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Abstract
When laying hen diets are enriched with omega-3 polyunsaturated fatty acids to generate value-added eggs for human consumption markets, concentrations of alphalinolenic, eicosapentaenoic, and docosahexaenoic acids (ALA, EPA, and DHA) in the yolk can reach 250mg/50g whole egg. Flaxseed, a rich source of ALA, is commonly used for omega-3 enrichment; however, the impact of dietary flaxseed source (extracted oil vs. milled seed) on fatty acid transfer to egg yolk in laying hens is unknown. Therefore, transfer of ALA, EPA, and DHA into egg yolk from extracted flaxseed oil or milled flaxseed was evaluated in Hy-Line W-36 laying hens over an 8 wk feeding period (25 to 33 wk old). Hens (n=132) were randomly housed with 3 birds/cage (4 replicates/treatment) for each of the 11 treatment groups. Diets were isocaloric and consisted of a control diet, 5 flaxseed oil diets (0.5, 1.0, 2.0, 3.0, or 5.0% flaxseed oil), and 5 milled flaxseed diets (calculated flaxseed oil concentration from milled flaxseed 0.5, 1.0, 2.0, 3.0, 5.0%). Increasing dietary concentrations of flaxseed oil and milled flaxseed resulted in increased ALA, EPA, and DHA concentration in egg yolk, and fatty acid deposition from flaxseed oil was 2 times greater compared to milled flaxseed when fed at the same dietary inclusions (P

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Comparative omega-3 fatty acid enrichment of egg yolks from first-cycle laying hens fed flaxseed oil or ground flaxseed

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Metabolism and Nutrition

ABSTRACT When laying hen diets are enriched with omega-3 polyunsaturated fatty acids to generate value-added eggs for human consumption markets, concentrations of alpha-linolenic, eicosapentaenoic, and docosahexaenoic acids (ALA, EPA, and DHA) in the yolk can reach 250mg/50g whole egg. Flaxseed, a rich source of ALA, is commonly used for omega-3 enrichment; however, the impact of dietary flaxseed source (extracted oil vs. milled seed) on fatty acid transfer to egg yolk in laying hens is unknown. Therefore, transfer of ALA, EPA, and DHA into egg yolk from extracted flaxseed oil or milled flaxseed was evaluated in Hy-Line W-36 laying hens over an 8 wk feeding period (25 to 33 wk old). Hens (n=132) were randomly housed with 3 birds/cage (4 replicates/treatment) for each of the 11 treatment groups. Diets were isocaloric and consisted of a control diet, 5 flaxseed oil diets (0.5, 1.0, 2.0, 3.0, or 5.0% flaxseed oil), and 5 milled flaxseed diets (calculated flaxseed oil concentration from milled flaxseed 0.5, 1.0, 2.0, 3.0, 5.0%). Increasing dietary concentrations of flaxseed oil and milled flaxseed resulted in increased ALA, EPA, and DHA concentration in egg yolk, and fatty acid deposition from flaxseed oil was 2 times greater compared to milled flaxseed when fed at the same dietary inclusions ($P<0.01$). Egg yolk EPA and DHA concentrations were not different due to oil or milled source ($P=0.21$); however, increasing dietary inclusion rates of flaxseed oil from either source increased yolk EPA and DHA ($P<0.01$). Hens fed either flaxseed oil or milled flaxseed resulted in reduced BW change as dietary concentrations increased ($P=0.02$). Feed efficiency increased as flaxseed oil increased in concentration, while feeding milled flaxseed decreased feed efficiency ($P=0.01$). Analysis of the nitrogen corrected apparent metabolizable energy of flaxseed oil resulted in 7,488 kcal/kg on an as-fed basis. Dietary flaxseed oil improved feed efficiency and increased ALA deposition into yolk compared

to a milled source, demonstrating flaxseed oil to be a viable alternative for ALA egg enrichment.

Key words: omega-3, ALA/EPA/DHA, layers, value-added egg, energy

INTRODUCTION

Consumer demand for value-added omega-3 fatty acid enriched egg products from US egg producers has risen steadily and now captures 10 % of the market share for shell egg and egg product retail market (USDA, 2016). Hens readily absorb and transfer omega-3 fatty acids from dietary sources for deposition into the yolk (Cherian and Sim, 1991). On average, it takes 2 wk for a laying hen to adjust to an omega-3 fatty acid enriched diet and reach a transfer plateau of dietary omega-3 fatty acid incorporation into developing ovarian follicles (Cherian and Sim, 1991; Nain et al., 2012). The beneficial anti-inflammatory properties for reducing health risks have been attributed to longer chain omega-3 fatty acids eicosapentaenoic (EPA; 20:5 omega-3 fatty acid) and docosahexaenoic acids (DHA; 22:6 omega-3 fatty acid; Cottin et al., 2011; Tur et al., 2012). Laying hens have the ability, although not efficient (< 6%), to elongate and desaturate alpha linolenic acid (ALA; 18:3 omega-3 fatty acid), an essential fatty acid and the predominant omega-3 fatty acid source in flaxseed, to the functional omega-3 fatty acids EPA and DHA (Burdge and Calder, 2006; Zivkovic et al., 2011; Gregory et al., 2013). Egg yolk fatty acid content is finite due to the 10 % total fat contained within an egg and reaches a plateau of saturation, which is directly influenced by the total ALA, EPA, and DHA omega-3 fatty acid composition within the diet (Cherian and Sim, 1991; Nain et al., 2012).

The fatty acid composition of an ingredient has a direct effect on fat utilization or deposition in poultry. Similar to other monogastric animals, poultry species have a limited endogenous enzymatic ability to modify the structure of dietary fatty acids compared to ruminant species, which contain ruminal microbes that highly modify dietary lipids, because poultry do not host the microbial populations responsible for the expression of

elongases and desaturases (Haug et al., 2014). During post-absorptive metabolism, long chain fatty acids such as ALA are added to triglycerides for long-term energy storage and contain the relatively unaltered fatty acids in adipocyte lipid droplets (Cherian and Sim, 1991). Longer chain fatty acids including omega-3 fatty acids EPA and DHA are almost exclusively deposited for storage in the form of phospholipids, particularly phosphatidylethanolamine in egg yolk (Jiang et al., 1991). Taking advantage of dietary omega-3 fatty acid deposition into yolk, producers are able to create value-added ALA, EPA, and DHA enriched eggs.

