Research Article

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Assessment of omega-3 and omega-6 fatty acid profiles and ratio of omega-6/omega-3 of white eggs produced by laying hens fed diets enriched with omega-3 rich vegetable oil

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Abstract: The aim of this study was to evaluate the levels of omega-3 (n-3) and omega-6 (n-6) fatty acids in egg and the ratio of n-6/n-3 of white eggs produced by laying hens (Hy-Line white) fed diets enriched with n-3 fats. In this study, alpha-linolenic acid (ALA) levels of the dietary treatments ranged from 0.3 to 6% energy. Grain-based diets containing a low linoleic acid (LA) content were selected to prepare a basal diet to optimize the conversion of ALA into n-3 long chain polyunsaturated fatty acids (LCPUFA). The results showed that the level of all n-3 LCPUFA in eggs improved (P < 0.01) by increasing the levels of dietary ALA. Importantly, eggs produced from laying hens fed diets containing 6% en ALA significantly increased (P < 0.01) the total of n-3 fats by approximately ninefold. Diets enriched with ALA significantly reduced (P < 0.01) the ratio of n-6/n-3 of the eggs. The n-6/n-3 ratio of eggs decreased from 7.17% in the 0.3% en ALA diet to 1.29% in the 6% en ALA diet. In conclusion, white laying hens fed ALA-enriched diets produced eggs higher in n-3 fatty acids and lower n-6/n-3 ratio, which provides an alternative n-3 rich food for consumers and have beneficial health effects.

Keywords: laying hen, alpha-linolenic acid, eggs, omega-3 fats, n-6/n-3 ratio

1 Introduction

Omega-3 polyunsaturated fatty acid (n-3 PUFA) is known to have health benefits for humans. Therefore, foods containing high omega-3 fatty acids are considered as functional foods. The consumption of omega-3 fatty acids, especially eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) is recommended by health authorities [1,2]. Fish is a major source of EPA and DHA; therefore, consumption of foods from marine sources and their products can help increase the recommended intake of omega-3 fatty acids. For example, for patients with documented coronary heart disease, the American Heart Association recommends consuming about 1 g/day of EPA and DHA, preferably from oily fish or from EPA and DHA supplementation [3]. However, because many people do not consume fish in their daily diet, it is necessary to provide alternative foods rich in omega-3 long-chain polyunsaturated fatty acid (n-3 LCPUFA).

The ratio of dietary omega-6/omega-3 fatty acids is also an important factor that needs to be considered to maintain the health of the human body. Today the diet of some populations contains excessive levels of omega-6 PUFAs and conversely has very low levels of omega-3 PUFAs, which causes a high omega-6/omega-3 ratio and is detrimental for health. Simopoulos [4] reported that the western diet has an omega-6/omega-3 ratio of around 15:1-16.7:1. Excessive consumption of omega-6 PUFA and very high omega-6/omega-3 ratios can increase the pathogenesis of many diseases, including cardiovascular disease (CVD), cancer, and inflammation and autoimmune disease. On the other hand, an increased intake of high omega-3 PUFA levels or a low omega-6/omega-3 ratio has a suppressive effect. The recommended omega-6/omega-3 ratio for preventing many pathological diseases caused by the current western diet is 3:1-4:1 [5], although the optimal ratio may vary depending on the disease. For example, a 4/1 ratio is associated with a 70%

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reduction in total mortality from CVD. In patients with colorectal cancer, the 2.5/1 ratio may reduce the proliferation of rectal cells. In women with breast cancer, a lower omega-6/ omega-3 ratio was associated with a reduced risk and a 2–3/1 ratio was able to suppress inflammation in patients with rheumatoid arthritis [6].

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 is already contained in the source into the egg. 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 [7,8].

