



Short communication

Effect of different microalgal n–3 PUFA supplementation doses on yolk color and n–3 LC-PUFA enrichment in the egg



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ABSTRACT

To examine the effect of different omega-3 polyunsaturated fatty acid (n–3 PUFA) doses on the long chain (LC) n–3 PUFA enrichment in the egg yolk, *Isochrysis galbana* was supplemented in nine different doses (from 0 to 400 mg n–3 PFAs per 100 g feed) to the diet of laying hens. A linear increase of the n–3 LC-PUFA enrichment was observed up to a dose of 120 mg n–3 PFAs per 100 g feed. Higher n–3 PUFA supplementation doses resulted in a decreased efficiency of n–3 LC-PUFA incorporation into the egg yolk. Pronounced color changes were observed due to transfer of carotenoids from the microalgal biomass to the egg yolk. However, a corresponding linear trend in yolk color changes was not observed for the low supplementation doses when compared to the n–3 LC-PUFA changes in the egg yolk. The a^* -value increased remarkably with supplementation of 3% *Isochrysis* (150 mg n–3 PFAs per 100 g feed) and remained stable with the higher supplementation doses.

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1. Introduction

Enrichment of eggs with omega-3 polyunsaturated fatty acids (n–3 PFAs) is possible through supplementation of the feed of laying hens with n–3 PUFA sources (e.g., flaxseed, fish oil or even microalgae) [1]. However, a clear distinction must be made regarding the enrichment of eggs with the shorter chain ($C \leq 18$) PUFA (α -linolenic acid or ALA) and n–3 longer chain PFAs or n–3 LC-PFAs (eicosapentaenoic acid or EPA and docosahexaenoic acid or DHA) [1]. Since several health benefits are related to the content of n–3 LC-PFAs instead of ALA, it is more interesting to raise the level of n–3 LC-PFAs in the egg [2–5].

ALA enriched eggs can be obtained by the supplementation of the diet of laying hens with flaxseed [1,6]. Enrichment of eggs with n–3 LC-PFAs can be achieved by supplementing the feed with fish oil, a source of EPA and DHA. A more recent trend is to replace fish oil with heterotrophic and autotrophic microalgae [1,4,5]. Supplementation of diets with autotrophic microalgae provides the most environmentally

sustainable approach to raising n–3 PUFA concentrations in eggs. Unlike heterotrophic microalgae, autotrophic microalgae only need light and CO₂ to form their biomass and the desirable fatty acids.

To the best of our knowledge, the effect of different microalgal n–3 PUFA supplementation doses on the n–3 LC-PUFA enrichment in the egg yolk has rarely been examined. Herber and Van Elswyk [7] supplemented 2.4% and 4.8% heterotrophically cultivated microalgae to the diet of laying hens, which correspond with a supplementation of 200 mg DHA/day and 400 mg DHA/day respectively. They noted that doubling the amount of microalgae in the diet did not result in a doubled n–3 LC-PUFA deposition in the egg yolk, thus leading to a decreased efficiency of n–3 LC-PUFA incorporation with higher n–3 PUFA supplementation doses. The same observation was made by Bruneel et al. [8] who supplemented two doses of the autotrophic microalgae *Nannochloropsis*, 5% and 10%, corresponding with an intake of 76 mg n–3 PFAs per 100 g feed and 152 mg n–3 PFAs per 100 g feed. They obtained an n–3 LC-PUFA enrichment efficiency of 25% and 20% for the respective doses of 5% and 10% *Nannochloropsis*. A lower n–3 LC-PUFA incorporation efficiency was also observed in our previous study [4], where four different microalgae in two different doses (125 mg and 250 mg ALA + EPA + DHA per 100 g feed) were supplemented to the hens' diet. Carvalho et al. [9] supplemented different amounts of DHA to the diet of the laying hens by the addition of salmon oil or the heterotrophic microalga *Schizochytrium*. These

Abbreviations: n–3 LC-PFAs, omega-3 long chain polyunsaturated fatty acids; ALA, α -linolenic acid; SDA, stearidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

