Review Article

Review on docosahexaenoic acid in poultry and swine nutrition: Consequence of enriched animal products on performance and health characteristics

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ABSTRACT

Omega-3 polyunsaturated fatty acids (n-3 PUFA) are linked to a variety of health benefits against human disorders and disease. However, the typical western diet is generally low in n-3 PUFA and high in n-6 PUFA, suggesting that the recommended intake of these essential fatty acids is seldom achieved. Therefore, dietary enrichment of animal meat and eggs with n-3 PUFA could help increase consumption of these fatty acids. Fish oils and microalgae (MA) are rich sources of long chain n-3 PUFA, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Feeding these marine products has been shown to increase DHA content of tissues and yolk, however, this may also lead to an increased requirement for anti-oxidants to prevent oxidative deterioration and associated negative sensory attributes. Nonetheless, increased DHA has been linked to promising results in animal growth, fertility, immunity and bone strength in both pigs and poultry. These findings suggest that feeding DHA-rich ingredients to mono-gastric can enrich human diets as well as providing additional benefits to the animal.

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1. Introduction

The typical western diet is deficient in omega-3 polyunsaturated fatty acids (n-3 PUFA), particularly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), while being abundant in omega-6 polyunsaturated fatty acids (n-6 PUFA), especially linoleic acid (LA, 18:2n-6), typically from the consumption of vegetable oils (Blasbalg et al., 2011). DHA is synthesised *de novo* from linolenic acid (LNA) by conversion to EPA, which is then further metabolised to DHA (Fig. 1). However, the desaturase enzymes used in this process are the same for both the n-3 and n-6 pathways (Schmitz and Ecker, 2008) and therefore the

rate of DHA synthesis is limited when the dietary LA to LNA ratio is high. At best, the conversion rate of LNA to long-chain n-3 PUFA is reported to be 4% (Shahidi and Ambigaipalan, 2018) in a typical western diet, indicating the importance of the ratio of LA to LNA on the supply of these essential fatty acids (FA).

Numerous reports have been published on the health benefits relating to n-3 PUFA and their effects on cardiovascular disease, diabetes, cancer, Alzheimer's disease, dementia, depression, retinal and neurological development, and immune function (Shahidi and Ambigaipalan, 2018). These potential health advantages of n-3 PUFA have led to a heightened interest in improving consumption of these essential FA. Oils containing n-3 PUFA originate primarily from marine sources, such as fish, shellfish and microalgae (MA). Lean fish such as cod store lipids in the liver, while fatty fish like salmon store lipid in the flesh. Due to a number of differences between species, such as metabolism, diet and growing conditions, the amount and composition of lipid will differ between fish. For example, cod liver oil contains greater amounts of EPA than DHA, while tuna oil is richer in DHA than EPA (Calder and Yaqoob, 2009). Therefore, not all sources of n-3 PUFA will provide the same quantity or composition of these essential FA. Nonetheless, due to

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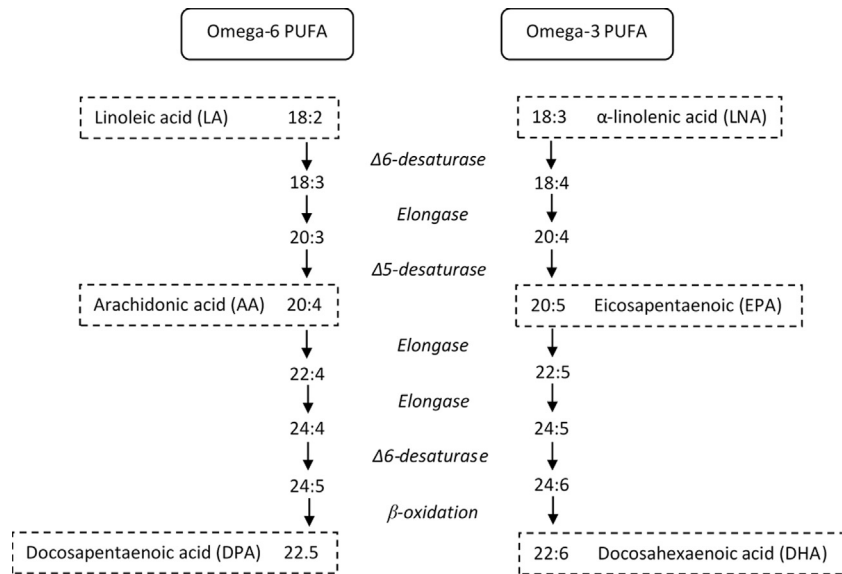
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Fig. 1. Polyunsaturated fatty acid (PUFA) metabolic pathway. Animals are unable to endogenously synthesise linoleic (LA) or α-linolenic acid (LNA) and therefore these essential fatty acids must be consumed from dietary sources. Endogenous desaturases and elongases are responsible for conversion of LA and LNA to the key metabolites: arachidonic acid (AA), docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Desaturase enzymes are the same for both omega-3 and omega-6 pathways, and therefore DHA production may be limited when the LA to LNA ratio of the diet is high.

the disproportionate consumption of poultry and pig meat in western diets compared with fish, such marine oils appear to be an attractive route to increase the level of n-3 PUFA in human diets. The European Food Safety Authority (EFSA) recommended that for a food to claim a source of omega-3 it must contain more than 0.30 g/100 g, or more than 0.60 g/100 g for high omega-3, in accordance to the recommended nutritional intake (2 g/day for an adult male) (Commission, 2005). Fortification of animal products such as dairy, meat and poultry products with n-3 PUFA for regular consumption has received considerable interest in recent years. However, consumer acceptability (Ganesan et al., 2014) and price (Watters et al., 2012) are some of the biggest challenges facing this movement within the livestock industry.

