© 2016 IJSRST | Volume 2 | Issue 6 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Red Crab (*Pleuroncodes Planipes*) Meal in Laying Hen Rations and Their Effect on Lipid Fraction and Oxidation of Egg Stored at Different Times and Temperatures

Carranco-Jáuregui ME[*,1], Calvo-Carrillo MC[1], Carrillo-Domínguez S[1], Fuente-Martínez B[2], Avila-González E[2]

[1]Animal Nutrition Department, Dr. Fernando Pérez-Gil Romo, National Institute of Medical Sciences and Nutrition Salvador Zubirán, Mexico City, Mexico

[2]Centro de Experimentación, Investigación y Extensión en Producción Avícola, Universidad Nacional Autónoma de México, Manuel M López S/N, Colonia Zapotitlan, Delegación Tláhuac, C.P. 13209, Ciudad de México, Mexico

ABSTRACT

The aim of this work was to evaluate the effect of including red crab meal (RCM) on laying hen performance, lipid fraction and oxidation of fresh and stored egg at different times and temperatures. 90 Isa-Brown hens, 32-week-old were randomly assigned to 2 treatments, with 5 replicates of 9 birds. The hens was fed sorghum-soybean basal diet (Control) or 4% red crab meal (RCM). Productive performance were evaluated daily. At the end 250 eggs were collected from each treatment group and stored as follows: fresh eggs, 15 and 30 days at 20°C and 4°C. Egg fatty acids, astaxanthin, peroxide index, TBAR's and yolk color were determined. No significant difference in productive performance among treatments. Levels of α-linolenic acid, linoleic acid, and arachidonic acid were significantly different ($P < 0.05$) and were decreased at 30 days/20°C of storage. Significant differences ($P < 0.05$) were reported for total lipids showing a three way interaction, indicating that the differences were due to the influence of these three variables, with 30 days/20°C of storage having the lowest value of total lipids. Significant differences can be seen ($P < 0.05$) in eicosapentaenoic acid, docosahexaenoic acid, and total n-3 in the treatment variable, with the highest values observed in the diets enriched with 4% RCM, compared to the control. However, for total n-6, a significant difference ($P < 0.05$) was observed in the storage time variable, which was lowest at 30 days. Astaxanthin increased compared with the control, but the values for eggs stored 30 days/20°C were lower ($P < 0.05$). However, significant differences were observed ($P < 0.05$) as well as a two way interaction for times and temperatures, with a decrease in yolk color at 30 days/20°C. TBARS not detected. Including 4% RCM in the diets of egg laying hens as a partial substitute for soybean meal did not affect any productive parameters and made it possible to obtain eggs enriched with n3 fatty acids.

Keywords: Red crab meal**,** Egg, Lipid fraction, Oxidation, Stored

I. INTRODUCTION

In Mexico, one of the most common natural resources used as an ingredient for animal feed is the crustacean known as "langostilla" (*Pleuroncodes planipes* Stimpson), which has a highly valued chemical composition for animal nutrition; however, there is currently no fishery that is dedicated to this resource. The proximate composition of "langostilla" varies depending on the area of capture, season, age of the organisms, etc., with the most abundant components being crude protein (21.2-54.7%), ash (12.8-35.9%), chitin (4.76-21.6%), ether extract (4.7-14%) and carotenoids (10-16 mg/100 g) [1]. Although fat content can vary, the fatty acid composition consists of mostly unsaturated fatty acids, especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. These fatty acids are present in the majority of marine products and are important for human health because of their antithrombotic and antitumor properties as well as their

effect in decreasing blood cholesterol levels, among other benefits [2]. The presence of astaxanthin (red pigment) is another characteristic that makes "langostilla" a resource of interest because it represents 95% of the carotenoids in this crustacean (astaxanthin esters, 83.5%; free astaxanthin, 12.1%, and β-carotene, 4.4%) [1, 3].

However, this resource is not intended for human consumption because of its small abdominal muscle. Rather, it has been studied mainly as a source of pigment in diets for aquatic organisms and poultry. From 1990 to date, alternative uses for "langostilla" are still being searched for, and one of the easiest ways is to use it is as meal [4, 5, 6].

In contrast, the poultry industry generates good quality food at low cost for human consumption; therefore, the laying hen eggs has achieved great prominence. Because the consumption of marine products in Mexico is very low, while the diet is very poultry high (22 kg/per capita) [7], it has been proposed that including red crab meal in the diet of laying hens can modify the concentration and quality of the lipid fraction of the egg, resulting in a product with added value. However, the egg reaches the consumer days after it was laid, and the consumer or the business that sells the eggs also store the eggs for different times and at different temperatures. As such, effects on product quality are not known, including whether total lipid content and n-6 and n-3 fatty acid, fat-soluble vitamin and astaxanthin levels are modified and whether oxidation occurs [8, 9]. Based on this concept, it is of utmost importance to maintain egg quality during storage once it is enriched. However, concrete data on this subject is lacking, which is why the objective of this study was to determine the effect of including red crab meal in rations for laying hens in productive variables, lipid fraction, and oxidation of fresh and stored eggs at different times and temperatures.

