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Physical and Chemical Changes in the Soluble Fraction of Eggs from Hens Fed With *Pleuroncodes Planipes* (Red Crab), Stored for Different Lengths of Time At Different Temperatures

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ABSTRACT

As an abundant resource on the coast of Baja California, Mexico, the crustacean known as *red crab* (*Pleuroncodes planipes*) may represent an alternative for use in the formulation laying hens' diet because of the crab's protein content. It has been reported that diets containing 4% red crab meal (RCM) generate eggs with satisfactory sensorial characteristics. However, changes in the physical and chemical characteristics of the water-soluble components that alter the biological value of eggs might occur during storage. Therefore the aim was to determine the effect of storage conditions on the water-soluble fraction of eggs from hens fed with *Pleuroncodes planipes* meal (RCM). To determine the effect of storage conditions on the water-soluble fraction of eggs from hens fed with a standard diet and with a diet with 4% (RCM), 90 hens were divided into two treatments: Control and RCM (4%). During four weeks of testing, production variables were evaluated. Eggs from each treatment were collected to assess the following factors: Haugh Units (HU), pH and levels of crude protein, essential amino acids and water-soluble vitamins after 0, 15 and 30 days at 4° and 20°C. The results were analyzed using a 2x2x2 factorial design, and the difference between means was compared using Tukey's test. The results showed that there was no significant difference in production performance between the treatment groups. During the storage period, there was a decrease in the CP content (49.8%/15 days – 49.2%/30 days) and HU (60.16/15 days – 52.62/30 days) and an increase in the pH (8.73/15 days – 9.32/30 days). The effects of temperature and egg storage time on the CP content (49.2%/20°C – 50.2%/4°C) and HU (56.82/20°C – 71.05/4°C) were different ($P < 0.05$). The effects of diet and egg storage time resulted in differences ($P < 0.05$) in the CP content (49.2 Control – 50.2 RCM) and HU (62.91 Control – 64.96 RCM). Adding 4% RCM to the diets of laying hens did not affect the studied productive parameters, but changes were observed in the water-soluble part of the egg in a double interaction (times and temperatures). The eggs presented changes inherent to the egg metabolism, modifying the variables studied.

Keywords: Red Crab Meal, Egg Quality, Storage

I. INTRODUCTION

Researchers at the Northwest Center for Biological Research (known by its Spanish acronym, CIBNOR) have been studying the biology, ecology and utilization of red crab (*Pleuroncodes planipes*) for several years, and the results show that this species can be considered the most abundant benthic decapod in Mexico. Data from Balart [1] and Aurioles and Pérez [2] reported an abundance of approximately 300,000-500,000 t/year and a maximum density of up to 40 red crabs/m^2 or higher for the coast of the Baja California peninsula. To date, this species has not been commercially exploited in Mexico. These authors suggest that 40,000 t of bentho-

pelagic red crabs could be captured during the initial phase of the fishery without decreasing the amount available; creating a sustainable fishery that allows the harvest of biomass for industrial exploitation. Therefore, it is important to develop technologies for red crab exploitation and to evaluate the resulting products in various applications. Today, this type of red crab is widely studied for its use in the poultry industry as an ingredient in poultry diets, where it constitutes an alternative source of protein, fatty acids and pigments [3, 4, 5, 6, 7].

Mexico ranks first in the word in egg consumption (22 kg per capita per year or 305 eggs per person per year) [8]. Furthermore, the Mexican Norm NMX-FF-079-SCFI-2004 [9] regarding poultry products, fresh chicken eggs and method specifications considers an egg to be fresh at the 15 days old; however, eggs are often stored for longer periods and the different temperatures. During this period, the air space within the egg increases due to loss of water, and a change in pH occurs. This change and the effect of the compounds in new raw materials added to the bird's diet can affect the chemical and physical quality of eggs [10, 11].

According to the results published by Carrillo et al. [6] and Carranco et al. [7], the inclusion of between 3 and 6% of RCM in the diets of laying hens is probably adequate for obtaining high-quality eggs because higher levels cause changes in the sensorial characteristics of eggs. Starting from the above inclusion criterion, this study was designed to determine whether the physical and chemical properties of the water-soluble portion of eggs from hens fed a standard diet and a diet with 4% of red crab meal undergo modifications when stored at 4° and 20° for 0, 15 and 30 days.

