Impact of different omega-3 polyunsaturated fatty acid (n-3 PUFA) sources (flaxseed, Isochrysis galbana, fish oil and DHA Gold) on n-3 LC-PUFA enrichment (efficiency) in the egg yolk.

Charlotte Lemahieu\textsuperscript{a,1,*}, Charlotte Bruneel\textsuperscript{a,1}, Eline Ryckebosch\textsuperscript{b,1}, Koenraad Muylaert\textsuperscript{b}, Johan Buyse\textsuperscript{c,1}, and Imogen Foubert\textsuperscript{a,1}

\textsuperscript{a}Research unit Food and Lipids, KU Leuven Kulak, Etienne Sabbelaan 53, 8500 Kortrijk, Belgium.

* Corresponding author: Charlotte Lemahieu

Phone: +32 (0) 56 24 61 72
Fax: +32 (0) 56 24 69 99
E-mail: charlotte.lemahieu@kuleuven-kulak.be

\textsuperscript{b}Research unit Aquatic Biology, KU Leuven Kulak, Etienne Sabbelaan 53, 8500 Kortrijk, Belgium.

\textsuperscript{c}Division of Livestock-Nutrition-Quality, KU Leuven, Kasteelpark Arenberg 30, 3001 Leuven, Belgium.

\textsuperscript{1}Leuven Food Science and Nutrition Research Centre (LFoRCE), KU Leuven, Kasteelpark Arenberg 20, 3001 Leuven, Belgium
ABSTRACT

Four different omega-3 polyunsaturated fatty acid (n-3 PUFA) sources (flaxseed, Isochrysis galbana, fish oil and DHA Gold) were supplemented to the diet of laying hens in such a way that the same amount of extra n-3 PUFA (120 mg per 100 g feed) was added to the diet and enrichment of egg yolk with n-3 PUFA was monitored. The obtained n-3 long chain (LC)-PUFA enrichment was not as efficient for all n-3 PUFA sources. The lowest enrichment efficiency (≈6%) was observed when flaxseed (α-linolenic acid source) was supplemented. Drastically higher n-3 LC-PUFA enrichment efficiencies were observed with supplementation of the n-3 LC-PUFA sources. However, for the n-3 LC-PUFA sources (fish oil, Isochrysis galbana and DHA Gold) differences in enrichment efficiencies were observed (≈55%, ≈30% and ≈45%, respectively), this because of different bio-accessibility of the n-3 PUFA and different n-3 PUFA profiles of the three sources.

KEYWORDS

n-3 LC-PUFA enrichment
Flaxseed
Fish oil
Isochrysis galbana
DHA Gold
Egg yolk
1. Introduction

Since the last decades, there is a growing interest to enrich food products with omega-3 polyunsaturated fatty acids (n-3 PUFA). Several health benefits, like the reduction of cardiovascular diseases and the development of visual and cognitive functions in foetus and young children, are associated with n-3 PUFA. Long chain (LC) n-3 PUFA, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), appear to have a much stronger effect than the shorter chain n-3 PUFA α-linolenic acid (ALA) (Bourre, 2005; Gogus and Smith, 2010; Harris, 2007; Jeyakumar, 2012; Jordan, 2010; Simopoulos, 1999; Trautwein, 2001; Yashodhara et al., 2009). The conversion of ALA to EPA and DHA in the human body is rather limited (Trautwein, 2001; Burdge, 2004; Komprda, 2012). Despite the proven health benefits, the intake of these n-3 PUFA, and more particularly n-3 LC-PUFA, does not meet the recommended intake of 250 mg per day in most countries worldwide (Burdge, 2004; Kris-Etherton et al., 2009; Meyer, 2011; Ruxton et al., 2004; Sioen et al., 2006; Sioen et al., 2009).

In this respect, enrichment of food products with n-3 LC-PUFA has received an increasing interest in the past few years (Fraeye et al., 2012; Morato et al., 2015; Stratulat et al., 2015).

