© 2018 IJSRST | Volume 4 | Issue 10 | Print ISSN : 2395-6011 | Online ISSN : 2395-602X
Themed Section: Science and Technology

Effect on Lipid Fraction of Egg Stored at different Times and Temperatures of Hens Fed with Shrimp Meal Litopenaeus SPP

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ABSTRACT

The aim of this work was to evaluate the effect of inclusion of shrimp meal (SM) on lipid fraction, oxidation, and pigment of fresh and stored eggs at different times and temperatures. Ninety Isa-Brown hens were randomly allocated to 2 treatments, with 5 replicates containing 9 laying hens per each replicate. The hens were fed sorghum-soybean basal diet and 20% SM. After 4 weeks 250 eggs were collected per treatment: 50/fresh; stored 50/20° and 50/4°C/15 days, 50/20° and 50/4°C/30 days. Egg fatty acids, astaxanthin, peroxide index, TBARS and yolk color were determined. The results were analyzed by ANOVA and 2x2x2 factorial and means by Tukey's test with a confidence level of 95%. The results showed that eggs with 20% SM were low in total lipids, n6 and n3, and astaxanthin compared to the control ($P < 0.05$). In general, all egg values stored 30 days/20°C were lower ($P < 0.05$). Peroxide index values were low, and TBARS not detected. The conclusion of this study indicates that shrimp meal up to 20% inclusion in diets for laying hens can be used in the formulation of balanced foods as a source of protein, fatty acids and astaxanthin.

Keywords : Egg, Lipid Fraction, Oxidation, Shrimp Meal, Stored

I. INTRODUCTION

The world shrimp catch is approximately 3.4 million ton/year, with Asia as the leading producer, making shrimp a very important product on the world market. However, the greatest economic interest is in the shrimp tail, not in the heads (cephalothorax), which are usually discarded, thereby causing an environmental impact [1, 2]. The high amounts of this waste, coupled with its rapid degradation capacity, has stimulated a great deal of interest in researchers, focusing on the determination of possible uses of these by-products for a dual purpose, the search for a beneficial exploitation economically and the other hand, reducing environmental impact [3]. The use and transformation of solid waste generate by-products such as shrimp cephalothorax, which can be used as raw material for feed and human and animal supplementation, since they are enriched with a high content of proteins, fatty acids, and astaxanthin, which are attractive to formulation of new food products [4].

In Mexico, waste of shrimp industry has not been used at an industrial level, and since 1990, alternatives have been sought for this use as source of protein, fatty

acids, and pigment [5]. The use of these by-products of the shrimp industry generates the possibility of developing balanced feed for animal consumption by providing added value, especially proteins, fatty acids and red pigments (astaxanthin) that can produce desirable coloration in egg yolk [2].

In Mexico, the consumption of marine products is very low due to cost and cultural factors, while that of poultry products, mainly eggs, is high because of the low cost and versatility in their preparation (consumption of 22.31 kg/per capita/year) [6]. Given this fact, it has been proposed to include SM in the diet for laying hens and thus obtain an egg with added value (fatty acids n3, n6 and red pigment). However, the egg reaches the consumer days later and is stored at different times and temperatures.Thus, some doubt exists concerning what is happening to the product during storage and whether the content of total lipids, n6 and n3 fatty acids, and astaxanthin may have been modified, as well as the possible oxidation of these compounds during storage when the product has been enriched with SM [7, 8]. Therefore, maintenance of the quality of the enriched egg during storage is important. However, no specific data exist on this subject, so the objective of this work was to determine the effect of including shrimp meal (SM) in rations for laying hens and the effect of this meal on the lipid fraction, and oxidation of the fresh egg and eggs stored at different times and temperatures.

II. METHODS AND MATERIALS

A. Shrimp meal (SM)

50 kg of SM was provided by Proteínas Marinas y Agropecuarias S.A. de C.V., Guadalajara, Jalisco, Mexico, and subsequently, the meal was transported to the Mexico City to the Departamento de Nutrición Animal Dr. Fernando Pérez-Gil Romo del Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, where it was stored in black plastic bags and frozen (-20°C) until use.

