



# Omega-3 Fatty Acid Enrichment Capacity in Egg Yolks from Laying Hens Fed either Corn Germ Oil or Corn Germ Meal

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## Abstract

Enrichment of omega-3 polyunsaturated fatty acids in egg yolk via diets alternation has been considered worldwide. The concentrations of alpha-linolenic (ALA), eicosapentaenoic (EPA), and docosahexaenoic acids (DHA) in the yolk can reach up to 250 mg/50 g whole egg. Corn germ meal (CGM), a rich source of ALA, is widely used for omega-3 enrichment; however, the impact of dietary corn germ source: corn germ oil (CGO) and CGM on fatty acid transfer to egg yolk in laying hens is still a little known. Therefore, this study was aimed to evaluate the transfer of ALA, EPA, and DHA into egg yolk from extracted corn germ oil or corn germ meal. A total of 132 Hy-Line W-36 laying hens (from 25 to 33 wks. old) 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 corn germ oil diets (0.5, 1.0, 2.0, 3.0, or 5.0% corn germ oil), and 5 corn germ meal diets (calculated corn germ oil concentration from corn germ meal 0.5, 1.0, 2.0, 3.0, 5.0%). Increasing dietary concentrations of corn germ oil and corn germ meal resulted in increased ALA, EPA, and DHA concentration in egg yolk, total fatty acid deposition from corn germ oil was 2 times greater than that of corn germ meal when fed at the same dietary inclusions ( $P < 0.01$ ) but EPA and DHA concentrations in egg yolk were not different due to oil or meal source ( $P = 0.22$ ); however, increasing dietary inclusion rates of corn germ oil from either source increased yolk EPA and DHA ( $P < 0.01$ ). Hens fed either corn germ oil or corn germ meal resulted in reduction of BW as dietary concentrations increased ( $P = 0.02$ ). Feed efficiency increased as corn germ oil increased in concentration, while feeding corn germ meal decreased feed efficiency ( $P = 0.01$ ). Analysis of the nitrogen corrected apparent metabolizable energy (AMEn) of corn germ oil resulted in 7,468 kcal/kg on an as-fed basis. Dietary corn germ oil improved feed efficiency and increased ALA deposition into yolk compared to that of the meal source, demonstrating that corn germ oil to be a viable alternative for ALA egg enrichment.

**Keywords:** Corn germ; Corn germ oil; Corn germ meal; Omega 3 fatty acid

## Introduction

Vietnam's corn output in 2017 was 5.5 million tons, of which 80% was used for animal feed. In recent years, the use of corn as a feed for the primary purpose of starch supply has not yet been addressed other nutrients such as lipids, especially fatty acids found in maize germination. Although the consumption of chicken eggs per capita is lower than that of developed countries, however, the demand for omega-3 enriched eggs has increased rapidly in recent years (MARD, 2016) [1]. Hens readily absorb and transfer omega-3 fatty acids from dietary sources for deposition into the yolk (Cassady et al. 2009). [2]. On average, it takes 2 weeks 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 (Cassady et al., 2009; Nain et al., 2012). [2,3]. 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). [4,5]. 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). [6-8]. The use of increasing levels of sesame oil decreased egg production, egg weight, feed intake and yolk colour - in group including 4.5% sesame oil, 74.77%, 61.58g, 120.09g, 6.58g, respectively (Hoan and Khoa, 2016) [9]. Egg yolk fatty acid content is finite due to the 10% total fat content 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 (Nain et al., 2012) [10]. The fatty acid composition of an ingredient has a direct effect on fat utilization or deposition in poultry. Similar to other mono gastric 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) [11]. 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 (Brunelli et al., 2010) [12]. Taking advantage of dietary omega-3 fatty acid deposition into yolk, producers are able to create value-added ALA, EPA, and DHA enriched eggs. Corn germ oil