Eggs are a very good food product to enrich with n-3 LC-PUFA. First of all, they are consumed by most people worldwide, as such or processed in other foodstuffs. Secondly, the fatty acid profile of the egg yolk, where the lipids are concentrated, is closely linked to the type of lipids consumed by the laying hens (Bourre, 2005; Fraeye et al., 2012). To raise the level of n-3 PUFA in the egg yolk, several n-3 PUFA sources can be supplemented to the diet of the laying hens: flaxseed, fish oil, DHA Gold (heterotrophic microalgae) or autotrophic microalgae (Fraeye et al., 2012). The supplementation of the ALA source flaxseed has already been studied intensively (Fraeye et al., 2012). Flaxseed enriched feed mainly results in ALA enrichment, up
to 200 mg per egg, but also substantial increases of the DHA content, up to 90 mg per egg, this with overdosing the amount of flaxseed (Aymond and Van Elswyk, 1995; Bean and Leeson; 2003; Fraeye et al., 2012). However, it is more interesting to enrich eggs with n-3 LC-PUFA, supplementation with a direct source of EPA and/or DHA. Fish oil, as source of EPA and/or DHA, for example, can be supplemented to the diet of the laying hens and mostly results in DHA enrichment in the egg yolk (up to ± 100 mg per egg), while the EPA content increases to a much lesser extent (Bovet et al., 2007; Cachaldora et al., 2008; Carrillo et al., 2008; Fraeye et al., 2012; Gonzalez-Esquerra and Leeson, 2000; Van Elswyk et al., 1995). The supplementation dose of fish oil, however, needs to be restricted, since inclusion levels of fish oil above 1.5% in the diet of laying hens leads to eggs which are no longer acceptable for human consumption, especially in Western countries (Gonzalez-Esquerra and Leeson, 2000; Van Elswyk, 1997) because they are described as ‘fishy’ eggs by sensory panels (Van Elswyk, 1997). Even when deodorized fish oil or microencapsulated fish oil is used, a negative impact on the sensory parameters of the egg is still observed (Gonzalez-Esquerra and Leeson, 2000; Lawlor et al., 2010). It has been suggested that an even higher enrichment of n-3 LC-PUFA can be obtained by the supplementation of heterotrophic microalgae, sold as e.g. DHA Gold, as source of DHA (Fraeye et al., 2012). Herber and Van Elswyk (1996), for example, observed a similar enrichment (± 130 mg n-3 PUFA per egg) by supplementation of 1.5% fish oil and 2.4% DHA Gold. However, DHA levels up to 200 mg per egg can be obtained by supplementation of these heterotrophic microalgae at higher doses (4.8%), while still obtaining eggs with an acceptable taste (Herber-McNeill and Van Elswyk, 1998).

Limited research has also been performed to investigate the effect of the supplementation of autotrophic microalgal species, as source of ALA or EPA and/or DHA, to the diet of the laying
hens. Supplementation of hens’ feed with autotrophic microalgae provides the most environmentaly sustainable approach to raise the n-3 LC-PUFA content in eggs. Autotrophic microalgae are the primary producers of these fatty acids. They only need light and CO₂ to produce their biomass, which is in contrast to heterotrophic microalgae which have to be fed organic molecules (Nuño et al., 2013). Depending on the autotrophic microalgal species used as feed supplement, different levels of n-3 LC-PUFA enrichment in the eggs can be obtained (Bruneel et al., 2013; Fredriksson et al., 2006; Lemahieu et al., 2013a; Nitsan et al., 1999).

There is a lack of studies that directly compare the effectiveness of different n-3 PUFA sources for enrichment of egg yolk with n-3 LC-PUFA. Moreover, the few comparative studies that are available do not supplement equal n-3 PUFA dosages. In this study, the four different n-3 PUFA sources: flaxseed, fish oil, DHA Gold and the autotrophic microalgal species Isochrysis galbana, were therefore supplemented to the diet of laying hens in one experimental set-up, which has, to the best of our knowledge, never been done before. Flaxseed, fish oil and DHA Gold are three n-3 PUFA sources which are commercially available and already used to enrich eggs with n-3 PUFA. In addition, based on the study of Lemahieu et al. (2013a), Isochrysis galbana was the most appropriate autotrophic microalgal species to enrich eggs with n-3 PUFA, so this microalgal species was included in this study. Moreover, the n-3 PUFA sources were supplemented in such a way that the same n-3 PUFA amount was added to the diet of the laying hens. This is important as earlier research showed that the supplemented n-3 PUFA amount has a drastic impact on the enrichment efficiency obtained in the egg yolk (Caston and Leeson 1990; Herber and Van Elswyk, 1996; Lemahieu et al., 2013a; Lemahieu et al., 2014; Van Elswyk, 1997;).
2. Materials and methods

2.1. N-3 PUFA sources

Four different n-3 PUFA sources were used in this study as a feed supplement: extruded flaxseed (AVEVE, Leuven, Belgium), Isochrysis galbana (Archimede Ricerche, Camporosso, Italy), fish oil (Inve België, Baasrode, Belgium) and DHA Gold (Bivit, Wevelgem, Belgium).