The recommended n-6:n-3 ratio is less than 4 (Wood et al., 2004), although for current animal products this ratio is between 10 and 15 (Kouba and Mourot, 2011). Commercially, poultry and swine diets typically include fats, derived from either animal or plant sources, alongside other ingredients to provide the recommended energy requirements to support health and growth of the animal. The type of fat used is largely driven by cost, but also other factors such as local availability, palatability, digestibility and performance response (Firman et al., 2008; Kanakri et al., 2018; Ravindran et al., 2016). Rarely does the composition of FA become a deciding factor on the type of fat fed. However, dietary supplementation of n-3 PUFA has been associated not only with the enrichment of animal products but also with some health benefits for both pigs and poultry (Swiatkiewicz et al., 2015), which may prompt greater consideration of dietary FA composition when formulating diets. Vegetable-based ingredients such as linseed meals/oils can be used to support the n-3 FA enrichment of animal diets (Kartikasari et al., 2012). However, LNA in linseed requires elongation and desaturation to generate EPA and DHA, a process which is limited in animals (Lopez-Ferrer et al., 2001). Therefore, attention has been given to supplementing diets with ingredients rich in DHA, since this is the final product in the de novo n-3 FA synthetic pathway. Fish oils can be a good source of DHA in the diet, although the intrinsically low oxidative stability of such ingredients can cause a rancid taste and odour in animal products (Hargis and

Van Elswyk, 1993). Therefore, advancements in the heterotrophic production of DHA enriched MA may offer an attractive opportunity for n-3 PUFA supplementation of animal diets (Barclay et al., 1994, 1998). In a marine environment, MA are a natural source of n-3 PUFA in the diets of fish, and therefore represent a more direct dietary source of these essential FA.

This review will cover the potential benefits of increasing DHA content of diets on growth performance, egg production, fertility, progeny performance, immunity and bone parameters, with particular focus on MA products. In addition it will consider the possible risks associated with increased exposure to products of lipid peroxidation that may adversely affect productively, as well as sensory quality and shelf-life of animal products.

2. Meat production

2.1. Growth performance

All animals require energy for maintenance and productive purposes, and therefore fats are essential in the diet to increase the caloric density of feeds to meet requirements. In addition to energy provision, the source of fat will also vary in composition in terms of saturation and chain length, all of which can affect functionality. Long chain PUFA are essential in the diet to promote optimal health, development and growth in the animal (Zhang et al., 2010). Digestion and nutrient absorption is one area of interest in this regard. It has been suggested that exposure to n-3 PUFA can increase glucose absorption in piglets (Gabler et al., 2007, 2009). This improvement was associated with increased n-3 PUFA content, 5′ adenosine monophosphate-activated protein kinase (AMPK) activity and abundance of glucose transporters (glucose transporter, type 2 [GLUT2] and sodium-dependent glucose transporter 1 [SGLT1]) in intestinal tissues. In rats, n-3 PUFA has been demonstrated to upregulate AMPK, a key sensor of energy status within cells (Suchankova et al., 2005). Once activated, AMPK increases translocation of GLUT2 to the brush boarder in mice (Walker et al., 2005), enhancing glucose uptake. It is possible that through such

mechanisms, performance of animals fed diets containing higher levels of n-3 PUFA could be enhanced.

Several studies have investigated the effect of enriching diets with n-3 PUFA on animal performance. Ribeiro et al. (2013) fed 7.4% DHA Gold (derived from *Schizochytrium* MA, Novus) to broilers from 21 to 35 days of age. Results showed a significant 195 g (22%) improvement in body weight gain of the DHA supplemented diets compared with the control without affecting feed intake. Despite the large effect on gain, FCR was not significantly influenced by treatment despite the calculated 26 points improvement with DHA. In a later study by Ribeiro et al. (2014), 7.4% DHA Gold was added to diets containing either standard (21%) or low (17%) crude protein (CP) and fed to broilers from 21 to 35 days of age. Adding DHA Gold to the standard CP diet significantly increased weight gain (by 188 and 319 g) and feed intake (by 145.2 and 251 g) and reduced FCR (by 57 and 44 points) at 28 and 35 days of age, respectively, compared to the non-supplemented standard CP diet. However, no effect of adding DHA Gold to the low CP diet was found. Although, it should be mentioned that birds fed the low CP diet performed significantly better than those fed the standard protein diet, possibly due to the inclusion of synthetic amino acids and increased energy from corn. It was also noted that pellets from the DHA Gold diet were more intact than control diets which may have led to greater feed intake and weight gain. However, other studies have also found no effect of feeding MA on broiler growth performance (Ao et al., 2015a; Rymer et al., 2010; Yan and Kim, 2013).

In a recent study, Konieczka et al. (2018) fed high (starter, 43.1; grower, 62.2; finisher, 51.1) and low (starter, 1.0; grower, 0.7; finisher, 0.5) n-6: n-3 ratios by varying inclusion level of fish/ linseed oil and corn oil in the diet, along with vitamin E added at 50 or 300 mg/kg to broilers. From 1 to 9 days of age, the low ratio reduced body weight of birds compared to the high FA ratio, while addition of higher vitamin E levels improved body weight, irrespective of FA ratio. FCR was significantly worse in birds fed low FA ratio diets, without an effect of vitamin E. From 9 to 42 days of age, FA ratio and vitamin E level had no effect on final body weight of birds, although FCR was numerically worse (around 8 points) for birds fed low FA ratio. Other studies have shown no negative effect of low n-6: n-3 ratios on broiler performance (Ibrahim et al., 2017; Qi et al., 2010). However, in these studies, the ratio was not as low as in the former study which might explain why some studies report negative effects of the low FA ratio, as n-6 also has an important function in birds. This might therefore suggest that supplementing diets with high concentrations of DHA via MA needs to take into account the balance with n-6 by keeping some vegetable oil in the diet.

Wei et al. (2013) found that feeding a diet containing MA DHA (7.5% Trevera, Novus International) to growing pigs enhanced muscle protein synthesis by increasing expression of muscle IGF-1 and insulin receptor activation, which may suggest a link to improved growth performance with n-3 PUFA supplementation. Body weight gain and FCR of pigs were numerically improved with 2.5% and 5.0% MA supplementation during the last 4 weeks of a 56-day trial (Sardi et al., 2006). MA also increased DHA levels in the loin and subcutaneous fat. However, Marriott et al. (2002) described no effect of feeding MA (Omega Tech, Boulder Colo) at 0.5% or 1.0% in starter (0–21 day) diets and 0.09% or 0.18% in grower (22–42 day) diets on feed consumption, ADG or FCR of pigs compared to the control. The much lower inclusion rate of MA in the latter trial may explain why performance affects were not evident. However, this inclusion rate was enough to cause a significant linear increase in DHA content of muscles. Pigs fed 1.6% MA (18% DHA) for the last 25 days prior to slaughter had improved weight gain and FCR compared to pigs fed 0.06% and 0.60% MA, respectively (Jon Meadus et al., 2011). However, carcass weight,

lean yield and pH were unaffected by treatment. It was also shown that expression of the adipose hormone, leptin, was significantly increased with increasing DHA inclusion rate. Leptin acts to lower feed intake in response to increased adipose cell numbers, although the concomitant improvement in both gain and FCR of pigs would suggest that feed intake was unaffected by treatment. In contrast, de Tonnac and Mourot (2017) found no effect of feeding 0.9%, 1.9% or 3.7% MA (DHA Gold, DSM) on the growth performance of pigs from 14 to 22 weeks of age. These studies suggest that there are potential performance benefits to feeding DHA to pigs and poultry. However, more importantly they show that increasing n-3 PUFA levels with the concomitant potential for increased oxidative stress does not bare a negative effect on performance when dosed at levels used in the aforementioned studies. Although, caution is advised when lowering the n-6:n-3 ratio to consider the importance of n-6 PUFA in the diet (Schmitz and Ecker, 2008).

