Effect of feeding hemp seed and hemp seed oil on laying hen performance and egg yolk fatty acid content: Evidence of their safety and efficacy for laying hen diets

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ABSTRACT Forty-eight 19-wk-old Bovan White laying hens were fed 1 of 5 diets containing either hemp seed (HS) or hemp seed oil (HO). The level of HO was 4, 8, or 12%, whereas the level was 10 or 20% for the HS. A set of 8 birds fed wheat-, barley-, and corn oil-based diets served as the control. Performance was monitored over 12 wk. Average hen-day egg production was not affected upon feeding of either HS or HO diets. Egg weight was higher than that of the controls for hens consuming the 20% HS diet ($P < 0.05$). Feed intake was lower than that of the controls for birds consuming the 4% HO diet but similar across other treatments. Final BW were not affected by diet, with the exception of being lower than that of the controls ($P < 0.05$) in hens consuming the 12% HO diet. The total egg yolk n-3 fatty acid content increased linearly ($P < 0.05$) with increasing dietary α-linolenic acid provision with the HS- or HO-based diets. A quadratic response ($P < 0.05$) was observed for docosahexaenoic acid levels in egg yolk in response to increasing dietary α-linolenic acid supply. The expression of hepatic fatty acid desaturase 1 and 2, key genes for the desaturation of long-chain polyunsaturated fatty acids, was significantly decreased (50–60% of controls; $P < 0.05$) as a result of feeding HS or HO diets. Based on the results from the current study, the inclusion of the hemp products HS or HO in the diets of laying hens up to a maximum level of 20 and 12%, respectively, does not adversely effect the performance of laying hens and leads to the enrichment of the n-3 fatty acid content of eggs.

Key words: egg quality, fatty acid, hemp product, laying hen, production

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INTRODUCTION

Hemp (*Cannabis sativa* L.) is an annual herbaceous plant belonging to the family Cannabinaceae (Turner et al., 1979), traditionally grown for fiber and seed production. In the past, the cultivation of hemp was prohibited due to the high content of Δ-9 tetrahydrocannabinol, a psychoactive substance present in the hemp plant. In 1998, regulatory changes undertaken by the Canadian government allowed for the legal cultivation of industrial hemp under a license (Health Canada, 2010). As per Health Canada guidelines, industrial hemp means the plants and plant parts of the genera *Cannabis*, the leaves and flowering heads of which do not contain more than 0.3% Δ-9 tetrahydrocannabinol (wt/wt), and includes the derivatives of such plants and plant parts. The increasing production of hemp and the availability of hemp seed (**HS**) and HS products create opportunities to use them in livestock rations.

Whole hemp seeds contain approximately 25% CP, 33 to 35% oil, and 34% carbohydrate, in addition to a broad range of vitamins and minerals (Darshan and Rudolph, 2000; Callaway, 2004; House et al., 2010). Hemp seed oil (**HO**) contains 75 to 80% polyunsaturated fatty acids (**PUFA**), including 60% linoleic acid and 17 to 19% α-linolenic acid (**ALA**) (Parker et al., 2003). The nutrient composition of hemp products provides evidence that these products may serve as potentially valuable livestock feed ingredients. However, to date, the use of hemp has not been approved for use in diets for any class of livestock in Canada due to a lack of data in support of safety and efficacy claims for these products.

Despite the potential of HS and HO to serve as feed ingredients in livestock production, there exists a single study in poultry (Silversides and Lefrançois, 2005) and a few studies in other species (Mustafa et al., 1999; Gibb et al., 2005; Hessle et al., 2008; Turner et al., 2008) that

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have used hemp as feed ingredients. One potential opportunity for hemp relates to the relatively high level of ALA (17–19%; Parker et al., 2003), as compared with other vegetable oils (<9%), with the notable exceptions being flax and chia. Dietary ALA undergoes a series of elongation and desaturation reactions, leading to the formation of the longer chain n-3 PUFA eicosapentaenoic acid (**EPA**), docosapentaenoic acid (**DPA**), and docosahexaenoic acid (**DHA**) that have purported beneficial effects for human health (Lewis et al., 2000; Belluzzi, 2002). As flaxseed has successfully been used in the diets of laying hens to produce n-3 enriched eggs, the level of ALA present in HO may provide additional options for the production of n-3 eggs.

The current study was designed to assess the effect of including HS and HO in diets for laying hens on measures of production, egg quality, and the corresponding fatty acid profile of egg yolks. A secondary objective of the study was to examine changes in the gene expression of fatty acid desaturase (**FADS**) 1 and 2 and elongation of very long-chain fatty acids protein 5 (**ELOVL5**), encoding enzymes critical for the desaturation and elongation of the longer chain PUFA.

MATERIALS AND METHODS

Birds and Housing

In total, forty-eight 19-wk-old Bovan White laying hens procured from a commercial supplier (ISA, Hendrix Genetics, Lockport, MB, Canada) were used in the study. Hens were allowed an adaptation period of 10 d before establishing the potential effect of feeding the HS- or HO-based diets on attainment of peak production. All of the birds were housed individually; the cage dimensions were 25.4 × 40.6 cm, providing 1,032 cm^2 of space per bird. The birds were kept in confinement housing under semicontrolled environmental conditions and were exposed to a 16-h photoperiod. Feed and water were available for ad libitum consumption. The birds were managed in accordance with recommendations established by the Canadian Council on Animal Care (CCAC, 1993) following an animal care protocol approved from the University of Manitoba's Animal Care Protocol Management and Review Committee.

Diets

Diets, based on wheat and barley, were formulated to meet the recommendations for Bovan White hens (NRC, 1994; Table 1) consuming 100 g of feed/d. The composition of the HS and HO used for the formulation of diets was based on certificate of analysis received from a commercial laboratory (Norwest Labs, Lethbridge, AB, Canada) sourced through Hemp Oil Canada, St. Agathe, MB, Canada (Table 2). The HS was also analyzed for CP (24.1%), crude fat content (30.4%, as-is basis), and DM (92.9%) in the laboratory. All treatment diets were designed to be isonitrogenous and isoenergtic, providing 13% crude fat to match the crude fat content associated with the highest inclusion rate of HS tested. The diets were analyzed in duplicate to determine the CP, crude fat, and the gross energy content. Nitrogen for CP analysis was measured using a nitrogen analyzer (NS-2000, Leco Corp., St. Joseph, MI). Dry matter was determined according to the method of AOAC (1990; method 925.09), and gross energy was determined using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). The diet samples were subjected to analyses of fat (method 920.39, AOAC, 1990). The diets were offered daily and were stored at 4°C during the course of the trial.

