

Camelina Meal Increases Egg n-3 Fatty Acid Content Without Altering Quality or Production in Laying Hens

Radhika Kakani · Justin Fowler · Akram-Ul Haq ·
Eric J. Murphy · Thad A. Rosenberger ·
Mark Berhow · Christopher. A. Bailey

Received: 21 June 2011 / Accepted: 10 January 2012 / Published online: 31 January 2012
© AOCS 2012

Abstract *Camelina sativa* is an oilseed plant rich in n-3 and n-6 fatty acids and extruding the seeds results in high protein meal (~40%) containing high levels of n-3 fatty acids. In this study, we examined the effects of feeding extruded defatted camelina meal to commercial laying hens, measuring egg production, quality, and fatty acid composition. Lohmann White Leghorn hens (29 weeks old) were randomly allocated to three dietary treatment groups ($n = 25$ per group) and data was collected over a 12 week production period. All the treatment groups were fed a corn soy based experimental diet containing 0% (control), 5, or 10% extruded camelina meal. We found no significant differences in percent hen-day egg production and feed consumed per dozen eggs. Egg shell strength was significantly higher in both camelina groups compared to the controls. Egg total n-3 fatty acid content increased 1.9- and 2.7-fold in 5 and 10% camelina groups respectively relative to the control. A similar increase in DHA content also occurred. Further camelina meal did not alter

glucosinolate levels and no detectable glucosinolates or metabolic product isothiocyanates were found in the eggs from either the 5 or 10% camelina groups. These results indicate that camelina meal is a viable dietary source of n-3 fatty acids for poultry and its dietary inclusion results in eggs enriched with n-3 fatty acids.

Keywords Laying hens · Camelina meal · Egg quality · n-3 Fatty acids · Egg enrichment

Abbreviations

AHA American Heart Association
ALA Alpha linolenic acid
DHA Docosahexaenoic acid
LNA Linoleic acid
NRC National Research Council

Introduction

There is an increasing interest in converting oil produced from oil seed crops into biofuels in response to the Energy Policy Act, 2005 [1]. However, oil seeds and oil seed meals are used as a source of crude protein and energy in poultry rations. Therefore, the increase in bio-fuel production has created a need to develop ways to use the meal produced following oil extraction that can benefit the poultry industry by producing alternative meals that do not compete with the human diet yet provide an adequate source of energy and protein.

Camelina sativa, also known as gold-of-pleasure or false flax, is an ancient crop native to Northern Europe and Central Asia. The plant belongs to the family Brassicaceae and is considered a versatile crop that requires a low amount of inputs [2, 3]. This annual oil seed crop can be grown with success under different climatic conditions,

R. Kakani (✉) · J. Fowler · A.-U. Haq · Christopher. A. Bailey
Department of Poultry Science, Texas A&M University,
Room 101 Kleberg Center, College Station,
TX 77843-2472, USA
e-mail: kakani@neo.tamu.edu

E. J. Murphy · T. A. Rosenberger
Department of Pharmacology, Physiology, and Therapeutics,
School of Medicine and Health Sciences, University of North
Dakota, Grand Forks, ND, USA

E. J. Murphy
Agragen, LLC, Cincinnati, OH, USA

M. Berhow
National Center for Agricultural Utilization Research,
USDA, Peoria, IL, USA

making it an ideal crop for bio-fuel feedstock. Camelina oil contains high amounts of unsaturated fatty acids with alpha-linolenic (ALA, 18:3n-3) and linoleic (LNA, 18:2n-6) acids accounting for approximately 35 and 15% of the total, respectively [2]. Camelina meal, the by-product of oil extraction, has a crude protein content of 40% and oil content of 10–12% oil of which approximately 5% of the remaining oil is enriched in n-3 fatty acid [4]. Camelina meal can be used as a substitute for soybean meal in animal feeds [4].

Glucosinolates are anti-nutritive compounds generally present in the plants belong to Brassicaceae family which may have negative effects on feed consumption [5, 6]. A reduction in feed intake was reported in turkey poults when camelina meal was included in turkey starter diets at a concentration of more than 5% [7]. In most foods consumed that have glucosinolates these compounds are generally rapidly converted to a variety of degradation products during the course of ingestion and digestion. The most prominent metabolites are isothiocyanates and nitrile forms which are generally believed to have potential biological effects [5, 6]. Low glucosinolate content of camelina makes it a desirable animal feed relative to other Brassicaceae species [4].

