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Krill Meal Enhances Antioxidant Levels and n-3 Fatty Acid Content of Egg Yolk from Laying Hens Fed a Low-Pigment Diet

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This study was conducted to determine effects of krill meal supplementation on production performance, egg quality, antioxidant substances, and fatty acid composition of egg yolk from hens fed a low-pigment diet. A total of 640 laying hens (Lohmann Brown), which were each 25 weeks old, were divided into 4 dietary treatment groups. Each treatment consisted of 8 replications with 20 laying hens per replication. The treatments were corn-soybean meal basal diet (CS), CS with 7.5% cassava meal (low-pigment; LP), and LP with 1.5 or 3% krill meal. All dietary treatments were formulated to be isocaloric (2,750 kcal/kg metabolizable energy) and isonitrogenous (17.5% crude protein). Birds were raised in an evaporative cooling system house for 8 weeks (25−33 weeks of age). Water was provided *ad libitum* and feed was provided according to breed requirement recommendations. The LP diets supplemented with krill meal had no effect on production performance and egg quality compared to those of the CS group ($P>0.05$). However, the LP diet caused a significant reduction in yolk color score, and astaxanthin, vitamin A, and vitamin E contents of egg yolk ($P<0.05$). However, the contents of these nutrients increased as the level of krill meal was increased in the diets ($P<0.05$). The highest yolk color score, and astaxanthin, vitamin A, and vitamin E contents were observed in laying hens fed 3% krill meal ($P<0.05$). The LP diet had no effect on n-3 fatty acid content; however, a significant reduction in the content of n-6 fatty acids, especially linoleic acid was observed ($P<0.05$). Further reduction occurred when higher level of krill was used in the diets ($P<0.05$). An increase in krill meal level significantly increased docosahexaenoic acid but not linolenic acid content of egg yolk. Krill meal, therefore, could be used to produce docosahexaenoic acid and antioxidant enriched eggs.

Key words: astaxanthin, krill meal, n-3 fatty acids, yolk color, vitamin A, vitamin E

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Introduction

Thailand is a major producer of cassava (*Manihot esculenta* L. Crantz) and also the world's leading cassava exporter (Kolawole *et al*., 2010). Cassava is widely used as the main energy source in animal diet. Many studies have demonstrated that cassava is a good energy source for poultry (Stevenson and Jackson, 1983; Ravindran and Blair, 1991). However, its utilization in the diet of laying hens is limited because it lacks pigments, which results in poor egg yolk color. The color of yolk is an important characteristic because it influences consumer preference. Although the degree of yolk color preferred by consumers differs in different parts of the world, deeper hues attract significant premiums in most markets. The food processing industry also prefers to use dark colored yolks rather than add artificial coloring agents. To improve yolk color, synthetic pigments such as chlorophyll red are generally added in the diets of laying hens. Such synthetic pigments, however, are costly and have a maximum allowable limit in feed. The European Union limits the addition of synthetic canthaxanthin such that it yields not more than 25 mg/kg canthaxanthin in the egg yolk. The reason is that this pigment could have a negative impact on human health, such as causing cancer (Commission Regulation, EC, No. 755/2008).

Astaxanthin (3,3′ -dihydroxy-β,β-carotene-4,4′ -dione) is a

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dark red pigment and a member of the carotenoid family. It is the major carotenoid found in algae and aquatic animals, including salmon, trout, shrimp, and krill (Britton, 1995; Fredriksson *et al*., 2006). Astaxanthin is a valuable compound not only for pigmentation but also for protection against lipid oxidation, inflammation, cardiovascular disease, and cancer (Palozza and Krinsky, 1992; Mortensen and Skibsted, 1997; Han *et al*., 2006). Astaxanthin has an antioxidant activity 10 times higher than that of β-carotene and 300 times more effective than α-tocopherol (Higuera-Ciapara *et al*., 2006; Rao *et al*., 2009). Most previous research with astaxanthin has focused on its pigmentation effect and not on its possible nutrient enrichment properties. Astaxanthin has been used successfully to improve the pigmentation of egg yolk, salmon, trout (Gobantes *et al*., 1997; Barbosa *et al*., 1999), and chicken meat (Takahashi *et al*., 2004). Dike *et al*. (1992) and Akiba (2000) found that the addition of low concentrations of astaxanthin-containing yeast, *Phaffia rhodozyma*, to the diet of laying hens enhanced the color of the egg yolk. However, there is little information on the dose response relationship between astaxanthin and yolk color.

