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ORIGINAL ARTICLE

Hempseed Products Fed to Hens Effectively Increased n-3 Polyunsaturated Fatty Acids in Total Lipids, Triacylglycerol and Phospholipid of Egg Yolk

M. Neijat[1] · M. Suh[2,3] · J. Neufeld[2] · J. D. House[1,2,3,4]

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Abstract Hempseed products represent potential alternative feed ingredients for poultry. However, their usage is not currently approved due to a lack of data to support their safety and efficacy. In this regard, the current study was conducted to assess the impact of dietary concentration of hempseed (HS) products and duration of their feeding to hens on the polyunsaturated fatty acid (PUFA) composition of egg yolk lipids. In the current study, 48 Lohmann LSL-Classic hens were individually housed in metabolism cages, in a completely randomized design, and provided one of six diets (wheat-barley-soybean-based) containing either HS (10, 20 and 30 %), hempseed oil (HO; 4.5 and 9.0 %) or no hempseed product (control) over 12 weeks. Increasing alpha-linolenic acid (ALA) intake via increasing dietary hempseed product inclusion, significantly ($p < 0.0001$) increased the n-3 PUFA contents of yolk total lipid. The values of ALA increased by 12-fold (152 ± 3.56 and 156 ± 2.42 mg/yolk) and docosahexaenoic acid (DHA) by twofold to threefold (41.3 ± 1.57 and 43.6 ± 1.61 mg/yolk) over the control, for the highest levels of HS and HO inclusion, respectively. Increasing levels of hemp products in laying hen diets proved effective in manipulating the fatty acid profile of the total lipid, triacylglycerol (TAG) and total phospholipid (PL) fractions of yolks, enhancing the n-3 fatty acids and reducing the n-6/n-3 ratio. The latter benefit was achieved within 4 weeks of feeding hens either HS- or HO-containing diets.

Keywords Egg yolk · Hempseed products · Fatty acid profile

Abbreviations

ADF	Acid detergent fibre
ALA	Alpha-Linolenic (C18:3n-3)
ARA	Arachidonic acid (C20:4n-6)
DHA	Docosahexaenoic acid (C22:6n-3)
DPA	Docosapentaenoic acid (C22:5n-3)
EPA	Eicosapentaenoic acid (C20:5n-3)
GLA	Gamma-linolenic acid (C18:3n-6)
HS	Hempseed
HO	Hempseed oil
LA	Linoleic acid (C18:2n-6)
MUFA	Monounsaturated fatty acid(s)
NDF	Neutral detergent fiber
PL	Phospholipid
PUFA	Polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)
TAG	Triacylglycerol

Introduction

Traditionally, North American laying hen diets are formulated on cereal grains and fat or oil sources that provide substantial levels of n-6 polyunsaturated fatty acids (PUFA), predominantly linoleic acid (LA, 18:2n-6), and only a small amount of n-3 PUFA [1–3]. As a result, classic

✉ J. D. House
j_house@umanitoba.ca

[1] Department of Animal Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

[2] Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

[3] Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB R3T 2E1, Canada

[4] Canadian Centre for Agri-Food Research in Health and Medicine, St. Boniface Research Centre, Winnipeg, MB R2H 2A6, Canada

eggs typically contain low levels of the n-3 PUFA, namely alpha-linolenic acid (ALA, 18:3n-3), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), and are virtually devoid of eicosapentaenoic acid (EPA) [4, 5]. As such, classic eggs make only minor contributions to recommended daily intakes [6] of these fatty acids in the general population. The fatty acid profiles of egg yolk lipids have been shown to be altered by adding natural sources of n-3 PUFA to the hens' feed [7–9]. Flaxseed, an oilseed that contains approximately 30 % lipid, is a rich source of ALA (53 % of total fatty acids in the oil) [10, 11], and is commonly used to enrich eggs with n-3 fatty acids [7, 12, 13]. Flaxseed is considered a minor crop in North America compared to other oilseeds such as canola and soybean [14]. However, given substantial interest for its direct consumption by humans, flaxseed has a high growth potential for use in functional foods for humans [15], thus potentially creating challenges for its utilization in animal feed. Flaxseed also contains anti-nutritive factors, including linamarin [16] and linatine [17]. These latter factors may lead to limitations in flaxseed use in hen diets in order to generate n-3 fatty acid-enriched eggs, with maximum inclusions levels of 15–20 % observed to influence hen body weight, egg size and production [18–20]. In addition, it has been reported that as little as 5 % inclusion of flaxseed in hen diets can cause negative organoleptic qualities, such as a "fishy taint" in eggs [21, 22]. Owing to these limitations, opportunity exists to evaluate alternative sources of n-3 fatty acids for laying hen diets.

The seed and oil of industrial hemp (*Cannabis sativa* L.) are rich sources of ALA, with this fatty acid comprising 19–22 % of the total fatty acid profile [23, 24]. Additionally, hempseed (HS) can be a valuable feed source for poultry, given its protein (24 %) and total fat (30 %) contents [24–26]. Hempseed oil (HO) is a rich source of PUFA (78 % of total fatty acids), with a linoleic acid (LA, 18:2n-6) to ALA ratio of approximately 3 to 1 [27]. This ratio is often cited as being ideal and better suited for human nutrition [28, 29]. Additionally, hemp oil contains approximately 4 % gamma-linolenic acid (GLA) [23], which may confer additional health benefits [30]. Despite these positive traits, the usage of industrial hemp products in Canada is not currently approved, owing to the need for safety and efficacy data on these components. The latter requirements stem from the potential for hemp to contain small amounts of tetrahydrocannabinol (THC), a psychoactive agent linked to cannabis varieties. Industrial hemp is grown under license from federal regulatory authorities [31], and these regulations are aimed at producing appropriate cultivars containing less than 0.3 % (by wt) THC. Hemp products are not currently registered as approved feed ingredients in North America, and approval requires submission of data in support of the safety and efficacy of hempseed products for use in livestock and poultry diets.

Previous research has shown that hempseed products can be used to generate eggs enriched with n-3 PUFA [24, 26, 32]. This previous work focused on the fatty acid composition of total yolk lipids, without considering the fatty acid composition of the major lipid classes, including the phospholipid (PL) and triacylglycerol (TAG) depots; yet each of these lipid fractions can be a potential food source in food industry. For example, egg phospholipids have been utilized in infant formulas [33]. Additionally, egg lipids could serve as a source of TAG molecules (based on their fatty acid composition) in the synthesis of structured lipids (composed of medium chain fatty acids and PUFAs) with improved nutritional benefits for ultimate use in food applications [34]. As such, the current study was designed to address the temporal changes in the fatty acid profiles, particularly the n-3 PUFA in chicken eggs as a function of increasing hemp oil, from either the seed or the extracted oil, in laying hen diets over a period of 12 weeks. Furthermore, the distribution of fatty acid profile in the major TAG and PL fractions within egg yolk was investigated.

