

Fatty Acid Profile and Protein Content of Eggs of Araucana Hens (“Blue-Eggs”) Compared with Other Commercial Laying Hens

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Abstract

Health benefits associated with consumption of eggs of Araucana hens have been reported in popular press because of they are supposed to have a low cholesterol and higher protein content respect to eggs of other hen races. The aim of this study was to assess the protein content and the lipid profile of eggs produced by Araucana (A) and Leghorn (L) hens, following the same feeding regimen. Conventional commercially-available eggs (C) were analyzed as well. Eggs were collected from local producers and stores. Egg protein and cholesterol content were analyzed using standard procedures of AOAC. Fatty acid composition was determined by gas chromatography. Results for water and protein (per 100g of egg) were very similar for A, L and C eggs: 74.59g, 76.18g and 76.63g, respectively, for water; and 12.44g, 12.54g and 12.07g for protein. Commercial eggs presented a lower fat content than other eggs: 9.50g with respect to 12.20g for Araucana eggs and 11.00g for Leghorn eggs. Araucana eggs contained higher amounts of cholesterol ($p < 0.05$). Araucana and Leghorn eggs showed very similar fatty acid profiles except for linoleic acid ($p < 0.05$), in which Leghorn eggs contained a higher percentage than did Araucana eggs, which presented the best omega-6/3 ratio. The small differences in protein content and fat composition observed in this study would not be a nutritional advantage in consumption of Araucana eggs over other types of eggs.

Keywords: Eggs; Araucana Hen; Fatty Acids; Protein; Cholesterol

Introduction

Eggs have always been traditionally considered a staple of the human diet. Over the years, they not only have been an inexpensive and ideal source of nutrients, they are also an indispensable and highly appreciated ingredient in many cuisines because of their functional properties and taste [1]. It is recognized that eggs contribute high-quality protein to the human diet, and they are rich in other substances of biological importance such as choline, lutein and zeaxanthin, and provide significant amounts of several vitamins such as A, D, E, B2, B12 and minerals such as P, Zn and Se [2].

The lipid fraction of eggs is about 9 - 10g per 100 g of raw egg [1,3] and its lipid profile corresponds with about 2/3 of unsaturated fatty acids and 1/3 of saturated fatty acids. Oleic, palmitic, stearic and linoleic acid are among the most abundant fatty acids [4]. The quality of dietary fat, as provided by eggs, has a profound influence on health. With respect to the effect of dietary fatty acids and total fat on health, especially focused on cardiovascular disease, some of the latest-referenced publications [5] draw on strong scientific evidence to claim that high total fat diets are innocuous, although beneficial for health when most of the fat is provided by monounsaturated and polyunsaturated fatty acids. Furthermore, consumption of animal products is not necessarily associated with increased risk of

cardiovascular disease (CVD) [6]. Regarding dietary polyunsaturated fatty acids, recent studies focused on the health benefits of the n-6/n-3 ratio have recommended a substantial reduction in n-6 intake [7] although it is firmly established that substitution of AGS by diets rich in n-6 reduces CVD [5]. This is based on the idea that the n-6 acid, linoleic acid, is the substrate for the synthesis of a variety of pro-inflammatory molecules associated with some chronic diseases.

Another item especially worth mentioning is egg cholesterol, whose mean values vary depending on the source, but usually range between 372 - 385 mg/100 per fresh egg [1,8,9]. The cholesterol content of eggs is a controversial theme in dietary recommendations, especially when referring to its effects on the serum cholesterol levels and its relation to the risk of CVD. There are many studies with surprisingly opposite results. To cite a few, the classic study of Hu., *et al.* 1999 [10] asserted that egg consumption has no effect on CVD in healthy people, whereas Li., *et al.* 2013 [11] observed an association between egg consumption and CVD. At the present, although controversy persists, there is a moderate trend to diminish the importance of cholesterol to CVD in relation to other factors such as fatty acid profile of the diet, body mass index, physical activity or genetic predisposition [12]. Specifically, in regards to the effects of dietary cholesterol, a recent systematic review and meta-analysis suggest that it is not statistically significantly associated with CVD [13]. However, for consumers, the longstanding accepted relationship between egg cholesterol and CVD is still deeply-rooted.