Krill are zooplankton invertebrates that are similar to small shrimps. There are about 85 species of krill, differing in size, which are found in the oceans around the Arctic, Antarctica and North America (Tou *et al*., 2007; Chen *et al*., 2009). Krill meal is also a good source of astaxanthin, vitamin A, vitamin E, and n-3 fatty acids (Savage and Foulds, 1987; Kolakowskan *et al*., 1994; Stine and Kirsten, 2015).

Published data on the use of krill meal as a pigment source in the diet of laying hens are scant. In the present study, graded levels of krill meal were evaluated as a source of pigment antioxidant and n-3 fatty acids when included in diets containing cassava meal, a local feed ingredient. It was hypothesized that such inclusion will enrich the functional antioxidant and fatty acid contents in egg yolk with no detrimental effect on laying hen performance and egg quality.

Materials and Methods

Ingredients

Cassava meal used to produce low-pigment diet in this study was purchased from a local supplier. Krill meal was obtained from a local supplier (Marine Leader Co. Ltd). It was made from whole ground and dried Antarctic krill (*Euphausia superba*). Particle size distribution of krill meal was measured using a sieve shaker (model RX-86, W. S. Tyler, Mentor, Ohio) equipped with six sieves (U.S. standard sieve Nos. 10, 20, 30, 40, 45, and 60) and a pan. The sieving procedure was similar to that described by ASAE Standards (1995). The procedure required 100 g of krill meal and 10 min sieving time. The sieve sizes determined the particle size categories, which are reported in g per 100 g retained on the smaller screen: 2.0 mm and larger (2.0 mm), 0.84 to 2.0 mm (0.84 mm), 0.60 to 0.84 mm (0.60 mm), 0.43 to 0.60 mm (0.43 mm), 0.35 to 0.43 mm (0.35 mm), 0.25 to 0.35 mm (0.25 mm), and less than 0.25 mm, respectively. Dry matter, crude protein, crude fat (EE), ash, calcium and phosphorus (AOAC, 2012) of krill meal samples were analyzed. The analyzed chemical composition and particle size of krill meal are shown in Table 1.

Table 1. **Nutrient composition of Antarctic krill (*Euphausia superba*)**

Nutrient composition	Value	Unit
Protein	58.4	g/100 g
Ash	10.5	g/100 g
Fat	23.2	g/100 g
Moisture	6.8	g/100 g
Calcium	19.1	g/kg
Phosphorus	13.8	g/kg
Particle size	800	micron
% Total Fat		
Astaxanthin	115.4	mg/kg
Vitamin A	1,170.0	μg/100 g
Vitamin E	9.6	mg/100 g
Total Omega 3	24.3	g/100 g
EPA (C20:5)	12.1	g/100 g
DHA (C22:6)	6.5	g/100 g
Total Omega 6	2.5	g/100 g

Animal Care and Dietary Treatments

The experimental protocol used in this study was conducted under the Animal Care and Use for Scientific Research of Kasetsart University guidelines (ACKU60-AGK-053). The feeding trial was conducted for a duration of 8 weeks. A total of 640, 25 weeks old, laying hens (Lohmann Brown) were used in this experiment. Hens were allocated to cages (4 birds per cage) and housed in a curtain-covered, evaporative cooled housing system. The lighting program consisted of 16 hours of light exposure per day. The diets were offered in mash form, and water was provided *ad libitum*.

The four dietary treatments were corn-soybean meal basal diet (CS), CS containing 7.5% cassava meal (Low-pigment; LP) and LP with 1.5 or 3% krill meal. All dietary treatments were formulated to be isocaloric (2,750 kcal/kg metabolizable energy, ME) and isonitrogenous (17.5% crude protein, CP) and met or exceeded the nutrient requirements for laying hens as recommended by the breeding company (Lohmann Tierzucht GmbH. Abschnede 64, 27472 Cuxhaven, P.O. Box 460, 27454 Cuxhaven, Germany) (Table 2). The experiment was conducted using a completely randomized design. Each treatment consisted of 8 replications with 20 birds per replication, which were housed in five adjacent cages (4 birds per cage).

