1 The cell wall of autotrophic microalgae influences
2 the enrichment of long chain omega-3 fatty acids
3 in the egg

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ABSTRACT

Two microalgal species, *Isochrysis galbana* and *Nannochloropsis oculata*, were supplemented to the diet of laying hens, in either non-disrupted or disrupted (by high pressure homogenization) form, to examine the effect of the presence of a cell wall on the omega-3 long chain polyunsaturated fatty acid (n-3 LC-PUFA) enrichment in the egg yolk. Only supplementation of *Nannochloropsis* led to significant differences in enrichment by supplementation of the two forms, with the highest enrichment obtained with the HPH-disrupted form. This confirms the presence of a rigid cell wall for this species, which reduces the bioaccessibility of the n-3 PUFA. The needed conversion reaction from EPA to DHA on the other hand did not seem to effect the enrichment, since the same n-3 LC-PUFA enrichment was obtained with the supplementation of the DHA source *Isochrysis* and the EPA source *Nannochloropsis*, the last in HPH-disrupted form.

Next to the microalgal species, also fish oil was supplemented to the diet of the laying hens. Fish oil resulted in a higher enrichment in comparison with the microalgal species, either non-disrupted or HPH-disrupted. This might suggest a difference in bioaccessibility of the n-3 PUFA when added as oil or biomass.

40 **KEYWORDS**

41 n-3 LC-PUFA enrichment

42 HPH disruption

43 *Nannochloropsis oculata*

44 *Isochrysis galbana*

45 Fish oil

46 Eggs

47

48 **ABBREVIATIONS**

49 n-3 LC-PUFA Omega-3 long chain polyunsaturated fatty acids

50 ALA α-linolenic acid

51 SDA Stearidonic acid

52 EPA Eicosapentaenoic acid

53 DPA Docosapentaenoic acid

54 DHA Docosahexaenoic acid

55 HPH High Pressure Homogenisation

56

1. Introduction

Supplementation of different omega-3 polyunsaturated fatty acid (n-3 PUFA) sources to the diet of laying hens has been gaining more and more interest the last years [1]. This because the health benefits of n-3 PUFA, and more in particular the n-3 long chain (LC)-PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) rather than the shorter chain n-3 PUFA α -linolenic acid (ALA), became more clear in the last decennia [2-10]. The health benefits related to these n-3 LC-PUFA, are, for example, the reduced risk of cardiovascular diseases with increased n-3 LC-PUFA intake [3,8-10]. In addition, the n-3 LC-PUFA DHA is very important for pregnant women since DHA is responsible for the development of the brain and visual functions of the fetus and the young children [7-8,10]. Nevertheless the proven health benefits, the intake of n-3 LC-PUFA in western countries is still below the recommended intake of 250 mg EPA+DHA a day, even below 100 mg EPA+DHA a day for countries like the USA, The Netherlands,... [11-13].

Since enrichment of food products will presumably be the best long term solution to increase the n-3 LC-PUFA intake by humans, more and more research is performed in this area. One of the food products that can be enriched with n-3 LC-PUFA are eggs. Even more, since they are consumed by most people worldwide, eggs could be a very promising source of n-3 LC-PUFA [1, 14]. The enrichment of eggs with n-3 LC-PUFA (EPA + docosapentaenoic acid (DPA) + DHA) can be obtained by supplementation of several n-3 PUFA sources to the diet of laying hens, such as flaxseed, fish oil, microalgae... [1]. However, from a sustainability viewpoint, it is more appropriate to supplement autotrophic microalgae to the diet of laying hens [1, 15]. These microalgae use light as energy source and inorganic carbon (CO₂) as carbon source to form their biomass [16-17]. Several of these autotrophic microalgal species contain a certain

80 amount of n-3 PUFA in their biomass [17-18]. Lemahieu et al. [15] supplemented four different
81 autotrophic microalgal species (*Phaeodactylum tricornutum*, *Nannochloropsis oculata*,
82 *Isochrysis galbana* and *Chlorella fusca*). Both *Phaeodactylum* and *Nannochloropsis* act as an
83 EPA source, while *Isochrysis* serves as a DHA source but also contains significant amounts of
84 ALA and stearidonic acid (SDA). *Chlorella*, on the other hand, is a typical example of an
85 autotrophic microalgal ALA source. The different microalgal species, although supplemented
86 to reach the same n-3 PUFA dose, caused different n-3 LC-PUFA enrichment (efficiencies) in
87 the egg yolk. The highest n-3 LC-PUFA enrichment efficiencies were obtained by the
88 supplementation of *Phaeodactylum* and *Isochrysis* (respectively 43% and 41% for the lowest
89 supplementation dose). A 20% lower n-3 LC-PUFA enrichment efficiency (approximately 23%
90 for the lowest supplementation dose) was obtained by the supplementation of
91 *Nannochloropsis*. *Chlorella*, as source of ALA, gave rise to the lowest n-3 LC-PUFA enrichment
92 efficiency in the egg yolk (12% for the lowest supplementation dose), presumably because of
93 the low conversion of ALA to n-3 LC-PUFA. Next to the different fatty acid profile of the
94 supplemented microalgal species, the difference in n-3 LC-PUFA enrichment efficiencies can
95 presumably also be explained by the difference in bioaccessibility of the n-3 PUFA in the
96 microalgal biomass. Several microalgal species possesses a thick and rigid cellulosic cell wall
97 which may reduce the bioaccessibility/digestibility of the microalgal biomass since it is not
98 digestible for humans and other ruminants [19-21]. For the microalgal species
99 *Nannochloropsis*, it is generally known that it consists of a very rigid, hard digestible cell wall
100 which contains an aliphatic, non-hydrolysable biopolymer, called algaenan [22-26]. For
101 *Isochrysis*, on the other hand, contradictory results were found whether or not this species
102 has a cell wall [27-28]. So, the different bioaccessibility of the n-3 PUFA could also be a possible

103 explanation for the different n-3 LC-PUFA enrichment efficiencies by the supplementation of
104 the diet with *Nannochloropsis* and *Isochrysis*.

105 To examine the effect of the cell wall of several microalgal species on the n-3 LC-PUFA
106 enrichment in the egg yolk, several methods can be used. First of all, cell wall degrading
107 enzymes such as glucanase and cellulase could be added to the diet of laying hens in addition
108 to the microalgal biomass [25, 29]. This was already performed by Nitsan et al. [29] where
109 *Nannochloropsis* was supplemented to the diet of laying hens with and without addition of
110 both enzymes. A little, but significant, increase in n-3 PUFA content in the egg was observed
111 with the supplementation of the diet with the cell wall degrading enzymes. A second way to
112 examine the effect of cell wall on the bioaccessibility of the n-3 PUFA could be the disruption
113 of the microalgal cells. Disruption of the cell wall of *Chlorella vulgaris* already showed an
114 increased digestibility of this microalgal biomass [21, 30]. Several methods can be used to
115 disrupt the microalgal cell wall, either mechanical or non-mechanical [31-33]. However, for
116 performing microalgal disruption on a large scale, only the mechanical disruption methods
117 can be used, and in particular high pressure homogenization (HPH) or bead milling [32]. Little
118 research on disruption of microalgal biomass by HPH has already been performed, this to
119 increase, for example, the lipid yield during extraction [34-35]. Olmstead et al. [34], for
120 example, performed HPH disruption of the highly resistant microalgal species *Nannochloropsis*
121 and observed higher lipid yields, obtained by hexane extraction, when the microalgal biomass
122 was subjected to HPH disruption.