II. METHODS AND MATERIAL

A. Red Crab Meal (RCM)

60 kg of RCM was provided by CIBNOR (The Northwest Center for Biological Research), Guaymas Unit, Sonora, Mexico. Subsequently, the meal was transported to the City of Mexico to the Department of Animal Nutrition Dr. Fernando Pérez-Gil Romo at the National Institute of Medical Sciences and Nutrition Salvador Zubirán. The meal was stored in black plastic bags and frozen (-20°C) until use.

B. Chemical Analysis (RCM)

The following analyses were performed on the RCM according to standardized techniques published by the Association of Official Analytical Chemists [10]: crude protein, total ash, ether extract, minerals (Ca, Na and Mg), gross energy of RCM, was determined a total combustion using an adiabatic bomb calorimeter (Parr 1755, Parr Instrument Company, Moline, Il, USA).

C. Total Lipid and Fatty Acid Analysis (RCM)

About 2 g of RCM, was taken for total lipid extraction following the method 923.07 [10]. Fatty acid methyl esters were prepared from total lipids extract following method 969.33 [10] by gas chromatograph method.

D. Astaxanthin Cuantification (RCM)

A methodology described previously, Surai and Speake [11], and Pérez-Gálvez et al [12] by spectrophotometry method.

E. Birds, Diets, and Housing

The procedure for the use of birds was according to The Technical Specifications for the Production, Care and use of Laboratory Animals [13].

This test was conducted at the CEIEPAV (Center for Teaching, Research and Extension in Poultry Production), in the School of Veterinary Medicine of the National Autonomous University of Mexico.

The sorghum-soybean meal-based diet and the 4% RCM diet were formulated to meet the nutrient requirements of the National Research Council [14] for laying hens through Nutrion Windows™ (Version 5.0 Pro), a computerized system for feed formulation (Guadalajara, Jal., Mexico) (Table I).

Table I. Experimental diet composition and calculated nutrient analysis

	Experimental diets	
Ingredients (g/kg diet)	Control	Red crab meal
Sorghum	673.957	701.255
Red crab meal	0.000	40.000
Calcium carbonate	107.159	92.204
Soybean meal	182.800	150.932
Calcium phosphate 1821	11.931	9.356
Vegetal oil	10.000	0.000
Salt (NaCl)	3.626	0.290
Premix[a]	2.500	2.250
L-Lysine HCl	2.426	0.000
DL-Methionine	2.249	1.163
Mycotoxin sequestrant	1.000	1.000
L-Threonine	0.703	0.000
Avelut powder[b]	0.500	0.500
Choline chloride 60%	0.500	0.500
Bacitracin-zinc	0.300	0.300
Avired[c]	0.200	0.000
Antioxidant	0.150	0.000*
Nutrient analysis		
ME (kcal/kg)	2,715	2,715
Crude protein, %	150.0	151.0
Methionine, %	4.56	3.94
Methionine + cysteine, %	6.90	6.90
Total calcium, %	41.78	39.81
Available phosphorus, %	3.40	3.40
Sodium, %	1.50	1.50
Lysine, %	8.60	9.84
Threonine, %	6.20	6.42
Tryptophan, %	1.91	1.74
Fatty acid composition, %		
Arachidonic acid (C20:4 n6)	0.29	0.97
α-linolenic acid (C18:3 n3)	3.23	7.24
Linoleic acid (C18:2 n6)	39.60	48.33
Eicosapentaenoic acid (C20:5 n3)	1.44	1.88
Docosahexanoic acid (C22:6 n3)	0.19	2.32
Astaxanthin (mg/100g)	----	0.46

[a] The premix provided the following per kg of diet: vitamin A (retinyl acetate), 12000 IU; vitamin D_3 (cholecalciferol), 2500 IU; vitamin E (DL-α-tocopheryl acetate), 30 mg; vitamin K_3 (menadione), 2 mg; vitamin B_1 (thiamine), 2.25 mg; vitamin B_2 (riboflavin), 7.5 mg; vitamin B_6 (pyridoxine), 3.5 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; D-pantothenic acid, 12.5 mg; biotin, 0.125 mg; f olic acid, 1.5 mg; Zn, 50 mg; Cu, 12 mg; I, 0.3 mg; Fe, 110 mg; Se, 0.1 mg; Mn, 110 mg. [b] Saponified xanthophylls of Aztec marigold (yellow, 15 ppm). [c] Red xanthophylls (canthaxanthin, 10 ppm). * Due to the carotenoids contained.