Analysis of Laying Hen Performance

All hens were weighed individually at the beginning and end of the experiment. Egg production and feed intake were recorded daily. In each 28-day period of the experiment, daily feed intake (FI), feed conversion ratio (FCR), hen-day egg production (HD) and hen-housed egg production (HH), were determined on a per replicate basis. FCR was expressed as gram of feed consumed per gram of egg produced.

Table 2. **Composition and analysis of experimental diets (as-fed basis)**

Ingredient	Diets			
	CS	LP	LP with 1.5% krill meal	LP with 3% krill meal
Corn	55.65	47.14	48.18	49.15
Soybean meal, 49%	24.59	25.59	23.72	21.85
Rice bran	5	5	5	5
Fat-extracted rice bran	3	3	3	3
Cassava meal	-	7.5	7.5	7.5
Krill meal	-	-	1.5	3
Soybean oil	0.94	0.98	0.48	0
DL- methionine	0.17	0.18	0.12	0.07
Choline chloride, 50%	0.01	0.01	0.01	0.1
Calcium carbonate ($CaCO_3$)	8.57	8.52	8.48	8.42
Monodicalcium phosphate, 21%	1.46	1.48	1.41	1.4
Salt	0.36	0.35	0.35	0.35
Vitamin-trace mineral premix*	0.25	0.25	0.25	0.25
Calculated analysis (%)				
GE, kcal/kg	3952.44	3928.49	3922.4	3944.22
Protein	17.84	17.75	17.82	17.91
Fat	3.79	3.88	3.96	3.21
Fiber	2.3	2.08	2.05	2.03
moisture	9.13	9.64	9.59	9.85
Ash	13.58	14.56	14.25	14.32
Calcium	3.95	3.94	3.48	3.99
Total phosphorus	0.97	0.92	0.94	0.98

* Supplied the following per kilogram of complete feed: vitamin A, 5,000,000 IU; vitamin D_3, 1,200,000 IU; vitamin E, 4,000 IU; vitamin K_3, 0.60 g; vitamin B_1, 0.8 g; vitamin B_2, 2.0 g; vitamin B_6, 1.2 g; vitamin B_{12}, 0.0025 g; pantothenic acid, 3.76 g; folic acid, 0.2 g; nicotinic acid, 5.0 g; biotin, 0.036 g; manganese, 24 g; zinc, 20 g; iron, 16 g; copper, 4 g; iodine, 0.80 g; cobalt, 0.08 g; selenium, 0.04 g.
CS＝Corn-soy basal diet
LP＝CS with 7.5% cassava meal
KM＝Krill meal
GE＝Gross energy

Egg mass was calculated by multiplying the egg weight by egg production.

Analysis of Egg Quality

On the last 3 days of each 28-day period, 3 eggs per replicate per day were collected for egg quality measurements. Each egg was weighed individually, and the quality was measured by the breaking out method. The proportion of egg components (yolk, albumen and shell) was recorded. Egg yolk color was evaluated visually by means of the Roche Yolk Color Fan (DSM, Basel, Switzerland). Shell thickness (without shell membrane) was measured by a micrometer (Coolant Proof Micrometer, Series 293-with Dust/Water Protection Conforming to IP65 Level). The quality of egg albumen was measured in Haugh units (HU), calculated using the formula indicated by Haugh (1937).

Chemical Analysis

Egg Yolk Fat Extraction

On the last 3 days of each 28-day period, representative samples of yolk from 3 eggs per replicate per day were obtained, pooled and stored at −20℃. Before fat extraction, egg yolk was thawed to room temperature. Five grams of egg yolk were weighed (wet weight) and added to 30 mL of extraction solution (chloroform/methanol: 2/1). For each extraction process, solvents were mixed in a round bottom flask. Egg yolk was added to the extraction solvent in the form of a thin squirt while mixing vigorously. Extraction was done at 25℃ for 30 minutes and then filtered using vacuum filtration and collected into a clean container (Folch *et al*., 1957). The oil was recovered by evaporation of the solvent mixture using a rotary evaporator and stored in a vial under −20℃ for astaxanthin, vitamin A, vitamin E and fatty acid profile analysis.