Experimental Approach

The hens were placed individually into metabolic cages and were randomly assigned to 1 of 6 dietary treatments: 1) control diet with no supplemented HO or HS (n = 8); 2) control diet + 4% HO (n = 8); 3) control diet + 8% HO (n = 8); 4) control diet + 12% HO (n = 8); 5) control diet + 10% HS (n = 8); and 6) control diet + 20% HS (n = 8). The HO and HS were provided as an in-kind contribution by Hemp Oil Canada. After the adaptation period, the diets were fed over a 12-wk period. All of the birds were weighed individually at the end of wk 4, 8, and 12, and feed consumption for each bird was measured for ADFI and feed conversion efficiency calculations. The feed conversion efficiency was calculated as grams of feed consumed per gram of egg produced. Daily egg production was recorded and average hen-day egg production calculated for wk 4, 8, and 12 of the study. Daily egg weights were also recorded for wk 4, 8, and 12 of the study. At the end of the 12-wk period, all of the birds were killed by cervical dislocation and liver samples collected for gene expression analysis.

Extraction and Analysis of Yolk and Diet Fatty Acids

Eggs were collected for a period of 3 consecutive days from all of the birds under each of the 6 treatments during wk 4 and 12 for egg yolk fatty acid analysis. The egg yolks were separated using an egg yolk separator and stored at −20°C until analyzed. The fatty acid composition of the test diets (Table 3) and the egg yolks were determined using standard gas chromatographic (Agilent Technologies, Santa Clara, CA) techniques of the fatty acid methyl esters (AOAC, 1990), using C17:1 fatty acid (Nu-Chek Prep Inc., Elysian, MN) as an internal standard. Fatty acids were extracted from the egg yolk according to the methods of Folch et al. (1957). The diet subsamples were also stored at −20°C until analyzed for fatty acids.

Table 1. The composition of the diets used to test the effect of including hemp seed (HS) or hemp seed oil (HO) on laying hen performance

	Diet					
Item	Control	4% HO	8% HO	12% HO	10% HS	20% HS
Ingredient (%)						
Soybean meal (CP 44%)	29.6	29.6	29.6	29.6	24.6	19.6
Wheat (CP 11%)	28.8	28.8	28.8	28.8	27.2	25.0
Barley (CP 14.2%)	15.0	15.0	15.0	15.0	15.0	15.0
Corn oil	12.0	8.0	4.0	0.0	8.8	5.9
Hemp seed	0.0	0.0	0.0	0.0	10.0	20.0
Hemp oil	0.0	4.0	8.0	12.0	0.0	0.0
Limestone	10.1	10.1	10.1	10.1	10.0	10.1
Vitamin mineral premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
Dicalcium phosphate	1.6	1.6	1.6	1.6	1.5	1.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine	0.014	0.014	0.014	0.014	0.000	0.000
L-Lysine-HCl	0.000	0.000	0.000	0.000	0.043	0.098
Composition (calculated unless noted)						
AME_n (poultry; kcal/kg)	2,998	2,998	2,998	2,998	2,988	2,988
Crude fat (%)	13.22	13.22	13.22	13.22	13.00	13.00
Crude fat (analyzed, %)	13.05	13.72	12.31	12.16	13.64	15.41
Linoleic acid (%)	6.96	7.06	7.16	7.26	6.91	6.99
α-Linolenic acid (%)	0.17	0.80	1.43	2.07	0.65	1.13
Ratio LA:ALA[2]	40.94	8.82	5.00	3.50	10.63	6.18
Ratio LA:ALA (analyzed)	37.89	8.45	4.66	3.23	11.13	6.47
CP (%)	19.00	19.00	19.00	19.00	19.00	19.00
CP (analyzed, %)	19.98	19.20	20.23	20.67	19.08	19.45
Calcium (%)	4.10	4.10	4.10	4.10	4.05	4.05
Total phosphorus (%)	0.66	0.66	0.66	0.66	0.73	0.80
Available phosphorus (%)	0.45	0.45	0.45	0.45	0.45	0.45
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15
Methionine (%)	0.30	0.30	0.30	0.30	0.30	0.32
Total lysine (%)	0.96	0.96	0.96	0.96	0.95	0.95
Threonine (%)	0.72	0.72	0.72	0.72	0.72	0.72
TSAA (%)	0.30	0.30	0.30	0.30	0.30	0.32

[1]Provided per kilogram of diet: 11,000 IU of vitamin A; 3,000 IU of vitamin D_3; 150 IU of vitamin E; 3 mg of vitamin K (as menadione); 0.02 mg of cyanocobalamin; 6.5 mg of riboflavin; 4 mg of folic acid; 10 mg of calcium pantothenate; 40.1 mg of niacin; 0.2 mg of biotin; 2.2 mg of thiamine; 4.5 mg of pyridoxine; 1,000 mg of choline; 125 mg of ethoxyquin (antioxidant); 66 mg of Mn (as manganese dioxide); 70 mg of Zn (as zinc oxide); 80 mg of Fe (ferrous sulfate); 10 mg of Cu (as copper sulfate); 0.3 mg of Se (as sodium selenite); 0.4 mg of I (as calcium iodate); and 0.67 mg of iodized salt. Ground wheat was used as a carrier for the vitamin mineral premix.

[2]LA = linoleic acid; ALA = α-linolenic acid.

