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The cell wall of autotrophic microalgae influences the enrichment of long chain omega-3 fatty acids 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, fish oil was also 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.

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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 over the last years [1]. This is 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 (CO_2) as carbon source to form their biomass [16–17]. Several of these autotrophic microalgal species contain a certain amount of n − 3 PUFA in their biomass [17–18]. Lemahieu et al. [15] supplemented four different autotrophic microalgal species (*Phaeodactylum tricornutum*, *Nannochloropsis oculata*, *Isochrysis galbana* and *Chlorella fusca*). Both *Phaeodactylum* and *Nannochloropsis* act as an EPA source, while *Isochrysis* serves as a DHA source but also contains significant amounts of ALA and stearidonic acid (SDA). *Chlorella*, on the other hand, is a typical example of an autotrophic microalgal ALA source. The different microalgal species, although supplemented to reach the same n − 3 PUFA dose, caused different n − 3 LC-PUFA enrichment (efficiencies) in the egg yolk. The highest n − 3 LC-PUFA enrichment efficiencies were obtained by the supplementation of *Phaeodactylum* and *Isochrysis* (respectively 43% and 41% for the

Abbreviations: n − 3 LC-PUFA, Omega-3 long chain polyunsaturated fatty acids; ALA, α-Linolenic acid; SDA, Stearidonic acid; EPA, Eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA, Docosahexaenoic acid; HPH, High Pressure Homogenization.

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lowest supplementation dose). A 20% lower n − 3 LC-PUFA enrichment efficiency (approximately 23% for the lowest supplementation dose) was obtained by the supplementation of *Nannochloropsis*. *Chlorella*, as a source of ALA, gave rise to the lowest n − 3 LC-PUFA enrichment efficiency in the egg yolk (12% for the lowest supplementation dose), presumably because of the low conversion of ALA to n − 3 LC-PUFA. Next to the different fatty acid profiles of the supplemented microalgal species, the difference in n − 3 LC-PUFA enrichment efficiencies can presumably also be explained by the difference in bioaccessibility of the n − 3 PUFA in the microalgal biomass. Several microalgal species possess a thick and rigid cellulosic cell wall which may reduce the bioaccessibility/digestibility of the microalgal biomass since it is not digestible for humans and other ruminants [19–21]. For the microalgal species *Nannochloropsis*, it is generally known that it consists of a very rigid, hard digestible cell wall which contains an aliphatic, non-hydrolysable biopolymer, called algaenan [22–26]. For *Isochrysis*, on the other hand, contradictory results were found whether or not this species has a cell wall [27–28]. So, the different bioaccessibility of the n − 3 PUFA could also be a possible explanation for the different n − 3 LC-PUFA enrichment efficiencies by the supplementation of the diet with *Nannochloropsis* and *Isochrysis*.

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

The purpose of this study was therefore to evaluate the n − 3 LC-PUFA enrichment efficiency when adding *Isochrysis* and *Nannochloropsis* to laying hens, in either non-disrupted or disrupted (using HPH) form. Next to the microalgal biomasses, also fish oil was supplemented 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: *I. galbana* and *N. 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 state of the art methods.

2.1.1. HPH disruption of the autotrophic microalgae

I. galbana and *N. 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), and 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 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 16 h 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

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