Ninety Isa-Brown laying hens (32 wk age, with mean body weight of 1560 ± 20 g) were selected from 150 hen flock based on similar weights and production rate. After a 2-wk of adaptation, the selected hens were divided into 2 groups of 45 birds each, which consisted of five replications of nine birds each. The experimental period lasted 4 wk (34 - 38 wk of age). The treatments consisted of a control diet, and a diet containing 4% RCM. Throughout the experimental periods, feed and water provided on *ad libitum* consumption. During this period, the productive parameters (egg production, egg weight, feed conversion and feed intake) were measured. At the end of 4 weeks, 250 eggs were collected from each treatment group and stored as follows: fresh eggs (50 eggs), 15 days at 20°C (50 eggs), 15 days at 4°C (50 eggs), 30 days at 20°C (50 eggs), and 30 days at 4°C (50 eggs).

F. Yolk Color

For the fresh eggs and the eggs stored at 20°C and 4°C for 15 and 30 days, the yolk color were determined using automated equipment (Technical Service TSS and Supplies, Inc., England, UK).

G. Eggs Chemical Analysis

Eggs from evaluating yolk color were mixed (yolk and albumen), and subjected to a lyophilization process to facilitate analysis: Total lipids and fatty acid profile and peroxide index [10], lipid-soluble vitamins (A and E) by HPLC [15], rancidity index colorimetric method TBAR'S (thiobarbituric acid reactive substances)[16] and astaxanthin [11, 12].

H. Statistical Analysis

The data of the productive parameters tests were analyzed using a completely randomized design (ANOVA), and the differences between means were tested using Tukey's test with a confidence level of 95%. The analyses were conducted using SAS, 2004 Version 9.1 ed., SAS Institute Inc., Cary, NC:

$$Yij = \mu + \tau i + Єij$$

where Yij = the response variable, μ = the experimental mean, τi = the effect of the i-th treatment, Єij = the experimental error.

The data of the physical and chemical analyses of the lipid fraction of the egg were analyzed according to a 2x2x2 factorial design with the following factors: treatment, time and temperature. Tukey's test was used to perform a pairwise comparison of the means with a $P < 0.05$, using SAS, 2004 Version 9.1 ed., SAS Institute Inc., Cary, NC:

$$Yijk = \mu + \alpha i + \beta j + {}_{\gamma k}(\alpha\beta)ij + (\alpha^{\gamma})_{ik} + (\beta^{\gamma})_{jk} + (\alpha\beta^{\gamma})ijk + Єijk(l)$$

where Yijk = response variable, μ = experimental mean, αi = effect of the i-th diet reatment, βj = effect of the j-th time treatment, ${}_{\gamma k}$ = effect of the k-th temperature treatment, (αβ)ij = effect of the interaction of treatment and time, $(\beta^{\gamma})_{jk}$ = effect of the interaction of time and temperature, $(\alpha\beta^{\gamma})ijk$ = effect of the interaction of treatment, time and temperature, Єijk(l) = experimental error.

III. RESULTS AND DISCUSSION

RCM Chemical Composition

The nutrient profiles of red crab meal (RCM) are reported in Table II. The gross energy content was 2.56 Kcal/g, crude protein (33.74%), crude fat (7.29%), total astaxanthin (5.41%) and saturated (39.16%), monounsaturated (15.22%) and polyunsaturated (12.74%) fatty acids.

Table II. Chemical composition and nutrient profile of red crab meal

Nutrient composition	RCM[1]
g/100g	
Moisture	9.13
Ash	20.24
Crude protein	33.74
Total carbohydrates	29.58
Ca	4.58
Na	10.45
Mg	14.02
Astaxanthin	5.41
Crude fat	7.29
Gross Energy Kcal/g	2.56
Fatty acid, mg/100g sample	
Saturated fatty acid	39.16
Myristic (14:0)	183.8
Palmitic (16:0)	599.43
Stearic (18:0)	115.24
Arachidic (20:0)	24.23
Behenic (22:0)	14.91
Lignoseric (24:0)	4.92
Monounsaturated fatty acid	15.22
Palmitoleic (16:1)	373.89
Oleic (18:1)	403.74
Erucic (22:1)	10.03
Nervonic (24:1)	13.54
Polyunsaturated fatty acid n3	8.73
α-Linolenic (18:3 n3)	11.38
Eicosapentaenoic (20:5 n3)	291.65
Docosahexaenoic (22:6 n-3)	171.95
Polyunsaturated fatty acid n6	12.74
Linoleic (18:2 n-6)	27.65
γ- Linolenic (18:3 n-6)	8.50
Arachidonic (20:4 n-6)	24.23
n-6/n-3 ratio	7.86

