Food Research 5 (Suppl. 2) : 57 - 64 (2021)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Omega-3 fatty acid levels and sensory quality of eggs following consumption of alpha-linolenic acid enriched diets

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Article history:
Received: 7 December 2020
Received in revised form:
Accepted: 10 June 2021
Available Online: 20 June 2021

Keywords:
Laying hen,
Alpha-linolenic acid,
Eggs,
Omega-3 fats,
Sensory quality

DOI:
https://doi.org/10.26656/fr.2017.5(S2).010

Abstract

The aim of the study was to increase the levels of omega-3 fatty acids in eggs, mainly in the form of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n -3) as EPA and DHA have beneficial health effects. This study tested whether the inclusion of a vegetable source of omega-3 (n-3) fat in the form of alpha-linolenic acid (ALA, 18:3n-3) in the diets of laying hens (Hy-Line brown) would improve n-3 fat accumulation, without altering the product performance or the sensory characteristics of eggs. In this study, the ALA levels of the diets were varied from 0.3 to 6% energy (%en). In order to optimize the conversion of ALA into n-3 long chain polyunsaturated fatty acids (LCPUFA), grain-based diets containing a low linoleic acid (LA, 18:2n-6) level were chosen as a basal diet, and the level of competing substrate, LA, in the dietary treatments was also kept constant. Results showed that increasing the levels of dietary ALA increased all n-3 LCPUFA (EPA, DPA, and DHA) in the eggs. Importantly, diets enriched with ALA did not impair the sensory quality of the eggs. In conclusion, brown laying hens fed ALA enriched diets produced eggs higher in n-3 fatty acids, and met the requirement needed for labelling as n-3 PUFA sources, which provides an alternative n-3 rich food for consumers.

1. Introduction

Foods rich in n-3 PUFA fatty acids are known to have health benefits for people. Therefore, health authorities recommend consuming n-3 fatty acids, especially eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA (Thompson *et al*., 2019; Rao *et al*., 2020). The American Heart Association recommends that patients with documented coronary heart disease take approximately 1 g/d EPA and DHA, preferably from oily fish although the supplementation of EPA and DHA could also be considered (Siscovick *et al*., 2017). However, because many people do not consume fish which is the main source of EPA and DHA in their daily diet, it is important to provide alternative foods rich in n-3 LCPUFA.

One of the efforts to improve the content of n-3 LCPUFA in the diet is by consuming eggs that contain high levels of omega-3 fats. To produce eggs high in omega-3 fatty acids, layer hen diets can be supplemented with ingredients rich in omega-3 fatty acids sourced from the sea. This method is considered effective because of the direct incorporation of n-3 LCPUFA that was already contained in the source into the egg. The use of fish meal or fish oil in layer hen diet to produce n-3 eggs has been widely studied and resulted in an increase in n-3 LCPUFA, especially EPA and DHA (Gonzalez-Esquerra and Leeson, 2000a; Lawlor *et al.*, 2010). For example, the inclusion of 60 g/kg menhaden oil in laying hen diet resulted in eggs containing about 45-60 mg EPA/egg and 150-200 mg DHA/egg (Gonzalez-Esquerra and Leeson, 2000a). However, some investigators reported that there was a decrease in the organoleptic quality of the final product, such as a fishy odor and fishy off-flavor with the use of fish products in layer hen feed (Bou *et al*., 2005; Chekani-Azar, Shahriar, Maheri-Sis, 2008).

