The alga *Sargassum spp.* as alternative to reduce egg cholesterol content

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In order to determine the effect of the addition of the sea alga *Sargassum spp.* on the egg cholesterol content in the diet of laying hens, 225 Leghorn hens were used at 19 weeks of age and distributed randomly into five treatments (0, 2, 4, 6, and 8 % of the sea alga). The experiment lasted five weeks, during which the productive variables were recorded. The physical quality was assessed in 75 eggs per treatment. Thirty-five pieces of each treatment were collected for the analysis of the egg cholesterol (yolk + albumin) through gas chromatography. The data were analyzed through ANOVA and the test of Tukey was applied to compare the means ($P < 0.05$). The results showed that with 4, 6, and 8 % of the sea alga the egg production was reduced and the yolk color was increased. The cholesterol concentrations (mg 100 g^{-1} fresh egg) were 416.28 (0 % sea alga), 396.77 (2 % sea alga), 363.35 (4 % sea alga), 309.05 (6 % sea alga), and 338.76 (8 % sea alga). It was concluded that the addition of 4, 6, and 8 % of the alga *Sargassum spp.* in the diet of laying hens reduces significantly the egg cholesterol content and affects favorably the yolk color.

Key words: *sea algae, eggs, lipids, hypocholesterolemic properties*

Several clinic and epidemiologic studies reveal that the cholesterol in the feeds has little effect on the plasma cholesterol (Hu *et al*. 1999). In fact, it has been proved that some individuals do not respond to the cholesterol level in the diet and that, even another section of the population can be genetically resistant to the rise of the blood cholesterol as response to that same level in the diet (McNamara and Nicolosi 1998). As consequence of these findings, it has been established that healthy individuals can consume one egg daily, not affecting their blood cholesterol levels, and not representing cardiovascular risk factor (Kraus *et al*. 2000). Still, many people avoid or reduce the egg intake.

With the aim of keeping the population consuming this feed, several studies have been conducted to reduce the egg cholesterol content in a way it does not represent an obstacle to the intake. A method to this aim is the addition of chemical compounds to the poultry diet, such as lovastatin (Wang and Pan 2003), mevastatin or compactita (Elkin 2007), dehydrated garlic (*Allium sativum*) (Chowdhury *et al*. 2002) and copper (Pesti and Bakalli 1998 and Balevi and Coskun 2004). Also, natural compunds were added such as the meal of alfalfa, barley, oats, pectin and cellulose (Beyer and Jensen 1991 and 1993) and plant sterols (Peterson 1951).

Sea algae constitute an alternative to reduce the cholesterol content in the egg, because they have compounds with hypocholesterolemic and hypolipidemic properties, such as polyunsaturated fatty acids, polysaccharides (agar, carrageenans, alginic acid, and fucans) and sterols (fucosterol, desmosterol, sargasterol, estigmasterol and beta sitosterol) (Rodríguez *et al*. 1995, Jiménez-Escrig and Goñi 1999, Nishide and Uchida 2003 and Yuan 2008).

The brown alga *Sargassum spp.* forms large spots in tropical and subtropical waters. It has been estimated that it has an available biomass of 183,000 t in the Peninsula of Lower California, Mexico (Casas 2009). It is an excellent source of minerals, vitamins, essential amino acids, polysaccharides, n3 and n6 fatty acids, and sterols (Carrillo *et al*. 1992, Gojón-Baez *et al*. 1998 and Yuan 2008). The objective of this study was determining the effect of the addition of the sea alga *Sargassum spp.* on the cholesterol content of the eggs.