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researchers concluded that the highest DHA incorporation efficiency was obtained at an intake of 120 mg to 180 mg DHA per 100 g feed. Remarkable decreases in incorporation efficiencies were observed with higher supplementation doses. However, in this study, no information was provided for supplementation doses lower than 20 mg DHA per 100 g diet. Nitsan et al. [10] supplemented very low doses of *Nannochloropsis*, corresponding to very low doses of n–3 PUFAs. Addition of 0.1% and 0.5% *Nannochloropsis* did not give rise to enrichment of DHA in the egg yolk [10]. Only 1% supplementation of *Nannochloropsis* gave rise to a higher n–3 PUFA content in the egg yolk. From the above, it is clear that supplementation of different microalgal doses, corresponding with different n–3 PUFA doses, has a significant effect on the n–3 LC-PUFA enrichment in the egg yolk.

An important difference regarding the use of fish oil and microalgae for supplementation of laying hens' feed with n–3 LC-PUFAs is that microalgae contain significant concentrations of carotenoids. Like the n–3 LC-PUFAs, carotenoids can be transferred to the egg yolk and influence the yolk color [4]. This can be advantageous, because it reduces the need for addition of synthetic carotenoids to the feed, which is a significant cost. However, it is important to take into account consumer preferences relating to the yolk color, as deeply red yolk colors are not desirable [5].

Unfortunately, in the abovementioned studies, only a few different n–3 PUFA supplementation doses were studied, making it difficult to draw conclusions on the general effect of doses on the n–3 PUFA and/or n–3 LC-PUFA enrichment (efficiency) in the egg yolk. Moreover, in most cases, no information was provided on the n–3 PUFA dose, making it difficult to compare different studies. Therefore, in this research, for the first time, a thorough study was performed to examine the effect of nine n–3 PUFA supplementation doses on the n–3 LC-PUFA incorporation (efficiency) in the egg yolk using supplementation of different doses of *Isochrysis galbana* into the diet of the laying hens. In addition to the n–3 LC-PUFA enrichment, the effect of the different *Isochrysis* doses on the egg yolk color was examined in this study, since earlier research has shown a carotenoid transfer from autotrophic microalgal biomass to the egg yolk [4].

2. Materials and methods

2.1. Microalgal biomass

To determine the impact of different supplementation doses on the n–3 LC-PUFA enrichment in the egg yolk, *I. galbana*, selected by Lemahieu et al. [4] as the most appropriate autotrophic microalgal species, was supplemented into the diet of laying hens. The *I. galbana* lyophilized biomass used in this study was obtained from Archimede Recherche (Italy).

The total lipid content and the fatty acid profile of the *Isochrysis* biomass were determined in triplicate by the method described in Ryckebosch et al. [11]. After the extraction and quantification of the total lipids, the fatty acids were methylated and the fatty acid methyl esters were gas chromatographically separated (Trace GC Ultra, Thermo Scientific, Interscience, Louvain-la-Neuve, Belgium), as described in Lemahieu et al. [4].

2.2. Animals and diets

72 ISA Brown laying hens (33 weeks of age, 't Munckeni, Wingene, Belgium) were housed in battery cages (two hens per cage) under environmentally controlled conditions (room temperature of 20 °C and a 16 h photoperiod). Feed and water were supplied for ad libitum consumption. The experiment started with an adaptation period of 14 days in which the hens could adapt to the new environmental conditions and the new commercially available control diet (AVEVE legmeel Total 277). The control diet consisted mainly of corn, wheat, sunflower cake, corn gluten, limestone, soybean meal, and palm oil. The control

diet contained no n–3 LC-PUFAs, and only a small amount of ALA (0.074%) was observed (Table 2).

After the adaptation period, the laying hens were randomly assigned to one of the nine treatment diets (n = 8 hens per treatment diet): a control diet, without supplementation of *I. galbana*, and eight diets in which the amount of *Isochrysis* was calculated to reach eight different levels of extra microalgal n–3 PUFA (ALA + stearidonic acid (SDA) + EPA + docosapentaenoic acid (DPA) + DHA) supplementation per 100 g feed. An overview of the different n–3 PUFA doses and the added levels of *I. galbana* is shown in Table 1. The supplementation of *Isochrysis* in different doses lasted 21 days. Earlier research by Lemahieu et al. [12] showed a maximum n–3 PUFA incorporation after 14 days of supplementation. However, to be sure of the maximum incorporation of the n–3 LC-PUFAs in this experiment, *Isochrysis* was supplemented for 21 days to obtain reliable results.