Egg yolk composition depends on the dietary nutrient provision. Altering the dietary fatty acid intake of a laying hen can either directly [8] or indirectly due to further acyl chain elongation and desaturation in the liver [9] alter the fatty acid composition of the egg yolk. Feeding poultry diets rich in n-3 fatty acids increases the content of these fatty acids in the egg yolk. [10–17]. Because camelina meal is a rich source of essential fatty acids, it can be incorporated in laying hen diets to enrich the n-3 fatty acid content in eggs.

The modern Western diet is low in n-3 fatty acids resulting in a n-6 to n-3 fatty acid ratio ranging from 15:1 to 20:1 [18], which is proposed to increase the incidence of cardiovascular disease, rheumatoid arthritis, and cancer [18, 19]. There is substantial evidence illuminating health concerns caused by low dietary intake of n-3 fatty acids as well beneficial effects of supplementing diets with long-chain n-3 fatty acids [20–23]. The American Heart Association (AHA) recommends increasing the intake of dietary n-3 fatty acids to reduce the risk of coronary heart disease [24]. Because the available sources of long-chain n-3 fatty acids are limited, additional sources of highly nutritious products must be made available. Because egg fatty acid composition can be increased by altering the poultry diet [8, 9] addition of n-3 rich meals will increase n-3 fatty acid content of eggs. Flax seed is traditionally used to enrich eggs with n-3 fatty acids [14–17], however alternative, lower-cost options are needed. Herein, we propose that extruded defatted camelina meal is an excellent alternative

to flax meal as a source of n-3 fatty acids and protein for laying hen diets. Further, we hypothesize that the inclusion of extruded defatted camelina meal containing 10% residual oil, at levels of 5 and 10% will not alter feed consumption, body weight, egg production, internal egg quality, or sensory quality of eggs, but will increase egg n-3 fatty acid content.

Materials and Methods

One hundred, 25 week-old Lohmann White Laying hens, purchased from Feather Crest Farms, were placed individually into 100 laying hen cages. After a three-week acclimation period, 25 birds were randomly allocated to each of the three treatment blocks at the start of the trial, for a total of 75. Individual bird weights (g) were recorded at day 0 and at the end of each 28 day period (3 periods in total). Birds were caged on one tier in a laying hen house with open windows on two sides and an exhaust fan on one end. An additional fan was placed at the opposite end of the house to provide positive airflow towards the exhaust fan. All the methods used in this study were approved by the Texas A&M University Institutional Animal Care and Use Committee (AUP 2007-250).

A commercial type, phase 1 laying hen diet was formulated according to NRC Poultry [25], (Table 1) including control diet (no camelina meal) and diets that contained 5 and 10% camelina meal. All diets were formulated to be iso-nitrogenous and iso-caloric relative to the basal control diet (Table 1). Diets were formulated based on actual chemical analysis of the mechanically pressed defatted camelina meal (supplied by Great Plain Oil and Exploration) used in this study (Table 2). Feed mixtures were made fresh just prior to each 28 day period. Feed and water were offered ad libitum. No concomitant drug therapy was used during the study. Daily observations were made with regard to general flock condition, temperature, lighting, water, feed, and unanticipated events in the house. Pens were also checked daily for mortality.

The treatment group/block was the unit of measure for egg production and feed consumption. There were 5 hens per treatment within each block for a total of 75 hens. Egg measurements were taken on individual eggs. Weekly egg production was recorded. Egg weight, shell thickness and breaking strength, measured in triplicate, were determined for all eggs laid on one day of each week. Feed consumed per dozen of eggs was calculated based on 28-day periods. During the 2nd feeding period, eggs were collected for determination of interior egg quality. Color and Haugh Units were determined using an Egg AnalyzerTM (Orka Food Technology) at time zero and after 1 and 2 weeks of storage at 4 °C.

Table 1 Diet composition and nutrient content

Ingredient	Control (%)	5% Camelina (%)	10% Camelina (%)
Corn	60.097	58.282	56.466
Dehulled Soybean meal	26.399	23.029	19.659
Camelina meal ^a	0.000	5.000	10.000
DL-Methionine	0.150	0.131	0.111
Fat, AV blend	1.558	1.792	2.026
Limestone	9.712	9.716	9.72
Mono-dicalcium phosphate	1.380	1.344	1.308
Salt	0.403	0.407	0.409
Trace minerals	0.050	0.050	0.050
Vitamin premix	0.250	0.250	0.250
Nutrient content			
Protein	18.00	18.00	18.00
ME (Kcal)	2,800.00	2,800.00	2,800.00
Crude fat	4.10	4.73	5.36
Crude fiber	2.35	2.73	3.11
Calcium	4.00	4.00	4.00
AV phosphate	0.40	0.40	0.40
Sodium	0.18	0.18	0.18
18:3 n-3	0.0616	0.2348	0.4081