Materials and Methods

Chemical analysis of the sea alga. One kilogram of sample was collected per part and ground in blade mill until obtaining 1-mm particle size, with the object of making by triplicate through the standardized methods of AOAC (2000), the following chemical analyses: humidity content (drying oven at 60 °C until constant weight, method 976.05), ashes (calcination at 550 °C in muffle, method 923.03), crude fiber (Soxthec, method 962.09), ether extract (Soxhlet equipment, method 920.39), and nitrogen content by micro-Kjeldahl (method 976.05). In order to calculate the protein content, a conversion factor of 6.25 was applied. In order to determine Ca, Mg, Na, K, Fe, Zn, and Cu, the algae were subject to acid digestion and the minerals were determined by atomic absorption spectrophotometry (method 968.08). P was quantified by colorimetry (method 999.11). Gross

Table 1. Composition of the experimental diets (%)

Ingredients	0%AM	2%AM	4%AM	6%AM	8%AM
Sorghum	56.55	57.00	54.41	51.95	49.40
Soybean	26.94	24.70	24.90	25.13	25.35
Sargassum spp.	0.00	2.00	4.00	6.00	8.00
Calcium carbonate	9.95	9.80	9.61	9.45	9.27
Soybean oil	3.82	3.94	4.47	5.01	5.56
Orthofosfate[1]	1.64	1.66	1.66	1.70	1.71
Methionine	0.16	0.16	0.12	0.08	0.04
Salt (NaCl)	0.46	----	----	----	----
HCL L-Lysine	0.08	0.44	0.44	0.46	0.46
Vitamin premix[2]	0.10	0.10	0.10	0.01	0.01
Micoad (klin-sil)[3]	0.10	0.01	0.10	0.01	0.01
Avelut powder 15[4]	0.05	0.05	0.05	0.05	0.05
Mineral premix[5]	0.05	0.05	0.05	0.05	0.05
Coline chloride 60	0.05	0.05	0.05	0.05	0.05
Avired[6]	0.02	0.02	0.02	0.02	0.02
IQ[7]	0.01	0.01	0.01	0.01	0.01
Furacyl[8]	0.01	0.01	0.01	0.01	0.01
Total	100	100	100	100	100

[1]Calcium orthophosphate P 21% min, Ca 13% min, FO.21% max. [2]Vitamins per kg: 10 000 000 IU vit. A, 30 000 000 IU vit. D_3, 20 000 IU vit. E, 2 500 g vit. K_3, 2 500g vit. B_1, 5g vit. B_2, 35g niacin, 10g D- pantothenic acid 4g pyridoxine, 1g folic acid, 10mg vit. B12, 200mg biotin, 1 000. [3]Mycotoxins catcher [4]Saponified xanthofils obtained from the cempasuchitl flower (yellow 15 ppm). [5]Mineral premixture (mg/kg of the diet): magnesium 120, zinc 100, iron 120, copper, 12, iodine 0.7, selenium 0.4, cobalt 0.2. [6]Red xanthofils (cantaxantina, 10 ppm). [7]Antioxidant. [8]Furazolidon-bacitracin-zinc.

energy was determined through Parr calorimetric pump. In order to quantify the cholesterol content in the alga, the method of Fenton and Sim (1991) was followed and 5-α-cholestan was used as internal standard.

Experimental assay. During five weeks of the assay, 225 Leghorn hens, of 19 weeks of age, were distributed into five treatments, according to random design (control, 2, 4, 6, and 8 % of sea alga) with five repetitions each. The diets were prepared with the Nutrion software (version 3.2) for laying hens, according to NRC (1994), substituting partially sorghum, soybean, and salt (Table 1). Water and feed were given *ad libitum*. The productive variables (egg weight, egg production, and feed intake) were measured daily and they were recorded weekly (Quintana 1999).

Egg physical quality. At the end of the period, 75 eggs were collected per treatment (15 per replicate) to evaluate the physical quality (height of albumin, Haugh units and yolk color) through QCM automated equipment (Technical Services and Supplies Inc.).

Quantification of egg cholesterol. At the end of the fifth week of experimentation, 35 eggs were collected randomly from each treatment (seven per replicate). The eggshell was separated and the yolk and the albumin were mixed until obtaining a homogenous sample. The aliquots of 1 g of whole egg were added KOH, ethanol and 5-α-cholestan (as internal standard) (Fenton and Sim 1991). The cholesterol quantification was performed in gas chromatographer Varian 3400 CX, equipped with capillary column DB-5 (3m x 0.25 mm of internal diameter), an auto-sampler and a detector of flame ionization. Nitrogen was used as gas carrier, at a flow of 30 mL min^{-1}. The temperatures were: column 280 ºC, injector 260 ºC and detector 280 ºC.