Egg Quality Assessment

Eggs collected on 3 consecutive days of wk 4, 8, and 12 of the study were stored at 4°C for measuring albumen height on the following day, according to the procedure described by Silversides and Lefrançois (2005). Shell thickness was measured with membranes intact using a thickness gauge micrometer (B. C. AMES Co., Waltham, MA) in which the eggshell thickness of a chip was taken from the equator region of the egg. Ten measurements were taken from the 3 eggs collected per hen, and these observations were averaged to determine the eggshell thickness for each hen. The obtained thickness values (in thousandths of an inch) were converted into micrometers by multiplying by 25.4. Specific gravity of the eggs collected from 3 consecutive days during wk 4, 8, and 12 was measured. Specific gravity measurements were conducted on eggs using the flotation measurement method as indicated by Holder and Bradford (1979).

Table 2. Nutrient composition of the hemp seed oil and hemp seed used for the formulation of the diets

Composition	Hemp seed oil	Hemp seed
AME_n[1] (poultry; kcal/kg)	8,812.0	4,300.0
Crude fat[2] (%)	100.0	30.4
CP[2] (%)	0.0	24.2
Total lysine[3] (%)	0.0	0.9
Calcium[3] (%)	0.0	0.2
Total phosphorus[3] (%)	0.0	1.2
Available phosphorus[3] (%)	0.0	0.2
Sodium[3] (%)	0.0	0.0
Chloride[3] (%)	0.0	0.0
Methionine[3] (%)	0.0	0.6
Threonine[3] (%)	0.0	1.0
Linoleic acid[3] (%)	56.0	17.0
Linolenic acid[3] (%)	17.0	5.2

[1]Values estimated based on proximate composition and comparisons to values for similar feed ingredients (NRC, 1994).

[2]Analyzed as per Materials and Methods section.

[3]As provided in the Certificate of Analysis from supplier.

Tissue Collection and RNA Isolation for mRNA Expression Analysis

Liver tissues were freshly harvested and stored in RNA stabilization solution (Applied Biosystems Inc., Foster City, CA). Four birds from each dietary treat-

Table 3. Fatty acid profile of the diets used in the study[1]

Fatty acid (mg/g of diet)	Control	4% HO	8% HO	12% HO	10% HS	20% HS
Palmitic ($C_{16:0}$)	9.57	8.25	6.90	5.56	9.18	8.68
Palmitoleic ($C_{16:1}$)	0.09	0.09	0.08	0.09	0.09	0.10
Stearic ($C_{18:0}$)	1.44	1.60	1.71	1.84	1.73	2.03
Oleic ($C_{18:1}$)	20.62	16.72	12.01	7.61	19.21	16.57
Linoleic ($C_{18:2}$)	45.43	45.66	43.86	41.43	48.52	50.59
γ-Linolenic ($C_{18:3, n-6}$)	0.01	1.06	2.06	2.91	0.88	1.82
α-Linolenic ($C_{18:3, n-3}$)	1.20	5.40	9.40	12.82	4.36	7.82
Arachidonic ($C_{20:4, n-6}$)	0.01	0.01	0.02	0.01	0.00	0.06
Eicosapentaenoic ($C_{20:5, n-3}$)	0.03	0.03	0.02	0.03	0.02	0.02
Docosapentaenoic ($C_{22:5, n-3}$)	0.00	0.00	0.00	0.00	0.00	0.00
Docosahexaenoic ($C_{22:6, n-3}$)	0.01	0.01	0.00	0.01	0.01	0.01

[1]HO = hemp seed oil; HS = hemp seed.

ment were chosen at random for individual RNA extraction that was performed according to the manual of RNeasy Mini Kit (Qiagen Canada Inc., Mississauga, ON, Canada). Total concentration of RNA samples was measured at 260 nm on a DU800 spectrophotometer (Beckman Coulter Canada Inc., Mississauga, ON, Canada). The absorbance ratio at wavelength 260:280 nm was within 1.8 to 2.1. The prepared RNA samples were then treated with TURBO DNA-free Kit (Applied Biosystems Inc.), to eliminate the possibility of genomic DNA contamination.

Reverse Transcription and Real-Time PCR Analysis

Reverse transcription and real-time PCR were conducted in a single tube using QuantiFast SYBR Green RT-PCR kit (Qiagen Canada Inc.). Briefly, 1 ng of total RNA was used as the template in 25-μL reactions. The contents of the 25-μL reactions included 12.5 μL of 2× QuantiFast SYBR Green RT-PCR master mix, 2.5 μL of primers, 0.25 μL of QuantiFast RT mix, 1 ng of total RNA, and variable volumes of RNase-free water that depended on the volume of total RNA added into the 25-μL reactions. The thermal cycling conditions were 10 min at 50°C for reverse transcription, 5 min at 95°C for PCR initial activation, 40 cycles of denaturation at 95°C for 10 s, and combined annealing/extension at 61°C for 30 s, and then followed by one 3-segment cycle of product melting (95°C/15 s, 60°C/1 min, and 95°C/15 s). Melting curves confirmed the specific amplification of *FADS1*, *FADS2*, *ELOVL5*, and β-actin. All samples were amplified in triplicate, and the mean was used for further analysis. The primers used in the real-time PCR were designed using Primer Premier 5 software (Premier Biosoft, Palo Alto, CA), and sequences for *FADS1*, *FADS2*, *ELOVL5*, and β-actin (internal control) are presented in Table 4.

The standard curves for the 4 genes were set up using serial dilutions of sample RNA. The mRNA expression of *FADS1*, *FADS2*, and *ELOVL5* was calculated with the relative standard curve method, which was based on the calculation of the starting copy amount of 3 target genes and β-actin using their respectively constructed standard curve. The results were expressed as a ratio with β-actin that was amplified in a separate PCR.