To determine the n-3 PUFA content of the different sources, the lipid fraction of the extruded flaxseed, Isochrysis galbana and DHA Gold was first extracted with chloroform:methanol (1:1, v:v) according to the method described by Ryckebosch et al. (2012). At the start of the extraction, an internal standard (C12:0) was added to calculate the amount of n-3 PUFA in the different sources. The fish oil was used as such, only the internal standard (C12:0) was added for quantification. The lipid fraction was then methylated according to Ryckebosch et al. (2012) and the n-3 PUFA profile was determined by gas chromatography as described by Lemahieu et al. (2013a). The results are shown in Table 1 and discussed in section 3.1.

2.2. Animals and diets

Forty ISA Brown laying hens (28 weeks of age, ‘t Munckenei, Wingene, Belgium) were housed in battery cages, two hens per cage, in an environmentally controlled room. The room temperature was set at 20 °C and the hens received 16 h of light per day. Feed and water were supplied ad libitum.

During the adaptation period of 14 days, the laying hens only received the commercially available standard diet (Legmeel Total 277, AVEVE, Wilsele, Belgium), in order to adapt to the new environment and the new diet. After these 14 days of adaptation, the 40 laying hens were
divided into five groups of eight hens. One of these groups, the control group, continued to receive only the standard diet, further referred to as control diet. The other four groups received the control diet supplemented with one of the four n-3 PUFA sources: extruded flaxseed, *Isochrysis galbana*, fish oil or DHA Gold. Earlier research by Lemahieu *et al.* (2014) showed that supplementation of 120 mg n-3 PUFA per 100 g feed, by addition of *Isochrysis galbana*, was the most optimal supplementation dose to reach the highest n-3 LC-PUFA enrichment efficiency. To make a correct comparison with the other sources, they were also supplemented to reach 120 mg extra n-3 PUFA per 100 g feed. Based on the fatty acid profile of the n-3 PUFA sources, obtained as described in 2.1 and shown in Table 1, the supplementation doses of the four n-3 PUFA sources were calculated: 0.56% extruded flaxseed, 2.03% *Isochrysis galbana*, 0.68% fish oil (which is below the maximum advised level of 1.5%) and 0.44% DHA Gold (all doses expressed on feed basis).

The supplementation of the different n-3 PUFA sources lasted 21 days (supplementation period). During this period, the average daily feed intake, the egg production and egg weight, and the mortality and the morbidity of the hens were registered on a daily basis.

### 2.3. Egg collection, storage and analysis

The eggs collected during the supplementation period were stored at -20 °C, until further analysis. The fatty acid profile of the eggs at the start and at the end of the supplementation period were determined according to the method described in Lemahieu *et al.* (2013a). Briefly, the lipid fraction of the egg yolk was extracted, after addition of an internal standard, with chloroform:methanol (2:1, v/v). The extraction was performed twice, and the combined chloroform:methanol extracts were washed with KCl (0.88%). Afterwards, the
chloroform:methanol was removed by rotary evaporation. The lipid fraction was then methylated according to Ryckebosch et al. (2012) and the n-3 PUFA profile was determined by gas chromatography as described by Lemahieu et al. (2013a).

2.4. Statistical analysis

The results were statistically evaluated by one way analysis of variance (ANOVA) and post-hoc Tukey’s test with $\alpha=0.05$ (Sigmaplot 11, Systat Software Inc., Chicago, IL, USA).
3. Results and discussion

3.1. n-3 PUFA composition of the four sources

The n-3 PUFA content and profile of extruded flaxseed, Isochrysis galbana, fish oil and DHA Gold is shown in Table 1. Extruded flaxseed was source of the shorter chain n-3 PUFA ALA (21.4 ± 0.7 %). Isochrysis was mainly source of stearidonic acid (SDA) (2.67 ± 0.07 %) and DHA (1.78 ± 0.04 %), but also contained significant amounts of ALA (1.36 ± 0.03 %). Fish oil, on the other hand, was mainly source of EPA (7.06 ± 0.15 %) and DHA (6.76 ± 0.16 %), and contained much lower amounts of ALA (1.105 ± 0.003 %) and SDA (1.35 ± 0.03 %). DHA Gold was source of DHA (26.4 ± 0.3 %) and contained only very small amounts of the other n-3 PUFA.