^a Mechanically pressed defatted camelina meal

Procedures Used to Measure Egg Lipid Content

Reagents

HPLC grade *n*-hexane, 2-propanol, and anhydrous methanol, methyl formate, and concentrated sulfuric acid were purchased from EM Science (Gibbstown, NJ). Reagent grade sodium methoxide (25 wt%) and ferric chloride hexahydrate was purchased for Sigma-Aldrich (St. Louis, MO). Fatty acid methyl ester and cholesterol standards were purchased from Nu-Chek-Prep (Elysian, MN). All samples were stored at -80°C and sample extracts were stored in *n*-hexane/2-propanol (3:2, by Vol.) at -20°C prior to use.

Sample Extraction

Yolk samples (0.8–1.0 g) from hard boiled eggs were extracted in *n*-hexane/2-propanol (3:2, by vol.) using a Potter–Elvehjem tissue homogenizer equipped with a Teflon pestle [26]. The final lipid extract was concentrated to zero under a steady-stream of N_2 at 45°C then re-dissolved in 40 mL *n*-hexane/2-propanol (3:2, by vol.) immediately prior to analysis.

Table 2 Nutrient profile of camelina meal

Nutrient name	Units	Amount
ME poultry	Kcal/kg	2,463.000
Crude protein	PCT	36.000
Crude fat	PCT	10.000
Crude fiber	PCT	11.000
Fatty acids		
Palmitic (16:0)	PCT	1.070
Palmitoleic (16:1)	PCT	0.020
Stearic (18:0)	PCT	0.300
Oleic (18:1)	PCT	2.400
Linoleic (18:2 n-6)	PCT	2.780
α -Linolenic (18:3 n-3)	PCT	3.520
Amino acids		
Cystine	PCT	0.808
Methionine	PCT	0.635
Lysine	PCT	1.760
Tryptophan	PCT	0.340
Threonine	PCT	1.530
Arginine	PCT	2.970
Histidine	PCT	0.880
Valine	PCT	1.910
Leucine	PCT	2.390
Isoleucine	PCT	1.350
Phenylalanine	PCT	1.530
Glycine	PCT	1.914
Phe + Tyr	PCT	2.260
Minerals		
Calcium	PCT	0.276
Phosphorus	PCT	1.140
Sodium	PCT	0.002
Potassium	PCT	1.660
Magnesium	PCT	0.515
Sulphur	PCT	0.931
Iron	mg/kg	160.000
Copper	mg/kg	9.250
Zinc	mg/kg	70.900
Manganese	mg/kg	33.000
Cobalt	mg/kg	0.300
Molybdenum	mg/kg	1.100

Fatty Acid Quantitation and Gas Chromatography

Fatty acid content in the yolk extract was measured in triplicate using a modified method [27]. Briefly, samples were concentrated to zero residual solvent under a steady-stream of nitrogen at 45°C then transesterified in 2.5% sodium methoxide at 40°C for 30 min. The reaction was

stopped with the addition of methyl formate and the fatty acid methyl esters were extracted with *n*-hexane. Triheptadecanoin was used as an internal standard to calculate fatty acid mass. Fatty acid content was measured using a Shimadzu 2010 gas chromatograph (Kyoto, Japan) equipped with a flame ionization detector and a capillary column (SP 2330; 30 m × 0.32 mm i.d., Supelco, Bellefonte, PA). Sample runs were initiated at a column temperature of 180 °C followed by a temperature gradient to 200 °C over 8 min starting at 2 min from the beginning of the run. The temperature was held at 200 °C until the end of the run at 20 min. Fatty acid methyl ester standards were used to establish relative retention times and response factors. The internal standard, methyl heptadecanoate, and the individual fatty acids were quantified by peak area analysis and linear regression analysis (Class VP 7.2.1 Datasystem, Kyoto, Japan). The detector response was linear, with correlation coefficients of 0.998 or greater within the sample concentration range for all standards.

Cholesterol Content

The cholesterol content in the yolk lipid extract was measured in triplicate using a colorimetric assay [28]. Briefly, an aliquot of the yolk lipid extract was concentrated to zero under a steady-stream of nitrogen at 45 °C then dissolved in 3 ml absolute ethanol (Pharmco, Brookfield, CT). A 3-ml aliquot of iron working reagent was added to the sample then vigorously mixed for 10 min to bring the reaction to completion. The absorbance of each sample was measured at 550 nm and the concentration was calculated by comparing sample absorbencies to freshly prepared standard curves using linear regression analysis.