2.2. Oxidative stability

It has been shown that lipid oxidation increases linearly with the concentration of PUFA, and the stability of PUFA deteriorates as the degree of unsaturation increases (Cortinas et al., 2005). Evidence suggests greater susceptibility of n-3 PUFA to oxidisation than n-6 PUFA (Li et al., 2013), although the reasons for this remain unclear. Lipid oxidation may in part, initiate in the gut prior to absorption and therefore any deleterious effects of dietary PUFA is likely to begin in the intestinal epithelium (Konieczka et al., 2018). MA are natural sources of carotenoids that have anti-oxidant properties (Gong and Bassi, 2016), and therefore may support oxidative stability to a greater extent than other n-3 PUFA sources. In a 21-day feeding trial, Rymer et al. (2010) found that supplementing 1.1%, 2.2% or 3.3% MA to 21-day-old broilers had no effect on oxidative stability of chicken breast muscle, as determined by aldehyde content. However, feeding 3.3% MA reduced oxidative stability in the leg muscle, which usually accumulates more fat than breast muscle, compared to the blended vegetable oil-fed control. No difference in feeding fish oil or MA on oxidative deterioration was shown. Other studies have also reported increased susceptibility to oxidation and discolouration of meat when feeding MA (Nute et al., 2007; Sirri et al., 2003). This might also suggest that the carotenoids associated with MA are not sufficient in mitigating oxidative instability and discolouration associated with increased n-3 PUFA in the diet. It was reported by Ruiz et al. (1999) that β-carotene, for example, only shows anti-oxidative properties when vitamin E reaches a certain level in tissues. Therefore, supplementation of additional anti-oxidants may be required to prevent oxidative deterioration when MA is added to the diet. For example, All-G-Rich (Alltech) is an MA product that contains ethoxyquin, a synthetic antioxidant added to protect against lipid peroxidation (Moore et al., 2002).

Increasing n-3 PUFA content of the diet increases vitamin E requirement of the animal as a result of increased susceptibility to peroxidation (Valk and Hornstra, 2000). Vitamin E is a lipid soluble vitamin known to exert its anti-oxidant properties by effectively scavenging peroxyl radicals and donating single electrons to form stable peroxide species (Fig. 2). In this way, vitamin E can prevent the peroxidation of lipid caused by increased PUFA levels. If the fats are oxidised before absorption they are not absorbed as effectively and thus Vitamin E can enhance digestibility by preventing oxidation before absorption. When added on top of a fish oil supplemented diet, vitamin E increased the content of DHA in egg yolk and breast muscle of the hen (Cherian et al., 1996) and reduced oxidative deterioration in the egg, liver, leg and breast muscle. It has been recommended that greater than 100 mg vitamin E/kg diet is required to protect meat from oxidative deterioration when
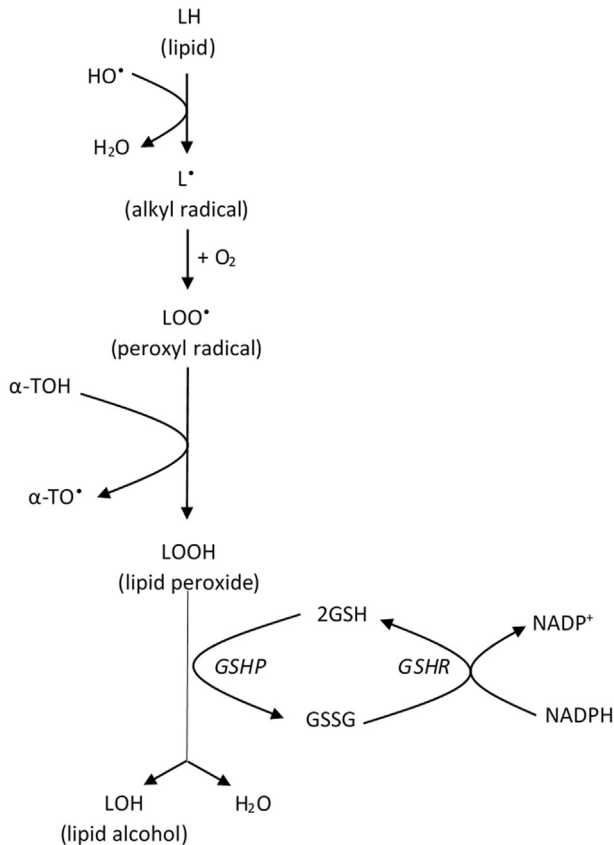
Fig. 2. Schematic of lipid peroxidation and scavenging reactions. Polyunsaturated fatty acids of the lipid membrane are highly susceptible to free radical damage. Reactive oxygen species, such as OH^-, take electrons from the lipid producing water and an alkyl radical. This fatty acid radical reacts readily with oxygen to generate a peroxyl radical. Vitamin E (α-TOH) donates an electron to the peroxyl radical to create a lipid peroxide, which is then further detoxified by glutathione peroxidase (GSHP) to produce a lipid alcohol and water. Glutathione reductase (GSHR) subsequently reduces glutathione disulphide (GSSG), consuming nicotinamide adenine dinucleotide phosphate (NADPH) and producing glutathione (GSH), which is then cycled back to GSHP.

reheated (Rymer and Givens, 2010). However, Konieczka et al. (2018) showed that addition of 300 mg/kg vitamin E improved oxidative stability of breast and thigh muscle of 43-day-old broilers, yet induced DNA damage in the duodenum and jejunum of 17-day-old broilers. This might suggest that vitamin E levels that significantly exceed the recommended levels induce pro-oxidative activity (Pearson et al., 2006) and this should be taken into consideration.

