JOURNAL OF FUNCTIONAL FOODS 5 (2013) 897–904

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Impact of microalgal feed supplementation on omega-3 fatty acid enrichment of hen eggs

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ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form
30 January 2013
Accepted 31 January 2013
Available online 6 March 2013

Keywords:
Omega-3 polyunsaturated fatty acids
Microalgae
Nannochloropsis
Eggs
Enrichment
Efficiency of deposition of n-3 LC-PUFA

ABSTRACT

In many Western countries, the average intake of the health beneficial omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is below the recommended level, raising interest in food enrichment with n-3 LC-PUFA. To that end, the impact of feed supplementation with EPA rich autotrophic microalgal biomass on n-3 LC-PUFA enrichment of eggs was studied. Hens were divided in three groups receiving different diets for 28 days: a standard diet (C) for laying hens, (C) supplemented with 5.0% spray dried *Nannochloropsis gaditana*, and (C) to which 10.0% of these microalgae were added. Microalgal EPA was hardly accumulated in yolk lipids, but preferentially converted to DHA and deposited in yolk phospholipids. The efficiency of deposition of microalgal n-3 LC-PUFA to eggs was rather low. Switching back to standard feed ensured that the n-3 LC-PUFA level obtained in enriched eggs decreased back to that of the control eggs. Moreover, the colour of egg yolk shifted from yellow to more orange-red, which is presumably due to transfer of microalgal carotenoids to egg yolk. Thus, the use of autotrophic microalgae as supplement for standard feed offers an alternative to current sources for the production of DHA enriched eggs.

1. Introduction

It is generally accepted that omega-3 polyunsaturated fatty acids (n-3 PUFA) have potential in the prevention and treatment of several diseases aside from their important role in neuronal development (Gogus & Smith, 2010; Jordan, 2010; Yashodhara et al., 2009). The health benefits are mainly ascribed to the long chain (LC) n-3 PUFA eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), rather than to the shorter chain n-3 PUFA α-linolenic acid (ALA, 18:3 n-3). The conversion of ALA to EPA and further to DHA is very limited and inefficient in the human body (Komprda, 2012), and especially in infants and elderly people (Lagarde, 2008; Nitsan, Mokady, & Sukenik, 1999; Trautwein, 2001). Various governments and health organizations recommend an average dietary intake of EPA plus DHA ranging from 140 to 600 mg per day (Komprda, 2012; Molendi-Coste, Legry, & Leclercq, 2011). Unfortunately, in many Western countries, the recommended dietary intake of n-3 LC-PUFA is rarely met by the majority of consumers (Sioen et al., 2006; Welch,

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1756-4646/$ - see front matter
http://dx.doi.org/10.1016/j.jff.2013.01.039

Shakya-Shrestha, Lentjes, Wareham, & Khaw, 2010). To that end, there is clearly a need for direct intake of EPA/DHA in the human diet, raising interest in food enrichment with these n-3 LC-PUFA. Hence, their inclusion into foods and specialty products has been in the forefront of research and development (Shahidi, 2009).

Eggs are an integral part of our diet either as food but also as food ingredient in many applications. Hence, eggs form an interesting food product to enrich with n-3 LC-PUFA. Moreover, it has been shown that the level and type of PUFA in eggs can be modified through dietary supplementation with n-3 PUFA (Baucells, Crespo, Barroeta, Lopez-Ferrer, & Grashorn, 2000; Cachaldora, García-Rebollar, Alvarez, De Blas, & Méndez, 2008). Different sources of n-3 PUFA can be used to enrich eggs. For the interested reader we refer to the review of Fraeye et al. (2012). Briefly, when hens' diet is supplemented with a traditional ALA rich plant source, such as flaxseed, eggs are mainly enriched with ALA, and to a lesser extent with n-3 LC-PUFA. The n-3 LC-PUFA levels in such 'ALA-enriched' eggs are generally below 100 mg/egg (Aymond & Van Elswyk, 1995; Bean & Leeson, 2003; Fraeye et al., 2012). However, when hens are fed fish oil (rich in EPA/DHA), egg yolk lipids are mainly enriched with DHA, whereas EPA is rarely detected and seems to be converted to DHA before it is deposited. In such 'DHA-enriched' eggs, the level of yolk DHA is generally below 100 mg/egg (Fraeye et al., 2012; Gonzalez-Esquerra & Leeson, 2000; Lawlor, Gaudette, Dickson, & House, 2010), which is rather low since similar DHA levels, *i.e.* maximum 100 mg DHA per egg, can be obtained when hens are fed with flaxseed. These rather low DHA levels obtained when hens are fed fish oil can be explained by the fact that the dosage of fish oil for feed supplementation needs to be restricted since n-3 LC-PUFA in fish oil are highly susceptible to oxidation and the formed oxidation products cause undesirable off-flavours in the enriched eggs (Gonzalez-Esquerra & Leeson, 2000; Gonzalez-Esquerra & Leeson, 2001).