Statistical Analysis

Data collected from wk 4, 8, and 12 of the study were analyzed using the Proc mixed procedure of SAS (SAS Institute, 2008). Data for the HS and HO were analyzed as 2 separate experiments, with both experiments employing the same data for the control diet in the statistical model. The ANOVA for the hen-day egg production, egg weight, yolk fatty acid, and BW included the main effect of the treatment, week, and treatment × week interaction. Contrasts of treatment effects were made between each dietary treatment. The yolk fatty

Table 4. Primers of *FADS1*, *FADS2*, *ELOVL5*, and β-actin for real-time PCR[1]

Gene	Primer sequence[2] (5′ to 3′)	Amplicon length (bp)	Reference
FADS1	F: CTTGGCGAACAAAAGAAGAAAT R: CCCAGTAAGGGCAGGTAGGT	200	XM_421052
FADS2	F: AACCATCGTCACTTCCAACATC R: CTTCAGCTTCTTCTTGCCGTAC	126	XM_421053
ELOVL5	F: ATTGGGTGCCTTGTGGTCA R: AGCTGGTCTGGAAGATTGTCA	180	XM_426204
β-actin	F: CAACACAGTGCTGTCTGGTGGTA R: ATCGTACTCCTGCTTGCTGATCC	205	X00182

[1]*FADS1*: fatty acid desaturase 1 encoding Δ-5 desaturase; *FADS2*: fatty acid desaturase 2 encoding Δ-6 desaturase; *ELOVL5*: a gene encoding elongation of very long-chain fatty acids protein 5; β-actin: internal control.

[2]F: forward; R: reverse.

Table 5. Egg weights, feed intake, final BW, feed conversion efficiency, and average hen-day egg production of hens consuming diets containing hemp seed (HS)[1]

						P-value		
Item	Week	Control	10% HS	20% HS	SEM	Trt	Week	Trt × week
Egg weight (g)	4	54.6	53.7	58.1				
	8	56.4	57.0	60.8				
	12	57.7	59.4	62.6				
	Overall	56.2[b]	56.7[b]	60.5[a]	1.14	<0.01	<0.01	0.86
Feed intake (g/d)	4	93.7	93.9	98.7				
	8	95.0	98.5	96.2				
	12	101.9	95.7	100.2				
	Overall	96.9	96.0	98.3	2.84	0.78	0.48	0.70
BW (kg)	4	1.49	1.50	1.54				
	8	1.52	1.53	1.57				
	12	1.54	1.53	1.58				
	Overall	1.52	1.52	1.56	0.05	0.57	0.66	0.99
Feed conversion efficiency (g of feed/g of egg)	4	1.77	1.69	1.71				
	8	1.68	1.79	1.59				
	12	1.76	1.71	1.61				
	Overall	1.74	1.73	1.63	0.04	0.22	0.85	0.60
Hen-day egg production (%)	4	97.8	96.4	97.7				
	8	99.9	95.8	100.0				
	12	99.9	97.8	100.0				
	Overall	99.2	96.7	99.3	1.06	0.09	0.34	0.89
Egg mass (g/hen per day)	4	53.4	51.9	56.7				
	8	56.4	55.3	60.7				
	12	57.7	58.7	62.6				
	Overall	55.9	55.3	60.0	1.33	0.01	0.01	0.94

[a,b]Values with different superscripts within a row indicate significant differences ($P < 0.05$).

[1]Values are means ± SEM for each treatment (Trt) group (24 observations for 10% HS, 23 observations for 20% HS, and 21 observations for the control). There was one mortality each under the control and the 20% HS group during the course of the trial.

acid data from wk 4 and 12 were also subjected to the Proc Reg procedure of SAS, and regression equations were obtained to predict the output of DHA as a function of the percentage of inclusion of either HO (4, 8, or 12%) or HS (10 or 20%) in the diets. Comparisons between wk 4 and 12 within each treatment group were performed if the treatment × week interaction was significant. At wk 12, data for ALA intake and total n-3 fatty acid or DHA output in eggs were subjected to regression analysis using SigmaPlot v. 11 (Systat Software Inc., San Jose, CA). Data for mRNA expression were subjected to ANOVA using PROC GLM of SAS (SAS Institute, 2008). Differences between treatments were assessed using the protected least squares difference method, and all statements of significance are based on $P < 0.05$.

RESULTS

For hens consuming diets containing graded levels of HS, egg weights were significantly affected by HS inclusion in the diet, with weights from the 20% HS diet being greater ($P < 0.05$) than those observed with either the control or the 10% HS diets (Table 5). With respect to other measures of production performance, the inclusion of HS in laying hen diets did not significantly affect feed intake, BW, feed conversion efficiency, hen-day egg production, or egg mass (Table 5). The inclusion of HO in laying hen diets did not affect egg weight (Table 6); however, feed intake was significantly lower ($P < 0.05$) relative to that of the controls for hens consuming the 4% HO diet (Table 6). Similar to the situation with the HS diets, BW, feed conversion efficiency, hen-day egg production, or egg mass were similar across the levels of HO inclusion (Table 6).

With respect to measures of egg quality, the addition of either HS (Table 7) or HO (Table 8) to the diet of the laying hens had no effect on eggshell thickness, albumen height, or specific gravity. Due to complications with the micrometer, values for albumen height are only available for wk 12. A significant effect of period was noted for both eggshell thickness and specific gravity, providing evidence of changes in these parameters as the hens age; however, the interaction with treatment was not significant.

The fatty acid composition of the egg yolks are presented in Table 9 (HS) and Table 10 (HO). In general, as the level of HS or HO inclusion in the diet increased, the concentrations of stearic acid, γ-linolenic acid, and the n-3 fatty acids (ALA, EPA, DPA, and DHA) increased. The overall increase in total n-3 content of the egg yolks appeared to be at the expense of oleic acid, which decreased at higher rates of inclusion of HS or HO. With respect to the n-3 fatty acids, ALA, EPA, and DPA increased significantly ($P < 0.05$) with each additional increment of either HO or HS. However, DHA levels increased and appeared to reach a plateau between 45 and 50 mg/yolk. This quadratic response observed for the DHA levels in the egg yolks in the HS

Table 6. Egg weights, feed intake, final BW, feed conversion efficiency, and average hen-day egg production of hens consuming diets containing hemp seed oil (HO)[1]