B. Chemical Analysis of SM

The following analyses were performed on the SM according to standardized techniques published by the Association of Official Analytical Chemists [9]: crude protein, total ash, ether extract, minerals (Ca, Na and Mg), and gross energy 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 of SM

About 2g of SM was taken for total lipid extraction using equal volumes 1 chloroform: 1 ethanol (v/v) following the method 923.07 [9]. Fatty acid methyl esters were prepared from following the method 969.33 [9] from total lipids extract using methanolic NaOH solution. Total lipid extract was trans methylated into fatty acid methyl esters with BF3 and separated by using a gas chromatograph (Varian 3380, Varian Inc, Lake Forest, CA, U.S.A). 1 µL was injected into a DB-23 capillary column (30m x 0.25mm i.d.; 0.25µm thickness; J&W Scientific No. 122-2332) with cianopropyl methylpolysiloxane as stationary phase. Nitrogen was used as the carrier gas at a constant flow rate of 2.0 mL/min. The following oven temperature program was used: 120ºC held for 1 min, increased to 200ºC/min, then to 230ºC/min, and 240ºC held for 10 min. Myristoleic acid (C14:1) (SIGMA M3650) was used as an internal standard. Peaks were separated using a flame-ionization detector (FID) and were identified and quantified with Star Chromatography Workstation (Varian Inc. Lake Forest, CA, USA Lake Forest, CA, USA) using pure standard mixture (F.A.M.E. Mix C4-C24 SUPELCO 18919).

D. Astaxanthin Quantification of SM

A methodology described previously, Surai and Speake [10], was used but employing acetone as extraction solvent. 1g of SM was extracted with 3.5 mL of solvent, vortexed for 1min and sonicated for 1min. The simple was centrifuged (9000 rpm during 6 min) collecting the upper phase, which subsequently analyzed by spectrophotometry. The solvent was

evaporated on a rotary evaporator and redissolved in hexane for quantify astaxanthin at 470 nm. A known concentration of astaxanthin standard was used to perform a calibration curve and calculate the concentration of astaxanthin [11].

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 [12].

This test was conducted at the Centro de Enseñanza, Investigación y Extensión en Producción Avícola (CEIEPAv) de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad Nacional Autónoma de México.

The sorghum-soybean meal-based diet and the 20% SM diet were formulated to meet the nutrient requirements of the National Research Council [13] for laying hens through Nutrion WindowsTM (Version 5.0 Pro), a computerized system for feed formulation (Guadalajara, Jal., Mexico). Diets were isoproteic (15% crude protein PC) and isoenergetic (2750 Kcal/kg) (Table I).

Ninety Isa-Brown laying hens (34 weeks of age) were divided into 2 groups of 45 birds each, which consisted of five replications of nine birds each. The experimental period lasted 4 weeks. The treatments consisted of a control diet, and a diet containing 20% SM. Throughout the experimental periods, feed and water available to allow for ad libitum consumption. At the end of 4 weeks, 250 eggs were collected from each treatment group and stored as follows: fresh eggs (50 eggs), 15 days/20°C (50 eggs), 15 days/4°C (50 eggs), 30 days/20°C (50 eggs), and 30 days/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 and Supplies TSS, Inc., England, UK). The system consists of a reflectance spectrophotometer (QCC Yolk color) connected to a digital scale of DMS Fan. The QCM + collects data from online tools and displays a reading, which is transferred to a computer equipped with egg quality-assurance software, with values ranging from 1 to 15, corresponding to the number 1 light yellow to 15 orange-yellow (DMS color fan).

G. Eggs Chemical Analysis

Eggs from evaluating yolk color were mixed (yolk and albumen), and subjected to a liophylization process to facilitate analysis: Total lipids, fatty acid profile, and peroxide index by AOAC [9], rancidity index colorimetric method TBARS (thiobarbituric acid reactive substances) [14] and astaxanthin [10, 11].

H. Statistical Analysis.

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:

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

Where: Yijk = response variable; μ = experimental mean; αi = effect of the i-th diet treatment; 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

A. Chemical composition of SM

Table II presents the results of the approximate chemical analysis and fatty acids for SM. The most

abundant fraction turned out to be the crude protein (36.07%), ash (29.86%), total carbohydrates (24.17%), and Ca (4.58%). The ether extract and astaxanthin were found in small amounts (0.88% and 0.34 mg/100g respectively). Notably, the SM contains 39.16% saturated fatty acids, with the highest concentrations being palmitic (21.72%) and stearic (12.45%) acids, whereas 47.97% of the unsaturated fatty acids comprise 19.28% monounsaturated and 28.69% polyunsaturated fatty acids. Of these, the monounsaturated oleic acid accounts for the greatest percentage (13.95%), whereas the polyunsaturated acids are present by docosahexaenoic acid (DHA) (7.22%) and eicosapentaenoic acid (EPA) (7.33%).

