v. 3, n. 1, 2023

Journal of
Agricultural
Sciences
Research

INCLUSION OF SEAWEED *GRACILARIA SP* AS A DIETARY PROTEIN ALTERNATIVE IN LAYING HENS

Hernán Rodríguez-Ríos
Universidad de Concepción, Facultad de Agronomía, Animal Production Department, Chillán, Chile

Jorge Campos-Parra
Universidad de Concepción, Facultad de Agronomía, Animal Production Department, Chillán, Chile

Rita Astudillo-Neira
Universidad de Concepción, Facultad de Agronomía, Animal Production Department, Chillán, Chile

Jesús Grande-Cano
Universidad Autónoma Metropolitana Iztapalapa, Área de Sistemas de Producción Agropecuarios. Ciudad de México, Mexico

Silvia Carrillo-Domínguez
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Colonia Belisario Domínguez Sección XVI, Delegación Tlalpan

Fernando Pérez Gil- Romo
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Colonia Belisario Domínguez Sección XVI, Delegación Tlalpan

Abstract: The recommendations for restricted egg consumption are aimed at the population with hypercholesterolemia, directing research to reduce their cholesterol. The objective was to evaluate the inclusion of red algae: *Gracilaria sp*. in the diet of laying poultries and its effect on productive variables and concentration of cholesterol in eggs. The alga was cultivated in the Atacama region, Chile, dried in the sun and crushed to be included in the diet. Two hundred 40-week-old laying hens were used, divided into 4 treatments and 5 repetitions, distributed in a randomized block design. The treatments were supplementation with seaweed at 0, 15, 30 and 45%. The following were evaluated: weekly feed intake, egg production, and feed conversion and egg mass were calculated. Cholesterol content was determined in 480 randomly collected eggs per week per repetition and treatment. Data were analyzed with ANOVA and Duncan's test ($p<0.05$). The treatments of 30 and 45% were suspended at the fifth and fourth week, respectively, due to decreased food consumption and cessation of egg laying. The cholesterol content of the yolk decreased from 23.36 to 17.27 mg g of yolk-1, due to the inclusion of the algae. The inclusion of the alga: *Gracilaria sp,* decreases the cholesterol level of the egg yolk, but alters the consumption of the poultries.
Keywords: Algae, eggs, cholesterol.

INTRODUCTION

The egg is a low-cost and highly nutritious food, which makes it a valuable component of a balanced and healthy diet with a low caloric intake (75 average calories per unit) (Dussaillant et al., 2017). Its lipids correspond mainly to monounsaturated fatty acids, and a low proportion of saturated fats (Dussaillant et al., 2017), with a cholesterol intake of ~210 mg of cholesterol per unit (~71% of the recommended daily allowance) (Krauss et al, 2000; Soriano-Maldonado et al., 2013; Miranda et al., 2015; Fuentes-García 2016). It is also one of the main sources of vitamin D, with important contributions of riboflavin, folate, selenium, vitamin A and B12 (Gray & Griffin 2009). Few studies have found a negative relationship between the daily intake of a unit of egg and adverse effects on the cardiovascular health of individuals (Nettleton et al., 2008; Djousse & Gaziano 2008; Djousse & Gaziano 2008b; Nakamura et al., 2004), and the contrast between the results may be due to the lack of analysis of variables derived from the diet, such as other sources of dietary cholesterol, intake of saturated fats, transesterified fats, fiber and total calories (Dussaillant et al., 2017). However, the population has received messages for years aimed at reducing egg consumption, as a predisposing source to cardiovascular diseases, despite being clear that the recommendation to reduce egg consumption must only be aimed only at the population with hypercholesterolemia problems (Elkin 2006; Oriondo-Gater et al., 2013). This restriction in egg consumption has oriented poultry research in obtaining eggs reduced in cholesterol and saturated fats or enriching them with beneficial compounds for health (Grobas & Mateos, 1996). Investigations that begin in the 90s with the studies by Naber (1990) that achieved decreases of 10% and continue until today.