[1] The values presented are means of 6 samples

The gross energy obtained in this study was 2.56 kcal/g, a similar value to that of meat meal (271.5 kcal/100g) that has also been used as an ingredient in diets for laying hens [17]. Crustaceans obtain their energy mainly from protein catabolism, so the metabolism of these organisms is different from land animals [17]. The value obtained for crude protein was lower than the values reported by Charley [18] for crab meal (47.2%) and by Castro et al [1] for red crab (39.9%). The value of crude fat was greater than that reported by [18] of 4.9%. This variability is likely due to the season of capture and the

fact that this meal was composed of males and females. Castro et al. [1] reported that females have a higher lipid content, requiring stored energy for reproduction. Norman et al. [19] determined the astaxanthin content in different species of shrimp tissue, which ranged from 6.5 to 9.8 mg/100 g, which was higher than observations in this study. The total concentration of carotenoids present in RCM reported by Spinelli et al. [20] was 10-16 mg/100 g; however, it is known that 95% of carotenoids consist of astaxanthin. Total fatty acids were similar to those reported by Carrillo et al. [4] and by Astiasarán and Martínez [21].

A. Production Variables

No statistically significant differences were observed ($P > 0.05$) between the two treatments for the productive variables quantified in this study (Table III), which is consistent with Carrillo et al., [4], who included 3% and 6% RCM in the diets of laying hens. However, these same authors state that levels higher than 6% could affect the palatability of the food, reduce egg production and increase water levels in the stool, causing diarrhea in birds.

Table III. The effect of dietary supplementation with red crab meal (RCM) 4% on the production performance of laying hens between 34 and 38 weeks of age[1].

	Egg production %	Egg weight g	Feed conversion ratio, g feed/g egg	Feed intake, g/hen/day
Control	88.35 ± 6.26	64.20 ± 1.30	2.07 ± 0.11	118.0 ± 2.54
RCM	87.22 ± 7.78	62.5 ± 1.23	2.07 ± 0.15	112.74 ± 4.2

[1] The data are means of five replicates of nine hens each. No significant differences were found ($P > 0.05$)

B. Total Lipids and Fatty Acids Profile

Unlike the water-soluble fraction of the egg, it is possible to achieve a significant change in the lipid raction content of eggs by utilizing marine products (fish oil, fishmeal and shellfish, seaweed, etc.) rich in fatty acids and carotenoids to supplement the diets of egg-producing hens [22].

Levels of α-linolenic acid (ALA) (18:3 n-3), linoleic acid (LA) (18:2 n-6) and arachidonic acid (AA) (20:4 n-6) were significantly different ($P < 0.05$) and were decreased at 30 days/20°C of storage (Table IV). Significant differences ($P < 0.05$) were reported for total lipids showing a three way interaction (treatment, time and temperature), indicating that the differences were due to the influence of these three variables, with 30 days/20°C of storage having the lowest value. It is worth mentioning that according to the technique employed, that use as extraction solvent (chloroform/methanol) another series of liposoluble compounds (phospholipids, triglycerides, free cholesterol, esterified cholesterol, vitamins and pigments) was extracted, whose difference can be attributed to the oxidation or loss of some of the compounds from the total lipidic fraction [4, 23].

Fatty acids found in the greatest quantity were ALA, LA, AA, EPA (20:5 n-3) and DHA (22:6 n-3) (Table IV). ALA, AL and AA had the same significant differences ($P < 0.05$) in the number of storage days, with 30 days having the lowest value. As these fatty acids are polyunsaturated, they are more susceptible to changes and oxidize easily. Additionally, eggs enriched with these fatty acids are also susceptible to lipid degradation, necessitating protection through antioxidants [4, 24]. It is possible that the differences in the results are due to the presence of vitamin E and carotenoids, which provide protection from the effect of oxidation, which will be discussed later.

In Table IV, significant differences can be seen ($P < 0.05$) in EPA, DHA and total n-3 in the treatment variable, with the highest values observed in the diets enriched with 4% RCM (12.7%, 542.8% and 705.6% respectively, compared to the control). However, for total n-6, a significant difference ($P < 0.05$) was observed in the storage time variable, which was lower at 30 days. Although there were no differences in EPA, DHA and total n-3, these were also lower for the same variable, possibly due to their oxidation. The diet with 4% RCM had the highest levels of these acids, coinciding with that reported by Carrillo et al [4]. These authors state that marine RCM is rich in these acids and

claim that the addition of these resources to the avian diet can enrich eggs.

The n6/n3 proportion in this study ranged from 7 to 11:1. A proportion less than n-6 to n-3 in poultry diets may decrease competition between ALA with LA by means of the enzymes involved in the bioconversion of n3 long chain fatty acids, resulting in their higher tissue content [2].