Milled or ground flaxseed, also known as linseed, is an ALA source and is used by poultry producers in the US for enriching commercial table eggs and meat products (Samman et al., 2009; Petrovic et al., 2012; Lopes et al., 2013). Flaxseed contains 7 times the amount of ALA compared to soybean and corn oil with 3 times less omega-6 linolenic acid content, properties which contribute to its use as an ALA supplement in poultry formulation (NRC, 1994). Whole flaxseed contains 30 to 40 % fat with 50 % of the fat composition consisting of ALA and 15 % linoleic acid (LA; 18:2 omega-6 fatty acid; Kratzer and Vohra, 1996). Flaxseed products effectively deliver ALA to poultry meat and eggs due to high concentration and bioavailability (Jia et al., 2008; Fraeye et al., 2012). Eggs from unsupplemented laying hens may contain 93 mg ALA and 173 mg total omega-3 fatty acid (ALA, EPA, and DHA)/50 g egg while adding 15 % dietary flaxseed can increase ALA and total omega-3 fatty acid content to 358 mg and 468 mg/50 g egg, respectively (Jia et al., 2008; Samman et al., 2009).

There appears to be a tolerable limit to how much flaxseed can be included in a ration, due to anti-nutrients or palatability, without negatively affecting bird performance

and feed efficiency. Digestion and absorption may be impaired due to various anti-nutrients found in flaxseed such as cyanogenic glycosides and phytic acid, or due to increased viscosity of ingesta caused by mucilage in flaxseed (Alzueta et al., 2003; Goyal et al., 2014). Laying hens fed diets with 20 % ground flaxseed, compared to 0 and 10 % treatments, resulted in inadequate weight gain, reduced egg production, and increased feed intake, which resulted in significantly reduced feed efficiency (Leeson et al., 2000). Anti-nutrients or reduced nitrogen corrected apparent metabolizable energy (AME_n) due to flaxseed in the diet were suspected as the cause for the adjustment in laying hen feed intake (Leeson et al., 2000).

Yet to be investigated is how the source of flaxseed ingredient may affect the efficiency of ALA transfer from the laying hen diet into the developing egg yolk. Even in further processed ingredients such as milled or ground flaxseed, the flaxseed still contains the components of the seed's cellular matrix that may trap some of the lipid fraction as seen with other seed types (Cassady et al., 2009), which may be a factor in reducing digestibility or availability for transfer of ALA from the milled flaxseed. Human studies comparing flaxseed in whole, milled, and oil sources delivering 6 g of ALA for 4 wk observed 77 % greater plasma ALA levels in flaxseed oil cohorts compared to milled flaxseed cohorts, while cohorts that consumed whole flaxseed did not observe increased plasma ALA levels (Austria et al., 2008). Cohorts from each treatment group experienced gastrointestinal discomfort, especially with cohorts consuming whole flaxseeds resulting in dietary non-compliance, which stresses the importance of how increased oil consumption effects intestinal microbiota, digestibility, and potential for discomfort (Austria et al., 2008).

In order to further investigate how the form of ALA source affects the fatty acid transfer rate from laying hen diet to the egg yolk, purified extracted flaxseed oil and conventional milled flaxseed ingredients were fed at increasing inclusions in experimental omega-3 fatty acid ALA enriched laying hen diets. Dietary AME_n of the flaxseed oil ingredient was determined to fill the gap in knowledge for future research and industry application. It was hypothesized that an extracted flaxseed oil ingredient would have an improved rate of ALA deposition to egg yolk compared to a milled flaxseed ingredient when supplemented in a laying hen ration during peak production, with no adverse effects on AME_n and performance.

MATERIALS AND METHODS

Animals and Housing

The Institutional Animal Care and Use Committee (IACUC) at Iowa State University (Ames, IA) approved the protocol and the experiment was conducted in accordance with university policies and the Ag Guide (2010). Single-Comb White Leghorn laying hens (n = 132, Hy-Line W-36, age = 25 wk old) were obtained from a commercial source (Bancroft, IA) as they were approaching peak production and reproductive efficiency. Laying hens were randomly placed into 3 tier conventional cages (Poultry Layer Cage, Safeguard, New Holland, PA), resulting in 3 hens/cage at a density of 696 cm^2/bird. Each single cage of 3 hens represented an experimental unit (EU). Treatments were assigned in a completely random design, allowing for 11 dietary treatments with 4 replicates/treatment. Hens were allowed *ad libitum* access to feed and water during the 8 wk experiment from 25 to 33 wk of age. Lighting started at 13.5L:10.5D with 30 minutes

of increasing light/wk until 15.5L:8.5D was achieved 4 wk into the experimental period. House temperature was maintained between 18 to 27° C for the 8 wk experimental period.

Flaxseed Source Enriched Diets

Experimental diets were formulated to meet or exceed the NRC (1994) requirements of commercial layer hens. Flaxseed oil diets were formulated by adding the purified flaxseed oil at the expense of soy oil in the diet (Table 1). Formulations were isocaloric and consisted of a control diet, 5 flaxseed oil diets, and 5 milled flaxseed diets (calculated flaxseed oil concentrations for treatment diets were 0.5, 1.0, 2.0, 3.0, and 5.0 %). A twelfth treatment diet contained 5.0 % added flaxseed oil with an antioxidant stabilizer (n = 12 additional hens, internal data not reported here). The milled flaxseed ingredient was created by grinding whole flaxseed through a 4.0 mm screen (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Company, Philadelphia, PA) with corn as a carrier. To obtain the calculated oil concentrations in the milled flaxseed diets, the milled flaxseed was included at 1.5, 3.0, 6.0, 9.0, and 15.0 % in the diet (Table 2).