II. METHODS AND MATERIAL

Red crab meal (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, where it was stored in black plastic bags and frozen (-20°C) until use.

A. Preparation of diets and test with birds

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 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 [13] for laying hens through Nutrion Windows™ (Version 5.0 Pro), a computerized system for feed formulation (Guadalajara, Jal., Mexico).

Ninety Isa-Brown laying hens (32 wk age, with mean body weight of 1560 ± 20 g) were selected from 300 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 (Control) and a diet containing 4% RCM. Throughout the experimental periods, feed and water available to allow for 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: day 0 (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).

B. Haugh Units (HU)

For the fresh eggs and the eggs stored at 20°C and 4°C for 15 and 30 days, the HU were determined using automated equipment (Technical Service and Supplies, Inc., England, UK). The system consists of a microprocessor (QCM+) connected to a digital scale and a micrometer for measuring albumen height. The QCM+ collects data from online tools and displays a reading, which is transferred to a computer equipped with egg quality-assurance software. The HU were calculated from the albumen height and the egg weight using the same processor.

C. Chemical Analysis

The following analyses were performed on the RCM according to standardized techniques published by the Association of Official Analytical Chemists [14]: crude protein (method 968.06), total ash (method 942.05), ether extract (method 920.39), minerals (Ca, Na and Mg) (methods Cap. 4-40), water-soluble vitamins (B_1, B_2 and niacin) by high performance liquid chromatography (HPLC) (methods 970.65 and 942.23), gross energy of RCM, was determined a total combustion using an adiabatic bomb calorimeter (Parr 1755, Parr Instrument Company, Moline, Il, USA), and the amino acid profile by HPLC (hydrolyze the sample into its component amino acids, and derivative the amino acids using AccQ-Fluor Reagent) (WatersAcc-Qtag Manual, Manual No. WAT052874, April 1993).

The pH of the egg samples fulfilling the desired age and storage conditions was performed using a Hanna Instrument pH-210 potentiometer, and the egg were subsequently mixed (yolk and albumen) and lyophilized. Using the lyophilized egg, analyses of crude protein, essential amino acids and water-soluble vitamins were performed according to the techniques described above.

D. 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 SPSS version 11.0 for Windows (SPSS Inc., Chicago IL, USA).

The statistical model was: $Yij = \mu + \tau i + Єij$, 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 water-soluble 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 confidence level of 95%, using SPSS version 11.0 for Windows (SPSS Inc., Chicago IL, USA).

The statistical model was: $Yijk = \mu + \alpha i + \beta j + \gamma_k + (\alpha\beta)ij + (\alpha^{\gamma})_{ik} + (\beta^{\gamma})_{jk} + (\alpha\beta^{\gamma})ijk + Єijk(l)$, where Yijk = the response variable, μ = the experimental mean, αi = the effect of the i-th diet treatment, βj = the effect of the j-th time treatment, γ_k = the effect of the k-th temperature treatment, $(\alpha\beta)ij$ = the effect of the interaction of treatment and time, $(\beta^{\gamma})_{jk}$ = the effect of the interaction of time and temperature, $(\alpha\beta^{\gamma})ijk$ = the interaction of treatment, time and temperature, Єijk(l) = the experimental error.

III. RESULT AND DISCUSSION

A. Chemical composition of the red crab meal (RCM)

The results of the approximate chemical analyses are presented in Table 1; the most abundant fractions were crude protein (33.74%), total ash (20.24%) and Mg (9.70 mg/100 g). Due to the high protein content found in this unconventional raw material, it was important to evaluate the content of essential amino acids. The experimental diets were formulated based on the chemical and amino acid compositions of the meal (Table 2).