During the supplementation period, some zootechnical parameters and egg quality parameters were registered on a daily basis: feed intake, egg production, mortality, morbidity, egg weight and yolk weight.

2.3. Egg collection, storage and analysis

The eggs were collected on a daily basis and stored at –20 °C. The eggs at the start and the end of the supplementation period were analyzed to determine the n–3 LC-PUFA content and the yolk color according to the methods described in Lemahieu et al. [4].

2.4. Statistical analysis

The results were statistically evaluated by one way analysis of variance (ANOVA) and post-hoc Tukey's test with $\alpha = 0.05$.

3. Results and discussion

3.1. Microalgal biomass

The fatty acid profile of *I. galbana* was determined in order to calculate the different supplementation doses (Table 1). The n–3 PUFA composition of *Isochrysis* is shown in Table 2. Predominantly DHA was observed as an n–3 LC-PUFA in the biomass but *Isochrysis* also contained high amounts of SDA. So, instead of only taking into account ALA, EPA and DHA as omega-3 fatty acids, as in the experimental design of Lemahieu et al. [4], all the omega-3 fatty acids (ALA, SDA, EPA, DPA and DHA) were taken into account to calculate the supplemented dose of *I. galbana* (Table 1). The added levels of *I. galbana* were varied between 0% and 8.1% to reach n–3 PUFA supplementation doses of 0 to 400 mg per 100 g feed.

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

Supplementation of *Isochrysis* to the diet of the laying hens resulted in changes of the n–3 PUFA content (Table 1); no drastic changes in other fatty acids were observed (results not shown). Fig. 1 shows the n–3 LC-PUFA content (EPA + DPA + DHA) in the egg yolk at the start of the supplementation period (day 0) and at the end of the supplementation period (day 21) for the different doses of microalgal n–3 PUFA supplementation. These results show, at day 21, a linear relationship between the two parameters until a dose of 120–150 mg n–3 PUFAs per 100 g feed. This linear increase of the n–3 LC-PUFA enrichment in the yolk was mainly caused by DHA enrichment, as DHA is preferentially incorporated in the egg yolk [4]. More than 90% of the n–3 LC-PUFA content in the egg yolk was attributed to the fatty acid DHA. A flattening of the curve was observed at higher n–3 PUFA supplementation doses, indicating a decrease in efficiency of the n–3 LC-PUFA incorporation. In Table 3, the efficiency of n–3 LC-PUFA incorporation in the egg for several microalgal supplementation doses is shown. To obtain this value, the ratio of the n–3 LC-PUFA enrichment in the egg

Table 1

Overview of the nine different microalgal supplemented n–3 PUFA (ALA + SDA + EPA + DPA + DHA) doses (in mg per 100 g feed) and the corresponding percentage of supplemented microalgal biomass (in %).

	Dose of microalgal ALA + SDA + EPA + DPA + DHA (mg per 100 g feed)	Supplemented microalgal biomass (%)
Diet 1	0	0
Diet 2	30	0.6
Diet 3	60	1.2
Diet 4	90	1.8
Diet 5	120	2.4
Diet 6	150	3
Diet 7	200	4.1
Diet 8	300	6.1
Diet 9	400	8.1

and the intake of n–3 PUFAs was calculated. The former value was obtained by correcting the n–3 LC-PUFA content in the egg at day 21 with the n–3 LC-PUFA content in the egg at day 0. The latter value was calculated by multiplying the different doses (for example 30 mg n–3 PUFAs per 100 g feed) by the mean feed intake of the laying hens in the respective groups [4]. The highest efficiency of n–3 LC-PUFA incorporation was observed with supplementation of 120 mg microalgal n–3 PUFAs per 100 g feed, although this value was not significantly different from the efficiencies obtained by supplementation of 60 mg and 90 mg microalgal n–3 PUFAs per 100 g feed. However, the total n–3 LC-PUFA enrichment is equally important. As supplementation of 120 mg n–3 PUFAs per 100 g feed (corresponding with the supplementation of 2.4% *Isochrysis* biomass) leads to the highest n–3 LC-PUFA enrichment in the yolk with the highest n–3 LC-PUFA incorporation efficiency, this supplementation dose can be considered as the optimal dose (Table 3).