It has also been suggested that high selenium can increase DHA concentration in tissues, potentially through anti-oxidative mechanisms (Haug et al., 2007; Pappas et al., 2012).

In a study by Pappas et al. (2005), it was demonstrated that addition of 5.5% fish oil to the diets of young broiler breeders reduced the quality of stored eggs in terms of Haugh units, while addition of selenium alleviated this effect. Selenium is an essential component of selenoproteins, including glutathione peroxidase (GSHP) and glutathione reductase (GSHR), which act as a part of the anti-oxidant protective system (Duthie et al., 2007) by working in synergy to catabolise reactive oxygen species (ROS) and prevent oxidative stress in cells. It has also been suggested that GSHP works in synergy with vitamin E by detoxifying hydroperoxides produced from peroxyl radical scavenging (Surai, 2002), as illustrated in Fig. 2. This facilitates the accumulation of higher concentrations of DHA in eggs, while limiting the negative effects on lipid peroxidation. However, Konieczka et al. (2015) found no effect of

increasing dietary selenium on oxidative stability of meat with higher PUFA content when vitamin E was added in excess of requirements. A similar response was also demonstrated by Taulescu et al. (2011), suggesting that response to vitamin E and selenium is not additive. However, it should be noted that organic forms of selenium are more bioavailable and efficiently absorbed in the small intestine than inorganic forms (Mahima et al., 2012), and this will have a greater effect on oxidative status (Ahmad et al., 2012).

2.3. Sensory characteristics of meat

Meat quality is characterised by several factors that are influenced by lipids, such as firmness, shelf life, juiciness, tenderness and flavour (Wood et al., 2004). Although, it is thought that juiciness and tenderness are influenced by total lipid content rather than lipid composition per se (Wood et al., 2008). As FA unsaturation increases, melting point reduces which has a negative effect on meat firmness. Moreover, oxidation increases, not only of lipids but also of fat soluble pigments, leading to a reduced shelf life of the meat product. Concerns have been raised over the reduced oxidative stability of meat in response to increasing dietary n-3 PUFA and consumer acceptability (Leskanich and Noble, 1997). Oxidative deterioration is responsible for the formation of peroxides and off-flavours which reduce the sensory characteristics of meat (Wood et al., 2004). The thiobarbituric acid reacting substances (TBARS) test is a commonly used method for determining lipid oxidative stability of food, by measuring the oxidation product malonaldehyde (Tarladgis et al., 1960, 1964). Values of TBARS above 0.5 indicate a critical level of oxidation products, as they generate rancid odours and taste that can be detected by consumers. Therefore, it is important to understand the relationship between n-3 PUFA fortification of meat products, antioxidant supplementation levels and acceptable meat quality characteristics.

Ribeiro et al. (2013) found no effect of supplementing 7.4% DHA Gold on pH, cooking loss, shear force, tenderness or juiciness of broilers. However, birds fed DHA Gold had increased off-flavour, and reduced flavour and overall acceptability, compared to the control. In this study, TBARS exceeded 0.5 in breast meat at 6 days of storage and in the thigh from 4 days when DHA Gold was fed. Conversely, TBARS remained below 0.5 in control fed birds throughout. Similar findings were also shown in a later trial (Ribeiro et al., 2014). The deterioration of meat flavour quality with MA supplementation was also reported by Mooney et al. (1998). Feeding 2.8% and 5.5% MA DHA to broilers for 28 days significantly increased DHA and reduced n-6 PUFA content of the breast meat relative to the control. However, 5.8% MA DHA also increased total saturated and mono-unsaturated FA in breast meat, suggesting a possible compensatory mechanism to maintain normal membrane function. Following 3 days of storage, pre-cooked meat TBAR and flavour scores were decreased with DHA supplementation of diets, although only 5.8% DHA was deemed unfavourable with increased storage time. Even so, TBARS values were relatively low and did not exceed 0.5 for any treatment. It was noted by Mooney et al. (1998) that the reduction in flavour score was less pronounced for MA than fish oil, which could be due to a number of possible reasons including the cell encapsulation of the oils providing some protection from oxidation prior to ingestion and greater anti-oxidant content, primarily carotenoids, providing protection against oxidative deterioration (Ribeiro et al., 2013).

Vossen et al. (2017) found that feeding 0.3%, 0.6% or 1.2% MA (DHA Gold, Alltech) to pigs significantly increased DHA content in the loin (10−20 mg/100 g) and dry cured hams (25−56 mg/100 g), compared to feeding soybean oil (2.4 mg/100 g loin and 4.5 mg/100 g dry cured ham) or linseed oil (4.3 mg/100 g loin and 9.1 mg/100 g dry cured ham). Colour parameters and oxidative stability in

the loins was unaffected by MA feeding. Conversely, oxidative stability as well as hardness and chewiness was reduced in dry cured hams with MA treatment compared to soybean oil. These findings were not overly surprising, since dry cured hams are particularly susceptible to oxidation due to their extended maturing time. However, no treatment effects were demonstrated for customer sensory analysis of the dry cured hams. This might be due to the relatively low inclusion rate of MA in the later pig study compared to the aforementioned poultry studies, which could have limited the degree of oxidative stress. These results suggest that the extent of n-3 PUFA induced oxidation may be dependent on the type of meat product and inclusion rate of MA.

2.4. White striping

White striping is a breast muscle defect associated with the appearance of white striations parallel to muscle fibres. This muscle defects are thought to ascend from insufficient oxygen supply and subsequent oxidative stress leading to a loss of protein structural integrity and consumer acceptability. An increase in the severity of white striping has been associated with an increase in intramuscular fat and a reduction in protein in the breast muscle (Petracci et al., 2014). Kuttappan et al. (2012) reported differences in FA composition between normal and severe degrees of white striping. Breast muscle with severe white striping had significantly lower levels of EPA and DHA, despite having a greater level of LNA. Therefore, increasing DHA in the diet may alleviate this effect, although it may also increase oxidative stress if not added in combination with antioxidants, as previously discussed. Research into this area is scarce, but should be considered.