Performance

Laying hens were monitored twice daily for the duration of the 8 wk experiment in accordance with IACUC policy. Eggs were collected daily for hen-housed egg production (HHEP; no mortality occurred, therefore hen-day egg production was not reported) calculation and average daily feed intake (FI) was determined by measuring weekly disappearance of feed: HHEP % = (# eggs laid ÷ # hens housed ÷ # days) × 100; FI = Start feed weight in kg – End feed weight in kg. Body weight (BW) was recorded at the start, 4

wk, and 8 wk. Egg weight (EW), egg mass (EM), and feed efficiency were measured weekly from 0 to 8 wk: Egg mass = Average egg weight in g × (HHEP % ÷ 100). Feed efficiency (FE) was reported as g eggs per kg FI: FE = Egg mass in g ÷ Feed intake in kg.

Egg Yolk Analysis

A pooled sample of 5 egg yolks from each EU was used to measure egg solids and yolk fatty acid profile at 4, 6, and 8 wk of the experiment. Fatty acid analysis of egg yolk, as previously described by Sun et al. (2013) and Nam et al. (2001) using gas chromatography (HP 6890, Hewlett Packard Co., Palo Alto, CA), was performed starting at 4 wk to allow an adjustment period for maximal transfer of fatty acids to egg yolk.

AME_n Experiment

After the initial 8 wk omega-3 fatty acid enrichment experiment, all birds (now 33 wk of age) were utilized in a 2 wk study to determine the AME_n content of the flaxseed oil, as this information is currently lacking in the literature. The laying hens (n = 144, including the unreported 12^{th} diet group) were removed from their respective cages and separated, randomly rearranged using the same 3 tier cages as previously described so that each cage contained 3 hens that were new cage-mates. This ensured that the previous flaxseed dietary enrichment would not adversely affect the AME_n experiment. Treatments were assigned in a completely random design allowing for 4 dietary treatments with 12 replicates per treatment for the AME_n experiment. Each EU consisted of 3 hens per cage with identical bird density as previously mentioned in the flaxseed enrichment experiment. Hens were managed as previously described with *ad libitum* access to feed and water for the 2 wk

AME_n experiment from 33 to 35 wk of age. Performance data during this 2 wk AME_n experiment was not reported.

A basal diet with titanium dioxide (0.30 %) and increasing levels of flaxseed oil (0.0, 3.0, 6.0, and 9.0 % added to the basal diet) were used to generate 4 AME_n treatment diets. Experimental diets were formulated to meet or exceed the NRC (1994) requirements of commercial laying hens (Table 3). These diets were fed for a 2 wk adjustment period, which served as a washout period for the previous flaxseed egg yolk fatty acid deposition experiment, in order to collect excreta on d 14 for AME_n determination and regression analysis.

Feed samples were ground through a 0.5 mm screen and subsequently dried for 24 h at 100° C for DM determination. Pooled excreta samples were dried at 75° C in a convection oven for 3 d and subsequently ground through a 1.0 mm screen as previously described by Ehr et al. (2015). The ether extract of the feed samples was determined by AOAC Official Method 920.39, traditional Soxhlet extraction using diethyl ether at the University of Missouri Agricultural Experiment Station Chemistry Laboratories (AESCL, Columbia, MO). For the feed and excreta samples, N concentration was determined by thermal combustion (TruMac N Analyzer, LECO Corp., St. Joseph, MI); gross energy (GE) was determined using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL); and Ti concentration was determined using the method outlined by Leone (1973). All samples of excreta and feed were analyzed in duplicate. The diet AME_n determination was calculated using the following formula from Leeson and Summers (2001) modified for Ti:

$$AME_n = GE_{Diet} - (GE_{Excreta} \times Ti_{Diet} \div Ti_{Excreta}) - 8.22 \times (N_{Diet} - N_{Excreta} \times Ti_{Diet} \div Ti_{Excreta}).$$

Statistical Analysis

Data were analyzed by repeated measures ANOVA using SAS (SAS 9.4, 2012, SAS Institute Inc., Cary, NC) with diet, week, and diet × week interaction included in the model. Orthogonal contrasts were used to test the response variables of the control against the 10 flax source supplemented diets and the 5 flaxseed oil vs. 5 milled flaxseed supplemented diets. There was only one control group for the experiment, therefore responses variables were tested for linear, quadratic, and cubic orthogonal contrasts of dietary oil % (0.5, 1.0, 2.0, 3.0, 5.0 %) and dietary oil % × flax source (flaxseed oil or milled flaxseed) interaction for the 5 flaxseed oil and 5 milled flaxseed supplemented treatments. For the purposes of this experiment, linear and quadratic functions were used to explain responses in the biological model. Linear fitment indicated progressive response to dietary inclusion and quadratic fitment represented an upper limit for response in relation to dietary inclusion. Cubic contrasts were included for the reader to make further inferences if desired. In all cases $P \leq 0.05$ was accepted as significant.

RESULTS

Enrichment Period Performance

During the dietary flax source enrichment period, no differences were observed for FI, EM, or egg solids for the orthogonal contrasts tested ($P \geq 0.07$). Quadratic contrast of oil % × flax source resulted in increasing then plateauing HHEP as flaxseed oil increased in dietary inclusion for the flaxseed oil dietary treatments ($P = 0.01$). As milled flaxseed increased in dietary inclusion, HHEP declined then plateaued ($P = 0.01$). Linear contrast of oil % × flax source resulted in increasing FE as dietary flaxseed oil was increased in the

flaxseed oil treatments ($P = 0.01$). Increasing milled flaxseed dietary inclusion resulted in decreasing FE ($P = 0.01$). Linear contrast of dietary oil % resulted in decreasing EW as increasing inclusion of either flax source (flaxseed oil or milled flaxseed equivalent) was added to the diet ($P = 0.05$). Gains in BW decreased as oil % increased in flaxseed oil or milled flaxseed equivalent diets linearly ($P = 0.02$). Change in BW decreased for milled flaxseed fed hens 24 times more compared to flaxseed oil fed hens as dietary inclusion of each respective flax source increased ($P = 0.02$; Table 4).