Analysis of Astaxanthin Content

Astaxanthin content was analyzed according to the method described by Pavasant *et al*. (2008) with some modifications. A mixture of acetonitrile/dichloromethane/ethanol (5/10/85) was used as the mobile phase. The sample in 1 mL of hexane was then solubilized. The filtrate was transferred to high-performance liquid chromatography (HPLC) equipment (Waters 2998 Photodiode Array Detector, U.S.A.) using C18 column (150×4.6 mm, Waters, Milford, MA, U.S.A.). The flow rate and detection wavelength were kept constant at 1.0 mL/min and 470 nm, respectively. Astaxanthin content was calculated from a calibration curve using an astaxanthin standard (Dr. Ehrenstorfer GmbH Co., Augsburg, Germany).

Analysis of Vitamin A Content

Vitamin A content was analyzed according to the method described by Cort *et al*. (1983). The sample was solubilized using H_2Cl_2 containing 0.001% trimethylamine/acetonitrile/methanol (300/700/0.5) as the mobile phase. The filtrate was transferred to high-performance liquid chromatography (HPLC) equipment (Waters 2475 Multi λ Fluorescence Detector, USA). The flow rate and detection wavelength were kept constant at 0.3 mL/min and 280 nm, respectively. Vitamin A content was calculated from a calibration curve using a vitamin A standard.

Analysis of Vitamin E Content

Vitamin E content was analyzed using the method described by AOAC (1990). The sample was solubilized using methanol/water (7/3) as mobile phase. The filtrate was transferred to high-performance liquid chromatography (HPLC) equipment (Waters 2998 Photodiode Array Detector, U.S.A.) using C18 column (150×4.6 mm, Waters, Milford, MA, U.S.A.). The flow rate and detection wavelength were kept constant at 1.0 mL/min and 298 nm, respectively. Vitamin E content was calculated from a calibration curve using tocopherol standard.

Analysis of Fatty Acid Content

The fatty acids were analyzed using the methods described by Folch *et al*. (1957) and AOAC (1990) with some modifications. The extracted lipids (100 mg) were dissolved in 5 mL toluene and methylated with 3 mL BF_3/MeOH. The fatty acid methyl esters were extracted with 3 mL hexane and 1 mL 10% NaCl. The solution was filtered through a mem brane filter (0.45 μm) and analyzed using gas chromatography [GC; CP9001, CHROMPACK, Netherlands; GC capillary column, 50 m×0.25 mm i.d., 0.2 μm; carrier gas, helium; detector, flame ionization detector; column temperature, 140−215℃ at a rate of 4℃/min]. Each fatty acid was calculated by comparing it to a standard fatty acid methyl ester mixture (Sigma-Aldrich, St. Louis, MO, USA) with the retention time under identical conditions. The fatty acid content was expressed as a percentage of each peak area.

Statistical Analysis

The data was analyzed using the General Linear Model of SAS (SAS, 2015). Treatment effect was considered to be significant at $P<0.05$. Mean values of variables having a significant F-test were separated using the Least Significant Difference test.

Results and Discussion

Laying Hen Performances

The effects of graded levels of krill meal inclusion on laying hen performances are summarized in Table 3. LP diet l had no effect on the feed intake, FCR, hen-day egg production, hen-housed egg production, egg weight and egg mass when compared to that of the CS group ($P>0.05$). When 7.5% cassava meal was incorporated in the diet, the level of corn was reduced because cassava is documented as a good energy source for poultry. However, Lei *et al*. (2017) indicated that cassava meal could replace up to 30% of corn in the diet of laying hens without any adverse effects on performance.

The inclusion of krill meal in the LP diet at 1.5 and 3% had no effect on overall laying hen performances when compared to those of the CS and LP groups ($P>0.05$). Krill meal contains approximately 280 mg chitin/100 g (Hansen *et al*., 2010) which is lower than that of red crab meal (890 mg/100 g) (Carrillo-Dominguez *et al*., 2005). The research conducted by Carrillo-Dominguez *et al*. (2005) indicated that red crab meal could be incorporated up to 6% in the diet with no adverse effect on feed intake of laying hens. These findings are in agreement with those of Nakpun (2013) who reported that the use of krill meal (*Euphausia superba*) up to 5% in isonitrogenous and isocaloric diets had no effect on feed intake and overall laying hen performances. Similarly, Rys and Koreleski (1979) also found that using up to 3% krill