3.2. n-3 (LC-)PUFA enrichment in the egg yolk

The supplementation of the different n-3 PUFA sources resulted in different enrichment patterns in the egg yolk (Table 2).

Feed supplementation with the ALA source flaxseed (120 mg extra n-3 PUFA per 100 g feed or 0.56 g flaxseed per 100 g feed) resulted in significantly higher amounts of ALA (19.2 ± 2.5 mg per egg) and DHA (33 ± 4 mg per egg) in the egg yolk in comparison with the ALA (9.9 ± 1.0 mg per egg) and DHA (21 ± 3 mg per egg) content in the eggs from the control group. This is in accordance with the results in the literature, although the absolute amounts cannot be compared as a different dose was supplemented (Caston and Leeson, 1990; Fraeye et al., 2012; Schiedeler and Froning, 1996; Van Elswyk, 1997). For example, diet supplemented with 15% of ground flaxseed increased ALA from 13 to 212 mg per egg and DHA from 28 to 90 mg per egg (Aymond and Van Elswyk, 1995). Based on the literature and the results obtained in this study, it can be concluded that ALA can be converted to DHA by the laying hens, but that
this is a rather inefficient process since also significant amounts of ALA were incorporated into the egg yolk, especially with very high supplementation doses of flaxseed (Aymond and Van Elswyk, 1995; Bean and Leeson, 2003). A second indication for the conversion of ALA to DHA by the laying hen is the slightly, but significantly, increased, docosapentaenoic acid (DPA) content (5.5 ± 3.0 mg per egg compared to 3.0 ± 0.5 mg per egg in the control group), as intermediate between ALA and DHA.

The three n-3 LC-PUFA sources (Isochrysis galbana, fish oil and DHA Gold) mainly resulted in DHA enrichment in the egg yolk, regardless of whether EPA or DHA was supplemented to the diet.

A DHA content of 92 ± 3 mg per egg was observed for the supplementation with fish oil (0.68 g fish oil per 100 g feed or 120 mg extra n-3 PUFA per 100 g feed). However, also a slightly, but significantly, higher EPA (3.5 ± 0.3 mg per egg) and DPA (10.0 ± 1.1 mg per egg) content was observed in comparison with the control group. This is, in general, in accordance with the results obtained by Cachaldora et al. (2008); Herber and Van Elswyk (1996) and Lawlor et al. (2010) although again exact comparison of enrichment amounts is not possible. It can thus be concluded that, with supplementation of fish oil, a conversion by the laying hens of EPA to DHA has occurred, since mainly DHA was observed in the egg yolk and an increase of the conversion product DPA was also obtained.

The DHA content obtained by the supplementation of DHA Gold (0.44 g DHA Gold per 100 g feed or 120 mg extra n-3 PUFA per 100 g feed) was very similar (not significant different) to the DHA content obtained by supplementation of fish oil (90 ± 5 mg DHA per egg compared to 92 ± 3 mg DHA per egg, respectively). No drastic changes were however observed for the
other n-3 PUFA, which is in accordance with literature and can be explained by the fact that
no conversion processes are needed. DHA can be incorporated in egg yolk in a direct way
(Cachaldora et al., 2005; Cachaldora et al, 2008; Cheng et al., 2004; Fraeye et al., 2012; Herber
and Van Elswyk; 1996).

Also mainly DHA enrichment (67 ± 3 mg DHA in the egg) was observed in the egg yolk with
supplementation of Isochrysis galbana (2.03 g per 100 g feed or 120 mg extra n-3 PUFA per
100 g feed), despite the high amounts of ALA and SDA supplemented (Table 1). This confirmed
once again that preferably DHA was deposited in the egg yolk. So, next to the direct
incorporation of the supplemented DHA also conversion of ALA and SDA occurred.

3.3. n-3 PUFA egg enrichment efficiencies by supplementation of different n-3 PUFA
sources

To compare the different sources, it is especially interesting to calculate the efficiency of the
n-3 (LC-) PUFA enrichment in eggs. This is calculated as described in Lemahieu et al. (2013a)
and the results are presented in Table 3.