3. Eggs and fertility

3.1. Egg production

Egg yolk comprises around 30% lipid, the composition of which varies depending on the hen's age, strain and diet (Grobas et al., 2001; Şahan et al., 2014). Reports indicate that including a source of n-3 PUFA in laying hen diets is an effective way of increasing content of these essential FA in egg yolk (Hargis and Van Elswyk, 1993). However, it has been suggested that increased n-3 PUFA in the diets of laying hens can have adverse effects on egg production and quality characteristics, although reports are inconsistent (González-Esquerra and Leeson, 2001). Herber and Van Elswyk (1996) fed 2.4% and 4.8% natural golden MA (Omega Tech Inc., DHA content 7.4% of total FA) to 24- and 56-week-old laying hens in two 4 week experiments. Egg production was significantly reduced at 4.8% inclusion in the 56-week-old birds, while 2.4% MA had no negative effect on egg production. Addition of MA had no effect on saturated or monounsaturated FA content of yolk. However, MA reduced n-6 and increased n-3 FA, and specifically DHA, in a dose dependant manner. It could be calculated that the efficiency of DHA deposition from MA to egg yolk was 74.8% and 48.9% in hens fed 2.4% and 4.8%, respectively. It has been noted that long chain n-3 PUFA accumulation in the yolk plateaus between 1.5% and 3.0% of fish oil inclusion in the diet (Van Elswyk, 1997; Van Elswyk et al., 1995). This might explain why efficiency of DHA deposition was lower with higher MA inclusion in this study, as long chain n-3 PUFA content of eggs reached a plateau. In a later experiment, Herber-McNeill and Van Elswyk (1998) reported enhanced yolk colour with 2.4% and 4.8% MA, by reducing lightness and improving redness of the yolk, while maintaining flavour acceptability. This was interesting, since similar n-3 FA levels in yolk from fish oil resulted in poor sensory quality (Van Elswyk et al., 1992, 1995),

which might suggest that the accompanying carotenoids were supporting oxidative stability of the PUFA supplied by MA.

More recently, Park et al. (2015) fed layers at 40 weeks of age 0.5% and 1.0% MA powder (DHA content 37.6% of total FA) for 6 weeks. From 40 to 43 weeks, egg production was not significantly affected by MA, however, from 43 to 46 weeks 1.0% MA increased egg production. MA had no effect on egg weight, although at 46 weeks, egg shell thickness was significantly improved with increasing MA concentration. LA content of the yolk decreased and DHA increased with increasing MA concentration, lowering the n-6:n-3 FA ratio. In another study, 45-week-old laying hens were fed diets containing dehydrated MA (All-G-Rich, Alltech) containing 16% DHA (Ao et al., 2015b). Supplementing 1%, 2% and 3% MA to layer diets increased DHA content of the egg yolk by 261, 469 and 528 mg/100 g, respectively, compared to the non-supplemented control. Egg production, egg weight and yolk percentage were not affected after 32 weeks of dietary MA supplementation. However, yolk lightness was significantly reduced and redness increased with 2% and 3% MA compared to the control after feeding diets for 25 weeks. Moreover, storage of eggs up to 30 days had no effect on oxidative stability of egg yolk when feeding any level of MA.

From these studies it is clear that feeding MA to hens improves DHA content of eggs, although the extent of deposition may be restricted. In general, egg performance results suggest limited evidence for adverse effects of increasing DHA content of the diet through MA supplementation. This may be accredited to the carotenoid content of MA supporting oxidative stability and thus mitigating any negative effects of increased n-3 PUFA levels. There is clearly an economic optimum inclusion rate for MA if the goal is to enrich eggs with n-3 PUFA while avoiding any adverse effects on egg production, and understanding this balance is paramount for acceptability of this approach.

3.2. Fertility

Semen quality is a major factor affecting fertility in pigs and poultry. Avian semen characteristically contains a high proportion of PUFA, particularly n-6 PUFA (Surai et al., 1998). However, the importance of n-3 PUFA in semen quality has been made evident in a number of studies. Kelso et al. (1997c) noted that a reduction in the number of spermatozoa and sperm motility in seminal plasma from ageing bulls was related to a reduction in the proportion of DHA in sperm phospholipid. In male broiler breeders, Kelso et al. (1997a) found that supplementing diets with 6% LNA maintained DHA levels in sperm phospholipid from 24 to 54 weeks of age, while feeding 6% LA only maintained DHA content until 39 weeks. However, dietary FA source had no effect on sperm motility or fertility up to 72 weeks of age.

Blesbois et al. (1997a) also found a clear relationship between dietary lipids and the FA profile of spermatozoa with male broiler breeders fed fish oil having a greater proportion of n-3 FA (9.6% vs. 4.3%) and lower n-6 FA (22.4% vs. 33.3%) than birds fed corn oil. Additionally, feeding fish oil significantly increased fertility rates (96%) compared to corn oil fed birds (91.6%). In another study by Blesbois et al. (1997b), similar findings were described, however, it was noted that the benefit of fish oil on fertility was only evident in fresh semen, while in cryopreserved semen not only was fertility lower than fresh, the effect of fish oil was actually negative, presumably due to oxidative stress acting out over time.

Surai et al. (2000) studied the effects of long-term feeding of high n-3 PUFA diets to male broiler breeders from 26 to 60 weeks of age. At 60 weeks, feeding fish oil rich in DHA (18.5%) significantly increased DHA content in the sperm phospholipid (4.3% vs. 1.6%) and decreased the proportion of arachidonic acid (8.7% vs. 11.2%), thereby reducing the n-6:n-3 ratio (5.0 vs. 18.1) compared to

feeding maize oil. Feeding fish oil with 40 mg/kg vitamin E significantly reduced vitamin E levels in the whole semen and increased *in vitro* susceptibility to peroxidation compared to maize oil. However, increasing the vitamin E inclusion level to 200 mg/kg enhanced vitamin E content of the semen and reduced *in vitro* susceptibility to peroxidation in roosters fed fish oil. Semen volume and the number of spermatozoa per ejaculate decreased from 26 to 60 weeks by around 50% in birds fed maize oil, while feeding fish oil prevented this age-related decline in spermatozoa. However, overall fertility (%) was unaffected by treatment. Kelso et al. (1997b) found that the maximum level of DHA in sperm phospholipid when feeding 3% fish oil to broiler breeders was <10% of total FA, which is still considerably lower than that found in humans (35.2%) and bulls (61.3%) fed diets without fish oil. This suggests that n-3 PUFA is probably less essential for avian spermatozoa efficacy compared to other species.