Table 3. **Effect of krill meal supplementation on laying hen performance***

Item	BW (kg)	FI (g/d)	FCR	HD (%)	HH (%)	Egg weight (g)	Egg mass (g)
CS	1.85	105.8	1.78	93.7	92.0	58.5	54.9
LP	1.84	108.6	1.83	95.4	92.9	58.7	56.0
LP with 1.5% KM	1.84	107.7	1.81	97.8	90.4	58.7	57.4
LP with 3% KM	1.85	109.3	1.85	93.6	90.0	58.9	55.1
SEM	0.12	1.41	0.02	0.69	0.63	0.17	0.48
P-value	0.63	0.84	0.78	0.13	0.35	0.91	0.25

* Each mean represents values from eight replicates (20 birds/replicate).
Laying hens 25 to 33 weeks of age.
BW=Body weight
FI=Daily feed intake
FCR=Feed conversion ratio
HD=Hen-day egg production
HH=Hen-housed egg production
CS=Corn-soy basal diet
LP=CS with 7.5% cassava meal
KM=Krill meal

meal (*Euphausia superba*) in diets had no effect on broiler and laying hen performances. These results indicated no negative effect of chitin on feed intake and laying hen performances when krill meal was used at low levels in the diet. However, feed intake of laying hens in the present study was numerically higher in LP and LP diet supplemented with krill meal. The change in bulk density of the diets could possibly be the cause. Cassava meal is known as a low bulk density (372.4 kg/m^3) feed ingredient compared to corn (606 kg/m^3) (American Feed Industry Association, 2005; Oladunmoye *et al*., 2010). Using cassava meal to produce LP diet caused a reduction in corn level since it substituted for the energy from corn. This could lower bulk density of the LP diet, causing higher feed wastage and higher average feed intake of laying hens.

Egg Quality

Albumen quality and the relative proportions of egg components were not influenced ($P>0.05$) by dietary treatments (Table 4). A previous study by Yang *et al*. (2006) also indicated that there was no effect of the astaxanthin supplements in corn-soy basal diet (1.3 mg/kg) on egg quality. However, the LP diet decreased the yolk color score when compared to that of the CS group. Yolk color increased ($P<0.05$) with an increase of krill meal level in LP diet. Krill meal is a good source of the dark-red pigment, astaxanthin. An increase in yolk color with krill meal inclusion suggests the efficient transfer of the pigment to egg yolk. Mammershoj (1995) found that diets containing 1.5−3.0 mg/kg astaxanthin resulted in a significant increase of yolk color. Elwinger *et al*. (1997) reported that yolk color score increased from of 5.8 to 11.8 when 0.5−3.0 mg/kg astaxanthin was supplemented in the diet of laying hens.

Astaxanthin Content of Egg Yolk

The astaxanthin content of egg yolk from hens fed CS and LP diet with 0, 1.5 and 3% krill meal differed ($P<0.05$; Table 5). As expected, the LP diet lowered the astaxanthin content of egg yolk compared with that of the CS group ($P<0.05$). Replacing corn with cassava would have lowered the

Table 4. **Effect of krill meal supplementation on egg quality***

Item	Yolk color (score)	Shell thickness (μm)	HU	Albumen weight (%)	Yolk Weight (%)	Shell weight (%)
CS	8.2^b	354.4	84.7	65.3	25.3	12.8
LP	7.1^c	353.9	83.6	65.6	25.4	12.9
LP with 1.5%KM	8.2^b	353.7	85.1	66.0	25.1	12.8
LP with 3%KM	8.6^a	350.1	85.6	65.1	25.0	13.8
SEM	0.06	0.01	0.48	0.25	0.04	0.13
P-value	<0.01	0.50	0.49	0.91	0.46	0.33

$^{a, b, c}$ Values within a column with different superscripts are significantly different ($P<0.05$).
* Each mean represents values from 144 eggs per treatment
CS=Corn-soy basal diet
LP=CS with 7.5% cassava meal
HU=Haugh unit
KM=Krill meal

Table 5. **Effect of feeding graded levels of krill meal on astaxanthin, vitamin A and vitamin E***