The highest n-3 LC-PUFA enrichment, accompanied by the highest n-3 LC-PUFA incorporation
efficiency, was obtained with the supplementation of fish oil (enrichment efficiency of ≈ 55%).
A 10% lower enrichment efficiency (≈ 45%) and significantly different with the enrichment
efficiency obtained by fish oil, was obtained with the supplementation of DHA Gold. This is in
contrast to the results obtained by Herber and Van Elswyk (1996) who supplemented 1.5%
fish oil and 2.4% heterotrophic microalgae, which led to approximately the same n-3 LC-PUFA
enrichment in the egg yolk. However, much more n-3 PUFA were added by the
supplementation of 1.5% fish oil in comparison with the supplementation of 2.4% microalgae.
This suggests a higher enrichment efficiency of supplementation with heterotrophic microalgae in comparison with fish oil, although the results are biased by a possible dose effect on the enrichment efficiency since the n-3 PUFA dose supplemented was not the same for both sources.

Supplementation of *Isochrysis* to the diet of the laying hens led to an enrichment efficiency of approximately 30%, which is significantly lower in comparison with fish oil and DHA Gold. But, this is also drastically lower in comparison with the research of Lemahieu *et al.* (2014) where the same microalgae was supplemented in the same n-3 PUFA dose (equivalent to 2.4% microalgal biomass) and an efficiency of 53% was observed. A possible explanation could be the percentage of DHA supplemented, which was somewhat (5%) lower in this study, since more SDA was present in the microalgal biomass. Different batches could also have a different digestibility, which could lead to different enrichment efficiencies. Next to this, also variation between different experiments can occur, which makes it important to compare the four different sources in the same study.

The lowest enrichment efficiency (≈ 6%) was observed with the supplementation of flaxseed. It is difficult to compare this efficiency with results obtained in literature since almost in all cases no information was given about the supplemented n-3 PUFA amount and as known, the supplemented n-3 PUFA dose has a significant influence of the enrichment efficiency.

Several suggestions can be made to explain the differences in enrichment efficiencies with the supplementation of the different n-3 PUFA sources. Based on the fatty acid profile of the n-3 PUFA sources, it could be expected that DHA Gold would lead to the highest enrichment efficiency since it is a direct source of DHA, which is the fatty acid preferentially stored in the
egg yolk. Cachaldora et al. (2006) also showed that supplementation of fish oil with different ratios of EPA/DHA leads to different incorporation efficiencies, with the highest efficiency obtained with the fish oil with the lowest EPA/DHA ratio, so, with the highest DHA content. However, in this study a higher n-3 LC-PUFA enrichment efficiency by the supplementation of fish oil was obtained. This can presumably be explained by the higher bio-accessibility of the n-3 PUFA, since these fatty acids were supplemented as oil. Based on the results obtained in this study, the bio-accessibility thus seems to have a greater impact on the enrichment efficiency than the type of n-3 LC-PUFA provided. However, to definitely conclude this, the n-3 LC-PUFA enrichment obtained by the supplementation of fish oil should be compared to the enrichment obtained by the supplementation of the oil of DHA Gold.

The n-3 LC-PUFA enrichment and the n-3 LC-PUFA incorporation efficiency obtained by supplementation of Isochrysis galbana to the diet of the laying hens was drastically lower in comparison with the supplementation of fish oil and DHA Gold. First of all, Isochrysis contained much higher amounts of ALA and SDA and a lower amount of DHA in comparison with fish oil and DHA Gold. This means that the percentage of DHA in the total supplemented n-3 PUFA was much lower for Isochrysis galbana and thus more conversion reactions are needed to raise the level of DHA in the egg yolk, which could decrease the efficiency of enrichment. Cachaldora et al. (2008) supplemented diets, rich in ALA and EPA/DHA, to laying hens by supplementation of flaxseed and fish oil and concluded that an excess of the n-3 LC-PUFA limits the conversion of ALA. The relative portions of the supplemented n-3 PUFA thus plays a crucial role in the n-3 LC-PUFA enrichment efficiency. Secondly, compared to fish oil, the lower efficiency could also partly be explained by the bio-accessibility. Isochrysis consist of a cell membrane/cell wall which could reduce the bio-accessibility in contrast to fish oil, where the
oil was supplemented as such (Wootton et al., 2007; Zhu and Lee, 1997). This could also be an explanation for the lower efficiency compared to DHA Gold. Different microalgae consist of different cell wall compositions which could affect the digestibility and lead to different enrichment efficiencies (Lemahieu et al., 2013a). This parameter, bio-accessibility, should, by the way, also be taken into account in the study of Cachaldora et al. (2008), since flaxseed and fish oil probably also lead to a different bio-accessibility of the n-3 PUFA.