TABLE I

Composition of red crab meal (*Pleuroncodes planipes*) used in this study

(g/100 g)[1]	RCM
Moisture	9.13 ± 0.07
Ash	20.24 ± 0.03
Ether extract	7.29 ± 0.01
Crude protein	33.74 ± 0.14
Total carbohydrates	29.58
Gross energy (kcal/100 g)	255.7 ± 0.08
Calcium	7.97 ± 0.05
Sodium	1.18 ± 0.08
Magnesium	9.70 ± 0.96
Amino acid composition g aa/100g protein	
Isoleucine	3.98 ± 0.01
Leucine	6.55 ± 0.01
Lysine	10.02 ± 0.02
Methionine	1.55 ± 0.01
Cystine	1.98 ± 0.02
Phenylalanine	4.31 ± 0.02
Tyrosine	4.08 ± 0.03
Threonine	3.55 ± 0.01
Valine	5.35 ± 0.03
Arginine	3.68 ± 0.02
Histidine	6.80 ± 0.02
Alanine	6.35 ± 0.03
Aspartic acid	9.18 ± 0.03
Glutamine	14.55 ± 0.01
Glycine	7.56 ± 0.01
Proline	5.15 ± 0.03
Serine	3.42 ± 0.02
Tryptophan	1.27 ± 0.02

[1] The values are the mean ± standard deviation of six samples.

TABLE II

Composition of the experimental layer diets

Ingredients (as fed g/kg)	Control	40 g/kg 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*
Calculated analysis (g/kg diet)		
ME (kcal/kg)	2715.1	2715.1
Crude protein	150.0	151.0
Methionine	4.56	3.94
Methionine + cystine	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

[a] The premix provided the following per kg of diet: retinyl acetate, 12000 IU; cholecalciferol, 2500 IU; DL-α-tocopheryl acetate, 30 mg; menadione, 2 mg; thiamine, 2.25 mg; riboflavin, 7.5 mg; pyridoxine, 3.5 mg; cyanocobalamin, 0.02 mg; D-pantothenic acid, 12.5 mg; biotin, 0.125 mg; folic 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.

B. Production variables

No statistically significant differences ($P > 0.05$) in the productive parameters measured were found (Table 3).

TABLE III

The effect of dietary supplementation with RCM on the production performance of laying hens between 34 and 38 weeks of age[1].

	Number of eggs/hen	Egg weight (g)	Feed conversion ratio (g feed/g egg)	Feed consumption (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 with the SD. No significant differences were found.

C. Crude protein content (CP), pH and Haugh Units (HU) in eggs

Table 4 shows the results of the response of RCM addition, days and temperature storage on the crude protein content (CP), pH and HU of the egg. Showing a significant difference ($P < 0.05$) in CP, pH and HU between days and temperatures storage.

With respect to temperature were found significant difference ($P < 0.05$) in CP and HU, but not in pH ($P < 0.05$). For the days of storage, there were differences ($P < 0.05$), with CP and HU decreasing and the pH increasing as storage time went on ($P < 0.05$).

The effect of temperature and egg storage time on CP, pH and HU, showing a significant difference ($P < 0.05$) for CP between temperatures and not between storage days. The pH and HU showed differences for either of the measured variables ($P < 0.05$).

The effects of diet and egg storage time on CP, pH and HU showing significant differences for both diet and storage days ($P < 0.05$).

TABLE IV

The effect of RCM addition storage days and storage temperature on egg Crude Protein content, pH and Haugh Units.

	Diet		
	Crude Protein content (g/100 g)	pH	Haugh Units
Control	49.2[b]	8.48[a]	62.91[b]
RCM	50.2[a]	8.53[a]	64.96[a]
SEM	0.27	0.06	1.56
Temperature			
Baseline*	48.8	7.35	76.14
20°C	49.2[b]	8.44[a]	56.82[b]
4°C	50.2[a]	8.50[a]	71.05[a]
SEM	0.19	0.04	1.1
Storage days			
15	49.8[ab]	8.73[b]	60.16[b]
30	49.2[b]	9.32[a]	52.62[c]
SEM[d]	0.27	0.06	1.56
Significance			
Diet	0.0002	0.4216	0.0158
Temperature	0.0037	0.0761	0.0001
Storage days	0.0033	0.0001	0.0001
Diet * Temperature	0.1101	0.1907	0.9504
Diet * Storage days	0.0001	0.5193	0.0055
Temperature*Storage days	0.0100	0.1343	0.0001
Diet *Temperature* Storage days	0.0515	0.1886	0.9767

[a,b,c] Means in each column followed by different subscript letters differ significantly.