The result of the crude protein was less than that reported by Morillo et al. [4] (46.79%) and Salas et al. [2] (40.67%) in shrimp cephalothorax meal. For the ash content was greater than that reported by these other authors (20.89% and 27.48%). This ash content may be due to the exoskeleton of the crustaceans being calcified and formed mainly by calcium and phosphorus [2, 4, 15]. The ether extract was found to be present in very small amounts, similar to that indicated by Charley [16] of 0.80% for shrimp meal, in contrast to other reported results ranging from 2.68% to 21% [2, 4, 17] . For these species, the stored fat allows them to have a greater fluidity, flexibility, and permeability of the cellular membrane at low temperatures, and when this diminishes in the water, a greater incorporation of polyunsaturated fatty acids occurs in the tissues. The crude energy obtained in this study was similar to that observed with meat meal (11.36 kJ/g) and higher than the value reported for crab meal (3.16 kJ/g). Importantly, crustaceans obtain energy mainly from the catabolism of proteins; therefore, the metabolism of these organisms is different from that of terrestrial animals [15]. Normen et al. [18] obtained an astaxanthin content in shrimp tissue of 0.650 mg/100 g for Litopenaeus vannamei, 0.980 mg/100 g for L. monodon, and 0.790 mg/100 g for L. japonicus. Those values are greater than those found in this work because those data are for whole shrimp. The data obtained are consistent with those published by Astiasaran and Martínez [19], who report that marine products are rich in fatty acids.

In general, in this study, the differences with the results of other authors may be due to other factors such as temperature, catch zone and depth, time of year, age, sex, reproductive status, and the process of obtaining the meal.

B. Total lipids in eggs

Table III presents the results of the total lipid content in the lyophilized egg, with significant differences ($P < 0.05$) for the variable time, with concentration of the lipid content decreasing at 30 days of storage.

At present, great interest exists in the lipid content of the feed and its composition. Unlike the water-soluble fraction of the egg, in the lipid fraction content, achievement of a significant change is possible when marine products (oils and meal from fish and crustaceans, seaweeds, etc.) rich in fatty acids and carotenoids are included in the diets of laying hens [20, 21, 22].

The total lipid content in the lyophilized egg, was decreasing at 30 days/20°C of storage. Notably, because of the technique used, a further series of liposoluble compounds were also extracted, most of which are found in egg yolk (63% lipids in dry matter, of which almost 30% are phospholipids, 63% are triglycerides, 4.9% is in the form of free cholesterol, 1.3% is esterified cholesterol, and 1% is made up of vitamins and pigments), so this difference was possibly due to the oxidation of these compounds from the lipid fraction [21, 22].

C. Fatty acids in eggs

Alpha-linolenic acid (ALA, C18:3 n3), linolenic acid (LA, C18:2 n6), arachidonic acid (AA, C20:4 n6) DHA (C22:6 n3), n3 total, and n6 total showed differences only between treatments, differences

between treatments, times and temperatures for EPA and in DHA only in times ($P < 0.05$). The n6: n3 ratio varied from 8:1 to 11:1 only in treatments (Table III), with a notable difference in the 20% inclusion.

As reported by Carrillo et al. [21], who note that the marine material is rich in fatty acids, these marine materials, when included in the diet of the birds, can enrich the egg with these fatty acids. For the time variable, differences were only observed for EPA and DHA, and differences were observed for the variable temperature only for EPA, showing a decrease in values for these two variables studied at 30 days/20°C storage. These fatty acids are more susceptible to changes because they are polyunsaturated and oxidize easily, and eggs enriched with them also become susceptible to lipid deterioration, which could be affected by being stored for different times and at different temperatures. Therefore, these eggs need protection by the addition of antioxidants [21, 22].

On the other hand, limited or contradictory evidence exists on the enrichment of eggs with n3 and n6 fatty acids and on the effects of these fatty acids on quality during storage. For example, according to one study, no alteration occurred in the fatty acid profile during cooking or storage for 7 weeks/25°C [23], while other studies have reported increased susceptibility to oxidation during storage and cooking [24]. However, enriching the egg yolk with vitamin E may be an effective way to significantly reduce rancidity (thiobarbituric acid-reactive substances, or TBARS) in eggs enriched with n3 fatty acids [25].

With a lower proportion of n6 to n3 in poultry diets, the competition between ALA and LA can be reduced by the enzymes involved in the bioconversion of n3 long-chain acids, resulting in higher content in tissues [26].

Humans evolved on a diet having an n6 to n3 fatty acid ratio of approximately 1:1; however, today, Western diets have a ratio of 10:1 to 20-25:1, indicating that these diets are deficient in n3 fatty acids compared to the diets on which humans evolved and established their genetic patterns. However, n6 and n3 are not synthesized in the human body and are important components in the total composition of cell membranes. Studies indicate that DHA is essential for normal functional development of the brain and retina, particularly in premature babies, and accounts for 40% of the membrane phospholipids in the brain. Both EPA and DHA have an effect on the function of membrane receptors and even the generation of neurotransmitters. Evidence exists that EPA and DHA could play a role in hostility and violence, in addition to the beneficial effects in substance abuse and alcoholism disorders. However, the high proportion of n6, much of it by LA, is far from optimal. The ratio of n6:n3 in the brain is 1:1 and 2:1 and consistent with data on the evolutionary aspects of diet, genetics, and studies with animal models. Therefore, an n6:n3 ratio of 1:1 to 2:1 should be optimal for health [27]. However, in this study the results were not consistent with the aforementioned relationship. Therefore, n6 fatty acids will have to be reduced to reach the optimum proportion in poultry products.