Humans evolved on an approximately 1:1 diet of n-6 to n-3 fatty acids. However, currently, Western diets have a ratio of 10:1 to 20-25:1 n-6 to n-3 fatty acids, indicating that these diets are deficient in n-3 fatty acids compared to the diets in which humans evolved and established their genetic patterns. It is worth mentioning that n-6 and n-3 are not synthesized in the human body and are important components in the overall functioning of cell membranes. Studies indicate that DHA is essential for the normal functional development of the brain and the retina, particularly in premature infants, and represents 40% of the membrane phospholipids in the brain. Both EPA and DHA have an effect on the function of membrane receptors as well as the generation of neurotransmitters. There is also evidence that EPA and DHA play a role in aggressive behavior as well as having beneficial effects addictions. However, the high proportion of n-6, largely LA, is far from being optimal. The n6/n3 relationship in the brain ranges from 1:1 to 2:1, which is consistent with data on the evolutionary aspects of diet, genetics, and studies with animal models. Therefore, a proportion of 1:1 to 2:1 n6/n3 should be the optimal ratio for health [2]. However, the n6/n3 ratio reported in this study is not adequate according to the recommendation by Simopoulos [2], which presents a challenge for reducing n-6 and reaching the optimum proportion in poultry products enriched with these fatty acids.

Table IV. Effect of inclusion of red crab meal (RCM) between treatments, days and storage temperatures on total lipids, fatty acids, total n-3, total n-6, and relationship n6/n3

	Total lipids	ALA n-3	LA n-6	AA n-6	EPA n-3	DHA n-3	Total n-3	Total n-6	n6/n3
	g/100g sample								
Treatments (T):									
Baseline*	42.9	175.2	4856.8	826.7	4.05	374.7	554.0	5683.6	10.2
Control	43.6[a]	155.9	4498.0	786.4	3.91[b]	320.4[b]	480.3[b]	5062.4	11.1[a]
RCM	41.3[b]	150.1	4321.3	728.5	12.7[a]	542.8[a]	705.6[a]	5284.5	7.1[b]
SEM	0.31	3.06	103.7	20.5	0.27	11.38	12.42	123.8	0.218
Times(Ti):									
Baseline*	42.9	175.2	4856.8	826.7	4.05	374.7	554.0	5683.6	10.2
15	43.2[a]	164.0[a]	4616.5[a]	795.3[a]	8.61	449.8	622.5	5411.9[a]	8.9
30	41.7[b]	142.0[b]	4202.8[b]	719.6[b]	8.01	413.3	563.3	4935.0[b]	9.3
SEM	0.31	3.06	103.7	20.5	0.27	11.38	12.42	123.8	0.218
Temperatures (TM):									
Baseline*	42.9	175.2	4856.8	826.7	4.05	374.7	554.0	5683.6	10.2
20°C	42.4	151.6	4353.9	749.5	8.26	426.0	588.9	5119.5	9.3
4°C	42.5	154.4	4465.4	765.5	8.37	437.1	597.0	5227.5	8.9
SEM	0.31	3.06	103.7	20.5	0.27	11.38	12.42	123.8	0.218
	P value								
(T)	0.0001	0.215	0.263	0.081	0.0001	0.0001	0.0001	0.240	0.0001
(Ti)	0.010	0.0009	0.022	0.031	0.156	0.052	0.009	0.026	0.217
(TM)	0.740	0.535	0.469	0.595	0.781	0.511	0.654	0.554	0.180
T x Ti	0.224	0.752	0.852	0.634	0.767	0.039	0.061	0.993	0.018
T x TM	0.155	0.541	0.726	0.723	0.445	0.740	0.638	0.761	0.448
Ti x TM	0.950	0.803	0.878	0.244	0.193	0.530	0.626	0.691	0.091
T x Ti x TM	0.019	0.002	0.077	0.295	0.198	0.044	0.010	0.086	0.119

*Baseline (fresh eggs without storing)
[a,b] Different letters in each column indicate significant differences ($P < 0.05$)
ALA = α-linolenic acid (C18:3); LA = Linoleic acid (C18:2); AA = Arachidonic acid (C20:4); EPA = Eicosapentaenoic acid (C20:5); DHA = Docosahexaenoic acid (C22:6); SEM = Standard Error of the Mean

C. Astaxanthin, Peroxide Index, TBAR'S and Yolk Color

Polyunsaturated fatty acids, because of their several double bonds, are susceptible to oxidation; the eggs enriched with these fatty acids are also susceptible to lipid degradation, and thus, protection with antioxidants is necessary. Astaxanthin (3,3'-dihydroxy-β-carotene-4,4'-dione) xanthophyll is responsible for the red pigmentation in salmon, trout, prawns, shrimp and flamingo have been shown to strongly inhibit lipid peroxidation activity by active forms of oxygen. Astaxanthin is produced by the algae *Haematococcus pluvialis* and the yeast *Phaffia rhodozyma*, which are the basis of zooplankton and krill feed. These species store pigment in their skin and fatty tissue, and because it is a fat-soluble pigment, it is incorporated into cell membranes [25]. Terao [26] found that canthaxanthin and astaxanthin were more effective antioxidants than ß-carotene in the stabilization of free radicals. Other studies [27] showed the function of astaxanthin as a potent *in vivo* and *in vitro* antioxidant in the inhibition of lipid peroxidation.