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is an industrial product characterized by high polyunsaturated fatty acids, low saturated fatty acids, essential amino acids, fatty acids, polyesters and tocopherols. Oil is a specialty trait for plant breeders in maize which is also useful and unique (A. Rajendran et al., 2017; Stringhini et al., 2009) [13,14]. The economic viability analysis favored soybean oil suggested that corn germ oil can replace soybean oil in diets formulated with sorghum, since it has higher apparent metabolizable energy and resulted in better breast composition (Marcella M. A., Joao Paulo R. B., 2016) [15,16]. Corn germ oil or corngerm meal is an ALA source and is used by poultry producers in the United States for enriching commercial table eggs and meat products (Samman et al., 2009; Petrovic et al., 2012; Lopes et al., 2013) [17-19]. In order to further investigate how the form of ALA source affects the fatty acid transfer rate from laying hen diet into egg yolk, purified extracted corn germ oil and corn germ meal ingredients were fed at increasing inclusion levels in experimental omega-3 fatty acid ALA enriched laying hen diets.

## Materials and Methods

### Animals and Housing

The experiment was conducted at Thuy Phuong Poultry Research Center under the National Livestock Research Institute - Vietnam in 2017. Single-Comb White Leghorn laying hens (n = 132, Hy-Line W-36, age = 25 wks. old) were obtained from a commercial source as they were approaching peak production and reproductive efficiency. Laying hens were randomly placed into single tier conventional cages, with 3 hens/cage, stock density of 696 cm<sup>2</sup>/bird. Each single cage of 3 hens represented an experimental unit. Treatments were assigned in a complete random design, allowing for 11 dietary treatments with 4 replicates/treatment. Hens were allowed ad libitum access to feed and water during the 8-week experiment from 25 to 33 wks. Of age. Experimental animals' management protocol was approved by Vietnamese Animal Ethic Committee (Convinced by Ministry of Agriculture and Rural Development -MARD - Vietnam).

### Corn Source Enriched Diets

Experimental diets were formulated to meet NRC (1994) [20] recommendation of commercial layer hens. Corn germ oil diets were formulated by adding the purified corn germ oil at the expense of soy oil in the diet (Table 1). Formulations were isocaloric and consisted of a control diet, 5 corn germ oil diets, and 5 corn germ meal diets (calculated corn germ oil concentrations for treatment diets were 0.5, 1.0, 2.0, 3.0, and 5.0%). The corn germ meal ingredient was created by grinding whole corn germ through a 4.0 mm screen with corn as a carrier. To obtain the analyzeoil concentrations in the corn germ meal diets, the corn germ meal 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-week experiment in accordance with MARD policy. All laid 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:  $HHEP \% = (\# \text{ eggs laid} \div \# \text{ hens housed} \div \# \text{ days}) \times 100$ ;  $FI = \text{Start feed weight in kg} - \text{End feed weight in kg} \div \text{Body weight (BW)}$  was recorded at the start, at 4 wks., and at 8 weeks. Average egg weight (EW), egg mass (EM), and feed efficiency were measured weekly from zero to 8 wks.:  $\text{Egg mass} = \text{Average egg weight in g} \times (HHEP \% \div 100)$ . Feed efficiency (FE) was reported as g eggs per kg FI:  $FE = \text{Egg mass in g} \div \text{Feed intake in kg}$ .

### Egg Yolk Analysis

A pooled sample of 5 egg yolks from each replicate was used to measure egg solids and yolk fatty acid profile at 4, 6, and 8 wks. of the experiment. Fatty acid analysis of egg yolk, as previously described by Sun et al. (2013) [21] and Nam et al. (2001) [22] using gas chromatography (HP 6890, Hewlett Packard Co., Palo Alto, CA), was performed starting at 4 wks. to allow an adjustment period for maximal transfer of fatty acids to egg yolk.