D. Astaxanthin, Peroxide index, and TBARS in eggs

Table IV shows that the highest value was for the control egg (0.466 g/100g) because natural red xanthophylls (capsantin) were added to the diet; However, with the addition of 20% of SM, it was not possible to exceed this value (0.416 g/100g) with only astaxanthin as a red pigment ($P < 0.05$). Differences ($P < 0.05$) were also observed in the time and temperature of storage, with the astaxanthin content decreasing between the fresh egg (0 days) and the egg at 30 days/20°C, so that the content of astaxanthin, which is susceptible to

oxidation by temperature and storage, was reduced with these variables.

In this study, we detected a significant difference ($P < 0.05$) between treatments and times for the peroxide index, and TBARS were not detected.

As previously mentioned, by having several double bonds, polyunsaturated fatty acids are susceptible to oxidation, and eggs enriched with these fatty acids are also susceptible to lipid deterioration, making protection using antioxidants necessary. Astaxanthin (3,3'-dihydroxy-β-carotene 4,4'-dione), the xanthophyll responsible for the red pigmentation of salmon, trout, lobster, shrimp, and flamingo, has also been shown to strongly inhibit lipid peroxidation by active forms of oxygen. Astaxanthin is produced by the algae Haematococcus pluvialis and the yeast Phaffia rhodozyma, which are the main food of zooplankton and krill; in turn, these species are preyed on by species that store the pigment in skin and fat tissue, as it is a liposoluble pigment incorporated in the cell membranes [28]. Other studies [29] have shown the role of astaxanthin as a potent antioxidant in vivo and in vitro for the inhibition of lipid peroxidation.

Differences were also observed in the time and temperature of storage, with the astaxanthin content decreasing between the fresh egg (0 days) and the egg at 30 days/20°C, so that the content of astaxanthin, which is susceptible to oxidation by temperature and storage, was reduced with these variables. Astaxanthin was not included in the control diet formulation; however, when the eggs from this diet were analyzed, they generated a response at the same wavelength as astaxanthin, which can be explained by the technique used for the quantification of astaxanthin, for which the wavelength used in the detector was 470 nm. According to Britton [30], another series of carotenoids have a similar chemical structure and are quantified at a wavelength equal to or similar to astaxanthin (canthaxanthin, β-carotene, lutein, zeaxanthin, and lycopene), and as lutein + canthaxanthin are incorporated into this diet, these pigments are reported.

From the physiological point of view, astaxanthin is involved in essential cellular functions such as acting as provitamin A, which is associated with the reproduction and development of embryos as well as with the protection of cells against oxidative effects; it also improves stress tolerance and increases the immune response [31], so its use in human consumption has recently been promoted due to these properties. In the case of birds, astaxanthin has been used to increase egg production and improve the health status of hens. It has also been used as egg yolk pigment [32].

The main source of "rancidity" in food originates in the autoxidation of the lipid components. Autoxidation is defined as the spontaneous oxidation of a substance in contact with molecular oxygen. Although the occurrence of "rancidity" is the most significant consequence of autoxidation of lipids, the deterioration in taste is not the only damage suffered by food in this process. Color is also affected through accelerated browning reactions, the nutritional value decreases, and toxic effects can be induced. Texture may also be modified as a result of lateral reactions between proteins and fat oxidation products. In short, oxidative deterioration of lipids can be considered a deteriorating factor that affects all aspects of food acceptability. One of the most important functions of vitamin E, astaxanthin, and BHT (butylhydroxytoluene) is as an antioxidant, so in this study, the peroxide index was determined in the eggs of the experimental diet to detect the formation of peroxides and "rancidity." However, as no data existed on rancidity in stored eggs, the peroxide index of 5.3 mEq peroxide/kg corresponded to fresh oil or as being within its period of "rancidity" induction [33].