A linear increase of n–3 PUFA content in the egg yolk by supplementation of different n–3 PUFA doses was also observed by Caston & Leeson [13], Schiedeler & Froning [14] and Van Elswyk [6], when flaxseed was used as the supplement. However, in these studies, no information was given about the exact amount of n–3 PUFA supplementation. So, it is hard to say if the linear increment corresponds to similar observations in this study. On the other hand, many studies, supplementing different amounts of n–3 PUFAs to the diet of laying hens, observed a decrease in efficiency of n–3 PUFA incorporation with increasing n–3 PUFA supplementation. Van Elswyk [6], for example, supplemented different doses of fish oil as a source of n–3 PUFAs, and observed no significant difference in n–3 PUFA content of the eggs from hens fed 15 g fish oil/kg or 30 g fish oil/kg. This means that starting from a certain dose, the n–3 PUFA content in the egg reached the maximum incorporation despite the increase of n–3 PUFAs in the diet of laying hens. However, no information was given about the fish oil composition, so the effective supplemented n–3 PUFA dose could not be determined. The same doses of fish oil (15 g/kg diet and 30 g/kg diet, corresponding to a supplementation of 462 mg n–3 PUFAs and 924 mg n–3 PUFAs per 100 g feed, respectively) were used by Cachaldora et al. [15]. The supplementation of these two doses of fish oil resulted in an efficiency of n–3 LC-PUFA incorporation of approximately 22% and 18% for the respective doses, which means

Table 2

n–3 PUFA composition (ALA, SDA, EPA, DPA and DHA) of the control diet, AVEVE legmeel Total 277, and *Isochrysis galbana* (in g/100 g biomass; mean $\pm$ SD; n = 3).

n–3 PUFAs	Control diet	<i>I. galbana</i>
ALA	0.074 $\pm$ 0.023	1.247 $\pm$ 0.016
SDA	0	1.830 $\pm$ 0.031
EPA	0	0.091 $\pm$ 0.004
DPA	0	0.023 $\pm$ 0.002
DHA	0	1.737 $\pm$ 0.052

that a higher dose also resulted in a lower efficiency of n–3 LC-PUFA enrichment. However, only two doses were tested so the general trend could not be derived.

The same trend was observed by supplementation of autotrophic microalgae as the n–3 PUFA source. Bruneel et al. [8] also observed a decreased efficiency of n–3 LC-PUFA incorporation for the highest n–3 PUFA supplementation dose (152 mg n–3 PUFAs per 100 g feed) in comparison with the lower dose (76 mg n–3 PUFAs per 100 g feed). This corresponds also with the study of Lemahieu et al. [4], who suggested that the highest incorporation efficiency can be obtained with a supplementation dose below 250 mg n–3 PUFAs per 100 g feed.

3.3. Yolk color analysis

Not only an enrichment of n–3 LC-PUFAs by supplementation of autotrophic microalgae into the diet of the laying hens, but also a remarkable yolk color change was observed [4]. The egg yolk color is influenced by the type and concentration of carotenoids present in the diet of the laying hen [16–18]. Therefore, supplementation of autotrophic microalgae, rich in carotenoids, results in changes of the yolk color [4]. The results of the yolk color analysis are shown in Table 4. The yolk color was measured with a colorimeter to obtain a CIE 1976 L^* , a^* , and b^* color space. The L^* -value corresponds to the degree of lightness, the a^* -value corresponds to the degree of redness, and the b^* -value, corresponds to the yellowness of the egg yolk [8]. In general, addition of *Isochrysis* to the diet of laying hens resulted in a decrease of the L^* - and b^* -values and an increase of the a^* -value. This corresponds with an increased darkness, reduced yellowness and increased redness of the egg yolk. Based on the research of Lemahieu et al. [4], the color shift by supplementation with *Isochrysis* can be explained by the enrichment of fucoxanthin derivatives in the egg yolk. Fucoxanthin has a typical brown color, which is darker than the yellow lutein and zeaxanthin pigments normally present in the egg yolk.

