocks from all age groups and two only one. Information on farm practices were also recorded. All type of information recorded from supermarket and farms can be found in APPENDIX B.5. Immediately after collection, eggs were transferred in polypropylene opaque boxes at Newcastle University and were stored at ambient temperature till further processing.

4.3.3. Pooled egg sample preparation

Three random supermarket eggs, one from each box, per system per supermarket were pooled and mixed. For EO farms with flocks of varying ages one random egg per age group or three eggs when there was only one age group, were used for pooling. Whole eggs were mixed, freeze-dried and used for dry matter, vitamin B_2 and B_9 and chemical element analysis. Using another three random eggs from each box, yolks were separated from white and carefully rolled on a blue absorbant tissue roll to remove any albumen residues. Yolks were mixed and used for fatty acid analysis, fat soluble vitamins (vitamin A, E and D) and carotenoid analysis. A small quantity of pooled yolks was also freeze-dried. Each sample was immediately frozen or/and freeze-dried and stored at -80°C till further analysis. All eggs for analysis were frozen or/and freeze-dried before their "best before date".

4.3.4. External and internal standard egg quality assessments

Within a week of collection the same number and type of eggs as for the pooled sample preparation from supermarkets and EO farms were transferred to The Lakes FR Egg Company, UK and assessed for shell colour (scale 1 brown -100 white), egg weight, albumen height, Haugh units (HU), yolk colour (DSM colour fan), shell weight, and shell density, using an TSS QCM+ microprocessor (York, UK). In parallel, egg weight, egg albumen, yolk and shell weight and proportion were measured and calculated. The egg was weighted and broken on the microprocessor. After all measurements were taken, the yolk was manually separated, rolled on absorbant blue tissue roll to remove any albumen residues and the weight recorded. Shells were thoroughly dried in a microwave and their weight was recorded. Albumen weight was calculated as the difference between egg weight and shell and yolk weight (Silversides and Scott, 2001). Proportions of each egg component were expressed as a percentage of whole egg weight. The mean of measurements on 3 eggs was used for each sample.

4.3.4. Dry matter

A homogenised portion of whole egg and yolk samples, prepared as described in section 4.*3.3. Pooled egg sample preparation*, was adequately freeze-dried for 6 days. Moisture content was

calculated as a percentage taking into account the weight loss before and after freeze-drying. Dry matter (DM) was calculated as the inverse of moisture.

4.3.5. Fatty acid analysis

Fatty acid analysis of eggs was performed as described in Chapter Three.3.3. on composite egg yolk samples.

4.3.6. Carotenoid, vitamin A and E analyses

4.3.6.1. Chemicals

High performance liquid chromatography (HPLC) grade acetonitrile, ethanol, methanol, chloroform, hexane, trimethylamine and potassium hydroxide (KOH, pellets) were purchased from Fisher Scientific (Loughborough, UK). Butylated hydroxytoluene (BHT), pyrogallol and analytical standards of tocopherols α, β and γ and retinol were obtained from Sigma-Aldrich (UK). Analytical standards of echinenone, lutein, zeaxanthin, canthaxanthin, β-cryptoxanthin, β-carotene and α-carotene were purchased from CaroteNature GmbH (Ostermundigen, Switzerland).

4.3.6.2. Standards and sample preparation

Echinenone was used as an internal standard. All procedures were undertaken under dim lighting to avoid degradation of analytical compounds. Stock solutions of standards retinol and tocopherols were dissolved in ethanol (containing 0.1% BHT), while carotenoids were dissolved in chloroform (containing 0.1% BHT). The concentration of standard stock solutions was measured after appropriate dilution by measuring the absorbance in either ethanol or hexane with a UV spectrophotometer as described by Liu et al. (2011). Calibration curves for each compound were then made with a range bracketing the expected concentrations.

Preparation of egg yolks was made according to Kurilich and Juvik (1999) and Hammershøj et al. (2010) with slight modifications. Prior to preparation, frozen egg yolk samples were thawed for an hour at room temperature. In a glass tube 0.2g±10μg of yolk was weighed, 1 ml of ultrapure water (18.2 Ω) was then added and vortexed. Two ml of ethanol of 5% pyrogallol, 1% BHT also containing the internal standard (around 0.01%) were added and vortexed. Saponification took place by adding 0.5 ml of KOH and after filling the tubes with nitrogen, incubating for 30 min at 45◦C. After that, tubes were cooled for 1 min in an ice bath, 1 ml of ultrapure water was added and vortexed. Two ml of hexane were added to each sample before being vortexed in an orbital shaker for 1 min. They were then centrifuged at 2000 g for 5 min, the upper hexane layer was removed and transferred to a new tube using a glass Pasteur pipette.

The extraction procedure with hexane was repeated and the hexane layers were dried down under a steady stream of nitrogen. Dried extracts were then reconstituted in 1 ml of HPLC mobile phase and filtered using a 0.45 µm syringe PTFE filter into a centrifuge tube. Aliquots of 300 µl were transferred to amber glass vials and immediately run in the HPLC, while being kept at 4 ◦C. Vials were then stored at -80 ◦C in case they were needed for repeat analyses.

4.3.6.3. HPLC analysis, compound identification and quantification

HPLC specifications used were as described by Liu et al. (2011), using a Shimadzu HPLC system coupled with a photodiode array detector (PDA) and a fluorescence detector (FD). The column used was a Phenomenex 250mm x 4.6mm at 30◦C. Briefly, the method used a linear gradient for 20 minutes. The mobile phase consisted of 65% acetonitrile, 35% methanol and 0.065% trimethylamine, with a flow rate of 1.5 ml/min. The injection volume was 20 µl.

Identification was done by comparing the retention times and spectral data of authentic standards obtained by the PDA with the relative retention times reported by Liu et al. (2011) and values of the compounds' retention times and spectral PDA data. Details of the retention times for each compound can be found at the chromatogram included in the APPENDIX B.5 Figure 1. 1. Spectral data on each compound are not included in this thesis but will be included in any future publication using these data. Quantification was made at 450 nm (carotenoids) and at 325 nm (retinol). For tocopherols FD was used at excitation and emission wavelengths of 295nm and 325 nm, respectively. The external standard calibration curves were used to quantify the area of each compound.

4.3.6.4. Compound content calculations

Total vitamin A and E contents were calculated according to egg compositional analysis reports by the Public Health England (2015). Total vitamin A was calculated as retinol equivalents equating to retinol + (beta-carotene equivalents/6). Retinol was calculated as all trans-retinol. Public Health England (2015) although specified that retinol should be calculated as all trans-retinol + (0.75 x 13-cis retinol), there was no quantification for 13-cis retinol and hence in our study only retinol was taken into account. Total vitamin E was expressed as a-tocopherol equivalents, calculated using the following conversion factors for vitamin E activity: α-tocopherol x 1.00, β-tocopherol x 0.40, δ-tocopherol x 0.01, γ-tocopherol x 0.10, α-tocotrienol x 0.30, β-tocotrienol X 0.05, γ-tocotrienol x 0.01. There was no quantification foreseen of tocotrienols and hence only tocopherols were taken into account. Results are expressed as µg/100 g yolk.

4.3.7. Vitamin D_3 analysis

Composite yolk samples from two sampling dates (July 2017 and January 2018) were air transferred in dry ice to the Research Group for Bioactives at the Technical University of Denmark in the framework of a partnership agreement for vitamin D analysis. Samples were shipped on dry ice and then stored at -80 ◦C upon arrival and analysed within two months from reception. Sample preparation and analysis were as described by Barnkob et al. (2019). Briefly, 1 g of egg yolk was saponified, and then cleaned-up by liquid-liquid extraction and solid phase extraction. Detection and quantification of vitamin D_3 and 25-hydroxyvitamin D_3 was made using an LC-MS/MS system. Precision for vitamin D_3 and 25-hydroxyvitamin D_3 was 5.3% and 4.6%, respectively. Quantification was performed by single determination accredited according to ISO17025. Total vitamin D was calculated as vitamin D_3 + (25OH vitamin D_3 * 5) according to Public Health England (2015) and expressed as µg/100 g yolk

4.3.8. Chemical elements

Freeze dried whole egg samples from all dates (except April 2017) were air transferred to ALS Food pharmaceuticals (Czech Republic) for elemental analysis. Elements analysed and respective limits of quantifications (LOQ) were: Magnesium (Mg) (3 mg/kg) Calcium (Ca) (10 mg/kg), Phosphorus (P) (2 mg/kg), Zinc (Zn) (2 mg/kg), Cobalt (Co) (0.5 mg/kg), Cadmium (Cd) (0.1 mg/kg), Barium (Ba) (0.5 mg/kg), Manganese (Mn) (0.1 mg/kg), Selenium (Se) (5 mg/kg), Chromium (Cr) (0.2 mg/kg), Arsenic (As) (2 mg/kg), Iron (Fe) (1 mg/kg). Detection and quantification were made using inductively coupled plasma optical emission spectrometry (ICP-OES-A). Methods were accredited according to ISO 11885 and results were expressed as µg/100g of fresh egg. The elements were chosen on the basis of the analytical method (ICP-OES-A) and if it could simultaneously analyse the element in question. Furthermore, iodine analysis although highly anticipated, the method proved complex and expensive to include.

4.3.9. Vitamin B_2 and B_9 analyses

Frozen whole egg samples from July 2017 and January 2018 were air transferred in dry ice to SGS institute Fresenius GmbH (Germany) for vitamin B_2 and B_9 analyses. Vitamin B_2 was analysed by HPLC-fluorimetry using protocol and expressed as mg/100g egg (BS EN 14152, HPLC/Fl). Vitamin B_9 was analysed according to microbiological methods using protocol (BS EN 14131:2003) and expressed as µg/100g egg. Both vitamins were chosen to be analysed because egg can potentially be a good source of them.

4.3.10. Statistical analysis

All analyses of variance (R Development Core team, 2009) derived from linear mixed-effects models were performed in the R statistical environment (R Development Core team, 2009). Management systems (OR, FR, CA and EO) and sampling date (B, C, D and E) were considered as fixed factors, while source of sampling (supermarket and farms) was considered as a random factor. The main effects of, and interactions between, the above factors were assessed. Pairwise comparisons of means ($P < 0.05$) were performed using post-hoc Tukey's honestly significant difference test. Vitamin B_2, B_9 and vitamin D_3, were examined across two contrasting dates (B and E). A comparative analysis between sampling date A (April 2017) when all eggs from supermarkets were from hens that were kept indoors and date E (April 2018) was also performed on external and standard quality, fatty acids and carotenoids. Only CA, FR, and OR eggs were considered for this analysis. The statistical model used was identical to the one described above.

4.4 RESULTS

All differences discussed here are statistically significant ($p<0.05$), unless specified otherwise.

4.4.1. External and internal standard egg quality assessments

Results for the external and internal standard egg quality can be found in Table 4. 2. Management had an impact on many of the egg characteristics explored, whereas date had very limited impact. Shell colour was lighter for OR and EO eggs compared with CA eggs, while FR eggs had a similar colour to all systems. At the same time, eggs had the most intense brown colour in April compared with July. Yolk were the most orange in FR eggs, followed by EO eggs by one unit less in the Roche scale and finally by three units less in OR and CA eggs. Albumen height and HU were both the highest in EO eggs, compared with the rest (+23% for albumen height and +14% for HU) and similar between CA, FR and OR eggs. The age of egg when analysed was at an average 7 days closer to their lay date for EO eggs compared with the rest. Across dates, there was a slight fluctuation on the age of eggs at analyses, but that was only a magnitude of an average of one day.

Concerning weights and relative proportions of the various components; egg and albumen weight varied between systems. Eggs were 4% heavier from OR and EO eggs compared with both CA and FR systems. Albumen was also greater in EO eggs (+4% compared with OR eggs and +8% compared with CA and FR). The relative proportion of shell only differed between FR and EO eggs, being 5% less in EO eggs, although neither differed from CA or OR eggs.

While management did not have an impact on the DM content of yolk or egg, they both changed slightly over time. DM in April was weakly associated with a slightly higher (+1%) percentage when compared with January, while both January and April were similar to July and October. When yolk DM was compared between dates when all birds were kept indoors (April 2017) and when the outdoor systems were under more normal conditions (APPENDIX B.5 Table 1.1), yolk DM was higher in eggs during April 2018 when birds were allowed to roam outside.

Interactions between management and date for all measurements were not significant.

Table 4. 2. Means ± SE and ANOVA P values for external and freshness characteristics, component proportions and dry matter assessments for eggs collected from supermarkets (caged, free-range and organic systems) and extensive organic farms in the UK, by management and date

	Management (M)				Date (D)				ANOVA - P values		
									Main Effects		Interactions
	Caged (CA) n=16	Free-range (FR) n=32	Organic (OR) n=32	Extensive organic (EO) n=19	Jul-17 n=25	Oct-17 n=25	Jan-18 n=25	Apr-18 n=24	M	D	M x D
Measurement[1]											
External and freshness characteristics											
shell colour[2]	25.4±0.8^{b}	28.4±0.9^{ab}	30.4±0.7^{a}	31.2±1.9^{a}	31.9±1.2^{a}	29.4±1.0^{ab}	28.4±1.0^{ab}	26.7±1.1^{b}	0.005	0.005	0.099
Shell density (g/cm^3)	87.3±1.0	87.9±0.6	87.0±0.8	85.7±1.0	87.6±0.7	86.9±1.1	87.2±0.7	86.6±0.6	0.375	0.852	0.426
yolk colour[3]	6.0±0.5^{c}	9.6±0.3^{a}	6.2±0.2^{c}	8.3±0.2^{b}	7.4±0.4	7.7±0.4	7.6±0.4	8.0±0.5	<0.001	0.395	0.410
albumen height (mm)	4.72±0.21^{b}	5.16±0.21^{b}	4.75±0.15^{b}	6.02±0.13^{a}	4.73±0.23	5.32±0.19	5.06±0.20	5.38±0.20	<0.001	0.080	0.214
Haugh Units (HU)	65.4±2.0^{b}	68.3±1.9^{b}	64.6±1.5^{b}	75.0±1.1^{a}	64.0±2.1	69.7±1.8	68.0±1.8	70.0±1.8	0.002	0.106	0.152
Age of egg (days)	14.6±0.6^{a}	15.6±0.4^{a}	16.0±0.6^{a}	8.6±0.3^{b}	14.3±0.8^{ab}	13.7±0.8^{b}	15.4±0.8^{a}	13.4±0.6^{b}	<0.001	0.021	0.460
Weights (g) and proportions (%)											
Egg weight	58.4±0.4^{bc}	58.2±0.2^{c}	60.1±0.7^{ab}	61.9±0.6^{a}	59.8±0.6	59.8±0.8	59.0±0.5	59.7±0.5	<0.001	0.705	0.835
Yolk weight	15.1±0.2	14.9±0.2	15.4±0.2	15.7±0.3	15.4±0.3	15.3±0.2	15.0±0.2	15.1±0.3	0.150	0.541	0.569
Shell weight	6.14±0.08	6.17±0.05	6.24±0.09	6.25±0.08	6.25±0.06	6.21±0.10	6.17±0.0a	6.18±0.07	<0.001	0.685	0.924
Albumen weight	37.2±0.2^{bc}	37.2±0.2^{c}	38.5±0.4^{b}	40.0±0.5^{a}	38.1±0.4	38.3±0.5	37.9±0.4	38.5±0.4	0.895	0.584	0.584
Yolk proportion	25.8±0.3	25.5±0.3	25.5±0.3	25.3±0.4	25.9±0.4	25.6±0.3	25.4±0.3	25.2±0.3	0.018	0.680	0.503
Shell proportion	10.5±0.1^{ab}	10.6±0.1^{a}	10.4±0.1^{ab}	10.1±0.1^{b}	10.5±0.1	10.4±0.1	10.5±0.1	10.4±0.1	0.482	0.487	0.539
Albumen proportion	63.7±0.4	63.9±0.3	64.1±0.3	64.6±0.3	63.7±0.3	64.0±0.4	64.1±0.3	64.4±0.3	0.150	0.541	0.569
Dry Matter (DM) (%)											
DM yolk	49.3±0.3	49.8±0.2	49.7±0.1	49.9±0.2	49.7±0.2^{ab}	49.8±0.2^{ab}	49.3±0.2^{b}	50.0±0.2^{a}	0.207	0.028	0.942
DM egg	24.0±0.1	24.1±0.1	23.8±0.1	24.4±0.2	23.8±0.2	24.1±0.2	24.3±0.2	23.9±0.2	0.156	0.150	0.140

[1] Values that do not share the same letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$); [2] 1 brown – 100 white; [3] yolk colour was measured with a DSM Yolk Colour Fan

4.4.2. Fatty acid profiles

The nutritionally relevant FA identified and reported in the tables 4.3 and 4.4 comprise more than 90% of the total FA detected in eggs. Results for the full FA profiles can be found in APPENDIX B.5 (APPENDIX B.5 Table 1. 2, APPENDIX B.5 Table 1. 3). The effect of management and date on the fat composition of eggs collected from supermarkets and EO farms in the UK between July 2017 and April 2018 are shown in Table 4. 3. Table 4. 4 presents results from the comparison of eggs from April 2017 when birds had no outside access and were confined into their housing with those from April 2018, when outside access was permitted.

Management system had a significant overall impact on the egg FA content and profile. The lowest lipid content was found for FR eggs, which differed only from EO (- 7%), although both these systems were similar to CA and OR eggs. Eggs compared between April 2017 and 2018, also revealed no difference between CA, FR and OR eggs in their lipid content.

Although total SFA did not differ between systems, individual FA contents of the minor C14:0 and the most abundant SFA, C16:0, were both different; C16:0 was lowest in EO eggs although not statistically different from OR eggs. Organic eggs were lower in C16:0 (-4%) than CA but not FR egg, while FR and CA eggs had similar contents. It has to be noted that whilst CA eggs had a statistically lower C16:0 content than FR and OR eggs when April dates were compared, numerically this difference was very small at <1%. Regarding C14:0, the content of this FA in eggs from OR systems (both OR and EO) were on average of 2% lower than that from conventional CA and FR systems.

Total MUFA showed the lowest content in OR eggs, had on average 8% less MUFA than CA and FR eggs, while having a similar content to EO eggs. At the same time EO eggs had a similar MUFA content to CA and FR eggs. The most abundant MUFA, OA was highest in CA eggs; +4% higher than in FR eggs and an average of +10% higher compared with eggs from both OR and EO systems. Although OR eggs had statistically less OA content than FR eggs, the content in EO and FR eggs were statistically similar. As for OA, c11C18:1 was the highest in CA eggs which was 7% higher than in eggs from FR and 21% higher than that in OR eggs (OR and EO). The differences between systems for c9C16:1 follow exactly the pattern as that for C16:0.

Total PUFA was the lowest in CA eggs; 10% less than FR eggs and an average of 26% lower than in OR and EO eggs. Whereas PUFA in OR eggs was higher than FR eggs, EO did not differ from FR eggs. These results were similar when eggs between April 2017 and April 2018 were compared. The most abundant PUFA, LA, was lowest in CA eggs although levels were statistically similar to those in FR eggs. At the same time the content in FR eggs was also similar

to EO but 15% lower than in OR eggs. The highest LA content was in OR eggs, whilst this did not differ from the content in EO eggs. Dominating the total n-6 FA, LA drove the pattern of the group of n-6 FA. For ALA, DHA, n-3>18C and total n-3 FA, OR (both OR and OE) eggs had a higher content compared with conventional eggs (either CA or FR); at +60%, +44%, +47% and +31%, respectively. Again, results were similar when eggs from April 2017 and April 2018 were compared, apart from ALA which was different between CA and FR eggs. The highest DPA content was found in OR eggs and although there were not distinct differences between eggs of different systems, the contents of this FA followed the pattern already described for n-6 FA. The ratio of n-6/n-3 FA was the lowest in OR and EO eggs and by 18% lower than FR eggs, while being statistically similar with CA eggs. Eggs from CA and FR systems were statistically similar.

The FA profile did not differ over time (with the sole exception of the n-6/n-3 ratio) although the total lipid content did show small but significant differences between dates. Date had no overall effect on egg FA composition except on total lipid content and the n-6/n-3 ratio. The lowest content was found for April eggs, which was statistically different only from January (-8%). Eggs from both these dates were similar to July and October. With regards to n-6/n-3 ratio, July eggs had the lowest ratio, which was statistically different only from April eggs (-17%). Again, eggs from both these dates were similar to those collected in October and January. A trend of n-6 FA being lower in July and October was found. When eggs from April 2017 were compared with eggs from April 2018, there was no difference in lipid content and minor differences in its composition with respect to the n-6/n-3 ratio. A lower ratio was found for April 2017 eggs when birds were confined to housing, driven by trends for lower LA and n-6 and more ALA in April 2017 eggs. Some other minor effects of season on were on unidentified FA and other minor FA including C20:3, C22:2 and C22:3 that are considered not particularly relevant for discussion and can be found in APPENDIX B.5 However, it is worth mentioning the year effect on C14:1 and its lower concentration in eggs when birds had access to the range in April 2018 (compared with April 2018), (APPENDIX B.5).

Table 4. 3. Means ± SE and ANOVA P values for yolk lipid content (%) and fatty acid (FA) profile (g/100g total FA) of eggs collected from supermarkets (caged, free-range and organic systems) and extensive organic farms in the UK by management and date

	Management (M)				Date (D)				ANOVA - P values		
									Main Effects		Interactions
	Caged (CA) n=16	Free-range (FR) n=32	Organic (OR) n=32	Extensive organic (EO) n=19	Jul-17 n=25	Oct-17 n=25	Jan-17 n=25	Apr-18 n=24	M	D	M x D
Lipid content	33.3±0.8^{ab}	33.4±0.6^{b}	34.3±0.5^{ab}	35.9±0.6^{a}	34.4±0.6^{ab}	34.5±0.6^{ab}	35.3±0.7^{a}	32.3±0.6^{b}	0.032	0.012	0.7077
Fatty acids[1]											
SFA											
C14:0	0.33±0.01^{a}	0.32±0.01^{a}	0.30±0.01^{b}	0.29±0.01^{b}	0.31±0.01	0.31±0.01	0.30±0.01	0.31±0.01	0.001	0.379	0.546
C16:0	25.3±0.1^{a}	24.8±0.2^{ab}	24.4±0.1^{bc}	23.9±0.2^{c}	24.8±0.2	24.6±0.2	24.5±0.2	24.5±0.2	<0.001	0.350	0.039
C18:0	7.92±0.08	7.60±0.25	7.79±0.08	7.84±0.45	7.95±0.11	7.45±0.32	7.73±0.33	7.92±0.09	0.874	0.403	0.308
MUFA											
c9C16:1	3.28±0.11^{a}	3.09±0.10^{ab}	2.80±0.11^{bc}	2.54±0.14^{c}	2.98±0.11	2.99±0.12	2.77±0.14	2.94±0.12	0.001	0.449	0.130
OA	41.0±0.6^{a}	39.4±0.4^{b}	36.7±0.3^{c}	37.8±0.5^{bc}	38.6±0.4	39.0±0.7	37.9±0.5	38.3±0.6	<0.001	0.546	0.338
c11C18:1	2.45±0.07^{a}	2.28±0.05^{b}	2.08±0.03^{c}	1.98±0.06^{c}	2.18±0.05	2.23±0.07	2.13±0.05	2.20±0.05	<0.001	0.539	0.159
PUFA											
LA	13.0±0.7^{c}	15.3±0.7^{bc}	17.9±0.3^{a}	17.4±1.1^{ab}	15.3±0.5	15.0±1.0	16.3±0.8	16.9±0.6	<0.001	0.115	0.461
ALA	0.62±0.03^{b}	0.73±0.04^{b}	1.12±0.056^{a}	1.04±0.04^{a}	0.85±0.05	0.847±0.08	0.93±0.07	0.965±0.07	<0.001	0.295	0.926
AA	1.74±0.04	1.83±0.07	1.74±0.03	1.97±0.10	1.73±0.03	1.79±0.08	1.92±0.09	1.80±0.04	0.104	0.290	0.199
DPA	0.12±0.01^{c}	0.13±0.01^{bc}	0.16±0.01^{a}	0.10±0.01^{ab}	0.14±0.005	0.14±0.01	0.14±0.01	0.14±0.01	<0.001	0.909	0.468
DHA	0.83±0.03^{b}	0.86±0.03^{b}	1.28±0.05^{a}	1.15±0.07^{a}	1.06±0.06	0.98±0.08	1.08±0.05	1.05±0.05	<0.001	0.461	0.485
FA groups											
SFA	33.9±0.2	33.1±0.3	32.1±0.8	32.4±0.6	33.4±0.2	32.7±0.4	31.9±1.1	33.0±0.2	0.238	0.373	0.735
MUFA	47.4±0.7^{a}	46.0±0.8^{a}	43.0±0.9^{b}	44.2±1.4^{ab}	44.4±0.6	45.8±1.2	45.4±1.4	44.0±0.6	0.011	0.507	0.664
PUFA	17.1±0.8^{c}	19.2±0.7^{b}	23.3±0.3^{a}	21.8±1.1^{ab}	20.2±0.6	19.7±1.0	21.3±0.8	21.6±0.7	<0.001	0.122	0.417
n-3	1.80±0.09^{b}	2.01±0.08^{b}	2.82±0.11^{a}	2.67±0.06^{a}	2.51±0.11	2.30±0.16	2.36±0.11	2.29±0.12	<0.001	0.365	0.391
n-3>18C	0.99±0.04^{b}	1.03±0.06^{b}	1.50±0.05^{a}	1.46±0.05^{a}	1.23±0.07	1.23±0.08	1.35±0.08	1.25±0.06	<0.001	0.530	0.486
n-6	15.2±0.7^{c}	17.6±0.7^{bc}	20.4±0.3^{a}	19.9±1.0^{ab}	17.6±0.5	17.3±0.9	18.8±0.8	19.3±0.6	<0.001	0.065	0.451
n-6/n-3	8.6±0.32^{ab}	9.1±0.34^{a}	7.5±0.28^{b}	7.5±0.40^{b}	7.2±0.22^{b}	8.0±0.42^{ab}	8.3±0.41^{ab}	8.7±0.31^{a}	0.002	0.014	0.323

[1] Values that do not share the same letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$) [2] SFA = saturated FA(C12:0, C14:0+9C13:1, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0); MUFA = monounsaturated FA (c9C14:1, t9C16:1, c9C16:1, c9C17:1, t6+t7+t8C18:1, t9C18:1, t11C18:1, c9C18:1 (OA),

c11C18:1, c13C18:1, c5C20:1, c8C20:1, c15C24:1; PUFA = polyunsaturated FA (t10t14C18:2, t8c13C18:2, c9t12C18:2, t9c12C18:2, ctmix10,14+12,16C18:2, c9c12C18:2 (LA), c9c15C18:2, c6c9c12C18:3(GLA),c 9c12c15C18:3 (ALA), conjugated linoleic acid (CLA), t11c13C18:2, unknown CLA(t,t), unknown CLA(t,t), c9c13c15C18:3, c11c14C20:2, c9c11c15C18:3, c8c11c14C20:3, c11c14c17C20:3, c5c8c11c14C20:4 (AA), c13c16C22:2, c5c8c11c14c1720:5(EPA), c7c10c13c16C22:4,c7c10c19C22:3, c4c7c10c13c16C22:5, c7,c10,c13,c16,c19C22:5(DPA), c4,c7,c10,c13,c16,c19C22:6 (DHA)); omega 3(n-3) = c9c15C18:2, ALA, c9c13c15C18:3, c9c11c15C18:3, EPA, c11c14c17C20:3, DPA, DHA; n-3<18C (very long chain n-3): EPA, c11c14c17C20:3, DPA, DHA; omega-6 (n-6) = c9t12C18:2, t9c12C18:2, LA, GLA, c11c14C20:2, c8c11c14C20:3, AA, c13c16C22:2, c7c10c13c16C22:4, C22:5n-6; c = cis; t = trans

Table 4. 4. Means ± SE and ANOVA P values for yolk lipid content (%) and fatty acid (FA) profile (g/100g total FA) of eggs from different management systems collected from supermarkets in the UK on April 2017 (all hens confined to housing) and April 2018 (free-range and organic hens had range access).

	Management (M)			Year (Y)		ANOVA - P values		
						Main Effects		Interactions
	Caged n=8	Free-range n=16	Organic n=16	Apr-17 n=20	Apr-18 n=20	M	Y	M x Y
Lipid content	31.0±1.0	32.1±0.7	32.7±0.6	32.4±0.5	31.8±0.7	0.823	0.518	0.822
Fatty acids[1]								
SFA								
C14:0	0.34±0.02^{a}	0.32±0.01ab	0.30±0.01b	0.32±0.01	0.31±0.01	0.077	0.913	0.577
C16:0	25.5±0.1^{a}	24.4±0.2^{b}	24.3±0.2^{b}	24.7±0.2	24.5±0.2	0.001	0.407	0.471
C18:0	7.75±0.10	7.92±0.08	7.71±0.11	7.75±0.08	7.86±0.09	0.291	0.390	0.413
MUFA								
c9C16:1	3.54±0.09^{a}	2.88±0.10^{b}	2.95±0.16^{b}	3.07±0.10	3.01±0.14	0.011	0.680	0.170
OA	41.7±0.6^{a}	39.4±0.6^{b}	37.3±0.4^{c}	39.4±0.5	38.7±0.6	<0.001	0.275	0.975
c11C18:1	2.56±0.08^{a}	2.23±0.04^{b}	2.12±0.06^{b}	2.25±0.07	2.26±0.05	<0.001	0.864	0.192
PUFA								
LA	12.0±0.7^{c}	15.9±0.7^{b}	17.9±0.4^{a}	15.3±0.7	16.6±0.7	<0.001	0.093	0.547
ALA	0.59±0.05^{c}	0.77±0.05^{b}	1.05±0.08^{a}	0.78±0.06	0.91±0.07	<0.001	0.075	0.741
AA	1.68±0.06	1.77±0.03	1.79±0.04	1.73±0.03	1.78±0.04	0.247	0.343	0.301
DPA	0.13±0.02	0.13±0.01	0.15±0.01	0.13±0.010	0.14±0.01	0.238	0.821	0.728
DHA	0.85±0.07^{b}	0.87±0.027^{b}	1.17±0.07^{a}	0.98±0.06	1.00±0.05	<0.001	0.736	0.902
FA groups								
SFA	33.9±0.1^{a}	33.0±0.2^{b}	32.7±0.1^{b}	33.1±0.2	33.0±0.2	0.001	0.670	0.247
MUFA	48.5±0.7^{a}	45.1±0.7^{b}	42.9±0.5^{c}	45.3±0.7	44.5±0.7	<0.001	0.285	0.750
PUFA	16.1±0.8^{c}	20.3±0.7^{b}	23.0±0.5^{a}	19.9±0.8	21.2±0.8	<0.001	0.137	0.608
n-3	1.82±0.12^{b}	2.01±0.08^{b}	2.61±0.15^{a}	2.25±0.12	2.17±0.13	<0.001	0.559	0.936
n-3>18C	1.01±0.08^{b}	1.05±0.03^{b}	1.39±0.08^{a}	1.16±0.07	1.19±0.06	<0.001	0.767	0.962
n-6	14.2±0.7^{c}	18.2±0.7^{b}	20.3±0.4^{a}	17.6±0.7	18.9±0.7	<0.001	0.095	0.533
n-6/n-3	7.9±0.40^{ab}	9.1±0.26^{a}	8.1±0.45^{a}	8.0±0.30	9.0±0.33	0.049	0.033	0.351

[1] SFA = saturated FA(C12:0, C14:0+9C13:1, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0); MUFA = monounsaturated FA (c9C14:1, t9C16:1, c9C16:1, c9C17:1, t6+t7+t8C18:1, t9C18:1, t11C18:1, c9C18:1 (OA), c11C18:1, c13C18:1, c5C20:1, c8C20:1, c15C24:1; PUFA = polyunsaturated FA (t10t14C18:2, t8c13C18:2, c9t12C18:2, t9c12C18:2, ctmix10,14+12,16C18:2, c9c12C18:2 (LA), c9c15C18:2, c6c9c12C18:3(GLA),c 9c12c15C18:3 (ALA), conjugated linoleic acid (CLA), t11c13C18:2, unknown CLA(t,t), unknown CLA(t,t), c9c13c15C18:3, c11c14C20:2, c9c11c15C18:3, c8c11c14C20:3, c11c14c17C20:3, c5c8c11c14C20:4 (AA), c13c16C22:2, c5c8c11c14c1720:5(EPA), c7c10c13c16C22:4,c7c10c19C22:3, c4c7c10c13c16C22:5, c7,c10,c13,c16,c19C22:5(DPA), c4,c7,c10,c13,c16,c19C22:6 (DHA)); omega 3(n-3) = c9c15C18:2, ALA, c9c13c15C18:3, c9c11c15C18:3, EPA, c11c14c17C20:3, DPA, DHA; n-3<18C (very long chain n-3): EPA, c11c14c17C20:3, DPA, DHA; omega-6 (n-6) = c9t12C18:2, t9c12C18:2, LA, GLA, c11c14C20:2, c8c11c14C20:3, AA, c13c16C22:2, c7c10c13c16C22:4, C22:5n-6; c = cis; t = trans

Interactions

The only meaningful interaction noticed was for the C16:0 FA (Figure 4. 1). No other interactions were significant, apart from some weak interactions for some unidentified FA and one minor n-3 FA that were considered of low nutritional relevance (APPENDIX B.5 Table 1. 2) and so are not included for discussion in this Chapter. Regarding the percentage of C16:0, in July and April, management system did not have an impact and eggs from all systems were similar in their C16:0 content. However, in October and January, the concentration in EO eggs was 7% lower than CA and FR eggs within those months and it was also constantly lower than CA and FR eggs in July and April (but similar with FR in April). When April 2017 was compared with April 2018, there were no meaningful or significant interactions and the significant C16:0 interaction was not shown.

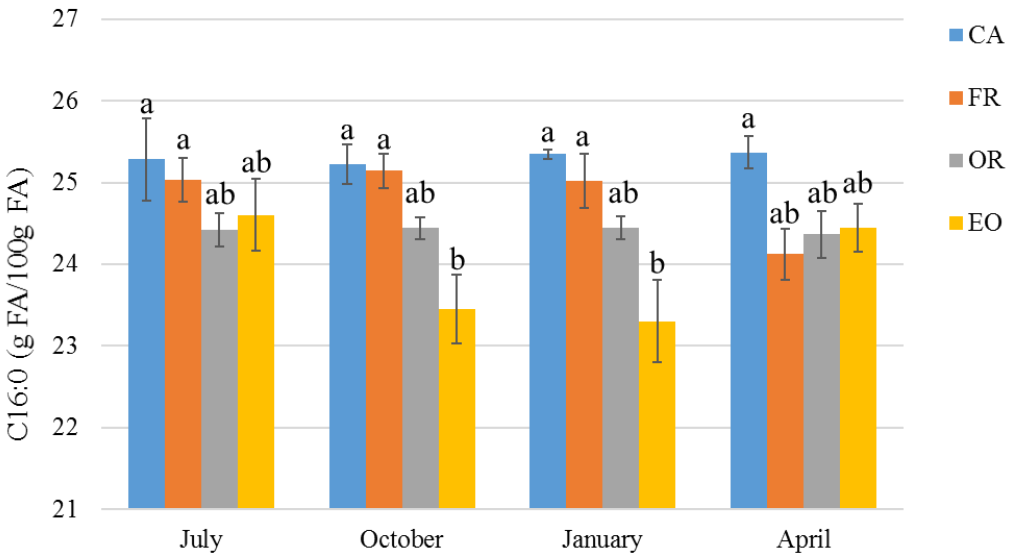

Figure 4. 1. Interactions means ± SE for the effects of management (caged (CA), free-range (FR), organic (OR) and extensive organic (EO)) and dates (July (2018) , October, January and April) on the percentage of C16:0 fatty acid (FA) (g FA/100g total FA) of yolks collected from supermarkets (CA, FR and OR systems) and EO farms in the UK. Bars labelled with different letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$)

4.4.3. Carotenoids, vitamin A and E

Typical chromatograms of the measured compounds are shown in APPENDIX B.5 Figure 1. 1. No β- or α-carotenes were detected in yolk samples. Results for the effect of management and date of eggs collected from supermarkets and EO farms in the UK between July 2017 and April 2018 are shown in Table 4. 5. Results from the comparison between eggs from birds confined

to housing in April 2017 and from those with outside access in April 2018 are shown in Table 4. 6.

Management had a significant impact on yolk carotenoids and vitamin A and E. Specifically, lutein, zeaxanthin, canthaxanthin and β-cryptoxanthin were the highest in EO eggs, followed by OR eggs which were 37%, 53%, 51% and 43% lower for lutein, zeaxanthin, canthaxanthin and β-cryptoxanthin respectively. The lowest contents were found in CA and FR eggs which were statistically similar and 67%, 88%, 83% and 77% lower for lutein, zeaxanthin, canthaxanthin and β-cryptoxanthin respectively, when compared with EO eggs. Unknown carotenoids 1 and 2 exhibited similar behaviour to the carotenoids described above, whereas minor unknown compounds 3 was the highest in FR eggs while similar across CA, OR and EO eggs. Similar relationships for management were noticed throughout the comparison of eggs between birds kept indoors and under normal conditions (Table 4. 6), except lutein content which was similar between FR and OR eggs.

Vitamin A content of the eggs was more consistent than the carotenoids although was lowest in CA eggs which were 9% lower than FR and OR eggs. At the same time, eggs from CA systems were statistically similar to EO eggs, while EO were statistically similar to FR and OR eggs. Total vitamin E, was the lowest in CA and FR eggs, by an average of 32% lower than in OR eggs. At the same time, eggs from OR systems were statistically similar to EO eggs, as well as CA and FR eggs. Similarly, α-tocopherol, the largest contributor to total vitamin E content, showed a similar pattern, except that the only statistical difference was between CA and OR eggs. No equivalent differences were shown in the indoor and outdoor comparison between the two April collection dates (Table 4. 6.). Minor tocopherols, γ- and δ-tocopherol, with lower vitamin A activity, showed a similar pattern to α-tocopherol; the highest contents were found for OR and OE eggs, while CA and FR were similar; γ-tocopherol was on average 98% higher and δ-tocopherol was on average 74% higher in OR and OE eggs, compared with both conventional egg types.

Date had a very limited impact. The only compounds changing over time were the minor tocopherols, γ- and δ-tocopherol. For γ-tocopherol, the lowest concentrations were found in July when it was on average 20% lower compared with all other months. The only difference for δ-tocopherol was between eggs in July and those collected in January when the concentrations were higher. When all birds were kept indoors compared with normal conditions, date had an effect only on vitamin A, which was 14% higher when the birds had access to the outside.

Table 4. 5. Means concentrations ± SE and ANOVA P values for the effect of management and date on yolk carotenoids, vitamin A and E (µg/100 g yolk) of eggs collected from supermarkets (caged, free-range and organic systems) and extensive organic farms in the UK

	Management (M)				Date (D)				ANOVA - P values		
									Main Effects		Interactions
	Caged (CA)	Free-range (FR)	Organic (OR)	Extensive organic (EO)	Jul-17	Oct-17	Jan-17	Apr-18			
	n=16	n=32	n=32	n=19	n=25	n=25	n=25	n=24	M	D	M x D
Carotenoids [1]											
Lutein	285±21^{c}	461±46^{c}	721±59^{b}	1142±99^{a}	622±72	723±103	632±62	613±95	<0.001	0.537	0.058
Zeaxanthin	32.1±3.5^{c}	84.7±10.9^{c}	220.1±22.9^{b}	467.4±37.4^{a}	199.3±33.4	212.2±42.5	174.4±33.4	187.4±40.8	<0.001	0.637	0.514
Canthaxanthin	12.8±1.2^{c}	23.0±2.1^{c}	52.2±5.1^{b}	106.1±8.2^{a}	48.4±7.6	48.6±9.4	44.6±7.1	45.3±8.4	<0.001	0.921	0.512
β-cryptoxanthin	1.47±0.26^{c}	4.15±0.46^{c}	7.12±0.91^{b}	12.48±1.30^{a}	6.57±1.11	6.67±1.21	5.90±1.03	5.94±1.08	<0.001	0.875	0.439
Unknown 1	28.0±2.1^{c}	49.2±4.9^{c}	75.9±4.6^{b}	145.7±12.0^{a}	69.2±8.2	83.6±12.9	68.9±6.6	69.8±11.7	<0.001	0.235	0.028
Unknown 2	27.3±2.1^{c}	44.0±4.0^{c}	72.8±5.0^{b}	116.4±8.6^{a}	62.9±7.0	71.0±10.2	64.1±5.9	59.8±8.7	<0.001	0.547	0.050
Unknown 3	22.4±10.4^{b}	66.7±15.5^{a}	5.22±1.08^{b}	13.92±2.32^{b}	26.44±9.3	20.18±8.9	32.34±12.2	39.53±16.0	<0.001	0.513	0.686
Vitamin A [2]	350±11^{b}	384±7^{a}	383±9^{a}	383±10^{ab}	375±10	389±8	378±9	368±10	0.008	0.418	0.315
Vitamin E [3]	1464±131^{b}	2123±130^{b}	2649±245^{a}	2574±227^{ab}	2191±233	2211±212	2424±221	2265±217	0.001	0.742	0.322
α-tocopherol	1387±122^{b}	2002±128^{ab}	2481±241^{a}	2350±225^{ab}	2066±228	2069±203	2257±212	2103±214	0.002	0.808	0.398
γ-tocopherol	773±101^{b}	1201±91b	1677±100^{a}	2230±214^{a}	1246±127^{b}	1415±179^{a}	1657±174^{a}	1621±127^{a}	<0.001	0.041	0.019
δ-tocopherol	42.4±4.3^{b}	53.9±3.6^{b}	83.4±5.3^{a}	83.7±7.3^{a}	55.7±4.4^{b}	63.4±6.^{ab}	77.4±7.5^{a}	72.9±4.9^{ab}	<0.001	0.010	0.199

1 Values that do not share the same letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$); 2 Vitamin A is the sum of retinol expressed as retinol equivalents, 3 Vitamin E is the sum of α- (x 1), γ- (x 0.1), and δ-tocopherol (x 0.01), expressed as α-tocopherol equivalents.

0

Table 4. 6. Means ± SE and ANOVA P values for yolk carotenoids, vitamin A and E (µg/100 g yolk) of eggs from different management systems collected from supermarkets in the UK on April 2017 (all hens confined to housing) and April 2018 (free-range and organic hens had range access).

	Management (M)			Year (Y)		ANOVA - P values[1]		
						Main Effects		Interactions
	Caged n=8	Free-range n=16	Organic n=16	Apr-17 n=20	Apr-18 n=20	M	Y	M x Y
Carotenoid[1]								
lutein	258±29^{b}	437±66^{ab}	528±39a	426±58	450±39	0.015	0.708	0.642
zeaxanthin	23.1±4.6^{b}	68.8±12.6^{b}	240±38.3^{a}	131.0±33.3	125.4±28.8	<0.001	0.863	0.630
canthaxanthin	10.9±1.2^{b}	20.1±2.7^{b}	57.1±8.9^{a}	33.5±7.5	32.6±6.3	<0.001	0.909	0.553
β-cryptoxanthin	1.32±0.37^{b}	3.7±0.57^{b}	11.3±2.48^{a}	7.50±2.14	5.03±1.10	0.002	0.223	0.143
Unknown 1	24.2±3.7^{b}	46.0±6.1^{b}	71.4±6.9^{a}	53.2±7.9	50.4±5.1	0.001	0.697	0.369
Unknown 2	25.1±3.1^{c}	41.9±5.8^{b}	56.8±3.3^{a}	44.5±5.5	44.4±3.8	0.001	0.984	0.874
Unknown 3	26.4±18.2^{b}	80.2±24.4^{a}	8.8±3.6^{b}	37.1±13.4	44.7±19.1	0.006	0.700	0.305
Vitamin A [2]	322±16^{b}	363±13^{a}	343±13^{ab}	324±8	370±12	0.017	0.008	0.172
Vitamin E [3]	1432±158	2490±198	2660±448	2515±344	2178±238	0.106	0.360	0.590
α-tocopherol	1362±152	2335±199	2475±441	2367±336	2025±232	0.150	0.345	0.633
γ-tocopherol	690±118^{b}	1544±128^{a}	1850±209^{a}	1470±209	1521±123	0.002	0.809	0.301
δ-tocopherol	38.6±5.4^{b}	67.8±5.7^{a}	83.7±7.7^{a}	65.2±7.8	71.4±5.4	0.003	0.480	0.978

[1] Values that do not share the same letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$); [2] Vitamin A is the sum of retinol expressed as retinol equivalents, [3] Vitamin E is the sum of α- (x 1), γ- (x 0.1), and δ-tocopherol (0.01), expressed as α-tocopherol equivalents

The only significant interaction found between management and date was for the carotenoid unknown 1 and for γ-tocopherol (Table 4. 5) and these interactions were not significant in the comparison of birds having access to the range or not. These interactions are shown in Figure 4. 2 and Figure 4. 3. Carotenoid unknown 1 demonstrated a similar behaviour within the systems and across the year although the magnitude of the difference between the systems was much greater for eggs collected in October and April than those from July and January. Statistically similar concentrations were found for CA, FR and OR yolks, with a numerical increase in the concentration among the aforementioned systems within each system and across the year. Eggs from EO systems were systematically higher than all other systems within each month, except for July, when EO eggs were similar to both FR and OR. However, even then, concentrations in EO eggs were numerically higher. The highest concentration of this carotenoid was found in October and April and the difference between management systems was the highest compared with July and January.