							P-value		
Item	Week	Control	4% HO	8% HO	12% HO	SEM	Trt	Week	Trt × week
Egg weight (g)	4	54.6	54.2	53.3	54.0				
	8	56.4	56.6	55.1	56.0				
	12	57.7	57.0	55.5	57.4				
	Overall	56.2	55.9	54.6	55.8	1.16	0.57	0.03	0.99
Feed intake (g)	4	92.8	79.0	96.6	90.7				
	8	94.1	86.2	80.4	90.2				
	12	101.0	78.6	91.9	85.3				
	Overall	96.0[a]	81.2[b]	89.6[a]	88.7[a]	4.60	0.03	0.88	0.37
BW (kg)	4	1.48	1.51	1.59	1.45				
	8	1.52	1.50	1.55	1.47				
	12	1.54	1.46	1.59	1.48				
	Overall	1.52	1.49	1.58	1.47	0.04	0.11	0.98	0.96
Feed conversion efficiency (g of feed/g of egg)	4	1.77	1.62	1.95	1.74				
	8	1.68	1.88	1.63	1.71				
	12	1.76	1.61	1.91	1.53				
	Overall	1.74	1.70	1.83	1.66	0.06	0.31	0.73	0.13
Hen-day egg production (%)	4	97.9	100.0	97.9	97.9				
	8	100.0	97.1	97.9	94.2				
	12	100.0	92.8	97.9	100.0				
	Overall	99.3	96.6	97.9	97.4	1.14	0.42	0.72	0.14
Egg mass (g/hen per day)	4	53.4	57.6	52.6	53.0				
	8	56.4	58.3	54.3	54.4				
	12	57.7	55.0	54.9	57.8				
	Overall	55.9	57.0	53.9	55.1	1.07	0.25	0.21	0.50

[a,b]Values with different superscripts within a row indicate significant differences ($P < 0.05$).

[1]Values are means ± SEM for each treatment (Trt) group (24 observations per treatment for 4% and 8% HO, 21 observations per treatment for control and 12% HO group). There was one mortality each under the control and the 12% HO group during the course of the trial.

and the HO diets was best explained by the equations $DHA = 17.1307 + 2.9179X - 0.0711X^2$ ($R^2 = 0.71$; $P < 0.01$) and $DHA = 18.0592 + 6.4593X - 0.3394X^2$ ($R^2 = 0.64$; $P < 0.01$), respectively, where X is equal to the percentage of hemp product inclusion in the diet. Irrespective of the treatment modality, increasing the content of ALA in the laying hen diet led to increases in both the individual n-3 fatty acids and the total n-3 fatty acid content. A linear increase in the total n-3 fatty acid content output in eggs (Figure 1) was observed with increasing ALA intake. The relative efficiency of output (i.e., conversion of dietary n-3 to egg n-3) was calculated to be 19.7%. For DHA, however, the data was curvilinear, with evidence of a plateau in DHA output in eggs (approximately 50 mg/d) at the highest ALA intakes.

The relative mRNA expression of *FADS1*, *FADS2*, and *ELOVL5* is presented in Table 11. Results revealed that the mRNA levels of *FADS1* were significantly reduced by 39% ($P < 0.05$) in hens fed the diet containing 12% HO, and *FADS2* mRNA decreased by 45 and 51% ($P < 0.05$) in hens fed the diet containing 8 and 12% of HO in comparison with the control diet, respectively. However, the expression of *ELOVL5* was unaffected ($P > 0.05$) by the treatment of HO. Compared with the control diet, *FADS2* mRNA expression was

Table 7. Shell thickness, absolute specific gravity of eggs, and albumen height from hens consuming diets containing hemp seed (HS)

						P-value		
Item	Week	Control	10% HS	20% HS	SEM	Trt	Week	Trt × week
Eggshell thickness[1] (μm)	4	382	369	387				
	8	348	309	306				
	12	301	349	340				
	Overall	344	343	344	20.0	0.99	0.07	0.57
Absolute specific gravity of eggs[1]	4	1.083	1.084	1.084				
	8	1.084	1.083	1.083				
	12	1.084	1.087	1.087				
	Overall	1.085	1.085	1.085	0.0007	0.99	<0.01	0.89
Albumen height[2] (mm)	12	7.7	8.1	7.9	0.32	0.73		

[1]Values are means ± SEM for each treatment (Trt) group (24 observations for 10% HS, 23 observations for 20% HS, and 21 observations for the control).

[2]Values are means ± SEM for each treatment group (8 observations for 10% HS, 7 observations for 20% HS, and 7 observations for the control). There was one mortality each under the control and the 20% HS group during the course of the trial.

Table 8. Shell thickness, albumen height, and specific gravity of eggs from hens consuming diets containing hemp seed oil (HO)

							P-value		
Item	Week	Control	4% HO	8% HO	12% HO	SEM	Trt	Week	Trt × week
Egg shell thickness[1] (μm)	4	382	324	400	392				
	8	348	312	310	225				
	12	301	312	335	259				
	Overall	344	316	349	292	22.5	0.34	<0.01	0.24
Absolute specific gravity of eggs[1]	4	1.083	1.084	1.087	1.084				
	8	1.084	1.085	1.084	1.085				
	12	1.088	1.088	1.088	1.088				
	Overall	1.085	1.084	1.084	1.088	0.0006	0.41	<0.01	0.43
Albumen height[2] (mm)	12	7.7	7.9	7.0	7.43	0.36	0.31		

[1]Values are means ± SEM for each treatment (Trt) group (24 observations per treatment for 4% and 8% HO, 21 observations per treatment for control and 12% HO group).

[2]Values are means ± SEM for each treatment group (8 observations for 4% and 12% HO, 7 observations for the control and 12% HO group). There was one mortality each under the control and the 12% HO groups during the course of the trial.

significantly ($P < 0.05$) declined in hens fed the diet containing 10% HS, but no remarkable changes ($P > 0.05$) were observed in hens fed the diet containing 20% HS, and both *FADS1* and *ELOVL5* were unaffected ($P > 0.05$) by the treatment of HS.