Egg Yolk Omega-3 Fatty Acid Deposition

Egg yolk total omega-3 fatty acid (ALA, EPA, and DHA) concentration increased linearly as oil % increased for flaxseed oil and milled flaxseed supplemented dietary treatments ($P < 0.01$). Increasing inclusion of flaxseed oil supplementation increased egg yolk total omega-3 fatty acid deposition 2 times more compared to equivalent inclusion of milled flaxseed dietary treatments ($P < 0.01$). The flaxseed oil treatments resulted in a linear equation of $y = 1.604x + 2.171$ ($R^2 = 0.880$) and milled flaxseed treatments resulted in a linear equation of $y = 0.783x + 2.310$ ($R^2 = 0.808$; Figure 1). Egg yolk EPA and DHA concentration was not different by flax source ($P = 0.26$). However, as oil % increased in flaxseed oil and mill flaxseed dietary treatments, egg yolk EPA and DHA concentration increased linearly ($P < 0.01$; Figure 2).

Oil AME_n Determination

The equation of the AME_n regression line was $y = 74.88x + 2750$, which was linear in fitment ($P < 0.01$; $R^2 = 0.941$). The slope of the regression line equated to the AME_n

value of the flaxseed oil ingredient, which was 7,488 kcal/kg on an as-fed basis (Figure 3). The mean analyzed values for AME_n determination of the experimental diets were 2,762, 2,948, 3,218, and 3,421 kcal/kg as the flaxseed oil concentration increased from 0.0 to 9.0 % for each diet, respectively.

DISCUSSION

This experiment was designed to evaluate the impact of structurally different (extracted oil vs. ground seed) dietary omega-3 fatty acid sources on egg yolk deposition and to determine the AME_n of purified flaxseed oil. No significant differences were observed in FI for hens fed diets containing milled flaxseed. If any were to be observed, the 5.0 % milled flaxseed treatment would have been expected to suppress FI due to anti-nutrients present in or palatability of the ground seeds (Table 4). However, the rate of BW gain of hens fed milled flaxseed declined 24 times more than hens fed flaxseed oil as dietary inclusions increased. The difference in rate of BW change may have been due to anti-nutrients present in the milled flaxseed causing impaired digestion or absorption of the dietary nutrients (Gonzalez-Esquerra and Leeson, 2000; Leeson et al., 2000).

Cyanogenic glycosides including linustatin, neolinustatin, and linmarin present in the milled flaxseed may have caused a reduction in BW change due to loss of effective intestinal epithelial cell absorptive function (Oomah et al., 1992; Feng et al., 2003; Kajla et al., 2015). In addition, phytic acid present in milled flaxseed may result in protein-mineral-phytic acid complexes that are not bioavailable, reducing BW gain by exacerbating impaired nutrient absorption (Erdman, 1979; Feng et al., 2003; Goyal et al., 2014). Trypsin inhibitors present in flaxseed may have played a minor role in decreasing nutrient

bioavailability, but the quantity found in flaxseed is insignificant compared to levels found in soybean (Bhatty, 1993; Feng et al., 2003). It has been well documented that mucilage, a water-soluble polysaccharide found in flaxseed, increases chicken intestinal content viscosity (Rodriguez et al., 2001). The increased viscosity inhibits nutrient digestion and absorption of the intestinal ingesta (Alzueta et al., 2003). Similar declines in performance were observed in FE for hens fed milled flaxseed diets as the inclusion increased. Data from this current work suggests that feeding hens milled flaxseed exerted physiologic effects impairing nutrient storage and anabolic activity compared to equivalent inclusions of supplemental flaxseed oil.

When analyzing the total omega-3 fatty acid (ALA, EPA, and DHA) transfer from the diet into the egg yolk, the highest dietary inclusions of flaxseed oil incorporated 66 % more total omega-3 fatty acids into yolks compared to milled flaxseed. This difference in rates was likely because the structural components of the cell wall entrapped the lipid fraction, as seen in other seeds types such as almonds (Ellis et al., 2004; Mandalari et al., 2008). The presence of anti-nutrients likely contributed to the decreased transfer of ALA due to reduced bioavailability or absorption of the dietary lipids. Extracted flaxseed oil treatments resulted in delivering dietary ALA to egg yolk without seed components interfering with intestinal utilization. In an experiment with up to 7 % dietary flaxseed oil inclusion, total omega-3 fatty acid deposition in the muscle tissue of broiler chickens was increased in a curvilinear manner (Kartikasari et al., 2012). The lack of quadratic fit or plateau in the work reported here suggests that the maximum saturation limit of total omega-3 fatty acid deposition to the egg yolk was not reached and would explain the linear fit of omega-3 fatty acid egg yolk content as dietary oil % increased in the flax source

treatment groups. EPA and DHA egg yolk inclusion was not different based on flax source enriched treatments suggesting that laying hens deposited modified portions of ALA to egg yolk at a finite but constant rate.

The AME_n of the flaxseed oil ingredient was determined after the 8 wk omega-3 fatty acid flaxseed source enrichment to egg yolk experiment, as few data are published regarding the metabolizable energy of flaxseed oil. The energy value found was 7,488 kcal/kg of flaxseed oil as fed, which was on the lower end of the predicted value. Flaxseed oil may be used as an energy source when formulating diets, but with less energy compared to the corn and soy oil values listed in the NRC (1994). Previous investigations of flaxseed AME_n reported different age-sensitive tolerances to dietary flaxseed by poultry. In an experiment feeding 10 % flaxseed supplemented diets to broiler chicks, significantly lower tolerance was observed manifesting as diarrhea compared to mature single comb white leghorn roosters fed flaxseed diets (Gonzalez-Esquerra and Leeson, 2000).

Processing also affects AME_n of flaxseed supplemented diets. Pelleting (4,578 kcal/kg) or crumbling (4,277 kcal/kg) significantly increases AME_n fed to roosters compared to mash (3,659 kcal/kg; Gonzalez-Esquerra and Leeson, 2000). Extrusion processing of an ALA feed supplement containing 60 % flaxseed improved AME_n by 18 % (Bean and Leeson, 2002). The additional pressure and heat of processing may release trapped oil from the matrix of the cell or the additional heat may destroy some of the toxic anti-nutrients in the feed (Calet, 1965; Gonzalez-Esquerra and Leeson, 2000). Therefore, pelleting and extrusion may be a method to offset the energy deficit of flaxseed oil compared to soy oil.