123 The purpose of this study was therefore to evaluate the n-3 LC-PUFA enrichment efficiency
124 when adding *Isochrysis* and *Nannochloropsis* to laying hens, in either non-disrupted or

125 disrupted (using HPH) form. Next to the microalgal biomasses, also fish oil was supplemented
126 to the diet of the laying hens, as a reference.

2. Materials and methods

2.1. N-3 LC-PUFA sources

Two different autotrophic microalgal species were used in this study: *Isochrysis galbana* and *Nannochloropsis oculata* (Archimede Ricerche, Camporosso, Italy). Both microalgal species were delivered as paste (approximately 20% dry matter). The obtained batches of both species were divided into two separate batches. One of the batches was only freeze dried (non-disrupted biomass). The other batch was subjected to HPH to realize a cell wall disruption before freeze drying (HPH-disrupted biomass). So in total, four different batches were obtained for the autotrophic microalgal biomass.

Fresh 18/12 refined fish oil was obtained from Inve Belgium (Baasrode, Belgium). This oil was fully processed according to the state of the art methods.

2.1.1. HPH disruption of the autotrophic microalgae

Isochrysis galbana and *Nannochloropsis oculata* were HPH-disrupted at 1000 bar using a Panda 2k high-pressure homogenizer (Niro Soavi, Parma, Italy), of which the in- and outlet were thermostated at 4 °C using a cryostat (Haake, Karlsruhe, Germany). Multiple passes were used: 3 passes for *Isochrysis* and 4 passes for *Nannochloropsis*.

2.1.2. Evaluation of disruption

The disruption of the microalgal biomass was evaluated by microscopical observation (Olympus BX 51 bright-field microscope (Olympus, Berchem, Belgium) with an infinity 2 camera) and lipid extraction yield. The lipids from the microalgal biomass (non-disrupted and HPH-disrupted) were extracted using different extraction solvents: chloroform/methanol

(1:1), hexane and hexane/isopropanol (3:2). The extraction of the lipid content in the microalgal biomass with chloroform/methanol was described by Ryckebosch *et al.* [36]. Briefly, the microalgal biomass (100 mg) was extracted four times with chloroform/methanol (twice 8 ml and twice 4 ml) and washed with water. After extraction, the solvents (chloroform and methanol) were removed by rotary evaporation. The lipid content was gravimetrically determined. The extraction procedures with the other two solvents were described by Ryckebosch *et al.* [37]. Briefly, the microalgal biomass (100 mg) was extracted four times with the solvent(mixture) (hexane or hexane/isopropanol). However, to evaluate the performed disruption by HPH, no extra disruption step, as mentioned by Ryckebosch *et al.* [37] i.e. bead beating, was performed during the extraction procedures using hexane and hexane/isopropanol. Again, after extraction, the solvent layers were combined and removed by rotary evaporation. The lipid content obtained by the different extraction solvents was gravimetrically determined.

2.2. Composition of n-3 PUFA sources

To calculate the supplementation dose, the n-3 PUFA content of the different n-3 PUFA sources was determined. Therefore, the lipid fraction of the microalgal biomass was first extracted with chloroform/methanol (1:1) according to the method described by Ryckebosch *et al.* [36]. At the start of the extraction, an internal standard (C12:0) was added to calculate the amount of n-3 PUFA. The fish oil was used as such, only the internal standard (C12:0) was added for quantification of the n-3 PUFA. The lipid fraction was then methylated according to Ryckebosch *et al.* [36]. Briefly, the lipids were resolved in toluene and 1% sulfuric acid in methanol was added. After overnight incubation at 50°C, a 5% sodium chloride solution and hexane were added. Ultimately, the methylesters in the hexane layer were analyzed by

gaschromatographical separation and detected with a flame ionization detector as described by Lemahieu *et al.* [15] The total fatty acid profile of the n-3 PUFA sources is shown in the supplementary Table 1.

2.3. Animals and diets

72 ISA Brown laying hens (27 weeks of age, 't Munckenei, Wingene, Belgium) were housed in battery cages, two hens a cage, in an environmentally controlled room. Hens received 16h light, room temperature was set at 20°C and feed and water were supplied *ad libitum*.

According to the studies of Lemahieu et al. [38-39], the experiment started with an adaptation period (14 days, commercially available control diet: Legmeel Total 277, AVEVE, Wilsele, Belgium) followed by the microalgal supplementation period (21 days). The control diet consisted mainly of corn, wheat, sunflower, cake, corn gluten, limestone, soybean meal, and palm oil. The control diet contained a 6.2% lipid fraction with no n-3 LC-PUFA and only a very small amount of ALA (0.074%) was measured. At the start of the supplementation period the laying hens were divided into six groups of 12 hens (6 cages per treatment). One group, the control group, continued to receive only the standard diet. The other five groups received the standard diet supplemented with one of the five n-3 PUFA sources: fish oil, HPH-disrupted or non-disrupted *Isochrysis*, HPH-disrupted or non-disrupted *Nannochloropsis*. The supplemented dose of these n-3 PUFA sources was calculated to add 120 mg microalgal n-3 PUFA (ALA+SDA+EPA+DPA+DHA) per 100 g feed (according to Lemahieu et al. [38]). So, based on the n-3 PUFA profile of the different sources (**Table 1**), the amount of biomass that had to be supplemented could be calculated (0.52% fish oil, 2.97% *Isochrysis* non-disrupted, 3.18%

192 *Isochrysis* HPH-disrupted, 4.00% *Nannochloropsis* non-disrupted and 4.31% *Nannochloropsis*
193 HPH-disrupted, all doses expressed on feed basis).

194 To evaluate the impact of the different n-3 PUFA sources on the zootechnical performance of
195 the laying hens, the average daily feed intake, the egg production and egg weight, and the
196 mortality and the morbidity of the hens were registered on a daily basis. Next to this, the body
197 weight of the laying hens was registered at the start and the end of the supplementation
198 period.

199 **2.4. Egg collection, storage and analysis**

200 The eggs were collected daily and stored at – 20°C. The eggs obtained at the start and the end
201 of the supplementation period were further analyzed. The fatty acid profile of the eggs (egg
202 yolks from one cage were pooled and the extraction was performed in triplicate on the pooled
203 egg yolk sample) was determined as described by Lemahieu et al. [15].