Among the food additives aimed at reducing the cholesterol content of eggs, seaweeds such as *Sargassum spp*. have achieved significant reductions with 4, 6 and 8% inclusion, decreasing cholesterol in the egg by up to 26% (Carrillo et al., 2012). In general, red marine algae present 5 to 44% protein, 1 to 5% lipids, with a composition of mainly unsaturated fatty acids, varying according to species, geographic area, season of the year, and environmental conditions (Gil-Saavedra 2016). Red marine algae are characterized by having abundant amounts of ash, phycocolloids, and sterols (Reiner et al., 1962; Jimenez-Escrig & Goñi, 1999; Yuan, 2008). Studies carried out by these authors have shown that sterols and phycocolloids have the ability to reduce blood cholesterol concentrations, since they have soluble carbohydrates (agar and carrageenan) that are part of dietary fiber, which reduces cholesterol levels in blood, because they interrupt the enterohepatic cycle of bile acids and prevent cholesterol from being synthesized de novo. On the other hand, the evaluation of *Porphyridium sp* in diets of 30-week-old White Leghorn laying poultries, the serum cholesterol of the poultries decreased by 28%, the decrease being attributed to the fiber present in the algae, which would be reducing the absorption of cholesterol at the intestinal level (Ginzberg et al., 2000). The seaweed: *Gracillaria sp* belonging to the group of red algae (Gil-Saavedra 2016) group of Rodophytas (Edding et al., 1987), could also be another alternative to achieve such a decrease in cholesterol in the egg. Few studies have been carried out on the use of this algae with laying hens, one of which is supplementation with 2% flour. *Gracilaria vermicullophylla* in order to evaluate its effect on the physical quality of the egg and the composition in fatty acids (Ozaki et al., 2013).

The objective of the present investigation was to evaluate if the inclusion of red algae (*Gracilaria* sp.) in the diet of laying poultries has an effect on the productive variables and the concentration of cholesterol in the egg yolk.

MATERIALS AND METHODS

The seaweed: *Gracilaria sp*. was cultivated in the coastal zone of the Atacama region, Chile (Lat. 27.0667°S, Long. 70.8333°W). This was planted directly on the seabed using a hand tool long enough to carry out the planting from smaller boats. The planting distance was between 50 and 80 cm at a depth of 3.54 m in a total area of 60 m^2, with a total of 1959 plants. The harvested algae were dried in the sun, and later crushed in a hammer mill (Wetmore Brand, USA) to be included in the diet. Table 1 shows the chemical composition of the algae as offered to poultries.

The bioassay was carried out at the poultry experimental station of the Faculty of Agronomy, Department of Animal Production, of the ''Universitdade de Concepción", Campus Chillán, Chile, Ñuble Region, for a period of two months. Two hundred 40-week-old laying hens, Hy-line strains, divided into 4 treatments and 5 repetitions (n=10), distributed based on a random block design, were used. Housing was in conventional laying cages with access to food and water ad libitum. The diets were elaborated based on the requirements indicated by the National Research Council (NRC, 1994). These included corn grain, wheat bran, soybean paste, calcium carbonate, DL methionine, vitamin mix, mineral mix, common salt, and were supplemented with 0, 15, 30, and 45% red marine algae: *Gracilaria sp*. (Table 2).

During the entire experimental period, the following productive variables were evaluated: feed consumption per week (g sem^{-1} $poultry^{-1}$), egg production (dozen sem^{-1}), and feed conversion was calculated (kg feed dozen eggs $^{-1}$) and egg dough (g egg of $poultry^{-1}$ day^{-1}).

To determine the concentration of cholesterol in the egg, a sampling of these was carried out by random collection of 3 eggs per week, per repetition and treatment, involving a total of 480 eggs (n total). The cholesterol content in the fresh yolk was determined using the technique described by Rodríguez-Ríos et al. (2020), using instrumental planar chromatography (HPTLC) with automated multiple development (AMD) chamber.

Statistical analysis was performed through an ANOVA analysis of variance for a completely randomized block design. For the comparison of means, Duncan's test was used with a significance of 5% ($p \leq 0.05$). The assumptions of the ANOVA were verified using the Shapiro Wilk Modified test for normality and Levene's test for the homogeneity of variance, both with a significance level of 5% ($p \leq 0.05$). The analyzes were carried out using the statistical software Infostat (Di Rienzo et al., 2008).

RESULTS AND DISCUSSION

Egg production decreased drastically with values ranging from 81 to 21% laid as the level of seaweed increased: *Gracilaria sp*. in the ration, detecting statistically significant differences ($p \leq 0.05$). The most notable productive result refers to the notorious effect that the rations including algae had on the behavior of the poultries: their productive capacity fell markedly, food consumption was drastically reduced and production ceased.