During the last 15 years, a number of research groups have investigated the possibility of feeding laying hens microalgae, since they are the primary natural producers of n-3 LC-PUFA. Khozin-Goldberg, Iskandarov, and Cohen (2011) and Ryckebosch, Bruneel, Muylaert, and Foubert (2012) reported an overview of LC-PUFA occurrence in microalgae species from different classes. Most reports about enrichment of eggs using microalgae as feed supplement deal with DHA rich heterotrophic microalgae which use organic compounds as a primary source of nutrition. The PUFA profile of eggs from hens fed those algae is very similar to that of eggs from hens fed fish oil (Cachaldora et al., 2008; Gonzalez-Esquerra & Leeson, 2001; Herber & Van Elswyk, 1996). However, DHA levels up to 200 mg/egg are reported by Herber-McNeill and Van Elswyk (1998). Next to heterotrophic microalgae, also autotrophic microalgae, which are able to use CO_2 to produce organic compounds with the aid of sunlight (Nuño et al., 2013), can be used to enrich eggs with n-3 LC-PUFA. However, to the best of our knowledge, only two studies were already published in which autotrophic microalgae, *i.e. Nannochloropsis*, are used as a feed supplement for laying hens to enrich their eggs with n-3 LC-PUFA (Fredriksson, Elwinger, & Pickova, 2006; Nitsan et al., 1999). *Nannochloropsis* has an interesting fatty acid profile since it contains only EPA as n-3 LC-PUFA and relatively low levels of n-6 LC-PUFA (Khozin-Goldberg et al., 2011). When hens were fed *Nannochloropsis*, microalgal EPA was not accumulated in egg yolk, but apparently converted and deposited in egg yolk as DHA (Fredriksson et al., 2006; Nitsan et al., 1999). However, it should be mentioned that the experimental standard feed of both studies was already rich in ALA to increase the n-3/n-6 ratio of the diet and, hence, to stimulate the conversion steps of the n-3 pathway. Furthermore, autotrophic microalgae contain, next to n-3 LC-PUFA, also other nutritionally interesting components, such as carotenoids. They can act as antioxidants to preserve the relatively unstable PUFA and thus increase lipid stability (Pangestuti & Kim, 2011). However, they also have an impact on yolk colour which can shift from yellow to more orange-red (Fraeye et al., 2012; Fredriksson et al., 2006; Nitsan et al., 1999).

From the above summary, it is clear that very little research has been done about the impact of autotrophic microalgae to enrich eggs with n-3 LC-PUFA. Nevertheless, these sustainable autotrophic microalgae are an interesting alternative source to further study in this field of research. However, it is important to determine the impact of EPA supplementation by adding *Nannochloropsis* to a commercially available standard feed for laying hens instead of adding it to an ALA-enriched feed. Furthermore, since the price of autotrophic microalgae is currently still high, the efficiency of deposition of microalgal n-3 LC-PUFA in egg yolk needs to be calculated. Furthermore, it has not yet been studied to what extent n-3 LC-PUFA are still deposited in eggs after returning the hens to a standard feed, and there is also little known about the egg quality characteristics and zootechnical performances of hens fed autotrophic microalgae. Thus, the aim of this study was to investigate the impact of the EPA rich autotrophic microalga *Nannochloropsis gaditana* as a supplement to a commercially available standard diet on n-3 LC-PUFA enrichment of egg yolk. The efficiency of deposition of microalgal n-3 LC-PUFA in egg yolk was, to the best of our knowledge, for the first time calculated for autotrophic microalgae. Moreover, the impact of returning to the basal feed, devoid of *N. gaditana*, on the level of n-3 LC-PUFA in eggs was assessed.

2. Materials and methods

All used chemicals and reagents were at least of analytical grade and purchased from Sigma–Aldrich (Bornem, Belgium), unless specified otherwise.

2.1. *Microalgal biomass*

Spray dried biomass from the autotrophic cultured *N. gaditana* was obtained from Clean Algae (Las Palmas de Gran Canaria, Spain). The composition of the microalgal biomass – as given by the supplier – is shown in Table 1. The total lipid content and the fatty acid profile of the microalgal biomass were determined in our laboratory by a modified method of Folch, Lees, and Sloane Stanley (1957) which is described in Section 2.5. The results are added to Table 1.