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 (18:3n-3, ALA) is flaxseed. Through this strategy, it is hoped that laying hens can convert ALA to DHA [9]. 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 also reported that the elevated levels of ALA and DHA in eggs increased by eight and two-fold, respectively [10,11]. 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. Nevertheless, the connecting line from flaxseed supplementation in poultry diets is inconsistent and difficult to infer. Vlaicu et al. [12] suggested that supplementation with flaxseed meal at 9% in combination with 3% of sea buckthorn meal contributed to the intensification of the ALA and DHA, along with notable improvements for total polyphenol contents and antioxidants. In addition, regarding the utilization of Hy-Line hens, Ehr et al. [13] inferred a significant improvement on ALA, DHA, and EPA along with two times higher fatty acid deposition on its egg yolk following feeding using flaxseed oil. However, some studies have reported that feeding flaxseed up to 10% level tends to limit the effectiveness of increasing the levels of n-3 fatty acids [14,15]. For example, Sari et al. [16] found that the addition of flaxseed at a level of 5% to laying hen diet could significantly increase the content of n-3 LCPUFA (EPA, DPA, and DHA). However, the use of flaxseed at levels of 10 and 15% did not significantly change the deposition of DPA and DHA compared to the use of 5% flaxseed. Most of the increase in n-3 PUFA in eggs was due to ALA enrichment, which increased from

0.7% in laying hens fed the control diet to 4.3, 5.4, and 9.0% in the flaxseed-supplemented diet at levels 5, 10, and 15, respectively. Therefore, it should be noted that as the level of flaxseed in the diet increases, the total content of n-3 fatty acids increases linearly in egg yolk. In addition, a diet high in omega-6 PUFA (18:2n-6, 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. [17] proved that EPA and DHA levels in broiler tissue decreased with the increase in LA levels in the feed while maintaining dietary ALA content. 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 [18].

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 [19] without negatively affecting the sensory quality of chicken meat. Many studies have evaluated the use of ALA-rich vegetable oils in laying and broiler chicken feeds on the fatty acid profiles of the products. The deposition of n-3 fatty acids in chicken products depends on the type of vegetable oil used. However, the use of feed formulas enriched with n-3 fatty acids (ALA) while maintaining omega-6 fatty acid (LA) content has not been widely reported. Therefore, the aim of this study is to evaluate the levels of n-3 and n-6 fatty acids in egg and the ratio of n-6/n-3 of white eggs produced by laying hens (Hy-Line white) fed diets enriched with n-3 fats by including dietary ALA levels while keeping LA constant.

2 Materials and methods

2.1 Location

The Fatty Acid Laboratory and the Sensory Evaluation Laboratory at the Waite Campus of the University of Adelaide were used for research purposes. The chickens were raised at the Pig and Poultry Production Center (PPPI, SARDI), Roseworthy Campus.

2.2 Birds, management, and diets

This research used 24 laying hens (Hy-Line white). The birds were placed at point of lay (18 weeks) and fed *ad libitum* for 4 weeks. These hens were allocated to three

experimental diets and each of the three dietary treatments was replicated eight times (n = 8 for each group). Before the chickens were reared, they were weighed and placed one chicken per cage (50 cm width × 55 cm depth × 50 cm height; Figure 1). For each experimental diet, eight cages were used as replications. This diet was specially designed for the layer hens used in this study (Ridley Agriproducts Pty Ltd, Murray Bridge, South Australia), with low LA levels and varying ALA levels. The dietary treatments were created by combining a standard diet with pure or mixed vegetable oils. The starter basal diet comprised (%): finely ground peas (20.00), finely ground triticale (19.73), finely ground wheat (19.20), barley (15.00), soybean meal (5.53), millrun (3.90), wheat mill vits (0.80), meat meal (2.30), canola meal expeller (3.00), limestone large (9.38), monodicalcium phosphate (0.26), sodium bicarbonate (0.18), salt (0.16), choline chloride 75% (0.06), alimet (0.24), layer/ pullet premix (0.20), Roxaphyll 112 (0.005), Avizyme 1210 (0.003), and Ronozyme P 5000 Layer (0.009). The ALA and LA contents of the basal diet were 4.5 and 37.8% of total fatty acids, respectively.