Item	Astaxanthin (mg/kg)	Vitamin A (μg/100 g)	Vitamin E (mg/kg)
CS	4.92^b	586.30^a	3.74^c
LP	3.65^d	514.28^c	3.38^d
LP with 1.5%KM	3.74^c	532.23^b	4.07^b
LP with 3%KM	5.13^a	598.95^a	4.18^a
SEM	0.01	0.01	0.01
P-value	<0.01	<0.01	<0.01

$^{a, b, c}$ Values within a column with different superscripts are significantly different ($P<0.05$).
* Each mean represents values from 144 eggs per treatments (pooled and stored at −20℃).
CS=Corn-soy basal diet
LP=CS with 7.5% cassava meal
KM=Krill meal

beta-carotene content, which is a precursor to astaxanthin synthesis (Fraser *et al*., 1997). The inclusion of 1.5% krill meal increased yolk astaxanthin content to a level higher than that of the LP group but lower than the CS group ($P<0.05$). The highest astaxanthin content was observed in hens that were fed the 3% krill meal diet ($P<0.05$). These findings coincided with those of Nakpun (2013), showing that the supplementation of krill meal up to 5% in the diet of laying hens increased astaxanthin content of egg yolk. Yang *et al*. (2006) also found that the supplementation of astaxanthin (1.3 mg/kg diet) increased the astaxanthin in egg yolk. Similar effects have been observed in experiments with aquatic animals. Torrissen (1989) reported that adding up to 190 mg astaxanthin per kg diet for 10 weeks caused an increase in astaxanthin content of salmon. Astaxanthin is considered a good source of natural pigment and a potent natural antioxidant. An increase in astaxanthin content of egg yolk would not only increase yolk color but also protect against yolk lipid peroxidation. Enrichment of astaxanthin in egg would improve egg quality, prolong shelf life and ultimately deliver a superior nutritional value for human health (Walker *et al*., 2012).

Astaxanthin (3,3′ -dihydroxy-β,β-carotene-4,4′ -dione) is a dark-red pigment and a member of the carotenoid family. It is the main carotenoid found in algae, such as *Haematococcus pluvialis*; yeast such as *Phaffia rhodozyma*; 3 aquatic animals including salmon, trout, and shrimp; and birds, such as flamingo and quail. Astaxanthin has a strong antioxidant activity, 10 times higher than that of β-carotene and 300 times more effective than α- tocopherol. Astaxanthin from *H. pluvialis* is an excellent compound not only for pigmentation but also for protecting against lipid oxidation,

Vitamin A Content of Egg Yolk

Vitamin A plays many important roles in animal and human health. It is essential for vision, reproduction, growth and maintenance of differentiated epithelia, and mucus secretions (Wald, 1968; Goodman, 1980). Evidence also demonstrates that vitamin A is involved in gene transcription, and aspects of immunity (Combs, 1999). Cassava meal is not only a poor source of pigment but also of vitamin A. It contains only 1 μg of vitamin A/100 g (Montagnac *et al*., 2009). Using cassava meal lowered the vitamin A content of LP diet and consequently vitamin A deposition of egg yolk when compared to that of the CS group ($P<0.05$; Table 5). Krill meal has higher vitamin A content (1,170 μg/100 g) than that of cassava meal (1 μg/100 g) (Montagnac *et al*., 2009; Hansen *et al*., 2010). The supplementation of krill meal in the LP diet significantly increased vitamin A content of egg yolk in a dose dependent manner ($P<0.05$). However, vitamin A content was lower in the LP diet with 1.5% krill meal than in the CS group. Using cassava meal to produce the LP diet caused a reduction of 8.5% of corn in the diet (Table 2). Although vitamin A content of corn (490 μg/100 g; Montagnac *et al*., 2009) is less than that of krill meal (1,170 μg/100 g; Table 1), the supplementation of 1.5% krill meal could not recompense the reduction of vitamin A content caused by the 8.5% reduction of corn in the LP diet.