Materials and Methods

Animals and Diets

Forty-eight Lohmann LSL-Classic (white-egg layers; initial mean weight of 1.41 ± 0.08 kg) at 19 weeks of age were individually caged (25.4 cm × 40.6 cm dimension, with provided floor space of 1032 cm^2/hen), and maintained under semi-controlled environmental conditions at the University of Manitoba poultry barn. Hens were adapted to the cages and diets over an initial 2-week period. During the first week of adaptation, hens were fed a commercial layer diet, and then transitioned to a 50:50 blend of the commercial and test diets in the second week. Following the adaptation period, the hens were allocated to one of six wheat-barley-soybean meal-based diets. The experiment included a control diet without hempseed product (corn-oil-based, 9.86 % of diet); three diets providing 10, 20, or 30 % hempseed (HS; containing 32.8 ± 1.05 % lipid); and two diets with 4.5 or 9.0 % hempseed oil (HO). The layer diets were isocaloric, isonitrogenous and isolipidic and formulated to meet the minimum recommendation of laying hens consuming 105–115 g of feed per day in accordance with the strain's management guide [35]. The composition and nutrient contents of the experimental diets are shown in Table 1. To avoid lipid peroxidation, diets were prepared in two batches, and in addition, an antioxidant, vitamin E, was

Table 1 Composition and calculated nutrients of experimental diets containing hempseed (HS) and hempseed oil (HO)

	Control	10 % HS	20 % HS	30 % HS	4.5 % HO	9.0 % HO
Ingredients (%)						
Wheat	35.0	33.4	31.9	30.3	35.0	35.0
Barley	15.0	15.0	15.0	15.0	15.0	15.0
Soybean Meal	21.5	16.9	12.2	7.60	21.5	21.5
Hemp Seed	0.00	10.0	20.0	30.0	0.00	0.00
Hemp Oil	0.00	0.00	0.00	0.00	4.50	9.00
Corn Oil	9.68	6.48	3.28	0.07	5.18	0.68
Vitamin-mineral premix[a]	2.50	2.50	2.50	2.50	2.50	2.50
Limestone	13.9	13.5	13.0	12.4	13.9	13.9
Dicalcium Phosphate	1.88	1.60	1.56	1.52	1.88	1.88
Salt	0.34	0.35	0.35	0.35	0.34	0.34
DL-Methionine	0.116	0.095	0.073	0.051	0.116	0.116
Lysine-HCl	0.083	0.128	0.173	0.218	0.083	0.083
Threonine	0.005	0.000	0.000	0.000	0.005	0.005
Calculated nutrient contents						
AMEn (Kcal/kg)	2800	2800	2800	2800	2800	2800
CP (%)	17.0	17.0	17.0	17.0	17.0	17.0
Crude fat (%)	11.0	11.0	11.0	11.0	11.0	11.0
Calcium (%)	5.48	5.30	5.09	4.87	5.48	5.48
Total phosphorus (%)	0.70	0.72	0.80	0.87	0.70	0.70
Available phosphorus (%)	0.50	0.45	0.45	0.45	0.50	0.50
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16
Chloride (%)	0.26	0.26	0.25	0.25	0.26	0.26
Methionine	0.36	0.36	0.36	0.36	0.36	0.36
Total lysine (%)	0.85	0.85	0.85	0.85	0.85	0.85
Threonine	0.60	0.60	0.61	0.62	0.60	0.60
Analyzed nutrient contents[b]						
DM (%)	91.9 ± 0.21	91.8 ± 0.25	91.9 ± 0.21	91.9 ± 0.30	92.1 ± 0.36	91.9 ± 0.30
Energy (gross energy, kcal/kg)	3805 ± 19.9	3848 ± 22.8	3848 ± 14.2	3873 ± 32.1	3806 ± 13.9	3797 ± 2.55
CP (%)	16.9 ± 0.58	17.8 ± 0.35	17.3 ± 0.37	16.8 ± 1.67	17.0 ± 0.72	17.7 ± 0.50
Crude fat (%)	11.6 ± 0.62	12.7 ± 0.90	12.5 ± 0.02	12.5 ± 0.44	12.0 ± 0.01	11.3 ± 0.28
Calcium (%)	5.65 ± 0.43	5.64 ± 0.41	5.29 ± 0.38	5.93 ± 1.04	5.50 ± 0.81	5.73 ± 0.15
Total phosphorus (%)	0.75 ± 0.03	0.75 ± 0.07	0.80 ± 0.01	0.86 ± 0.04	0.70 ± 0.04	0.75 ± 0.01
ADF (%)	2.91	5.45	7.38	8.83	2.84	3.27
NDF (%)	14.3	16.8	18.2	21.6	11.8	13.3

HS contained 32.8 ± 1.05 % fat

[a] Provided per kilogram of diet, vitamin-mineral premix contained: 11,000 IU of vitamin A; 3000 IU of vitamin D_3, 150 IU of vitamin E, 3 mg of vitamin K_3 (as menadione), 0.02 mg of vitamin B_{12}, 0.2 mg of biotin, 6.5 mg of riboflavin, 4 mg of folic acid, 10 mg of calcium pantothenate, 39.9 mg of niacin, 2.2 mg of thiamine, 4.5 mg of pyridoxine, 1000 mg of choline chloride, 125 mg antioxidant (ethoxyquin), 66 mg of manganese oxide, 70 mg of zinc oxide, 80 mg of ferrous sulfate, 10 mg of copper sulfate, 0.3 mg of sodium selenite, 0.4 mg of calcium iodate, 0.67 mg of sodium chloride (salt)

[b] Mean values ± SD

supplemented at levels of 150 IU/kg of diet. The lipid profile of the experimental diets is shown in Table 2. Animal usage and care was reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee, and the hens were managed in accordance with the recommendations established by [36].

Diet and Egg Yolk Fatty Acid Extraction

Diet samples (duplicates per treatment) from both batches and eggs (eight replicates per treatment) collected within the last 3 days of each period (week 4, 8 and 12) of the experiment were used for fatty acid extraction. Diet

Table 2 Fatty acid profile of experimental diets

Fatty acid (mg/g of diet)[a]	Control	10 % HS	20 % HS	30 % HS	4.5 % HO	9.0 % HO
SFA						
Myristic (C14:0)	0.041	0.041	0.045	0.045	0.040	0.047
Palmitic (C16:0)	8.53	7.70	6.35	4.90	7.00	5.60
Stearic (C18:0)	1.17	1.35	1.49	1.51	1.38	1.65
Total SFA	9.74	9.09	7.88	6.46	8.42	7.29
MUFA						
Palmitoleic (C16:1)	0.074	0.078	0.078	0.074	0.077	0.080
Oleic (C18:1)	17.8	14.3	10.4	6.01	12.6	7.62
Total MUFA	17.8	14.4	10.5	6.09	12.7	7.70
PUFA[b]						
Linoleic (LA, C18:2n-6)	38.1	39.2	38.6	36.3	39.0	40.9
Gamma-Linolenic (GLA, C18:3n-6)	0.01	0.92	1.83	2.57	1.30	2.70
Arachidonic (ARA, C20:4n-6)	ND	ND	ND	ND	ND	ND
Alpha-Linolenic (ALA, C18:3n-3)	0.96	5.00	8.95	12.2	6.64	12.8
Eicosapentaenoic (EPA, C20:5n-3)	ND	ND	ND	ND	ND	ND
Docosapentaenoic (DPA, C22:5n-3)	ND	ND	ND	ND	ND	ND
Docosahexaenoic (DHA, C22:6n-3)	ND	ND	ND	ND	ND	ND
Total PUFA	39.1	45.1	49.4	51.0	46.9	56.5
Ratio LA:ALA	39.8	7.83	4.32	2.98	5.88	3.18

