



PHYSICAL AND CHEMICAL CHARACTERIZATION OF EGGS FROM ARAUCANA HENS OF FREE RANGE FED IN ARGENTINA

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Abstract

The physicochemical properties and fatty acid composition of eggs of laying hens with free range feeding (Araucana) were studied and a comparison with two kinds of commercial eggs, brown-yellowish (BR) and white (WH) shell eggs, laid by hens fed with a conventional diet was made.

Protein content was similar for all kinds of eggs (15 g/100 g egg). The dry matter in Araucana hens eggs (26 g/100 g egg) and the lipid and cholesterol contents (35 and 0.65 g/100 g yolk, respectively) were higher than in commercial ones. The content of minerals in the whole eggs was similar for all eggs analyzed, sodium and potassium being the most abundant. In all samples, the

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fatty acid of highest concentration was oleic acid, followed by palmitic and stearic acids. The content of linolenic acid (C18:3, n-3) was similar in eggs of hens with both kinds of feeding. However, the linoleic acid amount of the n-6 series (C18:2, n-6) was significantly lower in Araucana hens eggs. The polyunsaturated/saturated fatty acids ratio was appropriate in all kinds of eggs but the n-6/n-3 fatty acids ratio was significantly lower in Araucana eggs. In conclusion, eggs of domestic hens may be qualified as nutritious and healthy.

Keywords: araucana hens; eggs composition; free range feeding; fatty acids; cholesterol; mineral content

Resumen

Se estudiaron las propiedades fisicoquímicas y la composición de ácidos grasos de huevos de gallinas ponedoras con alimentación libre (Araucana) y se realizó una comparación con dos tipos de huevos comerciales, huevos de cáscara marrón-amarillenta (BR) y de cáscara blanca (WH), puestos por gallinas alimentadas con una dieta convencional.

El contenido de proteínas fue similar para todos los tipos de huevos (15 g/100 g de huevo). La materia seca en huevos de gallina Araucana (26 g/100 g de huevo) y el contenido de colesterol y lípidos (35 y 0,65 g/100 g de yema de huevo, respectivamente) fueron mayores que en los comerciales. El contenido de minerales en los huevos enteros fue similar para todos los huevos analizados con un mayor porcentaje de sodio y potasio. En todas las muestras, el ácido graso más abundante fue oleico, seguido por los ácidos palmítico y esteárico. El contenido de ácido linolénico (C18:3, n-3) fue similar en los huevos de gallina con los dos tipos de alimentación. Sin embargo, el ácido linoleico de la serie n-6 (C18:2, n-6) se encontró en cantidad significativamente menor en huevos de gallina Araucana. La relación de ácidos grasos poliinsaturados/saturados fue apropiada en todas las clases de huevos pero la relación de ácidos grasos n-6/n-3 fue significativamente menor en los huevos de gallina Araucana. En conclusión, los huevos de gallinas domésticas pueden ser calificados como nutritivos y saludables.

Palabras clave: gallina Araucana; composición de huevos; alimentación libre; ácidos grasos; colesterol; contenido mineral

Introduction

The main goal of modern animal production is to improve efficiency to meet food demands. The objective has been reached, as the high consumption of animal origin products attests, and the concern now is quality rather than quantity. In the case of laying hen eggs, color of yolk and shell, weight, albumen density, bacteriological quality and production conditions are key aspects.

Laying hen eggs are one of the most common and abundant foodstuffs in the human diet and they contain important compounds for human consumption such as lipids, amino acids and vitamins. The yolk is rich in saturated fat, cholesterol and other fatty compounds like lecithin [1]. Small quantities of liposoluble (A, D) and water-soluble (thiamine, riboflavin) vitamins, and minerals like iron, phosphorus, zinc, selenium and sodium are found in eggs too [2].

Eggs produced by domestic species have a characteristic and uniform composition that may be influenced by diet [3]. Moreover, egg composition is affected by genetics, age, feeding programs and the type and amount of dietary lipids [4]-[6].

Eggs available in supermarkets generally come from animals with controlled feeding while a smaller fraction corresponds to eggs from animals with free food.

In Argentina, where free food is used in many egg producing farms, there is a layer named Araucana that presents particular characteristics: red eyes, small fleshy protuberances near the ears and blue eggs. This color is due to biliverdin, a pigment synthesized in the shell gland and deposited onto the egg shell. This egg is one of the most commonly consumed, especially in the Northwest of Argentina [7].

Hen egg lipids composition has received much attention in recent years because of the lipid association with coronary heart disease [8] as high cholesterol and saturated fatty acids content are detrimental for human health.