Vitamin E Content of Egg Yolk

The vitamin E content of egg yolk from hens fed the CS and LP diets with 0, 1.5 and 3% krill meal differed ($P<0.05$; Table 5). The LP diet significantly lowered vitamin E content of egg yolk when compared to that of the CS group ($P<0.05$). Cassava meal contains only 190 μg vitamin E/ 100 g (Montagnac *et al*., 2009). Using cassava meal lowered vitamin E content in the diet and subsequently vitamin E deposition in egg yolk. Krill meal used in this study contains a higher level of vitamin E when compared to that of cassava (Table 1). Tou *et al*. (2007) reported an even higher vitamin E content of krill meal (15 mg/100 g). When krill meal was supplemented in the diet of laying hens, vitamin E content of egg yolk significantly increased with an increase in krill meal level ($P<0.05$). Vitamin E is widely known as a substance that promotes health, prevents and cures disease. The mechanisms by which vitamin E might provide these benefits involve its antioxidant and anti-inflammatory functions, an inhibition of platelet aggregation and immune enhancement. Vitamin E can alleviate many diseases, for example, heart disease, cancer, eye disorders, and cognitive decline (Rengaraj and Hong, 2015). An increase of egg yolk vitamin E content due to krill meal supplementation would therefore have a beneficial effect on human health.

n-6 and n-3 Fatty Acids Content of Egg Yolks

Effects of feeding graded levels of krill meal on the n-3 fatty acids of egg yolk, namely alpha linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of egg yolk are summarized in Table 6. There was no significant difference in the DHA contents among the CS and LP diet groups ($P>0.05$). The inclusion of 1.5 and 3% krill meal in the LP diet increased the ALA and DHA contents ($P<0.05$). No significant differences in egg yolk EPA were observed among the dietary treatments ($P>0.05$). Similarly, Songarj (2013) also reported that algae supplements to diets, as a source of omega 3 fatty acids, did not increase EPA deposition in egg yolk.

LP diet and LP diets containing krill meal had no effect on arachidonic acid (AA) content ($P>0.05$). Corn and soybean meal contain high levels of n-6 fatty acids (Cleland *et al*., 1992; de Lorgeril *et al*., 1994; Renaud *et al*., 1995; de Lorgeril and Salen, 2000), especially linoleic acid (LA). The highest level of LA determined in egg yolks was from hens fed the CS diet, as expected. A lower ($P<0.05$) LA content was observed when cassava meal was incorporated into the diet and further reductions were observed with the inclusion of krill meal. However, there was no significant difference in the n-6/n-3 fatty acid ratio of egg yolk between the CS and LP diets. However, there was a significant reduction in the n-6/n-3 fatty acid ratio in egg yolk as the krill meal increased in the diets ($P<0.05$). This could be attributed largely to increases in DHA.

In conclusion, a low-pigment diet caused a reduction in yolk color score, and astaxanthin, vitamin A. and vitamin E contents of egg yolk ($P<0.05$) but did not influence other egg quality parameters. Addition of 1.5 and 3% krill meal in low-pigment diets improved the egg yolk color and increased

Table 6. **Effect of feeding graded levels of krill meal on omega 6, omega 3 fatty acid content and n-6/n-3 ratio of egg yolk***

Item	n-6 (mg/kg)		n-3 (mg/kg)			n-6/n-3
	LA	AA	EPA	DHA	ALA	
CS	673.2[a]	31.1	0.0	0.0[c]	5.50[b]	127.2[a]
LP	666.2[b]	31.0	0.0	0.0[c]	5.50[b]	126.7[a]
LP with 1.5%KM	663.9[c]	30.9	0.0	13.9[b]	5.56[ab]	35.7[b]
LP with 3%KM	663.1[c]	31.1	0.0	18.4[a]	5.61[a]	29.0[c]
SEM	0.3	0.11	0.0	0.01	0.01	0.15
P-value	<0.01	0.94	0.0	<0.01	<0.01	<0.01

[a, b, c] Values within a column with different superscripts are significantly different ($P<0.05$).
* Each mean represents values from 144 eggs per treatment (pooled and stored at −20℃).
LA=Linoleic acid
AA=Arachidonic acid
EPA=Eicosapentaenoic acid
DHA=Docosahexaenoic acid
ALA=Alpha linolenic acid
CS=Corn-soy basal diet
LP=CS with 7.5% cassava meal
KM=Krill meal

the contents of astaxanthin, vitamin A, vitamin E and n-3 fatty acid, especially DHA in the egg yolk. Hence, it can be concluded from the present study that krill meal is beneficial for producing antioxidant and n-3-enriched eggs.

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Conflicts of Interest

The authors declare no conflict of interest.

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