Concentration of γ-tocopherol showed a similar pattern in July and April and across different systems; eggs within systems were statistically similar, However, CA showed numerically lower concentrations systematically and FR eggs were similar to CA only in July. The highest concentration in eggs and the largest differences compared with the other eggs, were shown for EO eggs in October and January. Eggs from CA and OR systems had a stable concentration across the year without large variation across dates. Eggs from FR systems were statistically similar across dates with a slight numerical increase in January and especially in April.

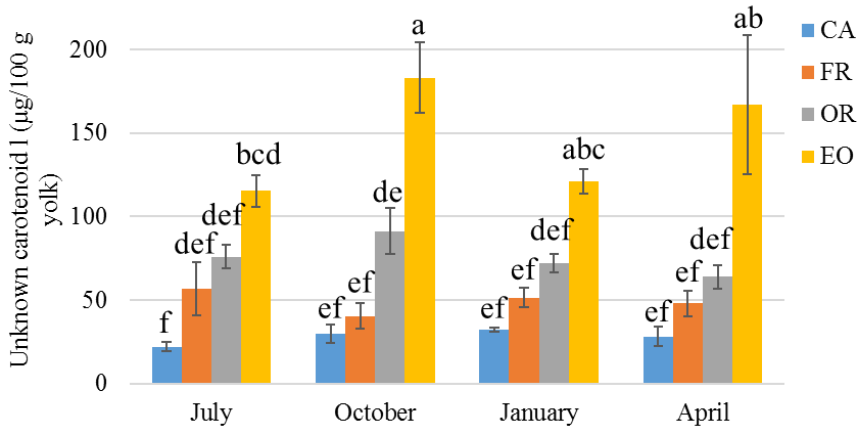

Figure 4. 2. Interactions means ± SE for the effects of management (caged (CA), free-range (FR), organic (OR) and extensive organic (EO)) and dates (July , October, January and April) on the percentage of unknown carotenoid 1 (µg/100 g yolk)of yolks collected from supermarkets (CA, FR and OR systems) and EO farms in the UK. Bars labelled with different letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$

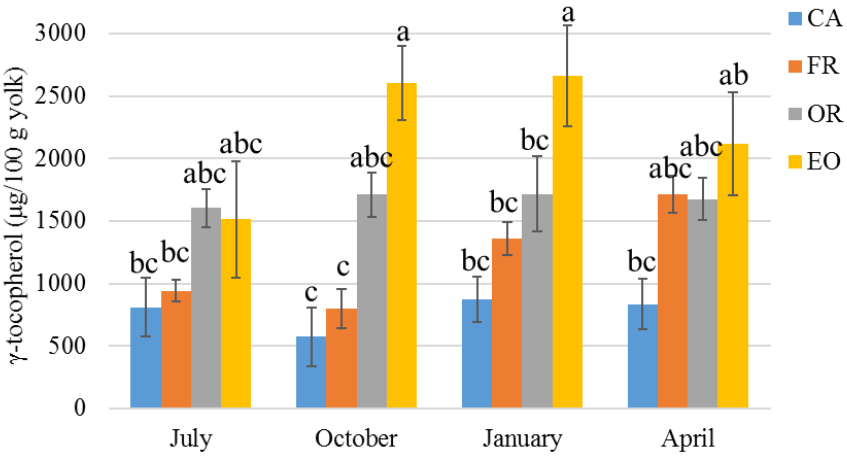

Figure 4. 3. Interactions means ± SE for the effects of management (caged (CA), free-range (FR), organic (OR) and extensive organic (EO)) and dates (July , October, January and April) on the percentage of γ-tocopherol (µg/100 g yolk)of yolks collected from supermarkets (CA, FR and OR systems) and EO farms in the UK. Bars labelled with different letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$

4.4.4. Vitamin D_3

The effect of management and date (July and January) on yolk vitamin D_3 contents can be found in Table 4. 7. Although management did not have a significant impact on vitamin D_3 there was a trend for 25-OH D_3 to be slightly lower in eggs from the EO system compared with the rest. On the contrary, significant differences were found for D_3, 25-OH D_3 and their combined concentrations between eggs collected in July and January. In general, concentrations were higher in July, with 54% more D_3 and almost 20% more 25-OH D_3 than in January. Total D_3 was overall 30% higher in July compared with January.

An interaction between management and date was shown for total D_3 potentially driven by a trend for an interaction for D_3 alone. As can be seen in Figure 4. 4, total D_3 concentration did not differ between the 2 months for CA, FR and OR eggs but, for EO eggs the January samples were 45% lower than those collected in July.

4.4.5. Vitamin B_2 and B_9

The effect of management and date (July vs January) on yolk vitamin B_2 and B_9 contents can be found in Table 4. 7. Contents of B_2 or B_9 in eggs did not different between management systems. Date had an impact on B_2 with 10% higher content in July. No differences were found for B_9 egg contents between dates. However, for the latter, an interaction between management and date was shown (Figure 4. 4). In July the B_9 content of EO eggs were significantly higher than that in CA and OR eggs. However, in January, EO eggs had a lower content than OR eggs but it was similar to CA eggs. Additionally, whereas for OR eggs, B_9 was significantly higher in January, for EO eggs, it was statistically higher by 137% in July. Within CA and FR eggs no variation between months was found.

Table 4. 7. Means ± SE and ANOVA P values for the effect of management and date on vitamin D_3 (µg/100g yolk), B_2 (mg/100g egg) and B_9 (µg/100g egg), of yolks and eggs collected from supermarkets (caged, free-range, organic) and extensive organic farms in the UK

	Management (M)				Date (D)		ANOVA - P values[1]		
							Main Effects		Interactions
	Caged	Free-range	Organic	Extensive organic	Jul-17	Jan-18	M	D	M x D
	n=8	n=16	n=16	n=10	n=25	n=25			
Compounds [2]									
total D_3	14.8±1.1	13.6±0.8	12.8±1.0	12.2±1.4	14.9±0.5	11.5±0.8	0.155	<0.001	0.047
vitamin D_3	5.5±0.67	4.4±0.55	4.4±0.54	4.8±0.78	5.7±0.38	3.7±0.39	0.400	0.002	0.062
25-OH D_3	1.9±0.09	1.8±0.09	1.7±0.11	1.5±0.14	1.9±0.07	1.6±0.08	0.073	0.013	0.270
B_2	0.39±0.01	0.41±0.01	0.42±0.01	0.41±0.02	0.43±0.01	0.39±0.01	0.264	0.001	0.375
B_9	29.1±3.2	30.7±2.8	34.3±3.1	26.9±3.9	32.6±1.6	29.1±2.8	0.264	0.116	0.020

1 Total vitamin D_3 is calculated as vitamin D_3+25-hydroxy 25-OHD_3 (x5)

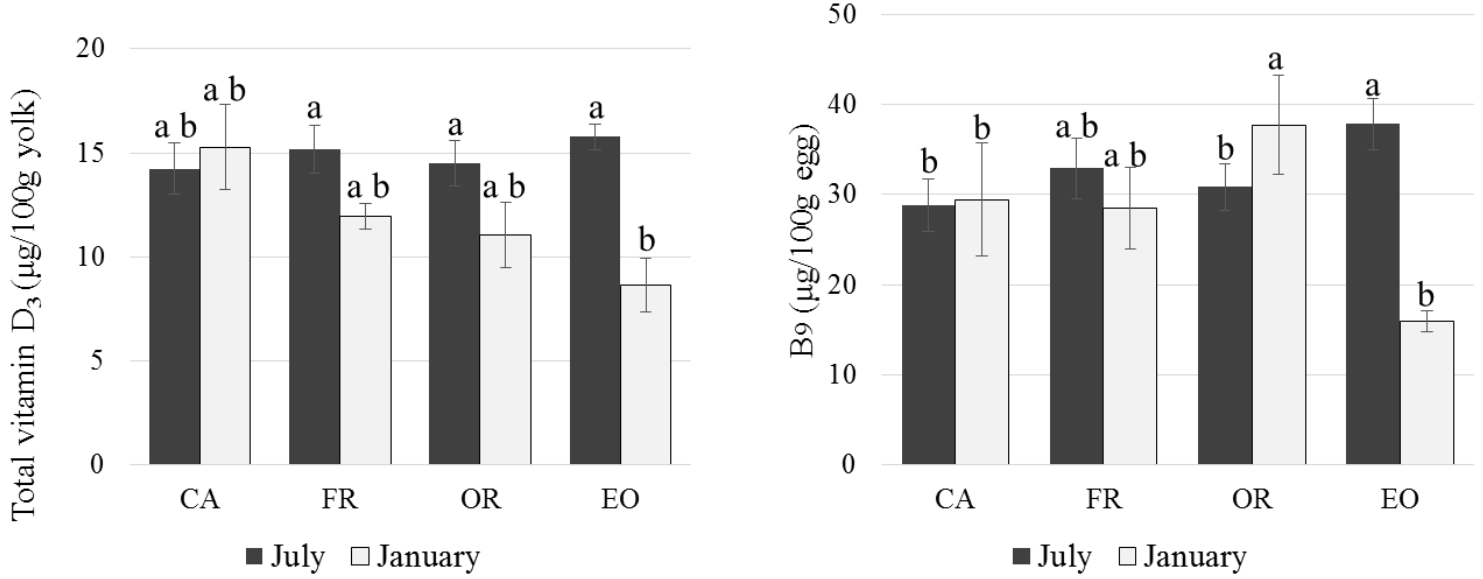

Figure 4. 4. Interactions means ± SE for the effects of management (caged (CA), free-range (FR), organic (OR) and extensive organic (EO)) and date (July vs January) on the concentrations of vitamin D_3 (µg/100g yolk) and B_9 (µg/100g egg), of yolks and eggs collected from supermarkets (CA, FR and OR systems) and EO farms in the UK. Bars labelled with different letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$

1

4.4.6. Chemical elements

Results of elemental analysis can be found in Table 4. 8. The analytical methods used in this study were not sensitive enough to detect and quantify cobalt, cadmium, selenium, chromium and arsenic.

Management had a significant overall impact on elements concentration, whereas date had very little effect. Barium content was generally very low but highest in CA eggs (0.07 mg/100 g fresh eggs), followed by FR and EO and last by OR eggs (0.05, 0.04 and 0.04 mg/100 g fresh eggs respectively). Calcium was highest in CA eggs and while similar to OR and EO eggs, it was significantly different and 9% higher from FR eggs only. Contents of Ca in FR eggs were numerically and statistically similar to that in OR and EO eggs. The magnesium content of eggs was statistically different only between CA and OR eggs, with CA having 7% higher content of Mg. Eggs from CA systems were similar to FR and EO, OR eggs were also statistically similar to FR and EO eggs. Phosphorus levels were the highest of all minerals and showed a trend for being greater in EO eggs compared with others. Zinc concentration was highest in CA, EO and statistically similar with FR eggs. At the same time, CA and EO eggs were not statistically different and averaged 6 % higher than OR eggs.

Date only had an impact on Mn, which was found in slightly lower concentrations in January compared with July and October, while being similar to the content in April.

The only interaction between management and date was a weak relationship ($P<0.05$) found for Mg, (Figure 4. 5). Whilst most groups did not differ from each other in Mg content across the sampling dates, exceptions were for low Mg in OR eggs laid in January and higher Mg in CA eggs laid in April.

Table 4. 8. Means ± SE and ANOVA P values for the effect of management and date on chemical elements (mg/100g) of eggs collected from supermarkets (caged, free-range and organic systems) and extensive organic farms in the UK.

	Management (M)				Date (D)				ANOVA - P values		
									Main Effects		Interactions
	Caged (CA)	Free-range (FR)	Organic (OR)	Extensive organic (EO)	Jul-17	Oct-17	Jan-17	Apr-18			
	n=16	n=32	n=32	n=19	n=25	n=25	n=25	n=24	M	D	M x D
Elements [1]											
Barium (Ba)	0.07±0.006^{a}	0.05±0.002^{b}	0.04±0.002^{c}	0.04±0.003^{bc}	0.05±0.003	0.05±0.004	0.05±0.003	0.06±0.003	<0.001	0.108	0.476
Calcium (Ca)	57.6±1.7^{a}	53.0±0.9^{b}	53.3±1.0^{ab}	55.8±1.0^{ab}	52.6±1.1	55.7±1.1	55.2±1.4	54.2±0.8	0.022	0.239	0.166
Iron (Fe)	1.90±0.05	1.91±0.03	1.86±0.04	1.93±0.05	1.86±0.05	1.94±0.04	1.88±0.03	1.91±0.04	0.509	0.801	0.116
Magnesium (Mg)	14.7±0.3^{a}	14.3±0.2^{ab}	13.7±0.2^{b}	14.1±0.2^{ab}	13.8±0.2	14.4±0.2	14.0±0.2	14.3±0.2	0.024	0.157	0.011
Manganese (Mn)	0.04±0.002	0.04±0.001	0.04±0.001	0.03±0.002	0.04±0.001^{a}	0.04±0.002^{a}	0.03±0.001^{b}	0.03±0.001^{ab}	0.513	0.003	0.643
Phosphorus (P)	224±4	219±3	218±4	231±4	215±4	229±4	222±4	222±3	0.079	0.160	0.129
Zinc (Zn)	1.36±0.03^{a}	1.27±0.03^{ab}	1.26±0.02^{b}	1.32±0.03^{a}	1.26±0.03	1.32±0.03	1.27±0.03	1.30±0.02	0.044	0.415	0.426

1 Values that do not share the same letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$); Cobalt (Co), Cadmium (Cd), Selenium (Se), Chromium (Cr) and Arsenic (As) were all below the limit of quantifications (LOQ) with the methods used.

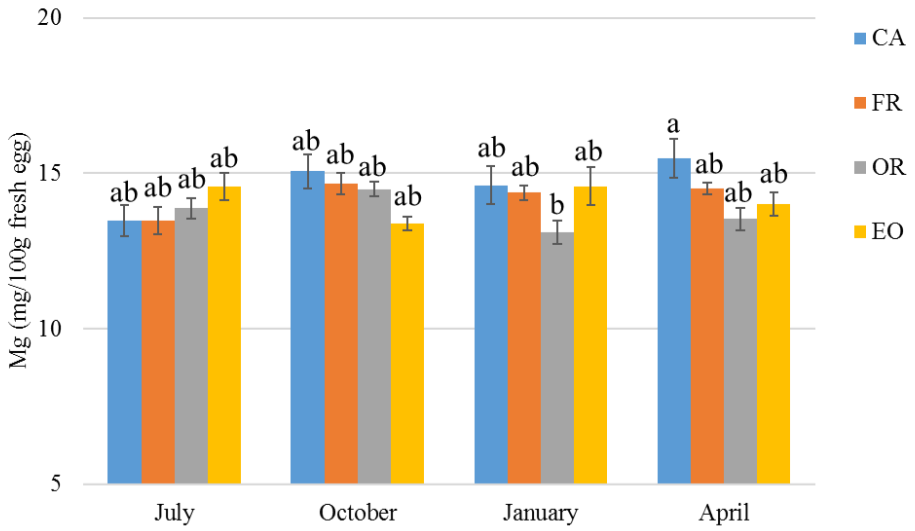

Figure 4. 5. Interactions means ± SE for the effects of management (caged (CA), free-range (FR), organic (OR) and extensive organic (EO)) and date (July, October, January and April) on the concentration of Magnesium (Mg), collected from supermarkets (CA, FR and OR systems) and EO farms in the UK. Bars labelled with different letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$

4.5 DISCUSSION

This study examined the nutritional differences between conventional indoor, conventional FR and OR eggs available at the supermarket retail level but also EO eggs available at farm gates. Extensive organic small scale farms were additionally, chosen to represent maximum foraging practices. Extensive foraging systems have been shown to be able to incorporate more nutritional compounds into the eggs (Mugnai et al., 2014) probably because of higher pasture availability per bird and more intense ranging behaviour. Although FR small-scale farms could have also been chosen, OR certification ensured maximum foraging practices and less variation between systems because of the clear difference between OR and conventional feed standards. Since there were differences in the results between OR and EO eggs, pooling these eggs under one OR group could mask system-specific effects and therefore was deemed unwarranted. Package information was the only background information used for supermarket eggs and hence the contributors of the variation are assumed to be the management practices overall. Dates were chosen to represent all seasonal changes related to ranging behaviour, pasture availability and forage intake. Analyses of standard shell and egg quality characteristics were also included in this study as complementary measurements that could be used to explore variation and patterns according to management systems. Overall, although, there is no information of the feed composition, the results of this retail study represent realistically the intake of egg nutrients consumers can have. Therefore such results can be readily used to calculate intakes and suggest recommendations.

4.5.1. External and internal standard egg quality assessments

External characteristics and standard egg quality, including proportions of egg parts are important aspects of the overall egg quality and consumer acceptance or preference. Shell colour is affected by many factors including bird genotype, age and rearing system (Samiullah et al., 2015). The results presented here indicated that OR and EO eggs had browner shell colour than CA eggs which is opposite to Kucukyilmaz et al. (2012) who showed that eggs from CA systems had more brown egg shell colour than FR and OR eggs. Contrary to our study, the latter study was experimentally set up and therefore other factors that could influence the egg colour (genotype) were controlled. The results from this study also showed a seasonal effect, where in April the egg shell was more brown compared with July. However, no information on genotype or age of the flocks which produced the eggs was available, it is not clear if this was a seasonal effect or potentially an age effect. The lack of management effect on shell density is in agreement with the literature (Campbell et al., 2017, Jones et al., 2010, Krawczyk et al., 2011). Shell density might be affected by shell weight and shell proportion. In this study although shell

weight was not affected by management or date similarly to Samman et al. (2009), shell proportion was shown to be higher for FR compared with EO eggs. However, the differences were very small. In contrast, CA and eggs from indoor systems have been reported to have higher shell weight than FR eggs (Jones et al., 2010, Krawczyk et al., 2011). Campbell et al. (2017), has shown that the shell proportion was the highest in the highest stocking density flocks. On the other hand, similar to the results from this experiment, Cherian et al. (2002) and Minelli et al. (2007), showed that the shell proportion between CA and OR eggs was similar. Lordelo et al. (2017), found no difference in shell proportion between CA FR and OR eggs. As for other parameters, age of birds has been shown to highly influence these qualities. Johnston and Gous (2007) has shown that shell density decreases with increasing age, while the opposite (Campbell et al., 2017, Minelli et al., 2007) and no age effect at all (Van Den Brand et al., 2004) has also been shown. Shell density is an important functional effect reassuring the egg reaching the consumer intact. Therefore, differences between systems are not ideal.

Another aspect of quality is the haugh units (HU) and the directly connected albumen height as this indicates the freshness of an egg. Both of these measurements followed the pattern led by the egg's age as clearly shown through literature (Silversides and Scott, 2001). The lack of management effects on HU units and albumen height has previously been shown (Hidalgo et al., 2008, Jones et al., 2010, Kucukyilmaz et al., 2012). However, according to Hidalgo et al. (2008) although they found no effect of management for albumen height, they found CA and FR eggs had similar HU values which were both higher than OR eggs. Concerning the whole egg, yolk and albumen weight, results on the effect of management vary through the literature. The results from the present study showing higher egg weight for EO and OR eggs followed by CA and FR eggs, are led by the differences in albumen weight. In contrast, Kucukyilmaz et al. (2012) and Lordelo et al. (2017) have shown higher egg weight for indoor eggs compared with FR and OR eggs. No differences between conventional and FR or OR eggs have also been reported by others in the literature (Jones et al., 2010, Kucukyilmaz et al., 2012, Samiullah et al., 2015, Samman et al., 2009). The results here on yolk weight and proportion are similar to those reported by Samiullah et al. (2015) and Krawczyk et al. (2011) who found no differences between conventional and OR systems. On the other hand, higher yolk proportion in conventional systems has also been documented (Cherian et al., 2002, Lordelo et al., 2017). For both the overall egg weight and yolk weight, most of the research agrees that these measures both increase with the bird's age (Johnston and Gous, 2007, Silversides and Scott, 2001, Van Den Brand et al., 2004). Genotype differences have also been documented (Kucukyilmaz et al., 2012, Van Den Brand et al., 2004, Jones et al., 2010). In contrast, it is interesting to note that albumen weight and proportion decreases with the increasing age of birds (Van Den Brand et al., 2004). Although the present study found differences in albumen weight, no management

effect was found for the proportion of albumen contrary to other studies that found CA eggs had higher (Kucukyilmaz et al., 2012) or lower albumen (Lordelo et al., 2017, Cherian et al., 2002, Samman et al., 2009) proportion than OR eggs.

Yolk colour is another aspect of the egg quality that is connected to consumer preferences and perception of quality. Intense yolk colour is directly connected to consumer choice of buying FR eggs over other alternatives (Pettersson et al., 2016). Skrivan and Englmaierova (2014) has shown higher scores for pasture-raised rather than indoor eggs. Campbell et al. (2017) showed that yolk colour was more orange with decreasing stocking density of FR flocks, attributing the difference to more and longer-lasting pasture for the lowest stocking density systems. Yolk colour is directly connected to the amount but also type of carotenoids in the yolk which come from the bird's diet (Mugnai et al., 2014). In this respect, even though the current results show that FR eggs had the highest score of yolk colour followed by EO and then by CA and OR eggs, results from this study indicated the carotenoid content increasing foraging intensity (CA<FR<OR<EO). However, from the individual carotenoids, it can be seen that one of the unknown-minor carotenoids was the highest in FR eggs, suggesting that this carotenoid might have contributed to a stronger red/orange shade in the yolk increasing the overall yolk colour. The effect of management will vary according to the diet formulation and pasture availability for foraging by the birds. In the current results, conventional FR eggs, being a prime product in the retail market, were probably supplemented with colour enhancers in their diet, although this cannot be confirmed due to lack of production data for the supermarket eggs. It is also important to have in mind that in OR feeding, synthetic colorants are not allowed. The analysis where year 2017 with no access at all to outdoor foraging was compared with year 2018 with normal FR access (APPENDIX B.5 Table 1. 1), showing no effect of year on yolk colour suggests that indeed the yolk colour is driven by the feed composition and the compounds in it might be the most influential, rather than those derived from fresh outdoor forage. Other retail studies have shown CA had a higher yolk colour score than OR eggs (Hidalgo et al., 2008, Lordelo et al., 2017), whereas Jones et al. (2010) and Van Den Brand et al. (2004) have shown that eggs from hens with outdoor access had higher colour scores than eggs from indoor systems. Some experimental trials on the other hand have reported higher scores for indoor rather than outdoor eggs (Kucukyilmaz et al., 2012, Samiullah et al., 2017), without though reporting the feed composition.

Similarly to the results of this study, the lack of management effect on dry matter content of yolk or egg is also reported by other studies (Hidalgo et al., 2008, Jones et al., 2010, Minelli et al., 2007). However, Filipiak–Florkiewicz et al. (2017) showed higher dry matter contents in OR compared with CA eggs. The weak effect of date on DM content of yolk suggests that yolk

DM content is higher when there is outdoor access, a suggestions reinforced also by the comparison of years with and without access to outdoors (APPENDIX B.5 Table 1. 1). It is interesting to note that DM has also been associated with age, although studies show contradicting results on the specific relationship either increasing or decreasing with increasing age (Minelli et al., 2007, Van Den Brand et al., 2004).

4.5.2. Fatty acids

Proportions of FA of UK eggs reported in this study are within the same range as proportions reported in the literature for eggs across Italy, Australia, Turkey, UK, Poland and Portugal (Public Health England, 2015, Filipiak–Florkiewicz et al., 2017, Hidalgo et al., 2008, Kucukyilmaz et al., 2012, Lordelo et al., 2017, Samman et al., 2009). The range of FA proportions of UK retail eggs in this study is also within the overall range reported for FR and OR eggs from Switzerland (Chapter Three, Table 3.2). A few differences were found and they were primarily identified for individual and total n-3 which were found in lower concentrations than in this study (Filipiak–Florkiewicz et al., 2017, Krawczyk et al., 2011, Lordelo et al., 2017). Reasons explaining this variation include the composition of the feeding regimes used in each country and potentially the season that the eggs were collected and will be overall discussed below.

Results of this study showing that the egg lipid content of FR eggs was lower than the that of EO eggs are in contrast with Samman et al. (2009) and Hidalgo et al. (2008) showing no effect of management practices on egg lipid content. However, OR rather than specifically EO systems were explored in the latter studies. Lack of management effects on egg lipid content was also identified in the results of Chapter Three (Table 3.2) between FR and OR eggs in Switzerland. The management effect between EO and conventional FR eggs shown in this study is difficult to explain as both FR and EO eggs had similar lipid content compared with the rest of the conventional and OR systems. As it has been suggested before, feed conversion efficiency depending on genotypes within systems could result in higher egg output and less fat deposition within the egg (Rizzi and Chiericato, 2010) and this could explain part of the variation between FR and EO egg lipid content assuming different genotypes between FR and EO systems. Indeed, the EO systems used in this study used multiple genotypes, which could potentially increase variation. The date effect noticed for lipid content in this study is in line with the results of Chapter Three (Table 3.2) showing higher lipid content in winter than in summer months. Reasons for this difference include potentially different feed intake between seasons and have been extensively discussed in Chapter Three (3.5.3.1. Lipid content). Even though the analysis between years with hens confined to housing compared with hens with normal FR access, indicated that access to outside did not affect the lipid content of eggs, this

analysis took place in April and throughout the year it is clear that lipid content is highest in the winter months.

Management, contrary to season, had a pronounced effect on FA composition of UK retail eggs. Starting with SFA, the most abundant FA in this group, C16:0, and the minor C14:0 were just slightly higher in both conventional than OR and EO eggs but this did not result in the total SFA content overall being higher. Results of this study both for the two individual FA or the total SFA content agree with the literature showing higher levels in systems with increasing intensity (Lordelo et al., 2017, Filipiak–Florkiewicz et al., 2017) and also with our results from Chapter Three (Table 3.2). However, in contrast, higher SFA in OR compared with CA eggs (but not because of differences in C16:0 content) has also been reported (Samman et al., 2009). To a limited extend C16:0 and SFA content can be influenced by the genotype used within each system as shown by the results of Chapter Three (Table 3.2) and other authors (Kucukyilmaz et al., 2012, Krawczyk, 2009). However, no information on genotype was available for this study and it can only be hypothesised that different genotypes within conventional and OR systems were used. On the other hand, there are studies that have reported similar C16:0 content in conventional (either CA or FR) and OR systems (Filipiak–Florkiewicz et al., 2017, Lordelo et al., 2017, Samman et al., 2009), which suggest that genotypes of laying hens may not be very different between systems and the choice of genotype on the country. The interaction seen for C16:0 showing significantly lower content of this FA in EO compared with conventional eggs during October and January only, suggests that a seasonal effect is affecting the overall content. As suggested by the results in Chapter Five (Section 5.4.3.), insect consumption can slightly increase the C16:0 content. During colder months, when the time that birds can roam outside while pasture is overall limited, insect consumption can be assumed to be restricted, lowering the C16:0 content in eggs in systems where insect consumption otherwise contributes to the overall content. Even if the differences reported by all studies were low, taking into consideration that it has been suggested to replace SFA and especially C16:0 with PUFA (Jakobsen et al., 2009), it is worth identifying differences between conventional and FR systems.

Concerning MUFA, similar to the results of the current study and the results of Chapter Three (Table 3.2) OA the most abundant MUFA, has been reported to be higher in systems with increasing intensity of production (CA>FR>OR) (Lordelo et al., 2017). In contrast, Filipiak–Florkiewicz et al. (2017) reported OA was higher in OR rather than CA eggs, whereas Samman et al. (2009) reported no differences between conventional and OR eggs. Although OA is the most abundant MUFA, it does not necessarily drive the overall MUFA levels as other minor MUFA levels might affect the outcome. The minor FA, c9C16:1 generally follows the pattern

of C16:0 and is also reported to be higher in CA than FR or OR eggs, even if there were no differences in C16:0 to start with (Filipiak–Florkiewicz et al., 2017, Lordelo et al., 2017). Desaturation activity (D-9) in the liver of broiler chicken of C16:0 and C18:0 leading to the respective minor monounsaturated FA have been shown to be affected by the level of fat in the diet, with higher activity in birds fed low fat diets (Infield and Annison, 1973). According to the results of Chapter Three (Table 3.2), while intakes per bird in OA and MUFA between systems were similar, MUFA and OA in yolks were higher in conventional eggs, suggesting that in laying chicken, parameters affecting the overall MUFA deposition potentially include the desaturation activity. However, in that study, lipid level of diets was similar and therefore parameters affecting MUFA concentration in eggs need further investigation.

Concentrations of all PUFA groups and the relevant individual FA, with the exception of AA, were significantly higher in OR eggs, in accordance with a large scale farm survey in the Netherlands (Chatzidimitriou et al., 2015) and the results of Chapter Three (Table 3.2). Eggs of EO systems were either similar to OR or similar to OR and FR eggs (in LA, PUFA and DPA), whereas FR were overall similar to CA eggs. The results from the present study for the dominant individual PUFA and total n-6 FA, LA are not in accordance with results from other retail studies, reporting non-significant differences between OR and conventional systems (Samman et al., 2009, Lordelo et al., 2017, Hidalgo et al., 2008) or even reporting higher levels in CA rather than OR eggs (Filipiak–Florkiewicz et al., 2017). With respect to OR eggs, having the highest LA and the lowest OA content between CA and FR eggs, as noticed in Chapter Three and as suggested by Kuksis (1992) LA has been deposited in egg yolk with simultaneous suppression of OA. Results from Chapter Three indicate that there is a relationship between dietary LA (which accounts for a high proportion of the FA of feed) and LA in yolk. Even though for this study there was no information on the respective diet composition, it is known that there is a potential impact of the type of feed. Depending on the production method of the feed (mechanical and solvent extraction methods for OR and conventional feed respectively), residual oil content might vary and it has been reported to be higher in OR feed. That could automatically lead to higher LA in OR feed as LA is one of the most abundant FA as shown before. Despite that n-3 FA only account for a minor proportion of yolk PUFA, the health benefits related to their dietary consumption and the relative n-6/n-3 ratio (Simopoulos et al., 2004) make n-3 FA, on top of n-6 FA, an important aspect of the overall egg nutritional composition. Additionally, the fact that the overall content in yolk can be increased by dietary manipulation of the feeding regime, make the eggs potentially an even more interesting human dietary source of n-3 FA. Omega-3 intake for hens is naturally enhanced through forage and grass consumption (Anderson, 2011, Mugnai et al., 2014), as well as insect consumption (Woods and Fearon, 2009). Therefore, one of the main hypotheses of this Chapter was that eggs

form systems with extensive pasture area for each hen, would exhibit higher n-3 FA yolk content. Mugnai et al. (2014b) showed that the n-3 FA content of eggs from conventional CA and OR systems were similar and only EO eggs had significantly higher content. However, results of the current study show that EO were similar to OR eggs and both types of eggs were higher in n-3 FA content as expected, similar to Lordelo et al. (2017). Conventional FR systems, even though they permit access to outside foraging have been criticized that because of the large size of the respective flocks, and that not all hens choose to go foraging outdoors (Richards et al., 2011, Gilani et al., 2014). In contrast, some studies report no differences between conventional and OR systems (Hidalgo et al., 2008, Samman et al., 2009, Kucukyilmaz et al., 2012). As seasonal variation affects not only the content of n-3 FA in pasture plants (Mugnai) but also the availability of it and the time available to hens for outdoor ranging, seasonal effects on yolk n-3 FA or interactions between systems and seasonal variation were anticipated. In this study, no effect of date was noticed across the year, except for the n-6/n-3 FA ratio, suggesting that intake of n-3 FA is consistently higher in OR and EO systems. However, the analysis comparing CA, FR and OR systems with and without outdoor access also failed to show an effect due to outdoor access, suggesting that OR feed is probably more rich in n-3 FA either because of different ingredients in it or because of higher n-3 FA in the OR grains used in OR feeds. On the other hand, April as the month of comparison of with or without access to outdoor might have been the weakest compared with other months in terms of n-3 FA and pasture availability so as to be able to show statistical significant interactions, as also suggested by the relationship of n-6/n-3. Overall, an n-6/n-3 ratio which would be more beneficial to human health was found for OR and EO compared with FR eggs even if OR and EO had more n-6 and n-3 FA. The date effect noticed for the ratio confirms the effect of pasture and forage intake on enhancing the egg nutritional quality.

4.5.3. Carotenoids and vitamin A and E

The sum of lutein and zeaxanthin represented around 80% of the total carotenoid content of the egg yolk. This is in agreement with proportions reported from the literature (van Ruth et al., 2011, Van Ruth et al., 2013). However, lower proportions of lutein and zeaxanthin (48-66%) and much higher percentages of canthaxanthin have been reported for conventional European eggs (van Ruth et al., 2011, Van Ruth et al., 2013). Lutein concentrations reported in this study are in the same range for conventional, OR and EO eggs reported by Mugnai et al. (2014b) and conventional and OR eggs by Schlatterer and Breithaupt (2006). Numerically, yolk lutein and zeaxanthin contents in this study were similar with the respective contents reported for UK eggs (Public Health England, 2015) but only if CA and FR are considered. Higher concentrations of carotenoids in yolks have been reported by Skrivan and Englmaierova (2014). Schlatterer and

Breithaupt (2006) reported much higher canthaxanthin levels (>10 times) than this study, whilst no canthaxanthin at all in OR eggs. Van Ruth et al. (2013) reported the canthaxanthin proportion at 5% for OR eggs in agreement with the results of the current study, but much higher proportions than the present results for conventional European eggs. Concentrations of β-cryptoxanthin in eggs, presented in this study are 5-10 times lower than the ones presented by Mugnai et al. (2014b) and 6 times less than the ones reported by Schlatterer and Breithaupt (2006) who only detected this carotenoid in OR eggs. According to these authors, it was the feed which was high in corn that increased the relative content of β-cryptoxanthin in eggs. In UK, it is common for diets not be rich in corn and therefore the present results are less than other studies. Van Ruth et al. (2013) reported β-cryptoxanthin in slightly higher proportions than our results, but it was still one of the minor egg carotenoids identified.

Although there was a clear management difference in carotenoids and vitamins, no date effect was shown suggesting that the specific management practices under each system are the strongest influence on the carotenoid and respective vitamin composition. Skrivan and Englmaierova (2014) who looked FR systems with and without grass availability, showed a significantly higher egg content of lutein and zeaxanthin concentrations in the eggs of grass-fed birds. Organic compared with conventional eggs generally have higher contents of both lutein and zeaxanthin (Schlatterer and Breithaupt, 2006, van Ruth et al., 2011, Van Ruth et al., 2013). However, Mugnai et al. (2014b) who compared, CA, OR and EO systems showed significantly higher contents of lutein and zeaxanthin only for EO eggs. Additionally, for zeaxanthin, these authors found an interaction with season, where in summer zeaxanthin levels were similar to all other systems. This is similar with the interaction shown from the current study for unknown carotenoid 1, where in summer, levels in EO eggs were similar to FR and OR (still higher than CA), whereas during the rest of the year, levels were always the highest in EO eggs. During summer months grass can dry out, supplying overall the lowest levels of carotenoids to birds. Mugnai et al. (2014b) measured the grass availability and its carotenoid concentration, in Italy, showing higher availability and concentration for winter, then spring, then autumn and last summer. However, these authors showed the highest lutein and zeaxanthin contents during autumn and spring and then winter, possibly because of the low accessibility of grass covered-by-snow during winter. Concerning canthaxanthin and β-cryptoxanthin, results vary in the literature. For canthaxanthin van Ruth *et al.* (2011, 2013), reported higher contents in conventional rather than OR eggs from Europe, whereas Schlatterer and Breithaupt (2006) did not see any differences between conventional eggs, while they did not detect this carotenoid in OR eggs. It is important to mention that potential toxic effects of canthaxanthin have been observed, mainly consisting of ocular lesions due to macular crystal formation, observed in animals, livestock and occasionally in human (Hueber et al., 2011). In human, cases

were observed after large ingestion of food supplements for cosmetic purposes (skin coloration) (Geoffrey, 1991). Therefore the highest concentrations in OR and EO eggs (0.52 and 1.06 mg/kg yolk) should be theoretically considered as negative to human health. However, the concentration in EO is still considered small compared to the maximum residue limits allowed (30 mg/kg) (EFSA 2010c). It has to be noted that canthaxanthin is a common synthetic feed additive for colour pigmentation, while it is also produced by microalgae (Guedes et al., 2011). Additionally egg yolk can potentially be a significant contributor to the canthaxanthin exposure (EFSA 2010c). Therefore potential higher supplementation either with feed supplements or algae extracts in egg production should be treated cautiously. For β-cryptoxanthin, Schlatterer and Breithaupt (2006) detected this carotenoid only in OR eggs, whereas Mugnai et al. (2014b) showed no differences between CA, OR and EO eggs similarly to van Ruth et al. (2011) who also showed lower levels in FR eggs. Overall, Van Ruth et al. (2013) demonstrated that variations in conventional eggs were higher than in OR eggs, which could explain the differences between studies. Pignoli et al. (2009) who looked into OR and conventional feed lutein levels, found higher lutein levels in conventional feed. However, when they measured lutein levels in yolks from CA and FR management systems, there was no difference. The authors also reported an effect of management, with FR eggs having overall higher contents of lutein, suggesting that even though feed levels of lutein in OR systems can be lower, FR practices can increase the final egg lutein levels.

Vitamin A and E concentrations were higher in eggs coming from FR and OR systems, compared with eggs from CA systems. This is in agreement with Karsten et al. (2010) who found that eggs from hens that foraged on pasture had higher concentrations of vitamin A and E than the eggs of CA hens. Skrivan and Englmaierova (2014) showed higher α-tocopherol and similar retinol content in eggs from grazing compared to non-grazing hens, while in pasture the authors did not detect retinol. On the other hand, Krawczyk et al. (2011) who examined two different genotypes under FR and CA management, found higher vitamin A content in one of the two genotypes under FR systems and similar vitamin E contents in eggs of both genotypes. Looking at vitamin E egg concentration of April 2017 and April 2018, there was no difference between systems or any interaction between year and system as expected. Taking into consideration that there was no date effect and hence the same results would have been expected irrespective of the time of the year, the lack of interactions could be explained by the lower number of samples used in this analysis. Higher contents of both vitamins during January were reported in conventional rather than OR eggs by Matt et al. (2009). Vitamin A and E in eggs from EO systems in this study were statistically similar to all systems, suggesting that EO systems have a more variable supply of vitamin E than the other systems. In contrast, Mugnai et al. (2014b), showed that EO eggs had higher contents of α-tocopherol than both OR and CA

during the whole year except winter where the concentrations were similar between all systems. The relative concentrations in eggs followed the same pattern as the concentrations in grass across seasons.

Having no information on the amounts of vitamins in the feed does not allow a full interpretation of the results of this study; whether it is the feed concentrations that vary or the variable forage material consumption within each system. Pignoli et al. (2009) who measured the feed concentration of tocopherols, even though they found higher α-tocopherol concentration in conventional compared to OR feed, there was no difference in yolk concentration between CA, conventional FR and OR systems, implying that in OR systems, α-tocopherol intake is also increased by other means. The current results on minor tocopherols γ and δ (both being higher in OR and EO systems) are not in accordance with Pignoli et al. (2009), who showed γ and δ tocopherols, although being higher in OR feed, yolk content was similar no matter the management system. In this context, the differences in the respective egg content and the effects of management between studies might be season dependent as indicated by the significant interaction seen in the current study.

4.5.4. Vitamin D_3

There is very limited information on Vitamin D egg yolk content and their specific forms of D_3 and 25-OH D_3. To our knowledge, this is the second study looking into seasonal variation of vitamin D_3 egg content across management systems in the UK market.

The current results for levels of both D_3 and 25-OH D_3 egg yolk content were similar to the ones reported by Guo et al. (2017b). However, levels of D_3 were 3-fold higher in the report of Public Health England (2015) and levels of 25-OH D_3 were 10-fold lower compared with the current results. However, despite these differences, total vitamin D_3 concentrations were similar to those in the current study. The large difference in the levels of both individual compounds between these studies, may lay to the fact that in the current study LC-MS/MS was used to quantify the D_3 contents, whereas the other study used HPLC. Compared with contents of Australian eggs (Dunlop et al., 2017), reported per 100g of whole egg, a yolk weight of 15.5 g is assumed (mean yolk weight of the current results) and 55g as the mean egg weight (without the shell), the current results for both D_3 (1.3 μg/100g egg) and 25-OH D_3 (0.5 μg/100g egg) are similar across FR eggs. However, for CA eggs, D_3 in UK eggs of this project, was almost 50% higher whereas 25-OH D_3 was 50% lower than Australian eggs, a pattern also reported for Australian commercial eggs by Liu et al. (2014). However, total D_3 concentration was within the same levels between UK and Australian eggs. The current results for both compounds are also within the range of US eggs reported by (Exler et al., 2013), although the mean for both

compounds in this study was slightly smaller than the Australian data. However, in that study, even though all eggs (in 2010) were collected in March/April there was a large variation among collection sites and geographical variation could also have an impact. On the other hand, Exler et al. (2013), collected large eggs, whereas in our study mainly medium eggs were used. Although the size of yolk does not differ greatly between eggs sizes, the fact that vitamin D is only found in yolk, means that even a small weight difference in yolk size, and could have an impact on total levels.

Vitamin D can be synthesised in skin exposed to ultraviolet (UV) radiation by humans and hens alike (Kühn et al., 2014, Borradale and Kimlin, 2009). The primary role of vitamin D has been relevant to calcium absorption and bone health (Borradale and Kimlin, 2009). The hydroxylated form of vitamin D_3, 25-OH D_3 may be up to five times more bioactive than vitamin D_3 (Cashman et al., 2012). Commercially, it is common that the compound feed for layers is supplemented with vitamin D and specifically D_3. Traditionally, vitamin D_3 has been used but lately the metabolite 25-OH D_3 has also been used. It has been shown that dietary supplementation with either of the compounds of vitamin D_3 can increase the relative egg deposition (Duffy et al., 2017, Mattila et al., 1999a, Mattila et al., 2011). Liu et al. (2014) showed that supplementing hens only with low levels of D_3 resulted in no 25-OH D_3 in egg. Duffy et al. (2017) quantified 25-OH D_3 in egg when only D_3 was added in the diet and showed no increase of 25-OH D_3 in egg when hens were supplemented either with only 37.5 or 75 μg of vitamin D_3 per kg of feed.