In Argentina, egg consumption has increased in these last years to the present 190 egg/human/year [9], but no data about chemical composition or cholesterol content of eggs are available, included those produced with a free range diet. Therefore, the aim of this work was to analyze the physicochemical properties and fatty acid composition, as well as cholesterol and mineral content, of eggs of free range hens (Araucana) and compare them with those of two kinds of commercial eggs laid by hens (Isa Brown and Lohman) fed with a conventional diet. The property comparison of eggs from different origin could be useful to establish the quality of Araucana hens' eggs and direct attention about the impact of their consumption on the nutritional state and cardiovascular health of consumers.

Materials and Methods

Sampling origin

Three kinds of eggs were used: brown-yellowish (BR) and white (WH) shell supermarket eggs from animals (Isa Brown and Loman, respectively) fed on a balanced diet and domestic farm eggs (DO) from free range fed birds (Araucana). Three batches of 12 eggs each were used for the study.

Laying hen diet

The diet used to feed laying hens (supermarket eggs) contains an average amount of 50% corn, 20-30% soybeans in the form of pellets or flour, vitamins, minerals and amino acids (Table 1). Isa Brown (brown-yellowish shell eggs) and Lohman (white shell eggs) birds were kept in cages (three each) where they had free access to conventional diet. They received additional artificial lighting to adjust 16 h daily light and 8 h dark. Domestic farm eggs were laid by 24-month-old Araucana birds fed in free-range conditions where the most abundant fully available grass in the breeding area is *Paspalum notatum* ("Horqueta" grass). The layers were kept in the nesting houses (200 m²), where they received daylight and were fed with corn and grass. Corn composition is shown in Table 2 [10], [11]. The characteristic blue egg of this species is shown in Figure 1.



Figure 1. Araucana's blue eggs.

Table 1. Balanced diet composition.

Corn	62.6
Soybean meal	16.4
Limestone	7.97
Soybean	8.21
Meat	4.24
Salt	0.26
Mineral +Vitamin suplement	0.15
DL-methionine	0.15
Coline	0.02

Table 2. Corn principal components.

Ingredients	Percentage *
Water	12.8
Protein	8.0
Fiber	2.0
Fat	4.7
Carbohydrates	70
Ash	1.2
Energy	280 kJ / 100 g

* Average data from Dale and Jackson [10] and Peplinski *et al.* [11].

Eggs classification

Based on quality, shell condition and appearance, eggs were classified as “AA” for their faultless shells, firm and thick albumen, round and raised yolk, practically free from defects. Grade “A” eggs have a firm enough albumen, round and raised yolk, clean and intact shell, almost without imperfections. “B” eggs show thinner albumen and smoother yolks than higher quality eggs.

Physical properties determination

The eggs of each batch were weighed with an analytical scale (Denver Instrument, PK-202, USA) with an error of ± 0.01 . Albumen, yolk and shell were also weighed separately.

The specific weight (sw) was obtained by placing eggs into increasing concentrations of saline solutions until they reached their flotation point. NaCl solutions of 1.070, 1.075 and 1.080 g cm⁻³ were used. Eggs were classified in four categories according to their sw: <1.070; 1.070-1.075; 1.075-1.080 and >1.080 [12].

Chemical composition

Dry matter was determined in a stove at 105 °C until reaching a constant weight.

Protein content was found by the Kjeldahl method [13], [14], digesting and distilling samples (Digester System 60300, Distiller System 65000, Labconco, Kansas City, MO, USA).

Lipid content was determined by the method of Boselli *et al.* [15]. The lipids were extracted using a mixture of chloroform and methanol (2:1 ratio) and after, the solvent was evaporated and the obtained residue was weighed.

Cholesterol determination was carried out by an enzymatic method (Colestat Enzymatic kit, Wiener lab, Argentina) after lipid extraction [15].

Fatty acids were quantified by gas chromatography of samples obtained by extraction [16] and derivatization [17]. One μL of fatty acid methyl esters dissolved in ethyl acetate was injected to an Agilent Technologies gas chromatograph (Model 6890N) equipped with a flame ionization detector and an automatic injector (Model 7683) into a HP-5 capillary column (30 m \times 0.32 mm \times 0.25 μm). The injector temperature was 270 °C. The initial oven temperature, 120 °C, was held for 1 min; then increased to 190 °C at 10 °C min⁻¹ and held for 4 min; finally, increased to 300 °C at 15 °C min⁻¹ and held for 3 min. The detector temperature was 330 °C. Nitrogen was used as a carrier gas at a 1 mL min⁻¹ flow rate. Fatty acid methyl esters were identified and quantified by comparison with the retention times and peak areas of methyl esters obtained from fatty acid standards (Sigma-Aldrich, Argentina).