Diets contain no hempseed product (control), *HS* hempseed or *HO* hempseed oil

[a] *SFA* saturated, *MUFA* monounsaturated, *PUFA* polyunsaturated fatty acids

[b] *ND* not detectable

samples (150 g each) were ground using a commercial grinding mill and stored at −20 °C until analyzed. The eggs were broken, the yolks carefully separated from the whites (albumen) using an egg separator, then individually weighed in plastic bags and stored at −20 °C until analyzed. Diet (1 g) and egg yolk samples (3 g) were used for the extraction of total lipids using chloroform/methanol (2:1, by vol) containing 0.01 % butylated hydroxytoluene (antioxidant) according to Folch et al. [37]. The extracted total lipids were weighed and reconstituted in hexane to a volume of 25 mL. From each extract, aliquots of a known volume (to contain 40–50 mg lipid) were dried under nitrogen, dissolved in toluene and methylated by heating in the presence of methanol containing 2 % (by vol) sulphuric acid [38]. The resulting fatty acid methyl esters (FAMEs) were extracted into iso-octane for determining the fatty acid profile using gas chromatography (GC). The major lipid classes (TAG and PL) in the total lipid extracts of egg yolk (obtained in week 12 of the trial, $n = 7$ per treatment) were separated by thin layer chromatography (TLC) on silica gel plates (Silica gel G, Uniplate™, Analtech, Inc) according to the method described in [39], with slight modification, using petroleum ether/diethyl ether/acetic acid (80:20:1, by vol), as the developing solvent. Approximately 2.4 mg of yolk lipid extract was applied on the TLC plates. Lipid classes were visualized with 0.1 % (wt/vol) 2, 7-dichlorofluorescein in methanol under UV light. Prior to methylation, the TAG fraction was saponified according to the method previously described in [40], and the PL fraction was directly esterified. Lipids were esterified using borontrifluoride in 14 % methanol, and fatty acids were quantified by GC using C17:1 (Nu-Chek Prep Inc., Elysian, MN) as the internal standard.

Fatty Acid Analysis

FAMEs were analyzed using a Varian 450 GC with flame ionization detector (FID) and equipped with a DB225MS column (30 m × 0.25 mm diameter and 0.25 μm film thickness; Agilent Technologies Canada Inc., Mississauga, Ontario). The temperature was 70 °C for 2 min, then raised to 180 °C at 30 °C/min, held for 1 min; raised to 200 °C at 10 °C/min, for 2 min; raised to 220 °C at 2 °C/min and held for 10 min and finally raised to 240 °C at 20 °C/min for 5 min. Total run time was 36.67 min, and samples (1 μL injection) were run with a 20:1 split ratio. Hydrogen was used as the carrier gas with a flow rate of 1.3 mL/min. Each fatty acid was identified by comparing its retention time to authentic standard samples of known composition (Lipid standards, Nu-Chek Prep, Inc., Elysian, MN, USA). The fatty acid content of egg yolk total lipid was calculated as concentration (mg/yolk) = [(peak area of a given fatty acid × concentration of internal standard (mg/mL)/peak area of internal standard) ×

dilution factor of extracted lipid] divided by yolk sample weight (g), then multiplied by total yolk weight per egg (g). For lipid classes, TAG and total PL of yolk, the peak area for an individual fatty acid was expressed as a percentage of the total peak area for all identified fatty acids in the lipid samples.

Statistical Analysis

The fatty acid content in the egg yolk total lipid and lipid classes were analyzed as a completely randomized design with the individual hen as the experimental unit. The HS (control (0), 10, 20 and 30 % levels) and HO (control (0), 4.5 and 9.0 % levels) treatments were analyzed as two separate experiments/trials, since the biological efficiency in the utilization of the seeds vs. the oils by the hens would be expected to differ. The same data for the control diet was applied in either treatment group. Feed intake was used as a covariate in all analysis, and this may influence the final control data sets between the two treatment groups. Except for lipid classes, the fatty acids in total lipid of egg yolk were run as repeated measure analysis using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC), following the model:

$$Y_{ijk} = \mu + d_i + h_{ij} + w_k + dw_{ik} + e_{ijk},$$

where μ = overall mean, d_i = fixed effect of diet (i = 1–4 for HS-containing diets or i = 1–3 for HO-containing diets), h_{ij} = random effect of hen within diet (j = 1–8, number of hens per treatment), w_k = fixed effect of week (k = 1–3), and dw_{ik} = interaction between diet and week (diet × week), and e_{ijk} = random error variation (residual error). Diet × week interactions were considered as fixed effects. Fatty acids in lipid classes were analyzed with a one-way ANOVA for the HS (control (0), 10, 20 and 30 % levels) or HO (control (0), 4.5 and 9.0 % levels) treatment groups (n = 7 for each diet), using the same statistical package. Normality of the data distribution was assessed using the Shapiro–Wilk test and data points with studentized residuals below or above ± 3.0 were considered to be outliers and were excluded from the analysis. Least squares means (LSM), adjusted using Tukey's significant difference test, were compared for significant difference ($p < 0.05$).

Results

Total Egg Yolk Lipid Content

Total lipid extracts from eggs were not statistically different between treatments for both treatment groups: HS [31.5, 34.3, 32.9 and 32.3 ± 1.27 (SE), $p = 0.51$; for control, 10, 20 and 30 % of diet HS inclusion, respectively]; and HO [31.5, 34.8 and 34.3 ± 1.37, $p = 0.20$ (SE); for control, 4.5 and 9.0 % of diet HO inclusion, respectively].

Fatty Acid Composition in the Total Lipid of Egg Yolk

With respect to the total lipid fraction, the egg yolk SFA [myristic (C14:0); palmitic (C16:0) and stearic (C18:0) acids] compositions, as a function of increasing levels of hempseed-derived products, are presented in Tables 3, 4 for the HS and HO treatment groups, respectively. There was a significant decrease in the levels of myristic ($p < 0.05$) and palmitic ($p < 0.001$) acids in eggs from hens receiving the HS-containing diets compared to the control (Table 3). With respect to HO treatments (Table 4), the myristic and palmitic acid levels in the egg yolk were significantly ($p < 0.01$ and $p < 0.05$, respectively) lower for hens fed the 4.5 % HO compared to the control or the 9.0 % HO. Additionally, in both the HS (Table 3) and HO (Table 4) groups, the level of stearic acid was significantly ($p < 0.0001$) greater in eggs obtained from hens consuming higher levels of HS (30 %) and HO (9.0 %) compared to those fed the control diet or lower levels of the hempseed products. However, overall, the inclusion of either HS (Table 3) or HO (Table 4) in the laying hen diets did not affect the levels of total SFA in the egg yolk. The contents of total MUFA (palmitoleic (C16:1) and oleic (C18:1) acids) in the egg yolk significantly ($p < 0.0001$) decreased with increasing inclusions of both HS (Table 3) and HO (Table 4) in the laying hen diets, particularly due to a significant reduction ($p < 0.0001$) in the levels of oleic acid for both treatment modalities. However, a highly significant ($p < 0.0001$) period effect observed in the levels of oleic acid in the egg yolk did not result in a significant diet × week interaction within each treatment in either the HS or the HO treatment settings (Tables 3, 4).

The inclusion of either HS (Table 5) or HO (Table 6) in the laying hen diets significantly influenced the total PUFA levels in the egg yolk, except for LA composition that was maintained at a similar level to the control. Although the levels of GLA in the total lipids of egg yolk were significantly ($p < 0.0001$) increased at higher levels of HS inclusions (at 20 and 30 %) in the diets compared to the control or at lower levels of the HS (10 %; Table 5), both levels of the HO (4.5 and 9.0 %) were effective in increasing the levels of this fatty acid in the egg yolks compared to the control (Table 6). Arachidonic acid levels were significantly decreased in the total lipid of egg yolk for both the HS ($p < 0.0001$) and the HO ($p < 0.001$) treatment groups compared to the control. In general, there was no significant treatment effect on the total n-6 fatty acids level in the total lipid of egg yolk in both treatment modalities (data not presented).