Statistical analysis. All the data were analyzed through ANOVA with random block design. The Tukey test was used to compare the means ($P < 0.05$). The procedure GLM of SAS, version v.6.12 (SAS Institute 1990), was used for the statistical analyses.

Resultados y Discusión

Chemical composition of the alga and of the diets. The results showed high content of mineral and nitrogen free extract in the alga, whereas the values for ether extract and crude protein were low (table 2). The diets in the experiment were isoproteic (17 % protein) and isocaloric (2 850 MC kg^{-1} of metabolizable energy).

Egg physical quality. The height of albumin and the Haugh units were not affected by including the sea alga in the poultry diet (table 3). However, the yolk color showed favorable increment as the sea alga level was increased in the diet. This rise was significant in the treatments with 4, 6, and 8 % of the sea alga (P < 0.05).

Productive variables. The feed intake was reduced significantly in the treatment with 2 % (P < 0.05), whereas the egg production diminished by including 4, 6, and 8 % of the sea alga in the diet (P < 0.05). Nevertheless, the egg weight and the feed conversion were not affected by this inclusion in the diet (Table 4).

Cholesterol concentration in the egg. The cholesterol concentration in the egg was reduced as the level of sea alga was increased in the diet. This reduction was significant in the treatments with 4, 6, and 8 % (P < 0.05). The reduction in the cholesterol concentration in the egg, compared with the control group, was as follows: for 6 % of sea alga inclusion, the reduction was of 26 %; for 4 %, it was of 13 %; and for 8 %, it was of 19 % (table 5).

Tabla 2. Chemical composition of *Sargassum spp*.

Indicator	*Sargassum spp..*
Humidity (g.100g^{-1})	7.40
Ashes (g.100g^{-1})	38.35
Crude protein (N x 6.25) (g 100g^{-1})	6.57
Crude fiber (g 100g^{-1})	6.55
Ether extract (g 100g^{-1})	1.05
Nitrogen free extract (g 100g^{-1})	40.08
Gross energy (MJ g^{-1})	10.48
Calcium (mg.100g^{-1})	3.21
Phosphorus (mg 100g^{-1})	0.1
Sodium (mg 100g^{-1})	20.1
Potasium (g 100g^{-1})	5.77
Magnesium (g.100g^{-1})	0.90
Copper (ppm)	1.0
Zinc (ppm)	1600.0
Iron (ppm)	3600.0
Cholesterol (mg 100g^{-1})	4.0
Total lipids (g 100g^{-1})	1.93
Fatty acids (% total FAs)	
Linoleic acid (C18:2 LA)	6.99
α-linolenic acid (C18:3 ALA)	2.65
Arachidonic acid (C20:4 AA)	9.83
Eicosapentaenoic acid (C20:5 EPA)	3.53
Docosahexaenoic acid (C22:6 DHA)	0.60

Discusion

Chemical composition of the sea alga. The figures agreed with Yuan (2008), in respect to the fact that most of the sea algae have high content of ash (8.4 - 43.6 % in dry weight) and nitrogen free extract (0.2-59.7 %), but low content of ether extract (0.92-5.2%), as well as a caloric value of < 1-3.1 kcal g^{-1} (< 4.18-12.95 MJ g^{-1}).

Egg physical quality. The increment in egg color, by adding 4, 6, and 8 % of sea alga to the poultry diet was possibly due to the presence of lutein, zeaxanthin and fucoxanthin, carotenoids commonly present in brown algae. Studies cited by Miyashita and Hosokawa (2008) revealed that fucoxantin, principal carotenoid of the brown algae, has anticancer and anti-inflammatory effects, and also acts against obesity. It has been proved that lutein and zeaxantin are important in the prevention of cataracts and macular degeneration in the retina.