In this study, a significant difference between temperatures and times was found. These results could confirm that the astaxanthin present in the egg with 20% SM possibly protected the lipid fraction of the egg from oxidation relative to the control, which was more susceptible to oxidation even though the latter also had carotenoids and antioxidants. Because the data obtained indicate only the formation of peroxides from fatty acid degradation, the level of rancidity (TBARS) was quantified; however, this level was not detected in the eggs analyzed, which could confirm the data obtained by the peroxide index. Stahl et al. [34] reported that the antioxidant activity of carotenoids in liposomes can inhibit the formation of TBARS in the order of lycopene > α-tocopherol > α-carotene > β-cryptoxanthin > zeaxanthin = β-carotene > lutein. In this same experiment, the carotenoid mixtures were more effective than the compounds alone, where a greater synergistic effect was expressed, which is linked to the presence of lycopene or lutein. Galobart et al. [35] studied the composition and oxidative stability of eggs enriched with n3 and n6 polyunsaturated fatty acids in fresh and dehydrated eggs and eggs stored for 0, 6, and 12 months as a function of the dietary supplementation with different compounds in the diets of laying hens. Experiments were designed with α-tocopherol acetate, canthaxanthin, and rosemary extract. The authors concluded that a minimum of 3 weeks of feeding of hens is necessary to achieve a better stabilization of the α-tocopherol in the eggs. The use of canthaxanthin and rosemary extract did not affect the deposition of the α-tocopherol in the egg, regardless of the doses of these compounds. Supplementation with canthaxanthin had no antioxidant effect on the eggs enriched with PUFA, as with the rosemary extract. In that study, they observed that n-3 were more susceptible to oxidation than n6.

E. Yolk color in eggs

The color of the yolk presented significant differences ($P < 0.05$) between treatments, times and temperatures (triple interaction) with the inclusion of 20% (8.7), compared to the control egg that presented the highest pigmentation (10.9) according to the DMS color fan, and this pigmentation was decreasing with the passage of time at 20ºC.

Shrimp by-product meal has carotenoid pigments (astaxanthin) that are used for pigmentation of salmon, trout, chicken skin and egg yolk. Nevertheless, astaxanthins present in the cephalotorax of shrimp are associated to protein, chitin, and mineral salts, forming stable complexes that are an obstacle for pigment absorption in these species and, therefore, there is low absorption of astaxanthin, which has an affect on final coloring [36, 37].

IV. CONCLUSION

The results suggest that shrimp by-product meal could be used in laying hen rations. The advantage is that it is a renewable resource, an economical raw material and, in addition, it is a good source of proteins, fatty acids and pigments.

Regarding temperature and storage time, the observed differences were due to the normal deterioration suffered by any perishable food during its prolonged storage.

V. REFERENCES

[1]. Guillet R. 2010. Estudio mundial sobre las pesquerias de camaron. Global study on shrimp fisheries]. FAO Fisheries Technical Document. No. 475. Roma.

[2]. Salas-Durán C, Chacon-Villalobos CH, Zamora-Sánchez L. 2015. The shrimp cephalothorax meal in rations for laying hens In SpanishAgronomia Mesoamericana, 26 (2): 333-343.

[3]. Belandria JC. 2009. Amino acid profile and pigment content of crab and shrimp waste

meals. In Spanish], Mc diss., Universidad del Zulia Venezuela.

[4]. Morillo N, Montiel N, Belandria J, Mújica F. 2006. Proximal characterization of processing waste of crustaceans (crab and shrimp) in the State of Zulia In SpanishVeterinaria Tropical, 31 (1-2): 71-83.

[5]. Casas VM, Ponce DG. 1999. Estudio del potencial pesquero y acuicola de Baja California Sur. Vol. II. SEMARNAP, Gob. Edo. BCS, FAO, INAPESCA, UABCS, CIB, CICIMAR y CET del MAR. Mexico. 651p.

[6]. UNA. 2018. Union Nacional de Avicultores. Indicadores Economicos. Parámetros al cierre de 2017, sobre las estadisticas de la industria avicola. Retrieved March 16, 2018. Available at www.institutonacionalavicola.org.mx.

[7]. Ahn D, Sell J, Chamruspollet L, Jeffrey M. 1999. Effect of dietary conjugated linolenic acid on the quality characteristics of chicken eggs during refrigerated storage. Poult. Sci. 78 (6): 922-928.

[8]. Guerra M. 2000. Factores que afectan la calidad del huevo. Rev. Agricultura. 4: 38-40.

[9]. AOAC. 2005. Official Methods of Analytical Chemist, 18th ed. Association of Official Analytical Chemists. Arlington VA, USA.

[10]. Surai PF, Speake BK. 1998. Distribution of carotenoids from the yolk to the tissues of the chick embryo. J. Nutr. Biochem. 9: 645-651.

[11]. Perez-Gálvez A, Negro-Balmaceda JJ, Minguez-Mosquera MI, Cascajo-Almenera MV, Garrido-Fernández J. 2008. Astaxanthin from crayfish (Procambarus clarkii) as a pigmentary in the feed of laying hens. Grasas y Aceites, 59:139-145.