Table 3 Total saturated (SFA) and monounsaturated (MUFA) fatty acids composition in egg yolk (mg/yolk) obtained from hens consuming diets containing increasing levels of hempseed (HS)

Fatty acids[1]	Week	Control	Effect of diet				Effect of week				*p* value		
			10 % HS	20 % HS	30 % HS	SE	Week 4	Week 8	Week 12	SE	Diet	Week	Diet × Week
$C_{14:0}$	4	9.03^{A}	7.17^{B}	7.19^{B}	6.93^{B}								
	8	8.38^{AB}	7.27^{B}	7.34^{B}	6.73^{B}								
	12	8.00^{B}	8.29^{A}	8.12^{A}	7.83^{A}								
	Overall	8.47^{a}	7.58^{b}	7.55^{b}	7.16^{b}	0.23	7.58^{b}	7.43^{b}	8.06^{a}	0.16	<0.01	<0.01	<0.01
$C_{16:0}$	4	757	664	620	608								
	8	795	713	669	636								
	12	759	769	709	703								
	Overall	771^{a}	716^{ab}	666^{bc}	650^{c}	17.4	662^{c}	703^{b}	735^{a}	12.5	<0.001	<0.0001	0.13
$C_{18:0}$	4	281	277	284	339								
	8	292	296	301	346								
	12	275	316	312	345								
	Overall	282^{b}	296^{b}	299^{b}	344^{a}	8.63	296^{b}	309^{a}	312^{a}	5.58	<0.001	<0.05	0.15
Total SFA		1062	1019	972	1000	22.2	966^{b}	1019^{a}	1055^{a}	16.2	0.053	<0.001	0.16
$C_{16:1}$	4	34.8	29.6^{B}	27.7	24.0^{B}								
	8	34.2	29.0^{B}	29.7	25.1^{B}								
	12	32.6	33.2^{A}	30.5	28.8^{A}								
	Overall	34.0^{a}	30.6^{ab}	29.3^{ab}	25.8^{b}	1.60	29.0^{b}	29.5^{ab}	31.3^{a}	0.92	<0.05	<0.05	<0.05
$C_{18:1}$	4	938	800	675	583								
	8	989	843	719	623								
	12	934	893	748	687								
	Overall	953^{a}	846^{b}	714^{c}	631^{d}	21.1	749^{b}	794^{a}	816^{a}	15.1	<0.0001	<0.01	0.31
Total MUFA		987^{a}	877^{b}	743^{c}	657^{d}	22.3	778^{b}	823^{a}	847^{a}	15.8	<0.0001	<0.01	0.28

Data are presented as least square means (LSM) ± standard error (SE)

[1] Myristic (C14:0), palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1), oleic (C18:1) acid

[a–d] Different superscripts between treatments (effect of diet) or periods (effect of week), within a row, are significantly different at $p < 0.05$

[A, B] Different superscripts within a treatment(diet), within a column, for each parameter are significant different ($p < 0.05$), comparison of diet by week interaction

A significant ($p < 0.0001$) increase in the n-3 fatty acid contents of the eggs (ALA, EPA, DPA and DHA) in both the HS (Table 5) and HO (Table 6) treatment groups were measured. A major increase (by 13-fold) was observed in the contents of ALA achieving 152 ± 3.56 mg/yolk for HS and 156 ± 2.42 mg/yolk for HO treatments at the highest level of inclusion compared to control treatment. Compared to the control group, hens consuming hempseed products continued to deposit higher levels of ALA in the egg yolk, with the greatest levels observed after 12 weeks and 8 weeks of feeding HS ($p < 0.0001$, Table 5) and HO ($p < 0.0001$, Table 6; Fig. 1), respectively. Among the long chain PUFA (LCPUFA), although EPA was not preferentially deposited in the egg yolk, upon feeding HS products to the laying hens, the levels of this fatty acid in the total lipid of egg yolk showed a significant ($p < 0.001$) linear association (regression) to the levels of ALA in the total lipid of egg yolk for HS ($EPA_{yolk\ (mg/yolk)} = 0.0159\ ALA_{yolk\ (mg/yolk)} - 0.0575$; $R^2 = 0.95$, $p < 0.001$) and HO treatments ($EPA_{yolk\ (mg/yolk)} = 0.0159\ ALA_{yolk\ (mg/yolk)} - 0.115$; $R^2 = 0.97$, $p < 0.001$), based on week 12 data.

The longer chain metabolites of ALA were significantly ($p < 0.0001$) increased in both the HS (Table 5) and HO (Table 6) treatment groups, although proportional increases, particularly in the amount of DPA and DHA did not reflect the corresponding increase in the levels of dietary ALA (inclusion levels of HS or HO). The level of DHA increased from 16.2 to 41.3 ± 1.57 mg/yolk and from 15.8 to 43.6 ± 1.61 mg/yolk for HS and HO treatment groups, respectively, at the highest level of inclusion. There was no significant period effect or an interaction with treatment in the accumulation levels of the n-3 LCPUFA of yolks derived from hens consuming either HS (Table 5) or HO (Table 6) products. The one exception was DHA in the HO treatment

Table 4 Total saturated (SFA) and monounsaturated (MUFA) fatty acids composition in egg yolk (mg/yolk) obtained from hens consuming diets containing increasing levels of hempseed oil (HO)

Fatty acids[1]	Week	Effect of diet				Effect of week				*p* value		
		Control	4.5 % HO	9.0 % HO	SE	Week 4	Week 8	Week 12	SE	Diet	Week	Diet × Week
$C_{14:0}$	4	8.98^{A}	7.02	8.15^{B}								
	8	8.34^{AB}	7.62	8.40^{AB}								
	12	7.96^{B}	7.93	8.90^{A}								
	Overall	8.43^{a}	7.52^{b}	8.48^{a}	0.21	8.05	8.12	8.26	0.17	<0.01	0.61	< 0.01
$C_{16:0}$	4	750	642	649								
	8	789	710	724								
	12	753	737	747								
	Overall	764^{a}	697^{b}	707^{ab}	16.7	681^{b}	741^{a}	746^{a}	13.1	<0.05	0.0001	0.098
$C_{18:0}$	4	281	284^{B}	311^{B}								
	8	292	319^{A}	347^{A}								
	12	274	321^{A}	349^{A}								
	Overall	282^{b}	308^{b}	335^{a}	7.63	292^{b}	319^{a}	315^{a}	5.64	<0.001	0.0001	<0.05
Total SFA		1055	1012	1051	21.2	980^{b}	1068^{a}	1068^{a}	17.4	0.31	0.0001	0.066
$C_{16:1}$	4	34.6	30.9	33.6								
	8	33.9	32.0	35.0								
	12	32.4	33.4	37.3								
	Overall	33.6	32.1	35.3	1.76	33.0	33.6	34.3	1.17	0.45	0.42	0.19
$C_{18:1}$	4	933	782	667								
	8	985	845	719								
	12	929	868	727								
	Overall	949^{a}	832^{b}	704^{c}	19.1	794^{b}	850^{a}	842^{a}	15.0	<0.0001	<0.01	0.25
Total MUFA		983^{a}	864^{b}	740^{c}	20.2	827^{b}	883^{a}	876^{ab}	15.8	<0.0001	<0.01	0.24

Data are presented as least square means (LSM) ± standard error (SE)

[1] Myristic (C14:0), palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1), oleic (C18:1) acid

[a–c] Different superscripts between treatments (effect of diet) or periods (effect of week), within a row, are significantly different at $p < 0.05$

[A, B] Different superscripts within a treatment (diet), within a column, for each parameter, are significant different ($p < 0.05$), comparison of diet by week interaction

group, where there was a significant period ($p < 0.01$) and week × treatment interaction ($p < 0.001$, Table 6) in week 8 and week 12 compared to that in week 4. The latter observation may relate to the fact that the accumulation of DHA in the egg yolk attains a maximal level.