The egg-mass parameter with different levels of inclusion of seaweed tended to decrease markedly ($p \leq 0.05$) as the level in the ration increased, varying between 50 and 12g (50, 36, 17 and 12 g for 0, 15, 30 and 45% inclusion, respectively), with significant differences between the four treatments ($p \leq 0,05$). *Gracilaria sp*, makes a contribution of 15% CP, values similar to the contribution that would be made by soybean bran (14.4% CP), which is recommended to be included in a maximum of 5% in layer diets. In the case of the use of *Gracilaria sp*, there are no reports of maximum allowed to date, and the results presented here suggest that their inclusion must not be greater than 10% and even less so as not to cause negative changes in the productive parameters, and to study whether a longer inclusion in the period production of hens causes significant decreases in yolk cholesterol. The productive results obtained agree with what has been pointed out by other authors in the sense that concentrations greater than 9% of seaweed in the diet of poultries cause a reduction in feed consumption and egg production, due to the high salt content. minerals and dietary fiber, which characterize marine algae (Jiménez-Escrig & Goñi, 1999). In the present investigation, the seaweed:*Gracilaria sp*, it was harvested, washed and dried in the sun, which caused the salt to concentrate, which could be the cause of the decrease in consumption in the treatments that included it. Carrillo et al. (2012), registered similar problems of food consumption and decreased egg production when they included 2, 4, 6 and 8% of brown algae. *Sargassum spp.*, in the diet of Leghorn hens for 5 weeks.

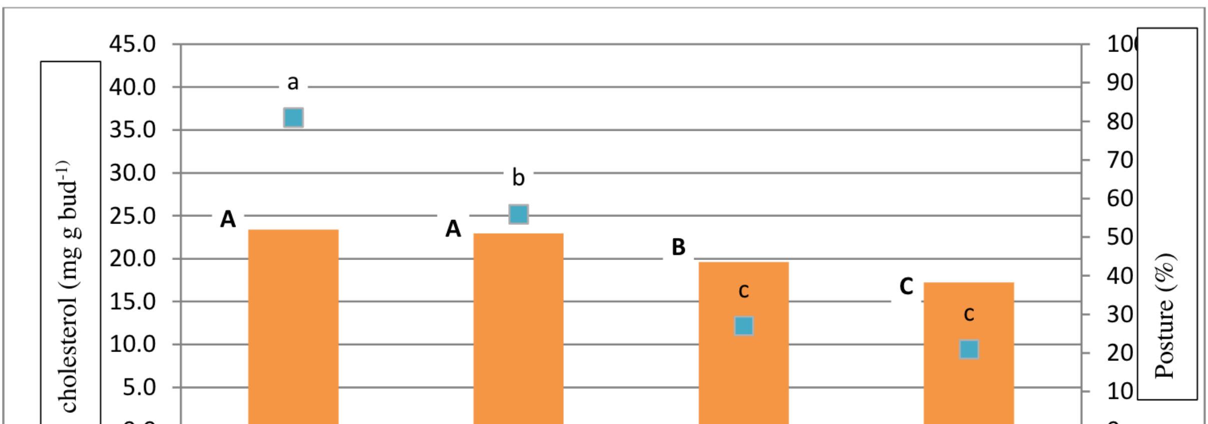
Feed intake tended to deteriorate as the inclusion of algae in the diet increased. In the case of the treatments with 30 and 45% algae in the diet, the trials with the poultries were suspended after the fourth and third week of experimentation, respectively, because feed consumption was drastically reduced and egg production ceased. (Table 3). Based on the information presented in the Hy Line Management Guide (2018), poultries must not consume more than 101g per day, accompanied by poor feed conversion per egg, which may be associated with management factors in general, or diet. deficient in metabolizable energy, especially in the treatments that included the algae.

In relation to the cholesterol content in the egg yolk, the treatments presented statistical differences ($p \leq 0.05$) with values of 23.36; 22.94; 19.60 and 17.27 mg g yolk-1 respectively, which implied a decrease in cholesterol, but affected egg production, with decreases of approximately 50% with the greater inclusion of the algae (Figure 1).