Productive variables. Although the principal object of this study was determining the effect of the addition of the alga *Sargassum spp.* to the poultry diet on the egg cholesterol, it was convenient to evaluate also its influence on the productive variables and the physical quality of the egg, because these are factors of great importance for producers and consumers. In this study, the decline in the egg production could be related to the cholesterol reduction obtained with the same treatments, because it is known that the cholesterol is related to reproductive aspects, by being precursor of estrogens (McNamara and Nicolosi 1998). In studies on dehydrated garlic (*Allium sativum*) in laying hens (Rahardja *et al.* 2010), a notable reduction in the egg cholesterol (34 %) was reported, together with the production increment, results opposed to those in this study.

These differences in egg production could be due to the different mechanisms of action of the components of the dehydrated garlic (*Allium sativum* L.) and of the *Sargassum spp.*. In garlic, two bioactive compounds have been identified: S-allylcisteine sulfoxide (alliin) and diallyl thiosulfinate (allicin), which have been proved to have hypocholesterolemic effects on blood. These effects are shown in the yolk content. It has been

Table 3. Physical quality of the egg from laying hens fed different levels of inclusion of the algae *Sargassum spp.* in the diet during a five-week period

Algae, %	Yolk color	Albumin height (mm)	Haugh Units (HU)
0	7.13^{c} ± 0.05	9.03 ± 0.07	95.80 ± 0.38
2	7.40^{c} ± 0.08	8.96 ± 0.10	95.56 ± 0.52
4	7.48^{b} ± 0.06	8.88 ± 0.10	95.66 ± 0.40
6	7.81^{a} ± 0.08	8.74 ± 0.08	94.69 ± 0.43
8	7.97^{a} ± 0.08	8.87 ± 0.07	95.58 ± 0.38

^{abc}Different letters in each column show significant difference (P < 0.05)

The mean and the standard error are presented in each column

Table 4. Productive variables in laying hens fed different levels of inclusion of the algae *Sargassum spp.* in the diet during a five-week period

Algae, %	Feed intake ($g\ hen^{-1}\ d^{-1}$)	Egg production (%)	Egg weight (g)	Feed conversion
0	96.80[a] ± 1.58	91.28[a] ± 1.20	53.61 ± 0.47	2.01± 0.06
2	93.11[b] ± 1.52	89.01[ab] ± 1.42	52.83 ± 0.32	2.00 ± 0.06
4	97.82[a] ± 1.29	87.10[b] ± 1.23	52.50 ± 0.26	2.19 ± 0.06
6	96.82[ab] ± 1.38	86.09[b] ± 1.56	53.34 ± 0.37	2.16 ± 0.07
8	97.71[a] ± 1.59	85.93[b] ± 1.51	52.30 ± 0.46	1.91 ± 0.09

[ab]Different letters in each column show significant difference ($P < 0.05$)
The mean and the standard error are presented in each column

Table 5. Cholesterol concentration in the egg of hens fed different levels of inclusion of the algae *Sargassum spp.* in the diet during a five-week period

Algae, %	Cholesterol ($mg\ 100g^{-1}$)	
0	416.28[a] ± 4.09	
2	396.77[a] ± 4.11	(5%)
4	363.35[b] ± 3.57	(13%)
6	309.05[c] ± 3.19	(26%)
8	338.76[b] ± 3.33	(19%)

[abc]Different letters in each column show significant difference ($P < 0.05$)
The mean and the standard error are presented in each column. The values in parentheses show the percentage of egg cholesterol content reduction as compared with the control

proposed that the presence of the enzyme 3-hydroxi-methyl-glutaril CoA reductase (HMG-CoA reductase) from garlic controls the rate of colesterol synthesis in the liver (Rahardja *et al*. 2010).