Mineral content (Ca, Mg, Fe, Na and K) in the whole egg and calcium in the shell were determined by using an atomic absorption spectrophotometer with flame ionization (Perkin Helmer Analyst 100, USA). Egg aliquots of 5 mL were dispensed into Petri dishes, frozen at -20 °C and dried in a chamber-type freeze-drier (Lyovac GT2; Leybold, Köln, Germany) for 16 h at 0.3 mbar to obtain less than 1% residual moisture. The dried samples were first desegregated with HCl and then used to quantify minerals in atomic absorption spectrometry.

Statistical analysis

All samples (n=12 for each type of egg) were analyzed in triplicate. Results were expressed as means \pm standard deviation (SD) and were statistically evaluated by the analysis of variance (ANOVA) test (Minitab Release 14 Statistical Software, 2003 Minitab Inc.). Differences were considered significant at $P < 0.05$ with Tukey's test.

Results and Discussion

Egg weight and specific weight

The three kinds of eggs used in this study were classified as “A” class for their quality. Egg weight results are shown in Table 3. Araucana free range eggs (DO) showed lower weight (56.9 ± 5.3 g) than brown (67.1 ± 6.9 g) or white (63.9 ± 4.7 g) eggs, the main difference being observed on the edible portion. Free range and white eggs showed similar yolk, albumen, and shell weights (≈ 32 , 58 and 10 g, respectively) and were significantly different from brown eggs (≈ 26 , 65 and 9 g, respectively). Whole egg weight determined in the present study is within the range observed by Cabrera *et al.* [18] in those of laying hens fed with different diets.

Eggs were separated in four categories depending on their specific weight (sw) as shown in Table 4. The eggs used in the present study were mainly classified as category 1 with a sw lower than 1.070 (69.4-83.3 %) while category 2, with a sw between 1.070-1.075, represented 8.4-16.7 % of the tested eggs. Eggs of Araucana and Isa Brown hens were classified in all categories, while Lohman hens' eggs were classified only in the first two, indicating a more homogeneous group.

Table 3. Egg weight analysis,

Sample	Balanced diet		Free range
	Isa (BR)	Lohman (WH)	Araucana (DO)
Egg weight (g)	67.1 ± 6.9^a	63.9 ± 4.7^a	56.9 ± 5.3^b
Edible portion (g)	56.8 ± 3.1^a	57.4 ± 4.5^a	50.5 ± 4.8^b
Yolk (g/100 g egg)	25.5 ± 2.2^b	31.7 ± 2.3^a	31.7 ± 5.1^a
Albumen (g/100 g egg)	65.4 ± 2.3^b	58.2 ± 2.4^a	57.8 ± 5.8^a
Shell (g/100 g egg)	9.1 ± 0.8^b	10.1 ± 0.3^a	10.5 ± 0.7^a

Means (n =12) within a row with different superscripts (a, b) differ, $P < 0.05$. BR: brown-shell egg; WH: white shell egg; DO: domestic farm egg.

Table 4. Percentage of egg varieties in different specific weight categories

Category	Balanced diet		Free range
	Isa (BR)	Lohman (WH)	Araucana (DO)
1	69.4	83.3	72.2
2	16.7	16.7	8.4
3	11.1	0	8.3
4	2.8	0	11.1

Results are expressed as percentage of total eggs. BR: brown-shell egg; WH: white shell egg; DO: domestic farm egg. sw: specific weight. 1: $sw < 1.070$; 2: $sw 1.070-1.075$; 3: $sw 1.075-1.080$ and 4: $sw > 1.080$.

Chemical composition

Table 5 shows the chemical composition of eggs. Dry matter varied between 23.0 and 26.1 g/100 g egg, being the highest value found in domestic eggs, with a significant difference from that of brown and white eggs. Protein content was similar in the three kinds of eggs, with a value near 15 g/100 g egg. In contrast, lipids content differed considerably. The highest value was determined in domestic eggs (35.0 g/100 g yolk or 11.1 g/100 g egg), followed by white (27.8 g/100 g yolk or 8.8 g/100 g egg) and brown eggs (19.8 g/100 g yolk or 5.1 g/100 g egg). This difference may be due to the corn and natural pasture diet fed to the Araucana domestic birds. However, lipids content may be also influenced by race [19]. The higher lipids value found in our study in Araucana hens' eggs may be a characteristic of its race, since Isa Brown and Lohman hens, fed with the same balanced diet laid eggs with different lipids content. Total fatty acid was 9.8 g/100 g yolk for Isa Brown layers, 7.7 g/100 g yolk for Lohman birds and 11.0 g/100 g yolk for Araucana hens.