The lack of management effects in this study is in accordance with Kühn et al. (2014) who did not show any differences between commercial FR and barn eggs sampled in September and January and with Dunlop et al. (2017) who did not find overall difference in vitamin D levels between FR and CA eggs. On the other hand, Kühn et al. (2014) showed that eggs from FR systems in which hens were exposed 9 hours sunlight per day, were higher in vitamin D than eggs from indoor systems. Guo et al. (2017b) showed that the D_3 egg content was higher in FR and OR eggs compared with indoor eggs and 25-OH D_3 egg content was higher in OR eggs compared with FR and CA eggs. In contrast, Matt et al. (2009), showed higher D_3 content in eggs from indoor compared to OR systems. However, the eggs were only sampled in January. Looking at the current results, it can be suggested that, the exposure to sun in FR systems can compensate for lower concentrations of vitamin D in feed in order to reach a stable egg vitamin D content as in CA eggs. This is supported by the interaction between date and season, showing equal vitamin D_3 content in all systems in July but numerically lower concentrations during January.

The current results showing higher D_3 and 25-OH D_3 contents in July, suggest that exposure to sun and ultraviolet light can indeed result in vitamin D synthesis and consequent deposition in eggs as suggested by Kühn et al. (2015). Authors from the same study also noticed that 25-OH D_3 reached a plateau in concentrations no matter the hours of exposure to ultraviolet light, much quicker than D_3, which is in agreement with our results as the weakest effect of date was noticed for 25-OH D_3. Looking at the interaction for total D_3 between management and date, looking at numerical differences and not only statistical significance, it is clear that a difference for D_3 content depending on the system exists only for systems that have access to the outside. This is in agreement with Guo et al. (2017b) who showed larger seasonal differences for both D_3 and 25-OH D_3 egg contents in FR and OR systems than for eggs from indoor systems. When Dunlop et al. (2017) looked into FR eggs from a low-stocking density farm (130 hens/ha)—these eggs had higher vitamin D_3 (but not 25-OH D_3) content than eggs from farms with higher stocking densities, suggesting that the hens from lower stocking densities could be exposed more to sunlight (assuming that the feed vitamin D content was similar). However, such a relationship between OR and EO eggs within the current study was not shown. Within the current study and within the results for each system, the fact that the only statistical difference between seasons was noticed for eggs of EO systems, suggests that these systems and to a lesser extent all FR systems, would benefit from winter supplementation if they want to keep their egg quality the same across seasons. Keeping egg vitamin D content high during winter months (for northern hemisphere), is potentially more important from a health perspective than during summer months, as sun exposure and human vitamin D synthesis is limited and therefore any supplementary dietary intake could positively boost plasma concentrations in humans.

4.5.5. Vitamins B_2 and B_9

To our knowledge, this is the first time that the impact of management or season on vitamins B_2 and B_9 in eggs has been investigated. Variation in water soluble vitamins and specifically, the effect of management has scarcely been investigated for any type of food commodity.

Results of concentration levels of B_2 (riboflavin) in eggs in our study (mean of 0.41 mg/100g egg) were slightly lower than results previously reported in the report by Public Health England (2015) (0.50 mg/100g egg). Contrary to the results here, with a lack of management effect, two studies showed milk under OR management with higher levels of riboflavin than milk coming from conventional systems (Kalač, 2011, Poulsen et al., 2015). However, this might be a result of silage consumption and its variation between dairy management systems. Poulsen et al. (2015) also showed seasonal variation in OR milk with even higher levels of riboflavin during winter than during summer. In contrast, although the current results also show seasonal variation, egg riboflavin content was higher in summer rather than winter. In this context, it is

known that deposition of riboflavin in egg yolk and albumen is dependent on dietary riboflavin (White et al., 1986) and that riboflavin is synthesized by green plants, yeast, fungi and some bacteria, meaning intake while grazing could have an impact on egg. Therefore, forage intake during summer could explain the variation. However, as suggested by Poulsen et al. (2015), taking into account that there was no difference between indoor and outdoor management, forage material intake solely cannot explain the seasonal variation and the higher ration of legumes in the diet during summer might explain the higher egg B vitamin content in summer. However, it would be expected to see an interaction between management and season as CA systems would not be affected by different feeding regimes across dates. This was not the case for the current results and therefore the effect of date needs more investigation.

Results of concentration levels of egg B_9 (folate) in the current study (mean 31 μg/100g egg) were considerably lower (-50%, -34%) than the ones reported in UK eggs from two different studies (Public Health England, 2015, Hoey et al., 2009). The current results were also lower (around -50%) than the levels reported in Swedish eggs (Strandler et al., 2011) when total folate was re-calculated using the average of 15.5 g of yolk weight from our study. Taking into account the fact that more than 95% of total folate is found in yolk (Public Health England, 2015) the differences in results might be down to the variation of egg yolk proportions used in those studies, although in general not large variations in yolk proportions have been reported. Similarly, to riboflavin, folate is found in high quantities in dark green leafy vegetables and legumes (Food Standards Agency 2002) and dietary folate can significantly increase yolk levels (Bunchasak and Kachana, 2009). Therefore it should be expected that there would be a seasonal variation in folate concentrations depending on leafy vegetables and legumes availability as demonstrated for human serum concentration in folate (Krajcovicova-Kudlackova et al., 2013). Indeed, EO eggs were highly affected by season with a much lower content in winter. As discussed before, EO systems possibly having higher legume ratios in their feeding regimes while grazing more extensively than other conventional systems are the most affected by seasonal variations. This is confirmed by the stable folate concentrations in CA eggs. However, the interaction also revealed an inverse relationship for OR eggs. Although numerically OR eggs folate content did not vary a lot the highest levels in winter could be explained by supplementary vitamin supplements during winter where systems are in higher danger of vitamin depletion. This however, needs further investigation.

4.5.6. Chemical elements

Apart from chick embryonic and bird development (Richards, 1997, Richards and Packard, 1996) there is considerable interest in the macro and micro element composition of eggs from

a human health perspective (Gupta and Gupta, 2014). Elements for which eggs can be a good source of, and analytical methods were adequate, have been selected for comment in this study.

Barium

Information on Ba concentration in eggs is very limited. The levels identified in one study in the Canary Islands (González-Weller et al., 2013), were similar to levels of OR and EO of this study. However, the current results are slightly higher than the ones reported in the UK eggs in the Total Diet Study in 2006 (Rose et al., 2010). Results of this study, showing higher Ba concentrations in CA eggs are opposite to those from Barbosa et al. (2014) and Borges et al. (2015) who showed FR eggs being higher in Ba than CA eggs and OR being higher than CA, respectively. Both studies of these authors took place in Brazil, whereas the current study took place in the UK, which suggests that the practices used in these management systems or/and the content of Ba in their soil and crops might be different than the one in the UK. It is also interesting to note that although FR were statistically similar to OR and EO eggs, both OR and EO eggs had numerically the lowest levels of Ba. Barium has been identified in various foodstuff with nuts having the highest levels, followed by cereals, vegetables and then by eggs (González-Weller et al., 2013, Rose et al., 2010). The fact that CA systems rely more than FR on a cereal diet, might explain the fact that CA eggs in the current study were higher in Ba.

Calcium

Levels of egg calcium in this study are slightly lower than the ones reported in the UK analysis for eggs and almost half of what Filipiak–Florkiewicz et al. (2017) reported for Polish eggs. The current results, showing higher Ca levels in eggs of CA, OR and EO systems than in eggs of FR systems ($P < 0.05$), are in line with Zhu et al. (2015) who showed that CA eggs were higher in Ca than FR eggs. In contrast, Szymanek et al. (2019) and Borges et al. (2015) showed higher Ca levels in OR compared with CA eggs, while the level of the OR eggs reported by these authors, was similar to the current results for EO eggs. Many studies have also reported no differences between conventional and OR systems (Filipiak–Florkiewicz et al., 2017, Kucukyilmaz et al., 2012b, Mizumoto et al., 2008, Bargellini et al., 2008). In systems with FR practices, calcium is likely to be digested by birds with the consumption of soil and small stones. The current results showing conventional FR eggs having lower Ca than CA and OR eggs, suggest that in overall FR systems, this is not always the case as birds are not necessarily outdoors as often as OR and EO birds. Therefore variation in FR systems can be higher compared with OR and EO systems, as demonstrated also by Szymanek et al. (2019). It is interesting to mention that vitamin D was not different between systems and therefore the reason of calcium variation in eggs is unlikely to be connected to variation of Ca and P absorption only because of vitamin D.

Iron

Concentration levels of Fe in the current study were similar to the report for the egg nutritional composition of UK eggs (Public Health England, 2015) but much lower (almost 10-fold) than the levels reported by (Filipiak–Florkiewicz et al., 2017). In this study, no effect of management was shown for Fe content in eggs, in line with other studies (Bargellini et al., 2008, Kucukyilmaz et al., 2012b, Mizumoto et al., 2008). However, Barbosa et al. (2014) and Borges et al. (2015) showed FR and OR eggs respectively, being higher in Fe than CA eggs. It is interesting to note that OR milk has been shown to have a higher Fe concentration than conventional (Srednicka-Tober et al., 2016b) possibly because of forage consumption. In contrast, Filipiak–Florkiewicz et al. (2017) showed that CA egg yolks had slightly lower Fe than OR egg yolks, but differences were very small.

Magnesium

Magnesium levels reported by Szymanek et al. (2019) were lower than the range of values reported for the current results whereas Filipiak–Florkiewicz et al. (2017) reported similar values to those reported here but only for CA and lower levels than those here for OR eggs. The current results were on average at similar levels with results for the nutritional composition of UK eggs (Public Health England, 2015). The only statistical difference for Mg egg concentration was shown for CA eggs which were higher than OR eggs similarly to a few studies from the literature (Bologa et al., 2013, Borges et al., 2015, Filipiak–Florkiewicz et al., 2017). Organic eggs were however similar to all other systems with FR practices; FR and EO. The contrary was shown by Szymanek et al. (2019) showing OR eggs having higher Mg levels. Results from a meta-analysis between conventional and OR crops have revealed OR crops being higher in Mg (Baranski et al., 2014). Various results have been reported throughout the literature, with no differences in Mg egg content between CA and OR or FR eggs being reported (Kucukyilmaz et al., 2012b, Mizumoto et al., 2008, Barbosa et al., 2014, Bargellini et al., 2008). The interaction for Mg in the current study shows no specific patterns and therefore is difficult to interpret.

Manganese

Manganese levels reported by Szymanek et al. (2019) were lower than the range of values reported for the current results. Results reported for UK eggs (Public Health England, 2015) were similar to those reported here. Results by Kucukyilmaz et al. (2012) on Mn egg concentration are similar to Giannenas et al. (2009) who showed similar levels of Mn between CA and OR eggs and Barbosa et al. (2014) showing no management effect between CA and FR systems. Similarly, to other elements, results through the literature vary. Contrary to the current results, showing no effect of management on Mn egg concentration, Zhu et al. (2015) reported

higher concentration for FR compared with CA eggs suggesting that industrial Mn waste in the air falls on the ground and later consumed by the birds under FR management. Similarly, Borges et al. (2015) showed higher Mn concentrations for OR eggs when compared to CA eggs. It is interesting to note that OR crops have been shown to have lower Mn concentration than conventional crops (Baranski et al., 2014) meaning that OR feeds could have naturally lower Mn content. In agreement with this, Filipiak–Florkiewicz et al. (2017) reported that CA eggs were higher in Mn than OR eggs both in yolk and in albumen. Giannenas et al. (2009), reported lower levels for eggs of backyard systems compared to CA and OR, probably because of not adding in their diet the mineral premix. The date effect noticed for the current results suggesting that in January and April there is slightly less Mn, could indicate that during these months ranging and consumption of soil (potentially along with Mn) under FR systems is more limited compared with July and October.

Phosphorus

Phosphorus levels reported in this study were around 20% higher than results previously reported for UK eggs (Public Health England, 2015). The current results are similar to some reported in the literature, showing no effect of management on egg P concentrations. However, there have also been some studies reporting much lower P concentration in OR compared with CA eggs (Kucukyilmaz et al., 2012b, Matt et al., 2009) suggesting that phosphorus might have been deposited and absorbed into the bones of birds under FR management because of increased physical activity. As for calcium, vitamin D in our study did not differ in yolks of different systems and therefore it can be assumed that vitamin D levels did not interfere with P absorption in the birds.

Zinc

Results reported for egg zinc levels in UK eggs (Public Health England, 2015) are similar but slightly lower than the results reported in this study. However, results in this study are almost 50% lower than the ones reported by Filipiak–Florkiewicz et al. (2017). The current results which showed the lowest levels of Zn in OR eggs and no differences between CA, FR and EO eggs are difficult to explain although results vary throughout the literature. Zhu et al. (2015) who measured higher Zn levels in CA compared with FR feed, found accordingly higher Zn content in yolks of CA compared with FR eggs. Kucukyilmaz et al. (2012b) and Giannenas et al. (2009) who measured similar levels in conventional (CA) and OR feed found higher levels in CA compared with OR eggs, similar to the current results. The authors suggest that the differences in yolk levels arise from the use of Zn from FR birds as a nutrient against the external stressors and hence deposing less Zn into their yolks. Contrary to the current results and other studies who showed CA being higher in Zn than OR eggs (Bologa et al., 2013, Borges

et al., 2015), Szymanek et al. (2019) showed higher Zn content in OR compared to CA eggs or even no difference between CA and OR yolks (Filipiak–Florkiewicz et al., 2017). However, the current results show EO eggs being similar to CA eggs, suggesting that birds within EO systems are either better adapted to external environmental stressors that might cause decreased deposition in OR systems, or that within EO systems extensive forage material intake can compensate the higher depletion of Zn. In this context, Giannenas et al. (2009) reported the highest Zn concentration in backyard system eggs, despite the fact that birds in these systems did not receive the mineral premix including Zn.

4.6 CONCLUSION

Apart from confirming the relationship of Haugh Units and freshness of eggs, for which eggs available at farm gates scored the highest values, no major effect of management practices or date was elucidated for egg part proportions and egg quality characteristics.

Eggs from OR and EO systems showed a FA profile more beneficial to human health, having higher FA proportions n-3 FA and lower n-6/n-3 ratio than FR eggs. Compared with CA eggs though, while OR and EO eggs had also higher n-3 FA, the ratio of n-6-/n-3 was similar (yet numerically higher).

Carotenoids were higher in eggs with increasing intensity of foraging within the systems, with EO eggs having the highest levels. Conventional systems CA and FR, gave eggs with similar levels of carotenoids and vitamin E, while vitamin A was higher in FR eggs (OR and FR) compared with eggs from CA systems. Eggs from EO systems were variable in vitamin A and E and were not different than any other system. The lack of interactions for FA and carotenoids between systems and dates highlights the weight of the impact that management practices have.

Although vitamin D did not differ between management systems, date had a clear effect confirming the positive impact of FR systems and the respective sun exposure of hens, to the overall vitamin D egg content. However, a higher variation in EO systems was noted, suggesting that winter supplementation within these systems would be beneficial if a standard quality of eggs needs to be maintained. Vitamins B_2 and B_9 did not vary between management systems, while a seasonal effect with higher levels in summer was shown for B_2, whereas B_9 was numerically higher during summer within FR systems. At the same time the fact that B_9 in OR eggs was higher in winter rather than summer, suggests that dietary supplementation in these systems might be tailored to reflect seasonal needs.

Micro and macro elemental composition of eggs vary according to management practices. However, the patterns of variation between conventional and FR or OR practices are not clear.

The strongest effect was shown for Barium for which eggs from CA systems had the highest concentration, yet overall in very low amounts.

CHAPTER 5. EFFECT OF INSECT MEAL ON EGG FATTY ACID COMPOSITION

5.1 SUMMARY

Replacing soybean cake in poultry diets will lower the dependency on high-protein feed imports and improve the overall sustainability of egg production systems. Insects, which are a staple feed for many wild birds and potentially for free ranging poultry, having high quality protein, are a promising alternative to soya. The objective of this study was to investigate the effects of replacing soybean cake with *Hermetia illucens* (black soldier fly) meal (HIM) in layers diets on egg fatty acid (FA) profile. A three-week feeding trial was carried out with 30 OR Lohman Selected Leghorn layers at 64-74 weeks old, randomly distributed to three feeding groups: (1) control feed containing 36 g/100 g soybean and no HIM, (2) feed containing 12 g/100 g HIM and 15.6 g/100 g soybean (H12) and (3) feed containing 24 g/100 g HIM replacing 100% of soybean (H24). The experiment was subsequently repeated three times, using different flocks of the same genotype at the end of their first laying cycle. Including HIM, increasingly replacing soybean cake in layers diet, significantly affected the egg FA profile. Complete replacement of soybean cake (H24) had a negative impact on egg FA composition with respect to consumer health as it increased SFA and decreased PUFA both n-6 and n-3 FA. However, the n-6/n-3 FA ratio was shown to be overall lower in eggs with HIM. On the other hand, eggs coming from diets with 12% HIM while replacing soybean cake by one third, had similar n-3 FA and MUFA concentrations to control eggs yet SFA were higher and PUFA lower.

Keywords: insect meal, Hermetia illucens, soybean replacement, egg composition, fatty acids

5.2 INTRODUCTION

The extensive use of soybean in livestock production poses several environmental issues, as for its production rain forests are being destructed, carrying overall a heavy burden for greenhouse gas emissions due to these land-use change (Semino et al., 2009). Use of soybean also means reliance on imports from non-European countries. Thus, in addition to questions about feeding a growing population, there are urges to find and establish alternative protein sources for poultry and egg production that could keep high production standards and contribute to global food security.

Insects are a staple feed for many wild birds and poultry and have always been known to be part of human's diet in some cultures. Their high level and good quality protein content along with their minimal land use necessary during production (Salomone et al., 2017) have made them good candidates to substitute soya in animal feed. The fact that insects can be fed on waste or by-products such as vegetable waste or biogas digestate (Spranghers et al., 2017), further adds to the sustainability of their inclusion in livestock production systems also creating added value products. Various insect species such as mealworm and locust have been investigated as potential feed ingredients in fish and poultry production and black soldier fly larva (*Hermetia illucens,* HI) is considered as promising (Makkar et al., 2014, van Huis, 2013) for use in livestock production. However, bioaccumulation of contaminants such as cadmium and arsenic by insects has been documented and therefore consumers' safety when introducing insects into feed should be addressed. Previous research has shown that the relatively poor quality of the fatty acid profiles in HI can be increased and manipulated to include desirable omega-3 FA (S. St-Hilaire et al., 2007). Moreover, various studies demonstrate that including HI into animal diets can change the FA profile of poultry meat (Schiavone et al., 2017) and fish (St-Hilaire et al., 2007b, Zhou et al., 2018). Therefore including HI and replacing soybean in poultry diets is worth investigating, to assess the impact in sustainability, and in this case, the potential impact on egg lipid composition. To our knowledge there is only one study reporting the complete replacement of soybean and the effect on the egg FA profile (Secci et al., 2018) which has shown limited effect on modifying the egg's FA profile

The aim of this study was to investigate the impact of two levels of HI meal (HIM) in layers diet including increasing levels of HIM, fed on vegetable waste, while completely replacing soybean cake on egg FA composition.

5.3 MATERIAL AND METHODS

5.3.1. Experimental set up

This study has been designed as part of a feeding trial looking into laying performance, described by Maurer et al. (2016). Briefly, a 3-week feeding trial was carried out in Switzerland with 30 OR Lohman Selected Leghorn classic white layers at 64-74 weeks old, randomly distributed to three feeding groups. Hens were adapted to the experimental feed for one week prior to the 3-week recording period. The experiment was subsequently repeated three times, resulting in four rounds, from July to December 2013 using different flocks of the same genotype and age of 80 weeks each time. During the trials, layers had access to a covered outdoor area but not to pasture or soil. Wild insect consumption could not be totally excluded but it would be insignificant comparing to the levels of HIM in feed.

5.3.2. Feed formulation and composition

Mixed isoenergetic feeds with three levels of HIM were produced by a commercial OR feed manufacturer and all components, except HIM, were certified OR: (1) control feed containing 36 g/100 g soybean cake and no HIM, (2) feed containing 12 g/100 g HIM and 15.6 g/100 g soybean cake (H12) and (3) feed containing 24 g/100 g HIM replacing 100% of soybean (H24). HIM was produced with *Hermetia illucens* larvae fed for 10 days on the control poultry feed and for 2-3 weeks with a wheat based, vegetarian by-products mainly coming from pasta industry, until being harvested before reaching the pre-pupal stage. The meal used was partly defatted, decreasing the fat content to 11%. The composition of the 3 mixed diets can be seen in Table 5.1.

5.3.3. Collection of eggs and feed samples

Ten eggs from each feeding group were sampled on the last day of the feeding period. After the eggs were collected, yolks were separated from the whites and placed in sterile plastic containers. The samples were kept at -20˚C until transported in ice at Newcastle University through airmail. Upon arrival the samples were stored at -20˚C until further analysis. Three replicates of each mixed diet fed to each flock were also sampled for each batch and delivered along with the egg yolks.

5.3.4. Fatty acid analysis of feed and eggs

Fatty acid analyses of feed and eggs were performed as described in Chapter Three.3.3 and details are not repeated here.

Table 5.1. Feed components and nutrient concentrations of mixed diets. Table adjusted from Maurer et al. (2016).

	Control	H12[a]	H24[b]
Feed compounds (g/100g fresh matter)			
Hermetia meal	0	12.0	24.0
Soybean cake	36.0	15.6	0
Corn/maize	35.0	40.9	34.3
Wheat	0	4.6	14.6
Wheat bran	3.1	2.2	2.1
Mixed bran	2.1	0	0
Sunflower cake	2.6	8.1	8.2
Alfalfa meal	3.1	3.0	3.0
Grass meal	2.1	2.2	2.1
Granulated cereals	4.1	0	0
Mineral, limestone, vitamins	11.8	11.3	11.7
Nutrient concentrations (g/100 g dry matter)			
Crude fat	4.50	5.72	6.44
Crude protein	20.0	20.3	21.4
Methionine	0.32	0.36	0.39
Lysine	1.20	1.03	1.01
Crude ash	14.0	13.4	13.7
Calcium	4.0	4.08	4.26
Phosphorus	0.53	0.52	0.53
Sodium	0.20	0.19	0.22
Chloride	0.22	0.23	0.28
Vitamin A[c]	3.6	4.0	4.6
Vitamin E[c]	60	65	75
Vitamin D[c]	0.058	0.065	0.075

[a]Feed with Hermetia meal at 12%, [b]Feed with Hermetia meal at 24%. [c] Concentrations of vitamins were not included in the publication but were provided after personal communication and are expressed in mg/kg of feed.

5.3.5. Calculated dietary fatty acid intakes from feed

Dietary intakes (g/hen/day) of individual FA and FA groups of flocks from different rounds and diets were calculated as follows using the feed intake values reported by Maurer et al. (2016):

FA intake (g) = total feed intake (g) × [feed lipid content (g/100 g feed)/100] × % of [FA (g/100 g total FA in feed)/100]

It is noted that a correction factor to allow for the fact that the total fat is not only FA has not been used as the diet used in this chapter was a mix of various cereals whose exact correction factors rest unknown.

5.3.6. Statistical analysis

Analyses of variance (ANOVA), derived from linear mixed-effects models were performed in R statistical environment (R Development Core team, 2009) using diet (Control, H12, H24) and round (round 1-4, corresponding to each round) as main, fixed factors and egg replicate as

random factor. Differences in diets' FA profile between the three different diets and the four different rounds were assessed by one way ANOVA using diet and round as fixed factors respectively. Pairwise comparisons of means ($P < 0.05$) were performed using post-hoc Tukey's honestly significant difference test.

5.4 RESULTS

5.4.1. General

Results on laying performance have been discussed by Maurer et al. (2016). However, it is worth mentioning that egg production was similar across all three diets and within the expected rate (79%-84%) for layers at this late stage of their laying cycle.

5.4.2. Diet fatty acid composition and estimated intake

Inclusion of HIM in the feed had a significant impact on the dietary FA profile (Table 5.2). Increasing the inclusion of insect meal considerably increased C12:0 and C14:0 content in the poultry diets. On the contrary, C16:0 decreased by 24% and 28% at H12 and H24 diets respectively, compared with control diet (but did not differ from each other). Likewise, C18:0 numerically decreased by increasing the insect meal but only H24 diet was statistically different (-70%) than the control diet.

Overall, SFA as a group was higher by 77% and 122% for the H12 and H24 diets respectively, than the control diet. A decrease in OA when including insect meal (-30% and -35% at H12 and H24 diets respectively) had a similar impact on MUFA content in the diets with H12 and H24 being 28% and 32% lower than the control diet, although the 2 diets including insect meal did not differ from each other. The dominant PUFA, LA was significantly lower at each level of insect meal; -23% at H12 and -47% at H24 compared with control. The same pattern can be seen for PUFA and n-6 FA, both driven by LA. Neither ALA nor n-3 FA concentrations in the diets were affected by the inclusion of insect meal. The long chain n-3 FA, EPA was higher in the diets with increasing insect meal whereas DHA was similarly but slightly lower than in the control diet, although at very low levels in all diets. The sum of EPA and DHA follows the pattern of the dominant EPA. The ratio of n-6/n-3 FA was 47% and 66% lower in H12 and H24 diets respectively when compared with control and did not differ from each other.

Table 5.3. shows the estimated dietary FA intake per hen per day when feed intake and the respective crude fat content of diets was taken into account. Although there were many similarities when compared with diet FA profile when expressed as a percentage of total FA content, some FA and groups of FA showed differences when expressed as estimated FA intake. More specifically, differences of C16, OA and LA of either H12 or/and H24 to control diet were less pronounced. Concerning PUFA, H12 diet showed a trend of a higher value compared with control diet, contrary to %FA expression where H12 was shown as statistically significantly lower than the control diet. With regards to n-3 FA, both for individual FA and group n-3 FA differences were revealed when expressed as the estimated FA intake. More specifically, ALA intake was higher in H12 diets compared with control and H24. Individual long chain n-3 FA followed the same patterns as the % FA expression whereas, when the sum EPA and DHA was considered, more pronounced differences in the same order of magnitude were shown. N-3 FA driven by ALA followed the same pattern as in the estimated FA intake expression mentioned above.

Table 5.2. Diet fatty acid (FA) profile (mean concentrations ± standard error of means in g/100g total FA) of control diet, diet with Hermetia meal at 12% (H12 and, diet with Hermetia meal at 24% (H24). Percent difference to control diet.

FA[2]	Control n=4	H12 n=4	H24 n=4	P value[1]	H12 ± to Control	H24 ± to Control
C12:0	0.23±0.05^{c}	23.82±1.48^{b}	34.88±0.58^{a}	<0.001	10257%	15065%
C14:0	0.17±0.012^{c}	3.30±0.17^{b}	4.75±0.18^{a}	<0.001	1841%	2694%
C16:0	16.79±0.51^{a}	12.78±0.15^{b}	12.08±0.15^{b}	0.015	-24%	-28%
C18:0	5.48±0.37^{a}	2.26±0.05^{a}	1.64±0.024^{b}	0.001	-59%	-70%
OA	30.42±1.53^{a}	21.42±0.37^{b}	19.64±0.13^{b}	0.001	-30%	-35%
LA	39.79±2.28^{a}	30.5±1.39^{b}	21.27±0.98^{c}	<0.001	-23%	-47%
ALA	1.87±0.23	1.89±0.032	1.49±0.03	0.813		
EPA	0.01±0.00^{c}	0.10±0.01^{b}	0.17±0.01^{a}	<0.001	900%	1600%
DHA	0.03±0.00^{a}	0.01±0.00^{b}	0.01±0.00^{b}	0.015	-70%	-74%
FA groups						
SFA	24.5±1^{c}	43.28±1.67^{b}	54.5±0.83^{a}	<0.001	77%	122%
MUFA	32.18±1.64^{a}	23.31±0.34^{b}	21.78±0.18^{b}	0.001	-28%	-32%
PUFA	42.63±2.22^{a}	32.95±1.38^{b}	23.3±0.95^{c}	<0.001	-23%	-45%
n-6	39.81±2.29^{a}	30.61±1.39^{b}	21.43±0.97^{c}	<0.001	-23%	-46%
n-3	1.97±0.23	2.04±0.04	1.70±0.041	0.713		
EPA+DHA	0.05±0.00^{c}	0.12±0.01^{b}	0.18±0.01^{a}	<0.001	140%	260%
n-6/n-3	20.99±2.32^{a}	15.05±0.92^{ab}	12.67±0.84^{b}	0.035	-28%	-40%

[1] Means with different superscripts are significantly different (P<0.05) according to Tukeys's honestly significant difference test [2] OA= oleic acid; LA = linoleic acid; ALA = α-linolenic acid; EPA = eicosapentanoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA =polyunsaturated fatty acids; n-6 = omega-6 fatty acids; n-3= omega-3 FA; n-6/n-3 = omega-6 to omega-3 ratio

Table 5.3. Estimated dietary fatty acid (FA) intake (mean concentrations ± standard error of means in g of FA per hen per day) from control diet, diet with Hermetia meal at 12% (H12 and, diet with Hermetia meal at 24% (H24). Percent difference to control diet.

Diet FA[1]	Control n=4	H12 n=4	H24 n=4	P value[1]	H12 ± to Control	H24 ± to Control
C12:0	0.01±0.002^{c}	1.79±0.11^{b}	2.4±0.04^{a}	<0.001	14601%	19696%
C14:0	0.01±0.00^{c}	0.25±0.013^{b}	0.33±0.01^{a}	<0.001	2699%	3606%
C16:0	0.88±0.03^{b}	0.96±0.01^{a}	0.83±0.01^{b}	0.001	9%	-5%
C18:0	0.29±0.02^{a}	0.17±0.004^{b}	0.11±0.002^{c}	<0.001	-41%	-60%
OA	1.59±0.08^{a}	1.60±0.03^{a}	1.35±0.01^{b}	0.015	1%	-15%
LA	2.08±0.119^{a}	2.29±0.10^{a}	1.47±0.07^{b}	0.003	10%	-29%
ALA	0.10±0.01^{b}+	0.14±0.002^{a}	0.10± 0.002^{b}	0.015	45%	6%
EPA	0.001±0.00^{c}	0.008±0.00^{b}	0.01±0.00^{a}	<0.001	1276%	1961%
DHA	0.002±0.00^{b}	0.001±0.00^{b}	0.001±0.00^{a}	0.002	-57%	-66%
FA groups						
SFA	1.28±0.05^{c}	3.24±0.13^{b}	3.76±0.06^{a}	<0.001	154%	194%
MUFA	1.68±0.09^{ab}	1.75±0.03^{a}	1.50±0.012^{b}	0.029	4%	-11%
PUFA	2.23±0.12^{a}	2.47±0.10^{a}	1.61±0.07^{b}	0.002	11%	-28%
n-6	2.08±0.12^{a}	2.29±0.10^{a}	1.48±0.07^{b}	0.003	10%	-29%
n-3	0.10±0.01^{b}	0.15±0.003^{a}	0.12±0.003^{b}	0.012	49%	14%
EPA+DHA	0.002±0.00^{c}	0.009±0.001^{b}	0.01±0.001^{a}	<0.001	265%	424%

[1]OA= oleic acid; LA = linoleic acid; ALA = α-linolenic acid; EPA = eicosapentanoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA =polyunsaturated fatty acids; n-3= omega-3 FA; n-6 = omega-6 fatty acids;n-6/n-3 = omega-6 to omega-3 ratio

As it can be seen from Table 5.4., there was no round effect on the FA profile of diets.

Table 5.4. Diet fatty acid (FA) profile (g/100g total FA) between rounds A, B, C and D. Experimental and controls diets were taken into account to calculate the mean at each round.

Diet FA[2]	Round A n=3	Round B n=3	Round C n=3	Round D n=3	P value[1]
C12:0	18.9±10.1	20.4±10.3	20.1±10.2	19.1±10.5	ns
C14:0	2.68±1.38	2.7±1.29	2.79±1.32	2.79±1.45	ns
C16:0	13.39±1.08	13.89±1.74	14.27±1.82	13.97±1.24	ns
C18:0	2.75±0.88	3.13±1.14	3.35±1.43	3.28±1.3	ns
OA	22.83±2.07	24.28±4.24	23.15±2.91	25.05±4.15	ns
LA	33.39±7.37	29.27±4.13	30.15±4.94	29.26±5.38	ns
ALA	1.79±0.12	1.61±0.15	1.66±0.16	1.95±0.29	ns
EPA	0.1±0.05	0.09±0.04	0.1±0.04	0.1±0.05	ns
DHA	0.01±0.01	0.02±0.013	0.02±0.01	0.02±0.01	ns
FA groups					
SFA	38.98±9.45	41.6±8.49	41.96±7.95	40.5±9.37	ns
MUFA	24.68±1.88	26.25±4.21	25.03±2.8	27.07±4.1	ns
PUFA	35.82±7.58	31.62±4.3	32.47±5.08	31.92±5.72	ns
n-6	33.5±7.33	29.36±4.09	30.25±4.91	29.35±5.34	ns
n-3	1.94±0.089	1.75±0.16	1.815±0.16	2.1±0.25	ns
EPA+DHA	0.11±0.04	0.11±0.04	0.12±0.03	0.11±0.04	ns
n-6/n-3	16.98±3.12	17.07±3.07	17.01±3.52	13.88±1.77	ns

[1] Significances were declared: ns = P>0.1. [2] OA= oleic acid; LA = linoleic acid; ALA = α-linolenic acid; EPA = eicosapentanoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA =polyunsaturated fatty acids; n-3= omega-3 FA; n-6 = omega-6 fatty acids; n-6/n-3 = omega-6 to omega-3 ratio

5.4.3. Egg fatty acid composition

Main Effects

The lipid content and fatty acid profiles of egg yolks from the 3 experimental diets are presented in Table 5.5. Linoleic acid was the dominant FA in yolks followed by OA and palmitic acid. Together these FA contributed to 53-86%of all FA across all diets. The level of insect meal in the diet did not affect the total egg lipid content but had a significant effect on its FA profile.

Saturated fatty acids

Increasing the level of insect meal raised the SFA as a group by 6% and 9% at H12 and H24 when compared with eggs from control diet respectively and lauric acid (C12:0) accounted for 0.2, 24 and 35% of the total FA in the control, H12 and H24 respectively. A similar increase was noticed for C16:0 but in this case, eggs from the 2 insect meal diets did not differ significantly from each other. The control diet was very low in C12:0 and low in C14:0 and both fatty acids were increased at each level of insect meal, having the most substantial effect at the first level of insect inclusion (a 50 fold increase over the control) and a smaller increment (+40%) when comparing H12 and H24. Stearic acid (C18:0) in eggs decreased slightly but significantly when including insect meal in the diet at either level but differences between eggs from H12 and H24 did not reach significance.

Monounsaturated fatty acids

The main MUFA, OA, was high in eggs from all diets and only differed significantly between eggs from the 2 diets including insect meal. MUFA as a group was by 7% higher only in eggs from H24 diets compared with control. Minor MUFA c9C16:1 concentration in eggs rose with increasing the level of insect meal in the diet by 54% and 75% at H12 and H24 diets respectively when compared with eggs from control diet. Similarly c11C18:1 increased by 10% and 18% at H12 and H24 diets.

Table 5.5. Means ± SE and ANOVA P values for the effect of diet on yolk lipid content (%) and fatty acid (FA) profile (g/100g total FA) of eggs.

	Diet			ANOVA - P values[1] Main Effects
	Control n=28	**H12 n=31**	**H24 n=31**	**Diet**
Lipid content	32.53 ± 0.87	35.07 ± 0.81	33.69 ± 1.01	0.127
SFA[2]				
C12:0	0.004 ± 0.00^{c}	0.2 ± 0.01^{b}	0.28 ± 0.02^{a}	<0.001
C14:0	0.23 ± 0.01^{c}	1.42 ± 0.07^{b}	1.87 ± 0.10^{a}	<0.001
C16:0	25.71 ± 0.18^{b}	27.34 ± 0.17^{a}	27.66 ± 0.2^{a}	<0.001
C18:0	8.91 ± 0.14^{a}	8.17 ± 0.08^{b}	8.11 ± 0.13^{b}	<0.001
MUFA				
c9 C16:1	1.89 ± 0.07^{c}	2.89 ± 0.10^{b}	3.3 ± 0.12^{a}	<0.001
OA	37.23 ± 0.46^{ab}	36.78± 0.31^{b}	38.17 ± 0.43^{a}	0.004
c11 C18:1	1.69 ± 0.03^{c}	1.86 ± 0.03^{b}	2.0 ± 0.04^{a}	<0.001
PUFA				
LA	18.26 ± 0.62^{a}	15.59 ± 0.38^{b}	13.04 ± 0.44^{c}	<0.001
ALA	0.54 ± 0.05^{a}	0.46 ± 0.02^{b}	0.34 ± 0.02^{c}	<0.001
AA	2.02 ± 0.03^{a}	1.78 ± 0.03^{b}	1.7 ± 0.04^{c}	<0.001
EPA	0.01 ± 0.001^{c}	0.02 ± 0.001^{b}	0.03 ± 0.001^{a}	<0.001
DPA	0.08 ± 0.003^{ab}	0.09 ± 0.003^{a}	0.08 ± 0.003^{b}	0.031
DHA	0.82 ± 0.02^{a}	0.80 ± 0.02^{ab}	0.74 ± 0.02^{b}	0.004
FA groups				
SFA	35.16 ± 0.23^{c}	37.39 ± 0.18^{b}	38.18 ± 0.18^{a}	<0.001
MUFA	41.62 ± 0.5^{b}	42.4 ± 0.35^{b}	44.40 ± 0.43^{a}	<0.001
PUFA	22.93 ± 0.67^{a}	19.9 ± 0.4^{b}	17.07 ± 0.47^{c}	<0.001
n-3	1.59 ± 0.07^{a}	1.50 ± 0.03^{a}	1.32 ± 0.04^{b}	<0.001
n-3>18C	0.93 ± 0.03^{a}	0.92 ± 0.02^{ab}	0.86 ± 0.03^{b}	0.012
n-6	21.24 ± 0.61^{a}	18.26 ± 0.38^{b}	15.58 ± 0.45^{c}	<0.001
n6/n3	13.58 ± 0.26^{a}	12.25 ± 0.27^{b}	11.92 ± 0.37^{b}	<0.001

[1]Means within Diet (D) and Round (R) with different superscripts are significantly different (P<0.05) according to Tukeys's honestly significant difference test. [2] SFA = saturated FA(C12+C11:1, C14:0+9C13:1, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0); MUFA = monounsaturated FA (c9C14:1, t9C16:1, c9C16:1, c9C17:1, t6+t7+t8C18:1, t9C18:1, t11C18:1, c9C18:1 (OA), c11C18:1, c13C18:1, c5C20:1, c8C20:1, c15C24:1; PUFA = polyunsaturated FA (t10t14C18:2, t8c13C18:2, c9t12C18:2, t9c12C18:2, ctmix10,14+12,16C18:2, c9c12C18:2 (LA), c9c15C18:2, c6c9c12C18:3(GLA),c 9c12c15C18:3 (ALA), conjugated linoleic acid (CLA), t11c13C18:2, unknown CLA(t,t), unknown CLA(t,t), c9c13c15C18:3, c11c14C20:2, c9c11c15C18:3, c8c11c14C20:3, c11c14c17C20:3, c5c8c11c14C20:4 (AA), c13c16C22:2, c5c8c11c14c1720:5(EPA), c7c10c13c16C22:4, c4c7c10c13c16C22:5, c7,c10,c13,c16,c19C22:5(DPA), c4,c7,c10,c13,c16,c19C22:6 (DHA)); n-3 = c9c15C18:2, ALA, c9c13c15C18:3, c9c11c15C18:3, EPA, c11c14c17C20:3, DPA, DHA; n-6 = c9t12C18:2, t9c12C18:2, LA, GLA, c11c14C20:2, c8c11c14C20:3, AA, c13c16C22:2, c7c10c13c16C22:4, C22:5n-6; c = cis; t = trans

Polyunsaturated fatty acids

The concentrations of most individual PUFA in egg decreased with increasing the inclusion of insect meal in the diet, with the exception of EPA which increased. The main PUFA, LA decreased by 15% and 29 % at H12 and H24 respectively compared with eggs from control diet. A similar pattern was noticed for the overall n-6 and PUFA (both groups dominated by LA). Minor arachidonic acid (AA) concentrations decreased with inclusion of the insect meal as well but the difference between eggs coming from the 2 diets with insect meal was smaller (4%) than for LA. Both n-3 and long chain n-3 FA were lowest in eggs from H24 diets - by17% and 8% respectively lower when compared with control. Alpha linolenic acid was lower in eggs coming from diets with insect meal by 15% and 37% respectively for H12 and H24 diets compared with control. Similarly, DHA was lowest in eggs from H24 diets (-10% to control), However, eggs from the lower level of insect meal (H12) did not differ from control eggs. Concentrations of DPA and EPA were relatively low in eggs from all diets, However, EPA showed a statistically significant increase with increments of insect meal in the diet. The n-6 to n-3 ratio were lower in eggs coming from insect meal diets by 10% and 12% for H12 and H24 diets respectively compared with control, with no difference between levels of insect meal included in the diets.

5.5 DISCUSSION

5.5.1. General

This study reports the effect of replacing soybean cake by HIM in diets for layers, on the egg FA composition. The study has been designed as part of an experimental trial looking into layers performance reported by Maurer et al. (2016).

5.5.2. Diet fatty acid composition

Even though experimental diets differed from the control by having higher contents of wheat and slightly more sunflower cake (to maintain consistent protein levels across treatments), the most prominent difference in diets was the partial and full replacement of soybean cake by insect meal. The high levels of C12:0 and C14:0 in the final diets are in line with other studies looking into diets' FA profile of HIM at comparable inclusion levels (Schiavone et al., 2017, Cullere et al., 2017). As sunflower tends to have less C16:0 than soybean (typically around 40% less in sunflower compared with soybean oil) (Oliveira et al., 2010), the lower levels of C16:0 (between 24-28%) in insect meal diets might have been partially driven by extra 55-56 kg of sunflower per tonne of these experimental diets. However, when feed C16:0 estimated intake was calculated only diet H24 was less than the control and only by 5%. The low levels of PUFA and MUFA found generally in *Hermetia illucens*, especially when compared with soybean

(Oliveira et al., 2010, Guil-Guerrero et al., 2018), can explain the decreased dietary levels of both groups – driven by their main respective FA (LA and OA). Similarly to C16:0, when both OA and LA intakes were calculated, only diet H24 supplied significantly less than the control. The changes on diet FA composition are in agreement with Schiavone et al. (2017) (C12:0: 1% in control and 21 and 38% in 3.% and 7% HI oil diets; LA 51% in control and 33% and 21% in 3.% and 7% HI oil diets). Schiavone et al. (2017) included HIM oil at 2 levels (3.5% and 7%) to conventional soybean diets not excluding soybean cake completely. Furthermore, having a much higher dietary fat content (around 10% across all treatments) and therefore this may not be fully comparable to this study. Contradictory results have been reported concerning EPA levels in diets including *Hermetia illucens*. However, as the n-3 FA content of insect meal depends on the substrate insects are grown on and can be generally manipulated (Barroso et al., 2017), our results will be further discussed in relation to their transfer in eggs. It should be noted that waste pasta from wheat is likely to be low in lipid and (like most seeds) dominated by n-6 rather than n-3 FA (Butler, 2014). It has to be noted that for the calculation of the diet FA intakes, a correction factor to allow for the fact that the total fat is not only FA has not been used. Although this is a weakness of the study and certainly not ideal as it does not give the exact and real amounts of FA, in the framework of this thesis the dietary FA intakes of the diet have been discussed with respect to their relative transfer to eggs and to their relative composition between diets.

5.5.3. Egg fatty acid composition

Even though inclusion of the defatted insect meal raised the fat content of the diets, the lipid content of the resulting eggs was unaffected. This is in line with Aida et al. (2005), who showed dietary treatments had no effects on egg lipid content which appears to be independent of the quantity and source of dietary lipid. It is also interesting to note similar yolks weights were reported by Maurer et al. (2016) relating to this study, along with similar feed intakes, further demonstrates the buffering capacity of hens with respect to egg lipid content under various dietary treatments. The study reported by Secci et al. (2018) is the only other study looking into complete replacement of 24% soybean cake in the control diet (versus 35% of soybean cake in our study) and with 17% inclusion of insect meal in the experimental diet (versus 12% for H12 and 24% for H24 in our study). Both their diets had around 4% of fat content (versus 4.%, 5-6.4% in our study) and reported 125.13 and 108.04 g/day feed intake for control and HIM diet (versus 116, 131 and 107 g/day in our study for control, H12 and H24 diets respectively).