The chemical compounds through which algae could reduce the cholesterol are the following:

Egg cholesterol concentration. The results obtained in the reduction of the egg cholesterol content agreed with those of Ginzberg *et al*. (2000), when supplementing the diet of laying hens for 10 d, with 5 and 10 % of *Porphyridium spp.*. In this research, the egg cholesterol concentration was reduced significantly (9.5 and 10 $mg\ g^{-1}$ of yolk vs 12.5 $mg\ g^{-1}$ in the control group).

This performance could be due to the effect of some chemical compounds of the algae, such as sterols, polysaccharides (diet soluble fiber), and fatty acids, on the egg cholesterol synthesis (Reiner *et al*. 1962, Jiménez-Escrig and Goñi 1999 and Yuan 2008).

a) Sterols. The presence of fucosterol, desmosterol, sargasterol, stigmasterol, beta-sitosterol and ergosterol has been reported in sea algae. The mechanisms through which these compounds reduce the egg cholesterol content are: 1) interference with the endogenous synthesis of cholesterol, 2) competition with the cholesterol for the absorption sites at the small intestine level, because they are structurally similar (Jimenez-Escrig and Goñi 1999 and Nishide and Uchida 2003).

b) Polysaccharides. The fraction of greatest importance in the polysaccharides, related to the cholesterol, is the diet soluble fiber. In sea algae, alginates, fucoidan, mannitol and laminaran have been reported as part of it (Yuan 2008 y Casas 2009). The hypocholesterolemic effect of these compounds is attributed to the formation of viscous systems in the small intestine that diminish the speed of the passage of nutrients such as glucose and lipids to the blood, and to the fact that the colloidal particles formed in the intestine with the water absorption retain the cholesterol and the biliary acids, forming a colloid of ionic type, that is later excreted in the feces (Kiriyama *et al*. 1968, Lamela *et al*. 1989 and Panlasigui *et al*. 2003).

c) Poly-unsaturated fatty acids. Apparently, the fatty acids (FA) present in the sea algae, not only modify the composition of fatty acids of the eggs from hens, but also may contribute to reducing the cholesterol levels. Some hypotheses have been made about the mechanism through which the FAs, mainly the n3, reduce the cholesterol. They are the stimulation of the synthesis of high-density lipoproteins (HDL), responsible for transporting the cholesterol from the tissues and the organs to the liver to be metabolized and removed; the reduction of the cholesterol absorption; the increase in the excretion of cholesterol and biliary acids; the reduction in the cholesterol synthesis and the rise in the activity of the enzyme that catalyzes the reaction between the lecithin and the cholesterol to produce lyso-lecithin and esters of cholesterol (Welch and Borlak 2008).

e) Minerals. Sea algae are an important source of minerals such as copper (4.7 $mg\ 100g^{-1}$) (Carrillo *et al*. 2002 and Yuan 2008), having in organic form greater bioavailability (Chapman and Chapman 1980). The mechanism through which copper reduces the cholesterol content in the egg can be attributed to the rise in the copper concentration in the liver, regulating indirectly the cholesterol biosynthesis through the drop in the reduced glutathione (GSH) and the rise in the oxidized glutathione (GSSG). It has been proved that the cell increment of GSSG decreases the activity of HMGCoA. This makes, at the same time, that the carbon flow through the mevalonate pathway can be reduced and, consequently, the cholesterol synthesis can be diminished (Konjufca *et al*. 1997, Gilbert and Stewart 1981 and Engle *et al*. 2001). It has also been proved that the algae are characterized by having high iodine content. In the case of the *Sargassum spp.*, contents of 0.02 mg $100g^{-1}$ of dry samples are reported (Yuan 2008). Studies in rats (Kaji *et al*. 1984) demonstrated that iodine has hypocholesterolemic properties.

Even when there is not consensus or definite evidence about the specific mechanism through which the sea algae reduce the egg cholesterol, it can be concluded, out of the results from this study, that the inclusion of the sea alga *Sargassum spp.* (6 %) in diets for hens under production reduces the egg cholesterol content, not affecting the productive variables and the egg physical quality, except its production. It is discarded the possibility that all the previously described mechanisms can act in a synergic form. Researches are suggested to deepen into this field to benefit public health.

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