The lowest n-3 LC-PUFA enrichment (efficiency) was obtained with the supplementation of extruded flaxseed to the diet of the laying hens. This could be expected since the conversion of ALA to the n-3 LC-PUFA is a rather limited process (Aymond and Van Elswyk, 1995). Since mostly ALA enrichment was observed in the egg yolk with supplementation of flaxseed (Fraeye et al., 2012), it is interesting to also evaluate the total n-3 PUFA incorporation efficiency, next to the n-3 LC-PUFA enrichment efficiency. However, the n-3 PUFA incorporation efficiency, which includes the ALA enrichment in the yolk, was only slightly higher (10 ± 8 %) than the n-3 LC-PUFA enrichment efficiency (6 ± 6 %) and still much lower than the n-3 PUFA efficiencies obtained with fish oil, Isochrysis galbana and DHA gold (respectively 54 ± 5 %, 30 ± 6 %, 45 ± 5 %). This means that also the ALA enrichment was rather inefficient in the egg yolk, not only for the n-3 LC-PUFA sources but also for flaxseed. This corresponds with literature, where was observed that the n-3 PUFA were preferentially stored as DHA in the egg yolk (Fredriksson et al., 2006; Nitsan et al., 1999). Only overdosing the amount of flaxseed in the diet of the laying hens leads to a significant higher increase of the ALA and DHA content in the egg (Aymond and Van Elswyk; 1995; Van Elswyk, 1997).

3.4. Zootchnical performance of the laying hens
Globally, no drastic influences of the different n-3 PUFA sources on the zootechnical parameters were observed (Table 4). In literature, the influence of feed supplementation on the zootechnical performance parameters are very contradictory for the different sources (Fraeye et al., 2012). This can probably be explained by different experimental setups, but, in most cases, also no drastic changes of the zootechnical performance parameters were observed (Fraeye et al., 2012).
4. Conclusion

The four different n-3 PUFA sources (flaxseed, *Isochrysis galbana*, fish oil and DHA Gold) supplemented, in such way to reach the same supplemented n-3 PUFA amount, to laying hens led to an increased level of n-3 LC-PUFA in the egg yolk. Mainly DHA enrichment was observed for all the sources. Only for the supplementation with flaxseed, also a significant increase of ALA was observed. However, the obtained level of enrichment was not the same for all n-3 PUFA sources, although the same amount of n-3 PUFA was supplemented. The lowest enrichment (efficiency) (= 6%) was observed when flaxseed was supplemented to the diet of the laying hens, this because of the inefficient conversion of ALA to DHA. Drastically higher n-3 LC-PUFA enrichments and enrichment efficiencies were observed with supplementation of n-3 LC-PUFA sources. Fish oil led to the highest efficiency (= 55%), followed by DHA Gold (= 45%) and *Isochrysis galbana* (= 30%). The differences in enrichment efficiency with these sources can be explained by the different bio-accessibility of the n-3 PUFA and the different n-3 PUFA profile of the three sources.
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>311</th>
<th>n-3 LC-PUFA</th>
<th>Omega-3 long chain polyunsaturated fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>312</td>
<td>ALA</td>
<td>$\alpha$-linolenic acid</td>
</tr>
<tr>
<td>313</td>
<td>SDA</td>
<td>Stearidonic acid</td>
</tr>
<tr>
<td>314</td>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
</tr>
<tr>
<td>315</td>
<td>DPA</td>
<td>Docosapentaenoic acid</td>
</tr>
<tr>
<td>316</td>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
</tbody>
</table>

| 317 |
ACKNOWLEDGEMENTS

This publication has been produced by the financial support of Flanders’ Food – IWT (OMEGA-EI) and the agency for Innovation by Science and Technology (IWT strategic research grant C. Lemahieu). We wish to thank everybody who has contributed to this project, especially the companies of the Flanders’ Food OMEGA-EI project, Archimede Ricerche (for delivery of Isochrysis galbana), Inve België (for delivery of the fish oil), Bivit (for delivery of DHA Gold), AVEVE (for delivery of flaxseed), ILVO (for making the different experimental diets), Stefaan Verhelle (’t Munckenei, for delivery of the laying hens), Marcel Samain and André Respen (for taking care of the laying hens).
REFERENCES


Table 1: n-3 PUFA content (% of the biomass; mean ± SD; n=3) of the four n-3 PUFA sources: extruded flaxseed, *Isochrysis galbana*, fish oil and DHA Gold.