Results reported here show that flaxseed oil is a well-tolerated, energy dense feed ingredient with a more efficient ability to transfer omega-3 fatty acid ALA to egg yolk as compared to ground flaxseed products containing a similar total omega-3 fatty acid content. Increasing dietary concentrations of both flaxseed oil and milled flaxseed resulted in increased ALA concentrations in egg yolk; BW gain was not as detrimentally affected by feeding flaxseed oil, but decreased with milled flaxseed feeding. Superior ALA deposition in egg yolk using flaxseed oil as compared to milled flaxseed warrants further investigation of the potential application of industrial-scale omega-3 fatty ALA acid oil enrichment for value-added egg products.

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TABLES

Table 1. Calculated and analyzed values for 11 control and experimental laying hen diets used to evaluate the transfer rate of dietary omega-3 fatty acids into the egg yolk from 25 to 33 wk of age.

Ingredient	Control	Flaxseed oil[1]					Milled flaxseed (% oil concentration)				
Calculated values (%)	**diet**	**0.5**	**1.0**	**2.0**	**3.0**	**5.0**	**0.5**	**1.0**	**2.0**	**3.0**	**5.0**
ME (kcal/kg)	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900
Corn	42.86	42.86	42.86	42.86	42.86	42.86	41.57	40.27	37.68	35.08	29.89
Soybean meal 48 % CP	34.20	34.20	34.20	34.20	34.20	34.20	33.99	33.78	33.37	32.95	32.12
DDGS	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Meat & bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Flaxseed (milled)	0.00	0.00	0.00	0.00	0.00	0.00	1.50	3.00	6.00	9.00	15.00
Flaxseed oil	0.00	0.50	1.00	2.00	3.00	5.00	0.00	0.00	0.00	0.00	0.00
Soy oil	5.86	5.36	4.86	3.86	2.86	0.86	5.87	5.89	5.92	5.95	6.01
Sodium chloride	0.41	0.41	0.41	0.41	0.41	0.41	0.40	0.40	0.40	0.40	0.39
DL-methionine	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.29
L-threonine	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.06
Limestone[2]	9.80	9.80	9.80	9.80	9.80	9.80	9.80	9.80	9.80	9.80	9.78
Dicalcium phosphate	1.94	1.94	1.94	1.94	1.94	1.94	1.93	1.93	1.92	1.91	1.89
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
V and M premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Omega-3 fatty acids (mg)[4]	473	710	946	1419	1892	2837	754	1036	1598	2161	3286
Omega-6 fatty acids (mg)[5]	4095	3911	3726	3356	2986	2247	4154	4217	4339	4461	4705
Omega-6:3 ratio	8.7	5.5	3.9	2.4	1.6	0.8	5.5	4.1	2.7	2.1	1.4
Analyzed values (%)											
Crude protein	21.51	22.48	21.84	22.29	20.49	22.25	21.01	21.33	22.16	22.26	22.40
Crude fat	6.67	7.08	6.90	7.01	6.49	7.39	7.79	8.53	9.27	10.90	13.36

Crude fiber	3.43	3.41	3.22	3.05	2.74	3.35	3.30	3.18	3.49	3.41	4.33
Moisture	9.51	9.05	9.68	8.88	9.64	6.06	9.50	8.70	9.10	8.81	8.03
Ash	13.68	14.10	13.47	13.58	11.71	12.01	12.35	11.60	12.10	11.90	12.59

[1]Extracted flaxseed oil was added at the expense of 5.86 % soy oil to generate individual diets that contained 0.5, 1.0, 2.0, 3.0, and 5.0 % extracted flaxseed oil, along with the 5.0 % flaxseed oil with antioxidant diet.

[2]Limestone added was a 50/50 mixture of small (≤ 2 mm) and large particle (> 2 mm).

[3]Vitamin and mineral premix provided per kg of diet: Selenium 200 µg; Vitamin A 6,600 IU; Vitamin D_3 2,200 IU; Vitamin E 14.3 IU; Menadione 880 µg; Vitamin B_{12} 9.4 µg; Biotin 33 µg; Choline 358 mg; Folic acid 1.1 mg; Niacin 33 mg; Pantothenic acid 8.8 mg; Pyridoxine 880 µg; Riboflavin 4.4 mg; Thiamine 1.1 mg; Iron 226 mg; Magnesium 100 mg; Manganese 220 mg; Zinc 220 mg; Copper 22 mg; Iodine 675 µg.

[4]Calculated dietary omega-3 fatty acid content (ALA, EPA, and DHA) mg per 100 g of completed feed.

[5]Calculated dietary omega-6 fatty acid content (LA and arachidonic acid) mg per 100 g of completed feed.

Table 2. Analyzed crude fat, fatty acid, and total omega-3 fatty acid concentrations in extracted flaxseed oil and milled flaxseed ingredients.

Fatty acid (C:double bond)	Flaxseed oil %	Milled flaxseed %
Myristic acid (14:0)	0.06	0.08
Palmitic acid (16:0)	5.72	6.06
Palmitoleic acid (16:1)	0.13	0.70
Margaric acid (17:0)	0.05	0.00
Stearic acid (18:0)	3.72	4.05
Oleic acid (18:1)	18.49	18.66
Vaccenic acid (18:1)	0.69	0.00
Linoleic acid (18:2)	15.19	15.24
Alpha-linolenic acid (18:3)	54.21	55.21
Arachidic acid (20:0)	0.58	0.00
Arachidonic acid (20:4)	0.09	0.00
Eicosapentaenoic acid (20:5)	0.16	0.00
Docosapentaenoic acid (22:5)	0.38	0.00
Docosahexaenoic acid (22:6)	0.46	0.00
Crude fat[1]	> 99	34
Total omega-3 fatty acid[2]	54.83	55.21

[1]Crude fat was the analyzed fat content of each feed ingredient. The fatty acid values represent the percent composition of the crude fat in each ingredient.