204 **2.5. Statistical analysis**

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

3. Results and discussion

3.1. Evaluation of the performed HPH disruption

First of all, the performed HPH disruption was evaluated by microscopic visualization of the *Isochrysis* and *Nannochloropsis* biomasses, either non-disrupted or HPH-disrupted (**Figure 1**). For the biomass of *Isochrysis*, a reduction in the amount of cells is seen when HPH disruption was performed. For *Nannochloropsis*, also a clear effect of HPH disruption was observed. The intact batch contained the normal cells of *Nannochloropsis*, but when disruption was performed, the cells were broken up and a lot of debris was formed, which can be seen on **Figure 1**. So, for both microalgal species, a clear disruption was observed.

Since it has been shown that cell disruption leads to higher extraction yields when using non-halogenated solvents, lipid extraction can also be a good method to evaluate the impact of the performed HPH disruption [36-37]. **Table 2** shows the lipid yield of the extraction of the microalgal biomass (non-disrupted or HPH-disrupted) with different extraction solvents: chloroform/methanol, hexane and hexane/isopropanol. The extraction with chloroform/methanol has already been shown to be the reference method to obtain the total lipid content of microalgae, independent of the cell wall permeability [36-37, 41]. Therefore, no difference in lipid yield, obtained by chloroform/methanol extraction, was observed between the non-disrupted and HPH-disrupted batches of the microalgal species *Isochrysis* and *Nannochloropsis* (**Table 2**). However, hexane and hexane/isopropanol extraction, on the other hand, have already been shown to be subject to the permeability of the cell wall [41]. For the batch of *Isochrysis*, no difference in lipid yield was observed between the non-disrupted and the HPH-disrupted batch, this for both the extraction solvents. Literature about

the existence of a cell wall for the microalgal species *Isochrysis* is very contradictory [27-28]. Since no difference in lipid yield was observed, even if disruption was performed, it can be suggested that (this) *Isochrysis* contains no cell wall or it has to be a very weak cell wall. For the batch of *Nannochloropsis*, on the other hand, a difference in lipid yield was observed for both hexane and hexane/isopropanol extractions between the non-disrupted and HPH-disrupted form. *Nannochloropsis* consists of a very rigid cell wall [22-24], which would lead to a low permeability of the solvent with the non-disrupted biomass and thus a higher lipid yield with the HPH-disrupted biomass of *Nannochloropsis*, which is in accordance with the study of Samarasinghe et al. [42]. These results suggest that with HPH a disruption of the biomass was performed, as was also seen in the microscopic images (**Figure 1**), and thus the effect of the cell wall on the n-3 LC-PUFA enrichment in the egg yolk can be examined.

3.2. Impact of the microalgal cell wall on n-3 LC-PUFA enrichment in the egg yolk

Supplementation with n-3 PUFA sources led mainly to changes in the n-3 PUFA profile of the eggs and less changes in the content of the other fatty acids (Supplementary Table 2). Only a (small) reduction of the C18:2 n-6 and C20:4 n-6 amounts was detected by supplementation of the different n-3 PUFA sources (Supplementary Table 1 and 2). Thus, to examine the effect of the microalgal cell wall on the enrichment of n-3 LC-PUFA in the egg yolk, the n-3 LC-PUFA content in the eggs, obtained by the supplementation of the diet with non-disrupted and HPH-disrupted biomass of *Isochrysis* and *Nannochloropsis* for 21 days, was evaluated. **Table 3** first of all shows the total n-3 LC-PUFA content (in mg/egg) in the eggs by supplementation of the diet with the different n-3 PUFA sources. However, more interesting is the n-3 LC-PUFA enrichment (in mg/egg), calculated by correcting the total n-3 LC-PUFA content in the egg at the end of the supplementation period with the total n-3 LC-PUFA content at the start of the

supplementation period. Based on the n-3 LC-PUFA enrichment in the egg, the n-3 LC-PUFA enrichment efficiency can be calculated by taking the ratio of the n-3 LC-PUFA enrichment to the effective intake of n-3 PUFA multiplied by 100 [15].

Supplementation of *Isochrysis*, which contains mainly DHA as n-3 LC-PUFA, in non-disrupted or HPH-disrupted form, led to the same ($\alpha < 0.05$) total n-3 LC-PUFA content, n-3 LC-PUFA enrichment and n-3 LC-PUFA enrichment efficiency. For both batches, the obtained n-3 LC-PUFA enrichment was mainly caused by DHA enrichment (**Table 4**) (approximately 71% of the total n-3 LC-PUFA enrichment) and lower amounts of EPA and docosapentaenoic acid (DPA) enrichment were observed (approximately 21% and 7% of the total n-3 LC-PUFA enrichment respectively) (**Table 4**), which is in accordance with Lemahieu et al. [15, 38]. Based on the obtained n-3 LC-PUFA enrichment efficiencies (**Table 3**), no effect of cell wall was thus observed with the supplementation of *Isochrysis*, since the same enrichment was obtained for the non-disrupted and HPH-disrupted biomass. This observation is in accordance with the results of the lipid extractions with hexane and hexane/isopropanol (**Table 2** and **3.1**) and can presumably be explained by the fact that no (rigid) cell wall or only a cell membrane is present in this microalgal species [27-28]. So, there are no issues of bioaccessibility of the n-3 PUFA when *Isochrysis* was supplemented to the diet of laying hens.

Supplementation of the diet with *Nannochloropsis*, on the other hand, resulted in significant differences of total n-3 LC-PUFA content and n-3 LC-PUFA enrichment when the biomass was supplemented in non-disrupted or HPH-disrupted form respectively (**Table 3**). The obtained n-3 LC-PUFA enrichment in both forms of *Nannochloropsis* was also mainly caused by the enrichment of DHA (approximately 73% of the total n-3 LC-PUFA enrichment) (**Table 4**), even since mainly EPA was supplemented (**Table 1**), which is in accordance with Lemahieu et al.