[d] Pooled standard error of mean *Baseline (fresh eggs without storing)

D. Amino acids and Water-soluble vitamin

There were no significant differences ($P > 0.05$) on the content of the studied amino acids or the water-soluble vitamins (B_1, B_2 and niacin).

In this study, the value obtained for crude protein (33.74%) in RCM was lower than the values reported by Charley [15] for crab meal (47.2%) and by Castro et al. [5] for red crab (39.9%). The RCM showed a high level of total ash (20.24%). However, this value is lower than that reported by other authors (28.9-33%) [16, 17, 18]. This discrepancy can be explained by the environment in which these crustaceans live, rich in minerals, in particular Mg, which is an element that is present in high concentrations in all crustaceans, ranging from 10 to 50 mg/100 g [19]. The value of ether extract (7.29%) was greater than that reported by [15] 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. [5] reported that females have a higher lipid content, requiring stored energy for reproduction.

The gross energy obtained in this study was 255.7 kcal/100g, 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 [16]. Crustaceans obtain their energy mainly from protein catabolism, so the metabolism of these organisms is different from land animals [16].

With respect to the essential amino acids for birds, most were found in higher quantities compared to those reported by Toma and Meyers [20]. Additionally, Quintero [21] published higher values for the amino acids reported in this study, with the exception of histidine, valine and lysine. According to the formulation of diets for birds [13], it was only necessary to add the amino acids methionine, threonine and lysine to the control diet, unlike the diet containing RCM, which is rich in lysine (10.02 g aa/100 g protein) and to which only methionine was added.

The objective of this study was to determine the effect of storage conditions on the water-soluble fraction of eggs from hens fed a standard diet and a diet containing 4% RCM. The pH has a value of 7.6 on a freshly laid egg and rises to 8.5 after 24 hours at 20°C, reaching values of 9 to 9.4 after several days. In this study, the pH increased as storage time went on (7.35 – 9.32), which is consistent with the pattern reported by Li -Chan et al. [22], who found that in a fresh egg (at the time of laying) the pH ranged from 7.6 to 8.5. The eggs stored for 15 days at different temperatures showed pH values between 7.35 and 9.32. These values are similar to those reported by these authors: the pH after three days of storage at 3°C was 9.18 and after 21 days was 9.4. The pH of the egg (mainly of the albumin) is an important factor in the control of the rheological properties of gels formed during the heat treatment (80°C). The pH depends on the balance between the dissolved carbon dioxide, bicarbonate ions and carbonate ions governed by the partial pressure of carbon dioxide from the external environment. The bicarbonate ion concentration increases as the carbonate concentration decreases [23]. In addition, the pH is related to the action of proteins and the reactivity of the sulfhydryl groups. As the pH increases in the egg during storage, the elasticity of the gel, the penetration force and the viscosity index decrease [24]. This change increases the fragility of the yolk by the dispersion of albumin, which could affect the functional properties of the egg through the formation of foam.

Both the physical and chemical egg qualities are important, especially when new ingredients are incorporated in the formulation of the birds' diet. Through various laboratory analyses, the changes experienced by the samples, both fresh and during storage, can be assessed, specifically regarding their physical (egg weight and HU) and chemical (protein, pH, amino acids and vitamins) characteristics, which influence their quality and the preference of the consumer. As reported by Grobas and Mateos [25], the water-soluble components of egg show little variation when the ingredients of the bird's diet are changed; however, these authors did not consider the changes due to the storage conditions and time.