The use of omega-3 source ingredients from plants in layer hen feed can be seen as an alternative to increase n-3 PUFA. One of the plant sources rich in n-3 PUFA, ALA is flaxseed. Through this strategy, it is hoped that

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eISSN: 2550-2166

laying hens can convert ALA to DHA (Ahmad *et al*., 2012). The use of 10% flaxseed (~ 33% ALA) in the layer hen diet was reported to result in higher n-3 fatty acid deposition compared to the control group. It was further reported that the elevated levels of ALA and DHA in eggs increased by 8 and 2-fold, respectively (Bean and Leeson, 2003; Hayat *et al.*, 2009). However, it was found that there was a higher level of ALA accumulation and lower accumulation of EPA and DHA with flaxseed-based feeding in layer hens compared to layer hens that were fed from sea sources, such as fish oil. Some studies have reported that feeding flaxseed up to the 10% level tends to limit the effectiveness of increasing the levels of n-3 fatty acids (Zuidhof *et al*., 2009; Al-Nasser *et al*., 2011). For example, Sari *et al.* (2002), found that there was no increase in the content of n-3 LCPUFA in eggs with the addition of 5, 10 and 15% flaxseed in chicken feed. In addition, a diet high in LA can inhibit DHA production due to competition between ALA and LA for the use of the same enzyme in metabolic pathways. A study conducted by Kartikasari *et al.* (2010) proved that EPA and DHA levels in broiler tissue decreased with increasing LA levels in the feed while maintaining dietary ALA constant. In addition, eggs produced from hens fed diets containing 10% flaxseed led to differences in sensory attributes including aroma, taste, and off-flavor compared to control eggs (Hayat *et al.,* 2010).

The use of ALA-rich vegetable oil in basal chicken feed against a background of low LA levels led to the significant accumulation of n-3 LCPUFA and total n-3 fatty acids (Kartikasari *et al*., 2012) without negatively affecting the sensory quality of chicken meat. There is little information regarding the use of ALA-rich vegetable oils in laying hens. Therefore, the aim of this study was to include the evaluation of the effectiveness of changes in dietary ALA levels while keeping LA constant at the incorporation of n-3 fatty acids and the sensory quality of eggs.

2. Materials and methods

2.1 Ethical considerations

Ethical approval for research activities was obtained from the Animal Ethics Committee of the South Australian Department of Primary Industry and the University of Adelaide. Research procedures followed and complied with the "Australian model code of practice for the welfare of animals: domestic poultry" (Standing Committee on Agriculture and Resource Management, 1995) and the "Australian code of practice for the care and use of animals for scientific purposes" (Australian Agriculture Council, 1997)

2.2 Location

Research activities were carried out at the Fatty Acid Laboratory and at the Sensory Evaluation Laboratory, Waite Campus, the University of Adelaide. The location for raising the chickens was at the Pig and Poultry Production Center (PPPI, SARDI), Roseworthy Campus, the University of Adelaide.

2.3 Birds, management, and diets

The experimental design of this research was a completely randomized block design using 8 replications for each ration. The dietary treatment was designed to maintain a constant LA level and increase the diet ALA level (%en). Three experimental diets were provided for 24 Hy-Line brown laying hens. After the chickens have been weighed, each chicken was placed in one cage (500 mm width × 550 mm depth × 500 mm height). Eight cages were used as replications for each experimental diet. This diet was specially formulated for the layer hens used for this study (Ridley Agriproducts Pty Ltd, Murray Bridge, South Australia), which was designed to have low LA levels and varying levels of ALA. The treatment diets were prepared by incorporating a basal diet with pure or mixed vegetable oils. The ALA content of the treated feed was 0.3% (low ALA), 3% (moderate ALA) or 6%en (high ALA) while maintaining a constant LA level of around 4%en (Table 1).

Table 1. Fatty acid composition of the experimental diets

Diets	Experimental diets		
	0.3 (low ALA)	3 (moderate ALA)	6 (high ALA)
Fat content (%)	8.5	8.5	8.6
ALA (%en)	0.3	3.2	6.2
LA (%en)	2.3	4.4	4.4
LA:ALA ratio	7.5	1.4	0.7
Fatty acids (%)[1]			
Total SFA[2]	18.4	13.7	15.7
Total MUFA[2]	66.8	42.3	24.3
18:2n-6 (LA)[2]	12.8	25.1	24.9
Total n-6	12.9	25.2	24.9
18:3n-3 (ALA)[2]	1.7	18.5	34.8
Total n-3	1.8	18.6	34.9
Total PUFA[2]	14.6	43.8	59.8

[1]Values are presented as % of total fatty acids.
[2]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; LA = linoleic acid; ALA = alpha- linolenic acid; PUFA = polyunsaturated fatty acid.