[15] and Bruneel et al. [40]. But, next to DHA, also small amounts of EPA and DPA enrichment were observed (approximately 11% and 16% of the total n-3 LC-PUFA enrichment respectively). The obtained difference in n-3 LC-PUFA enrichment for both forms of *Nannochloropsis* also led to significantly different enrichment efficiencies ($29 \pm 8\%$ for supplementation of the diet with non-disrupted biomass and $44 \pm 11\%$ for the HPH-disrupted biomass, **Table 3**). So, based on these results, the presence of a rigid cell wall presumably reduced the bioaccessibility of the supplemented microalgal n-3 PUFA, which led to a decreased n-3 LC-PUFA enrichment in the egg yolk. As already mentioned, the cell wall of *Nannochloropsis* is known to be very rigid since it contains an aliphatic, non-hydrolysable biopolymer, called algaenan [22-24]. The reduced bioaccessibility of the n-3 PUFA by the presence of a rigid cell wall was also observed by Nitsan et al.[29]. They supplemented 1% *Nannochloropsis* to the diet of the laying hens, whether or not combined with the addition of the enzymes cellulase and glucanase. Supplementation of the diet with these enzymes, to improve the microalgal biomass digestibility, resulted in a significant higher enrichment of DHA in the egg yolk, which also suggested the reduced bioaccessibility of the n-3 PUFA in the presence of a very rigid cell wall. However, it was not possible to calculate the n-3 PUFA enrichment efficiency since not all information was given, such as the effective supplemented n-3 PUFA amount and the exact amount of n-3 LC-PUFA enrichment obtained. Based on the results obtained in this study, also HPH disruption of the cell wall, leads to a drastic increase of the bioaccessibility of n-3 PUFA which resulted in higher n-3 LC-PUFA contents in the egg.

Next to the microalgal biomass, also fish oil was supplemented (same n-3 PUFA dose) to the diet of laying hens to enrich the eggs with n-3 LC-PUFA. The fish oil supplementation led to a total n-3 LC-PUFA content of 116 ± 13 mg per egg, an n-3 LC-PUFA enrichment of 79 ± 14 mg

per egg and an enrichment efficiency of 56 ± 10 % (**Table 3**). The obtained enrichment was mainly (but to a lesser extent compared to supplementation of microalgae) caused by DHA enrichment in the egg yolk (approximately 59% of the total n-3 LC-PUFA enrichment), but also a significant increase of the EPA content (approximately 32% of the total n-3 LC-PUFA enrichment) was observed (**Table 4**). The lower DHA incorporation and the higher EPA incorporation for fish oil in comparison with microalgal supplementation might be explained by the higher bioaccessibility of the n-3 PUFA supplemented as oil, leading to a faster incorporation in the egg yolk which could result in a higher EPA and lower DHA content. The EPA content increased to a much lesser extent (approximately 7% of the total n-3 LC-PUFA enrichment). The obtained n-3 LC-PUFA enrichment efficiency is in accordance with the study of Lemahieu et al. [39], where also fish oil was supplemented in the same n-3 PUFA supplementation dose, i.e. 120 mg n-3 PUFA from fish oil per 100 g feed, and an n-3 LC-PUFA enrichment in the egg of 76 ± 5 mg or an enrichment efficiency of 55 ± 4 % was observed.

Comparing the n-3 LC-PUFA enrichment efficiency obtained by the supplementation of fish oil (containing EPA and DHA) with those obtained by the supplementation of the microalgal sources, at least a 10% higher efficiency was observed with the supplementation of fish oil (**Table 3**). On the other hand, the same enrichment efficiency was obtained for the HPH-disrupted batch of *Nannochloropsis*, where mostly DHA enrichment in the egg yolk was observed, but only EPA was supplemented, as with the supplementation of the *Isochrysis* batches where already DHA was present in the biomass. Moreover, in the study of Lemahieu et al. [39], the enrichment efficiency of DHA Gold, heterotrophic microalgal biomass which contains mainly DHA, was shown also to be 10% when compared to fish oil, even though both n-3 PUFA sources were supplemented in the same n-3 PUFA dose. Based on these studies, the

influence of the needed conversion reactions from EPA present in the feed to DHA deposited in the egg seems to be rather limited. However, the bioaccessibility of the n-3 PUFA seems to play a role as fish oil leads to a higher bioaccessibility of the n-3 PUFA than autotrophic or heterotrophic microalgal biomass even if cell wall of the autotrophic biomass was disrupted. This may be explained by the fact that fish oil is supplemented as oil, in comparison with the supplementation of n-3 PUFA from microalgae which is supplemented as biomass. This hypothesis has been suggested for fish by other researchers since the supplementation of fish oil led to higher n-3 LC-PUFA enrichment in comparison to fish meal supplementation. Fish oil supplementation led to a DHA content of 90-120 mg per egg while supplementation of fish meal led to DHA contents of 60 – 80 mg per egg [43]. Unfortunately, no information was given about the supplemented n-3 PUFA dose, but, already an indication was given about the different bioaccessibility of oil versus biomass. Nitsan et al. [29] also supplemented non-disrupted *Nannochloropsis* biomass and oil and compared the n-3 LC-PUFA enrichment. Unfortunately, they did not supplement both forms of *Nannochloropsis* in the same n-3 PUFA supplementation dose, making it difficult to draw conclusions on the different bioaccessibility of n-3 PUFA supplemented as oil or biomass. Next to this, also the limited bioaccessibility plays an important role, due to the presence of a rigid cell wall, since non-disrupted microalgal biomass was used.

3.3. Zootechnical performance parameters

Feed intake, egg production, egg weight, yolk weight, body weight, mortality and morbidity were recorded as zootechnical performance parameters, to examine if the different n-3 PUFA sources and the difference in non-disrupted and HPH-disrupted biomass, had an effect on the laying hen (**Table 5**). Based on the results of these parameters no drastic effects of the

different n-3 PUFA sources, either as oil or in non-disrupted or HPH-disrupted form, were observed. The supplementation of the different n-3 PUFA sources did also not lead to mortality, morbidity or decreased body weight of the laying hens. Previous studies by Lemahieu et al. [15, 38-39] also observed no drastic influences of the supplementation of the diet with n-3 PUFA sources to the diet of the laying hens. Other studies, on the other hand, are very contradictory about the effect on the zootechnical performance parameters, which can be explained by the different experimental setups [1].

4. Conclusion

Supplementation of the diet with the microalgal biomass of *Isochrysis* and *Nannochloropsis*, either in non-disrupted or HPH-disrupted form, showed the effect of the presence of a (rigid) cell wall on the n-3 LC-PUFA enrichment in the egg yolk. For *Isochrysis*, no difference in n-3 LC-PUFA enrichment efficiency in the egg yolk was observed between the supplementation of the non-disrupted or HPH-disrupted form since this microalgal species does not contain a (rigid) cell wall or only a cell membrane. For *Nannochloropsis*, on the other hand, a higher enrichment efficiency was obtained by the supplementation of HPH-disrupted *Nannochloropsis* biomass, because the bioaccessibility of the n-3 PUFA was reduced by the presence of a very rigid cell wall.

Next to this, the same enrichment efficiency was obtained with supplementation of the *Isochrysis* biomass (DHA source) and the HPH-disrupted biomass of *Nannochloropsis* (EPA source), which means that the needed conversion reaction of EPA to DHA with supplementation of *Nannochloropsis* had no effect on the enrichment efficiency. However, the enrichment efficiency when supplementing fish oil was still higher than the efficiency

366 obtained by the microalgal species, even if the cell wall was disrupted. This can probably be
367 explained by a difference in bioaccessibility of n-3 PUFA when added as oil or biomass.