[2]Total omega-3 fatty acid was the sum of alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid.

Table 3. Calculated compositions and analyzed values of 4 laying hen diets used for the apparent metabolizable energy, nitrogen corrected (AME_n) assay fed from 33 to 35 wk of age.

	Basal	Flaxseed oil		
FSO[1] concentration (%)	**0.0**	**3.0**	**6.0**	**9.0**
ME (kcal/kg)	2800	-	-	-
Corn	60.18	58.37	56.57	54.76
Meat/bone meal	2.00	1.94	1.88	1.82
Soybean meal 48	25.01	24.26	23.51	22.76
Soy oil	1.33	1.29	1.25	1.21
Flaxseed oil	0.00	3.00	6.00	9.00
Sodium chloride	0.42	0.40	0.39	0.38
DL-methionine	0.15	0.15	0.14	0.14
Limestone[2]	9.24	8.96	8.68	8.40
Dicalcium phosphate	0.87	0.84	0.82	0.79
Titanium dioxide	0.30	0.29	0.28	0.27
Phytase	0.00075	0.00073	0.00071	0.00068
V and M premix[3]	0.50	0.49	0.47	0.46
Analyzed values (%)				
Dietary AME_n as-fed (kcal/kg)	2762	2948	3218	3421
Crude protein	18.02	17.72	17.13	16.76
Crude fat	2.95	4.84	7.89	10.80
Crude fiber	3.18	2.30	2.35	2.19
Moisture	10.14	9.99	9.68	9.61
Ash	14.04	14.29	11.21	10.63

[1]FSO = Flaxseed oil

[2]Limestone added was a 50/50 mixture of small (≤ 2 mm) and large particle (> 2 mm).

[3]Vitamin and mineral premix provided per kg of diet: Selenium 200 μg; Vitamin A 6,600 IU; Vitamin D_3 2,200 IU; Vitamin E 14.3 IU; Menadione 880 μg; Vitamin B_{12} 9.4 μg; Biotin 33 μg; Choline 358 mg; Folic acid 1.1 mg; Niacin 33 mg; Pantothenic acid 8.8 mg; Pyridoxine 880 μg; Riboflavin 4.4 mg; Thiamine 1.1 mg; Iron 226 mg; Magnesium 100 mg; Manganese 220 mg; Zinc 220 mg; Copper 22 mg; Iodine 675 μg.

Table 4. Laying hen performance[1] from 25 to 33 wk of age as affected by increasing dietary inclusion of flaxseed oil (FSO) or milled flaxseed (MFS).[2]

	Feed intake (g/bird/d)		HHEP (%)		FE (g eggs/kg feed)		Egg weight (g/egg)		Egg mass (g/bird/d)		Egg solids (%)		BW change (g)	
Oil (%)	**FSO**	**MFS**	**FSO**	**MFS**	**FSO**	**MFS**	**FSO**	**MFS**	**FSO**	**MFS**	**FSO**	**MFS**	**FSO**	**MFS**
Control 0.0	93.5		95.8		600		57.6		55.3		23.6		75.1	
0.5	92.3	92.9	91.4	98.3	583	640	58.7	59.4	53.8	58.4	23.3	23.5	54.8	79.2
1.0	94.4	93.1	96.9	96.6	597	603	58.0	57.9	56.2	56.0	23.5	23.4	78.6	74.8
2.0	93.8	92.7	98.3	97.7	586	606	55.8	57.3	54.9	56.1	23.7	23.6	80.5	38.1
3.0	94.5	93.1	96.4	95.1	593	589	57.7	57.5	55.8	54.7	23.4	23.5	77.2	58.8
5.0	90.8	93.8	96.2	96.9	602	591	56.4	57.2	54.3	55.5	23.0	23.5	61.1	24.2
SEM	1.59		1.23		11.5		0.98		1.05		0.22		11.71	
Contrast *P*-values														
Control vs. Flax	0.82		0.67		0.93		1.00		0.78		0.64		0.32	
FSO vs. MFS	0.97		0.18		0.07		0.39		0.10		0.53		0.04	
Linear														
Oil %	0.69		0.58		0.24		0.05		0.17		0.49		0.02	
Oil % × Flax	0.33		0.09		0.01		0.94		0.30		0.26		0.02	
Quadratic														
Oil %	0.37		0.24		0.17		0.23		0.77		0.19		0.37	
Oil % × Flax	0.20		0.01		0.29		0.92		0.07		0.28		0.14	
Cubic														
Oil %	0.94		0.03		0.78		0.13		0.97		0.43		0.73	
Oil % × Flax	0.94		0.15		0.46		0.64		0.61		0.67		0.12	

[1]Performance parameters measured were feed intake, hen-housed egg production (HHEP), feed efficiency (FE), egg weight (g/egg), egg

mass (g/bird/d), egg solids 4, 6, and 8 wk average (%), and body weight (BW) change from 25 to 33 wk of age.

[2]Hens were fed a control diet or one of the following omega-3 fatty acid enriched diets: purified flaxseed oil (FSO) treatments containing 0.5, 1.0, 2.0, 3.0, or 5.0 % FSO content, or milled flaxseed (MFS) treatments containing 0.5, 1.0, 2.0, 3.0, or 5.0 % FSO concentration (MFS included at 1.5, 3.0, 6.0, 9.0, or 15.0 % of the diet, respectively).