DISCUSSION

In certain jurisdictions, including Canada, the availability of hemp and hemp-seed products complete with a well-characterized nutrient profile provides a poten-

Table 9. Fatty acid composition of eggs produced by hens consuming diets containing hemp seed (HS)[1]

						P-value		
Fatty acid (mg/yolk)	Week	Control	10% HS	20% HS	SEM	Trt	Week	Trt × week
Palmitic ($C_{16:0}$)	4	648	646	656				
	12	809	868	817				
	Overall	728	757	737	38.7	0.87	<0.01	0.76
Palmitoleic ($C_{16:1}$)	4	29*	31*	29				
	12	47¥	44¥	34				
	Overall	38	37	31	3.1	0.19	<0.01	0.04
Stearic ($C_{18:0}$)	4	234	251	280				
	12	295	347	358				
	Overall	264[b]	299[ab]	319[a]	14.9	0.03	<0.01	0.63
Oleic ($C_{18:1}$)	4	857	847	823				
	12	1,069	1,134	1,020				
	Overall	963	990	921	45.6	0.60	<0.01	0.78
Linoleic ($C_{18:2}$)	4	829	855	939				
	12	1,097	1,204	1,220				
	Overall	963	1,029	1,080	51.3	0.27	<0.01	0.78
γ-Linolenic ($C_{18:3, n-6}$)	4	5.5	6.4	8.4				
	12	7.9	9.0	10.9				
	Overall	6.7[b]	7.7[b]	9.6[a]	0.56	0.01	<0.01	0.99
Arachidonic ($C_{20:4, n-6}$)	4	65.7	64.1	62.7				
	12	80.7	82.0	73.2				
	Overall	73.2	73.1	68.0	3.49	0.51	<0.01	0.65
α-Linolenic ($C_{18:3, n-3}$)	4	14.1	44.4	81.7				
	12	17.5	59.0	100.9				
	Overall	15.8[c]	51.7[b]	91.3[a]	3.23	<0.01	0.01	0.08
Eicosapentaenoic ($C_{20:5, n-3}$)	4	0.1	0.9	1.1				
	12	0.3	0.9	1.2				
	Overall	0.2[c]	0.9[b]	1.2[a]	0.08	<0.01	0.17	0.40
Docosapentaenoic ($C_{22:5, n-3}$)	4	1.6	3.1	4.2				
	12	1.9	4.2	5.4				
	Overall	1.8[c]	3.7[b]	4.8[a]	0.21	<0.01	0.01	0.16
Docosahexaenoic ($C_{22:6, n-3}$)	4	17.5	34.5*	42.1*				
	12	16.7	43.9¥	52.8¥				
	Overall	17.1[c]	39.2[b]	47.4[a]	1.64	<0.01	0.01	0.01
Total n-3	4	33.4	82.9*	128.9				
	12	36.4	108.0¥	160.2				
	Overall	34.9[c]	95.5[b]	144.6[a]	4.14	<0.01	<0.01	0.03

[a,b]Values with different superscripts within a row indicate significant differences ($P < 0.05$).

[1]Values are means ± SEM for each treatment (Trt) group (15 observations per treatment for control group and 20% HS group, 16 observations per treatment for 10% HS group).

*, ¥Denote the comparison of wk 4 and 12, respectively, in each treatment group if the $P_{\text{treatment} \times \text{week}}$ is significant.

Table 10. Fatty acid composition of eggs produced by hens consuming diets containing hemp seed oil (HO)[1]

							P-value		
Fatty acid (mg/yolk)	Week	Control	4% HO	8% HO	12% HO	SEM	Trt	Week	Trt × week
Palmitic ($C_{16:0}$)	4	648	637	649	626				
	12	803	709	746	806				
	Overall	725	673	697	716	26.2	0.50	<0.01	0.28
Palmitoleic ($C_{16:1}$)	4	29*	34	36*	32*				
	12	47¥	34	48¥	44¥				
	Overall	38^{ab}	34^{a}	42^{b}	38^{ab}	2.3	0.10	<0.01	<0.01
Stearic ($C_{18:0}$)	4	234	257	280	304				
	12	295	289	348	403				
	Overall	264^{c}	273^{c}	314^{b}	353^{a}	11.9	<0.01	<0.01	0.12
Oleic ($C_{18:1}$)	4	857	831	781	674				
	12	1,066	895	914	842				
	Overall	961^{a}	863^{b}	847^{b}	758^{d}	27.9	<0.01	<0.01	0.2
Linoleic ($C_{18:2}$)	4	829	721	849	903				
	12	1,090	963	1,063	1,278				
	Overall	960^{b}	842^{b}	956^{b}	1,090^{a}	43.2	0.01	<0.01	0.43
γ-Linolenic ($C_{18:3, n-6}$)	4	5.5	6.9	8.7	11.1				
	12	7.8	8.3	11.0	15.9				
	Overall	6.7^{d}	7.6^{cd}	9.8^{b}	13.5^{a}	0.50	<0.01	<0.01	0.13
Arachidonic ($C_{20:4, n-6}$)	4	65.7	66.0	60.6	63.7				
	12	79.7	74.1	69.8	77.5				
	Overall	72.7^{a}	70.0^{a}	65.2^{b}	70.6^{ab}	2.92	0.10	<0.01	0.61
α-Linolenic ($C_{18:3, n-3}$)	4	14.1*	53.6	98.9	158.2*				
	12	17.5¥	63.7	118.9	226.4¥				
	Overall	15.8^{d}	58.7^{c}	108.9^{b}	192.3^{a}	4.40	<0.01	<0.01	<0.01
Eicosapentaenoic ($C_{20:5, n-3}$)	4	0.1	1.0	1.8	2.5				
	12	0.3	1.2	2.0	3.2				
	Overall	0.2^{d}	1.1^{c}	1.9^{b}	2.8^{a}	0.13	<0.01	0.01	0.53
Docosapentaenoic ($C_{22:5, n-3}$)	4	1.6	5.2	4.7	7.1				
	12	1.9	5.5	5.9	7.6				
	Overall	1.8^{d}	5.4^{bc}	5.3^{b}	7.3^{a}	0.35	<0.01	0.15	0.59
Docosahexaenoic ($C_{22:6, n-3}$)	4	17.5	39.7	41.6	44.6				
	12	16.7	42.1	49.6	51.6				
	Overall	17.1^{c}	40.9^{b}	45.6^{a}	48.1^{a}	1.40	<0.01	<0.01	0.1
Total n-3	4	33.4	99.5	147.0*	212.4*				
	12	36.4	112.5	176.4¥	288.8¥				
	Overall	34.9^{d}	106.0^{c}	161.7^{b}	250.6^{a}	5.25	<0.0001	<0.0001	<0.01

$^{a–d}$Values with different superscripts within a row indicate significant differences ($P < 0.05$).