Another trend in food consumption, which is becoming commercial again, it is the health benefits associated with the consumption of eggs of Araucana hens, commonly called “blue eggs”. These eggs, supposedly, have more protein and less cholesterol than other eggs. Araucana are a breed of hen, originally from the Mapuche area in South Central Chile and Southwestern Argentina, with certain phenotypic characteristics, variable from worldwide and North America standards, that lays characteristically blue- to turquoise-colored eggs. Curiously, interest in this hen and its special eggs is not new. The low cholesterol content of this type of egg was already reported in the 1970s [14] at the same time that scientific papers demonstrated that cholesterol content of Araucana eggs yolks averaged about 4% higher than other hens, in this case, Leghorn [15].

Considering the controversial trends in human food consumption and the small number of scientific publications about Araucana eggs, we decided to analyze the nutritional content of Araucana eggs specifically focused on its protein content and fat composition. At the same time, these results were compared with the same values obtained for eggs produced by other commercial laying hens following the same feeding regimen. The intention of this study is to contribute to deeper knowledge of these types of eggs and to clarify misunderstandings about them, especially those related with their nutritional characteristics.

Materials and Methods

Sample collection

Eggs from Araucana (n = 48) and Leghorn (n = 36) hens were provided for three different local breeders which produce eggs for household consumption. Both, Araucana (A) and Leghorn (L), were jointly raised in the same small free-range systems with backyard flocks that provide a protected indoor shelter with outdoor access. Both also received the same food, comprised of a mixture of wheat, corn and vegetables, a calcium supplement and water ad libitum. As a reference point for values of protein and fat of commercial eggs and for a better understanding of the nutritional composition of eggs, samples of commercially-available conventional eggs (n = 24) (C) were taken in local food stores as well.

Chemical analysis

A dozen whole eggs without shells in each batch were homogenized. Samples were kept at -30°C until used. Samples were analyzed using standard procedures of AOAC [16] with the analytical numbers 925.30, 925.31, 925.32 and 994.10 for total solids, protein, fat and cholesterol analyses, respectively.

Fatty acid analysis of eggs was done by preparing lipid extraction and methylation according to the method described by Fernández, *et al.* 2015 [17]. The fatty acid methyl esters were analyzed and quantified on a Hewlett-Packard chromatograph (Model 6890, Hewlett-Packard, Wilmington, DE) equipped with an automatic injector (Model 7683, Hewlett-Packard) and a mass selective detector (Model 5973, Hewlett-Packard). Undecanoic acid (C11:0) was added as an internal standard. A Teknokroma TR-CN100 capillary GC column (60 m × 0.25 mm i.d. × 0.20 µm film thickness; Teknokroma Analítica S.A, Barcelona, Spain) were used and the peak areas in the chromatogram were calculated and normalized using response factors. All results concerning the fatty acid composition are expressed as a percentage, FAME g/100g of lipids. The repeatability of the method was between 85% to 99% for FAME with a concentration of over 1%. Standards were obtained from Supelco (Supelco, Bellefonte, PA).

Statistical analysis

All analyses were done in duplicate. Data were analyzed with the statistical program SPSS (Version 21; SPSS INC., Chicago, IL, USA). Measures of central tendency and dispersion were calculated. The normality of the sample distribution was evaluated with the Kolmogorov-Smirnov test, and the equality of variances was assessed with Levene’s test. A Student’s t-test and Mann-Whitney U test for independent samples were applied in order to determine statistical differences between Araucana and Leghorn hen eggs. Differences were considered significant at the level of $p < 0.05$.

Results and Discussion

Results for moisture, protein, fat and cholesterol content of eggs from Araucana (A) and Leghorn hens (L) are shown in table 1. In the same table, values for the same parameters for eggs taken from local stores (C) are included. It can be observed that these parameters presented similar values except for the cholesterol amount ($p < 0.05$).