[12]. NOM. 1999. Norma Oficial Mexicana NOM-062-ZOO-1999, Especificaciones Tecnicas para la Produccion, Cuidado y uso de los Animales de Laboratorio. Mexico, D.F.

[13]. NRC. 1994. Nutrient Requirement of Poultry. 8th. Ed. National Research Council. National Academy Press. Washington D.C., USA.

[14]. 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. Agr. Food Chem. 42 (9):1931-1937.

[15]. FAO. 1987. The Nutrition and Feeding of farmed fish and shrimp. A training manual. 1. The essential nutrients. Food Agriculture Organization. Brasilia, Brazil, Pp 132.

[16]. Charley H. 2004. Tecnologia de Alimentos. Procesos quimicos y fisicos en la preparacion de alimentos. Food Technology. Chemical and physical processes in the preparation of food Ed. Limusa, Mexico.

[17]. Fanimo AO, Oduguwa OO, Onifade AO, Olutunde TO. 2000. Protein quality of shrimp-waste meal. Bioresourse Technol. 72 (2):185-188.

[18]. Normen L, Shaw C, Fink A. 2004. Combination of phytosterols and omega-3 fatty acids: a potential strategy to promote cardiovascular health. Current Medical Chemistry Cardiovascular and Hematological Agents, 2: 1-12.

[19]. Astasarian I, Martinez J. 2000. Food, composition and properties. In Spanish2ª. Edition (Madrid, España, McGraw-Hill – Interamericana de España S. A.).

[20]. Hargis PS, Van Elswyk ME. 1993. Manipulating the fatty acid composition of poultry meat and eggs for the health conscious consumer. Worlds Poultry Sci. J. 49: 251-264.

[21]. Carrillo DS, Carranco JM, Castillo DRM, Castro GMI, Ávila GE, Perez-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). Poul. Sci. 84 (1):167-172.

[22]. Castillo BC, Vázquez VJL, González AM, Morales BE, Castillo DRM, Carrillo DS. 2005. Tuna oil as a source of omega-3 fatty acids in

laying hen eggs In SpanishGrasas y Aceites, 56 (2):153-159.

[23]. Bernal GME, De Mendoca CX, Mancini-Filho J. 2003. Oxidative stability of eggs enriched with omega 3 polyunsaturated fatty acids, against natural antioxidants. In PortugueseRevista Brasilia de Ciências Farmacêuticas, 39 (4): 425-432.

[24]. ku T, Kato H, Kunishige-Taguchi T, Hattori M, Wada K, Hayashi M. 1996. Stability of fat soluble components such as n-3 polyunsaturated fatty acids and physicochemical properties in EPA- and DHA-enriched eggs. Japanese Journal Nutrition Dietetics. 54 (2): 109-119.

[25]. Van Elswyk ME, Sams AR, Hargis PS. 1992. Composition, functionality and sensory evaluation of eggs from hens fed dietary Menhaden oil. J. Food Sci. 57 (2): 342-344.

[26]. Qi GH, Sim JS. 1998. Natural tocopherol enrichment and its effect in n-3 fatty acid modified chicken eggs. J. Agr. Food Chem. 46:1920-1926.

[27]. Simopoulos AP 2011. Evolutionary aspects of diet: The omega-6/Omega-3 ratio and the brain. Mol. Neurobiol. 44 (2): 203-2015.

[28]. Miki W, Otaki N, Shimidzu N, Yokoyama A. 1994. Carotenoids as free radical scavengers in marine animals. J. Mar Biotechnol. 2 (1): 35-37.

[29]. Kurshize M, Okimasu E, Inoue E, Utsuma K. 1990. Inhibition of oxidative injury of biological membranes by astaxanthin. Physiological Chemical Physiological Medicine 22 (1): 27-38.

[30]. Britton G. 1995. UV/visible spectroscopy. In: Britton, G.; Liaaen-Jensen, S. and Pfander, H., eds. Carotenoids. Basel, Birkhäuser Verlag. V 1B.

[31]. Putnam ME. 1991. Aquaculture and the environment. European Aquaculture Soc. Special Publication, 16: 245-263.

[32]. Miki W. 1991. Biological Functions and activities of animal carotenoids. Pure Appl. Chem. 63 (1): 141-146.

[33]. NMX-F-223-1985. Normas Mexicanas. Alimentos. Aceite vegetal comestible. Foods Edible vegetable oil. Direccion General de Normas, Mexico D.F.

[34]. Stahl W Junghans A, de Boer B, Driomina ES, Brivia K, Sies H. 1998. Carotenoid mixtures Project multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. FEBS Letters, 427 (2): 305-308.