The experimental diets were formulated by adding the basal diet (94%) with macadamia oil (6%) to provide a dietary treatment with a low ALA level. Mixed vegetable oil (6%) or pure oil high in n-3 fatty acids (6%) were used as a source of n-3 fatty acids (ALA) in the diets. A mixture of vegetable oils (6%) consisting of canola oil (60%) and flax-seed (40%) was used for a diet containing 3%en ALA

(moderate ALA). Flaxseed oil with a level of 6% was used to create diet with high ALA content. The fatty acid composition of vegetable oils supplemented in laying hen diets is presented in Table 1. The ALA content of the experimental diet was 0.3 (low ALA), 3 (moderate ALA), or 6% en (high ALA), while the LA level remained constant at around 4%en (Table 2).

2.3 Sample collection

All eggs laid by each hen in the last 3 days of the 28-day period were weighed individually. After cracking one egg from each hen, the albumen and yolk were weighed separately and recorded. On Day 28, 24 yolk samples (n = 8 egg yolks for each treatment) were stored at -20° C for analysis of the yolk fatty acid profiles.

2.4 Lipid extraction and fatty acid analysis

The total fat (TL) was extracted from the egg samples using a chloroform/methanol solution (2:1, v/v) as described by Folch et al. [21] and Tu et al. [22]. Fatty acid methyl ester (FAME) was synthesized using 1% H_2SO_4 in methanol at 70°C for 3 h. The resulting FAME was extracted with n-



Figure 1: Housing of laying hens in individual cages.

Table 1: Fatty acid composition of the vegetable oils included in the diets of laying hens

	Oils and blended oils ¹				
Fatty acids ²	Macadamia	Canola (% of tot	Flaxseed al fatty acid	Blended oils s)	
18:2n-6 (LA) ³	2.4	24.1	18.9	18.8	
Total n-6	2.4	24.3	19.0	18.9	
18:3n-3 (ALA) ³	0.1	9.4	47.0	23.4	
Total n-3	0.5	9.4	47.0	23.5	
Total n-7	20.5	3.6	0.9	2.6	
Total n-9	59.7	54.9	20.2	44.7	
Total SFA ³	16.7	7.7	12.8	10.3	
Total MUFA ³	80.4	58.5	21.1	47.3	

¹Composition of oils consisted of macadamia oil, diet 1; 60% canola and 40% flaxseed oil, diet 2; and flaxseed oil, diet 3.

²Data are mean values, n = 3.

³LA = linoleic acid; ALA = alpha-linolenic acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid.

heptane before being transferred to a GC vial containing anhydrous sodium sulfate. Methyl ester samples were stored at -20° C before being analyzed for fatty acids following the procedures of Kartikasari et al. [17] using GC (Hewlett-Packard 6890 GC) (CA, USA).

2.5 Statistical analysis

The fatty acid composition data of the samples were analyzed using GenStat's One Way ANOVA with a completely randomized design of three diets and eight replications (Release 14). The experimental unit was a replicate of eight adjacent birds that were fed as a group, resulting in eight cages for each diet and a total of 24 cages. The main effect of the diets (three levels) was investigated for the fatty acid profile of eggs, which was expressed as a percentage of total fatty acids. Eight replications were utilized by this study for each treatment feed group and were adequate to meet the sample requirements for statistical analysis. If there were significant differences between the dietary treatments, the analysis was followed by Tukey's multiple comparison test with a significance level of P < 0.05.

Ethical considerations: The South Australian Department of Primary Industries Animal Ethics Committee and the University of Adelaide provided their ethical permission for research endeavors under the project number H-071-2010. The research techniques followed and adhered with the Australian Model Code of Practice for the Welfare of Domestic Poultry (Standing Committee on Agriculture and Table 2: Composition of fatty acids in the dietary treatments

	Experimental diets				
ALA level (%en)	0.3 (Low)	3 (Moderate)	6 (High)		
Fat content (%)	8.5	8.5	8.6		
LA (%en)	2.3	4.4	4.4		
ALA (%en)	0.3	3.2	6.2		
LA/ALA ratio	7.5	1.4	0.7		
Fatty acids (%) ¹					
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 SFA ²	18.4	13.7	15.7		
Total MUFA ²	66.8	42.3	24.3		
Total PUFA ²	14.6	43.8	59.8		

¹Values are expressed as a percentage of total fatty acids.

²SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; LA = linoleic acid; ALA = alpha-linolenic acid; PUFA = polyunsaturated fatty acid [20].

Resource Management, 1995) and the Australian Code Of Practice For the Care and Use of Animals for Scientific Purposes (Australian Agriculture Council, 1997).

3 Results and discussion

The inclusion of essential n-3 fatty acids in animal diets plays a notable role in maintaining admissible n-6/n-3 ratio of its products. Our previous study suggested that the higher presence of ALA in chicken diets promoted a significant accumulation of essential n-3 LCPUFA in chicken tissue, mainly that of DPA, DHA, and EPA [19]. Nevertheless, studies focusing on the exploration of ALA inclusion in highly producing, versatile, and adaptable Hy-Line strain's diet are scarce. Thus, this study was expected to decrease this research gap by investigating the effect of increasing the levels of alpha-linolenic acid (ALA) in dietary treatments, namely from 0.3 to 6% of energy, while the levels of linoleic acid (LA) are maintained relatively low and constant. It should be noted that there is competition between ALA and LA in the use of desaturase and elongase enzymes in the metabolic pathway. Thus, this strategy was implemented as an effort to optimize the conversion of ALA into n-3 LCPUFA. On Day 28 of each dietary intervention, the fatty acid profiles of n-3 and n-6 white eggs were evaluated (Table 3). The use of omega-3 fatty acid-enriched laying hen feed specifically increasing the ALA content of the feed while maintaining a relatively constant LA, significantly increased the omega-3 fatty acid content of eggs,

ALA level (%en) Fatty acids (%) ³	Experimental diets				
	0.3 (Low)	3 (Moderate)	6 (High)	P-value	Significance ²
18:2n-6 (LA)	7.22 ^b	13.24 ^a	13.69 ^a	0.001	**
18:3n-6	0.00 ^c	0.07 ^a	0.06 ^b	0.001	**
20:3n-6	0.16 ^a	0.10 ^b	0.09 ^b	0.001	**
20:4n-6 (AA)	1.56 ^a	1.04 ^b	0.82 ^c	0.001	**
Total n-6	9.45 ^b	14.65 ^a	14.73 ^a	0.001	**
18:3n-3 (ALA)	0.25 ^c	4.48 ^b	9.24 ^a	0.001	**
20:3n-3	0.00 ^c	0.09 ^b	0.13 ^a	0.001	**
20:5n-3 (EPA)	0.00 ^c	0.11 ^b	0.19 ^a	0.001	**
22:5n-3 (DPA)	0.12 ^b	0.29 ^a	0.33 ^a	0.001	**
22:6n-3 (DHA)	0.85 ^b	1.76 ^a	1.71 ^a	0.001	**
Total n-3	1.32 ^c	6.80 ^b	11.60 ^a	0.001	**
n-3 LCPUFA	0.97 ^b	2.16 ^a	2.23ª	0.001	**
LA:ALA ratio	28.52 ^a	2.97 ^b	1.52 ^b	0.001	**
n6/n3 ratio	7.17 ^a	2.16 ^b	1.29 ^c	0.001	**

Table 3: Omega-3 and omega-6 fatty acid profiles and ratio of omega-6/omega-3 of white eqgs produced at Day 28 of dietary intervention¹

¹Values are mean values of eight observations per treatment. $^{(a-c)}$ Treatments with no common superscripts are significantly different (P < 0.05). $^{2}**P < 0.01$.

³LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; n-3 LCPUFA, omega-3 long-chain polyunsaturated fatty acid.

including ALA, EPA, DPA, and DHA (P < 0.01). Following an increase in the omega-3 fatty acid content of eggs, there was a significant increase in total omega-3 fatty acids (P < 0.01). ALA accounted for the majority of the increase in total n-3 fatty acids, rising from 0.25% in the feed with 0.3% en ALA content to 9.24% in the feed formula enriched with 6.0%en ALA. The inclusion of ALA-rich plant oils in laying hen feed significantly increased all n-3 LCPUFA (EPA, DPA, and DHA), total n-3 PUFA, and total PUFA content. Increasing the dietary ALA levels from 0.3 to 6.0% en linearly increased the EPA content, reaching the highest level of EPA in the diet with an ALA content of 6.0%en.