368

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REFERENCES

- [1] I. Fraeye, C. Bruneel, C. Lemahieu, J. Buyse, K. Muylaert, I. Foubert, Dietary enrichment of eggs with omega-3 fatty acids: A review, *Food Res. Int.* 48 (2012) 961-969.
- [2] A.P. Simopoulos, Essential fatty acids in health and chronic disease, *Am. J. Clin. Nutr.* 70 (1999) 560S–569S.
- [3] E.A. Trautwein, n-3 Fatty acids – physiological and technical aspects for their use, *Sciences-New York*. 103 (2001) 45-55.
- [4] J.M. Bourre, Where to find omega-3 fatty acids and how feeding animals with diet enriched in omega-3 fatty acids to increase nutritional value of derived products for human: what is actually useful, *J. Nutr. Health Aging*. 9 (2005) 232–242.
- [5] W.S. Harris, Omega-3 fatty acids and cardiovascular disease: a case for omega-3 index as an new risk factor, *Pharmacol. Res.* 55 (2007) 217–223.
- [6] B.M. Yashodhara, S. Umakanth, J.M. Pappachan, S.K. Bhat, R. Kamath, B.H. Choo, Omega-3 fatty acids: a comprehensive review of their role in health and disease, *Postgrad. Med. J.* 85 (2009) 84–90.
- [7] R.G. Jordan, Prenatal omega-3 fatty acids: review and recommendations, *J. Midwifery Womens Health*. 55 (2010) 520–528.
- [8] U. Gogus, C. Smith, n-3 Omega fatty acids: a review of current knowledge, *Int. J. Food Sci. Technol.* 45 (2010) 417-436.

- 398 [9] N. Siriwardhana, N.S. Kalupahana, M. Moustaid, Health benefits of n-3
399 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid, *Adv. Food Nutr.*
400 *Res.* 65 (2012) 211-222.
- 401 [10] P.C. Calder, Very long chain omega-3 (n-3) fatty acids and human health. *Eur. J. Lipid*
402 *Sci. Tech.* 116 (2014) 1280-1300.
- 403 [11] P.M. Kris-Etherton, J. A. Grieger, T.D. Etherton, Dietary reference intakes for DHA and EPA.,
404 *Prostaglandins. Leukot. Essent. Fatty Acids.* 81 (2009) 99–104.
- 405 [12] I. Sioen, S. De Henauw, J. Van Camp, J.-L. Volatier, J.-C. Leblanc, Comparison of the
406 nutritional-toxicological conflict related to seafood consumption in different regions
407 worldwide., *Regul. Toxicol. Pharmacol.* 55 (2009) 219–228
- 408 [13] B.J. Meyer, Are we consuming enough long chain omega-3 polyunsaturated fatty acids for
409 optimal health?, *Prostaglandins. Leukot. Essent. Fatty Acids.* 85 (2011) 275–80.
- 410 [14] J.M. Miranda, X. Anton, C. Redondo-Valbuena, P. Roca-Saavedra, J. A. Rodriguez, A.
411 Lamas, C.M. Franco, A. Cepeda, Egg and egg-derived foods: effects on human health and use
412 as functional foods, *Nutrients.* 7 (2015) 706–729.
- 413 [15] C. Lemahieu, C. Bruneel, R. Termote-Verhalle, K. Muylaert, J. Buyse, I. Foubert, Impact
414 of feed supplementation with different omega-3 rich microalgae species on enrichment of
415 eggs of laying hens, *Food Chem.* 141 (2013) 4051-4059.
- 416 [16] A. Richmond, *Handbook of Microalgal Culture*, Blackwell Science, Australia, 2014.
- 417 [17] E. Ryckebosch, C. Bruneel, K. Muylaert, I. Foubert, Microalgae as an alternative source
418 of omega-3 long chain polyunsaturated fatty acids, *Lipid Techn.*, 24 (2012) 128–130.

- 419 [18] E. Ryckebosch, C. Bruneel, R. Termote-Verhalle, K. Goiris, K. Muylaert, K., I. Foubert,
420 Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty
421 acids as an alternative for fish oil, *Food Chem.* 160 (2014) 393–400.
- 422 [19] E.W. Becker, *Microalgae – Biotechnology and Microbiology.*: Cambridge University
423 Press, Cambridge, U.K, 1994.
- 424 [20] E.W. Becker, Micro-algae as a source of protein., *Biotechnol. Adv.* 25 (2007) 207–210.
- 425 [21] A. Skrede, L.T. Mydland, Ø. Ahlstrøm, K.I. Reitan, Evaluation of microalgae as sources
426 of digestible nutrients for monogastric animals, *J. Anim. Feed Sci.* 20, (2011) 131–142.
- 427 [22] F. Gelin, I. Boogers, A.A.M. Noordeloos, J.S. Sinninghe Damsté, R. Riegman, J.W. De
428 Leeuw, J.W., Resistant biomacromolecules in marine microalgae of the classes
429 Eustigmatophyceae and Chlorophyceae: Geochemical implications, *Org. Geochem.* 26 (1997)
430 659-675.
- 431 [23] P. Blokker, S. Schouten, H. Van Den Ende, J.W. De Leeuw, J.S. Sinninghe Damsté, Cell
432 wall-specific ω -hydroxy fatty acids in some freshwater green microalgae, *Phytochem.* 49
433 (1998) 691-695.
- 434 [24] F. Gelin, J.. Volkman, C. Largeau, S. Derenne, J.. Sinninghe Damsté, J.. De Leeuw,
435 Distribution of aliphatic, nonhydrolyzable biopolymers in marine microalgae, *Org. Geochem.*
436 30 (1999) 147–159.
- 437 [25] H.G. Gerken, B. Donohoe, E.P. Knoshaug, Enzymatic cell wall degradation of *Chlorella*
438 *vulgaris* and other microalgae for biofuels production, *Planta.* 237 (2013) 239–253.

439 [26] M.J. Scholz, T.L. Weis, R.E. Jinkerson, J. Jing, R. Roth, U. Goodenough, M.C. Posewitz,
 440 H.G. Gerken, Ultrastructure and composition of the *Nannochloropsis gaditana* cell wall.
 441 Eukaryot Cell. 13 (2014) 1450–1464.

442 [27] C.J. Zhu, Y.K. Lee, Determination of biomass dry weight of marine microalgae, J. Appl.
 443 Phycol. 9 (1997) 189–194.

444 [28] E.C. Wootton, M. V Zubkov, D.H. Jones, R.H. Jones, C.M. Martel, C.A. Thornton, E.C.
 445 Roberts, Biochemical prey recognition by planktonic protozoa., Environ. Microbiol. 9 (2007)
 446 216–222.

447 [29] Z. Nitsan, S. Mokady, A. Sukenik, Enrichment of poultry products with omega3 fatty
 448 acids by dietary supplementation with the alga *Nannochloropsis* and mantur oil, J. Agric. Food
 449 Chem. 47 (1999) 5127-5132.