When looking in more detail at the effect of dose on the redness of the egg yolk, it can be seen that the a^* -value remained more or less the same up to a supplementation of 120 mg microalgal n–3 PUFAs per 100 g feed (2.4% *Isochrysis* supplementation). Starting from 150 mg n–3 PUFAs per 100 g feed, corresponding with 3% *Isochrysis* supplementation, a remarkable increase of the a^* -value was observed. Higher supplementation doses did not further increase the a^* -value. However, significant changes in L^* -, a^* - and b^* -values may be perceived differently by the human eye, so we therefore scored the yolk

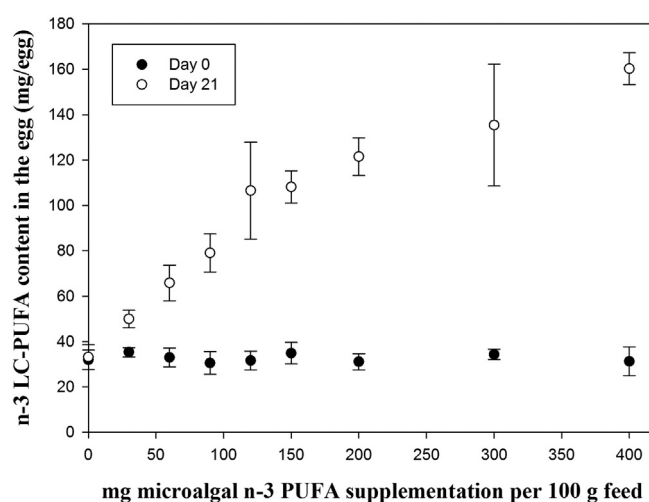


Fig. 1. n–3 LC-PUFA content (EPA + DPA + DHA) (in mg/egg, mean $\pm$ SD, n = 8) in the egg at the start and the end of the supplementation period with different n–3 PUFA doses from *Isochrysis galbana*.

Table 3

Incorporation efficiency* of the n–3 LC-PUFAs (in %, mean $\pm$ SD; n = 8) in the egg yolk for the different supplementation doses of *Isochrysis galbana*.^y

Microalgal n–3 PUFAs (mg per 100 g feed)	Intake of n–3 PUFAs (mg)	Enrichment of n–3 LC-PUFAs (mg)	Incorporation efficiency (%)
30	35.7 $\pm$ 0.1	14.7 $\pm$ 4.8 ^a	41.1 $\pm$ 13.4 ^b
60	68.6 $\pm$ 1.2	32.9 $\pm$ 6.0 ^b	47.9 $\pm$ 8.8 ^{bc}
90	107.0 $\pm$ 0.4	48.5 $\pm$ 6.6 ^c	45.3 $\pm$ 6.2 ^{bc}
120	142.0 $\pm$ 0.6	74.9 $\pm$ 6.0 ^d	52.8 $\pm$ 4.2 ^c
150	178.3 $\pm$ 0.7	73.3 $\pm$ 6.4 ^d	41.1 $\pm$ 3.6 ^b
200	237.6 $\pm$ 0.5	90.4 $\pm$ 5.6 ^e	38.1 $\pm$ 2.4 ^b
300	355.1 $\pm$ 2.4	101.1 $\pm$ 4.9 ^f	28.5 $\pm$ 1.4 ^{ab}
400	465.6 $\pm$ 2.8	129.0 $\pm$ 7.7 ^g	27.7 $\pm$ 1.7 ^a

* The incorporation efficiency was calculated by taking the ratio of the n–3 LC-PUFA enrichment (in mg/egg; mean $\pm$ SD; n = 8) to the n–3 PUFA intake (in mg, mean $\pm$ SD; n = 8) for the respective groups, multiplied by 100.