[1]Values are means ± SEM for each treatment (Trt) group (16 observations per treatment for 4% HO, 8% HO, 14 observations per treatment for 12% HO, and 15 observations for control group).

*, ¥Denote the comparison of wk 4 and 12, respectively, in each treatment group if the $P_{treatment \times week}$ is significant.

tial opportunity to use these products in livestock rations. Furthermore, the high ALA (17–19%) content of HO can position these potential ingredients as a means to enhance the fatty acid profile of table eggs, particularly in n-3 fatty acids. Despite this potential, the use of hemp has not been approved for poultry rations in Canada due to a lack of scientific evidence to support the safety and efficacy claims. Previous work by Silversides and Lefrançois (2005) reported the effect of feeding diets containing graded levels of hemp seed meal-based diets for 4 wk on production parameters and egg fatty acid composition of 42-wk-old DeKalb laying hens. The authors reported that the inclusion of hemp seed meal at 0, 50, 100, or 200 g/kg of diet did not significantly affect hen-day egg production or feed consumption, despite reductions in BW over the 4-wk duration of the study. The current study adds to this limited database by providing new information on the responses of laying hens when consuming diets containing either HS or HO.

Data from the current study provides evidence that the inclusion of either HO (up to 12% in the diet) or HS (up to 20% in the diet) did not lead to significant differences in feed intake, final bird weights, and average hen-day egg production when compared with birds consuming a control diet based on wheat and barley. Hens consuming the 4% HO diets did exhibit a significant depression in feed intake, but this did not influence hen-day egg production or egg weights. Given that higher levels of HO inclusion did not yield similar depressions, it could be inferred that the effect was transient and likely not specifically related to the dietary treatment. The lack of significant treatment × week interactions provides evidence that the inclusion of the hemp products did not alter the temporal patterns in changes in egg weights. A potential limitation of the current study relates to the sample size used in relation to the variability observed in the production data. The current studies provide relevant data in relation to the safety and efficacy of hemp products for supporting

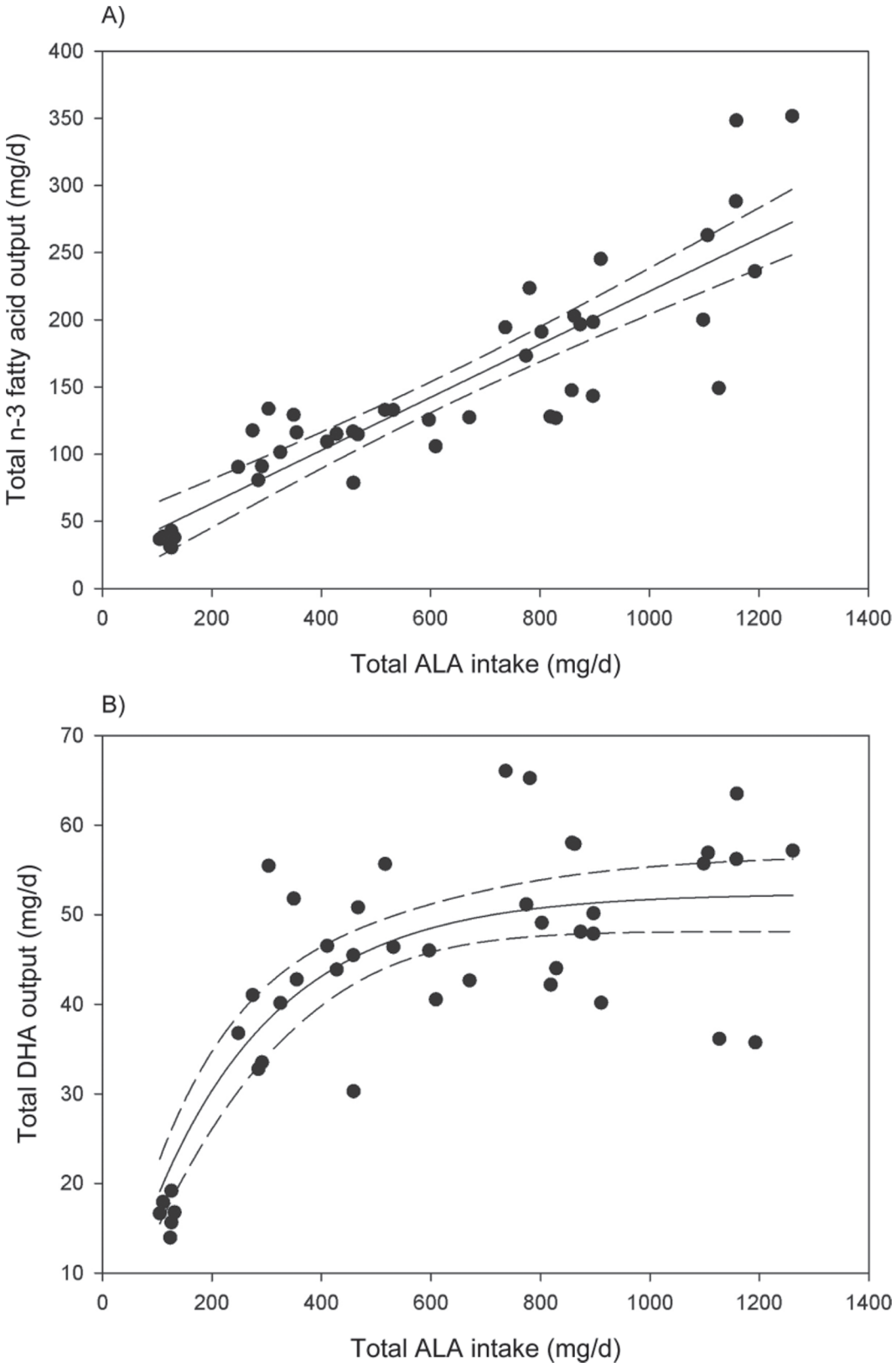

Figure 1. Relationship between dietary α-linolenic acid (ALA) intake (mg/d) and A) total n-3 fatty acid or B) total docosahexaenoic acid (DHA) output in eggs (mg/d) for hens consuming diets containing graded levels of either hemp seed or hemp seed oil. Regression equations: Total n-3 output (mg/d) = 23.91 + [0.20 × ALA intake (mg/d)], $r^2 = 0.78$; Total DHA output (mg/d) = $52.38\{1 - e^{(-0.0043)[\text{ALA intake (mg/d)}]}\}$, $r^2 = 0.67$. Individual data points are presented along with line of best fit (solid line) plus the upper and lower 95% confidence limits (dashed lines).