Saturated fatty acids

Total SFA account for between 35% and 38% of total FA in the eggs with C16:0 making the greatest individual contribution at 26%-28%. Lauric acid (C12:0) was substantially and significantly higher in eggs from hens fed insect meal, However, the actual level in the eggs (at <0.3% of total FA) cannot be classified as a food high in C12:0 and the relevance from a health impact is unlikely to be relevant. However, since insect meal is considered as a future feed ingredient, in this study we discuss the overall changes or trends in the egg FA profile by including insect meal. The limited capacity of hens to incorporate dietary C12:0 into the yolk is in agreement with Secci et al. (2018). However, they found no significant differences between eggs coming from control or insect fed hens (with 17% inclusion of insect meal). Furthermore, reported C12:0 values in eggs were 5-fold higher than ours, despite the C12:0 intake in our experimental diets being higher than that reported by Secci et al. (2018). Intake from our control diet was estimated at 0.01 g of C12:0 per hen per day whereas from control diet by Secci et al. (2018) at 0.07 g FA/hen/day. Similarly, from our H12 diet intake was estimated at 1.79 g of C12 per hen per day whereas from experimental diet by Secci et al. (2018) at 0.37g FA/hen/day. Analytical precision for C12:0 (and other FA found in low concentrations) could explain non detecting relative differences in C12:0 which was detected between 0.004 and 0.28% of total FA. Results here are more similar to Schiavone et al. (2017) who replaced soybean oil and Cullere et al. (2017) who also partially replaced soybean with black soldier fly fat into broilers and quail diets respectively. They both reported a considerable deposition of C12:0 into breast meat when including insect meal or lipid (in quail meat 100 fold increase was reported between control and a 10% HIM level diet). A pattern similar to C12:0 is noticed for C14:0 with results from Secci et al. (2018) agreeing with ours, reporting increased levels in eggs coming from insect fed hens. For both C12:0 and C14:0 it seems that while the relative concentrations in the lipid fraction were higher in the H12 eggs compared with control eggs, the transfer efficiency appeared to fall with further increments of insect meal in the H24 diet. Concerning the main SFA C16:0, although diets with insect meal were less rich than the control diet, eggs from H12 and H24 diets were higher in C16:0 compared with those from the control diet. Greater FA elongase activity in hens when fed diets rich in C12:0 and/or C14:0 could be hypothesized. The same pattern was noticed by Cullere et al. (2017), looking into breast meat FA profile using broiler chickens. On the contrary, Schiavone et al. (2017) also looking into breast meat did not notice variations either in diets or meat from including HIM, whereas Secci et al. (2018) looking into eggs, reported similar diet composition but a lower level of C16:0 in eggs coming from the insect meal diet. However, at the same time, account has to be taken of estimated intakes of C16:0 which for the H12 diet were higher than the control or H24 diets, and the intake from H24 was only 5% less than the control. Differences in the lipid content of these diets, driving

variation in FA intake, is a more plausible explanation for elevated concentrations of C16:0 in the resulting eggs, despite lower concentrations in the lipid fraction of the experimental diets. The slightly lower levels of C18:0 in H12 and H24 eggs is in contrast to Secci et al. (2018) who reported lower levels in control eggs, using the same layer genotype, while both studies in breast meat report no variation in C18:0 concentrations between eggs from insect and control diets (Cullere et al., 2017, Schiavone et al., 2017). These results suggest that the higher elongase activity hypothesized before for insect fed layers could be weaker for FA longer than C16:0. Finally, results from this study show that total SFA is higher in eggs with more insect meal in the diet, in agreement with both studies with broilers and breast meat (Cullere et al., 2017, Schiavone et al., 2017). On the contrary Secci et al. (2018) who although found higher SFA level in the insect meal diet, failed to show a similar relationship for eggs. However, the difference in total SFA intake from their aforementioned diet when compared with control was only 15% higher, whereas SFA intake from H12 in our study was 154% more than the control diet.

Monounsaturated fatty acids

The most abundant individual FA in eggs was OA (c9C18:1) responsible for 37%-38% of total FA and responsible for the greatest difference in MUFA concentrations, which varied between 42 and 44% of total FA. Differences in OA concentration in our study were relatively small compared with other studies and, although statistically different between H12 and H24 eggs (with H24 eggs being higher in OA) neither differ from the control eggs and concentrations seemed to follow no specific pattern. It is also interesting to note that OA concentration was significantly higher in control compared with both insect diets and also the estimated OA intake from the H24 diet was 15% lower than from the control diet. Yet in the eggs H24, levels were similar to control eggs and could hypothesize some type of elongase and/or desaturase activity. For instance, since hens' dietary intakes of C12 and C14 were higher in H12 and H24 diets than the control, it could be possible that elongases acted to transform these fatty acids in C16 and then to C18. Afterwards, Δ9 desaturase could have acted desaturating a high content of C18 to OA. It is interesting to note that LA intakes were higher in control and H12 diet compared to H24. Infield et al. (1973) wondered if a high intake of linoleic acid could hinder the desaturating activity on C18. That hypothesis could explain the higher desaturating activity in H24 eggs, resulting in equal OA to eggs from control diet.

Both Secci et al. (2018) and Cullere et al. (2017) showed no differences in OA levels in eggs and quail meat respectively. Since OA is by far the most abundant MUFA (responsible for 88-91% in this study), it is not surprising the concentrations of MUFA follows the pattern set by OA. Concerning minor MUFA c9C16:1 and c11C18:1, their concentrations in eggs are both

low (<3.5% of total FA) although increased with increasing level of insect meal in diets, with c9C16:1 showing greater relative increase. Both studies replacing soybean with insect meal for meat birds reported a similar pattern in FA profile of quail and broiler meat (Cullere et al., 2017; Schiavone et al., 2017). However, differences in c11C18:1 did not always reach statistical significance (Cullere et al., 2017). Contrary to our results, Secci et al. (2018) reported less of these 2 MUFA in eggs from hens fed HIM (-17% for c9C16:1 and -7% for c11C18:1). Overall, MUFA was higher in eggs from the highest level of insect fed hens, in agreement with the FA profile of insect fed quail meat (Cullere et al., 2017). On the contrary, Secci et al. (2018) reports a small but statistically higher MUFA content in eggs from their control diet. However, the level of insect meal reported by (Secci et al., 2018) was intermediate to the levels in our study, yet soybean was completely replaced as in the H24 diet in our study. Overall, it is interesting mentioning that although dietary MUFA content in feed was substantially lower when insect meal was included, the egg FA profiles were quite similar. Also, the estimated MUFA intake from the highest inclusion level H24 gave the lowest consumption, statistically similar to the control diet (and statistically lower than the H12 diet) but eggs ended up with higher MUFA levels than from the control and H12 diets.

Polyunsaturated fatty acids

The most abundant individual PUFA from all diets was LA at 18% of total FA for the control eggs but reduced with each increments of the insect meal. Additionally, for the LA content, the lower levels in eggs from the H24 diet were almost identical to the decrease in estimated LA intake relative to the control diet. However, the estimated LA intake from the H12 diet did not differ from the control, yet its concentrations in eggs were significantly less. Our results showing lower levels in eggs coming from diets with insect meal agree with Secci et al. (2018) and also with the meat studies by Cullere et al. (2017). Both studies reported egg, meat and diets including insect meal being lower in LA, n-6 FA and consequently total PUFA. However, Cullere et al. (2017) reported quail meat concentrations in LA were similar across control and insect meal diets. With regards to n-3 FA our results show less ALA in eggs from insect fed hens although, similarly to Cullere et al. (2017), the levels of ALA in the respective diets do not differ. Furthermore, even if estimated intakes are taken into account, ALA intake was higher at H12 diet, whereas H12 eggs are lower in ALA than the control. Although EPA in our results is present in higher levels with increasing inclusion of insect meal in the diet, concentrations of all long chain n-3 FA are relatively low and follow no specific pattern. These results are similar with Secci et al. (2018) and Schiavone et al. (2017) who found rather similar concentrations of long chain n-3, irrespective to including insect meal or not. Concerning the sum of long chain FA both the % FA profile in the diets and the estimate intake of these FA, represented as the

sum of EPA and DHA, were shown to be higher with increasing the insect meal in the diet. However, the FA egg content was significantly lower (but only by 8%) in the H24 eggs when compared with control diet, whereas H12 eggs were intermediate and similar to both the control and the H24 eggs.

Overall, n-3 FA were significantly lower only at the highest level of insect meal inclusion, although the n-6/n-3 ratio was also lower and hence more favourable, in eggs coming from insect meal diets. Insects used to formulate the meal in this study's diet were grown on a wheat based waste from pasta making, which is likely to be a poor source of n-3 FA compared with leafy vegetables. As mentioned, the FA profile of insects also depends on the growing substrate (Spranghers et al., 2017). Therefore using insects that have been fed a diet rich in n-3 FA like waste for fish production would be interesting to consider in the future, as a means of increasing further the n-3 FA egg content as shown by (St-Hilaire et al., 2007a). It is also important to highlight the difference in the lipid content of the diets in our study and the potential impact the absolute feed intake had on the actual FA intake from the respective diets.

5.6 CONCLUSION

Since insect meal is considered as a feed ingredient, in this study we discuss the overall changes or trends in the egg FA profile by including insect meal. Including HIM while increasingly replacing soybean cake in layers diet significantly affected the egg FA profile. However, it has to be noted that even if the dietary FA profiles were considerably different, the respective effects in the eggs were much smaller. Overall, complete replacement of soybean cake (H24) had a negative impact on egg lipid composition as it increased SFA and decreased PUFA both n-6 and n-3 FA. However, the n-6/n-3 FA ratio was shown to be overall lower in eggs with HIM, which could have a positive impact on consumer health. On the other hand, eggs coming from diets with 12% HIM replacing soybean cake by one third, had similar n-3 FA and MUFA to control diets yet SFA were higher and PUFA overall lower. Insect meal in this study was grown on wheat-based waste whereas different substrates for insect growth should be investigated in terms of ameliorating egg nutritional profile if replacing soybean cake.

CHAPTER 6. GENERAL DISCUSSION AND CONCLUSIONS

6.1 INTRODUCTION

This thesis has provided novel information on the effect of management systems on the nutritional quality of eggs. The hypothesis developed to account for the effects of OR management on egg nutritional quality is based on a framework of greater forage intake and FR behaviour. This framework proposes that better egg nutritional quality including amongst others the concentrations of n-3 FA, carotenoids such as lutein and zeaxanthin, vitamin A and E, is enhanced due to forage material consumption as herbage is rich in these nutrients (Mugnai et al., 2014a). Indeed, the fatty acid profile of non-ruminant animal products, including eggs, is essentially a direct reflection of their dietary intake, since limited transformation of dietary fatty acids occurs during digestion and absorption (Givens, 2005, Fraeye et al., 2012). Similarly, it has been shown that the relative content in eggs can be enriched in other nutrient compounds, such as carotenoids and vitamin A and E, via dietary enhancement through forage intake (Karsten et al., 2010). Additionally, there is a well-established relationship between UVB exposure of pigs and poultry and vitamin D deposition in their products (Barnkob et al., 2019, Schutkowski et al., 2013). Hence, it was hypothesized that a pasture based production system with an outdoor range, with higher exposure to UVB (compared with confined birds), will enhance the nutritional egg quality by increasing the overall egg vitamin D content. However, even though the nutritional composition of pasture plants is well established (Hammershøj and Johansen, 2016, Mugnai et al., 2014), the effects of management systems on egg nutritional quality have not been consistent across the literature. For example contrary to Karsten et al. (2010), Anderson (2011) showed no differences in egg vitamin A and E content from pasture-raised hens compared to CA hens. Finally, there is a dearth of information in relation to egg nutritional content in different management systems in relation to vitamin D content.

The hypotheses of this thesis are listed below:

1) Increasing intensity of forage material intake in hen management will result in enhanced egg nutritional composition. As pasture is a natural source of nutrients such as FA, carotenoids and vitamins, more of these compounds will be available for the hens in FR systems to uptake and deposit in their eggs.

2) Management practices between OR FR and conventional FR differ not only in feed origin but can also differ in the intensity of free-ranging behaviour and pasture availability. These differences can therefore affect further the final nutritional composition of the egg.

3) Seasonal variation can affect the availability and nutritional content of plants and invertebrates found in pasture. At the same time, seasonal variation can interact with management. It is expected that for systems where birds have access to pasture at times with higher pasture availability the egg nutritional profile would be more enriched. Additionally, seasonal variation will interact with management more within systems with intense free-ranging behaviour.

4) Different genotypes of brown and white laying hens will result in differing egg nutritional profiles. However, the literature on this topic has been rather contradictory.

5) Replacement of expeller soybean meal, an omega-6 FA source in the hens' diet, will affect the FA profile of the egg. The FA profile of insect meal, replacing soybean, will be very much dependant of the food substrate the insects were grown on.

In order to investigate the effect of management on egg nutritional quality, three major egg production systems were explored; caged (CA), conventional free-range (FR) and organic (OR). These were chosen to reflect the consumption and production reality at European and UK retail level (European Commission, 2018a, UK Industry data, 2018). To fully investigate the effect, these systems were looked at within a framework of a retail supermarket survey, a farm survey and a meta-analysis assessing all available published data generated so far having a similar hypothesis to this thesis. The meta-analysis allowed for a robust overview and quality appraisal of the available research so far, allowing a summary of research findings across studies (Gurevitch and Hedges, 1999, Koricheva et al., 2013). The farm survey was performed in order to fully control and investigate confounding parameters that could affect the egg nutritional quality, such as seasonal and genotypic variation. Finally, the supermarket survey was selected for a realistic overview of the nutritional quality actually available to the consumers and therefore allowing a critical evaluation of the impact of these different egg types on the human nutritional intake. In addition, in order to test the sensitivity of egg quality to seasonal variation, at least two contrasting seasons (winter and summer) were chosen (Chapter Two and Chapter Four), whereas where funding allowed, four seasons across a whole year were chosen to elucidate seasonal variation on egg quality.

In Chapter Five, the effect of alternative feed sources on egg fatty acid profile was investigated. The hypothesis here was that reducing or removing soya from the diet will change the egg lipid composition since the egg fatty acid profile reflects the fatty acid profile of the feed (Poureslami et al., 2012). In this Chapter, the impact of replacing soybean with insect meal on egg FA profile was looked within an experimental trial framework, for which, controlled conditions allowed

first producing the insect meal and then feeding the layers at the experimental setting in FiBL (Switzerland). The investigations of such an effect were deemed important as soybean is one of the most widely used ingredients in egg and poultry production, yet is considered unsustainable because of its high environmental impact (DiGiacomo and Leury, 2019, van Huis, 2015). At the same time insect meal is considered one of the most promising alternative protein sources for substituting soybean in hen diets (Sakkas et al., 2019b). The relationship of using insect-based ingredients in animal feeding and enhanced animal products in omega-3 FA has been repeatedly observed (St-Hilaire et al., 2007a, Wang and Shelomi, 2017). However, it is well established that the insect FA profile is highly variable, depending on the substrate the insects are grown on (Barroso et al., 2017, Meneguz et al., 2018). The objective of this part of the thesis was to investigate specifically the impact of replacing soybean with insect meal from insects grown on pasta-waste, on egg FA composition.

As each experimental Chapter (Two, Three, Four and Five) contain a comprehensive discussion of the results, the purpose of this general discussion will be to draw together the experimental results according to the broader impact of management system, season and other factors on the nutrients assessed. Afterwards, I will discuss the potential implications of these on human health and also suggest possible future work in the field of the nutritional quality of eggs.

6.2 OUTCOMES OF THE PHD

6.2.1. Egg nutritional quality

6.2.1.1. Effect of management

In Chapter Two the standardised mean difference metric taking into account all studies available in the literature, was measured to calculate the relative differences of egg FA contents (including docosahexaenoic acid (DHA) and n-3 FA), carotenoids (lutein, zeaxanthin and sum of carotenoids), vitamins A and E and cadmium content between OR and conventional systems. In Chapters Three and Four egg FA composition (both Chapters), carotenoids, vitamins A, E, D, B_2 and B_9 and some minerals (Chapter Four) were measured in order to be able to investigate actual differences between systems.

Fatty acids

Here I focus on differences in polyunsaturated FA (PUFA) and long chain n-3 FA concentrations, as these are particularly associated with human health benefits such as reducing circulating triglyceride concentrations (Abdelhamid et al., 2018) and lowering mortality, in older adults (Mozaffarian et al., 2013). Mention is also going to be made for saturated FA (SFA), as replacing SFA with PUFA has been shown to be associated with lower risk of cardiometabolic death (Micha et al., 2017).

The meta-analysis shows OR eggs were statistically higher in DHA and total n-3 FA compared to eggs from FR systems. On the other hand, when OR eggs were compared to eggs from indoor systems, the difference was not statistically significant, yet there was a trend for OR eggs to have slightly greater concentrations for both DHA and n-3. While several sensitivity analyses were considered, excluding the most influential study (relative to the overall result), no differences from the main analysis were identified, showing the robustness of the results. However, the sensitivity analysis excluding "lower quality" studies, changed the differences between OR and FR systems to "not significant". However, since over half of the studies were excluded, these results are possibly less reliable than the main analysis. For the comparison of OR to eggs from indoor systems, the fact that no changes were noticed with the sensitivity analyses, suggests that the robustness of these results and indeed the overall lack of difference in DHA and n-3 FA in OR and eggs from housed hens. However, if seasonal variation is responsible for extra differences in egg FA profile between systems, then unbalanced seasonality between studies contributing to the meta-analysis might have skewed results of OR to indoor eggs comparison to give no apparent difference. The fact that the studies included did not report sampling collection seasons, does not allow for further explanation. It does highlight however, the importance of correct and extensive reporting of experimental parameters within published studies. In Chapter Three, egg FA composition was compared between OR and FR eggs under Swiss FR systems. Eggs from OR systems were higher in total n-3 FA (+49%) and DHA (+30%) compared with FR eggs. Similarly, in Chapter Four, where egg FA composition was compared across EO, OR, FR and CA systems in the UK, both total n-3 FA and DHA were higher (+ 31% and +47% respectively) in the OR eggs (both OR and EO) compared with eggs coming from the conventional groups (both CA and FR).

Overall, results of this thesis show that eggs from OR systems, independently of the intensity of OR practices, were higher in n-3 and DHA than eggs from conventional systems regardless of whether they originated from conventional FR or CA systems. This supports the view of a positive OR management effect on egg FA, which is assumingly related to the effects of greater

forage material consumption than in comparison to conventional systems (Hammershøj and Johansen, 2016). Results of this thesis also come in agreement with those of the most recent study on the effect of management on egg composition, which showed that OR eggs are higher in DHA than those from either FR or indoor systems (Lordelo et al., 2017). This is expected since OR management allows for a hens to display more FR behaviour than conventional systems and certainly than indoor systems (Gilani et al., 2014). Studies assessing pasture or silage intake by hens show that foraging on pasture can account more than one third of the total feed intake in FR hens (Hammershøj and Johansen, 2016). Furthermore, according to the hypotheses put forward in this thesis, eggs from FR systems did not differ from eggs coming from CA system. It is well established that the large flock sizes of conventional FR systems do not induce substantial ranging behaviour and the available pasture is quickly depleted (Campbell et al., 2017) especially near the housing. Results from this thesis shows dietary n-3 FA from compound feed is also a significant driver (Figure 3.7) for egg FA content. However, while dietary FA intake of n-3 in OR and FR systems do not differ (Table 3.1), the egg n-3 FA content was higher in OR eggs. On the other hand, Mugnai et al. (2014a), found higher n-3 FA for EO systems when compared with OR and CA systems. The authors also reported that 6-18 g of fresh forage intake per hen per day (translated to 20 mg of C18:3 n-3 per hen per day) by hens in the OR systems did not increase egg n-3 content above that of eggs from indoor hens. Birds in the wild have been shown to incorporate higher levels of long chain n-3 FA into eggs, suggesting that not only quantity of vegetation but specific plant species diversity in the pasture might be driving higher levels of n-3 FA in eggs from OR or/and EO systems (Speake et al., 1998). Similar conclusions, showing mixed swards having a positive effect compared to pure swards, are also reported for cattle and the respective milk FA profile (Butler et al., 2008).

With respect to the OR and conventional diet, the mechanical methods for the removal of oil from soya and other oil seeds (contrary to the solvent extracted methods in the conventional systems), results in higher residual oil content in OR compared with conventional feeds. Cereals, being the main constituents of poultry diets, are relatively low (<30 g/kg) in total lipid but have a relatively high proportion of n-6 FA and specifically linoleic acid (LA–18:2), with the exception of rapeseed meal with a high proportion of OA (C18:1) and linseed (α-linoleic acid (ALA, C18:3) (Butler, 2014). Therefore, OR feed including soya, extracted under mechanical methods would have a higher input of n-6 FA than the conventionally prepared feed. This, could potentially explain the higher (although not statistically significant) LA content in the OR compared to the conventional feed in Chapter Three and the respective difference in eggs shown in both Chapters 3 and 4, showing OR eggs being clearly richer than

CA eggs in LA content. When it comes to the n-6/n-3 ratio, Chapter Four showed the lowest ratio for eggs of both OR systems, although statistically similar to CA eggs and similar to FR in Chapter Three. Despite the fact that UK OR eggs were both higher in n-6 and n-3 FA, the higher levels of n-3 FA were greater than the ones of n-6 FA, hence the lower n-6/n-3 ratio. It is interesting to note that the ratio of UK eggs was lower than that of eggs coming from Switzerland, possibly driven by the different formulation standards or primary ingredients used and the respective higher n-6 ratio in their diet.

In Chapter Three, similarly to Chatzidimitriou et al. (2015), SFA and the major C16:0 FA were lower (-3%) in OR eggs compared to FR eggs. On the other hand, in Chapter Four, no difference in egg SFA proportions were found across systems but the major SFA C16:0 was, as in Chapter Three, lowest in EO eggs (-4% than CA). Yet mainstream OR eggs in Chapter Four were similar to FR eggs but still the proportion of C16:0 was less than in CA eggs. Taking into account that the fat content of the diets were similar between systems, these results suggest that OR and possibly eggs from EO practices, can have a FA profile around 3% lower in SFA than the eggs from conventional systems. This trend seems to be consistent with the results of Filipiak–Florkiewicz et al. (2017) where although there was no significant difference in C16:0 content between OR and CA eggs, a similar magnitude of difference was noticed. In line with these results, and since C16:0 is the main SFA in egg yolks, a lower total SFA content was found for eggs from systems with n-3 FA rich diets (Oliveira et al., 2010). As suggested by Kuksis (1992), due to their biological importance to the embryo, yolk SFA is not prone to large variations. In addition, as shown by Cherian et al. (2002) the variation noticed in either SFA or C16:0 in eggs of OR or conventional systems is marginal and may be significant only if animal fat (high in SFA) is included in the hens' diets. The question whether these differences are relevant to human health remains open and will be addressed below.

Carotenoids

In the meta-analysis Chapter, the most robust analysis for carotenoids, with the most study points was for the sum of lutein and zeaxanthin, accounting in any case for more than 80% of the total carotenoids according to our results of Chapter four and reported by Public Health England (2015). The meta-analysis outcome with respect to carotenoids was robust as the same outcome was shown by sensitivity analyses, including analyses without imputed data or influential to the overall results data points. The results showed that the sum of lutein and zeaxanthin content in OR eggs was higher than that of conventional, either FR or CA. This is in agreement with the results from Chapter Four where OR eggs were higher in lutein and

zeaxanthin compared with FR and CA eggs. The magnitude of effect size and the respective confidence intervals of the meta-analysis were similar for both comparisons (OR with FR and OR with indoor eggs). Similarly in Chapter Four, eggs from OR systems were higher in lutein and zeaxanthin than eggs from FR and CA systems. With respect to the similar effect sizes and margins of exposures in the meta-analysis, one could speculate that the majority of studies used in both analyses used pooled eggs combining both conventional systems. Hence there was a pooled effect within both analyses, affecting the outcome the same way in both cases. In this respect, looking at OR compared with indoor eggs, when 7 out of 10 studies with pooled eggs from conventional systems were excluded, differences with OR eggs were not statistically significant. Although these results are in agreement with those of Mugnai et al. (2012) and Mugnai et al. (2014b), the conclusion that can be drawn taking into account only 3 studies is weak. However, even if these 3 studies failed to show differences between OR and indoor eggs, a significantly higher content of lutein and zeaxanthin was shown in EO compared with CA (and OR) eggs. According to Hammershøj and Johansen (2016) consumption of 50 g of fresh forage per hen a day would be adequate to increase the egg carotenoid content. In agreement with this view, only Mugnai et al. (2014b) measured a grass intake close to this, for hens in EO systems. Additionally, the same OR feed was given to hens of CA and OR systems to eliminate differences caused by feed composition and could explain why only EO eggs were higher in carotenoids than CA eggs in that study. To further explain the variation of egg carotenoid content in UK systems, feed analysis would be ideal. Our results from Chapter Four agree with Mugnai et al. (2014b), as EO were indeed higher in lutein and zeaxanthin than OR eggs. Contrary to the studies above, as mentioned, the OR eggs in Chapter Four were higher than the conventional eggs. The results of this thesis, support the view of the positive effect of OR management on egg carotenoid content as with the FA composition. Additionally, results of this thesis reveal a detailed picture of the egg carotenoid profile and how this varies across eggs from different UK management systems. It is important to note that the profile of egg yolk carotenoids may indeed vary between countries as feed ingredients and pasture plants may differ. Canthaxanthin and β-cryptoxanthin had a consistent pattern with lutein and zeaxanthin, being systematically higher in OR and EO eggs, suggesting that the majority if not all carotenoids came rather from the feed than from forage. Although in these results no β-carotene was detected, other studies report very low contents (Bunea et al., 2017, Skrivan and Englmaierova, 2014) and therefore it is not considered to have an impact on the overall carotenoid composition. Furthermore, it is interesting to highlight that even when samples of OR and FR eggs from hens with poor access to pasture were included (referring to April 2017 vs April 2018 analysis), OR eggs were still higher in carotenoids than FR and CA eggs, with

the exception of lutein. Although numerically higher in OR eggs, it lacked the statistical significance between FR and OR eggs. As also suggested in the absence of interactions between management systems and grazing (as a result of the normal access to a range in both FR and OR systems), and also between management and season, management is overall the most important factor affecting the egg carotenoid content, overruling other causes of variation.

Additionally, as shown by Bunea et al. (2017) differences in the overall carotenoid composition can be due to the various genotypes used in each system and their efficiency in transferring carotenoids from diets into the yolk. These authors showed hen breeds from light production lines specific for egg production having the highest carotenoids content (Italian breeds). In this respect, Pintea et al. (2012) comparing yolk carotenoid content of organically reared ISA brown and Araucana hens did not find any difference in egg carotenoid content even if Araucana yolk was higher in lipid content, suggesting a higher efficiency in carotenoid deposition by ISA brown hens. While in conventional CA and FR systems the genotypes used are more uniform, in OR environments, especially in EO systems, genotypes can vary (Leenstra et al., 2014). Therefore apart from higher carotenoid intake through forage material consumption, variation in hen strains within OR systems could potentially add to the higher carotenoid content in OR and EO eggs.

Vitamin A and E

In the meta-analysis chapter neither egg content of vitamin A nor vitamin E were statistically different between systems. This is in contrast with results from Chapter Four, where 1) although similar vitamin A content was shown for OR and FR eggs, they were both higher than CA eggs and 2) OR eggs were higher in vitamin E than eggs coming from FR or CA eggs. Contrary to our results, Skrivan and Englmaierova (2014) showed no differences between grazing and non-grazing hens in vitamin A content of eggs. Moreover, the meta-analyses revealed a trend of conventional eggs being higher than OR in both vitamins, in contrast to the UK retail survey (Chapter Four) where vitamin A content was statistically similar in OR and FR eggs, although OR eggs were numerically higher. Although the sensitivity analyses in the meta-analyses did not show deviations from the main analyses, the results were mainly based on too few studies for conclusions to be indisputable. Furthermore, the large confidence intervals for both vitamins, but especially for vitamin E, indicates large variation exists within all systems. This view is also supported by the Tukey's test between management systems in the UK farm survey showing EO eggs were similar to CA eggs.

According to Mugnai et al. (2014b) vitamin E was highest in EO eggs. Because this study had a common diet for all systems, with a vitamin E content much lower than that of forage material, it can be assumed that forage material intake drove any differences in the eggs. This is also supported by our results which showed eggs from hens without pasture access being lower than in eggs from hens with range access and hence forage intake. With respect to dietary vitamin supply both systems are supplemented with both the vitamins (European Commission, 2007) and it is well established that the egg content is directly correlated with dietary intake (Mendonça Jr et al., 2002). Therefore, the question lies on whether vitamin E intake via forage intake is high enough to make a difference and surpass the feed vitamin content. Mugnai et al. (2014b) showed that egg content in vitamin E were different only after an intake of vitamin E-relative to the forage intake of >800 μg hen/day. It has to be noted that vitamin E and A are fat soluble, therefore stored and no excreted. Therefore, the actual intake that results in a higher egg content may have been affected by the overall high vitamin E intake and status during precedent seasons. As vitamin E varies greatly amongst pasture plant species (Skrivan and Englmaierova, 2014), EO and OR systems are potentially more in danger of having an unbalanced supply according to seasonal variation. Also, plasma vitamin A and E levels, as well as carotenoids are modulated by exposure to pathogens and especially parasites (Sakkas et al., 2018). Therefore, FR systems with access to pasture and hence potential exposure to parasites, could decrease these nutrients content in eggs, especially in OR systems where routine use of coccdiostats are not allowed. However, results in Chapter Four indicate that this was not the case as FR eggs were higher than CA in vitamin A and OR eggs were higher than FR and CA in vitamin E. In this respect, another factor that could induce decrease such nutrients, would be stress levels of hens (Lauridsen, 2019), perhaps conventional hens have higher stress levels. However, whereas we would expect interactions between management and season, no such effect were noticed. Furthermore some EO flocks included in this study comprised of mixed hen genotypes and as suggested by Bunea et al. (2017), vitamin E content can vary greatly between hen strains, possibly explaining the wider variation within EO eggs compared with those from other OR or conventional systems. Furthermore, the results of this thesis support the views of Bunea et al. (2017) who showed positive correlations between carotenoid and vitamin E content.

Overall, results in this thesis show than in the UK market OR eggs have higher vitamin E content and that OR but also FR eggs have higher vitamin A content than eggs coming from CA systems. At the same time, it is clear that a non-stable supply of both vitamins is found for the smaller scale EO eggs.

Vitamin D

Effects of management and season in the vitamin D content of egg – both vitamin D_3 and 25-OH D_3, have been scarcely studied. Therefore, results in this thesis are considered important as they may advance the limited body of knowledge.

Vitamin D in birds, as in humans, is either synthesised in skin exposed to UV light (Kühn et al., 2014, Borradale and Kimlin, 2009) or consumed in the diet. Therefore, it is expected that FR hens may endogenously synthesize vitamin D because of exposure to sunlight, leading to a greater incorporation in eggs. Overall, both vitamin D_3 and 25-OH D_3 egg contents can be enhanced through dietary supplementation (Duffy et al., 2017, Mattila et al., 1999a, Mattila et al., 2011). With regards to 25-OH D_3, it has been quantified as an important proportion of the total vitamin D supply as it is about 5 times more effective in raising serum 25-OH D_3 than an equivalent amount of vitamin D_3 (Cashman et al., 2012). Its content is therefore multiplied 5 times to calculate the total vitamin D activity (Public Health England, 2015). However, if hens are only fed small quantities of D_3, little if any 25-OH D_3 would be found in eggs (Liu et al., 2014). In this respect, only when hens were supplemented with a combination of both D_3 and 25-OH D_3 the latter was higher than control eggs (Duffy et al., 2017) or even quantified when D_3 was in the diet but in low levels. Therefore, it seems important to supplement hens with 25-OH D_3. Additionally to the benefits for human health, such a supplementation has been shown to improve egg shell quality (McLoughlin and Soares, 1976) but only marginally (Yannakopoulos and Morris, 1979)). Furthermore from a produce's perspective, diets with 25-OH D_3 have been shown to improve overall bird performance both in the presence and absence of parasites (Sakkas et al., 2019a).

Looking at the results in this thesis, no effect of management was noticed as eggs from conventional and OR systems had similar levels of total and respective vitamin D compounds. This agrees with Kühn et al. (2014) and Dunlop et al. (2017) who did not show differences in egg vitamin D content from FR and indoor commercial layers, although in contrast with Guo et al. (2017b) who found FR being higher in total vitamin D than CA eggs and also OR being higher than FR eggs. Furthermore, contrary to this study, looking at the interaction results in this thesis between management and season, no differences are noticed between FR or OR and EO eggs within each season, suggesting a similar exposure of these commercial flocks to sun. However, taking into consideration the non-linear but obvious correlation between UVB exposure time and higher egg vitamin D_3 content, the greater difference of vitamin D_3 found between winter and summer for EO eggs (compared to the other systems) suggests EO hens may have greater exposure than other FR hens (FR and OR) during summer. Since the absolute

amounts of dietary vitamin D were unknown and assuming that all commercial systems have similar supplementation, it is interesting to note and discuss why the systems with range access have variations in their egg content and also, in the months with potentially greater sun exposure they do not differ from CA eggs. Assuming that the same feed is given in winter and summer, one would expect egg vitamin D content to be the lowest in eggs from CA systems especially in summer, as even 30 minutes of sun exposure increases Vitamin D in eggs (Kühn et al., 2015). However, the interaction discussed in Chapter Four suggests that if levels in eggs from CA systems are somewhat the baseline, it could be that because FR hens forage on pasture their dietary vitamin D intake in the offered feed is diluted and at the same time "re-gained" through sun exposure. On the other hand, Guo et al. (2017b), showed an interaction between management and supermarket, suggesting inconsistencies between management practices within systems such as the levels of vitamin D in the diet. It has to be noted though that OR and especially EO systems are more likely to have different types and levels of supplementation compared with conventional intensive systems. Finally, the lack of a relative interaction for 25-OHD_3 comes in agreement with Kühn et al. (2015) showing that, in response to UV light exposure, 25-OH D_3 content in eggs reaches a plateau much quicker than D_3. Similar to Guo et al. (2017b) egg vitamin D_3 variation between production systems here was higher than that observed for 25-OH D_3 concentration.

Contrary to expectations, my findings do not support the notion that management systems with FR practice have higher egg vitamin D metabolite content. However, the variation of egg vitamin D_3 content in FR systems across seasons and in comparison with eggs from CA systems, suggests that eggs from FR systems could benefit from further vitamin D supplementations through feed, to standardize egg nutritional value. It should be taken into account though, that limitations exist on this approach since the supplementation with both D_3 and 25-OH D_3 in poultry feed should not exceed legal limits (vitamin D_3: 75 μg/kg diet, 25-OH D_3: 80 μg/kg diet or total dietary concentrations of vitamin D_3 and 25-OH D_3: 80 μg/kg diet (EFSA, 2009b, European Commission, 2004, European Commission, 2009).

Vitamins B_2 and B_9

Effects of management on riboflavin (B_2) and folate (B_9) in eggs has not been studied before. Therefore, the results of this thesis can be considered crucial and novel when mapping the egg nutritional value across hen management systems. Only one study looking at the impact of OR and conventional feed has looked at B_9 egg levels and the results were similar to ours (Strandler et al., 2011). No differences were found in either of the egg's B vitamin contents across management systems. Even though the number of samples was only half that for analyses on FA and carotenoids, since statistical differences were significant for other vitamins on a comparable data set, it can be assumed statistical power sufficed to test the effect of management. As already mentioned in Chapter Four, the lack of differences are not in accordance with results from Poulsen et al. (2015). In their latter study, milk from OR dairying had higher content of B_2 than from conventional systems, possibly due to higher silage consumption. However, this was probably correlated with the impact of the rumen microbiota linked to silage feeding in dairy systems and since poultry have no known endogenous synthesis, the extrapolation of these results to poultry systems is likely irrelevant. Nevertheless, having in mind that B_2 is not only found in yeast or fermented food and feed products (EFSA et al., 2017) but also in green young plants, one might expect to see a higher B_2 egg content in OR systems as was the case for carotenoids. Yet the results of this study do not support this hypothesis. Similarly for B_9, which is also present in leafy vegetables and legumes (EFSA et al., 2014) an effect of management due to forage material intake was expected. For B_2, Squires and Naber (1993), found a difference in yolk content only when hens were fed 4.40 mg/kg of feed compared to 2.20 mg/kg of feed. As far as B_9 is concerned Buchanan et al. (2007) demonstrated that yolk content differed significantly only when hens were fed 10.3 mg/kg of feed compared to 0.8 mg/kg of feed with an intermediate dietary content of 4.31 mg/kg of feed not differing from the lowest level. Unfortunately, no information on herbage content on B_2 and B_9 is available to define the potential contribution from forage relative to these vitamin supplementation levels and with respect to differences which actually contribute to the overall egg vitamin content.

On the other hand, even though no clear effect of management was noticed for B_9, an interaction between management and contrasting seasons (winter and summer) was found. These results showed that EO eggs are higher in B_9 in summer than in winter and in parallel the summer content is higher than in CA eggs throughout the year, supporting the hypothesis that increased intake from the range (plants or/and insects) as usual in EO systems may increase B_9 in egg .

However, these results may be also connected with a higher legume ratio in the feeding regimes of EO systems during summer.

Overall, it is clear that OR systems can positively affect the folate egg content more than the conventional systems, possibly depending on the seasonal pasture and legume availability (without being lower than the conventional eggs at any point). At the same time, it is suggested that OR management should pay attention to their feeding regimes with respect to the vitamin contents to maintain egg quality at the highest possible standards. Taking into consideration that conventionally produced vitamin B_2 will be soon withdrawn from OR livestock diets as it is produced by recombinant microorganisms (*Candida famata, Bacillus subtilis and Ashbya gossypii*) (EFSA FEEDAP Panel., 2018, European Commission, 2018b), the availability of genetically modified organism-free riboflavin, obligatory in European OR agriculture, is a major issue. Further research should investigate the use of alternative ingredients to cover nutrient requirements in OR poultry production systems in an ever-changing regulatory framework.

Minerals

Cadmium and barium

Results in this thesis, specifically the meta-analysis, demonstrated that there is no differences in cadmium content of eggs between OR and conventional systems. The lack of variation in results from sensitivity analyses suggest the strength of this outcome. It has to be highlighted though that the most important uncertainty and weakness of this result is the fact that assumptions had to be made to impute values of cadmium reported to be below the LOQ. Although as already mentioned this type of imputations are common with such results (EFSA, 2010a), it gives bias and weakens the strength of the findings. According to the initial hypothesis of this thesis, since conventional crops including cereals that form a large part of hens' diet, are higher in cadmium (Baranski et al., 2014), eggs from conventional systems could incorporate higher quantities of cadmium. However, our results do not support this theory. As suggested, carry over of dietary cadmium into eggs is relatively low (Kan and Meijer, 2007, Sato et al., 1997). Furthermore, Waegeneers et al. (2009) speculated soil concentration rather than feed contamination with the heavy metal lead, is correlated with the egg content in FR systems. This suggests no overall differences in soil cadmium concentration across the studies involved in the meta-analysis. However, this has to be interpreted cautiously, as regional variation in contamination of soil, either by conventional fertilization regimes or possibly pollution, could play an important role in determining the egg cadmium content. The analysis

of the UK egg retail survey (Chapter Four) used quite a high LOQ and found all eggs lower than 0.01 mg/100 g. Yet, it has to be noted that the magnitude of the cadmium residues in other studies was 100 lower than the LOQ. Unfortunately, the lack of testing with a more sensitive limit of quantification is a weakness of this thesis to differentiate between systems however, with all results less than 0.01 mg/100 g, it suggests no safety concerns, regardless of systems. In conclusion, no clear differences in egg cadmium content coming from OR and conventional systems was noticed. With regards to barium, CA eggs were the highest when compared to FR and OR eggs in the UK retail survey. It was also the mineral with the most pronounced effect of management ($p<0.001$). Contrary to these results, FR and OR eggs from Brazil were shown to be higher in barium when compared to eggs from CA systems (Barbosa et al., 2014, Borges et al., 2015). Therefore, it seems that a regional/geological impact linked to the relative soil concentration of these metals should not be excluded. Overall, for egg quality to be stable and controlled, it seems important for the producers to test the concentration levels of both minerals in the soil were FR hens might range.

Calcium, phosphorus, magnesium and zinc

The UK retail survey (Chapter Four) show slightly higher calcium content in CA not statistically different though from OR and EO eggs. Taking into consideration the interaction between vitamin D and calcium metabolism it could be assumed that sun exposure in OR systems and therefore vitamin D increases or ameliorates the egg calcium content (Lips, 2012) in FR systems. However, our results with vitamin D in eggs do not show higher vitamin D in conventional or OR FR systems. Additionally, it is possible that hens in FR systems ingest calcium while picking forage material, invertebrates and soil when outdoors, again depending on the frequency of display of FR behaviours. Overall, results of this thesis show a lower calcium content of conventional FR eggs when compared to CA only. Similarly, for magnesium a weak management effect ($P<0.05$) showed a similar relationship but instead, CA eggs were higher than OR eggs only. No differences were noticed for phosphorus. In all cases, EO eggs were comparable to CA eggs. Takin into account that even if in systems with considerable forage intake the intake of dietary nutrients from the compound feed is diluted, extensive free-ranging behaviour on top of potentially specific management practices in EO can compensate for the controlled feeding regimes of hens in CA systems. Results in the literature are varying greatly and there is no consensus effect of management, while even if any effects are present differences are not large similar to our results.

A weak management effect ($P<0.05$) was noticed for egg zinc content, with OR eggs having slightly (6%) lower concentration than CA eggs and at the same time EO eggs were similar to

CA and conventional FR. Similarly to our results, Zhu et al. (2015) showed CA eggs being higher in zinc compared to eggs from outdoor systems, linked to a respectively higher zinc content in the feed of CA systems. With regards to zinc feed content, even in cases where the feed zinc was similar for different systems, CA eggs still showed higher levels of zinc than eggs from outdoor and OR systems (Giannenas et al., 2009, Kucukyilmaz et al., 2012b). Since cereals can be a good source of zinc (Rangan and Samman, 2012) FR birds consuming forage material could dilute the zinc intake from cereals in their overall diet. Another hypothesis made within these studies was that Zn is used as an antioxidant against stress in FR systems. This could be in line with the fact that many conventional FR birds do not differ much in outdoor behaviour than the CA counterparts and so eggs have zinc content similar to CA. Additionally, range conditions for EO provide more coverage and overall protection for birds than conventional or OR systems and hence potentially less environmental stress. This would explain why EO eggs have similar to CA egg zinc content. However, one could argue that the stress in CA systems could be higher than in FR environments (Bulmer and Gil, 2008). Overall, it seems that there is a trend for OR eggs to be slightly lower than CA eggs, whereas EO do not differ from CA eggs.

Manganese and iron

Neither iron nor manganese egg content were affected by management practices and forage intake, even though OR crops have been shown to be lower in Mn (Baranski et al., 2014). The results here agree with a few studies in the literature showing no difference between OR and conventional -either indoor or outdoor- systems (Barbosa et al., 2014, Giannenas et al., 2009, Kucukyilmaz et al., 2012b, Bargellini et al., 2008, Mizumoto et al., 2008). On the other hand, two studies one in Brazil, one in China, both show eggs from OR and outdoor systems having higher Mn egg content than conventional indoor systems (Borges et al., 2015, Zhu et al., 2015) and the study from Brazil also showed similar results for Fe .With regards to Mn, Zhu et al. (2015) who measured the Mn concentration and showed much higher levels for conventional indoor hens, suggested in the case of environmental pollution, FR layers would uptake the mineral through soil ingestion and potentially be higher than indoor eggs. Another study in Poland showed the exact opposite, meaning OR eggs were lower than CA eggs in Mn (Filipiak–Florkiewicz et al., 2017). The variation in the literature could be linked, as with other minerals, to the potential environmental impact and the relative Mn concentration in soil. Therefore, soil analysis would be important to be able to further explain the data available and control the nutritional egg quality.