### Apparent Metabolizable Energy, Nitrogen Corrected (AMEn) Experiment

After the initial 8-week omega-3 fatty acid enrichment experiment, all birds (now 33 wks. of age) were utilized in a 2-week study to determine the AMEn content of the corn germ oil, as this information is currently lacking in the literature. The laying hens (n = 144, including the unreported 12th 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 corn dietary enrichment would not adversely affect the AMEn experiment. Treatments were assigned in a completely random design allowing for 4 dietary treatments with 12 replicates per treatment for the AMEn experiment. Each unit consisted of 3 hens per cage with identical bird density as previously mentioned in the corn enrichment experiment. Hens were managed as previously described with *ad libitum* access to feed and water for the 2-week AMEn experiment from 33 to 35 wks. of age. Performance data during this 2-week AMEn experiment was not reported.

A basal diet with titanium dioxide (0.30%) and increasing levels of corn germ oil (0.0, 3.0, 6.0, and 9.0% added to the basal diet) were used to generate 4 AMEn treatment diets. Experimental diets were formulated to meet NRC (1994) [23] recommendation of commercial laying hens (Table 3). These diets were fed for a 2-week adjustment period, which served as a washout period for the previous corn egg yolk fatty acid deposition experiment, in order to collect excreta on d 14 for AMEn determination and regression analysis.

### 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  $\times$  week interaction included in the model. Orthogonal contrasts were used to test the response variables of the control against the 10 corngerm source supplemented diets and the 5 corn germ oil vs. 5 corn germ meal supplemented diets. There was only one control group for the experiment and, therefore,



**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 wks. of age.

Ingredient Control diet	Control diet	Corn germ oil					Corn germ meal (% oil concentration)				
		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
Corn germ meal	0.00	0.00	0.00	0.00	0.00	0.00	1.50	3.00	6.00	9.00	15.00
Corn germ 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	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	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)	474	712	946	1419	1892	2837	754	1036	1598	2161	3286
Omega-6 fatty acids (mg)	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 fibre	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

**Table 2:** Analyzed crude fat, fatty acid, and total omega-3 fatty acid concentrations in extracted corn germ oil and corn germ meal ingredients .

Fatty acid (C:double bond)	Corn germ oil %	Corn germ meal %
Myristic acid (14:0)	0.07	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)	3.73	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)	54.22	55.11
Arachidic acid (20:0)	0.58	0.00
Arachidonic acid (20:4)	0.09	0.00
Eicosapentaenoic acid (20:5)	0.17	0.00
Docosapentaenoic acid (22:5)	0.38	0.00
Docosahexaenoic acid (22:6)	0.47	0.00
Crude fat	>99	3.41
Total omega-3 fatty acid	54.88	55.11



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

Concentration (%)	Basal	Corn germ oil		
	0.0	3.0	6.0	9.0
ME (kcal/kg)	2800	-	-	-
Corn	60.19	58.36	56.58	54.77
Meat/bone meal	2.00	1.95	1.89	1.83
Soybean meal 48% CP	25.00	24.25	23.50	22.75
Soy oil	1.33	1.29	1.25	1.21
Corn germ oil	0.00	3.00	6.00	9.00
Sodium chloride	0.42	0.40	0.39	0.38
DL-methionine	0.16	0.15	0.14	0.14
Limestone	9.23	8.95	8.67	8.39
Dicalcium phosphate	0.86	0.84	0.82	0.80
Titanium dioxide	0.30	0.29	0.28	0.27
Phytase	0.00075	0.00073	0.00071	0.00069
Vitamin and Mineral premix	0.50	0.49	0.48	0.47
<b>Analyzed values (%)</b>				
Dietary AMEn as-fed (kcal/kg)	2764	2947	3218	3422
Crude protein	18.00	17.74	17.15	16.77
Crude fat	2.94	4.84	7.86	10.79
Crude fiber	3.16	2.30	2.34	2.19
Moisture	10.13	9.99	9.69	9.62
Ash	14.05	14.28	11.20	10.62

ME: Metabolic Energy; AMEn: Apparent Metabolic Energy nitrogen corrected.