	A eggs	L eggs	C eggs
Moisture (g)	74.59 ± 2.13	76.18 ± 1.30	76.63 ± 0.20
Protein (g)	12.44 ± 0.53	12.54 ± 0.15	12.07 ± 0.06
Fat (g)	12.20 ± 1.35	11.00 ± 0.47	9.50 ± 0.00
Cholesterol (mg)	451.24 ± 133.66 ^b	307.45 ± 57.79 ^a	316.30 ± 2.12

Table 1: Moisture, protein, fat and cholesterol content (per 100 g) of Araucana (A) and Leghorn hens eggs (L) breeding in the same feeding regime, and of commercial available conventional eggs (C). Data expressed as means ± standard deviation. ^{a,b}: Different superscripts indicate statistically significant differences between cholesterol of commercial laying hens eggs (C) and Araucana hens eggs (A) ($p < 0.05$).

For moisture content, all types of eggs present similar mean values between 74.6% - 76.6%. These results are representative of hen eggs and very similar values can be found in reference sources such as BEDCA net, 2015 [10] for food composition, 76.4%, or Encyclopedia of Human Nutrition [1] 76.15%. For protein and fat content, similar values for all type of eggs were obtained as well: 12% for protein content, 9.50% - 12.2% for egg fat. These results are also in agreement with values reported by other authors [1,10,18]. Protein content was very similar between Araucana and Leghorn eggs, as was found by Somes, *et al.* [19] in 1977, refuting the already-popular press claims that Araucana eggs had higher protein levels. At this point, we found it interesting to emphasize the high nutritional value of the protein of eggs, which, for a long time, had been considered a reference protein to evaluate nutritional quality of food protein until 2007, when FAO/WHO established new patterns of essential amino acids as a function of age [20].

Results for fat content are similar for Araucana and Leghorn eggs (12.2% and 11%). Cholesterol presented a statistically significant difference ($p < 0.05$) between them. The mean value of cholesterol content in Leghorn eggs was 382.9 mg per 100g of eggs, and for Araucana eggs it was 451.2 mg per 100 g of egg. Our results are in accordance with other published studies which compared Araucana eggs with other egg types, such as White-Leghorns and Sex-links in Somes., *et al.* 1997 [19] Lohmann Selected Leghorn and ISA Brown in Millet., *et al.* 2006 [21]. Pintea., *et al.* 2012 [2] compared Araucana with ISA Brown hen eggs, finding that the cholesterol content was not significantly different between them. These convergent results consistently refute the popular belief about the low cholesterol content of Araucana eggs.

With respect to the cholesterol content of eggs, we find it interesting, at this point, to make a brief review about the controversial effect of egg consumption over serum cholesterol in relation to CVD. With respect to this last point, the cholesterol content of eggs has been the cause of the complex evolution of egg consumption over the last years. It went from being a basic foodstuff in the 1950s and 1960s, to presenting a great decrease in consumption during the 1970s and 1980s, because of the classic diet-heart hypothesis. This suggested that an elevated intake of saturated fatty acid and cholesterol with a low intake of polyunsaturated fatty acid would increase serum cholesterol levels and would lead to the development of atherosclerosis [22]. As a result of this and because of the cholesterol content of eggs, a government and medical recommendation of restricted egg consumption in diet became a nutritional topic. At this time, although an intense controversy in modern scientific literature about the effects of egg intake over serum levels of cholesterol and CVD risk persists, even with opposite results for systematic reviews and meta-analysis [12,23], there is also a more global perspective of the influence of all types of dietary fats and other factors on health and CVD risk. There is some consensus that the effect of egg consumption on CVD risk is small [24] as compared with other factors, such as the fatty acid profile of the diet, which is more important than the amount of ingested fat, [25] the body mass index and the lifestyle [26].

Considering actual knowledge about the influence of egg consumption on serum cholesterol and the high nutritional value of protein from eggs, as commented in previous paragraphs, both aspects can be considered important enough to reinstate egg consumption in healthy diets.