The findings presented here demonstrated and confirmed previous studies that found that flaxseed is an energy-dense, well-tolerated feed element with a more effective ability to modify certain omega-3 fatty acid within the egg yolk, particularly ALA. A study by Ehr et al. [13] revealed that the concentrations of ALA in Hy-Line egg yolk rose due to the dietary inclusion of both milled and extracted flaxseed. A curvilinear increase in fatty acid deposition was previously found in both muscle tissue and egg yolks.

The findings of this study clearly show that there is a direct relationship between feed ALA levels and egg n-3 content, as evidenced by the accumulation of ALA and n-3 LCPUFA. Previous studies also reported that EPA, DPA, DHA, and total n-3 PUFA in eggs produced from laying hens

fed plant oil rich in ALA enhanced significantly [20]. In addition, while all n-3 LCPUFA content increased in ALArich diets, we observed that AA reduced. These findings support the previously held belief that the n-3 fatty acid composition of diets is directly responsible for the fatty acid profiles in egg yolks, and that alterations to the fatty acid composition of laying hen feed formulas can affect the yolk fatty acid profiles [23]. The increase in n-3 LCPUFA levels in eggs when high levels of ALA diet were used suggested that laying hens could desaturate and elongate ALA to n-3 LCPUFA in eggs. However, it appears that there are limitations in the conversion of ALA as a precursor of n-3 LCPUFA to n-3 LCPUFA, specifically DHA. The findings are consistent with the findings of other researchers who stated that there were limitations in converting feed ALA into DHA and n-3 LCPUFA [13,24]. When the EPA level of chicken eggs increased gradually from 0.00 to 0.19% by increasing the levels of dietary ALA from 0.3 to 6%en ALA, there appears to be a maximal ALA level for conversion to DHA. Diets with a 3%en ALA level increased the DHA content of the eggs by about two-fold; however, when the ALA content of the feed was increased to 6%en, there was no indication of a further increase in DHA. The findings in this study are consistent with those reported by Gibson et al. [25], who found that high PUFA supplementation in feed, including ALA and LA, can inhibit egg DHA production. Furthermore, competition between 18-carbon

PUFAs, LA, and ALA, in the use of desaturase enzymes in the synthetic pathway has no positive effect on egg DHA synthesis by increasing dietary ALA levels [25,26]. Several studies have found competition between LA and ALA in the use of the same desaturase and elongase enzymes for bioconversion to n-3 LCPUFA [27,28]. According to Kartikasari et al. [17] increasing the amount of LA in feed can reduce n-3 LCPUFA deposition in chicken tissue; however, increasing dietary ALA levels while maintaining low dietary LA levels can increase n-3 LCPUFA (EPA and DHA) deposition in chicken tissue [19]. This finding is consistent with previous research that found that increasing feed ALA can achieve the highest achievable DHA level [16]. For example, Grobas et al. [29] discovered that flaxseed oil supplementation from 5 to 10% did not change the DHA content of eggs, which is consistent with the current study's findings. The results clearly showed that increasing the EPA, DPA, and DHA content of eggs with the use of ALA-rich vegetable oils in the laying hen diet resulted in a significant increase in total n-3 LCPUFA. Total egg n-3 LCPUFA levels increased (P < 0.01) to about two times higher when ALA-enriched feed was used. This suggests that adding ALA-rich vegetable oil to feed in the presence of low LA levels can result in a significant accumulation of n-3 LCPUFA levels in eggs. The results obtained are beneficial to human health and provide an alternative source of omega-3 fatty acids. Another significant finding in this study is that laying hen feed containing high levels of ALA derived from plant oils can reduce the omega-6 to omega-3 ratio in eggs (P < 0.01), thereby providing functional food ingredients that are beneficial to health. The omega-6 to omega-3 ratios were 7.2, 2.2, and 1.3, respectively, for diet