**Table 4:** Egg yolk lipid fraction omega-3 fatty acid (%).

Ome-ga-3 type	Control diet	Corn germ oil (%)					Corn germ meal (% oil concentration)				
		0.5	1.0	2.0	3.0	5.0	0.5	1.0	2.0	3.0	5.0
EPA and DHA	1.12	1.54 <sup>b</sup> ±0.02	1.68 <sup>b</sup> ±0.01	1.85 <sup>a</sup> ±0.02	1.96 <sup>a</sup> ±0.02	2.12 <sup>a</sup> ±0.01	1.39 <sup>b</sup> ±0.01	1.48 <sup>b</sup> ±0.01	1.64 <sup>b</sup> ±0.01	1.92 <sup>a</sup> ±0.01	2.14 <sup>a</sup> ±0.02
Others	1.03	1.49 <sup>c</sup> ±0.02	2.18 <sup>c</sup> ±0.02	3.49 <sup>c</sup> ±0.02	5.61 <sup>b</sup> ±0.01	8.20 <sup>a</sup> ±0.02	1.00 <sup>c</sup> ±0.01	1.46 <sup>c</sup> ±0.01	2.09 <sup>c</sup> ±0.01	2.60 <sup>c</sup> ±0.02	3.07 <sup>c</sup> ±0.02
Total	2.15	3.03 <sup>c</sup> ±0.01	3.86 <sup>c</sup> ±0.11	5.34 <sup>c</sup> ±0.36	7.43 <sup>b</sup> ±0.81	10.32 <sup>a</sup> ±1.35	2.39 <sup>c</sup> ±0.09	2.94 <sup>c</sup> ±0.09	3.73 <sup>c</sup> ±0.10	4.52 <sup>c</sup> ±0.14	5.21 <sup>c</sup> ±0.20

Value in the same row with different subscription letters are different significant (p<0.01).

response variables were tested for linear, quadratic, and cubic orthogonal contrasts of dietary oil percentage (0.5, 1.0, 2.0, 3.0, and 5.0%) and dietary oil percentage × corn germ source interaction for the 5 corn germ oil and 5 corn germ meal 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 ≤ 0.05 was accepted as significant.

## Results

### Enrichment Period Performance

During the dietary corn germ source enrichment period, no

differences were observed for feed intake (FI), EM, or egg solids for the orthogonal contrasts tested (P ≥ 0.07). Quadratic contrast of oil percentage × corn source resulted in increasing then plateauing HHEP as corn germ oil increased in dietary inclusion for the corn germ oil dietary treatments (P = 0.01). As corn germ meal increased in dietary inclusion, HHEP declined then plateaued (P = 0.01). There is a positive correlation between FE and the ratio of corn oil added to diets (P = 0.01) but increasing corn germ meal ratio resulted in decreasing FE (P = 0.01). Linear contrast of dietary oil percentage resulted in decreasing EW as increasing inclusion of either corn source (corn germ oil or corn germ meal equivalent) was added to the diet (P = 0.05). Gains in BW decreased as oil percentage increased in corn germ oil or corn germ meal equivalent diets linearly (P = 0.02). The rate of supplementation of corn germ and corn germ oil was influential



but not in accordance with the change in hens' body weight ( $P = 0.02$ ; Table 5).

### Egg yolk omega-3 fatty acid deposition

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

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 corn germ oil (CGO) treatments containing 0.5, 1.0, 2.0, 3.0, or 5.0% CGO content, or corn germ meal (CGM) treatments containing 0.5, 1.0, 2.0, 3.0, or 5.0% CGO concentration (CGM included at 1.5, 3.0, 6.0, 9.0, or 15.0% of the diet, respectively). The CGM treatments resulted in a linear fit ( $P < 0.01$ ;  $R^2 = 0.808$ ) with an equation of  $y = 0.783x + 2.310$ . The CGO treatments also