The fatty acid profiles of Araucana, Leghorn and Commercial eggs are shown in table 2. With the chromatography technique used, 15 different fatty acids were found, most of them with an even number of carbon atoms. In table 2, it can be observed that major fatty acids for Araucana and Leghorn eggs were: oleic acid, 43.65% and 42.25%, palmitic acid, 23.97% and 23.11%, stearic acid, 12.35% and 12.39%, linoleic acid, 9.31% and 11.85%. Statistically significant differences ($p < 0.05$) were observed between Araucana and Leghorn eggs only for palmitic, linoleic, linolenic and eicosadienoic acid (Table 2). The highest difference between percentages of fatty acids for Araucana and Leghorn eggs was for linoleic acid: 9.31% versus 11.85%. Docosahexaenoic acid (C22:6n3) was not detected. These fatty acid profiles are similar to other published profiles for eggs from commercial laying hens such as in Alizadeh., *et al.* 2015 [4]; Popiela., *et al.* [27] 2013 and Beynen 2004 [19]. Oleic and palmitic acid are always the major fatty acids, with over more than 40% of the total fatty acid for oleic acid and more than 20%-near to 25%-for palmitic acid. In these cited references, linoleic acid is habitually the third major fatty acid, about the 14% of the total fatty acids, and stearic acid is the fourth, about the 9% of the total. In our results for commercial eggs, it can be observed that values obtained for linoleic and stearic acid are consistent with these references. However, for Araucana and Leghorn eggs, breeding in a non-commercial production system, the percentages are different, being higher for stearic acid and lower for linoleic acid. For Araucana and Leghorn eggs, stearic acid mean values were about 12.35% and for linoleic acid were 9.31% - 11.85%.

Published data for fatty acid composition of Araucana eggs is especially limited. Pintea., *et al.* 2012 [2] published a comparative study about the lipophilic compounds in eggs of ISA Brown and Araucana hens. The fatty acid profile obtained in their analysis for Araucana eggs was: 43.1% for oleic acid, 23.6% for palmitic acid, 11.87% for linoleic acid and 10.19% for stearic acid. Their results are very similar to ours, especially for oleic and palmitic acid. For stearic acid, the results are very similar for two types of eggs, which we observed as well. For linoleic acid, they also found a decrease in the percentage of this fatty acid in Araucana eggs with respect to ISA Brown eggs, although it not as large as we observed in our results.

Fatty acids	A eggs	L eggs	C eggs
Lauric (C _{12:0})	0.26 ± 0.13	0.26 ± 0.09	0.04 ± 0.00
Myristic (C _{14:0})	1.59 ± 0.54	1.55 ± 0.30	0.74 ± 0.05
Pentadecanoic (C _{15:0})	0.21 ± 0.07	0.18 ± 0.02	0.13 ± 0.01
Palmitic (C _{16:0})	23.97 ± 0.64 ^b	23.11 ± 0.69 ^a	23.73 ± 0.34
Palmitoleic (C _{16:1})	5.68 ± 0.79	5.29 ± 0.43	5.28 ± 0.36
Margaric (C _{17:0})	0.39 ± 0.15	0.46 ± 0.13	0.29 ± 0.01
Stearic (C _{18:0})	12.35 ± 1.32	12.39 ± 1.90	8.14 ± 0.34
Oleic (C _{18:1})	43.65 ± 1.62	42.26 ± 1.65	40.87 ± 0.43
Linoleic (C _{18:2n6})	9.31 ± 1.10 ^b	11.85 ± 2.35 ^a	18.46 ± 0.06
Arachidic (C _{20:0})	0.13 ± 0.04	0.10 ± 0.04	0.05 ± 0.01
Linolenic (C _{18:3n3})	0.82 ± 0.12 ^b	1.05 ± 0.10 ^a	1.04 ± 0.06
Elaidic/vaccenic (C _{9,11} C _{18:1})	0.14 ± 0.04	0.014 ± 0.03	0.07 ± 0.00
Eicosadienoic (C _{20:2})	0.08 ± 0.02 ^b	0.12 ± 0.03 ^a	0.18 ± 0.01
Eicosatrienoic (C _{20:3n3})	0.10 ± 0.02	0.13 ± 0.04	0.11 ± 0.01
Araquidonic (C _{20:4n6})	1.12 ± 0.32	1.00 ± 0.31	0.78 ± 0.08
Eicosapentanoic (C _{20:5n3})	0.21 ± 0.10	0.14 ± 0.02	0.14 ± 0.01
Fatty acids fractions	A eggs	L eggs	C eggs
SFA	38.90 ± 1.74	38.04 ± 1.82	33.11 ± 0.04
MUFA	49.32 ± 2.34	47.54 ± 1.32	46.14 ± 0.07
PUFA	11.78 ± 1.22	14.42 ± 2.52	20.75 ± 0.11
PUFA/SFA	0.30 ± 0.03	0.38 ± 0.08	0.63 ± 0.01
n-6	10.43 ± 1.22	12.85 ± 2.49	19.23 ± 0.02
n-3	1.21 ± 0.15	1.43 ± 0.15	1.46 ± 0.09
n-6/ n-3	8.73 ± 1.35	9.01 ± 1.65	13.25 ± 0.80