2.4 Sample collection

All eggs produced by each hen in the last 3 days of

the 28 days of week 4 and week 12 were weighed individually. One egg from each laying hen was then cracked and the albumen and yolk were weighed separately and recorded. A total of 24 yolk samples on day 28 (n = 8 egg yolks for each treatment) were stored at -20°C to analyze the yolk fatty acid profile. Eggs produced at week 12 were collected for testing the consumer preference for boiled eggs.

2.5 Lipid extraction and Fatty acid analysis

The total fat (TL) was extracted from egg samples following the procedure of Folch *et al*. (1957) using a chloroform/methanol solution (2:1, v/v). Fatty acids methyl ester (FAME) was prepared following the method of Tu *et al*. (2010) using 1% H_2SO_4 in methanol at 70°C for 3 hrs. The resulting FAME was extracted with n-heptane and then transferred to a vial gas chromatography (GC) containing anhydrous sodium sulfate. Methyl ester samples were stored at -20°C for fatty acid analysis using GC, Hewlett-Packard 6890 GC (CA, USA).

2.6 Consumer acceptance of hard-boiled eggs

The procedure for the study's consumer preference test was approved by the Human Ethics Committee of the University of Adelaide, Australia. After 12 weeks on the experimental diets, the eggs were collected and refrigerated (4°C) for 15 days for easy peeling (Parpinello *et al*., 2000). A total of 20 brown eggs from each of the 3 dietary treatments and 20 commercial brown eggs were used for boiled egg consumer evaluation. To prepare boiled eggs, 20 eggs from each treatment were put into a stainless pot containing cold water. The eggs were brought to a boil using gas and simmered for eight minutes (Parpinello *et al*., 2000), then cooled in cold running water. The boiled eggs were then peeled and divided into four portions. One-quarter of an egg from each diet was placed in a plastic container coded with randomly three-digit numbers and covered. Before the egg samples were evaluated by consumers, the containers were kept warm in an oven at about 40°C.

A group of consumers (n = 76) who had no allergies, liked and were willing to eat eggs, was recruited from both staff and students at the School of Agriculture, Food and Wine, the University of Adelaide. Before testing egg samples, consumers were asked to answer a questionnaire regarding their egg consumption behavior and demographic information. Panelists were then asked to rate their liking for the boiled egg samples by considering all characteristics including aroma and taste using a 9-point hedonic scale ranging from dislike extremely to like extremely (Wichchukit and O'Mahony, 2015). Consumer acceptance testing was conducted at the table in the open-plan cafeteria at the University of Adelaide's School of Agriculture, Food and Wine. The participants completed a questionnaire and rated their liking for the egg samples on the provided ballot papers. In order to clear the palate among the four samples, fresh water and fresh crackers were provided to each consumer.

2.7 Statistical analysis

The fatty acid composition data of the samples were analyzed by One-way ANOVA with a completely randomized design of three diets using eight replications using general analysis of variance on GenStat (Release 14). The experimental unit was a replicate consisting of eight adjacent birds which were fed as a group so that there were 8 cages for each diet and a total of 24 cages. The main effect of the diet (3 levels) was tested for the fatty acid profile of eggs, expressed as % of total fatty acids and mg/yolk. If there are significant differences between treatments, then the analysis was continued with Tukey's multiple comparison test with a significance level set at $P<0.05$. The consumer preference study was arranged in a completely randomized block design within two days of testing in which the individual assessors evaluated one egg sample. The data from the consumer acceptance study of boiled eggs were evaluated by One-way ANOVA in a randomized block design, with one block representing one assessor, using GenStat (Release 14). Attributes showing statistically significant differences were further analyzed using the Tukey test at the 95% confidence level, $P<0.05$.