450 [30] P. Janczyk, C. Wolf, W.B. Souffrant, Evaluation of nutritional value and safety of the
 451 green micro- algae *Chlorella vulgaris* treated with novel processing methods, Archiva
 452 Zootechnica. 8 (2005) 132–147.

453 [31] Y. Christi, M. Moo-Young, Review. Disruption of microbial cells for intracellular
 454 products, Enzyme Microb Tech. 8 (1986) 194-204.

455 [32] E. Molina Grima, E.-H. Belarbi, F.G. Acién Fernández, A. Robles Medina, Y. Chisti,
 456 Recovery of microalgal biomass and metabolites: process options and economics., Biotechnol.
 457 Adv. 20 (2003) 491–515.

458 [33] E. Günerken, E. D'Hondt, M.H.M. Eppink, L. Garcia-Gonzalez, K. Elst, R.H. Wijffels, Cell
 459 disruption for microalgae biorefineries, Biotechnol. Adv. (2015) accepted.

- 460 [34] I.L.D. Olmstead, S.E. Kentish, P.J. Scales, G.J.O. Martin, Low solvent, low temperature
461 method for extracting biodiesel lipids from concentrated microalgal biomass. *Bioresour.*
462 *Technol.* 148 (2013) 615–619.
- 463 [35] E.M. Spiden, B.H.J. Yap, D.R.A. Hill, S.E. Kentish, P.J. Scales, G.J.O. Martin, Quantitative
464 evaluation of the ease of rupture of industrially promising microalgae by high pressure
465 homogenization, *Bioresour. Technol.* 140 (2013) 165–171.
- 466 [36] E. Ryckebosch, K. Muylaert, I. Foubert, Optimization of an analytical procedure for
467 extraction of lipids from microalgae, *J. Am. Oil Chem. Soc.* 89 (2012) 189–198.
- 468 [37] E. Ryckebosch, S.P.C. Bermúdez, R. Termote-Verhalle, C. Bruneel, K. Muylaert, R. Parra-
469 Saldivar, I. Foubert, Influence of extraction solvent system on the extractability of lipid
470 components from the biomass of *Nannochloropsis gaditana*, *J. Appl. Phycol.* 26 (2013) 1501–
471 1510.
- 472 [38] C. Lemahieu, C. Bruneel, R. Termote-Verhalle, K. Muylaert, J. Buyse, I. Foubert, Effect
473 of different microalgal n–3 PUFA supplementation doses on yolk color and n–3 LC-PUFA
474 enrichment in the egg, *Algal Res.* 6 (2014) 119–123.
- 475 [39] C. Lemahieu, C. Bruneel, E. Ryckebosch, K. Muylaert, J. Buyse, I. Foubert, Impact of
476 different omega-3 polyunsaturated fatty acid (n-3 PUFA) sources (flaxseed, *Isochrysis galbana*,
477 fish oil and DHA Gold) on n-3 LC-PUFA enrichment (efficiency) in the egg yolk. In press: *J. Funct.*
478 *Foods* -doi:10.1016/j.jff.2015.04.021.
- 479 [40] C. Bruneel, C. Lemahieu, I. Fraeye, E. Ryckebosch, K. Muylaert, J. Buyse, I. Foubert,
480 Impact of microalgal feed supplementation on omega-3 fatty acid enrichment of hen eggs, *J.*
481 *Funct. Foods.* 5 (2013) 897–904.

- 482 [41] E. Ryckebosch, C. Bruneel, R. Termote-Verhalle, K. Muylaert, I. Foubert, Influence of extraction
483 solvent system on extractability of lipid components from different microalgae species, *Algal Res.* 3
484 (2014) 36–43.
- 485 [42] N. Samarasinghe, S. Fernando, B. Faulkner, Effect of high pressure homogenization on
486 aqueous phase solvent extraction of lipids from *Nannochloris oculata* microalgae, *J. Energy*
487 *Nat. Resources*, 1 (2012) 1-7.
- 488 [43] W. Barclay, R. Abril, P. Abril, C. Weaver, A. Ashorfd, Production of Docosahexaenoic
489 acid from microalgae and its benefits for use in animal feeds. In: A.P Simopoulos, *The Return*
490 *of ω 3 fatty acids into the food supply. Land-Based animal food products and their health*
491 *effects. World Rev Nutr Diet, Basel, Karger*, 83 (1998) 61-76..

492 **FIGURE CAPTIONS**

493 **Figure 1: Microscopic evaluation of the performed HPH-disruption on the microalgal**
494 **biomass of *Isochrysis* and *Nannochloropsis*.**

495

496

497 **TABLE**

498

499 **Table 1: Total lipid content as obtained with chloroform/methanol reference method (in mg/100 mg biomass; mean $\pm$ SD; n = 3 – analytical**
500 **replicates) and n-3 PUFA content (in mg/100 mg biomass; mean $\pm$ SD; n = 3) of the different n-3 PUFA sources: fish oil, *Isochrysis galbana* (non-**
501 **disrupted and HPH-disrupted) and *Nannochloropsis oculata* (non-disrupted and HPH-disrupted)**

	Fish oil	<i>Isochrysis</i>		<i>Nannochloropsis</i>	
		Non-disrupted	HPH-disrupted	Non-disrupted	HPH-disrupted
<i>Total lipid content</i>		25.6 $\pm$ 0.4	24.5 $\pm$ 1.2	24.8 $\pm$ 0.6	25.4 $\pm$ 1.1
<i>n-3 PUFA content</i> ¹					
ALA	1.45 $\pm$ 0.06	1.18 $\pm$ 0.02	1.12 $\pm$ 0.05	tr.	tr.
SDA	1.97 $\pm$ 0.09	1.52 $\pm$ 0.02	1.44 $\pm$ 0.06	- ²	-
EPA	10.8 $\pm$ 0.6	tr. ³	tr.	2.98 $\pm$ 0.11	2.76 $\pm$ 0.04
DPA	1.48 $\pm$ 0.10	tr.	tr.	-	-
DHA	7.40 $\pm$ 0.50	1.27 $\pm$ 0.05	1.13 $\pm$ 0.09	-	-

502 ¹ ALA = α -linolenic acid (C18:3 n-3), SDA = stearidonic acid (C18:4 n-3), EPA = eicosapentaenoic acid (C20:5 n-3), DPA = docosapentaenoic acid (C22:5 n-3) and
503 DHA = docosahexaenoic acid (C22:6 n-3).

504 ² - : not detected; detection limit: 0.1 ppm.

505 ³ tr.: indicates that the component is below 0.1 mg per 100 mg biomass.