Over the 12-week feeding period, the total n-3 PUFA composition of egg yolk lipids reflected that of the laying hen diets (primarily due to that of ALA content) in both the HS (Tables 5) and the HO (Tables 6) treatments (Fig. 1). Overall, the accumulation of total n-3 PUFA into the yolk significantly ($p < 0.0001$) increased as a function of increasing inclusion of the hempseed products in the laying hen diets. A period effect on the amounts of total n-3 PUFA in the egg yolks indicated a significant ($p < 0.001$, Fig. 1) gradual increase for the HS-containing diet (at 30 % level), with the highest levels observed at week 12 compared to that at week 4. For HO groups, a week × treatment interaction was observed for both levels of HO (4.5 and 9.0 %), as total n-3 PUFA were maintained significantly ($p < 0.05$) greater at week 8 compared to week 4 (Fig. 1). As a result of the enhanced amount of n-3 PUFA concurrent with decreased ARA levels (Tables 5, 6), the n-6 to n-3 ratio in the egg yolk obtained from HS and HO receiving hens gradually decreased ($p < 0.0001$), with the lowest values observed at 30 % HS and 9.0 % HO (Fig. 2). Although the n-6 to n-3 ratio in the egg yolk, as a function of hempseed product inclusion in the laying hen diets, was not influenced by period or the interaction with treatment effects, the control diet-fed hens produced eggs that showed a significant ($p < 0.0001$) period × treatment interaction in the n-6 to n-3 ratio in the egg yolk, indicating an increase in the ratio as time of feeding increased.

Table 5 Total polyunsaturated fatty acids (PUFA) composition in total lipid of egg yolk (mg/yolk) obtained from hens consuming diets containing increasing levels of hempseed (HS)

Fatty acids[1]	Week	Effect of diet					Effect of week				*p* value		
		Control	10 % HS	20 % HS	30 % HS	SE	Week 4	Week 8	Week 12	SE	Diet	Week	Diet × Week
LA(18:2n-6)	4	823	810^{B}	818	810^{B}								
	8	923	915^{A}	882	905^{AB}								
	12	835	970^{A}	944	999^{A}								
	Overall	860	898	881	905	23.1	815^{b}	906^{a}	937^{a}	16.2	0.59	<0.0001	<0.05
GLA(18:3n-6)	4	5.49	6.08	7.87	9.37								
	8	6.44	6.99	8.49	10.1								
	12	5.91	7.45	8.97	10.9								
	Overall	5.94^{c}	6.84^{c}	8.44^{b}	10.1^{a}	0.35	7.20^{b}	8.01^{a}	8.30^{a}	0.21	<0.0001	<0.0001	0.26
ARA(20:4n-6)	4	68.6	59.3	55.8	52.6^{B}								
	8	72.8	63.4	56.7	55.8^{AB}								
	12	68.6	66.9	59.1	63.4^{A}								
	Overall	70.3^{a}	63.2^{bc}	57.3^{cd}	56.8^{d}	1.61	59.1^{b}	62.2^{a}	64.5^{a}	1.11	<0.0001	<0.001	<0.05
ALA(18:3n-3)	4	13.4	53.2	99^{B}	135^{B}								
	8	13.7	59.2	110^{AB}	145^{B}								
	12	12.6	65.6	114^{A}	176^{A}								
	Overall	13.2^{d}	59.3^{c}	108^{b}	152^{a}	3.56	75.2^{c}	81.8^{b}	92.1^{a}	2.17	<0.0001	<0.0001	<0.0001
EPA(20:5n-3)	4	0.00	1.01	1.80	2.57								
	8	0.00	1.07	1.84	2.57								
	12	0.00	1.12	1.87	2.66								
	Overall	0.00^{d}	1.07^{c}	1.84^{b}	2.60^{a}	0.060	1.34	1.37	1.41	0.038	<0.0001	0.26	0.96
DPA(22:5n-3)	4	1.74	3.57	4.02	5.23								
	8	1.74	3.65	4.29	5.56								
	12	1.58	4.19	4.39	4.98								
	Overall	1.69^{c}	3.64^{b}	4.23^{b}	5.26^{a}	0.25	3.64	3.81	3.78	0.14	<0.0001	0.33	0.066
DHA(22:6n-3)	4	17.0	41.1	39.6	39.8								
	8	16.7	41.1	41.2	42.8								
	12	14.1	42.9	42.8	41.7								
	Overall	16.2^{b}	41.0^{a}	41.3^{a}	41.0^{a}	1.57	33.8	35.5	35.4	0.93	<0.0001	0.087	0.086

Data are presented as least square means (LSM) ± standard error (SE)

[1] *LA* linoleic acid, *GLA* gamma-linolenic acid, *ARA* arachidonic acid, *EPA* eicosapentaenoic acid, *DPA* docosapentaenoic acid, *DHA* docosahexaenoic acid

[a–d] Different superscripts between treatments (effect of diet) or periods (effect of week), within a row, are significantly different at $p < 0.05$

[A, B] Different superscripts within a treatment (diet), within a column, for each parameter, are significant different ($p < 0.05$), comparison of diet by week interaction

Fatty acid composition in the TAG and total PL lipid classes

The levels of fatty acid composition in both the TAG and the total PL fractions of the yolk are presented for both the HS and HO treatment groups (Tables 7, 8, respectively). Regardless of the percentage of inclusion of the hempseed products in the hen diets, the concentrations of SFA in yolk lipids (the TAG or the total PL) were not affected by treatment. As the level of HS or HO inclusion in the diet increased, the concentrations of MUFA significantly decreased in both the TAG ($p < 0.0001$) and the total PL ($p < 0.01$) lipid fractions compared to the control diet fed group. However, in the latter fraction, the decrease was only noted at higher levels of HS (30 %) or HO (9.0 %) inclusion. The level of LA significantly ($p < 0.001$) increased in the TAG fraction of the egg yolk for the HS treatment groups, but did not change across the HO treatment groups. The reverse was true for LA levels in the total PL fraction of the egg yolk (Tables 7, 8). Gamma-linolenic acid increased significantly ($p < 0.0001$) in both lipid fractions with each additional increment of either HO or HS.

Table 6 Total polyunsaturated fatty acids (PUFA) composition in total lipid of egg yolk (mg/yolk) obtained from hens consuming diets containing increasing levels of hempseed oil (HO)

Fatty acids[1]	Week	Effect of diet				Effect of week				*p* value		
		Control	4.5 % HO	9.0 % HO	SE	Week 4	Week 8	Week 12	SE	Diet	Week	Diet × Week
LA(18:2n-6)	4	818	763^{B}	816^{B}								
	8	912	873^{A}	964^{A}								
	12	827	914^{A}	970^{A}								
	Overall	852	850	917	22.1	799^{b}	916^{a}	903^{a}	16.9	0.070	<0.0001	<0.05
GLA(18:3n-6)	4	5.48	6.27	8.61								
	8	6.43	7.53	10.4								
	12	5.90	7.59	10.3								
	Overall	5.93^{c}	7.13^{b}	9.78^{a}	0.28	6.78^{b}	8.12^{a}	7.94^{a}	0.19	<0.0001	<0.0001	0.069
ARA(20:4n-6)	4	68.4	57.4	55.9								
	8	72.6	61.7	62.7								
	12	68.3	63.2	62.4								
	Overall	69.8^{a}	60.8^{b}	60.3^{b}	1.42	60.6^{b}	65.7^{a}	64.7^{a}	1.14	<0.001	<0.01	0.24
ALA(18:3n-3)	4	13.3	65.7^{B}	135^{B}								
	8	13.5	78.0^{A}	165^{A}								
	12	12.4	83.5^{A}	167^{A}								
	Overall	13.1^{c}	75.7^{b}	156^{a}	2.42	71.4^{b}	85.5^{a}	87.6^{a}	1.87	<0.0001	<0.0001	<0.0001
EPA(20:5n-3)	4	0.00	1.26	2.25								
	8	0.00	1.41	2.40								
	12	0.00	1.38	2.43								
	Overall	0.00^{c}	1.35^{b}	2.36^{a}	0.046	1.17^{b}	1.27^{a}	1.27^{a}	0.034	<0.0001	<0.05	0.24
DPA(22:5n-3)	4	1.68	3.45	5.07								
	8	1.67	3.89	5.07								
	12	1.52	3.77	5.35								
	Overall	1.62^{c}	3.70^{b}	5.17^{a}	0.25	3.40	3.54	3.55	0.16	<0.0001	0.41	0.24
DHA(22:6n-3)	4	16.9	37.1	40.6^{B}								
	8	16.6	40.3	45.1^{A}								
	12	14.0	40.5	45.1^{A}								
	Overall	15.8^{b}	39.3^{a}	43.6^{a}	1.61	31.5^{b}	34.0^{a}	33.2^{a}	1.01	<0.0001	<0.01	<0.001