Table V shows that the astaxanthin content in eggs with 4% RCM was 0.466%. Eggs from the control diet contained the carotenoids lutein (yellow) and canthaxanthin (red) (0.455%).

Table V. Effect of inclusion of red crab meal (RCM) between treatments, days and storage temperatures on astaxanthin, peroxide index and yolk color

	Astaxanthin g/100g	Peroxide index mEq O_2/100g	Yolk color
Treatments:			
Baseline*	0.523	279.3	10.1
Control	0.455[b]	242.9[a]	8.1[b]
RCM	0.466[a]	236.2[b]	10.9[a]
SEM	0.149	1.38	0.05
Times:			
Baseline*	0.523	279.3	10.1
15	0.466[a]	246.4[a]	9.6[a]
30	0.456[b]	232.7[b]	9.4[b]
SEM	0.149	1.38	0.05
Temperatures:			
Baseline*	0.523	279.3	10.1
20°C	0.455[b]	234.1[b]	9.2[b]
4°C	0.466[a]	245.0[a]	9.8[a]
SEM	0.149	1.38	0.05
		P value	
Treatments (T)	0.0006	0.009	0.0001
Times (Ti)	0.001	0.0001	0.0008
Temperatures (TM)	0.001	0.0005	0.0001
T x Ti	0.0001	0.0005	0.061
T x TM	0.01	0.0001	0.707
Ti x TM	0.125	0.0001	0.0001
T x Ti x TM	0.003	0.0001	0.061

*Baseline (fresh eggs without storing)
[a,b] Different letters in each column indicate significant differences ($P < 0.05$).
SEM= Standard Error of the Mean

The control diet did not include astaxanthin; however, analysis of eggs produced from this diet generated a response at the same wavelength as astaxanthin, possibly due to the technique used for the quantification of astaxanthin. The wavelength used in the detector was 470 nm. According to Britton [28], there is another series of carotenoids with a similar chemical structure that were quantified at a wavelength equal to or similar to astaxanthin (canthaxanthin, β-carotene, lutein, zeaxanthin and lycopene), and because lutein and canthaxanthin were included in this diet, these pigments are detected.

In this study, a three way interaction was found with eggs from the 4% RCM diet. These eggs had greater oxidative stability when stored at 15 days/4°C than control eggs. Considering that astaxanthin is susceptible to oxidation based on temperature and storage time, a decrease in astaxanthin quantity in the eggs at 30 days/20°C can be inferred.

From a physiological point of view, astaxanthin is involved in a number of essential cellular functions. Astaxanthin acts as provitamin A, which is associated with reproduction and embryonic development, as well as cellular protection against the effects of oxidation [29]. In addition, astaxanthin improves stress tolerance and increases the immune response [25]. In the case of birds, astaxanthin has been used to increase egg production and to improve the health of chickens and is the red pigment in egg yolk [5, 6].

Young and Lowe [30] examined the repressive effect of carotenoids against $O_2\beta^-$ reporting the effectiveness of astaxanthin, which had 100 times greater activity against $O_2\beta^-$ than vitamin E. It is known that one of the most important functions of vitamin E, astaxanthin and BHT is as an antioxidant, which is why in this study the peroxide index in the eggs of the experimental diets was determined to detect the formation of peroxides and "rancidity". However, because there is no information on rancidity in stored eggs, the peroxide index of 5.3, which corresponds to a fresh oil or one within its period of induction of "rancidity", was used as a reference during the discussion of the results [31].

This study showed a three way interaction with treatments, times and temperatures, all of which presented variables with significant differences ($P < 0.05$). This confirms that the astaxanthin present in eggs with 4% RCM possibly protected the lipid fraction of the egg from oxidation compared to the control, which was more susceptible to oxidation even though the latter also contained carotenoids and antioxidants. Because the data obtained only indicate the formation of peroxides from the degradation of fatty acids, the level of rancidity was quantified; however, this was not detected in the eggs analyzed, which confirmed the data obtained by the peroxide index.

Eggs contain vitamins A and E and natural carotenoids that possibly prevented the development of peroxides and rancidity in the analyzed samples. Stahl et al. [32] indicated that the antioxidant activity of carotenoids in liposomes inhibits the formation of TBAR'S in the following order: lycopene > α-tocopherol > α-carotene > β-cryptoxanthin > zeaxanthin = β-carotene > lutein. In this same experiment, carotenoid mixtures were more effective than single compounds, which expressed a greater synergistic effect in the presence of lutein or lycopene. When β-carotene was present, TBAR'S values in tissues were lower.