506

Table 2: Lipid yield (expressed as g lipids/100 g biomass; mean $\pm$ SD; n = 3 – analytical replicates) of *Isochrysis galbana* (non-disrupted and HPH disrupted) and *Nannochloropsis oculata* (non-disrupted and HPH disrupted) using different extraction solvent (systems): Chloroform/methanol (1:1), hexane and hexane/isopropanol (3:2).

	Chloroform/ methanol	Hexane	Hexane/ isopropanol
<i>Isochrysis</i> non-disrupted	25.6 $\pm$ 0.4 ^{b,1}	14.4 $\pm$ 0.7 ^{a,3}	25.4 $\pm$ 1.0 ^{b,3}
<i>Isochrysis</i> disrupted	24.5 $\pm$ 1.2 ^{b,1}	12.5 $\pm$ 1.3 ^{a,3}	24.0 $\pm$ 0.6 ^{b,3}
<i>Nannochloropsis</i> non-disrupted	24.8 $\pm$ 0.6 ^{c,1}	1.3 $\pm$ 0.5 ^{a,1}	5.3 $\pm$ 0.3 ^{b,1}
<i>Nannochloropsis</i> disrupted	25.4 $\pm$ 1.1 ^{c,1}	8.9 $\pm$ 0.5 ^{a,2}	17.9 $\pm$ 0.9 ^{b,2}

Results with the same letter in the same row are not significantly different ($p < 0.05$).

Results with the same number in the same column are not significantly different ($p < 0.05$).

Table 3: Total n-3 LC-PUFA content in the egg at the end of the supplementation period (in mg/egg; mean $\pm$ SD; n = 12), the enrichment of n-3 LC-PUFA in the egg (in mg/egg; mean $\pm$ SD; n = 12) and enrichment efficiency (in %; mean $\pm$ SD) for the different n-3 PUFA sources: fish oil, *Isochrysis* (non-disrupted and HPH disrupted) and *Nannochloropsis* (non-disrupted and HPH disrupted).

	Total n-3 LC-PUFA content (mg/egg)	n-3 LC-PUFA enrichment ¹ (mg/egg)	n-3 LC-PUFA enrichment efficiency ² (%)
Fish oil	116 $\pm$ 13 ^c	79 $\pm$ 14 ^c	56 $\pm$ 10 ^c
<i>Isochrysis</i> non-disrupted	94 $\pm$ 9 ^b	57 $\pm$ 11 ^b	40 $\pm$ 8 ^b
<i>Isochrysis</i> disrupted	96 $\pm$ 12 ^b	59 $\pm$ 13 ^b	42 $\pm$ 9 ^b
<i>Nannochloropsis</i> non-disrupted	77 $\pm$ 9 ^a	41 $\pm$ 11 ^a	29 $\pm$ 8 ^a
<i>Nannochloropsis</i> disrupted	100 $\pm$ 14 ^b	63 $\pm$ 15 ^b	44 $\pm$ 11 ^b

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

¹ n-3 LC-PUFA enrichment is calculated by correcting the total n-3 LC-PUFA content in the egg at the end of the supplementation period with the total n-3 LC-PUFA content at the start of the supplementation period

² n-3 LC-PUFA enrichment efficiency is calculated by taking the ratio of the n-3 LC-PUFA enrichment in the egg to the effective intake of n-3 PUFA by the laying hens multiplied by 100.

Table 4: Level of ALA, EPA, DPA and DHA (SDA was not detected in the egg yolk) in the eggs at the end of the supplementation period (in mg/egg; mean $\pm$ SD; n = 12) of the different treatment groups: the control group and the groups were fish oil, *Isochrysis* (non-disrupted and HPH disrupted) and *Nannochloropsis* (non-disrupted and HPH disrupted) was supplemented to the diet.

	ALA	EPA	DPA	DHA
Control	33 $\pm$ 5 ^a	-	7 $\pm$ 2 ^a	40 $\pm$ 4 ^a
Fish oil	33 $\pm$ 4 ^a	26 $\pm$ 5 ^c	11 $\pm$ 3 ^{bc}	80 $\pm$ 10 ^c
<i>Isochrysis</i> non-disrupted	35 $\pm$ 6 ^a	12 $\pm$ 4 ^b	8 $\pm$ 2 ^{ab}	75 $\pm$ 4 ^c
<i>Isochrysis</i> disrupted	36 $\pm$ 6 ^a	13 $\pm$ 4 ^b	9 $\pm$ 1 ^{abc}	75 $\pm$ 8 ^c
<i>Nannochloropsis</i> non-disrupted	31 $\pm$ 4 ^a	4 $\pm$ 1 ^a	12 $\pm$ 4 ^{cd}	61 $\pm$ 7 ^b
<i>Nannochloropsis</i> disrupted	33 $\pm$ 5 ^a	7 $\pm$ 1 ^a	15 $\pm$ 3 ^d	77 $\pm$ 11 ^c

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

Table 5: Zootechnical performance parameters and egg quality parameters for the 6 different treatment groups during the supplementation period: daily feed intake (in g, mean $\pm$ SD; n = 12), egg production rate (in %), egg weight (in g, mean $\pm$ SD, n = 12) and yolk weight (in g, mean $\pm$ SD; n = 12).

	Daily feed intake (g)	Egg production rate (%)	Egg weight (g)	Yolk weight (g)
Control	117.7 $\pm$ 1.1 ^a	95	58.9 $\pm$ 0.9 ^{cd}	13.9 $\pm$ 1.0 ^a
Fish oil	118.0 $\pm$ 0.8 ^{ab}	94	57.8 $\pm$ 1.1 ^{bc}	14.0 $\pm$ 1.0 ^a
<i>Isochrysis</i> non-disrupted	117.6 $\pm$ 0.7 ^a	99	56.8 $\pm$ 1.2 ^{ab}	13.8 $\pm$ 0.8 ^a
<i>Isochrysis</i> disrupted	117.5 $\pm$ 1.1 ^a	96	56.5 $\pm$ 1.2 ^a	13.6 $\pm$ 1.2 ^a
<i>Nannochloropsis</i> non-disrupted	118.9 $\pm$ 0.4 ^b	95	58.7 $\pm$ 0.8 ^{cd}	13.8 $\pm$ 1.0 ^a
<i>Nannochloropsis</i> disrupted	118.0 $\pm$ 0.4 ^{ab}	98	59.2 $\pm$ 1.2 ^d	14.5 $\pm$ 1.0 ^a

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

Supplementary information

Supplementary Table 1: Total fatty acid profile (in mg/100 mg biomass; mean $\pm$ SD; n = 3) of the different n-3 PUFA sources: *Isochrysis galbana* (non-disrupted and HPH-disrupted) and *Nannochloropsis oculata* (non-disrupted and HPH-disrupted) and fish oil.