Procedures for the Detection of Egg Glucosinolate Content

Methods: Sample Extraction

For HPLC analysis, typically between 0.25 and 0.5 g of defatted chicken feeds, defatted ground camelina seed meals, and defatted freeze dried whole egg powders were extracted with 2–5 mL of methanol. For glucosinolate recovery experiments, 100 µl of a 1.8 µg/µl solution of sinigrin was added to three 5% camelina feed meals and to egg powders. For isothiocyanate analysis, whole egg powders were extracted with dichloromethane.

HPLC Analysis and Quantitation

For intact glucosinolate quantitation, the extract was run on a Shimadzu (Columbia, MD) HPLC System. The column was a C18 Inertsil 250 mm × 4.6 mm; RP C-18, ODS-3,

5µ, column. The glucosinolates were detected by monitoring at 237 nm. The initial mobile phase conditions were 40% methanol/60% aqueous 0.005 M tetrabutylammonium bisulfate (TBS) at a flow rate of 1 ml/min. After injection the initial conditions were held for 40 min, and then up to 100% methanol over another 10 min. Intact glucosinolates were identified by retention time compared to standards and by LC–MS analysis. Extracts from egg powders were confirmed as not having any intact glucosinolates by HPLC and by LC–MS analysis.

Preparation of Glucosinolate Isothiocyanates

The seed meal was defatted with hexane in a Soxhlet extractor for 24 h, after which the residual seed meal was allowed to dry completely. Defatted seed meal (10-g samples) were mixed with 25 ml of 0.05 M Tris buffer, pH 10.0 to convert the glucosinolates into isothiocyanates. Fifty milliliters of dichloromethane was then added to each flask and the flasks were placed in an incubator shaker set at 25 °C and 200 rpm for 8 h. Following hydrolysis, 10 g of sodium chloride and 10 g of anhydrous sodium sulfate were added. The dichloromethane was decanted and filtered, the residual seed meal was extracted for an additional three times with excess dichloromethane. The combined crude dichloromethane extracts were analyzed by gas chromatography–mass spectrometry (GC–MS).

GC–MS Analysis of Isothiocyanates

Gas chromatography–mass spectrometry was performed using a Hewlett-Packard (HP) 6890 GC system attached to an Agilent Systems HP 5972A Mass Selective Detector. The column used was a fused silica HP-5MS capillary (0.25 µm film thickness, 30 m × 0.25 mm ID). The GC operating parameters were as follows: splitless injection mode; temperature programmed from 50 to 325 °C at 10 °C/min with a 5 min interval and final temperature hold; Helium carrier gas flow rate at 1.0 ml/min, with the injector temperature set at 325 °C. Spectra were compared with purified standards prepared in this laboratory. Dichloromethane extracts of camelina seed powders and meals and egg powders were examined by this method and no detectable isothiocyanates were found.

Sensory Evaluation of Eggs

A panel of 28 untrained evaluators (11–68 years of age), who were blinded to treatment groups did sensory evaluation of eggs. All the tasters ate half of a hard-boiled egg, rinsed their pallet and then ate another egg sample. All three treatment groups were eaten in random order. Panelists were asked to rate the taste and aroma of the eggs.

Statistics

Data were analyzed as a one-way ANOVA with 3 treatments using SPSS [29]. Significant differences among treatment means were separated using Duncan's multiple range test at $P < 0.05$.

The fatty acid content of the individual egg samples was measured then these values were used to calculate the average content of fatty acid per sample group. The fatty acid data were analyzed by using one-way ANOVA and a Tukey's post hoc test.

Results

Production

The egg production rate was greater than 95% for all groups with no significant difference in percent hen-day egg production (Table 3). Feed consumed per production of dozen eggs was 1,521, 1,460 and 1,472 g for Control, 5% camelina and 10% camelina groups, respectively. There was no statistical significant difference for the feed consumption and body weight (Table 3). No mortality was observed in any of the treatment groups during the experimental period.