FIGURES

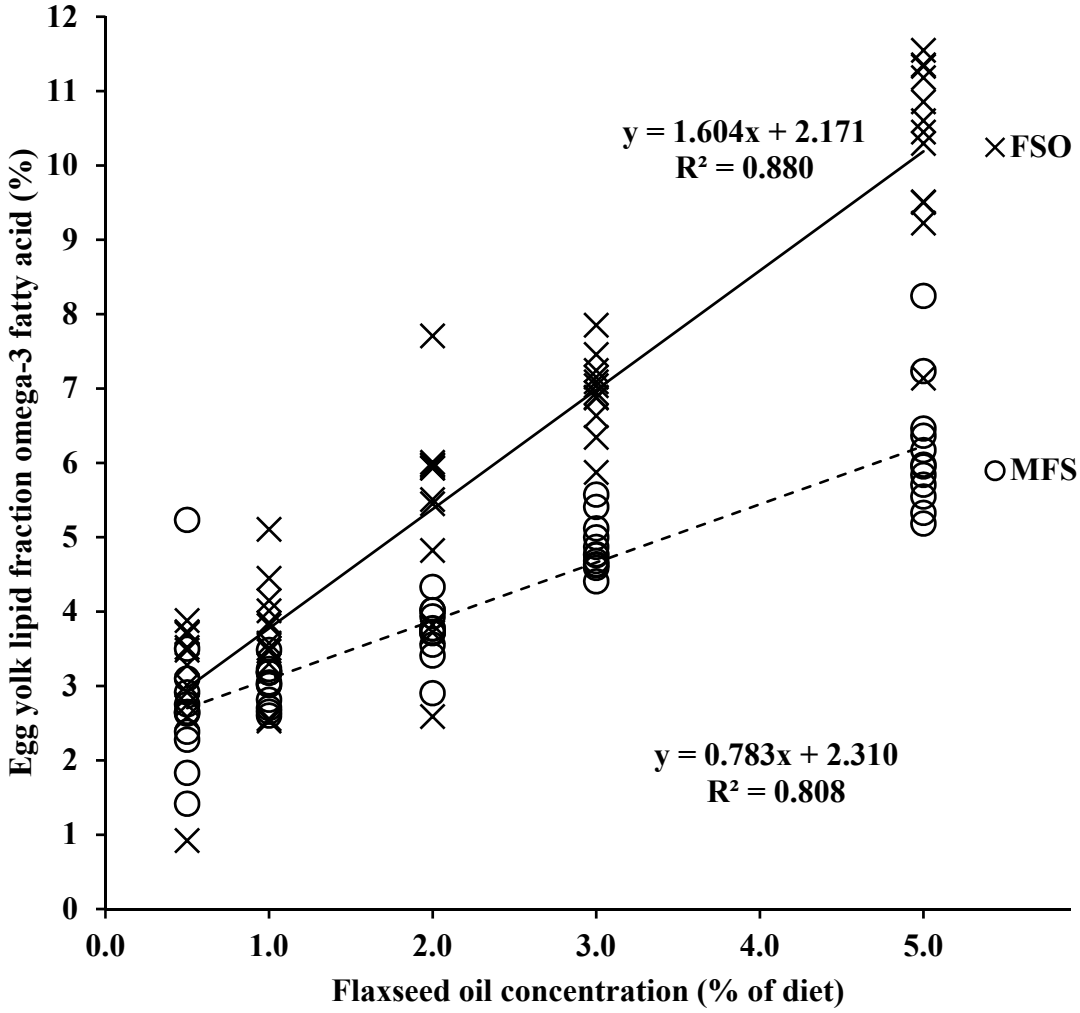

Figure 1. Egg yolk total omega-3 fatty acid content (ALA, EPA, and DHA) from hens fed a control diet, or one of the following experimental diets: purified flaxseed oil (FSO) treatments containing 0.5, 1.0, 2.0, 3.0, or 5.0 % FSO content, or milled flaxseed (MFS) treatments containing 0.5, 1.0, 2.0, 3.0, or 5.0 % FSO concentration (MFS included at 1.5, 3.0, 6.0, 9.0, or 15.0 % of the diet respectively). An experimental unit (EU) was a cage of 3 laying hens with 4 replicates for each of the 11 treatments (n = 132) fed for 8 wk (25 to 33 wk of age). Fatty acid analysis of yolks was performed on 4, 6, and 8 wk of the experiment using 5 eggs per EU and values in the figure represent the average of the 3

analyzed time points. The MFS treatments resulted in a linear fit ($P < 0.01$; $R^2 = 0.808$) with an equation of $y = 0.783x + 2.310$. The FSO treatments also resulted in a linear fit ($P < 0.01$; $R^2 = 0.880$) with an equation of $y = 1.604x + 2.171$. The FSO dietary treatments deposited 2 times more ALA, EPA, and DHA into egg yolk compared to MFS dietary treatments at equivalent flaxseed oil concentrations ($P < 0.01$).