[35]. Galobart J, Barroeta AC, Baucells MD, Guardiola F. 2001. Lipid oxidation in fresh and Spray-Dried eggs enriched with w3 and w6 polyunsaturated fatty acids during storage as affected by dietary vitamin E and Canthaxanthin supplementation. Poult. Sci. 80 (3): 327-337.

[36]. Hudon J. 1994. Biotechnological Applications of Research on Animal Pigmentation. Biotecnol Adv. 12: 49-69.

[37]. Leeson S, Summers JD. 2001. Nutrition of the chicken. 4th ed. University Books. Guelph, ON, Canada. 285 pp.

Table 1. Experimental diets composition and calculated nutrient analysis.

	Experimental diets	
Ingredient (g/kg)	Control	Shrimp meal (SM)
Sorghum	673.957	648.857
Shrimp meal (20%)	0.000	200.000
Calcium carbonate	107.159	82.920
Soybean meal	182.800	37.371
Calcium phosphate 1821	11.931	13.233
Vegetable oil	10.000	9.770
Salt	3.626	3.048
Vitamin/mineral premix[a]	2.500	2.500
L-Lysine HCl	2.426	0.000
DL-Methionine	2.249	0.000
Mycotoxin sequestrant[b]	1.000	1.000
L-Threonine	0.703	0.000
Yellow natural pigmen[c]	0.500	0.500
Choline chroride 60%	0.500	0.500
Bacitracin-Zinc	0.300	0.300
Red natural pigment[d]	0.200	0.000
Antioxidant[e]	0.150	0.000
Total	1000	1000
Nutrient analysis		
ME (MJ/kg)	11.51	11.51
Crude protein %	15.00	15.10
Methionine %	0.347	0.363
Methionine+cystine %	0.690	0.984
Total calcium %	3.45	4.89
Available phosphorus %	0.280	0.240
Sodium %	0.150	0.151
Lysine %	0.709	0.979
Threonine %	0.620	1.041
Tryptophan %	0.191	0.177
Fatty acid composition (g/100g)		
Total lipids	5.77	5.89
Arachidonic acid (C20:4 n6)	0.29	0.15
α-linolenic acid (C18:3 n3)	3.23	4.97
Linoleica cid (C18:2 n6)	39.60	41.19
EPA (C20:5 n3)[f]	1.44	0.87
DHA (C22:6 n3)[g]	0.19	1.51
Astaxanthin (mg/100g)[h]	---	0.23

[a]Vitamins and minerals mix (per kg for laying hens): Retinol, 3,600µg; Cholecalciferol, 62.5 µg; Tocopherol, 20 µg; Menadione, 2 mg; Thiamine, 2.25 mg; Riboflavin, 7.5 mg; Pyridoxine, 3.5 mg; Cyanocobalamin, 0.02 mg; Niacin, 45 mg; Pantothenic acid, 12.5 mg; Biotin, 0.125 mg; Co, 0.04g; Fe, 12.0g; I, 0.04g; Mg, 24g; Zn, 14g; Se, 0.04g; Cu, 0.6g. [b]Klinssil: mycotoxin sequestrant. [c]Natural pigment of Aztec marigold (yellow, 15ppm). [d]Red natural xanthophylls of chili of the genre capsicum (10ppm). [e]IQ: BHT (antioxidant). [f]Eicosapentaenoic acid. [g]Docosahexaenoic acid. [h]Natural red pigment that provides shrimp meal. ME= metabolizable energy.

Table 2. Chemical composition of shrimp meal (SM).

Nutrient composition	SM
g/100g	
Moisture	9.02
Ash	29.86
Crude protein	36.07
Ether estract	0.88
Total carbohydrates	24.17
Gross energy (Kjoules/g)	10.19
Calcium	4.58
Sodium	1.04
Magnesium	0.14
Astaxanthin (mg/100g)	0.34
Fatty acid (% of total fatty acid)	
Saturated fatty acids:	39.16
Myristic acid (C14:0)	1.18
Palmitic acid (C16:0)	21.72
Stearic acid (C18:1)	12.45
Arachidic acid (C20:0)	1.98
Behenoic acid (C22:0)	1.83
Monounsaturated fatty acids:	19.28
Palmitoleic acid	4.06
Oleic acid (C18:1)	13.95
Erucic acid (C22:1)	1.27
n3 Polyunsaturated fatty acids:	15.95
α-Linolenic acid (C18:3 n3)	1.40
Eicosapentaenoic acid (C20:5 n3)	7.33
Docosahexaenoic acid (C22:6)	7.22
n6 Polyunsaturated fatty acids:	12.74
Linoleic acid (C18:2 n6)	6.42
γ- Linolenic acid (C18:3 n6)	0.95
Arachidonic acid (C20:4 n6)	5.37
n6:n3 ratio	0.79

Each value represents the mean of 6 replicates.