The content of DHA in boar sperm has been positively correlated with motility, viability and normal morphology (Am-in et al., 2011). In contrast to the work of Blesbois et al. (1997a) in broilers, Paulenz et al. (1995) found minimal changes to the composition of boar sperm when feeding 75 mL/day fish oil (12.7% DHA) for 9 weeks. In a 28-week feeding trial, boars housed in research and commercial conditions were fed a diet containing fish oil with either 18% or 33% DHA (Castellano et al., 2010). The content of n-3 PUFA in boar sperm increased with feeding fish oil compared to animal fat, suggesting that longer supplementation is required to observe a change in FA composition of boar sperm. However, sperm production, morphology and motility were unaffected by treatment. In a more recent study (Murphy et al., 2017), boars fed 75 g MA (All-G-Rich, Alltech) per day on top of a basal diet increased semen volume and sperm numbers per ejaculate compared to pigs fed control diets. Feeding MA increased DHA content of sperm, while having no effect on DHA content of seminal plasma. However, increasing the inclusion rate of MA to 150 g/day gave no additional benefit to any measured parameter relative to control or 75 g/day MA fed pigs suggesting excess inclusion levels are counterproductive. Moreover, sperm quality, farrowing rate and litter size were unaffected by treatment. Rooke et al. (2001a) found a change in the composition of FA in sperm, specifically an increase in DHA and a reduction in n-6 docosapentaenoic acid (DPA), when feeding 3% fish oil. Seminal volume and concentration were unaffected by treatment, while sperm viability, motility and morphology were improved.

3.3. Progeny performance

Maternal nutrition plays a crucial role in the growth and development of animals, whether it is supplied through gestation, lactation or deposition into the egg. During the 21-day incubation period, the majority of yolk lipids are consumed by the developing embryo (Noble and Cocchi, 1990), primarily as a source of energy, while approximately 50% of total FA in yolk is incorporated into the newly hatched chick (Lin et al., 1991). Through manipulation of the maternal diet, it is therefore possible to alter the composition of FA transferred to the offspring and this presents a potential option for enriching hatching eggs with n-3 PUFA. The importance of early exposure to essential FA for development and growth is reviewed by Cherian (2015).

In a study by Macalintal et al. (2016), broiler breeders fed 1% MA (All-G-Rich, Alltech) plus EconomasE (proprietary blend designed to improve oxidative status), increased selenium, manganese and iron content of eggs. The DHA content of tissues from the progeny of breeder hens fed MA was greater than chicks from control-fed breeders. Moreover total n-3 FA concentrations were higher in the brain, liver and muscle tissues in chicks from MA-fed breeders. In another study, Kanakri et al. (2017) fed broiler breeders diets low

in n-3 PUFA (4% tallow), or high in LNA (4% linseed oil) or EPA + DHA (4% fish oil), and found that the n-3 PUFA content of egg yolk and day-old chick meat tissues corresponded closely to the n-3 PUFA content of the maternal diet. However, greater transfer of n-3 PUFA to chicks did not translate into an improved capacity for accumulating long chain n-3 PUFA in meat tissues in 42-day-old progeny chicks when fed high LNA diets post-hatch. Unfortunately, authors did not feed fish oil post-hatch and therefore the effect of directly supplementing long chain n-3 PUFA on LNA conversion at 42 days was not assessed. Overall, these results suggest that *in ovo* supply of n-3 PUFA can alter the FA composition of chicks, although subsequent feeding of n-3 PUFA post-hatch is required to continue supply of n-3 PUFA to birds.

Noble et al. (1986) postulated that poorer hatchability associated with eggs from young breeders is due to differences in yolk lipid composition between these early-lay birds and those at peak-lay. Feeding fish oil to the young (23 weeks) breeder has been demonstrated to reduce hatchability and day-old chick weight, while having no significant effect on embryonic mortality or fertility (Pappas et al., 2006). Addition of selenium alongside fish oil alleviated the negative effect on hatchability and day-old chick weight, which reaffirms the importance of combining an antioxidant with PUFA supplementation. However, fish oil was added at 5.5% of the breeder diet which might suggest that this inclusion level was too high. Cherian (2008) showed that whole egg, yolk and shell weight, as well as yolk colour was reduced with feeding higher n-3 PUFA (3.5% fish oil) compared to low n-3 PUFA diets (1.75% fish oil and 1.75% yellow grease) in hens from 26 to 62 weeks of age. However, albumen weight, shell thickness, haugh unit, fertility and hatchability were not significantly affected by treatment. Overall fertility was 98.6% and 97.4% for low and high n-3 eggs, respectively, compared to 96% for commercial eggs. Moreover, hatchability of fertile eggs was 80% and 84%, respectively, compared to 80% for commercial eggs, indicating favourable effects of increasing n-3 PUFA content of hen diets. In another study by Koppenol et al. (2015b), increasing DHA levels in the diet of broiler breeders from 24 to 58 weeks of age had no effect on fertility, hatchability, embryonic mortality or chick quality. However, a reduction in chick weight by 1.7 g and residual yolk weight was shown with higher DHA levels. It should be noted, however, that chick weight was on average 48.39 g vs. 46.68 g for control and high DHA broiler breeders, respectively, which is already heavier than expected by the breed standards (42 g). Offspring performance in terms of weight gain and FCR was unaffected by feeding higher DHA to broiler breeders. Again, these results suggest negligible undesirable effects of feeding higher n-3 PUFA, specifically DHA, on breeder and progeny performance. Potential reasons for some of the observed effects of n-3 PUFA on hatchability are discussed later.

In contrast to poultry, pigs rely on the trans-placental supply of nutrients to the developing foetus with glucose providing the main source of energy. Pigs are also litter bearing and thus maternal delivery of nutrients to each foetus can differ depending on their position along the uterine horn (Lima et al., 2018). Post-partum, maternal supply of nutrients to piglets is continued until weaning at around 28 days of age through lactational milk supply. Numerous studies have demonstrated that the modification of sow diets can influence supply and composition of FA to piglets during gestation and lactation (Lauridsen and Jensen, 2007; Rooke et al., 2000; Tanghe et al., 2014), thereby suggest a possible route for increasing n-3 PUFA transfer to piglets.