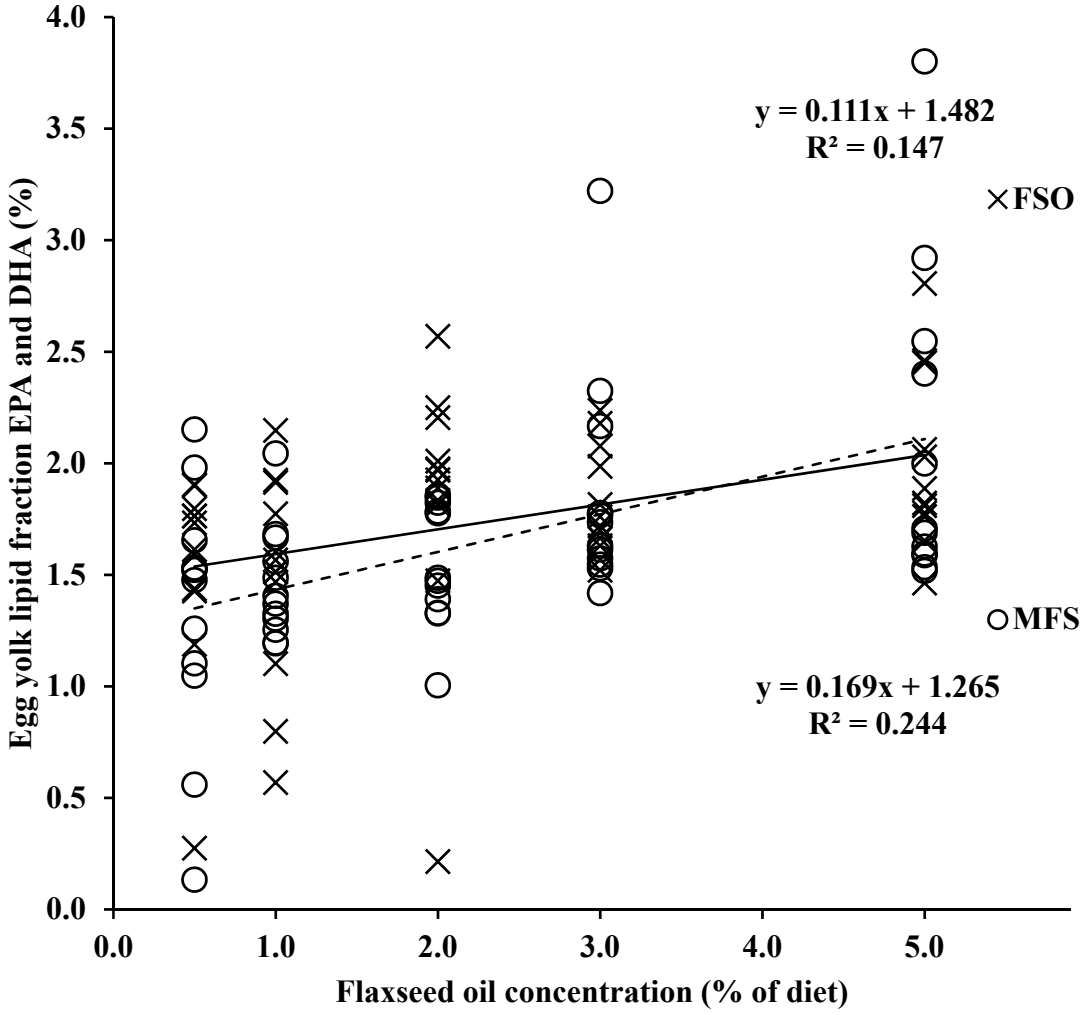

Figure 2. Egg yolk EPA and DHA content from hens fed a control diet, or one of the following experimental diets: purified flaxseed oil (FSO) treatments containing 0.5, 1.0, 2.0, 3.0, or 5.0 % FSO content, or milled flaxseed (MFS) treatments containing 0.5, 1.0, 2.0, 3.0, or 5.0 % FSO concentration (MFS included at 1.5, 3.0, 6.0, 9.0, or 15.0 % of the diet respectively). An experimental unit (EU) was a cage of 3 laying hens with 4 replicates for each of the 11 treatments (n = 132) fed for 8 wk (25 to 33 wk of age). Fatty acid analysis of yolks was performed on 4, 6, and 8 wk of the experiment using 5 eggs per EU and values in the figure represent the average of the 3 analyzed time points. The MFS treatments resulted in a linear fit ($P < 0.01$; $R^2 = 0.244$) with an equation of $y = 0.169x + 1.265$. The

FSO treatments resulted in a linear fit ($P < 0.01$; $R^2 = 0.147$) with an equation of $y = 0.111x + 1.482$. There was no difference between dietary flaxseed oil concentration by flax source (FSO or MFS) for response in egg yolk EPA and DHA deposition ($P = 0.21$).

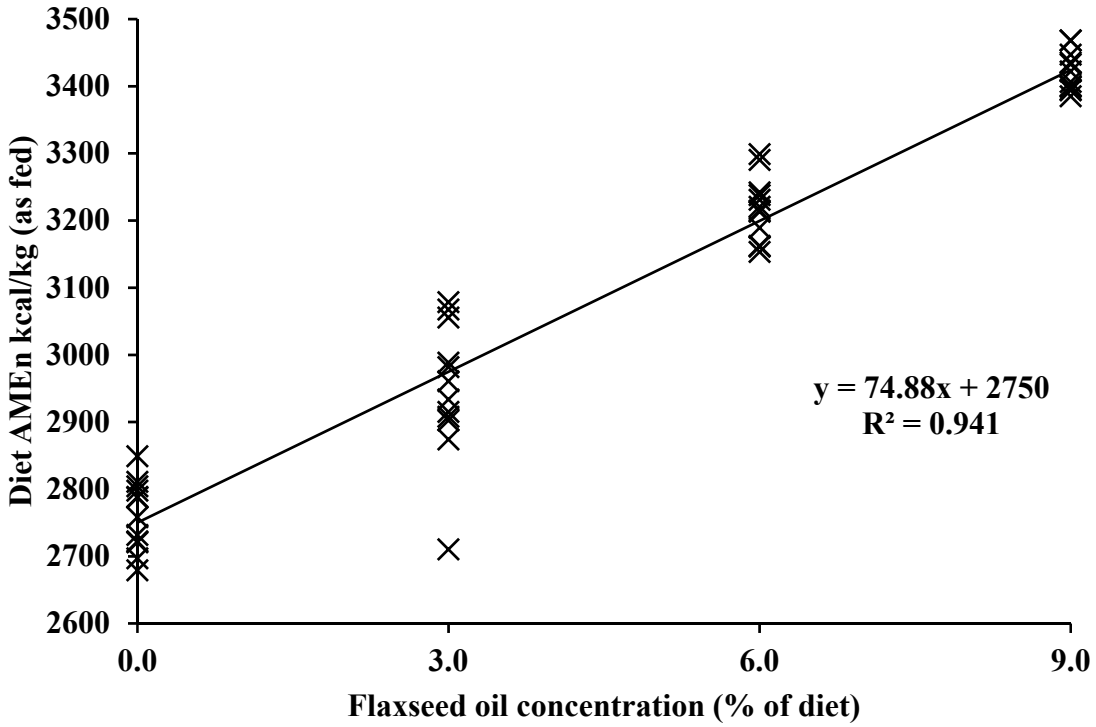

Figure 3. Dietary AME_n values on an as-fed basis for hens fed diets containing 0.0, 3.0, 6.0, and 9.0 % extracted flaxseed oil (FSO) added to a basal diet for 2 wk (33 to 35 wk of age). An experimental unit was a cage of 3 hens with 12 replicates for each of the 4 treatments (n = 144). The AME_n diets resulted in a linear fit ($P < 0.01$; $R^2 = 0.941$), where the slope of the line (y = 74.88x + 2750) equated to the AME_n value of the FSO. The FSO had an AME_n value of 7,488 kcal/kg on an as-fed basis.