Egg Quality Characteristics

There was no significant difference in egg weight between the Control and 10% camelina groups, however a significant reduction in the egg weight was observed in the 5% camelina group eggs (Table 4). We found a significant difference in the shell strength of eggs among all the groups (Table 4). Egg shell strength was significantly lower for the Control group and higher for the 5% camelina group. Shell thickness for the eggs from 5% camelina group was significantly higher than that of the Control and the 10% camelina groups (Table 4). Overall, shell strength and shell thickness were higher in the 5% group relative to the Control group, whereas shell strength was higher and shell thickness was lower in 10% group relative to control group. There was no significant difference among the treatments in yolk color when analyzed by the Egg analyzerTM at time

Table 4 Effect of camelina meal in laying hen diets on egg characteristics

	Egg weight (g)	Shell strength (Breaking force) (kg)	Shell thickness (mm)
Control	60 ± 4 ^a	4.5 ± 1.0 ^a	0.4019 ± 0.02 ^{a,b}
5% Camelina	58 ± 4 ^b	5.0 ± 1.1 ^c	0.4049 ± 0.02 ^a
10% Camelina	60 ± 5 ^a	4.7 ± 1.1 ^b	0.3997 ± 0.02 ^b

± Standard deviation

^{a,b,c} Means with no common superscript in the same column differ significantly ($P < 0.05$)

zero, one week of cold storage and 2 weeks of cold storage at 4 °C (Data not shown). However, when analyzed for Haugh Unit and Grade, at time zero, eggs from the 5 and 10% camelina groups were graded better [AA] relative to the control eggs [A]. We found no significant difference in the grade when measured after 1 and 2 weeks of cold storage at 4 °C (Table 5).

Egg Fatty Acid Composition

Detailed fatty acid profile of the eggs from three treatment groups is outlined in Table 6. There was a significant increase in the total n-3 fatty acids in the eggs from the 5 and 10% camelina groups, as compared to the Control group. The ratio of n-6 to n-3 fatty acids was also significantly lower in the 10% camelina group (4.3), relative to the 5% camelina group (6.0) and the Control group (12.4). Eggs from the 10% camelina group had on average of 78 mg of DHA per yolk, as compared to 59 mg/yolk from the 5% camelina group and 32 mg/yolk from the Control group. There was a nearly threefold increase in deposition of ALA in the 10% camelina group eggs when compared to the Control group eggs. Cholesterol content of the yolk did not differ significantly between the 5 and 10% camelina groups compared to the Control eggs (data not shown).

Glucosinolate Levels

There were no detectable camelina-derived glucosinolates found in eggs.

Table 3 Effect of camelina meal in laying hen diets on change in body weight, egg production and feed consumption

	Initial wt (g)	Ending wt (g)	Wt change (g)	% Hen day egg production	Feed (g) per dozen eggs
Control	1,438 ± 98	1,632 ± 106	194 ± 76	95.7 ± 3.4	1,521 ± 141
5% Camelina	1,453 ± 82	1,599 ± 82	149 ± 76	95.3 ± 3.2	1,460 ± 123
10% Camelina	1,453 ± 82	1,614 ± 118	161 ± 82	95.3 ± 3.4	1,472 ± 165

± Standard deviation

Table 5 Effect of camelina meal in laying hen diets on Interior Egg Quality—Haugh Unit & [Grade] (Egg AnalyzerTM)

	Time zero	One week cold storage	Two weeks cold storage
Control	71 ± 9 [A]	53 ± 8 [B]	54 ± 8 [B]
5% Camelina	75 ± 5 [AA]	57 ± 11 [B]	57 ± 11 [B]
10% Camelina	75 ± 4 [AA]	57 ± 6 [B]	56 ± 8 [B]

± Standard deviation

Table 6 Effect of camelina meal in laying hen diets on fatty acid profile of eggs

Fatty acid content mg/yolk	Control	5% Camelina	10% Camelina
C14:0	21.2 ± 4.9	11.1 ± 3.3*	8.7 ± 1.8*
C14:1n-5	14.6 ± 2.0	13.6 ± 3.8	11.8 ± 1.8
C16:0	1,168.9 ± 115.4	890.7 ± 156.3*	776.5 ± 100.8*
C16:1n-7	186.7 ± 25.5	133.9 ± 20.4*	106.6 ± 14.0***
C18:0	423.3 ± 51.8	381.2 ± 71.1	395.8 ± 54.1
C18:1n-9	2,028.7 ± 199.6	1,839.5 ± 303.2	1,855.1 ± 249.9
C18:2n-6	522.2 ± 71.4	519.8 ± 96.5	518.6 ± 49.2
C18:3n-6	8.0 ± 1.3	6.5 ± 1.8	7.2 ± 1.2
C18:3n-3	24.6 ± 4.9	50.9 ± 12.8*	73.3 ± 8.0***
C20:1n-9	23.6 ± 3.4	31.2 ± 7.5*	38.4 ± 5.6*
C20:2n-6	8.3 ± 1.1	10.4 ± 2.5	12.8 ± 2.0*
C20:3n-6	10.7 ± 1.5	10.7 ± 1.8	11.9 ± 1.3
C20:4n-6	158.9 ± 18.6	127.6 ± 23.2	129.4 ± 15.4
C20:5n-3	1.0 ± 0.2	1.5 ± 0.4	2.8 ± 0.6
C22:4n-6	5.7 ± 0.6	4.7 ± 0.9	5.0 ± 1.2
C22:5n-6	24.6 ± 1.8	12.5 ± 2.8*	9.6 ± 2.6*
C22:5n-3	2.6 ± 0.4	5.2 ± 1.5*	7.2 ± 1.3***
C22:6n-3	31.8 ± 3.9	59.1 ± 10.5*	77.6 ± 11.2***
Total	4,665.4 ± 456.5	4,110.1 ± 684.5	4,048.3 ± 464.6
Total n-6	738.4 ± 90.7	692.3 ± 125.6	694.5 ± 68.3
Total n-3	60.0 ± 8.1	116.7 ± 23.7*	161.0 ± 19.5***
n-6/n-3	12.4 ± 0.8	6.0 ± 0.2*	4.3 ± 0.1***