Overall, results for egg mineral composition did not reveal any clear management effects but rather suggested that the variation in results within the systems of this study and the results in the literature are potentially linked to the intensity of FR behaviour and the soil mineral concentrations.

6.2.1.2. Effect of season

In mid to higher latitude regions including temperate areas, climates are characterized by significant seasonal variations in temperature and precipitation, influencing both plant growth and potentially bird behaviour. Since the climatic dynamics are associated with the geographical latitude, the effect of seasonal variation in this thesis will be mainly linked to the European type of dynamics. Patterns observed in the extremes (winter and summer) could be extrapolated to other regions as well. The meta-analysis in Chapter Two used season as a moderator to further explain the variation of egg nutritional quality between OR and conventional systems. In the meta-analysis, seasons were considered winter or summer depending on the egg sampling date. Results within each study were considered winter between November and March and summer between April and October for summer (for north hemisphere while the opposite was applied for the southern hemisphere). However, from the studies included in the meta-analysis only one third reported information on sampling season so the impact of season was only available for a few compounds. In the study for Chapter Three, eggs were sampled in winter (January) and summer (July), whereas in Chapter Four, this was expanded to in winter (January), spring (April), summer (July) and autumn (October).

Fatty acids

In the meta-analysis chapter, meaningful analyses looking into differences within seasons, was possible mainly for summer, as for winter there were generally only two studies available for each comparison. In both cases, results were similar to the main results of the meta-analysis; OR being higher in DHA and n-3 FA than FR eggs, while no differences were shown between OR and CA eggs. The only possible comparison within winter was between OR and CA, looking into n-3 FA, where three studies showed a shift from, a positive trend to a negative trend with respect to OR, suggesting that in winter it is likely for OR eggs to be lower in n-3 FA compared to CA eggs. Yet numbers of these studies looking at seasonal variation would be the absolute minimum to draw conclusions. When OR eggs were compared to pooled conventional eggs (both FR and CA eggs), in summer (7 studies) OR eggs were higher both in DHA and n-3 FA, whereas no difference was noticed during winter (5 studies). Overall, the

results suggest an interaction between management and season, with FR eggs being more variable than CA eggs, especially in summer, where they have been shown to be lower than OR eggs in n-3 FA. Similarly to the results of the meta-analysis, the Swiss farm survey (Chapter Three) showed higher DHA and n-3 FA contents of OR eggs compared with FR egg during summer and, also during winter. Therefore, taking these findings together, we would expect OR eggs to have higher n-3 FA content than FR eggs in winter also. However, the main effect of season on DHA, n-3 and total PUFA content ($P<0.01$) revealed a rather unexpected seasonal effect, showing eggs during winter with higher contents of these FA than during summer, yet the differences were numerically small (5%, 3% and 7% respectively). Overall, the hypothesis of higher forage intake and associated FA deposition in eggs during summer could not be confirmed. In line with these results, forage composition and forage material intake reported by Mugnai et al. (2014a), revealed a higher n-3 FA content and consumption in winter rather than in summer. Additionally, Gilani et al. (2014) showed a wider ranging activity in winter rather than in May. Yet, results by Mugnai et al. (2014a) also showed that summer eggs in are higher in DHA but only from EO systems with very large available pasture area, suggesting that in summer there are additional sources of n-3 FA, possibly insects and vertebrates. Alternatively, as the pedo-climatic conditions may vary greatly between regions and years, there is the possibility that the winter pastures might be covered in snow and hence less accessible to hens. This notion might explain the variation in seasonal differences. Furthermore, rather than the seasonal effect *per se*, to further explain the results of chapter 3 it is probably relevant to discuss the potentially confounding effect of flock age. In this study, summer related to older hens with reduced egg production which might explain differences in egg FA composition. Yet, redundancy analysis in Chapter Three showed that age of the flocks is not significantly driving the FA composition, in agreement with the literature (Richards et al., 2011, Anderson, 2011). Contrary to results from the Swiss farm survey, the UK retail survey, did not reveal a seasonal effect for the egg FA composition, neither when years with and without access to pasture (restricted due to avian influenza) were compared. Additionally, no interactions between management and season were shown as expected from the meta-analysis. In parallel, the yolk lipid content was higher in winter (in both Chapters), suggesting not only a greater fat deposition in winter but potentially a significant impact of dietary FA profile on egg FA composition.

With regards to SFA, a lack of seasonal effect was also noticed in both these Chapters. However, it is interesting to mention an interaction for C16:0 between management and season

with less in OR eggs during winter and more during summer, possibly in relation to insect consumption and fatty acid synthesis and elongation (as suggested in Chapter Five).

Overall, no strong effect of season was noticed with the influences of management possibly overruling the impact of season on egg FA composition.

Carotenoids, vitamin A and E

Breitsameter et al. (2014) showed hens prefer pasture swards with softer-leave grass according to their picking behaviour. Mugnai et al. (2014a) measured grass availability and its carotenoid concentration, showing higher availability and carotenoids in winter, followed by spring, then autumn and lastly summer. Additionally, Karsten et al. (2010) showed different nutritional profile for various crops and therefore, both seasonal variation and management of crop rotation of the ranging area could affect the final yolk concentrations.

Meta-analysis results were only very limited, showing OR eggs higher in the sum of lutein and zeaxanthin content than CA eggs during summer, confirming the meta-analysis effect of management; OR being higher than FR and than indoor eggs. No seasonal effect was noticed in the results from the retail supermarket survey. Furthermore, no interactions between management and season were noticed either, suggesting that as for FA, the effect of management is the strongest, overruling any variation due to seasonal differences. Even though not reaching significance, egg content of lutein and zeaxanthin as shown from Chapter Four was the highest in October across the year, in agreement with Mugnai et al. (2014a) who showed the highest lutein and zeaxanthin egg content for spring and autumn within EO systems. With regards to vitamin E, conducting a meta-analysis was possible only comparing OR and CA eggs (both showing wide internal variation) found no difference between management systems neither in winter nor summer. These results are not in line with the UK retail survey, showing no seasonal effect but a difference between OR and CA eggs, with OR eggs having higher vitamin E content. Vitamin A was clearly shown to be affected by access to pasture as eggs from April 2018 had higher contents than April 2017 when all birds were confined to housing. These results come in agreement with Skrivan and Englmaierova (2014) who although showed pasture access raised the carotenoid and vitamin E content in eggs, their concentrations did not show seasonal variation.

Overall, the results of this thesis suggest that the carotenoid intake linked to forage material intake and offered feed intake is stable across management systems, irrespectively of seasons.

Vitamin D

Results of this thesis showed a strong effect of season ($P<0.001$) on total vitamin D content with eggs during July having almost 30% more than eggs in January. Typical duration of sunlight is much longer in summer than in winter. This agrees with studies showing hens exposure to UVB light can increase the egg vitamin D content (Kühn et al., 2015, Kühn et al., 2014). An interaction for total vitamin D_3 was also shown between season and management systems, suggesting that for systems with outdoor access, vitamin D_3 content tends to be numerically lower during winter, especially in EO farms where total vitamin D_3 in eggs is statistically lower. While levels of vitamin D remain consistent in CA eggs, it was clear that in FR eggs there is seasonal variation. This is in agreement with Guo et al. (2017b) who showed larger seasonal differences for both D_3 and 25-OH D_3 egg contents in FR and OR systems than for eggs from indoor systems. Such variation could either come from differences in feed vitamin content between systems - with CA feed having the highest vitamin D content and FR systems compensating by sun exposure at least during summer- or from impaired vitamin D absorption by birds in FR systems, again topped up by sun exposure during summer but unable to fully compensate during winter. In this respect, Sakkas et al. (2019a), showed lower plasma 25-OHD_3 in broilers infected with parasites when compared to healthy birds. Since hens from FR systems are potentially more exposed to parasites and in case of OR systems not receiving coccidiostasts, they maybe more prone to parasitic infections (Wuthijaree et al., 2017). The interaction between management and season could be further investigated if the feed vitamin D content was known and even further, if plasma vitamin D levels were taken. Contrary to our results Guo et al. (2017b), in a UK retail survey, did not show seasonal variation for vitamin D_3 but, in line with our results, did show an effect for 25-OH D_3. These authors also report an interaction for vitamin D_3 between management and season, however, a clear pattern for variation between months was not given.

Overall, results from this thesis, support the hypothesis that hens exposed to sun can enhance their egg vitamin D content, especially during summer months when there is adequate sun exposure. However, it is highlighted that FR systems, especially extensively OR systems could benefit from higher supplementation particularly during winter to keep their egg vitamin D stable.

Vitamin B_2 and B_9

This is the first study reporting seasonal differences in egg vitamin B content between different management systems and seasons. Results show a seasonal impact on egg riboflavin (B_2)

content being 10% higher in July than in January ($P<0.01$). Since B_2 is not only synthesized by microorganism but also by plants, it was assumed that forage intake would actually increase the respective egg content. Yet only a main effect was noticed, while no interaction between systems and season was shown. Concerning egg folate (B_9) content, an interaction between management and season revealed a seasonal impact especially on EO systems ($P<0.05$); the most variable system with a distinctively higher content in summer than in January. Even though eggs from OR systems showed the opposite to EO eggs in their relationship between seasons, the differences within OR throughout the seasons were much smaller. It is evident that similar to vitamin D, EO systems are more vulnerable to seasonal variation than other systems and it seems important to increase supplementation in winter to keep the standard quality stable. Additionally, as no seasonal variation was noticed within CA and FR systems, in which hens are likely to have less or no forage material intake than OR systems, it is concluded that forage itnake must be indeed relevant to egg folate content, as expected since green leafy plants are a good source of B_9 (EFSA et al., 2014).

Overall, results of this thesis can be considered the base for nutritional databases where production methods and seasonal variation are taken into account.

Minerals

The effect of season on mineral content of eggs was very limited. An effect was only noticed for Mn ($P<0.01$) suggesting that in January and April eggs have slightly less Mn, which could be a result of poorer ranging and consumption of soil (potentially a source of Mn) under FR systems. Yet differences are small (at the levels of 0.01 mg/100g egg) and unlikely to have an impact on human health. A weak interaction was also noticed for Mg ($P<0.05$) but again no conclusion can be drawn as no patterns for any of the systems could be extracted and the actual differences were only limited. Overall, no seasonal variation was found for the mineral composition of UK eggs.

6.2.1.3. Effect of genotype on egg fatty acid composition

The effect of genotype on egg fatty acid composition was only looked within the Swiss farm survey. Inclusion in the meta-analysis was not possible because of the lack of data reported on layer genotype. It is therefore recommended that studies reporting compositional analytical results should report hen genotype information, even at the level of white or brown eggs in order to further analyse the available data.

A effect of genotype on some limited FA, including the main SFA C16:0, C18:0 and therefore total SFA, one minor n-3 FA DPA and one other minor PUFA, AA were shown from the farm survey. The results showed brown eggs have less SFA and C16:0, are in agreement with Johansson (2010) who considered similar genotypes to this study's (Lohman) and Anderson (2013) who also looked into brown and white genotypes but with two different commercial layer lines than this study's; Hy-Line and Bovans. Rizzi and Chiericato (2010) who also looked into Hy-Line genotypes, found brown eggs were lower in total SFA but only because they had less C18:0 but not C16:0. These authors also showed a lower arachidonic acid (n-6 FA) proportion in brown egg and at the same time a higher docosopentanoic acid (DPA, n-3 FA), in line with the results of this thesis. As the same elongases and desaturases enzymes are antagonizing for either n-6 or n-3 FA synthesis, the balance of lower arachidonic (an n-6 FA) and slightly but higher DPA content for brown eggs suggest a preference for n-3 FA synthesis by brown genotypes. However, it cannot be excluded that this is a result of an interaction with other management practices including ranging and forage intake.

Indeed, interactions were shown for SFA and n-3 FA between season and genotype. Total proportion of SFA in eggs, possibly driven by C18:0 presenting similar patterns, suggest both brown and white eggs having greater variation within FR systems (rather than OR systems) during winter. Interactions for total n-3, including both ALA and DHA, between season and genotype reveal brown eggs differ between seasons while white eggs are stable in these FA. This pattern possibly elucidates variation in ranging behaviour of hens (Icken et al., 2008), rather than in metabolic capacity for FA synthesis and deposition, explaining why n-3 content of brown eggs is affected more by season than white eggs.

The lack of interactions between management and egg colour genotype suggests that strains used in this study were similarly suited to both conventional and OR FR systems. Lack of interactions between genotypes for brown or white egg and diet previously shown for CA systems (Millet et al., 2006, Bean and Leeson, 2003) also suggest the genetic similarity with respect to FA metabolism between commercially available birds. Yet for some less commercial genotypes used in this thesis, interactions between management in FR environments were shown (Rizzi and Chiericato, 2010). Therefore, it is worthwhile to further investigate whether within FR environments, where possible benefits of forage intake are shown, genotypes that could exploit this management to give a better egg FA profile are used.

Eggs from brown-egg genotypes systematically had lower SFA and higher DPA proportions than white-egg genotypes. Overall, results of this thesis demonstrate that brown genotypes exhibit a higher proportion of n-3 FA and possibly stronger synthesis capacity for long chain

n-3 FA over n-6 FA. It was also shown that hens in FR systems vary more in their intake and deposition of SFA in their yolk irrespective of hen genotype. Yet, during summer the differences in FA composition between systems and genotypes are smaller than in winter, suggesting that ranging behaviour and forage material consumption under the factors investigated is likely to reduce FA variance coming from offered feed.

6.2.1.4. Effect of insect meal on egg fatty acid composition

Under European law, insects and their products to be used in feed are considered as livestock and 'animal by-products' respectively (European Commission Regulation (EC) No. 1069/2009). Since the food crisis and risk associated with spongiform encephalopathies in bovine animals (BSE), production and utilisation of insect based products in livestock production was traditionally blocked by regulatory standards commonly known as "feed ban". Under European Commission (insect-based products are considered as 'Novel Food' for which an application to the European Commission, followed by a scientific evaluation by EFSA, is needed before the product is authorized. Furthermore, at European level, insects may only be fed with materials of vegetal origin.

Results of this thesis support the feasibility to partially replace soybean in poultry feeding regimes by insect meal. Soldier fly (*Hermetia illucens*, HI) larvae, grown on pasta by-products was fed to layers late in their production cycle, replacing half or all of soybean in the diet. Replacing soybean in poultry feeding regimes is an important step towards sustainability of egg and poultry production. Deforestation and high greenhouse gas emissions are some of the negative aspects of current extensive soybean use (Semino et al., 2009, Leinonen et al., 2013). Furthermore, European production of soybean cannot cover the demand neither in the UK nor in Europe and therefore replacement of soybean should help minimize the dependency on imports (Boerema et al., 2016). In recent years, insects have been discussed as an interesting alternative to soybean protein source, mainly because of their high quality protein content (Yi et al., 2013) their feed conversion efficiency (van Huis, 2015) and the potential to grow on food by-products or wastes (Meneguz et al., 2018) while modifying their FA composition depending on the substrate. Since egg FA composition is highly dependent on the dietary FA as previously shown (Fraeye et al., 2012) and also reported in Chapter Three of this thesis, the potential to use insects in poultry feed is additionally discussed from an egg nutritional perspective.

Importantly, it was shown that while modifying egg FA composition there was no difference in egg production rates (Maurer et al., 2016), when soybean is partially substituted by insect meal, grown on such a substrate and fed at 12% (H12) and 24% (H24) of the total diet. From a

nutritional perspective and in line with the results of Secci et al. (2018), egg PUFA proportion was depressed by feeding this insect meal. It is interesting to note that even though the estimated LA intake in this study between the control and the H12 group did not differ, the LA concentration in egg was significantly less in H12 compared to control eggs. Cullere et al. (2017) also showed lower egg PUFA and hypothesized a greater Δ-9 desaturase activity in insect supplemented birds compared with control diets. Since Δ-9 desaturase's preferred substrates are C16 and C18 (rather than MUFA or PFA), the fact that C16:1 and c11C18:1 are higher in eggs from insect fed layers support this hypothesis. With regards to n-3 FA, even though dietary intakes did not differ greatly when soybean was completely replaced, lower egg n-3 FA was noticed. Even when long chain n-3 FA intakes were higher in high insect meal diets, respective proportion in eggs was lower. This conflicts with results from Chapter Three, showing dietary n-3 FA as an important driver for egg n-3 FA profile and it might suggest some type of inhibited enzyme activity or impaired transfer efficiency of these FA in the yolk. Yet, when soya was only partially replaced, n-3 FA were similar to control diet, in line with Secci et al. (2018).

With regards to SFA, eggs from layers fed insect meal had higher C16:0 and total SFA. This pattern is in agreement with studies showing higher SFA in chicken meat after being fed HI (Cullere et al., 2017, Schiavone et al., 2017). The only study looking into egg FA composition after HI was fed to layers, cannot be used as comparison since, unlike this study, no significant difference in SFA intake was measured between diets (Secci et al., 2018). In this study, in line with the results of Cullere et al. (2017) although the C16:0 intake was similar between diets, the proportion was higher in eggs from insect fed layers, suggesting higher FA elongase activity in hens when fed diets rich in C12:0 and/or C14:0.

This study did not consider potential contaminants transferred into eggs but insects might be a source of chemical or other contaminants. Therefore, although the use of insect meal in feed is advised it ought to be produced with strict manufacturing and product quality procedures in place. A high transfer capacity of cadmium from contaminated growing substrate into HI larvae then into eggs has previously been documented (Purschke et al., 2017, Biancarosa et al., 2018). Since OR crops have been shown to have lower cadmium load (Baranski et al., 2014), substrate for insect growing could be more beneficial if coming from OR crops, both for the insect production and the egg nutritional quality. EFSA scientific opinion (EFSA, 2015) on the risk profile related to production and consumption of insects as food and feed concluded that when allowed feed materials are used as substrates, the possible occurrence of contaminants is not

expected to exceed the standards. Yet uncertainties surrounding insect meal production due to the luck of knowledge and collected data make imperative the data collection or generation.

Overall, results of this study suggest that completely replacing soybean in layers' diets with 24% insect meal grown on pasta by-products has a negative impact on egg lipid profile, with less PUFA and n-3 FA but also more SFA. Although the insect growth substrate of pasta-waste proved sufficient to keep the n-3 FA stable in the intermediate layer diet, lower PUFA and higher SFA in eggs were also shown. Further research on alternative insect growing substrates with enhanced FA profile should be considered for the chain of egg production. Finally, since the layers of this study did not have access to pasture, it would be interesting to see the interactions between the offered insect meal with forage and live invertebrate consumption.

The recent EU Regulation 2017/893, allows seven insect species including soldier fly, HI to be included in the formulation of feeds, so far only for aquaculture. However, the road for including insects in monogastric animal nutrition is under consideration and may not be long before insect meal is allowed. Therefore, studies investigating the substrate and insect species combination that would better suit egg production and egg nutritional quality are needed.

6.2.2. Implications for human health

Egg intake can contribute to human intake of several essential nutrients and antioxidants. Apart from the contributions of these compounds to specific metabolic functions or the protection against the development of age-related macular degeneration, their role in balancing oxidative damage and contributing to antioxidant defence in the human body may be of importance (Andersen, 2015). Therefore, increasing the intake of antioxidants, vitamins and nutrients through egg consumption may potentially enhance the nutritional capacity of the human body. According to Public Health England and the results from the National Diet and Nutrition Survey (Public Health England, 2016, Public Health England, 2018), in the UK an increase in nutrient intake depending on the population group should be considered as positive as the intake of many groups were below the lower reference nutrient intake.

Eggs, apart from being rich in fat soluble vitamins and essential fatty acids, are also rich in SFA. In this respect, nutritional recommendations are to reduce SFA consumption, and ideally substitute dietary SFA with PUFA or MUFA (EFSA, 2010b). Therefore, the intake of nutrients which have a positive impact on health should be discussed in line with the total SFA and especially C16:0 content of eggs. Additionally, egg consumption has been traditionally negatively perceived. The evidence on the association of egg consumption with CVD has been largely based on the high cholesterol content of eggs. Egg consumption has previously been

linked to increased risk of coronary heart disease because of the increase of ratio of total to HDL-cholesterol concentrations in people with high egg consumption (Weggemans et al., 2001). Dietary cholesterol is now known to be relatively less important to overall cholesterol blood concentrations, yet depending on multiple factors like gender (Vincent et al., 2018), which is more strongly related to SFA intake which promotes synthesis of cholesterol in the liver (Soliman, 2018). In addition, more recent evidence generally questions the relationship between egg consumption and CVD (Zazpe et al., 2011, Dehghan et al., 2020), especially among healthy individuals (Guo et al., 2017a, Geiker et al., 2018). Consequently, egg consumption and the overall potential benefit to human health should be promoted despite the high cholesterol yolk content.

Most of the nutrients concentrations researched in the framework of this thesis and in particularly within the UK retail study (Chapter Four), agree well with the composition profile of eggs reported in the McCance and Widdowson's Composition of Foods Dataset (Public Health England, 2015). Exceptions include vitamins B_2 and B_9 which were found in lower concentrations than in the aforementioned report and further research to update and confirm actual levels in the UK egg market is be required.

In the context of this thesis, significant differences for the majority of nutrients were shown between management systems. Therefore, implications to human health because of variation in egg nutritional profile, will be principally compared between management systems. However, where the main effect was other than the overall management effect, or where the most significant effect was noticed within an interaction, implications of these differences will be also discussed. The relative differences in daily nutrient intakes will be discussed in relation to the overall contribution of consuming eggs to the dietary reference values (DRVs) for the relevant nutrients set at European level by EFSA.

With respect to calculating nutrient intakes, egg concentration for respective nutrients will be taken from Chapter Four results as these represent the most recent and representative compositional profiles for eggs in the UK market. With regards to the population group of interest, adults (18-65 years old) as well as young children (4-10 years old) will be discussed. Very young children (1.5-3 years old) will be also considered. With regards egg intake, to better reflect the potential impact of egg choice, the contribution to DRVs will be examined with respect to consumption of one egg per day (sum of albumen and yolk weight) from each system per day. Egg consumption figures from the National Diet and Nutrition Survey (NDNS) (Bates et al., 2014) will also be used but only for the calculation of intake of the contaminant barium.

Overall, intakes of individual compounds were estimated as:

- FA intake (g/d) = Egg weight (g/day) × egg yolk lipid content (g fat/100 g egg) × egg FA concentration (% of total FA)/100 × 0.83 (correction factor representing % of FA in total yolk fat) (Posati et al., 1975) × correction factor to express yolk as egg edible weight (yolk weight/yolk + albumen weight[1])
- Vitamin and mineral intake (g/d) = Egg weight (g/day) × egg vitamin concentration (g/g egg) × correction factor to express yolk as egg edible (yolk weight/yolk + albumen weight[1]) for vitamins A, E and D

Even though egg consumption is on the rise, the calculation method used is considering the differences of the consumption of one whole egg coming from various systems. Ideally, the relative value of organic eggs compared with those from a conventional system could be assessed by examining the relative contribution in a whole diet situation. The limitation to approach the absolute chronic intake and impact of the relative differences is therefore acknowledged. Indeed, children and especially young children would consume much less of an egg compared to adults. According to NDNS (Bates et al., 2014) UK egg consumption for young children it is 9 g egg/day, for children 4-10 years old 10g egg/day and for adults 20g egg/day. Therefore, it is possible that using the above methods will result in overestimating the impact for these population groups in case of significant relative differences. However, using the method described above a clear idea of the potential impact of consuming eggs from different systems can be given especially in case of high egg consumers.

The table below (Table 6.1) shows the contribution to nutrient intakes (DRV) of consuming one egg per day, coming from CA, FR , OR or EO systems, for each age group, while in the case of barium the contribution to the WHO health guidance value (WHO, 2004) taking into account the egg intake per age group. Default values for human body weight were used as suggested by EFSA (2012b) (adults: 70kg, children 1-3 years old: 12kg and children 4-10 years old: 23 kg).

Differences in DHA egg concentration depending on the production system potentially could have a significant impact on human health. Consuming one OR egg, from either OR or EO over conventional, either FR or CA, would add about 8% to the AI. Since the recommended value is the same across age groups, no differences across age groups were noticed. On the contrary, even though the egg concentrations of both vitamin A and E were always higher in OR eggs when compared with eggs coming from CA systems, the differences in the contribution to the

[1] The specific values of yolk and albumen weight for each treatment were used (Table 4.2).

respective DRVs, were minimal, especially for adults. Within the younger age groups choosing OR eggs over CA eggs, would contribute an extra 2-3% to the respective PRIs. Given the critical development and need for nutrients in these stages of life, it could be argued that even this small systematic difference could have a long term impact on health. As far as mineral intake is concerned, no differences greater than 1% between eggs of different management systems were detected. Therefore, it can be concluded that choosing OR over conventional eggs would have no measurable impact on contribution to DRVs for the minerals reported. As far as the contaminant barium is concerned, the choice of OR over conventional eggs does not have an impact in any population groups.

Concerning the impact of egg choice on SFA, having in mind that the recommendations for the respective intake is to keep this as low as possible EFSA (2010c) and looking at the actual intake of C16:0 (g) (results not shown), there was no difference even if there was an effect of management on egg SFA content. Finally, even if there are currently no recommended dietary values for carotenoids and specifically for lutein and zeaxanthin, the significantly higher amounts found in eggs from OR over conventional production systems, especially for EO eggs, make them a better choice for consumers especially in the older age groups where benefits for delayed macular degeneration has been shown (Abdel-Aal et al., 2013).

As already discussed, season had a greater impact on some nutrients than management. Results of this thesis can be considered the base where nutritional recommendations should take into account variation within agricultural management or seasonal variation. With regards to vitamin B_9, the interaction between management and season on egg concentrations shows a potential impact on human health for the younger age groups only. While in July, it is better to consume EO eggs, in winter time such consumption will be disadvantageous. This observation could have an impact on dietary recommendations as previously reported that in winter human serum folate levels are lower than those in summer or autumn, especially in non-vegetarians (Krajcovicova-Kudlackova et al., 2013).

Table 6.1. Contribution of dietary reference values from daily consumption of 1 egg per day from different production systems (caged (CA), free-range (FR), organic (OR) and extensive organic (EO).

	%Contribution of one egg per day to dietary reference values (DRVs) per age group											
	1.5-3 years old				4-10 years old				19-64 years old			
Compounds and DRVs [a]	CA	FR	OR	EO	CA	FR	OR	EO	CA	FR	OR	EO
DHA % AI (250 mg/day), both genders	14%	14%	23%	21%	14%	14%	23%	21%	14%	14%	23%	21%
vitamin A %PRI (250, 400, 750 µg/day), male [b]	21%	23%	24%	24%	13%	15%	15%	15%	7%	8%	8%	8%
vitamin A %PRI (250, 400, 650 µg/day), female	21%	23%	24%	24%	13%	15%	15%	15%	8%	9%	9%	9%
vitamin E % AI (6, 9, 13 mg/day), male	2%	4%	5%	4%	2%	4%	5%	4%	2%	2%	3%	3%
vitamin E % AI (6, 9, 11 mg/day), female	2%	4%	5%	4%	2%	4%	5%	4%	2%	3%	4%	4%
B_9 % PRI (120, 200, 330 µg/day), both genders, July [b]	13%	14%	14%	18%	8%	8%	8%	11%	5%	5%	5%	7%
B_9 % PRI (120, 200, 330 µg/day), both genders, January [b]	13%	13%	17%	8%	8%	8%	10%	5%	5%	5%	6%	3%
Calcium (Ca) % PRI (450, 800, 1000 mg/day), both genders	7%	6%	6%	7%	4%	3%	4%	4%	3%	3%	3%	3%
Magnesium (Mg) % AI (170, 230, 350/day), male	3%	3%	3%	3%	3%	3%	3%	3%	2%	2%	2%	2%
Magnesium (Mg) % AI (170, 230, 300/day), female	3%	3%	3%	3%	3%	3%	3%	3%	3%	2%	2%	3%
Zinc (Zn) %PRI (4.3, 7.4, 14 -LPI 900 mg/day), male	17%	15%	16%	17%	10%	9%	9%	10%	5%	5%	5%	5%
Zinc (Zn) %PRI (4.3, 7.4, 11 -LPI 900 mg/day), female	17%	15%	16%	17%	10%	9%	9%	10%	6%	6%	6%	7%
	% Contribution of relative chronic egg intake to health guidance values											
Barium (Ba) % TDI (5 µg/kg of body weight), both genders	3%	2%	2%	2%	2%	1%	1%	1%	1%	1%	1%	1%

[a] Docosahexaenoic acid (DHA), population reference intake (PRI), adequate intake (AI), levels of phytate intake (LPI), tolerable daily intake (TDI) per population group when different

[b] for the age group of 4-10 years old, DRVs for 7-10 years old were used

Vitamin D_3 and B_2 were affected by seasonal variation. Table 6.2. shows the effect of season on the egg vitamin D_3 and B_2 intake with respect to their relative contribution to DRVs after consumption of one egg per day.

Table 6. 2. The effect of season on intake of vitamin D_3 and B_2 and contribution towards dietary reference values from daily consumption of 1 egg from different production systems.

Age Group				
Compounds and DRVs [a]	1.5-3 years	4-6 years	7-10 years	19-64 years
D_3 % AI (15 μg/day), July	15%	15%	15%	15%
D_3 % AI (15 μg/day), January	12%	12%	12%	12%
B_2 % PRI (0.6, 0.7, 1, 1.6 mg/day), July	38%	33%	23%	14%
B_2 % PRI (0.6, 0.7, 1, 1.6 mg/day), January	34%	29%	21%	13%

[a] Population reference intake (PRI), adequate intake (AI), considering both genders per population group when different

For both vitamins, eggs during July would contribute more to the respective DRVs, although the differences are small at less than 5 percentage units. However, in the case of B_2 such a difference was only relevant for younger group ages, while for adults contribution were very similar. Overall, no meaningful differences could be generalized between summer and winter eggs on vitamin D_3 and B_2 intakes. It should be mentioned however that for B_9, vitamin D_3 within EO, eggs were much lower in winter than in summer and this should be kept in mind, especially when population Vitamin D supplementation could be even more important during winter, when sun exposure is lowest at higher latitudes (Hayes et al., 2016).

It should be noted that for nutrients such as carotenoids, vitamins A and E, as well as vitamin D, storage and/or cooking might affect their contents in eggs, hence, the actual intake per egg might be lower than considered here. Concerning vitamin D, Mattila et al. (1999b) looking into storage at room temperature and cooking (boiling), showed only minor loses of vitamin D_3 compounds (<10%). On the other hand, Jakobsen and Knuthsen (2014) reported slightly higher losses for boiling, but higher for other methods of cooking. Results from the nutrient analysis in eggs (Public Health England, 2015) showed only losses in fried eggs. Concerning vitamin E, no effect of storage on lipid stability in fresh and stored eggs was noticed (Franchini et al., 2002). Skrivan and Englmaierova (2014), showed that the oxidative stability of egg yolks coming from hens with access to pasture increased, compared to eggs from hens with no access to pasture, possibly due to the combined effect of α-tocopherol and carotenoids present in the

pasture. In parallel, supplementing the diets of hens with α-tocopherol has been shown to increase the antioxidant stability of n-3 rich eggs (Galobart et al., 2001, Cherian et al., 1996), suggesting that eggs rich in vitamin E, in this case OR over conventional eggs, would better protect the overall oxidant stability. In this respect, whereas no storage effect on egg carotenoid content has been reported, cooking has been reported to decrease the carotenoid egg content (Nimalaratne et al., 2016). As far as water soluble vitamins, B_9 in eggs has been reported to be stable after cooking (Bassett and Samman, 2010). On the contrary results from the egg Nutrient analysis (Public Health England, 2015) showed a slight negative effect of cooking on vitamin B_9 (either boiling or frying) but in general no other significant losses (except vitamin D in fried eggs). Overall, cooking processes should be considered and standardized if the optimum intake of egg nutrients is to be included when discussing nutrient recommendations through egg consumption.

As a conclusion, choosing OR over conventional eggs would systematically contribute more to the health recommendations of increased omega-3 FA, DHA consumption, while no difference would occur with respect to SFA intake. Furthermore, choosing OR over eggs from CA birds could potentially have slight benefits to achieving vitamin A and E intake recommendations in young children, while they would systematically offer more carotenoids, being especially beneficial for older age groups. Seasonal variations in egg D_3 and B_2 were not shown to have a large impact on human health, but because of the observed variations, potentially higher contributions of these nutrients could be observed in summer (compared with winter), especially where young children are concerned.

6.3 FUTURE DIRECTIONS

The thesis presented provides novel findings in the field of production management systems and egg quality. Nonetheless, there are points to be considered and future directions to be taken that are briefly presented below.

Chapter Two, the meta-analysis showed that OR compared with conventional FR eggs were higher in n-3 FA. However, when OR were compared with eggs from indoor systems, no such relationship was established. According to the main hypotheses that the increasing free-range behaviour and forage intake in OR systems is responsible for higher n-3 FA, OR eggs should also be higher in omega-3 FA than eggs from CA systems, in accordance with the results of the retail egg survey (Chapter Four). Therefore, the results between OR and indoor eggs within a meta-analysis framework suggest that there is the need for further investigation. Meta-analysis within a Bayesian network would be able to look the inter-relationships between all systems and not separately to each conventional system but only if necessary information is reported. In general, we need to improve the quality of studies as well as reporting production systems details for any work considering the content of nutrients of interest to human health in eggs. Developing recommendations for conducting and reporting comparative studies on food compositional parameters to minimise heterogeneity should be proposed at a global level to ensure the quality of future studies generated.

Chapter Three looked into the effect of genotype on FA composition and did not find large differences between commercial brown and white egg layers. However, it has been shown that as far as carotenoids are concerned, genotype can have a significant impact, especially in OR egg production systems (Bunea et al., 2017). Therefore, further research could be initiated on identifying and establishing layer genotypes that could have higher egg nutritional content with similar egg production and economic standards than the ones currently used.

Chapter Four showed variation between winter and summer affects egg concentration of vitamins D_3, B_2, and B_9. First of all, as this was one of the very few studies looking into seasonal variation of D_3 and the first one of vitamin B in eggs, it would be interesting that more comparative studies are generated to confirm and validate the results. Secondly, to fully elucidate the seasonal effect it would be crucial to look across all seasons. In parallel, for vitamins D_3 and B_9 it was shown that EO systems were especially affected by seasonal variation. Therefore, efforts to standardize the nutritional quality between seasons and especially within the most affected systems, would be necessary if nutritional recommendations

would propose consuming eggs from various egg production systems. However, we could as well accept that natural variation may exist, especially in production systems like EO, and it could be preferably at consumer levels where adaptation could be suggested, meaning consumers tailoring their diet or general nutrient supplementation levels according to seasonal concentrations of eggs. Furthermore, the regulatory framework regulating the approval of feedstuffs suitable for OR livestock production will ban using B_2 produced by genetically modified organisms, the standard up-to-date. Therefore, studies investigating the ability of alternative feedstuff to contribute to OR diets are warranted.

Furthermore, egg could be an important source of iodine and selenium (Gray, 2018), although low bioavailability especially for iodine has been reported for these nutrients (Lipiec et al., 2012). As this thesis did not look into the respective concentrations and since it has been previously shown that management could affect the respective concentrations (Bargellini et al., 2008), also comparative studies looking into seasonal variation should be considered for iodine and selenium. Additionally, further analyses of heavy metal concentrations between systems should be carried out to generate a more complete the nutritional profile of eggs for comparison between production systems. In parallel, within FR systems, since many of heavy metals and more generally minerals in eggs depend on the background levels of the respective compounds in feed and soil, future studies could exploit soil analysis data and correlate them with the relative egg concentration levels in order to understand the sources but also predict the respective egg levels. Finally, within the framework of precision livestock farming (Wathes et al., 2008), it could be interesting to model the effects of management and seasonal variations, including the available soil mineral concentrations and/or forage intake, in order to tailor the nutritional needs of the flocks.

Chapter Five which examined the impact of replacing soybean meal with the alternative protein source insect meal established that it is viable from a production perspective to feed *Hermetia illucens* meal that has been fed on pasta waste. However, from the literature, the nutritional egg composition has greater potential to be enhanced, by exploiting the substrate itself as a means of dietary modification of the farmed insects (Barroso et al., 2017). Moreover, insect meal should be also investigated as the source of soon-to be withdrawn B_2 in OR production systems. If the substrate is also industrially produced food waste that could go into the food chain, that would be a very important step towards improving sustainability in the poultry industry. It is important to have in mind that a gap in risk assessment data on the safety of using insects as feed exists and therefore a bottleneck for current commercialization – not to mention the block by legislation. However, it has been concluded that both biological and chemical hazards in the

end product of an-insect-produced food, would depend on the specific production methods, the substrate used, the stage of harvest, and the insect species amongst others (EFSA, 2015). Therefore, the standardization of the nutritional quality but also chemical safety of the substrate used to grow the insects to be fed is an area of very interesting research.

6.4 GENERAL CONCLUSIONS

- This thesis provides evidence that management and to a lesser extent season and genotype can affect the egg nutritional quality.
- It also provides evidence that nutritional benefits of OR agriculture, are associated with increased levels of omega-3 fatty acids and carotenoids in eggs. It further shows seasonal variation in vitamin D_3, B_2 and B_9, is especially affecting extensively OR systems in case of D_3 and B_9, suggesting that if stable qualities are needed, winter flock supplementation would be beneficial.
- In addition it provides evidence that insect meal (*Hermetia illucens*) grown on industrial wheat-based pasta waste can partially substitute soybean meal without negatively affecting egg production. However, the egg fatty acid composition was partially compromised with higher saturated and less polyunsaturated FA in the experimental than the control eggs.
- Overall, this thesis contributes to the experimental evidence that management of egg production systems affect the nutritional quality. It further suggests that choosing OR over conventional eggs could have a positive impact to human health by substantially contributing more to DHA, vitamin A and E recommended intakes.

Future studies of standardized high quality should correlate soil and vegetation mineral concentrations with egg composition for birds with access to outdoor ranges. New comparative studies should be generated reporting differences in water soluble vitamins and heavy metals. Additionally, regarding alternative systems, further alternative sources of insect-growing substrates to be used as feed in egg production systems, should tested in relation to the final egg nutritional composition.

Results of this thesis can be considered crucial when mapping the nutritional value of food and food systems. The results are also of interest to the producers, as any variation in the egg quality is relevant to the final quality of their product and its value.

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APPENDICES

APPENDIX A.1

Questionnaire of farms and flocks included in the farm survey

Date of visit

Weather (air temperature, precipitation)

Visit done by:

Genotype

Age at visit

Rearing

Rearing system

Outside access yes / no

Laying-period

Date of placement layer house

Age at placement

Hens been locked up inside system yes/no

Hens been locked up inside system how many days?

Flock size

Group size

Housing system: ground stable / aviairies / cage

Supplements water

Supplements feed

Management during lay

Time popholes open

Daily outside access yes/no

max % hens seen outside ever of this flock

Roughage fed yes / no

Roughage fed type

Roughage fed daily / weekly / monthly / irregular

Roughage fed gram /hen/ day

Litter at arrival of hens in laying farm yes/no

Type of litter at arrival of hens in laying

Litter added daily / weekly / monthly / irregular

Additional grains given in litter area yes/no

Additional grains given in litter area amount/hen/day

Additional stones given in litter area yes/no

Additional stones given in litter area amount/hen/day

Additional shells given in litter area yes/no

Additional shells given in litter area amount/hen/day

Performance current flock

Number of eggs per placed hen until 60 wk

floor eggs at age of farm visit

Feed intake per hen until 60 weeks in grams

Method of moulting

Outside area

Description of the outdoor area

Impression of the use of the outdoor area (only around stable/ half of the surface/ whole surface)

APPENDIX A.2

Effect of management practices on fatty acid composition of chicken eggs: a farm survey in the Netherlands

Implications OR management of hens was associated with a slightly better egg fatty acid (FA) profile than FR management. Outdoor roaming of layers may increase omega-3 FA (n-3) concentrations in eggs from both systems.

Introduction Eggs' n-3 content has been shown to vary across different management practices although results are rather contradictory (e.g. Rizzi and Marangon, 2012), However, increasing dietary n-3 is considered desirable for human health (Simopoulos, 2002). The influence of management practices and their interactions on egg FA composition need further investigation, especially in FR conditions where n-3, α-linolenic, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) could be enhanced by higher forage intakes. This study investigated the effect of different management practices on egg FA profile from FR and OR flocks in the Netherlands (NL).

Material and methods Eggs were collected between August 2012 and June 2013 from FR (FR, n=10) and OR (OR, n=12) flocks in the NL comprised of differing hen genotypes: 5 Lohmann Brown Lite (LBL), 6 Lohmann Brown Classic (LBC), 4 Hyline Silver, 3 Dekalb White, and 4 flocks of other hen genotypes. Eggs (n=25 per flock) were randomly collected, pooling 5 yolks to yield 5 replicates per flock. Analysis of FA methyl esters (FAME) was carried out by gas chromatography using an Agilent CP-Sil 88 column (100m x 0.25mmID x 0.20µm film thickness). Peaks were identified using a 52 FAME external standard. Two separate statistical analyses, using either a subset (analysis of variance, by linear mixed models) or the complete data set (multivariate redundancy analysis, RDA), were performed. Analysis of variance was carried out in R statistical software, using management (FR, n=6 flocks, OR n=5 flocks) and genotype (LBC, LBL) as fixed factors and flock as a random factor. Each management system was represented by 3 LBC and 3 LBL flocks (only 2 for OR). The RDA using the CANOCO package assessed the influence of other management practices [age of layers in weeks (AGE), roughage intakes (Roughage), percentage bare soil in the range area (BareSoil) and proportion of hens roaming outdoors (HensRoam)] on egg FA profile.

Results OR eggs had less C16:0, oleic acid (OA) and monounsaturated FA (MUFA) but more linoleic acid (LA), αlinolenic (ALA), PUFA, n-6 and n-3 than FR eggs (Figure 1a). Management had no impact on EPA and saturated FA (SFA) content or the n-6:n-3 ratio, but

OR eggs had more DPA and DHA (results not shown). Genotype did not influence the FA profile and had no interaction with management. The RDA (Figure 1b) indicated that older layers were associated with more OA, C16:0 and MUFA. Contents of PUFA, n-6 FA and LA were positively affected by roughage in the diet. Higher percentage of layers roaming outside, possibly resulting in a more bare soil, was positively associated with ALA, DPA and n-3 and negatively with n-6:n-3 ratio. Management practices were only weakly associated with EPA, DHA and SFA.

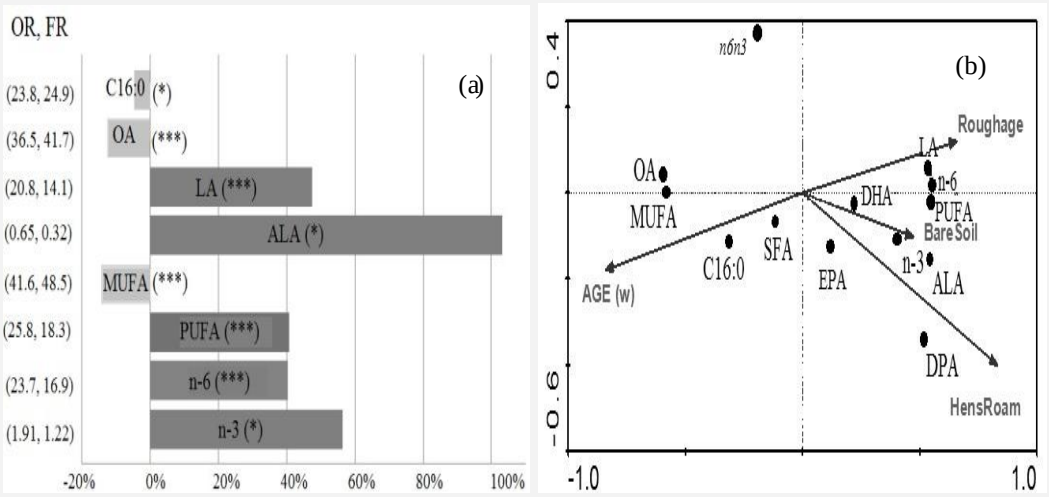

Figure Appendix A2 1. (a) Means (% of total FA) and differences in relative proportions (%) of individual FA and FA groups in eggs from OR compared with FR management. Significances were declared at ***P<0.001, *: P<0.05 (b) Biplot from RDA showing the relationship between management practices (drivers as arrows) and egg FA profile (response variables as dots)

Conclusion While genotype had no impact on egg FA profile, OR eggs had higher levels of nutritionally beneficial n-3. However, management did not influence the less desirable SFA or n-6:n-3 ratio. Outdoor roaming was positively associated with higher n-3 and negatively to n-6:n-3 ratio, highlighting the potential desirable impact of pasture on egg FA profile.