Table 11. Hepatic *FADS1*, *FADS2*, and *ELOVL5* mRNA expression in hens consuming diets containing either hemp seed oil (HO) or hemp seed (HS)[1]

	Fold changes relative to control (control = 1.0)		
Diet	*FADS1*	*FADS2*	*ELOVL5*
Control	1.00^{y}	$1.00^{a,x}$	1.00
10% HS	0.88	0.56^{b}	0.71
20% HS	0.72	0.76^{ab}	0.79
4% HO	0.77^{yz}	0.70^{yz}	0.96
8% HO	0.88^{yz}	0.55^{z}	1.08
12% HO	0.61^{z}	0.49^{z}	0.62

^{a,b}Values (n = 4 per dietary treatment) not sharing a common superscript within a column, within a hemp treatment differ significantly ($P < 0.05$). HS uses a and b superscripts.

$^{x–z}$Values (n = 4 per dietary treatment) not sharing a common superscript within a column, within a hemp treatment differ significantly ($P < 0.05$). HO uses x, y, and z superscripts.

[1]*FADS1*: fatty acid desaturase 1 encoding Δ-5 desaturase; *FADS2*: fatty acid desaturase 2 encoding Δ-6 desaturase; *ELOVL5*: a gene encoding elongation of very long-chain fatty acids protein 5.

laying hen production but larger studies will be necessary to derive commercially relevant production data.

Silversides and Lefrançois (2005) reported no effect of feeding hemp seed meal on albumen height or weight on any of the 3 egg components (yolk, shell, and albumen). In the current study, the feeding of up to 20% HS and 12% HO did not significantly affect eggshell thickness, specific gravity, or albumen height. Overall, the data from the current study supports the contention that laying hen diets can contain up to 20% HS and 12% HO without affecting measures of hen productivity or specific measures of egg and eggshell quality. Additional support for the use of hemp products in laying hen diets is realized by way of data supporting the lack of negative effects on the sensory parameters of the resultant eggs (Goldberg et al., 2012).

The inclusion of HS or HO dramatically reduced the linoleic acid:ALA ratio of the test diets from 38:1 in the control diet to 3.2:1 for the 12% HO diet (highest ALA diet; Table 1) and produced significant shifts in the n-3 content of the egg yolk lipids. For both the HO and the HS diets, the total n-3 content of the eggs increased with increasing inclusion of the hemp products, and these increases were observed across the 4 n-3 fatty acids measured: ALA, EPA, DPA, and DHA.

The intake of ALA appeared to be the primary determinant of the total n-3 fatty acid content of table eggs. However, the curvilinear relationship observed between ALA intake and total DHA output suggests a limitation in the conversion of ALA to DHA at intakes above approximately 600 mg/d of ALA. The biological explanation for this relationship is not readily apparent but may be related to the competition between substrates for access to the desaturase and elongase enzymes (Tu et al., 2010). Conversion of ALA to DHA occurs through the sequential actions of Δ-6 desaturase (encoded by *FADS2*), elongase (encoded by *ELOV5*), and Δ-5 desaturase (encoded by *FADS1*). Delta-6 desaturase is responsible for converting ALA (18:3 n-3) to 18:4 n-3, 18:2 n-6 to 18:3 n-6, 24:5 n-3 to 24:6 n-3, and 24:4 n-6 to 24:5 n-6. Therefore, the potential for substrate competition for the *FADS2* product, as well as the *FADS1* and *ELOV5* products, is substantial, within both the n-3 and n-6 fatty acid series. To partially address this issue, we evaluated the effect of consumption of the hemp products (and subsequently ALA intake) on the expression of 3 principal biosynthetic genes involved in PUFA metabolism: *FADS1*, *FADS2*, and *ELOVL5*. The mRNA expressions of *FADS1* and *FADS2* were reduced by the inclusion of HO or HS in laying hen diets, supporting a notion that enzymes involved in PUFA metabolism are regulated by dietary fatty acid composition. Previous work in rats (Tu et al., 2010) found that diets low in PUFA stimulated the expression of *FADS2* and *ELOVL2* when compared with higher PUFA diets; however, the endogenous synthesis of the n-3 long-chain PUFA from the precursor ALA appeared to be regulated independently of changes in the expression of the enzymes. The latter study provided evidence that the n-3 long-chain PUFA synthesis is regulated more by substrate competition for existing enzymes than by an increase in their mRNA expression. Further studies are needed to elucidate how fatty acid composition regulates gene expression and the biosynthesis of PUFA in poultry species.

Based on the results from the current study, it could be broadly concluded that the inclusion of HS and HO in the diets of laying hens up to a maximum level of 20 and 12%, respectively, does not have an adverse effect on the performance of laying hens, and these products can be included in the diets of laying hens without compromising their safety and efficacy. Given the ALA content of hemp, inclusion of HS and HO in hen diets led to increases in the n-3 fatty acid profile of eggs; however, the conversion of ALA to DHA was limited at higher ALA intakes. The current data provide evidence in support of safety and efficacy claims for the use of hemp-seed products in the diets of laying hens and the potential for these diets to serve as alternative sources of ALA for the production of eggs with higher levels of total n-3 content. However, to derive commercially relevant production data, studies employing larger sample sizes will be necessary in the future.

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