± Standard deviation

* Significant from 0% camelina, $P < 0.05$ ** Significant from 5% camelina, $P < 0.05$

Sensory Evaluation

No overt reactions were noted, i.e., facial expressions of poor tasting eggs or really bad tasting eggs, by the panelists. Two individuals noted that the 10% camelina group had a bit of a fishy taste.

Discussion

In this study, we evaluated the use of extruded defatted camelina meal as a protein source rich in n-3 fatty acids on

egg production and quality. In a previous study, when *Camelina sativa* seed oil is added at a level of 5% in laying hen diets, the n-3 fatty acid content of the eggs is increased [30]. Also, the functional properties and sensory quality of the eggs were not negatively affected by the 5% concentration of seed oil demonstrating that the camelina seed oil group scored better than the test group fed with flax seed oil diet [30]. This investigation suggests that *Camelina sativa* seed oil can increase n-3 fatty acid levels in eggs without imparting the same off flavor and odor as flax meal and oil. This is consistent with the results observed in this study.

Camelina meal as a potential feed ingredient in turkey diets at levels above 5% is not recommended as reduction in feed consumption occurs [7]. Feeding camelina meal at levels of 5 or 10% to broilers to enrich tissue n-3 fatty acid content, depresses feed intake and feed conversion ratio during starter phase (0–14 days) while camelina meal did not have any adverse effect on the sensory quality of the meat [31]. Camelina seed does not depress feed consumption at a levels up to 15% in rabbits [32]. Similarly, in our study there was no reduction in the percent hen day production, feed consumption or was there a reduction in hen body weight in the camelina meal fed groups. Because percent hen day egg production is an excellent indicator of health and well-being, the lack of change suggests that neither level of camelina meal had adverse effects on health.

Pekel et al. [33] reported that increasing levels of camelina meal in laying hen diets results in decreased feed consumption, egg production and body weight, however this effect was not observed when camelina meal is fed up to 9% between 22 and 34 weeks. In another report, feeding camelina meal to laying hens at concentrations of 5, 10, and 15% results in a reduction in hen-day egg production at the 15% concentration [34]. This group also reported that yolk weight expressed as a percentage of egg weight was lower for the 10 and 15% camelina groups, but albumin weight expressed as a percentage of egg weight, was higher for these same eggs. Herein, we report that feeding camelina meal up to 10% between 28 and 40 weeks and had no detrimental effects on performance parameters or on egg quality.

We observed a significant difference in the egg weight, shell thickness and shell strength in the 5% camelina group eggs relative to the control group. Our results of egg weight, shell thickness are in contrast to the other study [34], who reported no significant difference in egg weight or shell thickness. The observed reduction in egg weight, increased shell strength and shell thickness in 5% camelina group in the present study may be due to individual bird variation, unknown nutrient interactions or other unexplainable genetic factors because there was no strain difference in the birds used in this study.

As hypothesized, when camelina meal with approximately 12% residual oil is included in hen diets, there is a concomitant increase in total n-3 fatty acid composition from 0.32% in the control eggs to 2.54, 2.69 and 2.99% in 5, 10 and 15% camelina group eggs, respectively [34]. In our study, feeding camelina meal with residual oil content of 10% resulted in a total n-3 fatty acid composition of 2.5 and 3.7% in 5 and 10% camelina group eggs relative to the 1.1% of the control eggs. The net change in total n-3 fatty acids was not the result of a reduction in n-6 fatty acids (Table 6), indicating that inclusion of camelina meal in the diets provided adequate n-6 fatty acid intake. Similar to the other study [34], we also observed a net reduction in saturated fatty acids, but these changes all occurred in a manner that did not alter egg production or egg quality. Diets with moderate levels of ALA and high levels of LA produced increased deposition of DHA in the egg yolk, but not EPA [35]. In the study [35], authors suggested that the increased deposition of DHA may be due to the saturation of the different Δ -unsaturases depending on the level of dietary fat or due to impairment in the conversion of EPA to DHA. However, in the present study we also observed an accretion in the deposition of EPA, but to a very low extent compared to the deposition of DHA. This may be due to the specific action of desaturases to the different dietary source of fat or to the concentration of dietary fat.