Rooke et al. (2001b) found that feeding 1.75% fish oil to sows during pregnancy resulted in improved piglet growth during the first 35 days postpartum than piglets from sows that were fed a control diet. Rooke et al. (2001c) also found that feeding 1.65% fish oil to sows through gestation and lactation increased gestation

length and reduced birth weight of piglets, while having no effect on litter size. However, pre-weaning mortality was significantly reduced in piglets from sows fed fish oil compared to control diets. Long-chain PUFA have been associated with improved cardiovascular function (Tsuburaya et al., 2011) and thus might partly explain why reductions in mortality were shown in progeny of sows fed fish oil, even though birth weight was reduced. Luo et al. (2013) reported no effect on birth weight, but improved daily gain (from 0 to 21 days of age) in piglets from sows fed 7% fish oil. Following weaning, piglets from sows fed a control diet were offered a diet containing 7% fish oil. These piglets had a poorer growth rate from 35 to 70 days of age than control diet fed piglets from sows fed fish oil, but not different from that of control fed piglets from control fed sows or fish oil fed piglets from fish oil fed sows. This might suggest that feeding 7% fish oil to piglets is too high, and palatability may have played a part in this response, more so for pigs that were not previously exposed to maternally supplied n-3 PUFA from fish oil.

Newly hatched chicks are highly dependent on maternal antibodies supplied in the egg yolk for immune defence until around 14 days of age when the chick becomes immunocompetent (Cherian, 2015). It has been demonstrated that chicks hatched from hens fed low LA:LNA ratio had higher concentrations of circulating immunoglobulin G (IgG) than chicks from hens fed a high ratio, suggesting that increasing n-3 FA content of the hen's diet can affect passive immunity of the progeny (Wang et al., 2004). Moreover, suppressed cell-mediated immune responses have been shown in chicks from hens fed high n-3 PUFA levels (Wang et al., 2000a, b). These results suggest that in ovo supply of n-3 PUFA may alter passive immunity in progeny chicks. However, the fact that the chicks in the latter studies were continued on a diet with a similar dietary fat source as the hen may also have played a role in this immune response. This potentially confounding effect is suggested by Koppenol et al. (2015a), who showed that feeding hens high n-3 PUFA diets had minimal effect on passive immunity in chicks raised on a standard diet.

In sows, a number of studies have demonstrated that supplementing diets with n-3 PUFA can modulate immune status of piglets (Swiatkiewicz et al., 2015). Fritsche et al. (1993) showed that feeding 7% fish oil to sows during gestation and lactation significantly increased n-3 PUFA in the immune cells of 21d piglets and reduced basal pro-inflammatory eicosanoid release by 60% to 70% in vitro. Such a response if reflected in vivo may result in more moderate inflammatory responses which could benefit animal performance. A study by Farmer et al. (2010), reported that feeding 3.5% linseed oil in late gestation and through lactation improved antibody response to ovalbumin vaccine in piglets that gained weight during the first 24 h postpartum, suggesting a relationship with colostrum consumption. Although no growth performance benefits were observed in piglets from sows offered diets with increasing dietary n-3 PUFA levels, mortality in piglets was reduced. Luo et al. (2013) reported a decline in pro-inflammatory cytokine expression in muscles of piglets from sows fed 7% fish oil. However, feeding fish oil post weaning increased expression of pro-inflammatory cytokines in the spleen, with the authors suggesting that a moderate supply of n-3 PUFA from maternal milk may be beneficial, while feeding too high a concentration post-weaning to piglets can have the opposite effect. Thus the role of n-3 supply and its ratio with n-6 on immune status may differ when fed to the sow rather than the weaned piglet itself, perhaps implicating the combination with feed and no milk as being important in determining the response observed.

Other studies have been able to show that prenatal exposure to n-3 PUFA can enhance intestinal glucose absorption in weanling piglets. Gabler et al. (2007) fed 1% fish oil (Gromega365; JBS United, 13% DHA, 13% EPA) or 0.14% MA (S-type Gold Fat, Advanced BioNutrition, 40% DHA, 2% EPA) to sows during the entire gestation and lactation period. Results indicated that increasing n-3 PUFA in the diet by feeding either fish oil or MA increased DHA content of the milk compared to the control, and subsequently DHA content of the jejunum and muscle tissue of progeny piglets. Moreover, ex vivo active glucose transport in jejunum samples was approximately 4 and 6 folds greater in piglets from sows fed fish oil and MA, respectively, compared to the control. Expression of glucose transporter genes (GLUT2 and SGLT1) and AMP-activated protein kinase activity in the jejunum also tended to increase in piglets from fish oil and MA fed sows. Muscle glycogen was also significantly greater (46 vs. 34 mg/g wet tissue) in piglets from sows fed algal DHA compared to the control, but not in piglets from fish oil fed sows. Later, Gabler et al. (2009) found that feeding n-3 PUFA via 1% fish oil (Gromega365) to sows during gestation and not through lactation resulted in enhanced glucose absorption in piglets. It was also demonstrated that supplying DHA to intestinal preparations taken from piglets of sows fed a control diet (low n-3 PUFA) increased glucose absorption. Given the marked effects of fish oils and n-3 sources on glucose absorption it is surprising that the weight gain and FCR data is not more universally positive, however it does suggest that improved immune status and animal performance and reductions in mortality where noted may be as a result of improved energy supply to the enterocytes and the animal as a whole.

4. Immune response

A meta-analysis of poultry studies by van der Most et al. (2011) showed that selection for rapid growth considerably compromises the immune response of chicks to different immune-challenges. However, the addition of different sources of dietary PUFA can be an efficient tool for modifying immune function (Calder, 2001). The FA composition of immune tissues is closely related to the composition of the dietary fat source used (Fritsche et al., 1991b). Incorporation of unsaturated FA into immune cell membranes improves cell flexibility (Pike, 2003), and modulates functionality of specific immune cells (Calder, 2007). The type of PUFA present in these tissues can modulate eicosanoid production (Craig-Schmidt et al., 1987), and thus inflammatory response, suggesting the importance of n-6:n-3 balance for an optimal functioning of the immune system. Fritsche et al. (1991a) described suppression of spleen lymphocyte proliferation and improved humoural immune response by enhanced antibody production in chicks fed diets containing n-3 PUFA. Additionally, Selvaraj and Cherian (2004b) demonstrated increased anti-bovine serum albumin (BSA) antibody concentrations in the blood and delayed hypersensitivity inflammatory response in broilers fed diets containing n-3 PUFA (3.5% linseed or fish oil), due to a reduction in pro-inflammatory cytokines. It would be expected such a response would release a significant amount of nutrients from the inflammatory process and thus enable a greater proportion of digested nutrients to be directed towards growth. Indeed increased body weight and feed intake was noted in n-3 PUFA fed birds compared to n-6 PUFA (conjugated linoleic acid [CLA] and sunflower oil). A similar response was seen by the same authors in laying hens (Selvaraj and Cherian, 2004a), and it was also noted that yolk anti-BSA antibody content was higher in hens fed 3% fish oil compared to CLA and animal fat or sunflower oil. Puthpongsiriporn and Scheideler (2005) also showed improved humoural response through increased antibody production to Newcastle disease and infectious bursal disease in pullets fed low n-6:n-3 PUFA ratio (linseed) diets, without any impact on performance. Moreover, Guo et al. (2004) reported greater anti-BSA antibody production and blood lysozyme activity, and lower in vitro leucocyte production of prostaglandin E_2 (PGE_2) in hens fed diets rich in n-3 PUFA (linseed or fish

oil) than hens fed n-6 PUFA (maize oil), without any impact on performance. Results of these trials suggest that increasing n-3 PUFA in the diet can improve humoural immune response and reduce cell-mediated responses, thereby positively influencing immune function. However, this response does not always result in an improved growth performance (Swiatkiewicz et al., 2015).