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APPENDIX B.1

B.1.1 STUDY INFORMATION FORM

All information extracted from each study should be copied into an electronic spreadsheet

Study information

Study ID:

Source:

Publication Year:

Author:

Author contact details:

Type of publishing:

□ Published □ Unpublished

If Published:

□ Peer reviewed □ Non peer reviewed

Type of study:

□ Book □ Technical report □Conference paper □ Thesis □ Journal □ Other

Type of study research:

□ Basket study □ Farm Survey □ Experimental Trials

Study characteristics

Objective:

Study design:

OR certification:

□ Yes □ No

If yes, body of certification:

Type of control (s):

□ CA □ Furnished cages □ Barn □ FR □ other

Breed:

Age at sampling:

Flock size:

Location:

Month:

Type of OR (s):

□ OR □ Biodynamic □ other

Breed:

Age at sampling:

Flock size:

Location:

Month:

Feed composition data:

□ Yes □ No

Sample Information

Unit:

□ Whole egg □ Egg yolk □ Egg white

Pooled samples:

□ Yes □ No

If yes, how many were pooled:

In which form was the egg analysed:

□ Raw □ Freeze-dried □ other

Nutritional composition variables assessed in the study (specify which particular in parentheses):

□ Fatty acid composition

□ Carotenoids □Lutein □Zeaxanthin □Lutein/Zeaxanthin □Other

□ Tocopherols

□ Minerals

□ Vitamin B complex

□ Cholesterol

□ Dioxins

□ Protein Content (□on whole egg □ on egg yolk □on egg white)

□ Other

Other measurements

□ White % □ Yolk % □ Shell %

□ Albumen/Yolk ratio

□ Egg weight □ Yolk weight

□ Shell strength

□ Haugh Units

□ Dry Matter

□ Other

Effect size information

Sample size, mean, measure of variation and page of each outcome should be copied into the electronic dataset.

Inclusion criteria: □ All met □ Other

Exclusion criteria: □ None □ Other

B.1.2 QUALITY ASSESSMENT FORM

Type of study:

□Basket survey □Farm Survey □Experimental Trial □Other

Research Objectives

Is the research aiming to explore the compositional differences in eggs due to OR and conventional management?

□ Yes □ No

Baseline confounding

Is it published or not?

□ Yes □ No Add 1 point if 'no' is chosen

If yes, is it peer reviewed?

□ Yes □ No Add 1 point if 'no' is chosen

Treatment confounding

Was the sampling design balanced between OR and conventional samples?

□ Yes □ No Add 1 point if 'no' is chosen

Has the experimental design taken into consideration and balance any potential moderators (e.g. genotype)? Not applicable for basket studies

□ Yes □ No

Was there an OR certification (only for farm surveys and experimental trials)?

□Yes □ No

If you chose 'No', was it stated that OR practices were used?

□ Yes □ No □ Other Add 1 point if 'no' is chosen

Outcome measurements

Were the samples properly stored until analyses and are the methods of analyses appropriate and described explicitly (or citing the relevant reference)?

□ Yes □ No Add 1 point if ‘no’ is chosen

Are the result units clearly displayed (e.g. g/per egg or g/ per yolk)

□ Yes □ No Add 1 point if ‘no’ is chosen

Selective reporting

Were there any values/outliers excluded from the analysis?

□ Yes □ No

If yes, was it justified?

□ Yes □ No Add 1 point if ‘no’ is chosen

Are the data to be used for the effect size calculation explicitly reported (mean value, measure of variability, sample size)

□ Yes □ No

If you chose ‘No’, have the raw values been retrieved form the authors?

□ Yes □ No Add 1 point if ‘no’ is chosen

Table Appendix B.1.1. Overall, score of quality assessment for each individual study

Risk of bias categories	**Points**		
Baseline confounding /2			
Treatment confounding /3			
Reliable outcome measurements /2			
Selective reporting /2			
	Yes	No	Unclear
Overall, risk of bias			

Each point is giving a positive outcome. A study will be considered as of “biased” and eventually of lower quality when risk of bias is either positive or unclear for at least one of “baseline confounding”, “treatment confounding” and “selective reporting” or negative for “reliable outcome measurements”.

APPENDIX B.2

Calculations for weighted meta-analyses

Calculations used are as described by Średnicka-Tober et al. (2016). The standardized mean difference (SMD) from a single study will be calculated in random-effect model using standard formulas within "metafor" as follows:

$$SMD = \frac{\bar{X}_O - \bar{X}_C}{S_{within}} \times J$$

where $\bar{X}o$ is the mean value for experimental group (OR), $\bar{X}_C$ is the mean value for control group (conventional), S_{within} is the pooled standard deviation of the two groups, and J is a factor used to correct for small sample size. J is calculated as:

$$J = 1 - \frac{3}{4 \times (n_C + n_O - 2) - 1}$$

where n_O and n_C are OR and conventional sample sizes.

S_{within} is calculated as:

$$S_{within} = \sqrt{\frac{(n_O - 1)S_O^2 + (n_C - 1)S_C^2}{n_O + n_C - 2}}$$

where S_O and S_C are the standard deviations in individual systems (OR and conventional) respectively.

The pooled SMD (SMD_{tot}) across all studies was calculated as:

$$SMD_{tot} = \frac{\sum_{i=1}^{n}(\frac{1}{v_i} \times SMD_i)}{\sum_{i=1}^{n}(\frac{1}{v_i})}$$

Where v_i is a sampling variance estimated as:

$$v_i = \frac{n_C + n_O}{n_C \times n_O} + \frac{SMD^2}{2 \times (n_C + n_O)}$$

The pooled or summary effect (SMD_{tot}) was calculated for all nutrient compounds reported in a minimum of 3 studies, following procedures advocated by Lipsey and Wilson (2001b).

Calculations for summing means and standard deviations of two independent variables

$\mu_{X+Y} = \mu_X + \mu_Y$, $\sigma_{X+Y} = \sqrt{{\sigma_X}^2 + {\sigma_Y}^2}$, Where μ is the average and σ the sampling variance

APPENDIX B.3

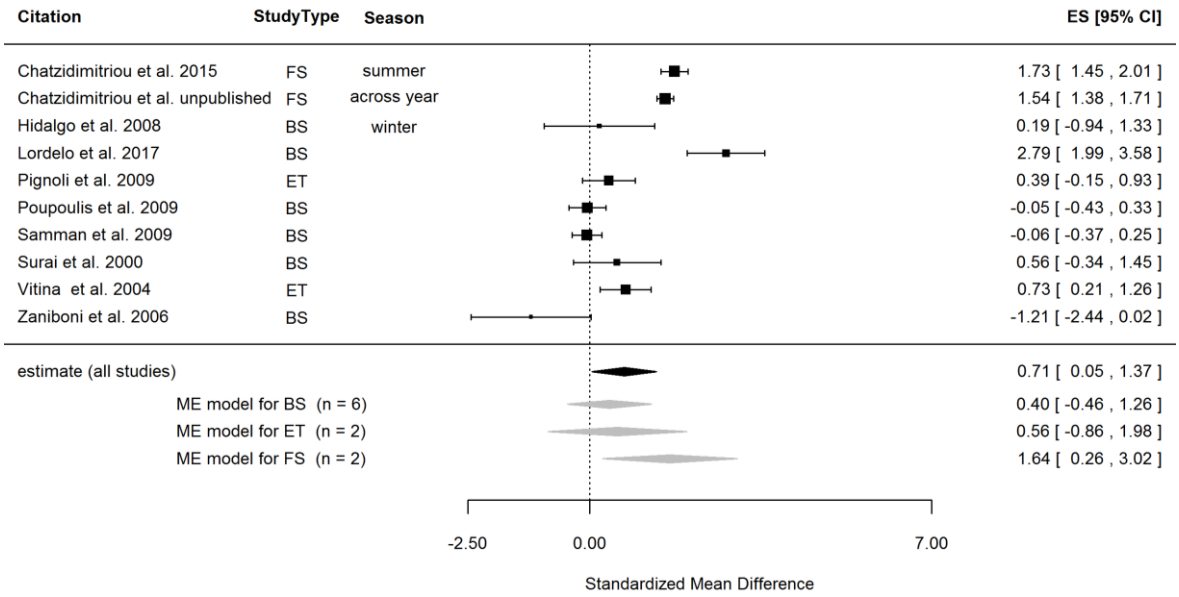


Figure APPENDIX B.3. 1. Forrest plot of standardised mean differences (SMD) in docosahexaenoic acid (DHA) content in OR compared with eggs from conventional FR systems, including study type as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=95%; P value (Q statistics) <0.001.

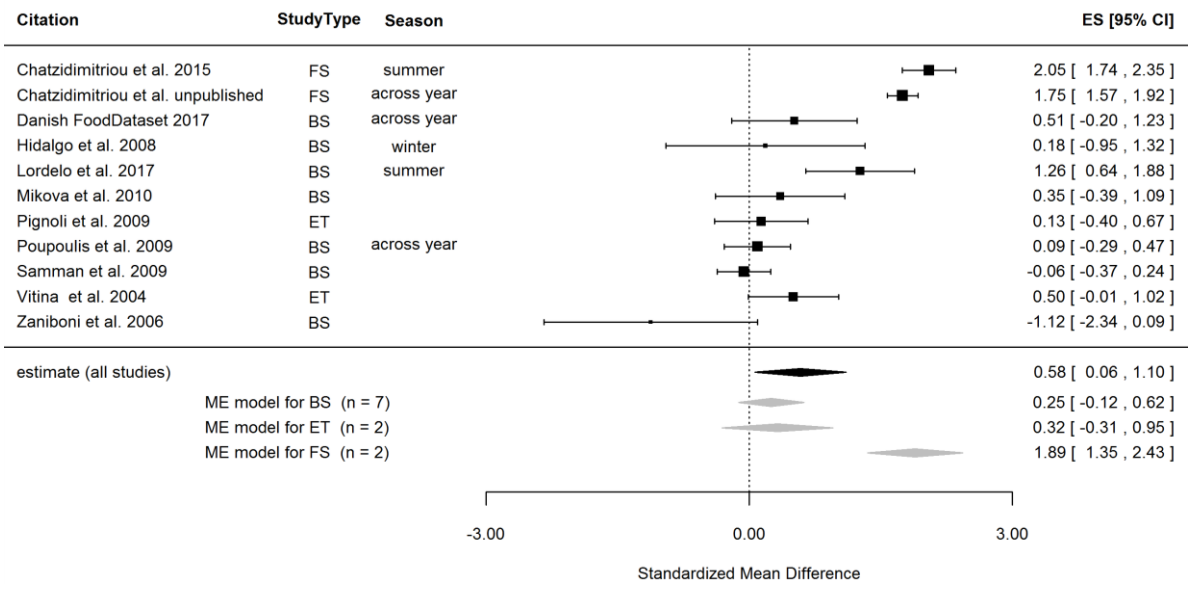


Figure APPENDIX B.3. 2. Forrest plot of standardised mean differences (SMD) in-3 FA cotnent in eggs from OR compared with eggs from conventional FR systems, including study type as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=72%; P value (Q statistics) <0.01

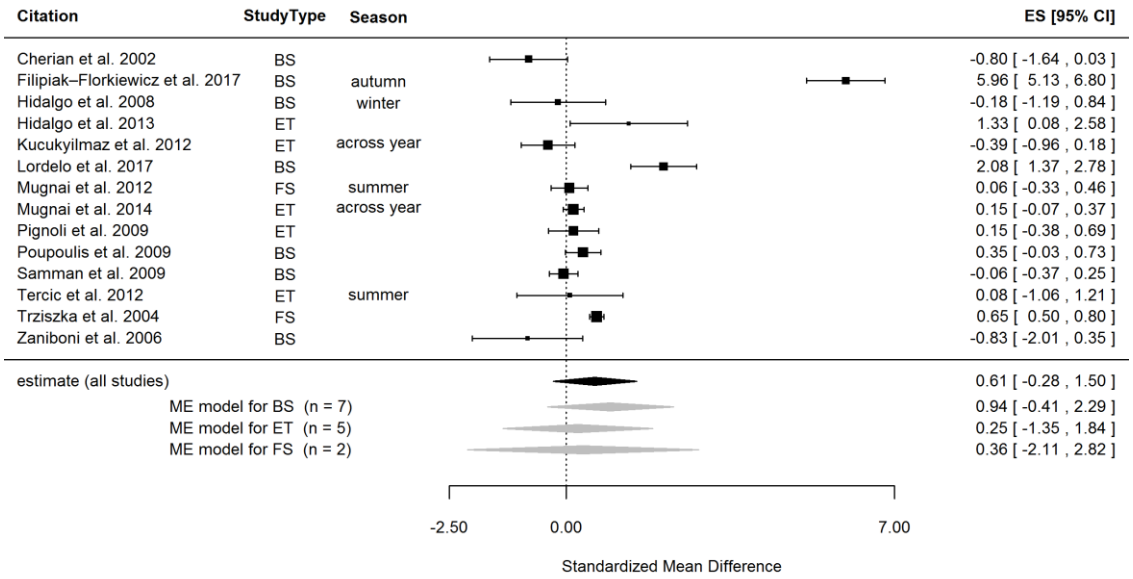


Figure APPENDIX B.3. 3. Forrest plot of standardised mean differences (SMD) in DHA content in eggs from OR compared with eggs from indoor systems, including study type as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=98%; P value (Q statistics) <0.001

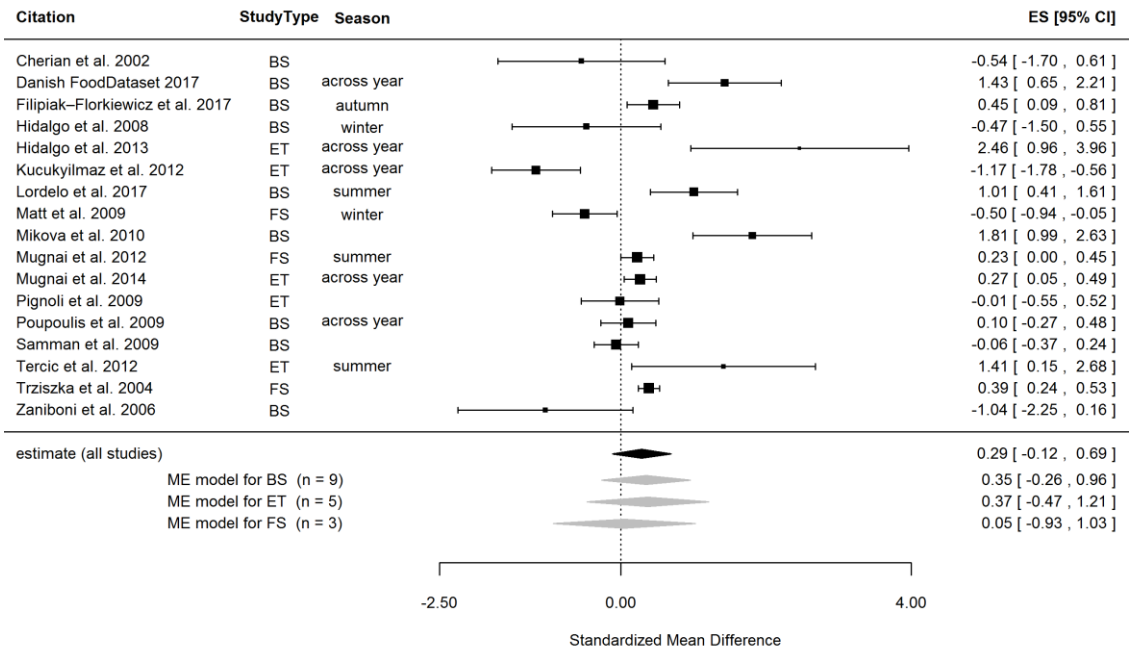


Figure APPENDIX B.3. 4. Forrest plot of standardised mean differences (SMD) in-3 FA content in OR compared with eggs from indoor systems, including study type as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=94%; P value (Q statistics) <0.001

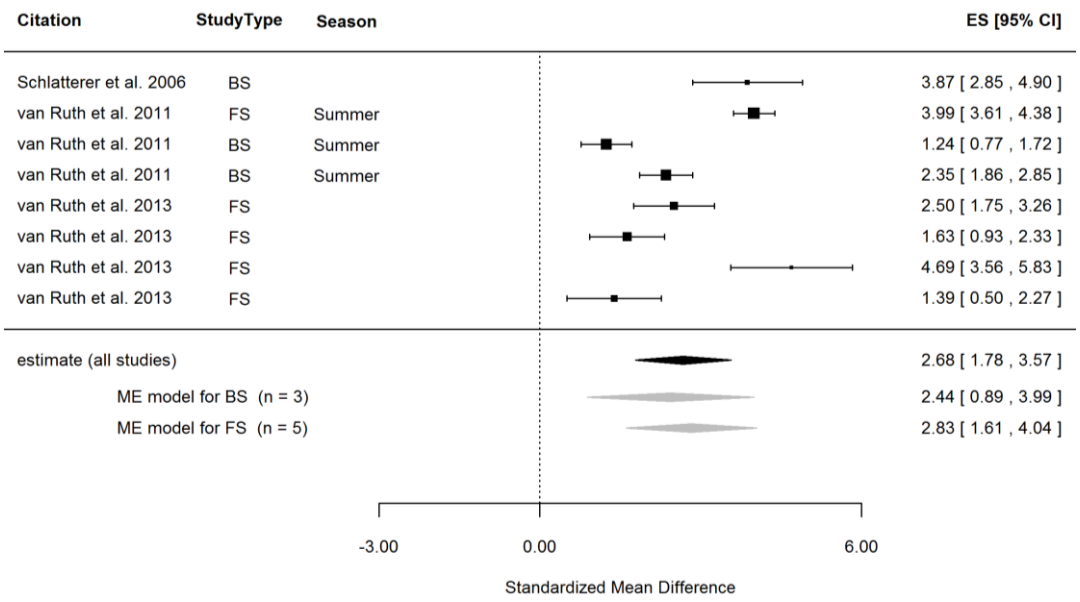


Figure APPENDIX B.3. 5. Forrest plot of standardised mean differences (SMD) in the sum of lutein and zeaxanthin (lut/zea) content in OR eggs compared with eggs from conventional FR systems, including study type as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=94%; P value (Q statistics) <0.001

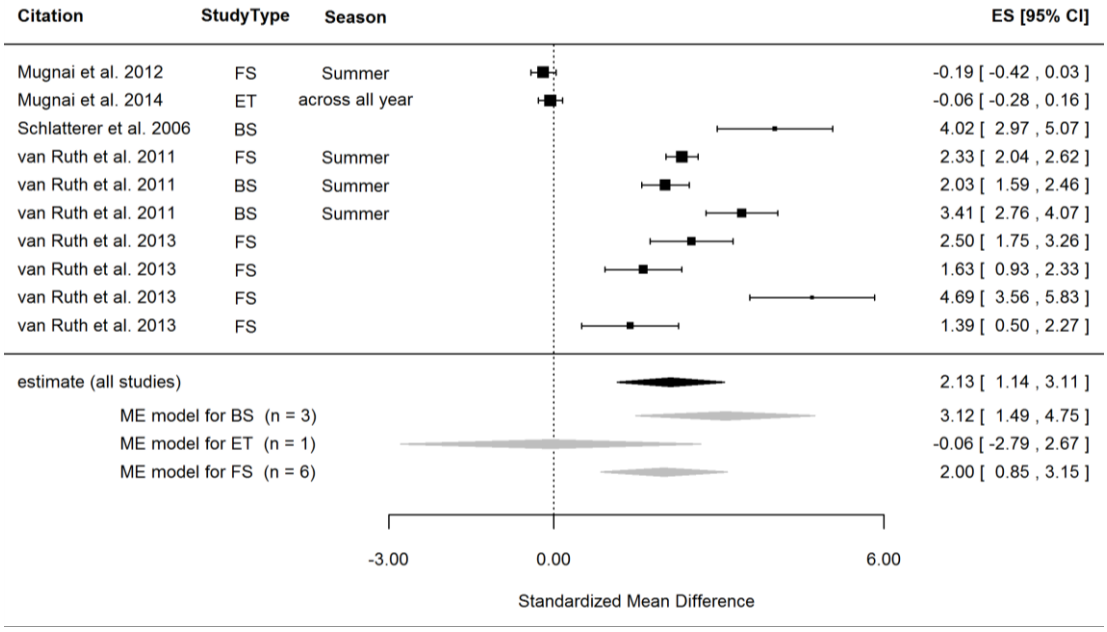


Figure APPENDIX B.3. 6. Forrest plot of standardised mean differences (SMD) in the sum of lutein and zeaxanthin (lut/zea) content in OR compared with eggs from indoor systems, including study type as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=97%; P value (Q statistics) <0.001

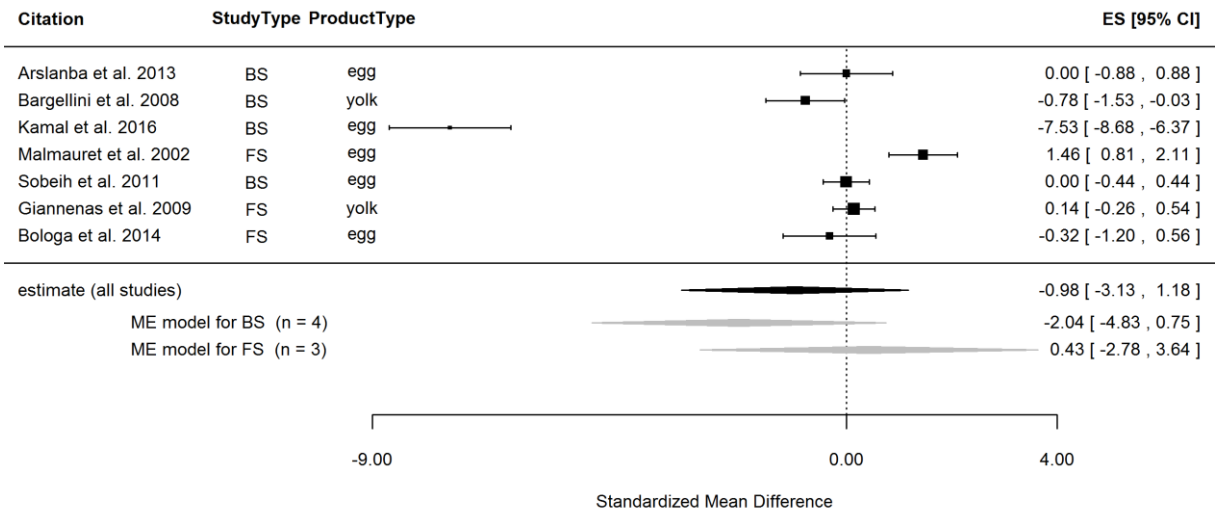


Figure APPENDIX B.3. 7. Forrest plot of standardised mean differences (SMD) in cadmium content in OR eggs compared with eggs from indoor systems, including study type as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=97%; P value (Q statistics) <0.001

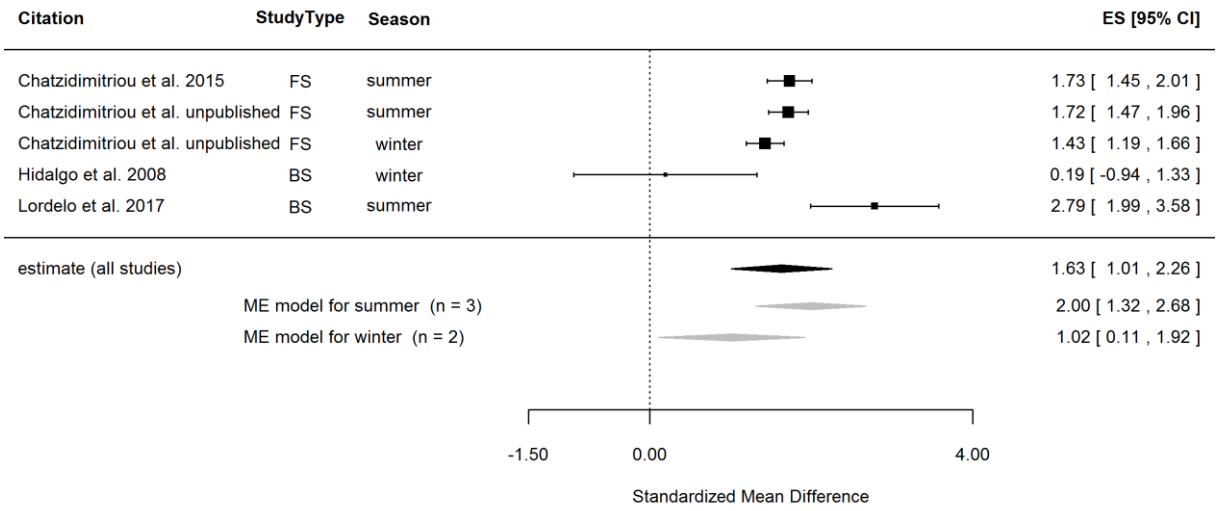


Figure APPENDIX B.3. 8. Forrest plot of standardised mean differences (SMD) in docosahexaenoic acid (DHA) content in OR compared with eggs from conventional FR systems, including egg collection season as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=88%; P value (Q statistics) <0.001

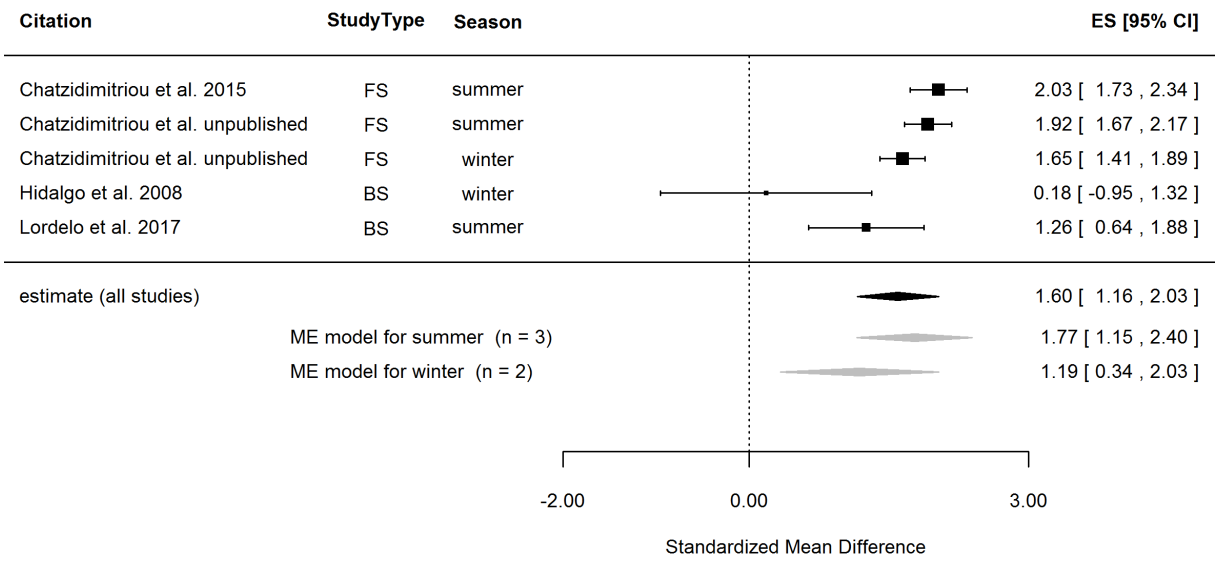


Figure APENDIX B.3. 9. Forrest plot of standardised mean differences (SMD) in n-3 FA cotnent in eggs from OR compared with eggs from conventional FR systems, including egg collection season as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=71%; P value (Q statistics) <0.05

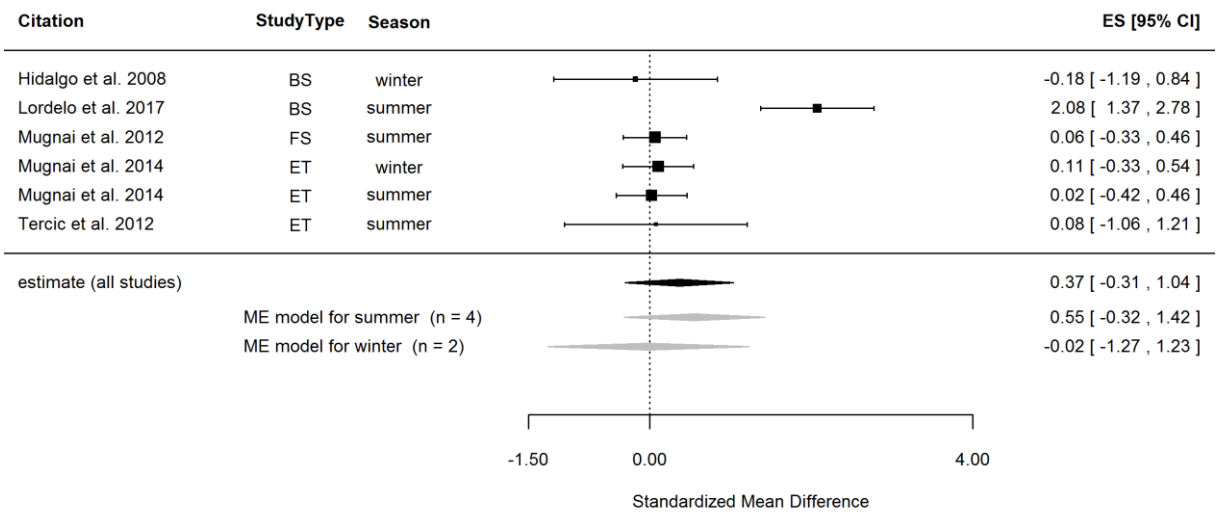


Figure APPENDIX B.3. 10. Forrest plot of standardised mean differences (SMD) in docosahexaenoic acid (DHA) content in OR compared with eggs from indoor systems, including egg collection season as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=94%; P value (Q statistics) <0.001

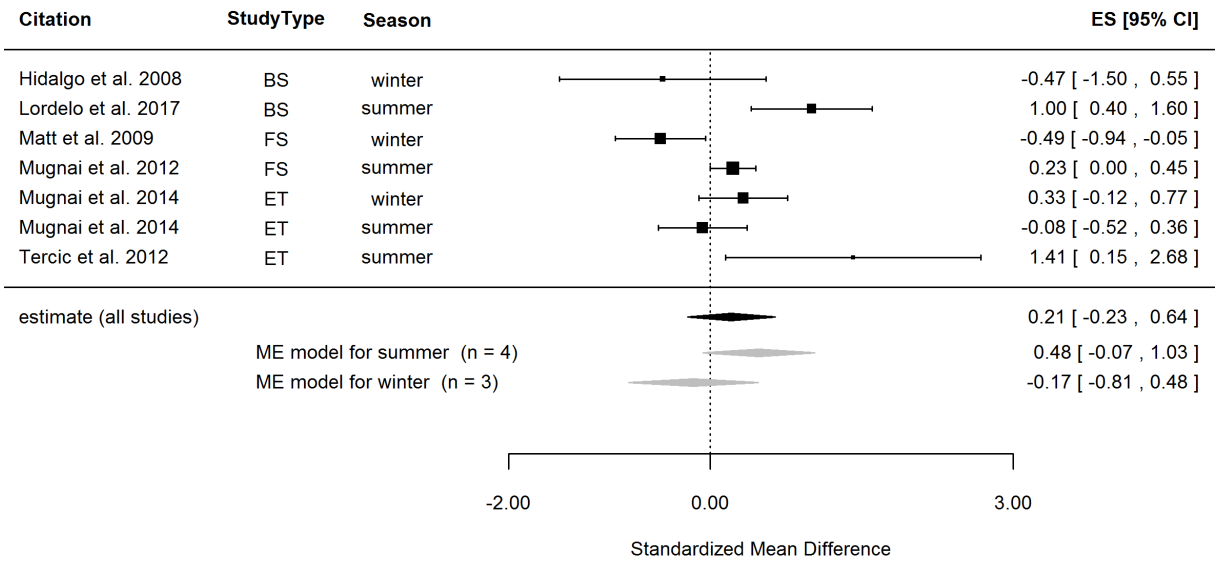


Figure APPENDIX B.3. 11. Forrest plot of standardised mean differences (SMD) in n-3 FA content in OR compared with eggs from indoor systems, including egg collection season as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=77%; P value (Q statistics) <0.001

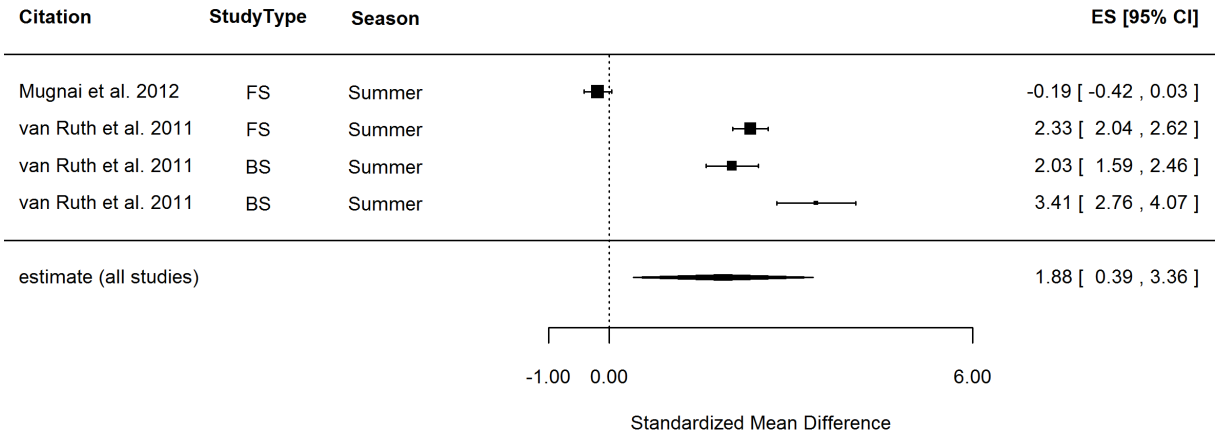


Figure APPENDIX B.3. 12. Forrest plot of standardised mean differences (SMD) in the sum of lutein and zeaxanthin (lut/zea) content in OR eggs compared with eggs from indoor systems, including egg collection season as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=98%; P value (Q statistics) <0.001

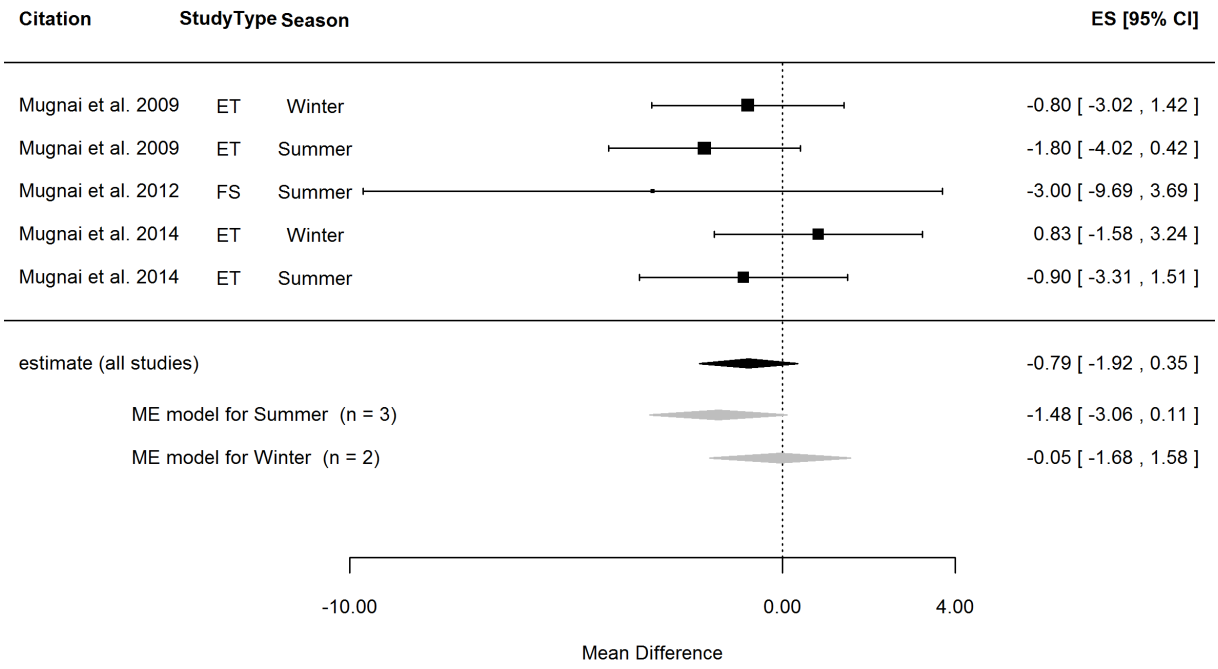


Figure APPENDIX B.3. 13. Forrest plot of mean differences (MD) in the sum of carotenoids content (%) in OR eggs compared with eggs from indoor systems, including egg collection season as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2< 1%; P value (Q statistics) >0.1

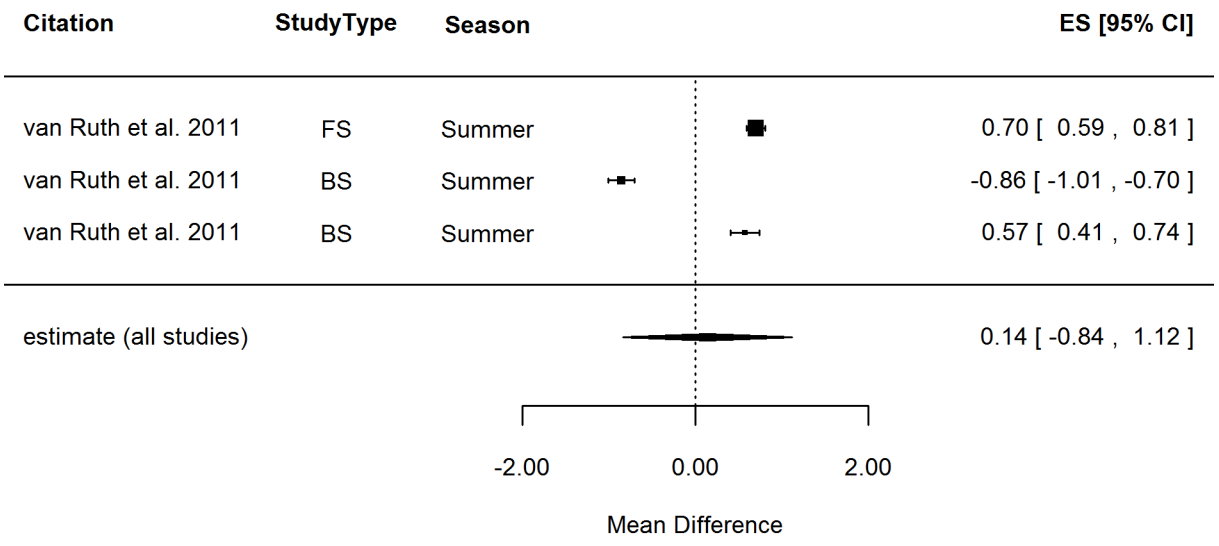


Figure APPENDIX B.3. 14 Forrest plot of mean differences (MD) in β-carotene content (%) in OR eggs compared with eggs from conventional FR systems, including egg collection season as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=99%; P value (Q statistics) <0.001

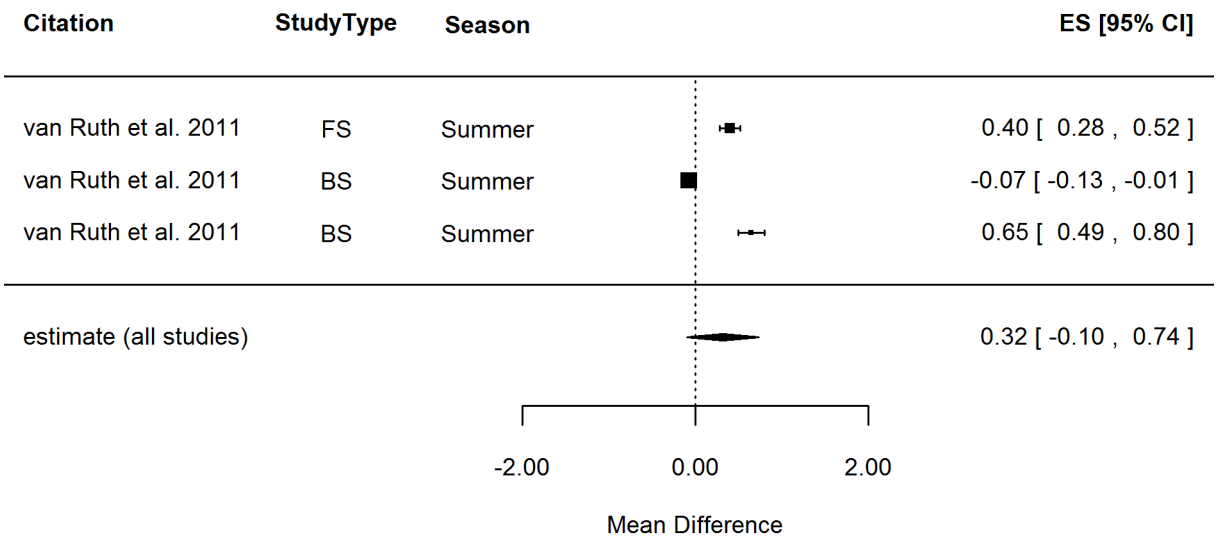


Figure APPENDIX B.3. 15. Forrest plot of mean differences (MD) in β-carotene content (%) in OR eggs compared with eggs from indoor systems, including egg collection season as a moderator. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant.. I^2= 98%; P value (Q statistics) > 0.001

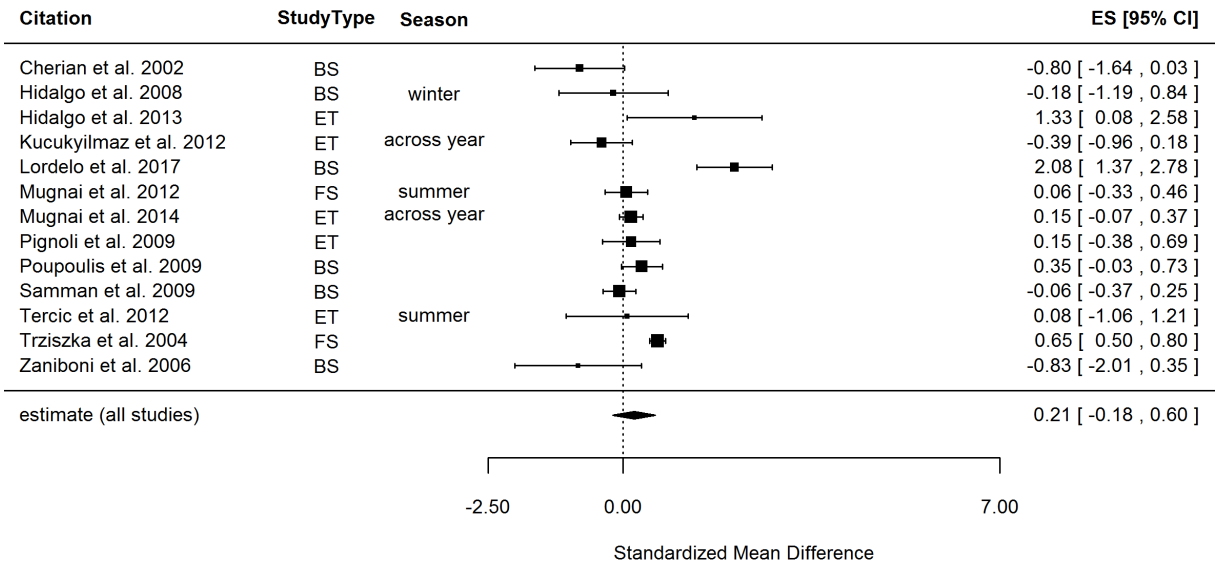


Figure APPENDIX B.3. 16. Forrest plot of standardised mean differences (SMD) in DHA content in eggs from OR compared with eggs from indoor systems, excluding influential points (study Filipiak–Florkiewicz et al. (2017)) . Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2= 91%; P value (Q statistics) < 0.001