Table 2: Lipid profile of Araucana (A) and Leghorn hens eggs (L) feeding in the same breeding regime, and of commercially available conventional eggs (C).

Data expressed as means of each singular fatty acid in percentage over total detected and identified fatty acids ± standard deviation. ^{a,b}: Different superscripts indicate statistically significant differences between Araucana (A) and Leghorn (L) hens eggs (*p* < 0.05).

In fatty acid fractions (Table 2), the major differences between types of eggs were observed mainly between Araucana and Leghorn as compared to commercial eggs. For SFA (saturated fatty acid), percentages for Araucana and Leghorn eggs were very similar, 38.90% and 38.04%, but greater than commercial, 33.11%. Interestingly, organic eggs are reported to have higher levels of saturated fatty acid, as in our results, especially with respect to commercial eggs of intensive production [28]. Pintea, *et al.* 2012 [2], found lower contents of SFA in Araucana eggs, about 35.28%. For MUFA (monounsaturated fatty acid), our values were similar for Araucana, Leghorn and commercial eggs, 49.32%, 47.54% and 46.14%, which is very similar to results published by Pintea, *et al.* 2012 [2]. The PUFA fraction (polyunsaturated fatty acid) presented the major differences between egg types: 11.78% for Araucana eggs, 14.42% for Leghorn eggs and 20.75% for commercial eggs. With respect to Omega-6 (n-6) and Omega-3 (n-3) series, n-3 series was presented in similar amounts,

1.45% - 1.21%, but the low content of the n-6 series in Araucana eggs, with respect to Leghorn and, especially to commercial eggs, constituted the major difference, although not statistically significant, for Araucana and Leghorn eggs: 10.43% for A eggs, 12.85% for C eggs and 19.23% for C eggs. For the same fractions, Pintea, *et al.* 2012 [2], found values of 0.87 for n-3 and 14.80 for n-6. Lastly, our values for the n-6/n-3 ratio for Araucana, Leghorn and commercial eggs were: 8.73, 9.01 and 13.25. The n-6 and n-3 series constituted a favorable aspect of the Araucana egg consumption. This would be due to the fact that a high ingestion of n-6 fatty acids and a high ratio of n-6/n-3 has been linked to the pathogenesis of many diseases such as CVD, cancer and inflammatory diseases [29] and Araucana eggs would contribute less to the dietary intake of the n-6 series than other types of eggs. Millet, *et al.* 2006 [21] observed a more favorable ratio of n-6/n-3 in Araucana eggs, and Pintea, *et al.* 2012 [2] affirmed, based on their experiment, that the genetic factor appears to be determinant for the differences observed in the fatty acid composition in Araucana and ISA Brown eggs.

Conclusion

Among the breeds tested in this study, raised with the same feeding regimen, Araucana eggs presented an almost identical protein content to that of Leghorn eggs. Cholesterol content presented statistically significant differences between breeds, with higher values for Araucana eggs. Fatty acid profiles were very similar. Linoleic acid was the fatty acid which showed the major difference between breeds, with a higher percentage in Leghorn eggs. Araucana eggs presented the best omega 6/3 ratio. The greatest differences were observed with commercial eggs taken from food stores. These results contribute to a better understanding of the composition of Araucana eggs.

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Conflict of Interest

There is no financial interest or conflict of interest.

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