In contrast, the yolk color of eggs with 4% RCM in the diet reported 10.9 on the Roche yolk color fan, different from the control at 8.1. However, significant differences were observed ($P < 0.05$) as well as a two way interaction for times and temperatures, with a decrease in color at 30 days/20°C.

Pérez-Gálvez et al. [12] studied the deposition of astaxanthin in egg yolk after feeding hens for a period of 14 days with a diet that included 20% red crab meal (*Procambarus clarkii*), as well as carotenoids lutein, zeaxanthin, and canthaxanthin. These authors mention that the increase in astaxanthin concentration in egg yolk was significant from the fourth day onward (8.19 mg/kg), and maximum deposition was reported at 9 days (22.24 mg/kg) and decreased over the 14 days of the trial (21.2 mg/kg). In this report, they conclude a good red coloration in the egg yolk can be obtained by including red crab meal in the diets of laying hens, as well giving it utility as a byproduct of marine origin instead of artificial pigment.

However, no scientific reports that discuss possible modifications that could occur in the color of egg yolk and its storage. In conclusion, including 4% RCM in the diets of egg laying hens did not affect any productive parameters and made it possible to obtain eggs enriched with n3 fatty acids. Regarding egg yolk pigmentation, RCM may be an option as a pigment in aviculture to reinforce the yellow pigment, and as a natural by-product, it can replace synthetic pigments. Astaxanthin can be considered a natural antioxidant in the protection of the lipid fraction. On the other hand, it is recommended that eggs should be consumed no more than 15 days after acquiring them and kept refrigerated if possible. Packaging conditions and therefore oxygen availability of egg powders after drying can be important factor for the observed changes, so it would be of interest for further studies to establish this effect.

IV.CONCLUSION

Including 4% RCM in the diets of egg laying hens did not affect any productive parameters and made it possible to obtain eggs enriched with n3 fatty acids. Regarding egg yolk pigmentation, RCM may be an option as a pigment in aviculture to reinforce the yellow pigment, and as a natural by-product, it can replace synthetic pigments. Astaxanthin can be considered a natural antioxidant in the protection of the lipid fraction. On the other hand, it is recommended that eggs should be consumed no more than 15 days after acquiring them and kept refrigerated if possible.

V.REFERENCES

[1] Castro GM, Carrillo DS, Pérez-Gil RF, Calvo CC. 1995. Red crab chemical composition and technological processes. In: Aureoles-Gamboa, D. and Balart, E.F. (Eds.). La Langostilla: Biología, Ecología y Aprovechamiento. Centro de Investigaciones Biológicas del Noroeste, S.C. México, Pp. 163-177. (in Spanish).

[2] Simopoulos AP. 2011. Evolutionary aspects of diet: The Omega-6/Omega-3 ratio and the brain. Mol. Neurobiol. 44:203-15.

[3] Hinostroza GL, Bjerkeng B. 2002. Astaxanthin from the red crab langostilla (Pleuroncodes planipes): optical R/S isomers and fatty acid moieties of astaxanthin esters. Comparative Biochemistry and Physiology Part B: Biochem. Mol. Biol. 437-44.

[4] Carrillo DS, Carranco JM, Castillo DRM, Castro GMI, Ávila GE, Pérez-Gil RF. 2005. Cholesterol and n-3 and n-6 Fatty Acid Content in Eggs from Laying Hens Fed with Red Crab Meal (Pleuroncodes planipes). Poult. Sci. 84:167-72.

[5] Carranco JME, Calvo CC, Carrillo DS, Ramírez CR, Morales BE, Sanginés GL, Fuente MB, Ávila GE, Pérez-Gil RF. 2011a. Crustacean meal in laying hen rations. Effect on productive variables and sensory evaluation of eggs stored in different conditions. Cuban J. Agric. Sci. 45:171-75.

[6] Carranco JME, Calvo CC, Carrillo DS, Ramírez CR, Morales BE, Sanginés GL, Fuente MB, Ávila GE, Pérez-Gil RF. 2011b. Crustacean meal in laying hen rations. Effect on the physical quality of the egg