resulted in a linear fit ( $P < 0.01$ ;  $R^2 = 0.880$ ) with an equation of  $y = 1.604x + 2.171$ . The CGO dietary treatments deposited 2 times more ALA, EPA, and DHA into egg yolk compared to MFS dietary treatments at equivalent corn germ oil concentrations ( $P < 0.01$ ). Egg yolk EPA and DHA content from hens fed a control diet, or one of the following experimental diets: Purified corn germ oil (CGO) treatments containing 0.5, 1.0, 2.0, 3.0, or 5.0% CGO content, or CGM treatments containing 0.5, 1.0, 2.0, 3.0, or 5.0% CGO concentration (CGM included at 1.5, 3.0, 6.0, 9.0, or 15.0% of the diet, respectively). An experimental unit was a cage of 3 laying hens with 4 replicates for each of the 11 treatments ( $n = 132$ ) fed for 8 wks. (25 to 33 wks. of age). Fatty acid analysis of yolks was performed at 4, 6, and 8 wks. of the experiment using 5 eggs per unit and values in the figure represent the average of the 3 analyzed time points. The CGM treatments resulted in a linear fit ( $P < 0.01$ ;  $R^2 = 0.244$ ) with an equation of  $y = 0.169x + 1.265$ . The CGO 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 corn germ oil concentration by corn germ source (CGO or CGM) for response in egg yolk EPA and DHA deposition ( $P = 0.21$ ).

### Oil AMEn Determination

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

**Table 5:** Laying hen performance<sup>1</sup> from 25 to 33 wks. of age as affected by increasing dietary inclusion of corn germ oil (CGO) or corn germ meal (CGM).

n = 4	Feed intake (g/bird/d)		HHEP %		FE (g eggs/kg feed)		Egg weight (g/egg)		Egg mass (g/bird/d)		Egg solid (%)		BW change (g)	
	CGO	CGM	CGO	CGM	CGO	CGM	CGO	CGM	CGO	CGM	CGO	CGM	CGO	CGM
Oil (%)	CGO	CGM	CGO	CGM	CGO	CGM	CGO	CGM	CGO	CGM	CGO	CGM	CGO	CGM
Control	93.6		95.7		600		57.5		55.4		23.7		75.2	
0.5	92.1	92.9	91.4	98.3	582	641	58.6	59.3	53.7	58.4	23.4	23.5	54.7	79.0
1.0	94.3	93.2	96.7	96.6	596	604	58.0	57.6	56.0	56.0	23.5	23.4	78.6	74.7
2.0	93.7	92.6	98.4	97.6	587	605	55.9	57.3	54.9	56.1	23.6	23.6	80.3	42.4
3.0	94.7	93.1	96.3	95.2	594	589	57.6	57.6	55.9	54.7	23.4	23.5	77.6	58.0
5.0	90.9	93.7	96.3	96.8	604	590	56.5	57.5	54.6	55.5	23.2	23.5	62.2	24.8
SEM	1.58		1.24		11.5		0.99		1.07		0.22		11.73	
Contr. P-values														
Control vs. Corn	0.81		0.68		0.94		1.00		0.79		0.65		0.33	
CGO vs. CGM	0.17		0.06		0.38		0.10		0.52		0.05		0.17	
Linear														
Oil %	0.68		0.57		0.23		0.06		0.18		0.47		0.03	
Oil % × Corn	0.30		0.09		0.02		0.93		0.30		0.25		0.01	
Quadratic														
Oil %	0.38		0.25		0.17		0.24		0.76		0.19		0.38	
Oil % × corn	0.21		0.02		0.28		0.90		0.09		0.29		0.13	
Cubic														
Oil %	0.93		0.02		0.77		0.14		0.95		0.42		0.72	
Oil % × corn	0.95		0.15		0.45		0.63		0.61		0.66		0.13	



slope of the regression line equated to the AMEn value of the corn germ oil ingredient, which was 7,468 kcal/kg on an as-fed basis. The mean analyzed values for AMEn determination of the experimental diets were 2,762, 2,948, 3,218, and 3,421 kcal/kg as the corn germ oil concentration increased from 0.0 to 9.0% for each diet, respectively. Dietary AMEn values on an as-fed basis for hens fed diets containing 0.0, 3.0, 6.0, and 9.0% extracted corn germ oil (CGO) added to a basal diet for 2 wks. (33 to 35 wks. of age). An experimental unit was a cage of 3 hens with 12 replicates for each of the 4 treatments (n = 144). The AMEn 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 AMEn value of the CGO. The CGO had an AMEn value of 7,468 kcal/kg on an as-fed basis.