Data are presented as least square means (LSM) ± standard error (SE)

[1] *LA* linoleic acid, *GLA* gamma-linolenic acid, *ARA* arachidonic acid, *EPA* eicosapentaenoic acid, *DPA* docosapentaenoic acid, *DHA* docosahexaenoic acid

[a–c] Different superscripts between treatments (effect of diet) or periods (effect of week), within a row, are significantly different at $p < 0.05$

[A, B] Different superscripts within a treatment (diet), within a column, for each parameter, are significant different ($p < 0.05$), comparison of diet by week interaction

While the ARA levels in the egg yolk were not affected by the levels of HS in the diets of the hens for both the TAG and the total PL fractions, decreased levels ($p < 0.01$) of the fatty acid were noted with the HO treatments in both lipid fractions.

As a percentage of total fatty acids, the levels of ALA in the egg yolk represented a greater ($p < 0.0001$) fraction of the TAG than the total PL in both the HS and HO treatment group (Tables 7, 8). Whereas the incorporation of DPA and DHA was observed mainly in total PL fractions of the egg yolks from both the HS and HO treated hens, EPA was highly ($p < 0.0001$) contained in the TAG fractions (data not presented). Although the DHA levels in the TAG fraction of egg yolk were not affected by the levels of inclusion of the hempseed products in the hen diets, this fatty acid reached a plateau in the total PLs of the egg yolk for both the HS and HO groups.

The total n-6 PUFA level increased significantly ($p < 0.001$) with increasing levels of HS in the egg yolk TAG fraction. A similar but nonsignificant pattern of

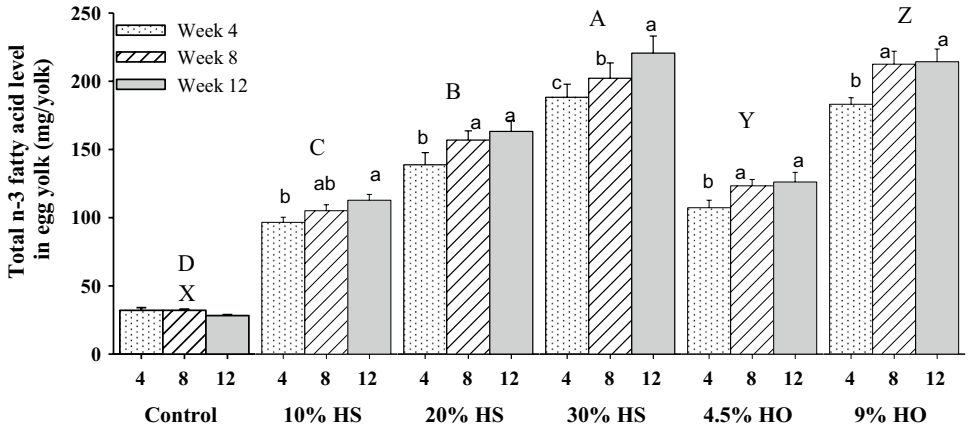

Fig. 1 Total n-3 fatty acid, sum of ALA, EPA, DPA and DHA (mg/yolk) in egg yolk as a function of period (week 4, 8 and 12) of hens fed diets containing varying levels of hempseed (HS: 10, 20 and 30 % of diet) or hempseed oil (HO: 4.5 and 9.0 % of diet). Data point in each period is a group mean ± SE (n = 8 hens per treatment). Letters denote significant differences ($p < 0.05$) between periods within a treatment and between diets within a treatment group, for either HS or HO vs. control

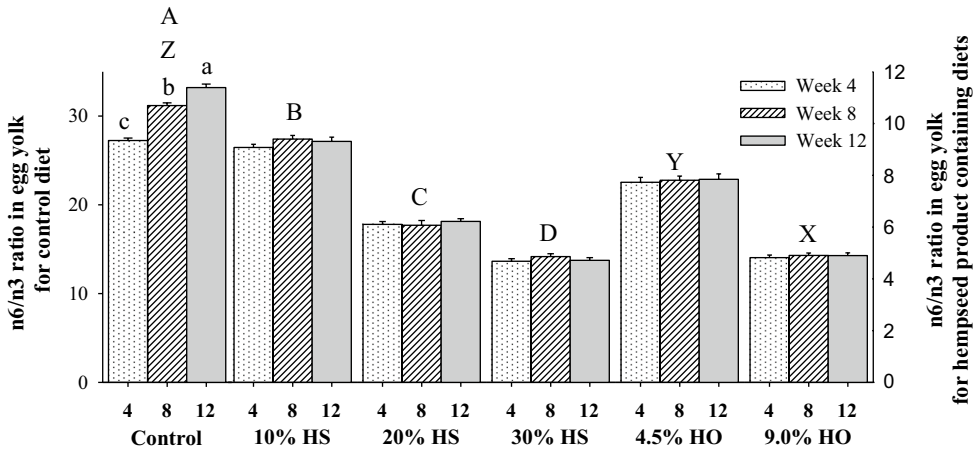

Fig. 2 n6/n3 ratio in egg yolk as a function of period (week 4, 8 and 12) of hens fed diets containing varying levels of hempseed (HS: 10, 20 and 30 % of diet) or hempseed oil (HO: 4.5 and 9.0 % of diet). The data point in each period is a group mean ± SE ($n = 8$ hens per treatment). Letters denote significant differences ($p < 0.05$) between periods within a treatment and between diets within a treatment group, for either HS or HO vs. control

increase was observed for total n-6 level in the TAG of the egg yolk obtained upon HO feeding (Table 7). The total n-6 levels in the total PL of egg yolk decreased significantly with increasing levels of either HS ($p < 0.05$) or HO ($p < 0.001$) in the total PL fraction (Table 8). Total n-3 increased significantly ($p < 0.0001$) due to the inclusion of hempseed products in both the TAG (Table 7) and total PL (Table 8) fractions of the egg in comparison to those obtained from the control fed hens. Furthermore, the inclusion of hempseed products (HS or HO) in the diets significantly ($p < 0.0001$) decreased the n-6 to n-3 ratio in both the TAG (Table 7) and total PL (Table 8) fractions of the egg in comparison to those obtained from the control fed hens. However, in the latter lipid fraction, no differences in the ratio were observed between the hempseed-containing diets. The highest reduction in the ratio, compared to the control, was achieved in TAG, rather than in the total PL fraction.