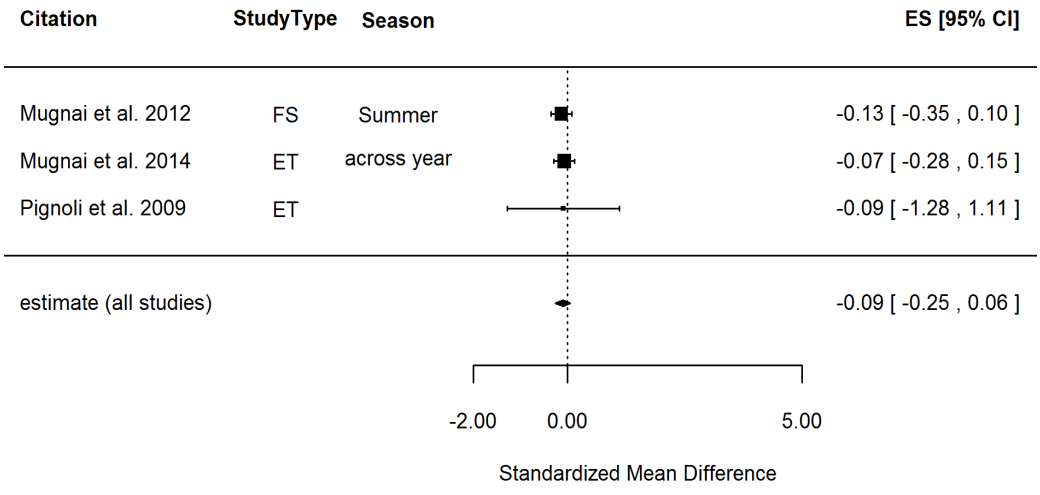


Figure APPENDIX B.3. 17. Forrest plot of standardised mean differences (SMD) in lutein content in eggs from OR compared with eggs from indoor systems, excluding influential points (study by Schlatterer and Breithaupt (2006)). Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2= <0%; P value (Q statistics) >0.1

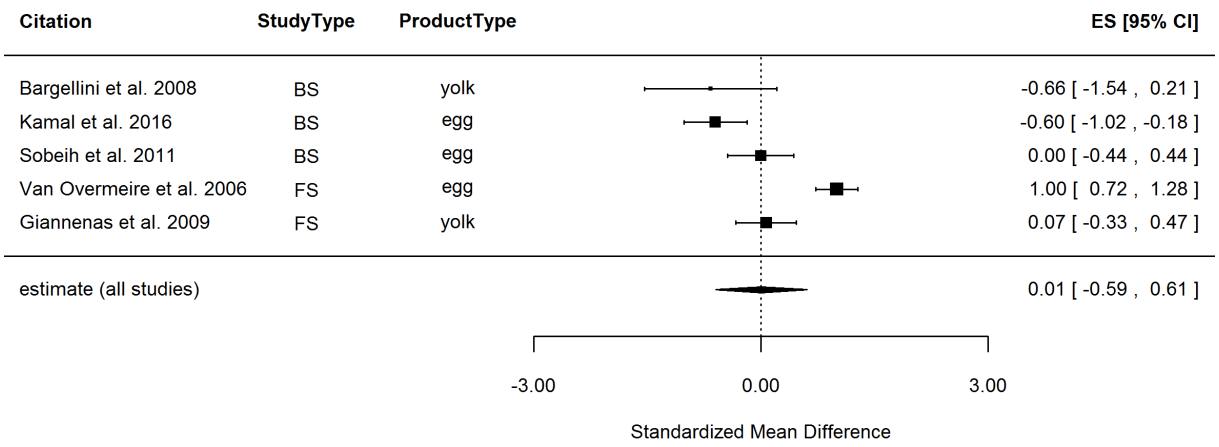


Figure APPENDIX B.3. 18. Forrest plot of standardised mean differences (SMD) in cadmium content in eggs from OR compared with eggs from conventional FR systems, excluding influential points (study by Malmauret et al. (2002)) . Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2= 90%; P value (Q statistics) < 0.001

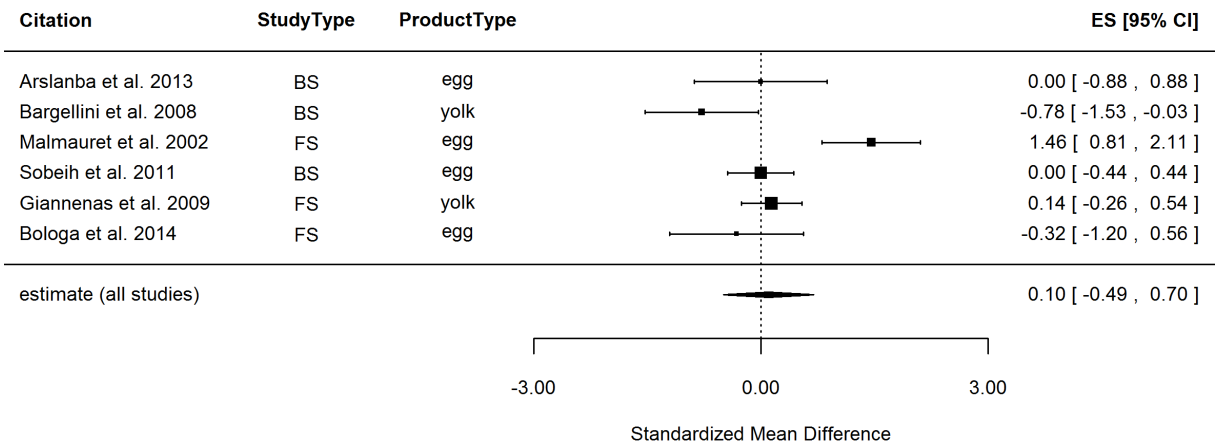


Figure APPENDIX B.3. 19. Forrest plot of standardised mean differences (SMD) in cadmium content in eggs from OR compared with eggs from indoor systems, excluding influential points (study by (Kamal et al., 2016)). Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2= 82%; P value (Q statistics) < 0.001

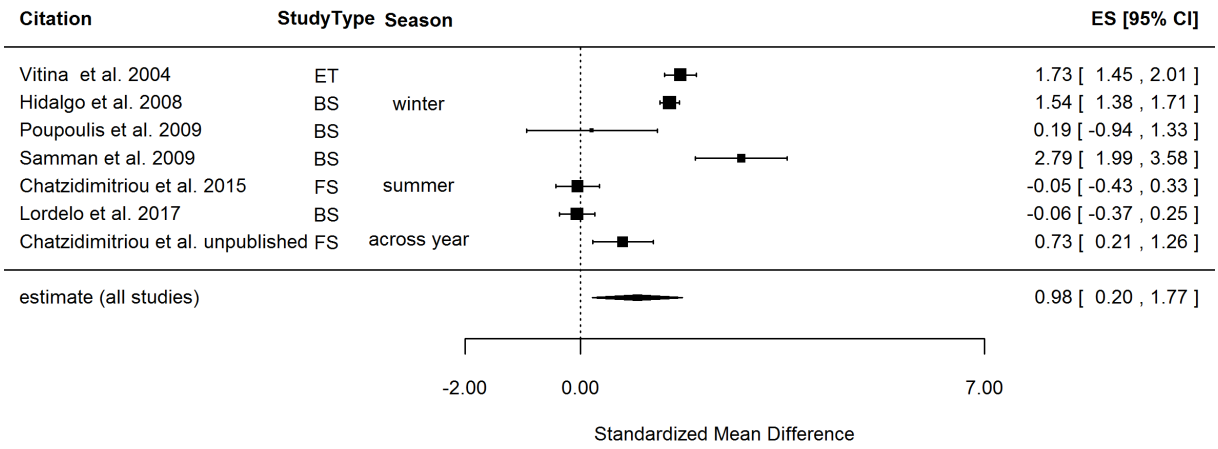


Figure APPENDIX B.3. 20. Forrest plot of standardised mean differences (SMD) in DHA content in eggs from OR compared with eggs from FR conventional systems, excluding studies where imputation methods where used to calculate missing values (Pignoli et al., 2009, Surai et al., 2000, Zaniboni et al., 2006). Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2= 97%; P value (Q statistics) < 0.001

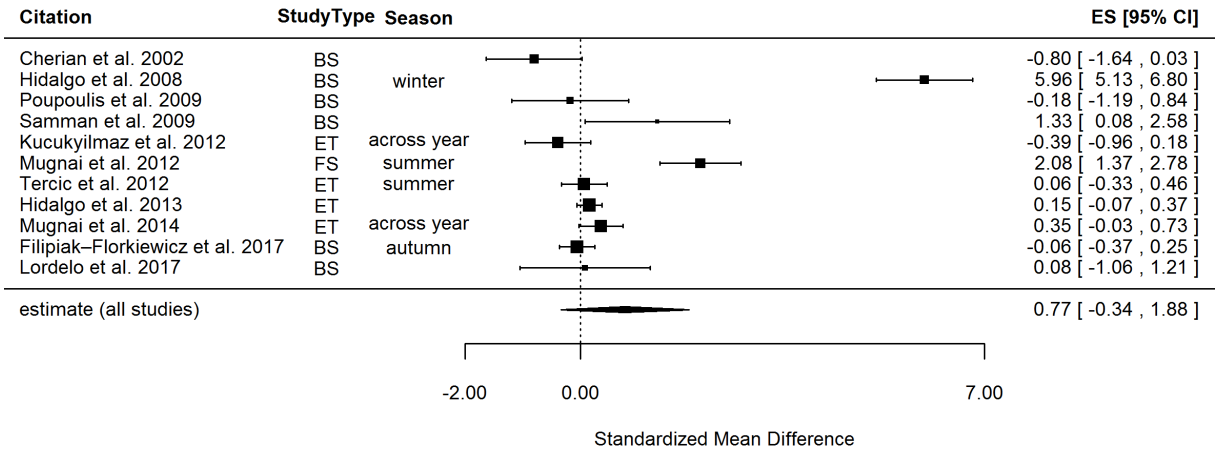


Figure APPENDIX B.3. 21. Forrest plot of standardised mean differences (SMD) in DHA content in eggs from OR compared with eggs from indoor systems, excluding studies where imputation methods where used to calculate missing values (Pignoli et al., 2009, Trziszka et al., 2004, Zaniboni et al., 2006). Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2= 98%; P value (Q statistics) < 0.001

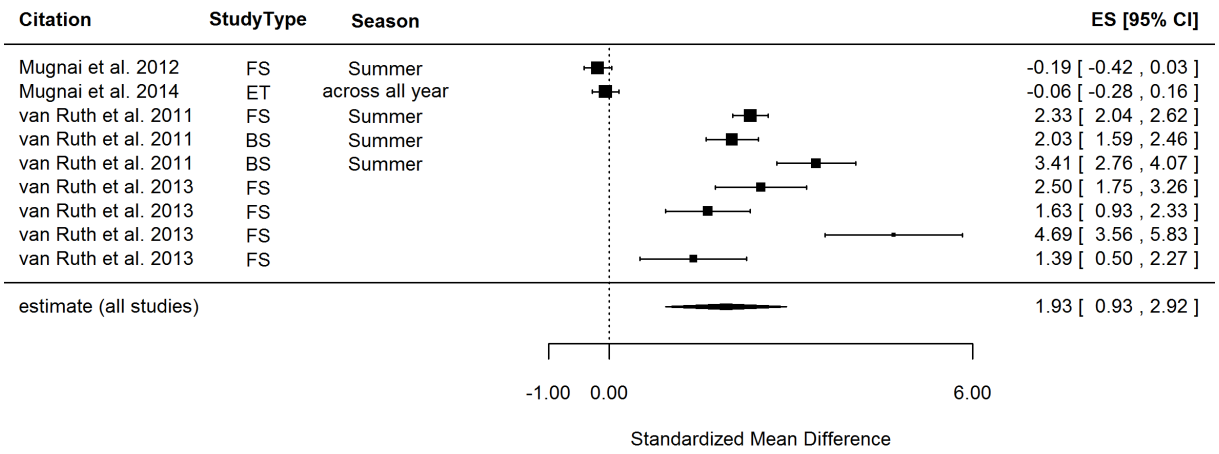


Figure APPENDIX B.3. 22. Forrest plot of standardised mean differences (SMD) in the sum of lutein and zeaxanthin (lut/zea) content in OR compared with eggs from indoor systems, excluding studies where imputation methods where used to calculate missing values (Schlatterer and Breithaupt, 2006). Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2= 98%; P value (Q statistics) < 0.001

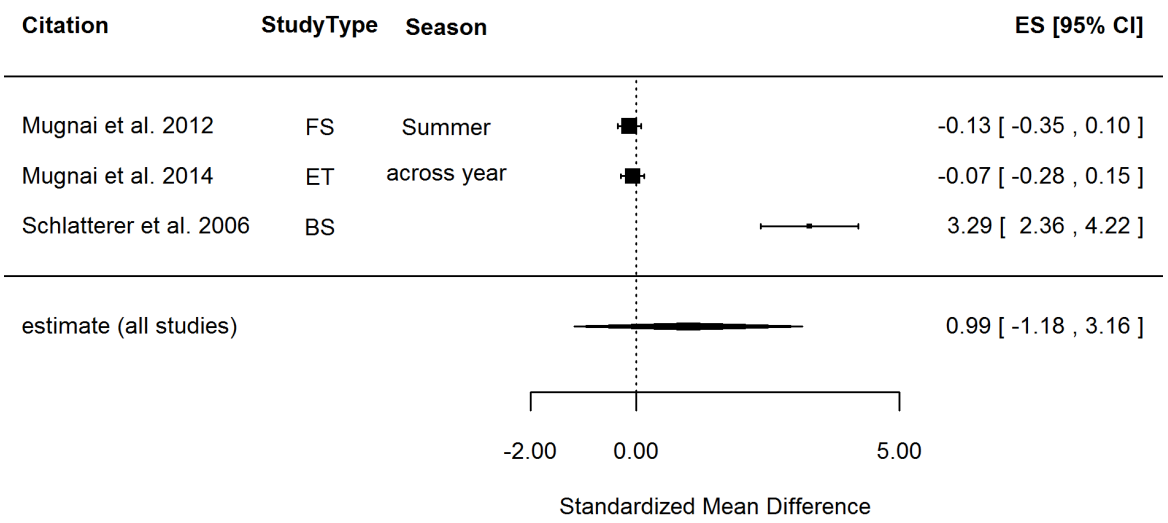


Figure APPENDIX B.3. 23. Forrest plot of standardised mean differences (SMD) for the sum of lutein and zeaxanthin (lut/zea) content in OR compared with eggs from indoor systems, excluding studies where imputation methods where used to calculate missing values (Pignoli et al., 2009). Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2= 99%; P value (Q statistics) < 0.001

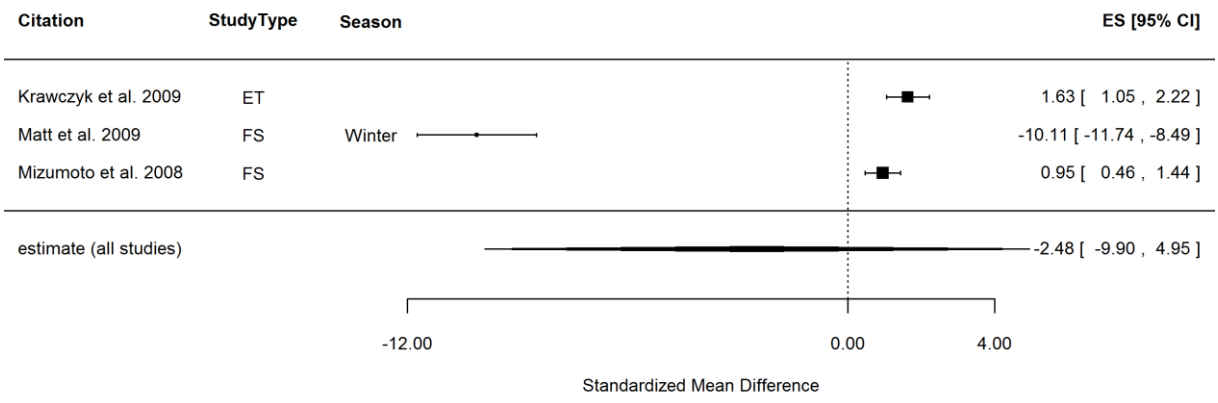


Figure APPENDIX B.3. 24. Forrest plot of standardised mean differences (SMD) in vitamin A content in eggs from OR compared with eggs from indoor systems, excluding studies where imputation methods where used to calculate missing values (FOODDATASET, DENMARK). Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2= 99%; P value (Q statistics) < 0.001

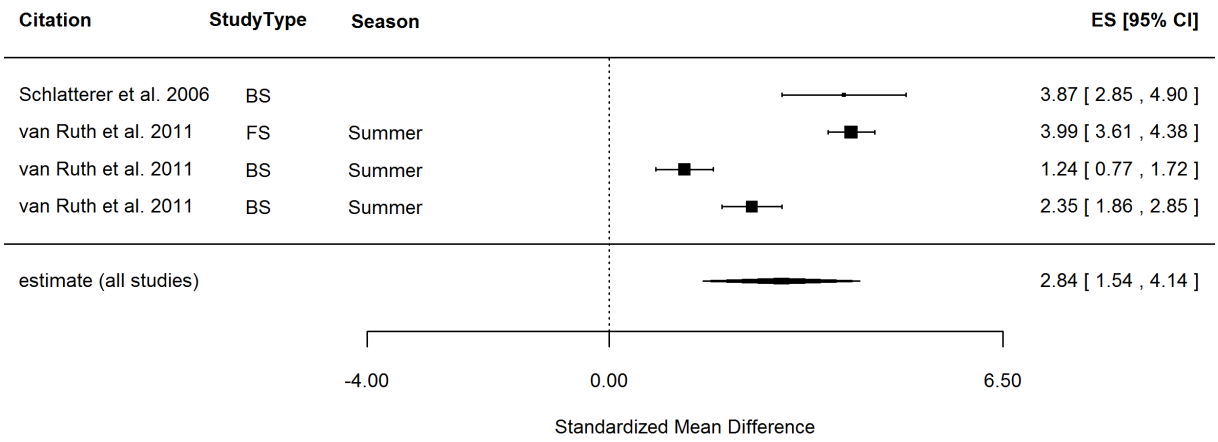


Figure APPENDIX B.3. 25. Forrest plot of standardised mean differences (SMD) in sum of lutein and zeaxanthin content in eggs from OR compared with eggs from conventional FR systems excluding studies with alternative management systems (Van Ruth et al., 2013). Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=94%; P value (Q statistics) <0.001.

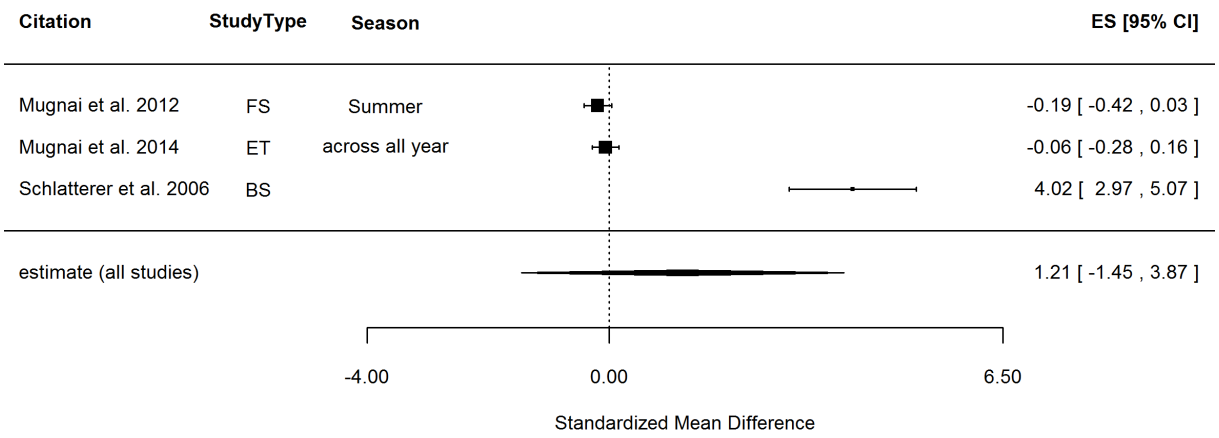


Figure APPENDIX B.3. 26. Forrest plot of standardised mean differences (SMD) in sum of lutein and zeaxanthin content in eggs from OR compared with eggs from indoor systems excluding studies with alternative management systems (Van Ruth et al., 2013, van Ruth et al., 2011). Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=98%; P value (Q statistics) <0.001.

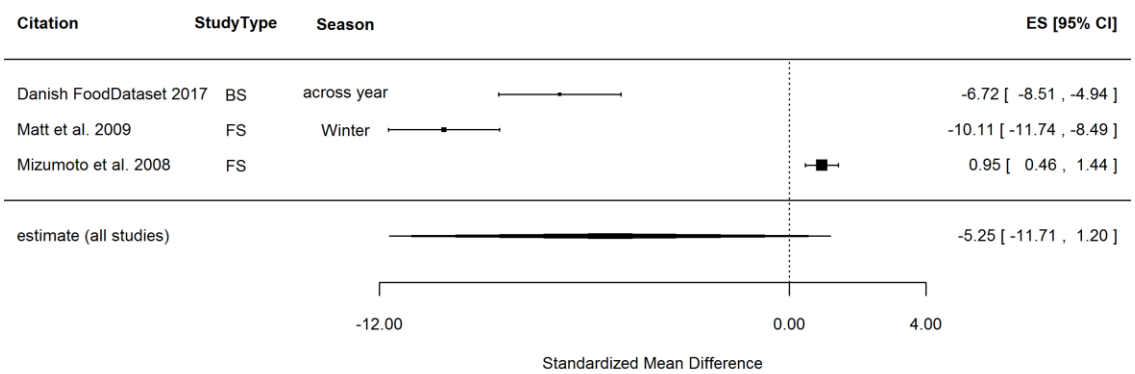


Figure APPENDIX B.3. 27. Forrest plot of standardised mean differences (SMD) in vitamin A content in eggs from OR compared with eggs from indoor systems excluding studies using alternative management systems (Krawczyk, 2009). Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=97%; P value (Q statistics) <0.001

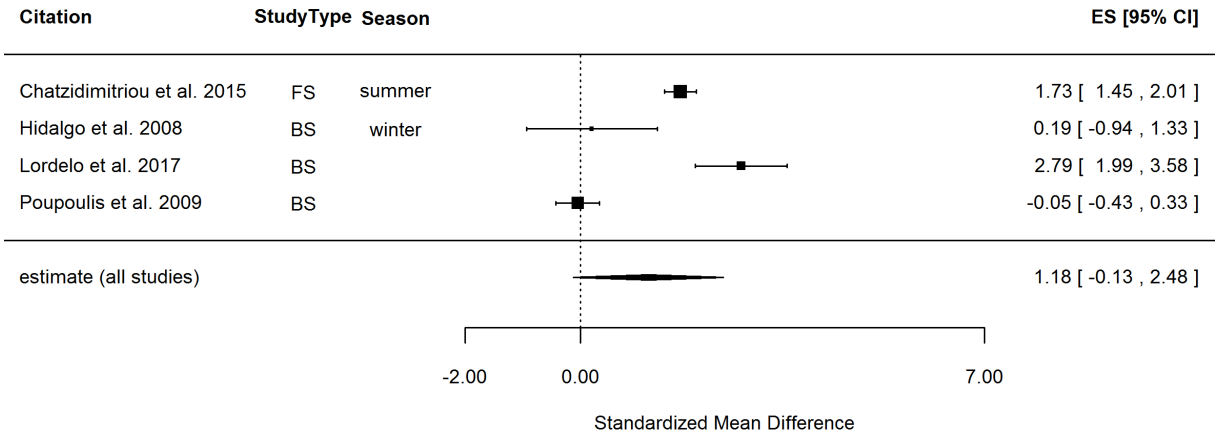


Figure APPENDIX B.3. 28. Forrest plot of standardised mean differences (SMD) in docosahexaenoic acid (DHA) content in OR compared with eggs from conventional FR systems excluding studies scored as low quality. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=96%; P value (Q statistics) <0.001

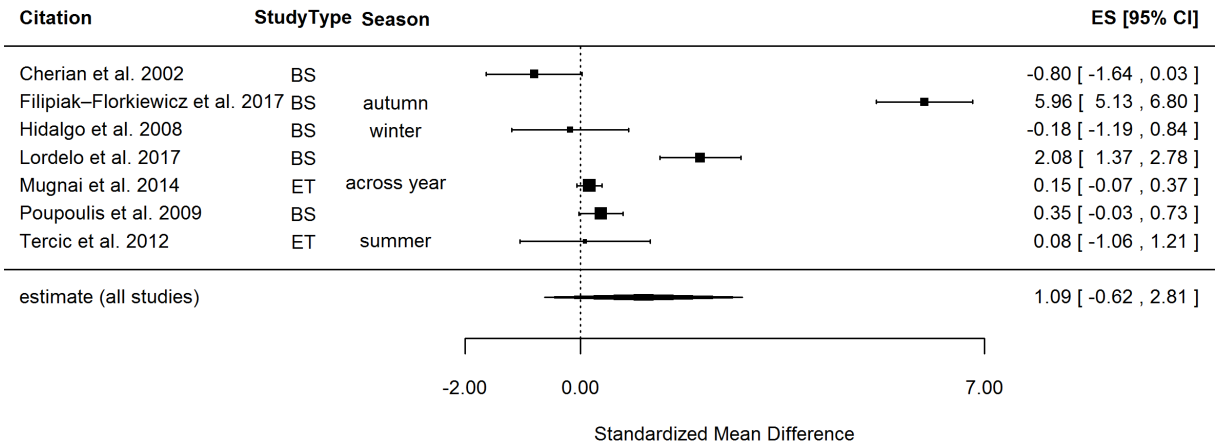


Figure APPENDIX B.3. 29. Forrest plot of standardised mean differences (SMD) in docosahexaenoic acid (DHA) content in OR compared with eggs from indoor systems excluding studies scored as low quality. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=99%; P value (Q statistics) <0.001

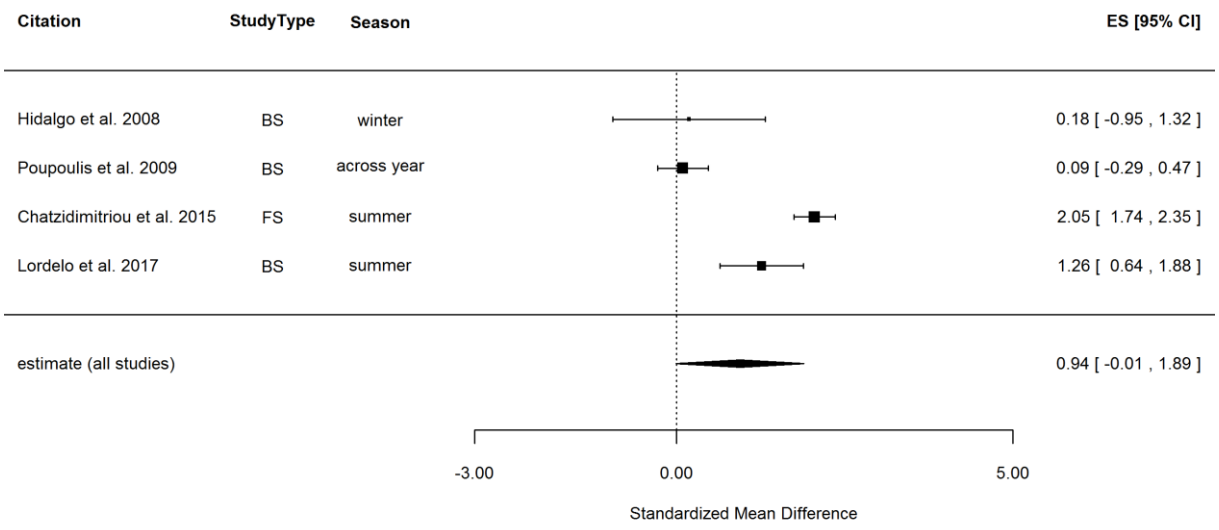


Figure APPENDIX B.3. 30. Forrest plot of of standardised mean differences (SMD) in omega-3 FA content in OR compared with eggs from conventional FR systems excluding studies scored as low quality. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=93%; P value (Q statistics) <0.001

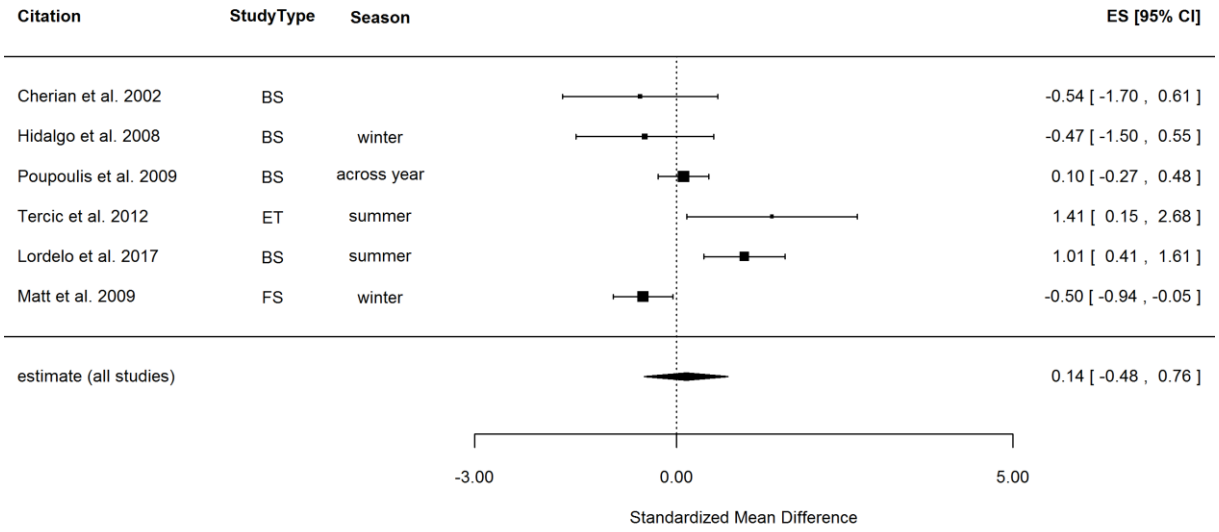


Figure APPENDIX B.3. 31. Forrest plot of of standardised mean differences (SMD) in omega-3 FA content in OR compared with eggs from indoor systems excluding studies scored as low quality. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=80%; P value (Q statistics) <0.001

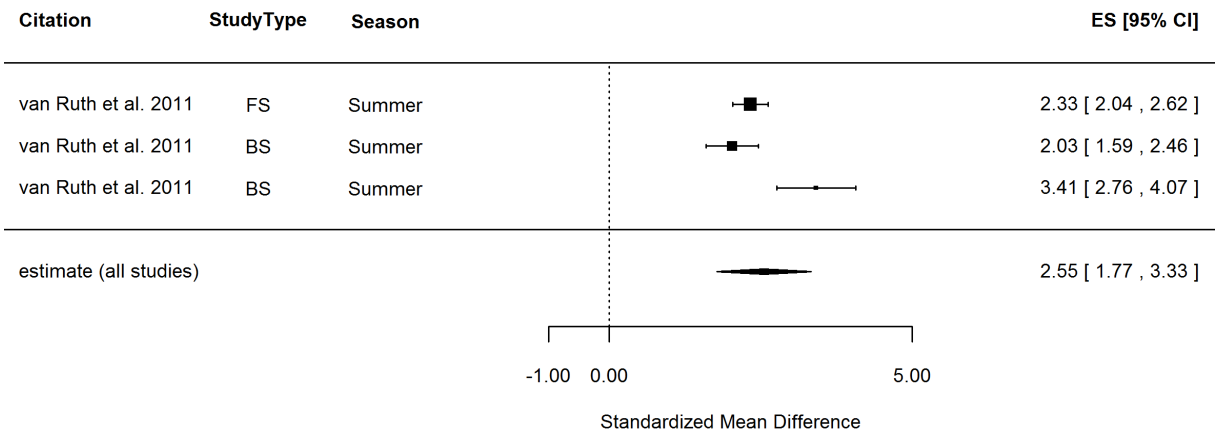


Figure APPENDIX B.3. 32. Forrest plot of standardised mean differences (SMD) in the sum of lutein and zeaxanthin content in OR compared with eggs coming from indoor systems, excluding studies scored as low quality. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=89%; P value (Q statistics) <0.001

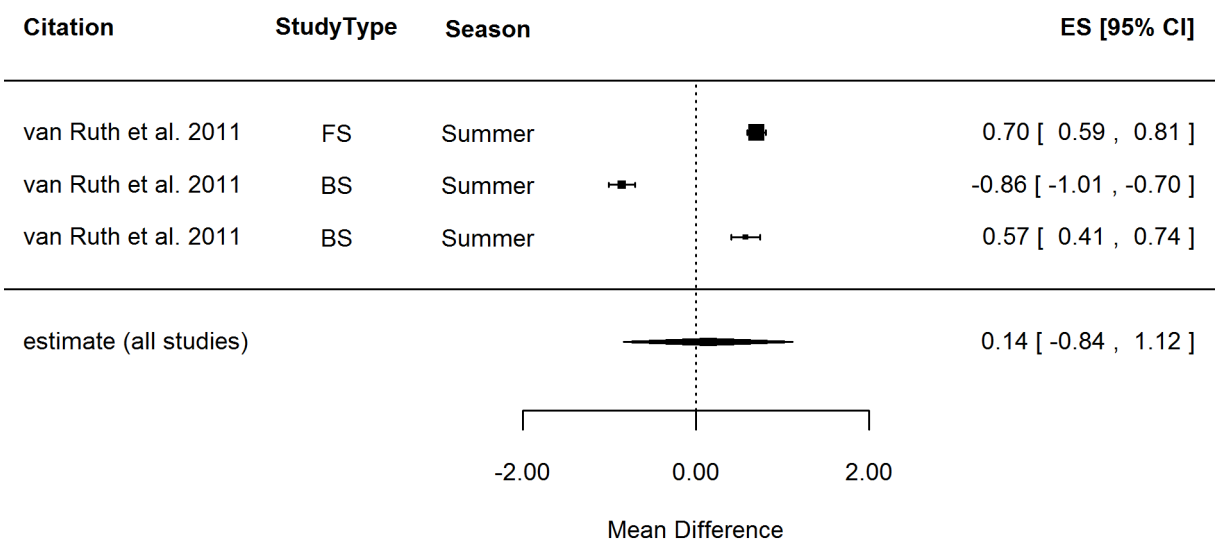


Figure APPENDIX B.3. 33. Forrest plot of mean differences (MD) in β-carotene content (%) in OR eggs compared with eggs from indoor systems, excluding studies scored as low quality. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=99%; P value (Q statistics) <0.001

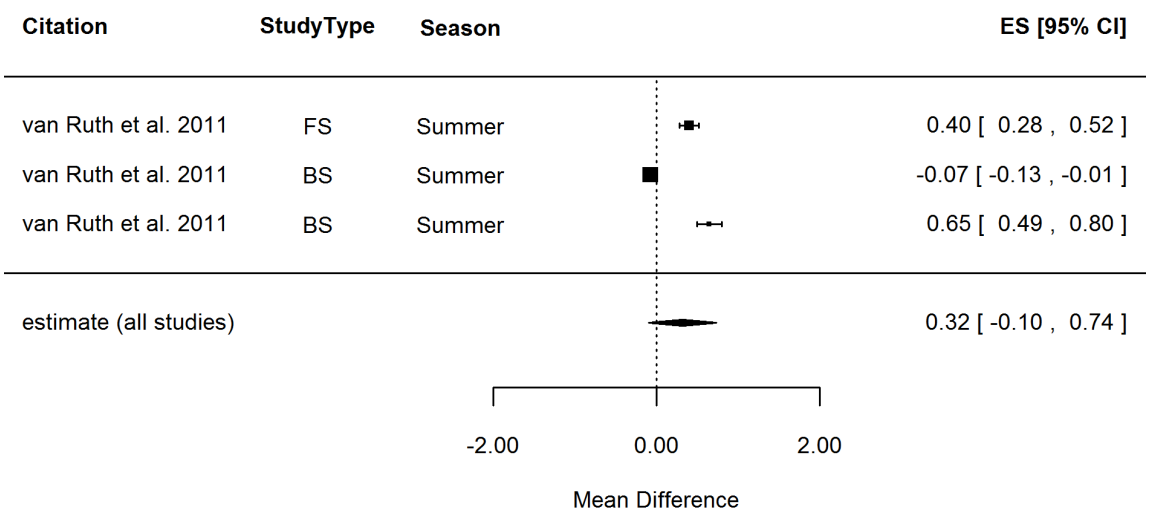


Figure APPENDIX B.3. 34. Forrest plot of mean differences (MD) in β-carotene content (%) in OR eggs compared with eggs from indoor systems, excluding studies scored as low quality. Data points are reported as their respective publication reference reporting the study type (BS: basket study; FS: farm survey; ET: experimental trial) and sampling season whenever reported. Values of the effect size (ES) are SMD, with 95% confidence intervals (CI) represented by horizontal bars. The weight of each study is represented by the size of the square at the centre of the CI. When CI included the value of zero then the P value was > 0.05 and not statistically significant. I^2=98%; P value (Q statistics) <0.001

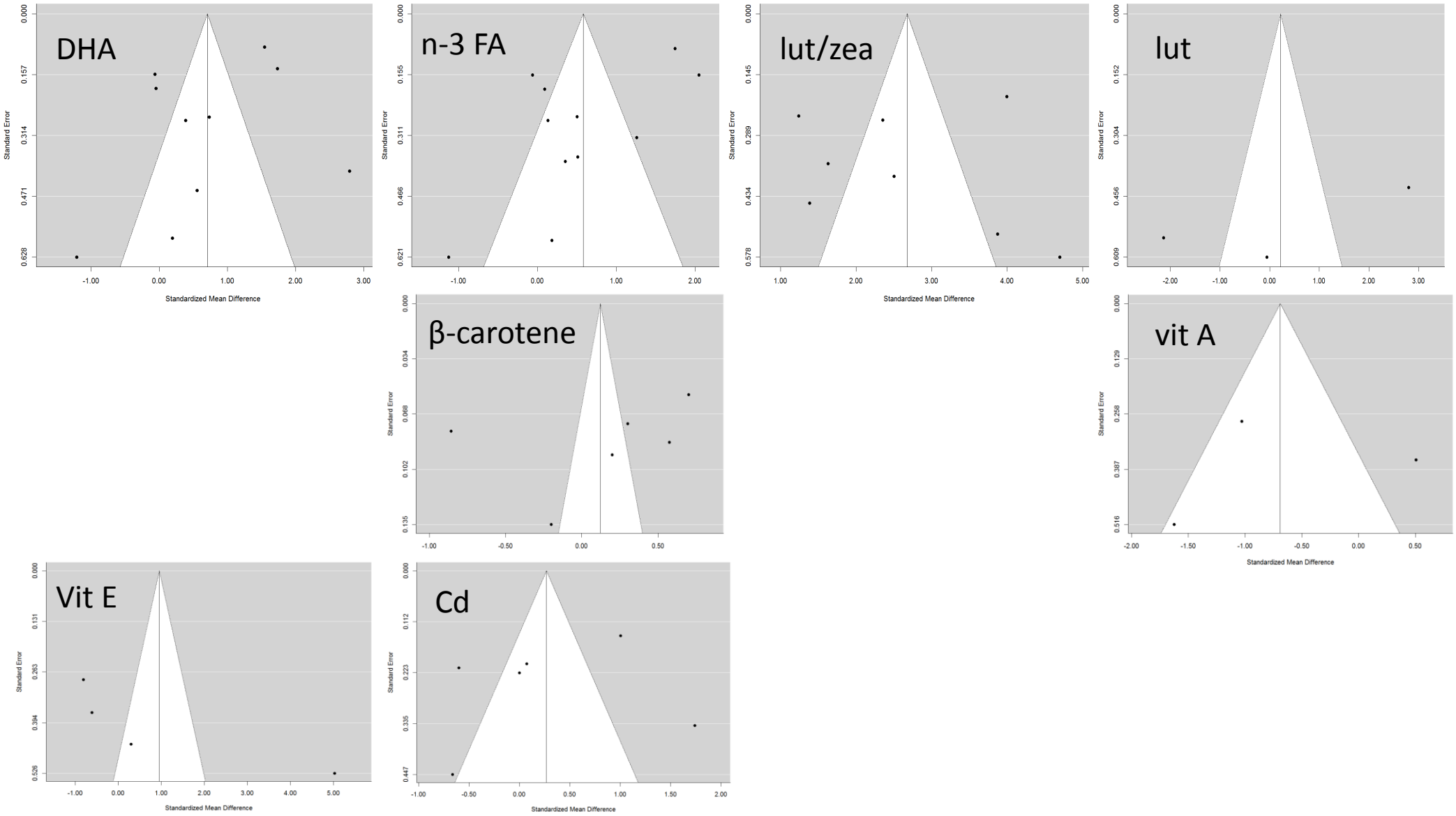


Figure APPENDIX B.3. 35. Funnel plots for egg content of docosahexaenoic acid (DHA), omega-3 FA (n-3 FA), lutein and zeaxanthin as a sum (lut/zea), lutein (lut), β-carotene, vitamin A (vit A), vitamin E (vit E) and cadmium (Cd) comparing OR and conventional FR systems. Study points are represented by dots.