3. Results and discussion

3.1 Fatty acids profiles of eggs

The fatty acid composition of eggs produced on day 28 of each dietary intervention was evaluated (Table 2). The use of plant oils rich in ALA content in the laying hens' feeds significantly increased all n-3 LCPUFA (EPA, DPA, and DHA), total n-3 PUFA, and total PUFA content. The EPA content of chicken eggs fed a diet containing 0.3, 3, and 6%en ALA were 0.0, 0.1, and 0.2% of the total fatty acids, respectively. The DPA content of eggs increased ($P<0.001$) to about 4 times higher with the enrichment of layer hens' feed with ALA compared to those not supplemented with ALA. The increase in total n-3 is at the expense of monounsaturated fatty acids (MUFA) accumulation. Importantly, the feed enriched with ALA levels did not cause a difference in the saturated fatty acid (SFA) content of eggs while the polyunsaturated fatty acids (PUFA) content significantly increased by about 2.5 times.

The results presented in this study indicate a major change in the fatty acid profile of egg yolks by the ALA

Table 2. Fatty acid profiles of eggs produced at day 28 of dietary intervention[1]

ALA Level (%en)	Experimental diets			P-Value	Significance[2]
	0.3 (low)	3 (moderate)	6 (high)		
Fatty acids (%)[3]					
16:0	20.12	20.14	20.18	0.99	NS
18:0	7.16[b]	7.68[b]	8.52[a]	0.001	**
Total SFA	27.87	28.21	29.05	0.108	NS
16:1n-7	4.91[a]	2.09[b]	2.24[b]	0.001	**
18:1n-9	50.19[a]	43.94[b]	39.06[c]	0.001	**
18:1n-7	4.36[a]	2.19[b]	1.70[c]	0.001	**
Total MUFA	61.27[a]	49.96[b]	44.22[c]	0.001	**
Total n-9	52.04[a]	45.53[b]	40.17[c]	0.001	**
Total n-7	9.27[a]	4.29[b]	3.94[b]	0.001	**
18:2n-6 (LA)	6.82[b]	12.81[a]	13.33[a]	0.001	**
18:3n-6	0.00[c]	0.09[a]	0.06[b]	0.001	**
20:3n-6	0.16[a]	0.11[b]	0.09[b]	0.001	**
20:4n-6 (AA)	1.61[a]	1.09[b]	0.78[c]	0.001	**
Total n-6	9.12[b]	14.30[a]	14.33[a]	0.001	**
(ALA)					
18:3n-3	0.24[c]	4.45[b]	9.54[a]	0.001	**
20:3n-3	0.00[c]	0.08[b]	0.14[a]	0.001	**
(EPA)					
20:5n-3	0.00[c]	0.11[b]	0.18[a]	0.001	**
(DPA)					
22:5n-3	0.12[b]	0.38[a]	0.43[a]	0.001	**
(DHA)					
22:6n-3	0.89[c]	2.21[a]	1.93[b]	0.001	**
Total n-3	1.35[c]	7.32[b]	12.22a	0.001	**
Total PUFA	10.47[c]	21.63[b]	26.56[a]	0.001	**
n-3 LCPUFA	1.01[b]	2.71[a]	2.54[a]	0.001	**
LA:ALA ratio	28.24[a]	2.88[b]	1.41[b]	0.001	**
n6:n3	6.77[a]	1.95[b]	1.18[c]	0.001	**

[1]Values are means of eight observations per treatment. Values in the same row with no common superscript are significantly different (P<0.05).

[2]**P < 0.01; NS, not significant.

[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid, ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