Discussion

The present study was conducted to examine the effects of dose and duration of feeding hempseed products on the fatty acid composition of egg yolk lipid and major fractions. In general, increasing levels of hemp products in diets for the laying hen effectively increased the n-3 PUFA profile of the total lipid, TAG and total PL fractions of yolks. As has been well documented, the inclusion of plant-based sources of n-3 PUFA in laying hen diets leads to increases in the n-3 LCPUFA content of egg yolk total lipids [5, 7, 9]. As ALA is the primary plant n-3 fatty acid, it stands to reason that this fatty acid should also reflect the major form of n-3 PUFA in the egg yolk. This has been previously observed for total egg yolk lipids for hens consuming hempseed products [24, 32]. Flaxseed oil contains about 50 % ALA [19]. On average, hens fed flaxseed up to 15 % [19, 41] or up to 20 % [42] in the diet produced

Table 7 Egg yolk triacylglycerol (TAG) fatty acid profiles (% of total fatty acid, by wt) after feeding hens with diets containing hempseed product

Fatty acids	Hempseed (HS)						Hempseed oil (HO)				
(%, by wt)	Control	10 % HS	20 % HS	30 % HS	SE	*p* value	Control	4.5 % HO	9.0 % HO	SE	*p* value
Total SFA[A]	31.6	31.7	32.1	32.5	0.48	0.50	31.6	31.4	33.0	0.52	0.082
Total MUFA[B]	35.2^{a}	31.8^{b}	28.8^{c}	22.5^{d}	0.76	<0.0001	35.2^{a}	31.5^{b}	25.9^{c}	0.57	<0.0001
PUFA											
LA(18:2n-6)	29.2^{c}	30.7^{bc}	31.4^{b}	34.3^{a}	0.67	<0.001	29.2	30.0	31.6	0.74	0.093
GLA(18:3n-6)	0.189^{c}	0.216^{c}	0.261^{b}	0.326^{a}	0.012	<0.0001	0.189^{c}	0.213^{b}	0.289^{a}	0.007	<0.0001
ARA(20:4n-6)	0.478	0.413	0.433	0.390	0.023	0.078	0.478^{a}	0.403^{b}	0.364^{b}	0.021	<0.01
ALA(18:3n-3)	0.445^{d}	2.27^{c}	3.76^{b}	6.69^{a}	0.25	<0.0001	0.445^{c}	2.97^{b}	5.69^{a}	0.18	<0.0001
EPA(20:5n-3)	0.022^{c}	0.055^{b}	0.068^{b}	0.102^{a}	0.008	<0.0001	0.022^{c}	0.053^{b}	0.094^{a}	0.006	<0.0001
DPA(22:5n-3)	0.043^{b}	0.090^{a}	0.099^{a}	0.092^{a}	0.009	<0.001	0.043^{c}	0.079^{b}	0.108^{a}	0.008	<0.0001
DHA(22:6n-3)	0.197	0.322	0.387	0.309	0.045	0.052	0.197	0.385	0.367	0.060	0.087
Total n-6[C]	29.9^{c}	31.3^{bc}	32.0^{b}	35.0^{a}	0.69	<0.001	29.9	30.6	32.3	0.74	0.097
Total n-3[D]	0.71^{d}	2.68^{c}	4.72^{b}	7.15^{a}	0.28	<0.0001	0.71^{c}	3.48^{b}	6.26^{a}	0.181	<0.0001
Ratio n-6/n-3	44.9^{a}	11.7^{b}	7.56^{c}	4.92^{c}	0.81	<0.0001	44.9^{a}	8.84^{b}	5.19^{c}	1.01	<0.0001

Data represents least square means (LSM) ± standard error (SE), $n = 7$ per treatment. Different superscripts within a treatment group within a row are significantly different at $p < 0.05$

[A] *SFA* myristic, (C14:0), palmitic (C16:0) and stearic (C18:0)

[B] *MUFA*: palmitoleic (C16:1) and oleic (C18:1)

[C] Total n-6: *LA* linoleic acid, *GLA* gamma-linolenic acid, *ARA* arachidonic acid

[D] Total n-3: *ALA* alpha-linolenic acid, EPA, *DPA* docosapentaenoic acid, *DHA* docosahexaenoic acid

Table 8 Egg yolk total phospholipid (PL) fatty acid profiles (% of total fatty acid, by wt) after feeding hens with diets containing hempseed product

Fatty acids	Hempseed (HS)						Hempseed oil (HO)				
(%, by wt)	Control	10 % HS	20 % HS	30 % HS	SE	*p* value	Control	4.5 % HO	9.0 % HO	SE	*p* value
Total SFA[A]	48.0	48.2	48.0	48.2	0.38	0.98	48.0	47.3	48.2	0.35	0.27
Total MUFA[B]	18.9^{a}	18.2^{a}	18.4^{a}	17.2^{b}	0.29	<0.01	18.9^{a}	18.9^{a}	17.6^{b}	0.32	<0.05
PUFA											
LA(18:2n-6)	21.7	20.8	20.8	20.9	0.26	0.062	21.7^{a}	20.6^{a}	19.4^{b}	0.38	<0.01
GLA(18:3n-6)	0.153^{d}	0.199^{c}	0.234^{b}	0.349^{a}	0.013	<0.0001	0.153^{c}	0.216^{b}	0.325^{a}	0.012	<0.0001
ARA(20:4n-6)	6.41	5.79	5.77	5.86	0.19	0.0788	6.41^{a}	6.03^{b}	5.49^{b}	0.16	<0.01
ALA(18:3n-3)	0.139^{d}	0.339^{c}	0.494^{b}	0.723^{a}	0.009	<0.0001	0.139^{c}	0.391^{b}	0.618^{a}	0.015	<0.0001
EPA(20:5n-3)	0.000^{d}	0.039^{c}	0.060^{b}	0.088^{a}	0.003	<0.0001	0.000^{c}	0.055^{b}	0.083^{a}	0.003	<0.0001
DPA(22:5n-3)	0.126^{c}	0.268^{ab}	0.248^{b}	0.318^{a}	0.023	<0.001	0.126^{c}	0.230^{b}	0.338^{a}	0.012	<0.0001
DHA(22:6n-3)	1.46^{b}	3.82^{a}	4.04^{a}	3.85^{a}	0.18	<0.0001	1.46^{b}	3.88^{a}	4.02^{a}	0.150	<0.0001
Total n-6[C]	28.3^{a}	26.8^{b}	26.8^{b}	27.1^{b}	0.33	<0.05	28.3^{a}	26.9^{b}	25.2^{c}	0.41	<0.001
Total n-3[D]	1.73^{b}	4.47^{a}	4.84^{a}	4.98^{a}	0.21	<0.0001	1.73^{c}	4.56^{b}	5.06^{a}	0.155	<0.0001
Ratio n-6/n-3	16.7^{a}	6.02^{b}	5.54^{b}	5.52^{b}	0.41	<0.0001	16.7^{a}	5.95^{b}	5.01^{b}	0.41	<0.0001

Data represents least square means (LSM) ± standard error (SE), n = 7 per treatment. Different superscripts within a treatment group within a row are significantly different at $p < 0.05$

[A] SFA: myristic, (C14:0), palmitic (C16:0) and stearic (C18:0)

[B] MUFA: palmitoleic (C16:1) and oleic (C18:1)

[C] Total n-6: *LA* linoleic acid, *GLA* gamma-linolenic acid, *ARA* arachidonic acid

[D] Total n-3: *ALA* alpha-linolenic acid, EPA, *DPA* docosapentaenoic acid, *DHA* docosahexaenoic acid

eggs containing about 322 and 90 mg/egg of total n-3 and DHA, respectively [19, 20]. On the other hand, hempseed oil and hempseed containing 18 and 7 % ALA, respectively [27], incorporated approximately 160–250 mg/egg total n-3 and 41–50 mg/egg DHA, at 20–30 % HS and 9.0–12 % HO inclusions in the current as well as previous studies [24]. This efficiency is related to the fact that DHA reaches a plateau, so further increases in ALA intake does not translate into a subsequent proportional increase in the level of DHA. Although a comparative assessment of the economics between the two sources may also be useful, hempseed products have yet to be approved for utilization in poultry and other livestock diets.