<table>
<thead>
<tr>
<th></th>
<th>Flaxseed</th>
<th><em>Isochrysis</em></th>
<th>Fish oil</th>
<th>DHA Gold</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA</td>
<td>21.4 ± 0.7</td>
<td>1.36 ± 0.03</td>
<td>1.105 ± 0.003</td>
<td>0.019 ± 0.0002</td>
</tr>
<tr>
<td>SDA</td>
<td>-</td>
<td>2.67 ± 0.07</td>
<td>1.35 ± 0.03</td>
<td>0.093 ± 0.009</td>
</tr>
<tr>
<td>EPA</td>
<td>-</td>
<td>0.082 ± 0.005</td>
<td>7.06 ± 0.15</td>
<td>0.36 ± 0.04</td>
</tr>
<tr>
<td>DPA</td>
<td>-</td>
<td>0.022 ± 0.002</td>
<td>1.34 ± 0.05</td>
<td>0.121 ± 0.010</td>
</tr>
<tr>
<td>DHA</td>
<td>-</td>
<td>1.78 ± 0.04</td>
<td>6.76 ± 0.16</td>
<td>26.4 ± 0.3</td>
</tr>
</tbody>
</table>
Table 2: Level of the different n-3 PUFA (ALA, EPA, DPA and DHA, in mg/egg, mean ± SD, n=8) in the egg at the end of the supplementation period obtained by feeding with extruded flaxseed, *Isochrysis galbana*, fish oil and DHA Gold.

<table>
<thead>
<tr>
<th>Source</th>
<th>ALA</th>
<th>EPA</th>
<th>DPA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.9 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>3.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flaxseed</td>
<td>19.2 ± 2.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5 ± 3.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Isochrysis</em></td>
<td>12.6 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.94 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.9 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fish oil</td>
<td>11.6 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5 ± 0.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.0 ± 1.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>92 ± 3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHA Gold</td>
<td>12.6 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90 ± 5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Within each n-3 PUFA, results with the same letter for the different sources are not significantly different (p < 0.05).
Table 3: Incorporation efficiency of the n-3 LC-PUFA in the egg yolk for the supplementation of the four different n-3 LC-PUFA sources (in %, mean ± SD, n=8): Flaxseed, *Isochrysis galbana*, fish oil and DHA Gold. The incorporation efficiency is calculated by taking the ratio of the enrichment of n-3 LC-PUFA in the egg (in mg mean ± SD, n=8) to the actual n-3 PUFA intake (in g; mean ± SD, n=8), multiplied with 100.

<table>
<thead>
<tr>
<th>Actual n-3 PUFA intake (mg)</th>
<th>Enrichment of n-3 LC-PUFA (mg)</th>
<th>Enrichment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flaxseed</td>
<td>140 ± 2</td>
<td>8 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Isochrysis</em></td>
<td>143.0 ± 0.2</td>
<td>43.4 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fish oil</td>
<td>138 ± 2</td>
<td>76 ± 5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHA Gold</td>
<td>141.1 ± 1.2</td>
<td>64 ± 6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results with the same letter in the same column are not significantly different (p < 0.05)
Table 4: Zootechnical performance parameters and egg quality parameters for the four n-3 PUFA sources during the supplementation period: feed intake (in g, mean ± SD; n = 8), egg production rate (in %), egg weight (in g, mean ± SD, n=8) and yolk weight (in g, mean ± SD; n = 8).

<table>
<thead>
<tr>
<th>n-3 PUFA source</th>
<th>Feed intake (g)</th>
<th>Egg production rate (%)</th>
<th>Egg weight (g)</th>
<th>Yolk weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>118.3 ± 0.6&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>98.0</td>
<td>59.8 ± 1.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14.5 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flaxseed</td>
<td>116.5 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.6</td>
<td>62.1 ± 1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.1 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isochrysis</td>
<td>119.2 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>97.6</td>
<td>58.9 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.4 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fish oil</td>
<td>118.0 ± 0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>99.2</td>
<td>58.7 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.8 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHA Gold</td>
<td>117.6 ± 1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>98.2</td>
<td>60.7 ± 0.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>15.5 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results with the same letter in the same column are not significant different (p < 0.05)