ALA contents of 0.3, 3, and 6% en ALA. Some populations' diets are high in omega-6 PUFAs but low in omega-3 PUFAs, resulting in a high and unhealthy omega-6/omega-3 ratio. Simopoulos [4] reports that the western diet has an omega-6/omega-3 ratio of 20:1 or even greater, whereas previously during evolution the ratio was 1:1. For example, current populations in the UK and northern Europe have a diet with an omega- 6/omega-3 ratio of 15:1, and the current populations in the US have a ratio of about 17:1 [4]. Omega-6/omega-3 ratios of 3:1-4:1 have been reported to prevent the pathogenesis of many diseases caused by the current western diet [5]; however, the target ratio of 1:1–2:1 seems consistent with studies related to the evolutionary aspects of diet, neurodevelopment, and genetics. According to the findings of this study, laying hens supplemented with ALA can produce eggs with an omega- 6/omega-3 ratio of 2:1-1:1. The obtained ratio indicates a balanced omega-6/omega-3 fatty acid ratio, which is important for health and the prevention of cardiovascular heart disease and possibly other chronic diseases. On the other hand, profiles of LCPUFA in Hy-Line white eggs after ALA inclusion in this study have potential as a proposed substance for histone deacetylase inhibitor (HDACi). Ediriweera [30] explained that histone deacetylase is an essential enzyme that plays a pivotal role in maintaining gene expression and various cellular functions, in which its altered functionalities are associated with serious diseases including neurodegenerative disorders, CVD, and cancer. Meanwhile, fatty acids derived from the chicken egg yolk were proven to exert robust antioxidant and HDACi effect. Another finding from this study was that a diet high in omega-3 fats (ALA) caused significant changes in the fatty acid profile of eggs, resulting

ALA level (%en) Fatty acids (%) ³	Experimental diets				
	0.3 (Low)	3 (Moderate)	6 (High)	<i>P</i> -value	Significance ²
16:0	20.67	20.22	20.78	0.577	NS
18:0	6.41 ^c	7.78 ^b	8.55 ^a	0.001	**
Total SFA	28.67 ^b	29.38 ^{ab}	30.69 ^a	0.012	**
16:1n-7	4.90 ^a	1.75 ^c	2.28 ^b	0.001	**
18:1n-9	49.44 ^a	43.76 ^b	37.83 ^c	0.001	**
18:1n-7	4.35 ^a	2.04 ^b	1.69 ^c	0.001	**
Total MUFA	60.16 ^a	48.97 ^b	42.81 ^c	0.001	**
Total n-9	52.95 ^a	45.05 ^b	38.72 ^c	0.001	**
Total n-7	9.24 ^a	3.97 ^b	3.79 ^b	0.001	**
Total PUFA	10.78 ^c	21.45 ^b	26.34 ^a	0.001	**

Table 4: SFA, MUFA, and PUFA of white eggs produced at Day 28 of dietary intervention¹

¹The values are the averages of eight observations per treatment. ^(a-c) Treatments with no common superscripts are significantly different (P < 0.05). ^{2**}P < 0.01; NS, not significant.

³SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty.

in an increase in PUFA levels (P < 0.01) and a decrease in MUFA levels (Table 4). As can be seen, the PUFA content increased by approximately 2.4 times when laying hens were fed diets containing 6%en ALA (Table 4).

4 Conclusion

The optimal level associated with n-3 LCPUFA deposition was found to be 3%en ALA; however, ALA can be added to commercial laying hen feed up to 6%en ALA and can increase the total n-3 fatty acids and PUFA. The findings of this study suggest that vegetable oil can be used as an alternative to marine sources of n-3 fats. Layer hens fed a moderate and high ALA diet produce eggs that are higher in n-3 LCPUFA and have a lower ratio of n-6 to n-3 fatty acids, providing consumers with an alternative diet rich in n-3 fats that can help them approach the recommended dietary n-6 to n-3 fatty acid ratio for human health.

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