In contrast, limited information exists as to the nature of the distribution of the various n-3 PUFA between the lipid classes in egg yolk as a function of dietary supply [43]. For total, as well as TAG and PL fractions, yolk ALA increased significantly in response to increasing dietary ALA from hemp products. The TAG was more responsive, increasing approximately 13-fold to 15-fold, over the control, at the highest level of HS or HO inclusion (6.69 ± 0.25 and 5.69 ± 0.18 % total fatty acids, respectively), consistent with the observed increases in total ALA content of egg yolk. As TAG represents approximately 65 % of the total lipid in egg yolk [44], close agreement in enrichment patterns is not unexpected. The ALA in the PL fraction, on the other hand, while enriched, was increased by only fourfold over the control. This was in accordance with the findings by [43], where moderate increases in the levels of ALA were reflected in the major PL sub-classes, but were dominantly contained in the TAG fraction. Consistent with our findings at the highest levels of HS or HO inclusion, the latter authors observed similar amounts of ALA and total n-3 PUFA in the egg yolk TAG fractions (6.9 and 7.1 % total fatty acids, respectively). While not reported, the total n-3 fatty acid content of PL (PC and PE) fractions, particularly in the PC, were closely related when hens consumed hempseed products, with PC: 4.08 ± 0.14 and PE: 10.8 ± 0.44 % of total fatty acids (unpublished data), to that of flaxseed, with PC: 6.1 and PE: 16.8 % of total fatty acids [43].

While ALA enrichment of the yolk lipid fractions demonstrated dose-dependent increases, differential responses were observed for the n-3 LCPUFA in the 12-week feeding regime. The levels of EPA and DPA, both precursors for DHA synthesis, in general, increased in response to graded ALA intakes from hemp products, particularly in the PL fraction of egg yolk. The presence of EPA, DPA and DHA in yolk lipids reflects endogenous synthesis from ALA and deposition in the yolk, since the diets were essentially devoid of these fatty acids. Docosahexaenoic acid is derived from ALA through the sequential actions of desaturase and elongase enzymes, as well as beta-oxidation with both EPA and DPA serving as intermediates [45]. The observed pattern of responses in DHA accumulation provides insight into n-3 fatty acid metabolism within the hen. As previously demonstrated in total yolk lipids [24, 26], increasing ALA intake by hens in response to dietary hemp inclusion results in increased DHA levels, but the response is saturable. The current data extends these results by providing evidence that both the TAG and PL fractions respond in a similar fashion. Further mechanistic studies are required to elucidate the biological rationale for this plateau. Previous research examining changes in n-3 LCPUFA in total egg yolk lipids, in response to graded levels of microencapsulated fish oil in a 21-day feeding trial, demonstrated significant capacity for the deposition of preformed DHA into total yolk lipids, with no evidence of a plateau being reached [46]. Furthermore, even though EPA intake exceeded DHA intake by a factor of 1.5 in the latter study, egg yolk deposition of EPA (total lipids) was approximately 25 % of that observed for DHA, suggesting alternative hepatic handling of these preformed fatty acids.

Consumer demand for n-3 enriched eggs has driven interest in the examination of alternative dietary sources of n-3 PUFA for laying hens. As hemp oil, either as a component of hempseed or as the extracted oil, contains approximately 19 % of its fatty acid profile as ALA, it can potentially serve as a source of dietary ALA for laying hens. The total n-3 PUFA in the egg yolk increased by about sevenfold when using 30 % HS (209 ± 4.74 mg/yolk) and 9.0 % HO (207 ± 3.88 mg/yolk) compared to the level observed when hens were fed the control diet (30.7 ± 4.74 mg/yolk) (Fig. 1). The highest enrichment levels of total n-3 PUFA (primarily due to the changes of ALA content) in the eggs was achieved at week 8 for the HO (4.5 and 9.0 % levels) treatment groups and at week 12 for HS (at 30 % inclusion), although no statistical difference in enrichment beyond week 8 was observed at lower levels of HS inclusions. These results are in accordance with previous research findings [47] indicating maximum accumulation levels of n-3 PUFA in egg yolk after 8 weeks of experimental feeding, although stability in egg yolk n-3 PUFA deposition may follow 4 weeks of feeding [48].

With respect to the total n-6 PUFA, with hempseed being a relatively rich source of GLA, the egg content of this fatty acid in both the total lipids and the major lipid classes closely resembled the changes in the laying hen diets. Overall, for the n-6 PUFA in the total lipid of egg yolk, greater accumulations were primarily associated with prolonged feeding of the diets, as evident by significant diet by time interactions. Although dietary LA levels were similar between treatments, LA accumulation was observed to increase in the TAG and decrease in the PL fraction, but did not reflect in similar changes in the levels in ARA, accumulating more in the PL fraction than the TAG of egg yolk.

Given the absence of ARA in the laying hen diets, egg yolk ARA reflects endogenous synthesis in the liver and deposition to eggs. The levels of ARA in the various egg yolk lipid fractions decreased with increasing hemp product inclusion, indicating the two desaturase enzymes (delta-6 and delta-5) and one elongase (elongase 5) [49] also favor n-3 fatty acid metabolism, as found in other animal models [50].

On the other hand, while the PUFA profile of egg yolk was greatly influenced by the levels of the primary fatty acid in the hens' diets (i.e., increasing HS or HO levels of inclusion in the diets reflecting corresponding increases in the amounts of dietary ALA), the total SFA in the egg yolk was not influenced by either the HS or the HO levels of inclusion. These results are in agreement with other studies utilizing flaxseed that provided evidence that the overall SFA levels in the egg yolk are not majorly influenced by dietary manipulations [7, 19]. However, a significant impact on SFA content of egg yolk in other lipid feeding trials has also been reported [5, 13]. Although a previous study [4] in which fish oil and flaxseed was included in hen diets showed greater reductions in the amount of palmitic acid (predominant SFA) during prolonged feeding, in the current study, no diet × week interaction was noted in this fatty acid in the total egg yolk lipid across the dietary treatments. Consistent with the total egg yolk lipid, the overall levels of total SFA in the both the TAG and PL lipid classes were not influenced by the dietary treatments.

The levels of MUFA in both the total lipid and the TAG fractions of egg yolk decreased with increasing levels of inclusion of either hemp product, by reflecting the fatty acids of the diets. However, this was not the case for the PL fraction, where reductions were observed, relative to controls, only for the highest levels of HS and HO inclusion. In general, changes in MUFA occurred primarily via changes in oleic acid, the predominant MUFA in both the diets and the eggs in this study. These data may support previous findings that provided evidence of a depressive effect of PUFA-rich diets on the desaturation of palmitic (C18:0) to oleic (C18:1) acid by Δ9-desaturase [51]. However, given the reduction in the oleic acid levels in the diet with increasing hemp product inclusion, the results may reflect a simple response to dose. As such, the PL fraction appears resistant to manipulation of its MUFA content [43] indicating the differential incorporation of the fatty acid between PL and TAG. Overall, palmitic acid, oleic acid, and LA represent the major fatty acids in yolk total lipid, TAG and PL fractions.

In summary, compared to the control, hempseed products improved the nutritional value of the egg, resulting in decreased n-6 to n-3 PUFA ratio, increased total n-3 PUFA, and selectively incorporated higher levels of DHA in particular into the PL fraction. Although overall, the utilization of hempseed products in laying hen diets results in maximum total n-3 PUFA enrichment achievable by week 8; egg enrichment with n-3 PUFAs is mainly due to the dosage of dietary inclusion rather than time effect (duration of feeding).

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