enriched feed, which can be summarized as an increase in PUFA levels and a decrease in MUFA. This clearly shows that there is a direct relationship between feed ALA levels and the n-3 content of eggs, especially accumulated as ALA and n-3 LCPUFAs. These results confirm the established idea that the composition of the n -3 fatty acids in dietary fat is directly responsible for the type of fatty acid content in egg yolk and that modifying the fatty acid composition of the diet can manipulate the fatty acid profile of the egg yolk (Neijat *et al*., 2016). An increase in egg n-3 LCPUFA content when high ALA levels were included in the diet indicated that laying hens have the ability to desaturate and elongate alpha-linolenic acid to n-3 LCPUFA in eggs. However, it appears that there is a limited conversion of ALA to n-3 LCPUFA (Zhang *et al*., 2017) and in particular, the capacity of layer hens to convert ALA to DHA (Ehr *et al.*, 2017). The findings of this study indicated that egg DHA accumulation was higher (P<0.001) in eggs produced from laying hens fed with ALA levels of 3 and 6%en compared to those fed with low ALA (0.3%en). However, we observed that the highest egg DHA levels were achieved when chickens were fed at the 3%en ALA level. The increase in DHA content of eggs from chickens that were fed with the addition of 3 and 6%en ALA was 2.5 and 2.2 times, respectively. The deposition of EPA (0.2%) and DPA (0.4%) in eggs observed in this experiment was relatively low, which may be due to the limited ability to accumulate EPA and DPA in egg fat. This finding is as reported by Gonzalez-Esquerra and

Leeson (2000b) where the levels of EPA, DPA and DHA from chicken eggs fed a diet supplemented with 2% menhaden oil containing 3.9% EPA, 1% DPA, and 1, 4% DHA were 0.3, 0.2, and 1.7%, respectively. These results correspond to the amount of DHA in eggs, 87 mg/egg, identical to the levels found in egg laying hens that were fed 3%en ALA. This suggests that vegetable oil rich in ALA (n-3 PUFA) added to feed against a background of low LA levels can lead to a significant accumulation of n -3 LCPUFA levels in eggs.

In this study, the accumulation of DHA gradually doubled with the use of layer hen feeds enriched with 3 and 6%en ALA. There appears to be a maximal ALA level for conversion to DHA. Layers fed 3%en ALA produced the highest amount of DHA (2.2%); however, when the dietary ALA level exceeds 3%en, there was no further increase in the DHA level. These findings are consistent with reports that a diet enriched with high levels of PUFAs, including ALA and LA, can inhibit DHA production (Gibson *et al*., 2013). In addition, no positive response in egg DHA synthesis with increased feed ALA levels could be explained as a result of competition between 18-carbon PUFA, LA and ALA, in the use of Δ-desaturase enzymes in the synthetic pathway (Gibson *et al*., 2011; Gibson *et al*., 2013). Competition in utilizing the same desaturase and elongase enzymes between LA and ALA for bioconversion to n-3 LCPUFA is well known (Barceló-Coblijn and Murphy, 2009; Nain *et al*., 2012). This is as reported by Kartikasari *et al*. (2010) where the accumulation of n-3 LCPUFA in chicken tissue was reduced with an increase in the amount of LA in the feed; however, n-3 LCPUFA levels (EPA and DHA) enhanced by increasing the levels of dietary ALA while holding dietary LA in a low level (Kartikasari *et al*., 2012). These findings are consistent with previous studies which found that increased feed ALA could achieve the maximum attainable DHA level (Sari *et al*., 2002). For example, a study conducted by Grobas *et al*. (2001) found that the DHA content of eggs did not change with flaxseed oil supplementation from 5 to 10%, which is consistent with the observations of the current study.

The fatty acid composition of eggs expressed in mg/ egg yolk was also evaluated in this study (Table 3). ALA, n-3 LCPUFA, total n-3 in egg yolk were accumulated more with ALA-enriched feed, while the evaluation of AA levels showed a decrease. The increase in ALA, EPA, DPA and total n-3 occurred linearly ($P<0.01$). However, the analysis showed that the highest DHA levels (87 mg/egg yolk) were found in laying hens

Table 3. Fatty acid profiles of eggs produced at day 84 of dietary intervention expressed in mg/yolk or egg[1]