stored in different conditions. Cuban J. Agric. Sci. 45: 177-81.
[7] UNA. 2014. Unión Nacional de Avicultores. Indicadores Económicos. Parámetros al cierre de 2014, sobre las estadísticas de la industria avícola. Retrieved September 21, 2015. Available at www.institutonacionalavicola.org.mx.
[8] Ahn DU, Sell JL, Chamruspollet M, Jeffrey M. 1999. Effect of dietary conjugated linolenic acid on the quality characteristics of chicken eggs during refrigerated storage. Poult. Sci. 78:922-28.
[9] Guerra M. 2000. Factors affecting egg quality. Agricultura. 4:38-40. (in Spanish).
[10] AC. 2005. Official Methods of Analytical Chemist, 18th ed. Association of Official Analytical Chemists. Arlington VA, USA.
[11] Surai PF, Speake BK. 1998. Distribution of carotenoids from the yolk to the tissues of the chick embryo. J. Nutr. Biochem. 9:645–51.
[12] Pérez-Gálvez A, Negro-Balmaceda JJ, Mínguez-Mosquera MI, Cascajo-Almenera MV, Garrido-Fernández J. 2008. Astaxanthin from crayfish (Procambarus clarkii) as a pigmentary ingredient in the feed of laying hens. Grasas y Aceites. 59:139-45.
[13] NOM. 1999. Norma Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. México, D.F. (in Spanish).
[14] NRC. 1994. Nutrient Requirement of Poultry. 8th. Ed. National Research Council. National Academy Press. Washington D.C., USA.
[15] Salo-VaEaEnaEnen P, Ollilainen V, Mattila P, Lehikoinen K, Salmela-MoElsaE E, Piironen V. 2000. Simultaneous HPLC analysis of fat-soluble vitamins in selected animal products after small-scale extraction. Food Chem. 71:535-43.
[16] Nickos AB, Dimitrios JF, Georgios EP, Vassilios NV, Antonios JM, Antonios GT. 1994. Rapid, Sensitive, and Specific Thiobarbituric Acid Method for Measuring Lipid Peroxidation in Animal Tissue, Food, and Feedstuff Samples. J. Agric. Food Chem. 42: 1931-37.
[17] FAO. 1987. The Nutrition and Feeding of farmed fish and shrimp. A training manual. 1. The essential nutrients. Food Agriculture Organization. Brasilia, Brasil, Pp 132.
[18] Charley H. 2004. Tecnología de alimentos. Procesos químicos y físicos en la preparación de alimentos. Limusa. México. 603 pp.
[19] Norman L, Shaw C, Fink C, Awad A. 2004. Combination of phytosterols and omega-3 fatty acids: a potential strategy to promote cardiovascular health. Curr. Med. Chem. 2: 1-12.
[20] Spinelli J, Lehman L, Wieg D. 1974. Composition, processing and utilization of red crab (Pleuroncodes planipes) as an aquacultural feed ingredient. J. Fish Res. Board Can. 31:1025-29.
[21] Astasarian I, Martínez J. 2000. Alimentos, composición y propiedades. Ed. McGraw Hill Internacional. Madrid, España.
[22] Hargis PS, Van Elswyk ME. 1993. Manipulating the fatty acid composition of poultry and eggs for the health conscious consumer. World's Poult. Sci J. 49:251-64.
[23] Castillo BC, Vázquez VJL, González AM, Morales BE, Castillo DRM, Carrillo DS. 2005. El aceite de atún como fuente de ácidos grasos omega-3 en el huevo de gallina. Grasas y Aceites. 56:153-59. (in Spanish).
[24] Bernal GME, De Mendoca CX, Mancini-Filho J. 2003. Estabilidad oxidativa de huevos enriquecidos con ácidos grasos poliinsaturados omega 3, frente a antioxidantes naturales. Rev. Bras. Cienc. Farmaco. 39:425-32.
[25] Miki W, Otaki N, Shimidzu N, Yokoyama A. 1994. Carotenoids as free radical scavengers in marine animals. J. Mar. Biotech. 2:35-7.
[26] Terao J. 1989. Antioxidant activity of α-carotene-related carotenoids in solutions. Lipids. 24:659-661.
[27] Kurshize M, Okimasu E, Inoue E, Utsuma K. 1990. Inhibition of oxidative injury of biological membranas by astaxanthin. Physiol. Chem. Phys. Med. 22:27-38.
[28] Britton G. 1995. UV/visible spectroscopy. In: Britton G.; Liaaen-Jensen S. and Pfander H., eds. Carotenoids. Basel, Birkhäuser Verlag. V 1B. p. 21-28.
[29] Putnam M. 1991. Aquaculture and the environment. European Aquaculture Soc. Special Publication, 16:245-63.
[30] Young AJ, Lowe GM. 2001. Antioxidant and Prooxidant propierties of carotenoids. Archives of Biochem. Biophysics. 385:20-7.
[31] NMX-F-223-1985. Normas Mexicanas. Alimentos. Aceite vegetal comestible. Foods Edible vegetable oil. Dirección General de Normas. México D.F. (in Spanish).
[32] Stahl W, Junghans A, de Boer B, Driomina E, Briviba K, Sies H. 1998. Carotenoid mixtures protect multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. FEBS Lett. 427:305-8.