Many studies were conducted to investigate the effects of n-3 rich dietary sources in laying hen diets. Using fish meal and fish oil in laying hen diets, both high in n-3 fatty acid content, produced n-3 enriched eggs [11–13, 36–38]. Similarly flax seed meal added to laying hen diets also increases the n-3 fatty acid content although is reported to impart a fishy odor in the egg [14–17]. Herein we propose that camelina meal is a better alternative to flax meal because it is a crop that is much less weather dependent, has more consistent yields, and is cheaper to produce [39]. Thus, camelina meal may offer a less expensive feed ingredient to enrich n-3 fatty acid content in eggs, compared to flax meal.

Addition of camelina meal to laying hen diets at a concentration of 5 and 10% didn't have any detrimental effects on feed consumption and egg production; and there were no detectable glucosinolates in eggs from either treatment groups. Isothiocyanates and nitrile forms are the metabolites of glucosinolates and these are the forms that could possibly have been accumulated in the eggs, but we found no indication of their presence.

Our results indicate that camelina meal can be added as an ingredient to laying hen diets as a source of energy, protein and n-3 fatty acids. Addition of mechanically pressed defatted camelina meal to laying hen diets up to a concentration of 10% did not have any detrimental effects on feed consumption, on hen body weight, on egg production, on

interior quality of eggs and on sensory quality of eggs, while producing n-3 fatty acid enriched eggs.

Acknowledgments This study was funded by Great Plain Oil and Exploration. Camelina meal used in this study was supplied by Great Plain Oil and Exploration.

References

1. US Department of the Interior (Accessed June 2011) <http://www.doi.gov/pam/EnergyPolicyAct2005.pdf>
2. Zubr J (1997) Oil-seed crop: *Camelina sativa*. *Ind Crop Prod* 6:113–119
3. Putnam DH, Budin JT, Field LA, Breene WM (1993) Camelina: a promising low-input oilseed. In: Janick J, Simon JE (eds) *New crops*. Wiley, New York, pp 314–322
4. Pilgeram AL, Sands DC, Boss D, Dale N, Wichman D, Lamb P, Lu C, Barrows R, Kirkpatrick M, Thompson B, Johnson DL (2007) *Camelina sativa*, a Montana omega-3 and fuel crop. In: Janick J, Whipkey A (eds) *Issues in new crops and new uses*. ASHS Press, Alexandria
5. Acamovic T, Gilbert C, Lamb K, Walker KC (1999) Nutritive value of *Camelina sativa* meal for poultry. *Br Poult Sci* 40:S27–S41
6. Tripathi MK, Mishra AS (2007) Glucosinolates in animal nutrition: a review. *Animal Feed Sci Technol* 132:1–27
7. Frame DD, Palmer M, Peterson B (2007) Use of *Camelina sativa* in the diets of young turkeys. *J Appl Poult Res* 16:381–386
8. Watkins BA, Feng S, Strom AK, DeVitt AA, Yu L, Li Y (2003) Conjugated linoleic acids alter the fatty acid composition and physical properties of egg yolk and albumen. *J Agric Food Chem* 51:6870–6876
9. Cherian G, Wolf FW, Sim JS (1996) Dietary oils with added tocopherol: effects on egg or tocopherol, fatty acids, acid oxidative stability. *Poult Sci* 75:423–431
10. Juneja LR (1997) Egg yolk lipids. In: Yamamoto T, Juneja LR, Hatta H, Kim M (eds) *Hen eggs*. CRC Press, Boca Raton, pp 73–98
11. Adams RL, Pratt DE, Lin JH, Stadelman WJ (1989) Introduction of omega-3 polyunsaturated fatty acids into eggs. *Poult Sci* 68(Suppl 1):166
12. Huang ZB, Leibovitz H, Lee CM, Miller R (1990) Effect of dietary fish oil on w-3 fatty acid levels in chicken eggs and thigh flesh. *J Agric Food Chem* 38:743–747
13. Nash DM, Hamilton RMG, Hulan W (1995) The effect of dietary herring meal on the omega-3 fatty acid content of plasma and egg lipids of laying hens. *Can J Anim Sci* 75:247–253
14. Caston L, Leeson S (1990) Research note: dietary flaxseed and egg composition. *Poult Sci* 69:1617–1620
15. Aymond WM, Van Elswyk ME (1995) Yolk thiobarbituric reactive substances and n-3 fatty acids in response to whole and ground flaxseed. *Poult Sci* 74:1358–1394
16. Scheideler SE, Froning GW (1996) The combined influence of dietary flaxseed variety, level, form, and storage conditions on egg production and composition among vitamin E-supplemented hens. *Poult Sci* 75:1221–1226
17. Gonzalez-Esquerra R, Leeson S (2001) Alternatives for enrichment of eggs and chicken meat with n-3 fatty acids. *Can J Anim Sci* 81:295–305
18. Simopoulos AP (2000) Human requirement for n-3 polyunsaturated fatty acids. *Poult Sci* 79:961–970
19. Leaf A, Weber PC (1987) A new era for science in nutrition. *Am J Clin Nutr* 45:1048–1053