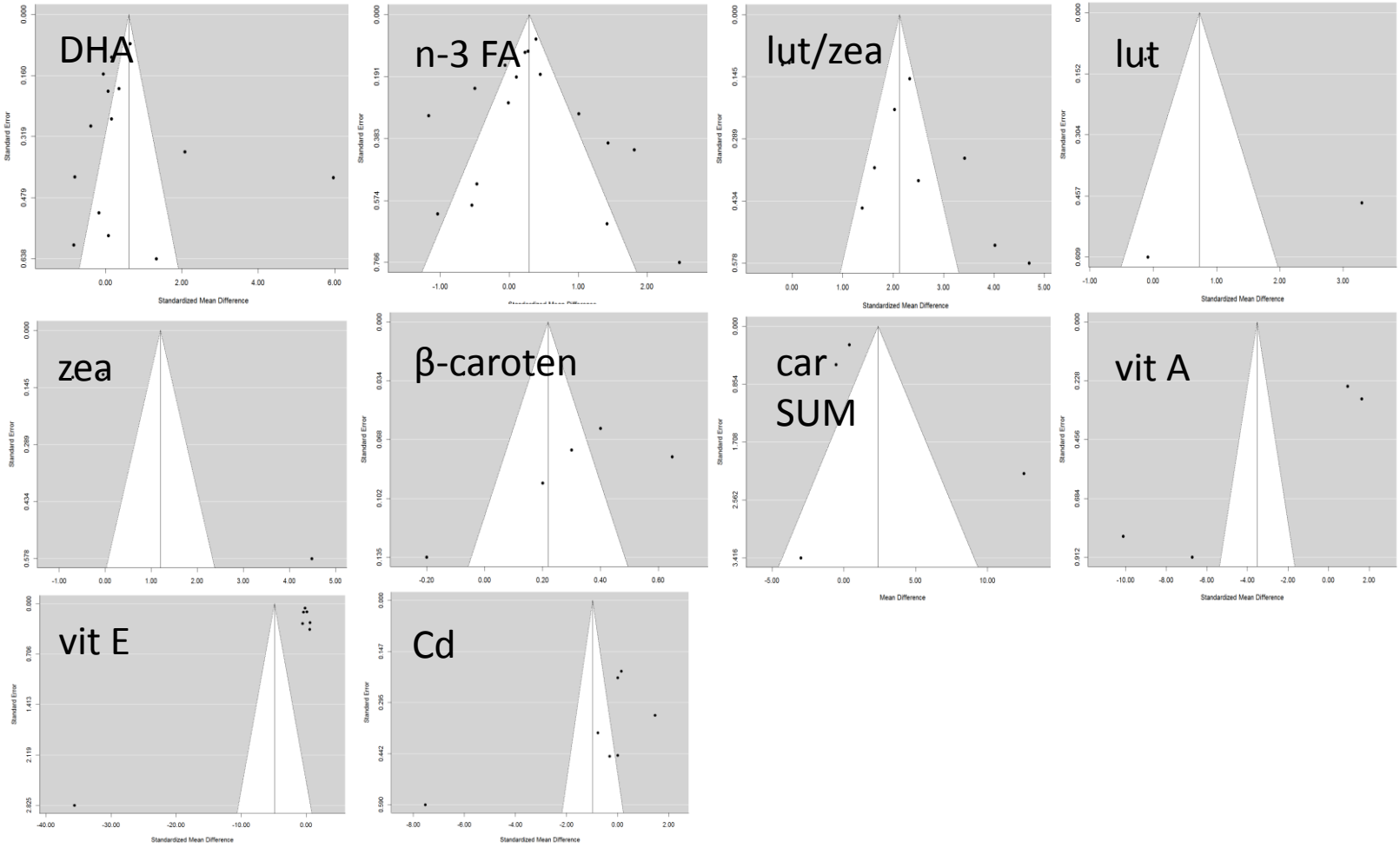


Figure APPENDIX B.3. 36. Funnel plots for egg content of docosahexaenoic acid (DHA), omega-3 FA (n-3 FA), lutein and zeaxanthin as a sum (lut/zea), lutein (lut) , β-carotne, sum of carotenoids (Carot SUM), vitamin A (vit A), vitamin E (vit E) and cadmium (Cd) comparing OR and indoor systems. Study points are represented by dots.

APPENDIX B.4

APPENDIX B.4. Table 1. Dietary intakes (g/hen/day) calculated at 60 weeks of age and diet fatty acid (FA) profile (g/100g total FA) of feed[a] used at free-range (FR) and organic (OR) flocks in Switzerland

	Management		
	FR n=7	OR n=6	ANOVA P-value
Feed Intake	119.8±3.7	118.3±6.8	0.846
Grain Intake	1.7± 0.9	8.6±3.0	0.037
Total Feed Intake	121.5±3.0	126.9±7.0	0.472
Diet FA[1]			
C14:0	0.64±0.20	0.17±0.02	0.051
C16:0	21.89±1.74	16.98±0.81	0.035
C18:0	6.50±0.85	6.46±0.47	0.966
OA	30.85±1.32	30.13± 1.11	0.691
LA	32.61±3.43	37.91± 1.64	0.213
ALA	1.63± 0.30	2.41±0.20	0.061
DHA	0.03±0.01	0.05±0.01	0.059
FA groups			
SFA	30.94±2.16	25.50±1.31	0.064
MUFA	32.90±1.40	32.12±1.20	0.686
PUFA	35.45±3.58	41.67±1.64	0.164
n-3	1.99±0.33	2.77±0.20	0.077
n-6	32.83±3.43	38.24±1.62	0.205
n-6/n-3	17.58±1.43	14.08±0.85	0.069
n-3>18C	0.12± 0.01	0.15±0.01	0.071

[a] Grain FA were not taken into account for diet FA profile in this table [1] OA= oleic acid; LA = linoleic acid; ALA = α-linolenic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA =polyunsaturated fatty acids; n-3= omega-3 FA; n-6 = omega-6 fatty acids;n-6/n-3 = omega-6 to omega-3 ratio; n-3>18C = omega-3 fatty acids with more than 18 carbon atoms.

APPENDIX B.5

Egg Farm Questionnaire

Date of Visit

Date of visit
Weather (air temperature, precipitation)
Nearest Weather station
Visit done by:
Age of flock at visit (weeks)
How many different flocks on the farm?
Flock size(s)
Genotype(s); are they always the same?

Rearing

Rearing on farm yes/no
Hens come from same hatchin and rearing group
Outside access (during rearing) yes / no
Outside access from what age

Laying-period

Date of placement at layer house
Age at placement (weeks)
Same food as at rearing farm yes/no
Feather condition at arrival at laying farm good/moderate/bad
Feather condition at day of visit good/moderate/bad
Behaviour at arrival on laying farm good/moderate/bad
Behaviour at t day of visit on laying farm good/moderate/bad
If problem behaviour at arrival, what problem(s)?
Litter at arrival of hens in laying farm yes/no
Litter type
Litter added daily / weekly / monthly / irregular
Hens been locked up inside house yes/no
Hens been locked up inside house how many days, when?
Feed intake at start of production good/moderate/bad
Did hens receive grit (stones for digestion)
Number of males
Sq.m. per bird (outside);
Floor type
Ventilation natural / mechanical / combination

Vaccinations/medications

Treatments during laying period
Vaccination for; age and frequency
Supplements water
Supplements feed

Management during lay

Time pop holes open
Are the birds in long before they are closed or you have to chase them in
Daily outside access yes/no
% hens of this flock roaming outside, assessment
% hens of this flock roaming outside, does it vary much? t (farmer's estimation)
% hens of this flock roaming outside the day before the visit (farmer's estimation)

Roughage fed yes / no
Roughage fed type
Roughage fed daily / weekly / monthly / irregular
Roughage fed gram /hen/ day
Additional grains given in litter area yes/no
Additional grid given in litter area yes/no
Additional shells given in litter area yes/no
Feed composition

Performance current flock

Number of eggs per placed hen yesterday
Feed intake
Planned slaughter age
Plan to moult the flock yes/no
Have they been moulted
Moulted flocks in the past

Observations inside stable

litter in stable: dry manure / original litter still recognisable
Stable climate in stable good/moderate/bad
Amount of light (much/sufficient/little)

Observations current flock

Feather cover
Percentage hens wounded
Fear of hens for visitors

Outside area

Description of the outdoor area (trees, coverage, _____); assessement
Bare soil % (eaten by hens?)
Trees
Vegetation height (small, medium, high)
Is it planted
Rotation
Sward composition
Impression of the use of the outdoor area (only around stable/ half of the surface/ whole surface)
Vegetation alternative use; silage?
Wildlife challenges
Other livestock on range?

Egg Marketing

Where/ how are eggs supplied?

Table of external and freshness characteristics, component proportions and dry matter assessments

APPENDIX B.5 Table 1. 1. Means ± SE and ANOVA P values for the effect of management and year on egg external and freshness characteristics, component proportions and dry matter assessments of eggs collected from supermarkets in the UK. During April 2017 all hens were kept indoors.

	Management (M)			Year (Y)		ANOVA - P values[1]		
						Main Effects		Interactions
	Caged n=8	Free-range n=16	Organic n=16	Apr-17 n=20	Apr-18 n=20	M	Y	M x Y
Measurement 2								
External and freshness characteristics								
shell colour	26.5±1.5[a]	27.0±1.0[a]	30.8±1.4[a]	30.4±1.1	26.5±0.9	**	†	ns
yolk colour	6.8±0.8[b]	10.4±0.4[a]	6.6±0.4[b]	8.3±0.5	8.0±0.6	***	ns	ns
albumen height	5.41±0.25	5.41±0.25	4.84±0.15	5.22±0.17	5.14±0.20	ns	ns	ns
Haugh Units	71.2±2.1	70.6±2.1	65.1±1.7	69.0±1.5	68.1±1.9	†	ns	ns
Shell density	87.2±0.7	87.1±0.9	88.4±1.1	88.5±0.9	86.8±0.7	ns	ns	ns
Age of egg*	12.6±0.5	14.0±0.6	14.8±0.7	13.8±0.6	14.3±0.5	†	ns	ns
Weights (g) and proportions (%)								
Egg weight	59.1±0.4	59.6±0.5	61.1±0.9	60.7±0.6	59.5±0.6	ns	ns	ns
Yolk weight	15.1±0.5	15.8±0.4	15.7±0.3	16.1±0.3	15.1±0.3	ns	†	ns
Shell weight	6.18±0.06	6.21±0.06	6.40±0.12	6.38±0.08	6.18±0.08	ns	ns	ns
Albumen weight	37.8±0.6	37.6±0.4	39.0±0.7	38.2±0.6	38.2±0.4	ns	ns	ns
Yolk proportion	25.6±0.8	26.5±0.5	25.7±0.4	26.5±0.5	25.4±0.3	ns	ns	ns
Shell proportion	10.5±0.1	10.4±0.1	10.5±0.1	10.5±0.1	10.4±0.1	ns	ns	ns
Albumen proportion	63.9±0.8	63.1±0.5	63.8±0.5	62.9±0.5	64.2±0.3	ns	ns	ns
Dry Matter (DM) (%)								
DM yolk	49.0±0.3	49.1±0.3	49.4±0.2	48.5±0.2[b]	49.9±0.1[a]	ns	**	ns
DM.egg	24.1±0.3	24.4±0.2	24.0±0.2	24.5±0.2[a]	23.9±0.2[b]	*ns*	*	*ns*

[1] Significances were declared: *** = P<0.001; ** = P<0.01; * = P<0.05; † =0.05<P<0.1; ns = P>0.1. 2 Values that do not share the same letters are significantly different (Tukey's honestly significant difference test, P < 0.05); 3 (1 brown – 100 white); 4 yolk colour was measured with a DSM Yolk Colour Fan

APPENDIX B.5 Table 1. 2. Means ± SE and ANOVA P values for the effect of management and date on yolk lipid content (%) and fatty acid (FA) profile (g/100g total FA) of eggs collected from supermarkets (caged, free-range and organic systems) and extensive organic farms in the UK.

	Management (M)				Date (D)				ANOVA - P values[1]		
									Main Effects		Interactions
	caged	Free-range	Organic	Extensive organic	Jul-17	Oct-17	Jan-17	Apr-18	M	D	M x D
	n=16	n=32	n=32	n=19	n=25	n=25	n=25	n=24			
Fatty acids[1]											
C12:0	0.012±0.002[a]	0.016±0.002[a]	0.015±0.002[a]	0.011±0.002[a]	0.013±0.002a	0.012±0.002a	0.014±0.002a	0.016±0.001a	0.206	0.367	0.951
C14:0	0.327±0.007[a]	0.317±0.005[a]	0.298±0.005[b]	0.291±0.006[b]	0.312±0.005a	0.309±0.007a	0.299±0.006a	0.311±0.008a	0.001	0.379	0.546
C14:1	0.091±0.012[a]	0.074±0.003[ab]	0.071±0.008[ab]	0.059±0.004[b]	0.079±0.008a	0.067±0.004a	0.080±0.010a	0.064±0.004a	0.079	0.277	0.569
C15:0	0.089±0.005[a]	0.086±0.004[a]	0.090±0.004[a]	0.078±0.005[a]	0.087±0.005a	0.087±0.004a	0.084±0.003a	0.087±0.005a	0.500	0.945	0.852
C16:0	25.3±0.1[a]	24.8±0.2[ab]	24.4±0.1[bc]	23.9±0.2c	24.8±0.2a	24.6±0.2a	24.5±0.2a	24.5±0.2a	0.000	0.350	0.039
t9C16:1	0.034±0.003[a]	0.032±0.002[a]	0.041±0.003[a]	0.032±0.003[a]	0.034±0.002ab	0.034±0.003ab	0.031±0.002b	0.042±0.003a	0.059	0.054	0.108
Unknown 1	0.750±0.023[a]	0.756±0.019[a]	0.737±0.028[a]	0.791±0.038[a]	0.730±0.020a	0.802±0.017a	0.728±0.040a	0.763±0.025a	0.646	0.146	0.206
c9C16:1	3.28±0.11[a]	3.09±0.10[ab]	2.80±0.11[bc]	2.54±0.14[c]	2.98±0.11a	2.99±0.12a	2.77±0.14a	2.94±0.12a	0.001	0.449	0.130
Unknown 2	0.024±0.003[a]	0.026±0.004[a]	0.022±0.002[a]	0.030±0.008[a]	0.024±0.002a	0.032±0.006a	0.025±0.005a	0.019±0.001a	0.513	0.141	0.024
C17:0	0.161±0.006[b]	0.166±0.005[b]	0.200±0.006[a]	0.196±0.010[a]	0.176±0.007a	0.178±0.007a	0.191±0.008a	0.183±0.008a	0.000	0.312	0.291
c9C17:1	0.118±0.005[b]	0.129±0.003[ab]	0.140±0.004[a]	0.127±0.006[ab]	0.133±0.006a	0.129±0.004a	0.136±0.003a	0.124±0.005a	0.003	0.218	0.149
C18:0	7.92±0.08[a]	7.60±0.25[a]	7.79±0.08[a]	7.84±0.45[a]	7.95±0.11a	7.45±0.32a	7.73±0.33a	7.92±0.09a	0.874	0.403	0.308

t9C18:1	0.105±0.011[a]	0.104±0.008[a]	0.097±0.010[a]	0.108±0.012[a]	0.121±0.013a	0.104±0.009a	0.086±0.007a	0.098±0.010a	0.879	0.140	0.899
OA	41.0±0.6[a]	39.4±0.4[b]	36.7±0.3[c]	37.8±0.5[bc]	38.6±0.4a	39.0±0.7a	37.9±0.5a	38.3±0.6a	0.000	0.546	0.338
c11C18:1	2.45±0.07[a]	2.28±0.05[b]	2.08±0.03c	1.98±0.06c	2.18±0.05a	2.23±0.07a	2.13±0.05a	2.20±0.05a	0.000	0.539	0.159
LA	13.0±0.7[c]	15.3±0.7[bc]	17.9±0.3[a]	17.4±1.1[ab]	15.3±0.5a	15.0±1.0a	16.3±0.8a	16.9±0.6a	0.000	0.115	0.461
C20:0	0.017±0.002[a]	0.025±0.003[a]	0.021±0.002[a]	0.028±0.004[a]	0.023±0.002ab	0.027±0.003a	0.025±0.004ab	0.015±0.002b	0.150	0.051	0.769
GLA	0.081±0.005[b]	0.084±0.003[b]	0.105±0.004[a]	0.104±0.007[a]	0.090±0.004a	0.086±0.005a	0.101±0.006a	0.100±0.006a	0.000	0.050	0.126
c5C20:1	0.032±0.003[a]	0.031±0.003[a]	0.030±0.002[a]	0.032±0.005[a]	0.029±0.003a	0.036±0.003a	0.033±0.004a	0.026±0.001a	0.973	0.143	0.166
c8C20:1	0.011±0.002[a]	0.014±0.002[a]	0.009±0.001[a]	0.013±0.004[a]	0.014±0.002a	0.012±0.002a	0.014±0.004a	0.007±0.001a	0.186	0.088	0.533
ALA	0.620±0.053[b]	0.730±0.044[b]	1.119±0.056[a]	1.044±0.064a	0.851±0.054a	0.847±0.081a	0.932±0.065a	0.965±0.070a	0.000	0.295	0.926
c11C20:1	0.228±0.006[a]	0.222±0.008[a]	0.211±0.004[a]	0.206±0.013[a]	0.216±0.006a	0.213±0.011a	0.216±0.009a	0.220±0.004a	0.270	0.946	0.081
CLA	0.013±0.002[a]	0.013±0.001[a]	0.011±0.001[a]	0.009±0.002[a]	0.012±0.002ab	0.013±0.002ab	0.014±0.002a	0.007±0.001b	0.266	0.048	0.439
Unknown 7	0.036±0.003[a]	0.043±0.003[a]	0.034±0.003[a]	0.033±0.003[a]	0.035±0.003a	0.038±0.003a	0.035±0.003a	0.040±0.002a	0.070	0.565	0.818
Unknown 8	0.021±0.002[a]	0.017±0.001[ab]	0.014±0.001[b]	0.012±0.001[b]	0.016±0.002a	0.018±0.002a	0.015±0.001a	0.014±0.001a	0.000	0.261	0.049
t11c13C18:2	0.033±0.002[a]	0.032±0.002[a]	0.022±0.001[b]	0.021±0.002[b]	0.026±0.002a	0.028±0.002a	0.026±0.002a	0.028±0.002a	0.000	0.847	0.163
unknown CLA(t,t)	0.010±0.002[a]	0.009±0.001[a]	0.010±0.002[a]	0.006±0.001[a]	0.014±0.002a	0.010±0.001ab	0.006±0.001b	0.007±0.001b	0.218	0.000	0.079
unknown CLA(t,t)	0.016±0.002[a]	0.012±0.001[a]	0.017±0.003[a]	0.012±0.002[a]	0.017±0.003a	0.015±0.002a	0.012±0.001a	0.013±0.001a	0.131	0.284	0.052
unknown CLA(t,t)	0.022±0.002[a]	0.019±0.002[a]	0.023±0.003[a]	0.015±0.001[a]	0.025±0.004a	0.020±0.002a	0.017±0.002a	0.018±0.002a	0.183	0.153	0.352
c9c13c15C18:3	0.014±0.007[a]	0.011±0.005[a]	0.017±0.007a	0.015±0.011[a]	0.024±0.009a	0.015±0.008a	0.012±0.006a	0.005±0.001a	0.925	0.310	0.177
c11c14C20:2	0.137±0.009[c]	0.159±0.008[b]	0.182±0.004a	0.179±0.011[ab]	0.164±0.006a	0.157±0.010a	0.170±0.010a	0.176±0.008a	0.000	0.237	0.120

unknown CLA(t,t)	0.053±0.005[a]	0.043±0.003[ab]	0.030±0.002[c]	0.035±0.005[bc]	0.044±0.003a	0.044±0.005a	0.037±0.003ab	0.030±0.002b	0.000	0.009	0.101
Unknown 9	0.069±0.005[ab]	0.072±0.004[a]	0.053±0.003[b]	0.057±0.004[ab]	0.065±0.005a	0.058±0.003a	0.063±0.005a	0.063±0.003a	0.002	0.604	0.432
C22:0	0.024±0.006[a]	0.026±0.003[a]	0.026±0.003[a]	0.028±0.007[a]	0.026±0.004a	0.030±0.005a	0.026±0.004a	0.023±0.003a	0.977	0.618	0.041
c8c11c14C20:3	0.122±0.007[b]	0.120±0.004[b]	0.148±0.006[a]	0.137±0.007[ab]	0.146±0.008a	0.129±0.006b	0.130±0.006b	0.124±0.004b	0.001	0.042	0.839
c11c14c17C20:3	0.018±0.001[b]	0.022±0.002[b]	0.029±0.002[a]	0.031±0.002[a]	0.029±0.002a	0.019±0.002b	0.026±0.002ab	0.027±0.002ab	0.000	0.007	0.819
AA	1.74±0.04[a]	1.83±0.07[a]	1.74±0.03[a]	1.97±0.10[a]	1.73±0.03a	1.79±0.08a	1.92±0.09a	1.80±0.04a	0.104	0.290	0.199
c13c16C22:2	0.008±0.001[a]	0.011±0.002[a]	0.011±0.001[a]	0.014±0.003[a]	0.010±0.001b	0.017±0.002a	0.010±0.002b	0.008±0.001b	0.110	0.009	0.205
EPA	0.014±0.002[c]	0.020±0.002[bc]	0.035±0.004[a]	0.033±0.004[ab]	0.026±0.003a	0.024±0.003a	0.028±0.004a	0.028±0.004a	0.000	0.775	0.855
C24:0	0.018±0.002[a]	0.021±0.002[a]	0.022±0.002[a]	0.035±0.009[a]	0.019±0.002a	0.029±0.007a	0.022±0.002a	0.024±0.002a	0.146	0.395	0.610
c7c10c19C22:3	0.126±0.037[a]	0.150±0.030[a]	0.152±0.029[a]	0.119±0.016[a]	0.331±0.021a	0.156±0.028b	0.032±0.002c	0.041±0.003c	0.510	0.000	0.011
c7c10c13c16C22:4	0.141±0.007[a]	0.156±0.005[a]	0.146±0.004[a]	0.155±0.009[a]	0.148±0.006a	0.141±0.005a	0.160±0.007a	0.154±0.005a	0.241	0.125	0.622
uknown 10	0.362±0.022a	0.315±0.016[a]	0.178±0.015[b]	0.179±0.016[b]	0.268±0.025a	0.245±0.022a	0.253±0.023a	0.242±0.022a	0.000	0.637	0.776
DPA	0.121±0.006[c]	0.127±0.005[bc]	0.157±0.005[a]	0.160±0.012[ab]	0.144±0.005a	0.139±0.008a	0.142±0.007a	0.143±0.009a	0.000	0.909	0.468
uknown 12	0.089±0.022[a]	0.112±0.019[a]	0.121±0.023[a]	0.166±0.062[a]	0.220±0.015a	0.189±0.050a	0.037±0.003b	0.036±0.003b	0.533	0.001	0.136
DHA	0.831±0.032[b]	0.856±0.028[b]	1.277±0.047[a]	1.146±0.066[a]	1.056±0.060a	0.982±0.075a	1.084±0.048a	1.053±0.051a	0.000	0.461	0.485
uknown 14	0.034±0.019[a]	0.014±0.002[a]	0.034±0.011[a]	0.043±0.027[a]	0.058±0.018a	0.040±0.020ab	0.008±0.001b	0.011±0.002ab	0.445	0.032	0.118
uknown 15	0.288±0.077[a]	0.272±0.040[a]	0.344±0.058[a]	0.300±0.043[a]	0.599±0.042a	0.396±0.052b	0.111±0.008c	0.099±0.007c	0.374	0.000	0.162
SFA	33.9±0.2[a]	33.1±0.3[a]	32.1±0.8[a]	32.4±0.6[a]	33.4±0.2a	32.7±0.4a	31.9±1.1a	33.0±0.2a	0.238	0.373	0.735
MUFA	47.4±0.7[a]	46.0±0.8[a]	43.0±0.9[b]	44.2±1.4[ab]	44.4±0.6a	45.8±1.2a	45.4±1.4a	44.0±0.6a	0.011	0.507	0.664

PUFA	17.1±0.8[c]	19.2±0.7[b]	23.3±0.3[a]	21.8±1.1[ab]	20.2±0.6a	19.7±1.0a	21.3±0.8a	21.6±0.7a	0.000	0.122	0.417
Unknown	1.67±0.12[a]	1.63±0.06[a]	1.65±0.11[a]	1.61±0.11[a]	2.02±0.06a	1.82±0.08a	1.42±0.12b	1.29±0.03b	0.969	0.000	0.328
SFA/PUFA	2.04±0.09[a]	1.80±0.08[ab]	1.38±0.04[c]	1.58±0.11[bc]	1.70±0.06a	1.81±0.11a	1.58±0.10a	1.57±0.06a	0.000	0.094	0.443
n-3 FA	1.80±0.09[b]	2.01±0.08[b]	2.82±0.11[a]	2.67±0.06[a]	2.51±0.11a	2.30±0.16a	2.36±0.11a	2.29±0.12a	0.000	0.365	0.391
n-6 FA	15.2±0.7[c]	17.6±0.7[bc]	20.4±0.3[a]	19.9±1.0[ab]	17.6±0.5a	17.3±0.9a	18.8±0.8a	19.3±0.6a	0.000	0.065	0.451
n-3/n-6	0.119±0.004[a]	0.134±0.021[a]	0.138±0.005[a]	0.167±0.031[a]	0.143±0.004a	0.149±0.024a	0.146±0.027a	0.119±0.005a	0.472	0.677	0.390
n-6/n-3	8.57±0.32[ab]	9.1±0.34[a]	7.53±0.28b	7.48±0.40[b]	7.17±0.22b	8.00±0.42ab	8.33±0.41ab	8.71±0.31a	0.002	0.014	0.323
EPA+DHA	0.846±0.033[b]	0.877±0.029[b]	1.312±0.050a	1.179±0.066[a]	1.082±0.062a	1.007±0.077a	1.112±0.052a	1.081±0.054a	0.000	0.431	0.526
EPA+DPA+DHA	0.967±0.038[b]	1.004±0.032[b]	1.468±0.052a	1.4±0.067[a]	1.225±0.064a	1.146±0.080a	1.254±0.057a	1.224±0.060a	0.000	0.432	0.540
n-3>18C	0.985±0.038[b]	1.03±0.063[b]	1.498±0.053a	1.459±0.047[a]	1.255±0.065a	1.234±0.081a	1.347±0.081a	1.251±0.060a	0.000	0.530	0.486
trans	0.222±0.012[a]	0.214±0.010[a]	0.211±0.016a	0.196±0.014[a]	0.234±0.019a	0.216±0.012a	0.187±0.010a	0.206±0.011a	0.707	0.138	0.654

1 Significances were declared: *** = P<0.001; ** = P<0.01; * = P<0.05; † =0.05<P<0.1; ns = P>0.1; Values that do not share the same letters are significantly different (Tukey's honestly significant difference test, P < 0.05) 2 SFA = saturated FA(C12:0, C14:0+9C13:1, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0); MUFA = monounsaturated FA (c9C14:1, t9C16:1, c9C16:1, c9C17:1, t6+t7+t8C18:1, t9C18:1, t11C18:1, c9C18:1 (OA), c11C18:1, c13C18:1, c5C20:1, c8C20:1, c15C24:1; PUFA = polyunsaturated FA (t10t14C18:2, t8c13C18:2, c9t12C18:2, t9c12C18:2, ctmix10,14+12,16C18:2, c9c12C18:2 (LA), c9c15C18:2, c6c9c12C18:3(GLA),c 9c12c15C18:3 (ALA), conjugated linoleic acid (CLA), t11c13C18:2, unknown CLA(t,t), unknown CLA(t,t), c9c13c15C18:3, c11c14C20:2, c9c11c15C18:3, c8c11c14C20:3, c11c14c17C20:3, c5c8c11c14C20:4 (AA), c13c16C22:2, c5c8c11c14c1720:5(EPA), c7c10c13c16C22:4,c7c10c19C22:3, c4c7c10c13c16C22:5, c7,c10,c13,c16,c19C22:5(DPA), c4,c7,c10,c13,c16,c19C22:6 (DHA)); omega 3(n-3) = c9c15C18:2, ALA, c9c13c15C18:3, c9c11c15C18:3, EPA, c11c14c17C20:3, DPA, DHA; n-3<18C (very long chain n-3): EPA, c11c14c17C20:3, DPA, DHA; omega-6 (n-6) = c9t12C18:2, t9c12C18:2, LA, GLA, c11c14C20:2, c8c11c14C20:3, AA, c13c16C22:2, c7c10c13c16C22:4, C22:5n-6; c = cis; t = trans

APPENDIX B.5 Table 1. 3. Means ± SE and ANOVA P values for the effect of management and year/outside access on yolk lipid content (%) and fatty acid (FA) profile (g/100g total FA) of eggs collected from supermarkets in the UK on April 2017 (no outside access) and April 2018 (outside access as normal).

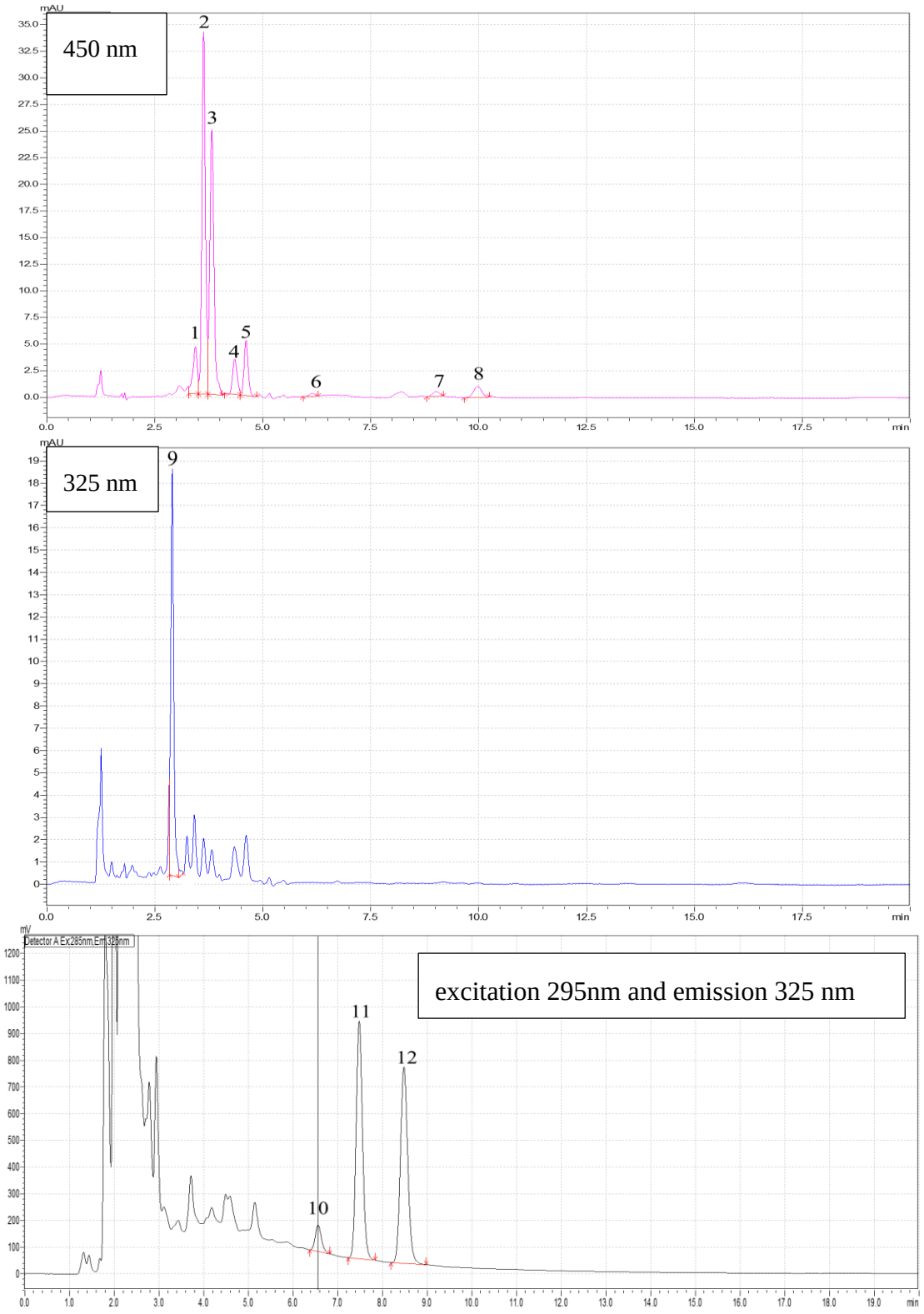
						ANOVA - P values[1]		
	Management (M)			Year (Y)		Main Effects		Interactions
	Caged	Free-range	Organic	Apr-17	Apr-18	M	Y	M x Y
	n=8	n=16	n=16	n=20	n=20			
Fatty acids[2]								
C12:0	0.016±0.002[a]	0.018±0.002[a]	0.018±0.001[a]	0.019±0.002	0.017±0.001	0.823	0.518	0.822
C14:0	0.338±0.017[a]	0.317±0.006[ab]	0.300±0.010[b]	0.315±0.008	0.314±0.009	0.077	0.913	0.577
C14:1	0.088±0.006[a]	0.071±0.004[a]	0.070±0.006[a]	0.081±0.004	0.067±0.005	0.086	0.046	0.453
C15:0	0.083±0.011[a]	0.091±0.007[a]	0.084±0.006[a]	0.085±0.006	0.088±0.006	0.742	0.664	0.511
C16:0	25.5±0.1[a]	24.4±0.2[b]	24.3±0.2[b]	24.7±0.2	24.5±0.2	0.001	0.407	0.471
t9C16:1	0.034±0.004[ab]	0.034±0.003[b]	0.048±0.005[a]	0.035±0.004	0.044±0.004	0.038	0.092	0.958
Unknown 1	0.778±0.031[a]	0.812±0.032[a]	0.791±0.023[a]	0.812±0.019	0.781±0.027	0.833	0.355	0.693
c9C16:1	3.54±0.09[a]	2.88±0.10[b]	2.95±0.16[b]	3.07±0.10	3.01±0.14	0.011	0.680	0.170
Unknown 2	0.020±0.003[a]	0.023±0.003[a]	0.023±0.004[a]	0.027±0.003	0.018±0.002	0.748	0.117	0.064
C17:0	0.148±0.007[b]	0.187±0.007[a]	0.190±0.009[a]	0.183±0.008	0.178±0.008	0.005	0.626	0.495
c9C17:1	0.127±0.012[a]	0.133±0.006[a]	0.133±0.005[a]	0.136±0.005	0.127±0.005	0.800	0.300	0.014
C18:0	7.75±0.10[a]	7.92±0.08[a]	7.71±0.11[a]	7.75±0.08	7.86±0.09	0.291	0.390	0.413
t9C18:1	0.105±0.020[a]	0.110±0.013[a]	0.107±0.010[a]	0.114±0.010	0.101±0.011	0.973	0.431	0.669
OA	41.7±0.6[a]	39.4±0.6[b]	37.3±0.4[c]	39.4±0.5	38.7±0.5.	0.000	0.275	0.975
c11C18:1	2.56±0.08[a]	2.23±0.04[b]	2.12±0.06[b]	2.25±0.07a	2.26±0.05	0.000	0.864	0.192
LA	12.0±0.7[c]	15.9±0.7[b]	17.9±0.4[a]	15.3±0.7	16.6±0.7	0.000	0.093	0.547
C20:0	0.013±0.003[a]	0.019±0.003[a]	0.022±0.005[a]	0.024±0.004	0.014±0.002	0.336	0.075	0.366
GLA	0.071±0.007[b]	0.084±0.004[b]	0.103±0.006[a]	0.086±0.007	0.092±0.003	0.004	0.391	0.253

c5C20:1	0.027±0.003[a]	0.028±0.004[a]	0.031±0.004[a]	0.031±0.004	0.027±0.002	0.761	0.469	0.832
c8C20:1	0.013±0.004[a]	0.018±0.009[a]	0.010±0.002[a]	0.020±0.007	0.007±0.001	0.665	0.132	0.540
ALA	0.587±0.050[c]	0.768±0.050[b]	1.053±0.076[a]	0.784±0.057	0.908±0.074	0.000	0.075	0.741
c11C20:1	0.230±0.007[a]	0.221±0.004[a]	0.215±0.005[a]	0.215±0.005	0.226±0.003	0.092	0.062	0.936
CLA	0.011±0.004[a]	0.011±0.002[a]	0.012±0.002[a]	0.015±0.002	0.007±0.001	0.730	0.047	0.203
Unknown 7	0.033±0.004ab	0.043±0.003a	0.033±0.003b	0.033±0.003	0.040±0.002	0.030	0.065	0.522
Unknown 8	0.015±0.002a	0.016±0.002a	0.016±0.002a	0.017±0.002	0.015±0.001	0.883	0.492	0.220
t11c13C18:2	0.037±0.004a	0.030±0.002ab	0.028±0.002b	0.031±0.003	0.030±0.002	0.063	0.752	0.896
unknown CLA(t,t)	0.005±0.001b	0.014±0.002a	0.009±0.002ab	0.012±0.002	0.008±0.001	0.032	0.075	0.903
unknown CLA(t,t)	0.012±0.003a	0.015±0.002a	0.016±0.002a	0.016±0.002	0.013±0.001	0.754	0.309	0.303
unknown CLA(t,t)	0.019±0.003a	0.015±0.002a	0.019±0.003a	0.016±0.002	0.019±0.002	0.236	0.222	0.372
c9c13c15C18:3	0.008±0.002a	0.006±0.001a	0.005±0.001a	0.006±0.001	0.006±0.001	0.435	0.819	0.555
c11c14C20:2	0.123±0.010b	0.167±0.011a	0.183±0.007a	0.154±0.009	0.174±0.009	0.003	0.111	0.335
unknown CLA(t,t)	0.055±0.007a	0.039±0.004b	0.029±0.002b	0.045±0.004	0.032±0.002	0.000	0.009	0.034
Unknown 9	0.061±0.005a	0.060±0.005a	0.055±0.004a	0.053±0.003	0.064±0.004	0.585	0.073	0.964
C22:0	0.010±0.003b	0.019±0.003ab	0.022±0.003a	0.013±0.001	0.024±0.003	0.013	0.009	0.032
c8c11c14C20:3	0.109±0.004b	0.115±0.004b	0.135±0.004a	0.122±0.004	0.122±0.004	0.001	0.906	0.732
c11c14c17C20:3	0.019±0.002b	0.028±0.003ab	0.030±0.003a	0.028±0.003	0.026±0.002	0.044	0.439	0.202
AA	1.68±0.06a	1.77±0.03a	1.79±0.04a	1.73±0.03	1.78±0.04	0.247	0.343	0.301
c13c16C22:2	0.011±0.002a	0.013±0.004a	0.014±0.003a	0.017±0.003	0.009±0.001	0.809	0.113	0.315
EPA	0.016±0.003b	0.022±0.003b	0.037±0.006a	0.028±0.004	0.026±0.004	0.005	0.754	0.901

C24:0	0.023±0.004a	0.023±0.002 a	0.025±0.003 a	0.024 ±0.00 2	0.024 ±0.00 2	0.849	0.885	0.992
c7c10c19C2 2:3	0.154±0.042a	0.149±0.031 a	0.135±0.028 a	0.250 ±0.01 3	0.039 ±0.00 3	0.055	0.000	0.261
c7c10c13c1 6C22:4	0.136±0.008a	0.156±0.004 a	0.149±0.007 a	0.143 ±0.00 5	0.155 ±0.00 5	0.165	0.157	0.576
unknown 10	0.364±0.024a	0.298±0.016 b	0.177±0.018 c	0.266 ±0.02 1	0.260 ±0.02 4	0.000	0.696	0.742
DPA	0.126±0.018a	0.128±0.009 a	0.147±0.008 a	0.134 ±0.01 0	0.137 ±0.00 7	0.238	0.821	0.728
unknown 12	0.096±0.025a	0.092±0.019 a	0.089±0.016 a	0.147 ±0.01 2	0.035 ±0.00 3	0.559	0.001	0.404
DHA	0.853±0.067b	0.871±0.027 b	1.172±0.073 a	0.975 ±0.06 3	1.001 ±0.05 4	0.000	0.736	0.902
uknown 14	0.013±0.003a	0.016±0.004 a	0.021±0.005 a	0.023 ±0.00 5	0.012 ±0.00 2	0.553	0.053	0.277
uknown 15	0.226±0.049a	0.210±0.037 a	0.203±0.037 a	0.328 ±0.02 4	0.093 ±0.00 7	0.190	0.000	0.558
SFA	33.9±0.1a	33.0±0.2b	32.7±0.1b	33.1± 0.2	33.0± 0.2	0.001	0.670	0.247
MUFA	48.5±0.7a	45.1±0.7b	42.9±0.5c	45.3± 0.7	44.5± 0.7	0.000	0.285	0.750
PUFA	16.1±0.8c	20.3±0.7b	23.0±0.5a	19.9± 0.8	21.2± 0.8	0.000	0.137	0.608
Unknown	1.61±0.09a	1.57±0.07a	1.41±0.06a	1.71± 0.05	1.32± 0.03	0.000	0.000	0.828
SFA/PUFA	2.15±0.11a	1.66±0.07b	1.43±0.04c	1.72± 0.08	1.61± 0.07	0.000	0.153	0.560
n-3 FA	1.82±0.12^{b}	2.01±0.08^{b}	2.61±0.15^{a}	2.25± 0.12	2.17± 0.13	0.000	0.559	0.936
n-6 FA	14.2±0.7^{c}	18.2±0.7^{b}	20.3±0.4^{a}	17.6± 0.7	18.9± 0.7	0.000	0.095	0.533
n-3/n-6	0.129±0.008^{ab}	0.111±0.003 b	0.129±0.008 a	0.129 ±0.00 6	0.115 ±0.00 5	0.049	0.057	0.568
n-6/n-3	7.89±0.40^{a}	9.13±0.26^{a}	8.13±0.45^{a}	7.98± 0.30	8.98± 0.33	0.049	0.033	0.351
EPA+DHA	0.870±0.065^{b}	0.893±0.029 b	1.209±0.077 a	1.003 ±0.06 6	1.027 ±0.05 7	0.000	0.735	0.915
EPA+DPA+ DHA	0.995±0.081^{b}	1.021±0.032 b	1.357±0.083 a	1.136 ±0.07 1	1.164 ±0.06 2	0.001	0.744	0.948
n-3>18C	1.01±0.08^{b}	1.05±0.03^{b}	1.39±0.08^{a}	1.16± 0.07	1.19± 0.06	0.000	0.767	0.962
trans	0.219±0.024^{a}	0.214±0.013 a	0.230±0.013 a	0.227 ±0.01 3	0.216 ±0.01 1	0.714	0.555	0.686

1 Significances were declared: *** = P<0.001; ** = P<0.01; * = P<0.05; † =0.05<P<0.1; ns = P>0.1; Values that do not share the same letters are significantly different (Tukey's honestly significant difference test, P < 0.05) 2 SFA = saturated FA(C12:0, C14:0+9C13:1, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0); MUFA = monounsaturated FA (c9C14:1, t9C16:1, c9C16:1, c9C17:1, t6+t7+t8C18:1, t9C18:1, t11C18:1, c9C18:1 (OA), c11C18:1, c13C18:1, c5C20:1, c8C20:1, c15C24:1; PUFA = polyunsaturated FA (t10t14C18:2, t8c13C18:2, c9t12C18:2, t9c12C18:2, ctmix10,14+12,16C18:2, c9c12C18:2 (LA), c9c15C18:2, c6c9c12C18:3(GLA),c 9c12c15C18:3 (ALA), conjugated linoleic acid (CLA), t11c13C18:2, unknown CLA(t,t), unknown CLA(t,t), c9c13c15C18:3, c11c14C20:2, c9c11c15C18:3, c8c11c14C20:3, c11c14c17C20:3, c5c8c11c14C20:4 (AA), c13c16C22:2, c5c8c11c14c1720:5(EPA), c7c10c13c16C22:4,c7c10c19C22:3, c4c7c10c13c16C22:5, c7,c10,c13,c16,c19C22:5(DPA), c4,c7,c10,c13,c16,c19C22:6 (DHA)); omega 3(n-3) = c9c15C18:2, ALA, c9c13c15C18:3, c9c11c15C18:3, EPA, c11c14c17C20:3, DPA, DHA; n-3<18C (very long chain n-3): EPA, c11c14c17C20:3, DPA, DHA; omega-6 (n-6) = c9t12C18:2, t9c12C18:2, LA, GLA, c11c14C20:2, c8c11c14C20:3, AA, c13c16C22:2, c7c10c13c16C22:4, C22:5n-6; c = cis; t = trans

Chromatogram of carotenoids, vitamin A and E



APPENDIX B.5 Figure 1. 1. Chromatograms and their retention time (RT) of carotenoids (450 nm), vitamin A (retinol) (325nm) and tocopherols (excitation and emission 295nm and 325 nm)1: unknown 1 (RT: 3’47), 2: lutein (RT: 3’65), 3:zeaxanthin (RT: 3’84) 4:unknown 2 (RT: 4’37), 5: canthaxanthin (RT: 4’65), 6: unknown 3 (RT: 6’19), 7: β-cryptoxanthin (RT: 9’02), 8: echinenone (internal standard) (RT: 10’00), 9: retinol (RT: 2’92), 10: δ-tocopherol (RT: 6’57), 11: γ-tocopherol (RT: 7’49), 12: α-tocopherol (RT: 8’49)