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

color using Roche values as well. A significant increase of the Roche value was observed starting from a supplementation of 150 mg microalgal n–3 PUFAs per 100 g feed corresponding with 3% *Isochrysis* biomass supplementation. Doses of ≥ 200 mg n–3 PUFAs per 100 g feed even reached a Roche value of ≥ 15 . In Belgium, egg yolks with a definite orange tinge (corresponding to a Roche value of 12–13) are desired. Higher Roche values (corresponding to deeply red egg yolk) lead to a reduced consumer acceptability [5]. In the present study, supplementation with up to 3% *Isochrysis* resulted in acceptable yolk color.

Leeson & Caston [16] examined the effect of different doses of lutein supplementation to the diet of the laying hens. They observed a dramatic increase of the yolk color, which was, however, little affected by the supplementation dose. The yolk color leveled off at a^* -values between 13 and 14 and was unaffected by dietary lutein supplements above 250 ppm. This trend corresponds to the results obtained in this study: no further increase of the a^* -value was observed when adding more than 3% *Isochrysis* biomass (a^* -value of approximately 19). However, this is in contrast with the yolk color results obtained by Bruneel et al. [8] and Fredriksson et al. [19], who supplemented *Nannochloropsis* in two different doses. The a^* -values increased significantly with increased supplementation of *Nannochloropsis*. This observation was also made by Grashorn and Steinberg [20] who supplemented different levels of canthaxanthin to the diet of laying hens and observed increased a^* -values with increasing canthaxanthin concentration. The different observations made by different researchers can presumably be explained by the different amounts and types of carotenoids supplemented to the diet, for example lutein versus canthaxanthin. In addition, the different matrix in which they are supplied can also have an influence, for example, the supplementation of the pure carotenoids or addition of, for example, microalgal biomass, where bioaccessibility is an important parameter to take into account.

Table 4

Yolk color values (Roche and CIELAB-values; mean $\pm$ SD; n = 8) of the egg yolks at the end of the supplementation period (day 21) for the different microalgal n–3 PUFA supplementation doses.^x

Microalgal n–3 PUFAs (mg per 100 g feed)	Roche value	Colorimeter		
		L*	a*	b*
0	12 $\pm$ 1	65.7 $\pm$ 3.8 ^{cd}	7.7 $\pm$ 3.2 ^a	46.2 $\pm$ 5.6 ^{ab}
30	12 $\pm$ 0	66.5 $\pm$ 2.3 ^d	11.1 $\pm$ 1.3 ^a	51.1 $\pm$ 1.9 ^b
60	13 $\pm$ 1	63.0 $\pm$ 4.9 ^{bcd}	13.8 $\pm$ 4.4 ^{ab}	51.5 $\pm$ 5.4 ^b
90	13 $\pm$ 0	61.4 $\pm$ 3.7 ^{bcd}	13.0 $\pm$ 2.0 ^a	47.4 $\pm$ 3.0 ^{ab}
120	13 $\pm$ 1	59.1 $\pm$ 4.0 ^{abcd}	13.1 $\pm$ 2.7 ^a	45.2 $\pm$ 5.0 ^{ab}
150	14 $\pm$ 1	58.3 $\pm$ 3.3 ^{abc}	19.6 $\pm$ 5.5 ^b	45.7 $\pm$ 4.2 ^{ab}
200	15 $\pm$ 1	55.2 $\pm$ 5.2 ^{ab}	20.0 $\pm$ 5.3 ^b	44.9 $\pm$ 7.3 ^{ab}
300	>15	53.0 $\pm$ 8.3 ^a	20.1 $\pm$ 4.9 ^b	41.7 $\pm$ 7.9 ^a
400	>15	51.5 $\pm$ 4.4 ^a	18.9 $\pm$ 3.0 ^b	38.6 $\pm$ 6.2 ^a

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

3.4. Zootechnical performance of the laying hens and egg quality parameters

No drastic impact on the zootechnical performance parameters (feed intake, egg production, mortality and morbidity) was observed by supplementation of different doses of n–3 PUFAs to the diet of laying hens (Table 5). Only a significantly lower feed intake was observed with the supplementation of 60 mg microalgal n–3 PUFAs per 100 g feed. However, this can be explained by the fact that one hen got sick in this experimental group. Also no drastic effects were observed on the egg and yolk weight (Table 5). It can thus be concluded that different supplementation doses have no impact on the zootechnical performance of the laying hens and the egg quality parameters, which is in accordance with Bruneel et al. [8] and Lemahieu et al. [4].