Despite the negative effect of the inclusion of the seaweed on the productive parameters, there was a notable reduction in cholesterol in the yolk, a positive aspect that confirms the hypocholesterolemic property of seaweed and agrees with the results obtained by Carrillo et al. (2012). Some authors (Reiner et al., 1962; Jiménez-Escrig & Goñi, 1999) suggest that the mechanism by which algae reduce cholesterol could be the presence of sterols and phycocolloids present in these marine resources. Sterols have the ability to lower blood cholesterol levels by competing with ingested cholesterol at absorption sites. On the other hand, Brown et al. (1999), consider that the phycocolloids present in the algae decrease the cholesterol content in the blood, because they interrupt the entero-hepatic cycle of bile acids and prevent cholesterol from being synthesized again. In hens, cholesterol requirements are covered almost exclusively by de novo synthesis carried out in the liver, therefore most of the cholesterol and lipids in the egg originate in this organ (Elkin 2006).

CONCLUSION

1. Based on the proposed objectives, it is concluded that the inclusion of marine algae (*Gracilaria sp*) in the diet of laying poultries at different levels produced a significant decrease in cholesterol in the egg yolk, but with 30 and 45% inclusion the productive parameters were negatively affected.



	0%	15%	30%	45%
cholesterol	23.37	22.93	19.60	17.24
Posture	81	56	27	21

Figure 1. Percentage of laying and cholesterol content in egg yolk, in laying poultries according to percentage of inclusion of Gracilaria sp. Lowercase letters equal in posture do not differ by Duncan's test, at 5% probability, while uppercase letters equal in cholesterol content do not differ by Duncan's test, at 5% probability.

REFERENCES

BROW, L.; RESNER, B.; WILLET, W.W.; SACKS, F.M. Cholesterol-lowering effects of dietary fiber: a meta-analysis. **The American Journal of Clinical Nutrition**, 69(1): 30-42, 1999.

CARRILLO, D.S.; BAHENA, A.; CASAS, V.M.; CARRANCO, J.M.E.; CALVO, C.C.; ÁVILA, G.E.; PÉREZ-GIL, R.F. The marine algae *Sargassum* spp. as an alternative for reducing the cholesterol content of eggs. **Cuban Journal Of Agricultural Science**, (46)2:1-6, 2012.

Di RIENZO, J.A.; CASANOVES, F.; BALZARINI, M.G.; GONZALEZ, L. M.; TABLEDA, M.; ROBLEDO, C.W. InfoStat, versión 2008. **Grupo InfoStat, FCA**, Universidad Nacional de Córdoba, Argentina, 2008.

DJOUSSE, L.; GAZIANO, J.M. Egg consumption and risk of heart failure in the Physicians' Health Study. **Circulation**, 117(4):512-6, 2008.

DJOUSSE, L; GAZIANO, J.M. Egg consumption in relation to cardiovascular disease and mortality: The Physicians' Health Study. **The American Journal of Clinical Nutrition**, 87(4):964-9, 2008b.

DUSSAILLANT, C.; ECHEVERRÍA, G.; ROZOWSKI, J.; VELASCO, N.; ARTEÍA, G.; ROZOWSKI, J.; VELASCO, N.; ARTEAGA, A.; RIGOTTI, A. Consumo de huevo y enfermedad cardiovascular: una revisión de la literatura científica. **Nutrición Hospitalaria**, 34(3):710-718, 2017.

EDDING, M.; MACCHIAVELLO, J.; BLACK, H. 1987. Culture of *Gracilaria sp* in outdoors tanks: productivity. Book series **Developments in Hydrobiology,** 151/152: 369-373, In Twelfth International Seaweed Symposium Proceedings of the Twelfth International Seaweed Symposium held in Sao Paulo, Brazil, July 27–August 1, 1986. DOI: 10.1007/978-94-009-4057-4_55.

ELKIN, R.G. Reducing shell egg cholesterol content. I. Overview, genetic approaches, and nutritional strategies. **World's Poultry Science Journal**, 62, 665- 687, 2006.

FUENTES-GARCÍA, A. Consumo de huevos y riesgo cardiovascular. **Nutrición Hospitalaria**, 33(Supl. 4):41-43, 2016.

GIL-SAAVEDRA, M. Algas como una alternativa en la nutrición en avicultura. 2016. Tesis para optar el Título Profesional de Médico Veterinario. **Universidad Nacional Mayor de San Marcos. Facultad de Medicina Veterinaria**, Escuela Profesional de Medicina Veterinaria. Lima, Perú.