ALA Level (% en)	Experimental diets			Significance[2]
	0.3 (low)	3 (moderate)	6 (high)	
Fatty acids (%)[3]				
Total SFA	1220	1251	1270	NS
Total MUFA	2687[a]	2188[b]	1928[c]	**
18:2n-6 (LA)	297[b]	584[a]	592[a]	**
20:4n-6 (AA)	71[a]	46[b]	31[c]	**
Total n-6	381[b]	644[a]	633[a]	**
18:3n-3 (ALA)	12[c]	200[b]	405[a]	**
20:5n-3 (EPA)	0[c]	5[b]	8[a]	**
22:5n-3 (DPA)	5[b]	16[a]	17[a]	**
22:6n-3 (DHA)	38[c]	87[a]	75[b]	**
EPA+DHA	38[b]	92[a]	83[a]	**
n-3 LCPUFA	43[b]	108[a]	100[a]	**
Total n-3	59[c]	313[b]	513[a]	**
Total PUFA	440[c]	957[b]	1146[a]	**

Hedonic test ALA Level (%en)	Experimental diets				
	Commercial	0.3	3	6	
Consumer Acceptance	6.0[a]	6.4[a]	5.6[a]	5.4[b]	**

[1]Values are means of eight observations per treatment. Values in the same row with no common superscripts are significantly different ($P<0.05$).

[2]*$P<0.05$; **$P<0.01$; NS, not significant.

[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid, ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

eISSN: 2550-2166

that were fed with ALA content of 3%en, indicating that the DHA levels in eggs had reached the maximum level. The feed containing the highest ALA (6%en) increased the EPA, DPA and DHA content of eggs by about 8, 3 and 2 times, respectively. The use of moderate (3%en) and high (6%en) ALA led to an increase in total n-3 to 313 and 513 mg/yolk (Table 3), respectively. This suggests that chickens fed moderate and high ALA enriched diets can produce eggs that achieve the minimum requirements needed for egg labeling as a source of n-3 PUFA (300 mg/egg) (Nain *et al*., 2012).

3.2 Consumer acceptance of hard-boiled eggs

The consumers in this study (n = 76) were between 19 and 65 years old and were gender-balanced. About 70% of consumers with tertiary qualifications and 74% had no formal food science or consumer education or training. The majority were students (65%) and 80% of all consumers had no experience in the food industry. Almost all consumers (96%) consumed boiled eggs and 70% reported consuming boiled eggs at least once a month. No consumer reported having an egg allergy. Importantly, the evaluation of consumer acceptance of boiled eggs showed that based on taste and flavor there was no difference in consumer preferences between commercial eggs purchased from local supermarkets and eggs from chickens fed a diet enriched with up to 3%en ALA. The score average of the evaluation was 6 which indicates a "like slightly" rating (Table 3). It appears that eggs produced from hens fed dietary treatments with the addition of 6%en ALA levels were significantly less liked than all other eggs; however, all the eggs were acceptable. This finding was in agreement with the results of previous investigators (Hayat *et al*., 2010).

4. Conclusion

The findings of this study suggest that the diet enriched with 3%en ALA was found to be optimum with respect to n-3 fatty acid accumulation and the consumer acceptance of the boiled eggs; however, ALA can be added to commercial laying hen feed up to 6%en ALA and increase all n-3 fats. Eggs from hens fed both the moderate (3%en) and high (6%en) levels of ALA reached 300 mg of total n-3 PUFA/egg, which is the minimum content needed for labelling the eggs as an n-3 PUFA source. Importantly, the dietary inclusion of ALA assessed did not alter the consumer acceptance of the boiled eggs compared to the commercial eggs. The findings of this study indicate that vegetable oils could be an alternative ALA source to marine sources. As laying hens fed moderate and high ALA diets resulted in eggs higher in n-3 LCPUFA without influencing consumer acceptance of the eggs, this provides an alternative food rich in n-3 PUFA for consumers and may help approach the recommended intake for human health.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank Dr. John Carragher for his suggestions and constructive comments and the technical support staff at the SARDI Pig and Poultry Production Institute (PPPI), Roseworthy Campus for help in conducting the animal experiments. We thank Ms. Ela Zielinski for the technical assistance and advice in the fatty acid analysis. We acknowledge the financial support from the Directorate General of Higher Education, Ministry of National Education Indonesia, the University of Adelaide, and the South Australian Department of Further Education, Employment, Science and Technology, Australia.

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