20. Connor WE (2000) Importance of n-3 fatty acids in health and disease. *Am J Clin Nutr* 71:171–175
21. Simopoulos AP (1999) Essential fatty acids in health and chronic disease. *Am J Clin Nutr* 70:560–569
22. Kang JX, Leaf A (1996) Antiarrhythmic effects of polyunsaturated fatty acids. *Recent studies. Circulation* 94(7):1774–1780
23. Leaf A, Kang JX, Xiao YF, Billman GE (2003) Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. *Circulation* 107(21):2646–2652
24. Kris-Etherton PM, Harris WS, Appel LJ (2002) For the Nutrition Committee. AHA scientific statement. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 106:2747–2757
25. Nutrient Requirements of Poultry, Ninth Revised Edition (1994) National Research Council. National Academy Press, Washington
26. Radin NS (1981) Extraction of tissue lipids with a solvent of low toxicity. *Methods Enzymol* 72:5–7
27. Willis S, Samala R, Rosenberger TA, Borges K (2009) Eicosapentaenoic and docosahexaenoic acids are not anticonvulsant or neuroprotective in acute mouse seizure models. *Epilepsia* 50: 138–142
28. Bowman RE, Wolf RC (1962) A rapid and specific ultramicro method for total serum cholesterol. *Clin Chem* 8:302–309
29. SPSS 14.0, SPSS Inc., Chicago, IL
30. Rokka T, Alen K, Valaja J, Ryhanen EL (2002) The effect of a *Camelina sativa* enriched diet on the composition and sensory qualities of hen eggs. *Food Res Int* 35:253–256
31. Ryhanen EL, Perttila S, Tupasela T, Valaja J, Eriksson C, Larkka K (2007) Effect of *Camelina sativa* expeller cake on performance and meat quality of broilers. *J Sci Food Agric* 87:1489–1494
32. Peiretti PG, Mussa PP, Prola L, Meineri G (2007) Use of different levels of false flax (*Camelina sativa* L.) in diets for fattening rabbits. *Livest Sci* 107:192–198
33. Pekel AY, Patterson PH, Hulet RM, Acar N, Cravener TL, Dowler DB, Hunter JM (2009) Production performance of two strains of laying hens fed different levels of camelina meal and flaxseed. *Poult Sci* 88(Suppl 1):31
34. Cherian G, Campbell A, Parker T (2009) Egg quality and lipid composition of eggs from hens fed *Camelina sativa*. *J Appl Poult Res* 18:143–150
35. Grobas S, Mendez J, Lazaro R, De Blas C, Mateos GG (2001) Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. *Poult Sci* 80:1171–1179
36. Herber SM, Van Elswyk ME (1996) Dietary marine algae promotes efficient deposition of n-3 fatty acids for the production of enriched shell eggs. *Poult Sci* 75:1501–1507
37. Nash DM, Hamilton RMG, Sanford KA, Hulan HW (1996) The effect of dietary menhaden meal and storage on the omega-3 fatty acids and sensory attributes of egg yolk in laying hens. *Can J Anim Sci* 76:377–383
38. Abril R, Barclay W (1998) Production of docosahexaenoic acid-enriched poultry eggs and meat using an algae-based feed ingredient. In: Simopoulos AP (ed) *The return of w-3 fatty acids into the food supply. I. Land-based animal food products and their health effects. World Review in Nutrition and Dietetics*, vol 83. Basel, Switzerland
39. Moloney AP, Woods VB, Crowley JG (1998) A note on the nutritive value of camelina meal for beef cattle. *Ir J Agric Food Res* 37:243–247