	<i>Isochrysis</i>		<i>Nannochloropsis</i>		Fish oil
	non-disrupted	disrupted	non-disrupted	disrupted	
C14:0	1.99 $\pm$ 0.07	1.95 $\pm$ 0.06	0.53 $\pm$ 0.02	0.51 $\pm$ 0.01	5.36 $\pm$ 0.19
C16:0	1.20 $\pm$ 0.02	1.18 $\pm$ 0.06	2.98 $\pm$ 0.10	2.82 $\pm$ 0.02	13.27 $\pm$ 0.52
C16:1	0.85 $\pm$ 0.03	0.77 $\pm$ 0.04	2.85 $\pm$ 0.11	2.72 $\pm$ 0.01	5.69 $\pm$ 0.22
C18:0	2.07 $\pm$ 0.05	2.01 $\pm$ 0.10	0.47 $\pm$ 0.02	0.44 $\pm$ 0.01	2.60 $\pm$ 0.12
C18:1	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.10 $\pm$ 0.01	0.09 $\pm$ 0.01	16.13 $\pm$ 0.70
C18:2n-6	0.59 $\pm$ 0.02	0.57 $\pm$ 0.02	0.30 $\pm$ 0.01	0.29 $\pm$ 0.01	3.84 $\pm$ 0.18
C18:3 n-3	1.18 $\pm$ 0.02	1.12 $\pm$ 0.05	tr. ¹	tr.	1.45 $\pm$ 0.06
C18:4 n-3	1.52 $\pm$ 0.02	1.44 $\pm$ 0.06	- ²	-	1.97 $\pm$ 0.09
C20:0	0.41 $\pm$ 0.04	0.41 $\pm$ 0.02	-	-	2.48 $\pm$ 0.14
C20:4 n-6	tr.	tr.	0.48 $\pm$ 0.02	0.46 $\pm$ 0.01	-
C20:5 n-3	tr.	tr.	2.98 $\pm$ 0.11	2.76 $\pm$ 0.04	10.8 $\pm$ 0.60
C23:0	0.24 $\pm$ 0.01	0.22 $\pm$ 0.02	-	-	-
C22:5 n-3	tr.	tr.	-	-	1.48 $\pm$ 0.10
C22:6 n-3	1.27 $\pm$ 0.05	1.13 $\pm$ 0.09	-	-	7.40 $\pm$ 0.50

¹ tr.: indicates that the component is below 0.1 mg per 100 mg biomass.

² - : not detected; detection limit: 0.1 ppm.

Supplementary Table 2: Total fatty acid profile (in mg/per egg; mean $\pm$ SD; n = 12) of the eggs at the end of the supplementation period for the different treatment groups: the control group and the supplementation with the different n-3 PUFA sources: fish oil, *Isochrysis galbana* (non-disrupted and HPH-disrupted) and *Nannochloropsis oculata* (non-disrupted and HPH-disrupted).

	<i>Isochrysis</i>				<i>Nannochloropsis</i>	
	Control	Fish oil	non-disrupted	disrupted	non-disrupted	disrupted
C14:0	13 $\pm$ 1 ^a	15 $\pm$ 2 ^{ab}	16 $\pm$ 2 ^b	16 $\pm$ 2 ^b	13 $\pm$ 2 ^a	15 $\pm$ 2 ^{ab}
C16:0	1055 $\pm$ 56 ^a	1066 $\pm$ 55 ^a	1042 $\pm$ 87 ^a	1038 $\pm$ 86 ^a	1035 $\pm$ 72 ^a	1070 $\pm$ 73 ^a
C16:1	124 $\pm$ 14 ^a	133 $\pm$ 13 ^{ab}	134 $\pm$ 22 ^{ab}	133 $\pm$ 17 ^{ab}	136 $\pm$ 14 ^{ab}	150 $\pm$ 19 ^b
C18:0	350 $\pm$ 25 ^a	350 $\pm$ 29 ^a	347 $\pm$ 13 ^a	330 $\pm$ 27 ^a	333 $\pm$ 28 ^a	349 $\pm$ 32 ^a
C18:1	1605 $\pm$ 124 ^a	1638 $\pm$ 67 ^a	1604 $\pm$ 109 ^a	1567 $\pm$ 108 ^a	1549 $\pm$ 98 ^a	1605 $\pm$ 120 ^a
C18:2n-6	847 $\pm$ 76 ^b	790 $\pm$ 77 ^{ab}	710 $\pm$ 89 ^a	701 $\pm$ 193 ^a	763 $\pm$ 80 ^{ab}	775 $\pm$ 79 ^{ab}
C18:3n-6	6 $\pm$ 1 ^a	5 $\pm$ 1 ^a	5 $\pm$ 1 ^a	5 $\pm$ 1 ^a	5 $\pm$ 1 ^a	5 $\pm$ 1 ^a
C18:3 n-3	33 $\pm$ 5 ^a	33 $\pm$ 4 ^a	35 $\pm$ 6 ^a	36 $\pm$ 6 ^a	31 $\pm$ 4 ^a	33 $\pm$ 5 ^a
C20:1	7 $\pm$ 1 ^a	7 $\pm$ 1 ^a	6 $\pm$ 1 ^a	6 $\pm$ 1 ^a	6 $\pm$ 1 ^a	7 $\pm$ 1 ^a
C20:2	7 $\pm$ 1 ^a	6 $\pm$ 1 ^a	6 $\pm$ 1 ^a	7 $\pm$ 1 ^a	7 $\pm$ 1 ^a	7 $\pm$ 1 ^a
C20:3n-6	6 $\pm$ 1 ^a	6 $\pm$ 1 ^a	6 $\pm$ 1 ^a	6 $\pm$ 1 ^a	6 $\pm$ 1 ^a	6 $\pm$ 1 ^a
C20:4n-6	80 $\pm$ 5 ^b	65 $\pm$ 7 ^a	66 $\pm$ 5 ^a	65 $\pm$ 6 ^a	72 $\pm$ 7 ^a	71 $\pm$ 7 ^a
C20:5n-3	- ¹	26 $\pm$ 5 ^c	12 $\pm$ 4 ^b	13 $\pm$ 4 ^b	4 $\pm$ 1 ^a	7 $\pm$ 1 ^a
C22:4	7 $\pm$ 2 ^a	5 $\pm$ 1 ^a	5 $\pm$ 1 ^a	5 $\pm$ 1 ^a	5 $\pm$ 1 ^a	5 $\pm$ 1 ^a
C22:5n-3	7 $\pm$ 2 ^a	11 $\pm$ 3 ^{bc}	8 $\pm$ 2 ^{ab}	9 $\pm$ 1 ^{abc}	12 $\pm$ 4 ^{cd}	15 $\pm$ 3 ^d
C22:6n-3	40 $\pm$ 4 ^a	80 $\pm$ 10 ^c	75 $\pm$ 4 ^c	75 $\pm$ 8 ^c	61 $\pm$ 7 ^b	77 $\pm$ 11 ^c

¹ - : not detected; detection limit: 0.1 ppm

Results with the same letter in the same row are not significantly different (p<0.05)