GINZBERG, A.; COHEN, M.; SOD-MORIAH, U.A.; SHANY, S.; ROSENSHTRAUCH, A.; ARAD, S. Chickens fed with biomass of the red microalga *Porphyridium sp*. Have reduced blood cholesterol level and modified fatty acid composition in egg yolk. **Journal of Applied Phycology,** 12: 325–330, 2000.

GRAY, J.; GRIFFIN, B. Eggs and dietary cholesterol - Dispelling the myth. **Nutrition Bulletin,** 34: 66-70, 2009

GROBAS, S.; MATEOS, G.G. Influencia de la nutrición sobre la composición nutricional del huevo. XII Curso de Especialización FEDNA. Madrid 7 y 8 de noviembre de 1996. Disponible en: https://www.researchgate.net/publication/28180440Influencia_de_la_nutricion _sobre_la_composicin_nutricional_del_huevo. 1996.

JIMÉNEZ-ESCRIG, A., GOÑI, I. Evaluación nutricional y efectos fisiológicos de macroalgas marinas comestibles. **Archivos Latinoamericanos de Nutrición,** 49(2): 114-119, 1999.

KRAUS, R.M.; ECKEL, R.H.; HOWARD, B.; APPEL, L.J.; DANIELS, S.R.; DECKELBAUM, R.J.; ERDMAN, J.W.; KRIS-ETHERTON, P.; GOLDBERG, I.J.; KOTCHEN, T.A.; LICHTENSTEIN, A.H.; MITCH, W.E.; MULLIS, R.; ROBINSON, K.; WYLIE-ROSETT, J.; JEOR, S.; SUTTIE, J.; TRIBBLE, D.L.; BAZZARRE, T.L. AHA Dietary Guidelines. Revision 2000: A statement for Healthcare Professionals from the Nutrition Committee of the American Heart Association. **Circulation** 102: 2284-2299, 2000.

MIRANDA, J.M.; ANTÓN, X.; REDONDO-VALBUENA, C.; ROCA-SAAVEDRA, P.; RODRÍGUEZ, J.A.; LAMAS, A.; FRANCO, C.M.; CEPEDA, A. Egg and egg-derived foods: Effects on human health and use as functional foods. **Nutrients,** 7(1):706-29, 2015.

NABER, E.C. Cholesterol content of eggs: Can and must it be changed? **Feedstuffs,** 62(5): 1, 47, 50-52, 1990.

NAKAMURA, Y.; OKAMURA, T.; TAMAKI, S.; KADOWAKI, T.; HAYAKAWA, T.; KITA, Y.; OKAYAMA, A.; UESHIMA, H. Egg consumption, serum cholesterol, and cause-specific and all-cause mortality: The National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged, 1980 (NIPPON DATA80). **The American Journal of Clinical Nutrition**, 80(1):58-63, 2004.

NETTLETON, J.A.; STEFFEN, L.M.; LOEHR, L.R.; ROSAMOND, W.D.; FOLSOM, A.R. Incident heart failure is associated with lower whole-grain intake and greater high-fat dairy and egg intake in the Atherosclerosis Risk in Communities (ARIC) study. **Journal of the American Dietetic Association**, 108(11):1881-7, 2008.

NRC. Nutrient Requirements of Poultry. 9th Revised Edition, 1994. **National Research Council**, Washington, D.C. 1994.

ORIONDO-GATER, R.L.; BERNUI-LEO, I.; VALDIVIESO-IZQUIERDO, L.R.; ESTRADA-MENACHO, E. Relación entre colesterol dietario, consumo de huevo y perfil lipídico en adultos aparentemente sanos, según grupos de edad. **Anales de la Facultad de Medicina**, 74(1):27-30, 2013.

OZAKI, H.; KAWAHARA, M.; NOGAMI, R.; YAMADA, Y.; TAKAHASHI, H. Supplemental red alga, *Gracilaria vermiculophylla*, from a Brackish Japanese Lake, strengthens egg shells and improves the Haugh unit of eggs in laying hens. **Journal of Fisheries & Livestock Production**, 2(1): 110-115, 2013.

REINER, E.; TOPLIFF, J.; WOOD, J.D. Hypocholesterolemic agents derived from sterols of marine algae. **Canadian Journal of Biochemistry and Physiology**, 40: 1401-1406, 1962.