## Discussion

This experiment was designed to evaluate the impact of structurally different (extracted oil vs. meal) dietary omega-3 fatty acid sources on egg yolk deposition and to determine the AMEn of purified corn germ oil. No significant differences were observed in FI for hens fed diets containing corn germ meal. If any were to be observed, the 5.0% corn germ meal treatment would have been expected to suppress FI due to anti-nutrients present in or palatability of the corn germ meal (Table 5). However, the rate of BW gain of hens fed corn germ meal declined 24 times more than hens fed corn germ oil as dietary inclusions increased. The difference in rate of BW change may have been due to anti-nutrients present in the corn germ meal causing impaired digestion or absorption of the dietary nutrients (Gonzalez-Esquerra and Leeson, 2000; Leeson et al., 2000) [24,25].

Cyanogenic glycosides including linustatin, neolinustatin, and linmarin present in the corn germ meal may have caused a reduction in BW change due to loss of effective intestinal epithelial cell absorptive function (Feng et al., 2003; Kartikasari et al., 2012) [26,27]. In addition, phytic acid present in corn germ meal may result in protein-mineral-phytic acid complexes that are not bio available, reducing BW gain by exacerbating impaired nutrient absorption (Rajendran et al., 2017) [28]. Trypsin inhibitors present in corn germ may have played a minor role in decreasing nutrient bioavailability, but the quantity found in corn germ is insignificant compared to levels found in soybean (Feng et al., 2003) [29]. It has been well documented that mucilage, a water-soluble polysaccharide found in corn germ, increases chicken intestinal content viscosity (Rodriguez et al., 2001) [30]. The increased viscosity inhibits nutrient digestion and absorption of the intestinal ingesta (Alzueta et al., 2003) [31]. Similar declines in performance were observed in FE for hens fed corn germ meal diets as the inclusion increased. Data from this current work suggests that feeding hens corn spout exerted physiologic effects impairing nutrient storage and anabolic activity compared to equivalent inclusions of supplemental corn germ 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 corn germ oil incorporated 66% more total omega-3 fatty acids into yolks compared to corn germ meal. 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) [32]. The presence of anti-nutrients likely contributed to the decreased transfer of ALA due to reduced bioavailability or absorption of the dietary lipids. Extracted corn germ 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 corn germ 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) [33]. 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 percentage increased in the corn germ source treatment groups. EPA and DHA egg yolk inclusion was not different based on corn germ source enriched treatments, suggesting that laying hens deposited modified portions of ALA to egg yolk at a finite but constant rate. The AMEn of the corn germ oil ingredient was determined after the 8-week omega-3 fatty acid corn germ source enrichment to egg yolk experiment, as few data are published regarding the metabolizable energy of corn germ oil.

The energy value found was 7,468 kcal/kg of corn germ oil as fed, which was on the lower end of the predicted value. Corn germ 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) [34]. Previous investigations of corn germ AMEn reported different age-sensitive tolerances to dietary corn germ by poultry. In an experiment feeding 10% corn germ meal supplemented diets to broiler chicks, significantly lower tolerance was observed manifesting as diarrhea compared to mature single comb white leghorn roosters fed corn germ diets (Rajendran et al., 2017) [35]. 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) [36,37]. Therefore, pelleting and extrusion may be a method to offset the energy deficit of corn germ oil compared to soy oil.

## Conclusion

From obtained results, it is concluded that corn germ oil is a well-tolerated, energy dense feed ingredient which had an efficient ability to transfer omega-3 fatty acid ALA into egg yolk as compared to that of ground corn germ products containing a similar total omega-3 fatty acid profile. Increasing dietary concentrations of both corn germ oil and corn germ meal increased ALA concentrations in egg yolk; feeding corn germ oil did not affect BW gain, but feeding corn germ meal. Superior ALA deposition in egg yolk from corn germ oil source as compared to that of corn germ meal suggested a 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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