In hens, daily consumption of the amino acids needed to procedure egg protein is more important than the total amount of protein. The amino acids that are important for the birds were quantified in RCM. Methionine, lysine and threonine are considered the limiting amino acids in poultry diets; in RCM, the lysine content was greater than that of methionine, such that it was only

necessary to add methionine to the diet. Therefore, the reduction of one of these amino acids negatively affects the production of albumin in the eggs, decreasing the size of the egg over time. Egg protein is considered high quality, and the essential amino acid ratio depends on the genetic line of the bird. Egg protein has been used as a reference for other types of food for human consumption. Therefore, it is important that the bird's diet contains appropriate levels of essential amino acids for maintaining biological and commercial egg quality [26].

The Mexican Norm NMX-FF-079-SCFI-2004 (regarding poultry products, fresh chicken eggs and test methods specification) [9] states the physical characteristics and specifications to be met by "classified fresh chicken eggs" that are produced and/or sold within the country. In this standard, five categories for fresh chicken eggs based on the egg weight and size are recognized and should be applied to all classifications of consumption (Extra Large = greater than 64 g, Large = 60-64 g, Medium = 55-60 g; Small = 50-55 g, and Pewee = less than 50 g). According to these categories, both the control eggs (63.8 g/initially to 60.1 g/20°C and 63 g/4°C) and the RCM eggs (64.2 g/initially to 61.4 g/20°C and 63.2 g/4°C) had weights that were within the "Large egg weight" category after 30 days in storage.

Likewise, fresh chicken eggs for cooking are classified in grades according to the following specifications: the scale of Haugh Units (HU) ranges from 0 to 110. The interpretation of these units is an aid to determine the laying time: lower values indicate older eggs. Thus, this classification is as follows: Mexico Extra = more than 60 HU; Mexico 1 = from 61 to 70 UH; Mexico 2 = from 31 to 60 UH and, out of classification = less than 30 HU. In general, both the control eggs and the RCM (62.91 and 64.96 HU, respectively) were classified as Mexico 1 (>5.5 mm height and albumin and >70 HU for eggs freshly laid), decreasing after 15 and 30 days at 4°C to rank as Large eggs (>4.2 mm and 61-70 HU) and after 30 days at 20°C as Medium eggs (>3.0 mm and 31-60 HU). In this study, a decrease in HU was observed as an effect of diet, storage time and temperature; this decrease was most notable for eggs stored for 30 days at 20°C. This change is explained by natural phenomena that occur in a perishable product that is not consumed at the laying time. This change can be understood as a decrease in albumen quality that is reflected in the HU and manifested by the liquefaction of the dense albumen, which results in the loss of the internal structure and the spatial organization of the layers of the albumen and the yolk. The mechanisms responsible for this change are complex and not well defined, but all involve alterations of proteins. The phenomena can be described as follows [27]: a) partial destruction of the lysozyme-ovomucin complex due to the deactivation of lysozymes; b) destruction of the electrostatic bonds between the amino groups of the lysine residues of the lysozyme and the carboxyl groups of the sialic acid in the ovomucin when the pH increases; c) dissociation of ovomucin subunits, mainly of β, which is favored by the passage of divalent ions from the albumin to the yolk of the eggs during storage; d) modification of the structure of the ovomucin, especially the β subunit, which changes the type of the lysozyme-ovomucin complex, due to the increase in pH and; e) partial degradation of the O-glycosidic bonds, which results in the solubilization of ovomucin-β and releases hexoses, hexosamines and sialic acid by an alkaline β-elimination process.

The above mentioned phenomena are related to the release of carbon dioxide from the interior of the egg, which tends to balance its concentration with the partial pressure of surrounding air, resulting in an increase in pH. The changes accelerate significantly when the egg is stored at room temperature. This acceleration occurred in eggs stored at 20°C for 15 and 30 days; when they were opened, it was observed that the yolk had moved from its original position (in the center), shrunk and flattened, and that the cell membrane (vitelline) broke easily. All of these changes are associated with alterations in the proteins present in the albumin [27]. Note that although there were no differences in the composition of water-soluble vitamins, their values become important in nutritional labeling [28].

IV. CONCLUSION

We conclude that the productive parameters are not affected by an addition of 4% RCM to the diets of laying hens. However, changes were observed in the CP content, pH and HU due to the chemical and physical changes that occur in the eggs stored at 20°C for 30 days.

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