RODRÍGUEZ-RÍOS, H.; CAMPOS-PARRA, J.; ASTUDILLO-NEIRA, R.; GRANDE-CANO, J.; CARRILLO-DOMÍNGUEZ, S.; PÉREZ GIL-ROMO, F. *Amaranthus cruentus* L. as a food alternative in laying hens to reduce cholesterol in eggs. **Chilean Journal of Agricultural & Animal Sciences,** 36(1): 78-85, 2020.

SORIANO-MALDONADO, A.; CUENCA-GARCÍA, M.; MORENO, L.A.; GONZÁLEZ-GROSS, M.; LECLERCQ, C.; ANDROUTSOS, O.; GUERRA-HERNÁNDEZ, E.J.; CASTILLO, M.; RUIZ, J.R. Ingesta de huevo y factores de riesgo cardiovascular en adolescentes; papel de la actividad física. Estudio HELENA. **Nutrición Hospitalaria**, 28(3): 868-877, 2013.

YUAN, Y. Marine algal constituents. In: Colin, B., Fereidon, S. (Eds.). **Marine Nutraceuticals and Functional Foods**. CRC Press, Boca Raton, Florida. pp 259-295, 2008.

Nutrients	**Seaweed:** *Gracilaria sp.*
Dry matter	87,9
Ashes	30,2
Ethereal extract	0,3
Crude protein	14,6
Crude fiber	1,4
Calcium	1,0
Available phosphorus	0,5
Methionine + Cystine *	-
Metabolizable energy (kcal Kg^{-1}) *	-
Aspartic	6,0
Glutamic	8,0
Serine	4,0
Wisteria	5,0
Histidine	1,0
Arginine	4,0
Threonine	5,0
Alanine	6,0
Proline	5,0
Tyrosine	4,0
Valine	10,0
Methionine	1,0
Isoleucine	7,0
Leucine	9,0
Phenylalanine	9,0
Lysine	9,0

* Values not determined analytically.

Table 1. Chemical composition (dry matter basis percentage) and algae amino acid: *Gracilaria sp.*

	Inclusion of the seaweed: *Gracilaria* in the diet (%)			
	0	15	30	45
Ingredients				
Corn	64,10	52,40	40,50	28,70
gracilaria sp,	0,00	15,00	30,00	45,00
soybean meal	24,60	21,40	18,20	15,00
oyster Shell	9,00	9,00	9,00	9,00
salt	0,30	0,30	0,30	0,30
tricalcium phosphate	1,80	1,80	1,80	1,80
vitamin mix [1]	0,05	0,05	0,05	0,05
mineral mix [2]	0,05	0,05	0,05	0,05
DL Methionine	0,10	0,10	0,10	0,10
Total	100,00	100,00	100,00	100,00
Chemical composition of ingredients				
E. metabolizable (kcal Kg^{-1})	2.733,0	2.637,0	2.541,0	2.445,0
crude protein (%)	15,08	15,22	15,36	15,50
total Met+Cis [3](%)	0,68	0,62	0,56	0,50
total lysine (%)	0,93	1,01	1,09	1,17
total calcium (%)	3,66	3,65	3,64	3,63
available phosphorus (%)	0,45	0,43	0,41	0,39

[1] Vitamin mixture (in 1000 kg of diet): Vit A 8,000,000 IU, Vit D3 3,300,000 IU, Vit E 20 g, Vit K (menadione) 2.5 g, Thiamin (B1) 2.5 g, Riboflavin (B2) 5, 5 g, Niacin (B3) 30 g, Pantothenic Acid (B5) 8 g, Pyridoxine (B6) 4 g, Biotin (B7) 75 mg, Folic Acid (B9) 0.9 g, Cobalamin (B12) 23 mg, Choline 110g. [2] Mineral mix (in 1,000 kg of diet): Zinc 80 g, Iron 40 g, Copper 8 g, Iodine 1.2 g, Selenium: 0,22 g. [3] Met+Cis total (%):Methionine plus total cysteine.

Table 2. Composition and nutritional contribution of the diets used

Percentage Inclusion	Consumption (g $poultry^{-1}$ day^{-1})	Conversion (kg feed dozen $eggs^{-1}$)
0 %	127 a	1,89 a
15 %	137 a	3,18 b
30 %	100 b	9,81 c
45 %	67 c	13,47 d

(1) Means followed by equal letters, lowercase in columns, do not differ by Duncan's test, at 5% probability

Table 3. Feed consumption and average feed conversion of 8 weeks of laying poultries with different percentages of inclusion of *Gracilaria sp.*