4. Conclusion

Supplementation of different n–3 PUFA doses of *Isochrysis* to the diet of laying hens linearly increased the n–3 LC-PUFA content in the egg yolk up to a supplementation dose of 120 mg microalgal n–3 PUFAs per 100 g feed. Higher supplementation doses resulted in a flattening of the amount of n–3 LC-PUFA incorporation, reflecting a decreased efficiency of n–3 LC-PUFA enrichment in the egg yolk. Due to the carotenoid rich biomass that was supplemented, drastic yolk color changes were observed, albeit there was no linear increase of the a^* value observed. Between 30 and 120 mg n–3 PUFAs per 100 g feed, more or less the same a^* -value was observed, while from 150 mg n–3 PUFAs per 100 g feed (3% microalgal biomass) a jump of this value was observed.

Based on the n–3 LC-PUFA enrichment in the egg yolk and the n–3 LC-PUFA incorporation efficiency, 120 mg n–3 PUFAs per 100 g feed can be considered as the optimal supplementation dose since higher n–3 PUFA supplementation doses (up to 400 mg n–3 PUFAs per 100 g feed) lead to decreased n–3 LC-PUFA incorporation efficiencies in the egg yolk. To obtain this dose, 2.4% of *Isochrysis* biomass has to be supplemented, which maintains an acceptable yolk color.

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Table 5

Zootechnical performance parameters and egg quality parameters during the supplementation period for the nine different supplementation doses: feed intake (in g, mean $\pm$ SD; n = 8; except for the group of 60 mg per 100 g feed, n = 7 since one hen got sick), egg production rate (in %), egg weight (in g, mean $\pm$ SD; n = 8 except, for the group of 60 mg per 100 g feed, n = 7 since one hen got sick) and yolk weight (in g, mean $\pm$ SD; n = 8, except for the group of 60 mg per 100 g feed, n = 7 since one hen got sick).^x

Algal PUFAs (mg per 100 g feed)	Feed intake (g)	Egg production rate (%)	Egg weight (g)	Yolk weight (g)
0	117.2 $\pm$ 0.7 ^a	91.8	61.19 $\pm$ 0.83 ^{abc}	15.15 $\pm$ 1.56 ^a
30	119.1 $\pm$ 0.4 ^b	98.2	60.58 $\pm$ 0.77 ^{ab}	14.91 $\pm$ 0.74 ^a
60	117.3 $\pm$ 1.2 ^a	96.8	60.99 $\pm$ 1.00 ^{abc}	15.75 $\pm$ 0.91 ^a
90	118.9 $\pm$ 0.5 ^b	97.6	62.94 $\pm$ 1.32 ^d	15.43 $\pm$ 1.42 ^a
120	118.4 $\pm$ 0.5 ^b	98.2	61.35 $\pm$ 0.79 ^{abcd}	15.94 $\pm$ 0.55 ^a
150	118.8 $\pm$ 0.5 ^b	98.2	62.25 $\pm$ 0.74 ^{cd}	16.01 $\pm$ 0.77 ^a
200	118.8 $\pm$ 0.3 ^b	96.4	61.72 $\pm$ 1.45 ^{bcd}	16.10 $\pm$ 0.96 ^a
300	118.4 $\pm$ 0.8 ^b	90.5	61.59 $\pm$ 0.99 ^{bcd}	15.95 $\pm$ 0.85 ^a
400	116.4 $\pm$ 0.7 ^a	95.2	59.80 $\pm$ 0.78 ^a	15.70 $\pm$ 0.33 ^a

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

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