Table 3. Effect of inclusion of shrimp meal (SM) between treatments, days and storage temperatures on total lipids, fatty acids, total n3, total n6, and relationship n6:n3

	TFA g/100g	AA n6	ALA n3	LA n6	EPA n3	DHA n3	Total n3	Total n6	n6:n3 ratio
					(mg/100g)				
Treatment									
Baseline*	41.6	774.7	157.5	4490.4	4.75	331.9	494.2	5265.2	10.6:1
Control	43.6	786.4[b]	155.9[b]	4498.0[b]	3.91[b]	320.4[b]	480.3[b]	5284.5[b]	11.1:1[a]
SM	44.6	915.8[a]	263.7[a]	6267.9[a]	11.7[a]	562.7[a]	838.2[a]	7183.7[a]	8.6:1[b]
SEM	0.35	19.4	7.08	161.05	0.28	15.5	20.91	173.7	0.19:1
Time									
Baseline*	41.6	774.7	157.5	4490.4	4.75	331.9	494.2	5265.2	10.6:1
15 days	45.2[a]	863.0	212.3	5421.2	8.32[a]	479.2[a]	698.9	6284.3	9.2:1
30 days	43.0[b]	839.2	207.3	5344.7	7.33[b]	403.8[b]	619.5	6183.9	10.4:1
SEM	0.35	19.4	7.08	161.05	0.28	15.5	20.91	173.7	0.19
Temperature									
Baseline*	41.6	774.7	157.5	4490.4	4.75	331.9	494.2	5265.2	10.6:1
20°C	43.5	847.3	206.6	5348.8	8.45[a]	440.9	654.8	6196.2	9.7:1
4°C	44.6	854.9	213.0	5417.1	7.20[b]	442.2	663.6	6272.0	9.9:1
SEM	0.35	19.4	7.08	161.05	0.28	15.5	20.91	173.7	0.19
					***P* values**				
Treatment (T)	0.078	0.001	0.0001	0.0001	0.0001	0.001	0.0001	0.0001	0.0001
Time (Ti)	0.002	0.410	0.632	0.745	0.038	0.008	0.027	0.693	0.002
Temperature (Te)	0.061	0.789	0.544	0.772	0.014	0.955	0.772	0.765	0.508
T x Ti	0.035	0.042	0.158	0.212	0.006	0.971	0.562	0.165	0.583
T x Te	0.652	0.648	0.547	0.966	0.006	0.439	0.412	0.928	0.092
Ti x Te	0.0004	0.370	0.676	0.930	0.022	0.219	0.433	0.854	0.553
T x Ti x Te	0.015	0.444	0.213	0.232	0.223	0.007	0.015	0.233	0.007

*Baseline (fresh eggs without storing).

[a, b] Different letters in each column indicate significant differences ($P < 0.05$).

SEM= standard error of the mean.

TFA= total fatty acids; AA= Arachidonic acid; ALA= alpha linolenic acid; LA= Linoleic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid.

Table 4. Effect of shrimp meal (SM), treatment, storage days, and storage temperature on astaxanthin, peroxide index, and yolk color in dry freezing eggs.

	Astaxanthin (g/100g)	Peroxide index (mEq peroxide/100g)	Yolk color (DMS color Fan)
Treatment			
Baseline*	0.580	247.7	10.8
Control	0.466[a]	242.9[b]	10.9[a]
SM	0.416[b]	255.4[a]	8.7[b]
SEM	0.001	1.70	0.06
Time			
Baseline*	0.580	247.7	10.8
15 days	0.483[a]	242.1[b]	9.9[a]
30 days	0.399[b]	256.2[a]	9.7[b]
SEM	0.001	1.70	0.06
Temperature			
Baseline*	0.580	247.7	10.8
20°C	0.431[a]	250.8	9.4[b]
4°C	0.451[b]	247.5	10.1[a]
SEM	0.001	1.70	0.06
		P values	
Treatment (T)	0.0001	0.0008	0.0001
Time (Ti)	0.0001	0.0004	0.037
Temperature (Te)	0.0001	0.207	0.0001
T x Ti	0.0001	0.0001	0.013
T x Te	0.0001	0.0001	0.890
Ti x Te	0.0001	0.079	0.434
T x Ti x Te	0.0001	0.0001	0.0001

*Baseline (fresh eggs without storing).

[a, b] Different letters in each column indicate significant differences ($P < 0.05$).

SEM= standard error of the mean.