5. Bone parameters

Wilkins et al. (2011) estimated that 15% to 55% and 30% to 95% of laying hens in cage and non-cage systems, respectively, suffer from keel bone breakage due to a combination of poor bone strength and housing system. This welfare issue is therefore likely to worsen in response to the 2012 EU directive (1999/74/EC) banning conventional battery cages. In recent years, there has been growing evidence on the benefits of dietary fats on bone health. A number of studies performed in rats indicate the protective effect of n-3 PUFA on age-induced bone loss by reducing local catabolic factors (PGE$_2$ and NO) and increasing systemic anabolic factors (IGF-1) and calcitrophic hormones (PTH and calcitriol), thereby reducing the rate of bone turnover (Shen et al., 2006, 2007). A high dietary ratio of n-6 to n-3 PUFA has been positively correlated with depressed bone formation rates (Watkins et al., 2000). A study by Reinwald et al. (2004) revealed that n-3 deficient rats had reduced structural integrity of the tibiae, with repletion of n-3 FA accelerating bone modelling indicating a compensatory effect. Therefore, there is potential to reduce bone breakage in laying hens, and even bone defects in broilers, by supplementation diets with n-3 PUFA.

Tarlton et al. (2013) fed linseed to laying hens from 6 commercial free range flocks to increase dietary LNA levels. Results showed that the incidence of keel bone fractures in the older bird was reduced by 62 and 42% compared to control fed hens at 50 and 70 weeks, respectively. Moreover, the higher LNA diet also improved bone mineral density and mechanical performance. However, it should be noted that in this trial linseed was not the only ingredient altered in the higher LNA diet, the source of Ca and P was also different, which would likely also have an effect on bone characteristics. In another study by Toscano et al. (2015), control fed birds had a greater incidence of keel fracture, measured by dissection, when fed a diet with a low n-3 (n-6:n-3 ratio of 8.9), compared to higher n-3 diets (n-6:n-3 of 1.3) composed of fish oil-linseed. However, feeding even lower n-6: n-3 ratio of 0.69 had no benefit on the occurrence or severity of fracture compared to the control suggesting an oversupply of n-3 completely undermines any benefits. In a 12-week feeding trial, Johnston et al. (2010) fed pullets corn oil, linseed or a proportion of the diet replaced with 10%, 20% or 50% MA. DHA level in the diet ranged from 0 to 0.94%. Strength of the tibia and humerus improved at 50% MA inclusion level, while femur strength was increased at all algal inclusion rates, compared to the controls. This suggested that feeding MA in replacement of linseed or corn oil can improve bone strength in layers.

Bone weakness is not only an issue in laying hens but also fast growing chickens due to the great weight-bearing load put onto the legs. Ao et al. (2015a) reported that feeding 1% or 2% MA (All-G-Rich, Alltech, 16% DHA) significantly improved humerus breaking strength in 19-day-old broilers, compared to birds fed 0 or 0.5% MA. In a long-term trial, feeding 5% fish oil for 7 months increased tibia ash, mineral content and mechanical properties in quail compared to feeding soybean oil or animal fat (Liu et al., 2003). In this study, body weight and feed intake were unaffected by treatment.

6. Conclusions

Enriching animal products directly with DHA appears a promising route to improve consumption of these essential FA, particularly in western populations. Supplementing diets with vegetable and fish sources of n-3 PUFA has been explored, although conversion of LNA to long chain n-3 PUFA is limited in animals, and response cannot be specifically related to increased EPA or DHA levels. However, advancements in MA production with high specific DHA content provide an opportunity to assess the direct effect of DHA on enrichment of animal products. In addition to the enrichment of tissues with DHA, feeding MA rich in DHA appears to also provide additional benefits to the animal. Growth and egg performance results suggest limited evidence for adverse effects of increasing DHA content of the diet, and beneficial effects have been often reported. However, caution is advised when lowering the n-6:n-3 ratio to consider the importance of n-6 PUFA in the diet. Moreover, increasing DHA content of tissues can be at the expense of oxidative stability and reduced consumer acceptability. Natural carotenoids in MA may provide a certain level of anti-oxidant activity, however additional supplementation of other anti-oxidants, such as vitamin E, might be necessary to prevent oxidative deterioration with higher levels of DHA. A number of studies have demonstrated improved semen quality and fertility by increasing DHA content of sperm phospholipid through dietary supplementation. While maternal feeding of DHA may have a limited effect on fertility and hatchability of eggs, positive effects on progeny performance in terms of immunity and growth are proposed for both poultry and pigs. Dietary supplementation of DHA also has the potential to improve bone strength in poultry, indicating a possible welfare benefit. Although, much of the previous work has been completed using linseed or fish oil as well as MA, the benefits of increasing n-3 PUFA, and specifically DHA, are apparent irrespective of dietary source. It is clear from the literature that the n-6:n-3 ratio is important, however studies investigating the effect of this ratio compared to total levels of these PUFA is limited. This questions whether the ratio becomes less important when total levels of both n-3 and n-6 PUFA are in sufficient quantities. It is also unknown as to whether an excess of n-3 presents a greater oxidative stress problem than an excess of n-6, or whether both are equally problematic. Regardless, upper limits of inclusion for n-3 and n-6 may depend upon the dietary content of the other. Also, a greater understanding is required as to what influence specific PUFA have on the ratio, with potential consideration of the n-6 Docosapentaenoic acid (DPA): n-3 DHA ratio which may be more relevant, since these are the final products of the PUFA synthetic pathways. These questions present opportunities for further research into this area, to establish a better understanding of how to optimise n-3 enrichment of animal products.

Conflicts of interest

All authors declare no conflict of interests.

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