Cholesterol levels also varied among eggs. Samples from hatcheries showed values of 0.45 and 0.50 g/100 g yolk each one, lower than that of eggs provided by farms with free range Araucana hens (0.65 g/100 g yolk). It has been claimed that Araucana eggs have less cholesterol than hatchery ones; nevertheless some authors reported that Araucana eggs have more cholesterol content than those of other breeds [20]. This last finding is in agreement with the data reported in our study. On the other hand, it is known that cholesterol level in eggs is high, but it may be diet dependent. It is probable that free range hens consume a higher lipid concentration than do hatchery hens (the corn and soybean seeds present in balanced food provide 2.94 and 1.64 % of lipids [21] respectively, to see Table 1 and 2) because they eat corn kernels with 4.7 % and *Paspalum notatum*, a graminaceous plant with a lipid content of about 2% [22]. However, the main cholesterol variation is observed when additional oils are supplemented to the diet [23]. Milinsk *et al.* [24] found high cholesterol content (1.0 g/100 g yolk) when hens were fed a diet rich in n-3 fatty acids, being this value 35 to 55% higher than those reported in our study. Values found in Isa Brown, Lohman and Araucana eggs represented 115, 159 and 206 mg /100 g egg respectively, being all of them within the range recommended for a daily consumption (less than 300 mg/day) by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).

Table 5. Chemical composition

Component	Unit	Balanced diet		Free range
		Isa (BR)	Lohman (WH)	Araucana (DO)
Dry matter	g/100g egg	23.0 ± 1.5 ^a	24.2 ± 2.0 ^a	26.1 ± 2.5 ^b
Proteins	g/100g egg	15.0 ± 0.8 ^a	14.7 ± 0.7	14.8 ± 0.7
Lipids	g/100 g yolk	19.8 ± 3.1 ^a	27.8 ± 5.3 ^b	35.0 ± 6.9 ^c
Total fatty acids	g/100 g yolk	9.8 ± 4.0 ^a	7.7 ± 3.1 ^a	11.0 ± 5.7 ^a
Cholesterol	g/100 g yolk	0.45±0.05 ^a	0.50±0.04 ^a	0.65±0.05 ^b

Results are expressed as mean ± standard deviation (SD). Means within a row with different superscripts (a, b) differ, P < 0.05. BR: brown-shell egg; WH: white shell egg; DO: domestic farm egg.

Mineral content was also determined in the present study (Table 6). Calcium, the main mineral component of the shell, presented an average value of 36-40 mg/100 g egg in eggs provided by hatcheries and farms with free range hens. Magnesium was close to 8 mg/100 g egg in all samples. Iron was 1.6 mg/100 g egg for hens fed a balanced diet and 2.0 mg/100 g egg for domestic (free range) birds. Sodium and potassium contents were similar for all kinds of eggs and values varied from 80-97 mg/100 g egg and 108-116 mg/100 g egg, respectively. Shell calcium was 38 % for all types of eggs. In general, the values were somewhat below those reported by Grobas and Mateos [4], which found 55 mg/100 g egg of Ca, 11 mg/100 g egg of Mg, 2-3 mg/100 g egg of Fe and 135 mg/100 g egg of Na and K.

Table 6. Mineral composition of eggs

Mineral (mg/100g egg)	Balanced diet		Free range
	Isa (BR)	Lohman (WH)	Araucana (DO)
Calcium	37.4 ± 7.9 ^a	40.0 ± 1.8 ^a	35.9 ± 3.4 ^a
Magnesium	8.3 ± 1.4 ^a	8.8 ± 1.1 ^a	8.4 ± 1.4 ^a
Iron	1.6 ± 0.3 ^a	1.6 ± 0.2 ^a	2.0 ± 0.3 ^a
Sodium	96.8 ± 8.2 ^a	88.6 ± 9.0 ^a	80.0 ± 18.4 ^a
Potassium	108 ± 9 ^a	108 ± 7 ^a	116 ± 11 ^a
Shell mineral (g/100g)			
Calcium	37.6 ± 1.1 ^a	37.8 ± 1.1 ^a	38.2 ± 1.1 ^a

Results are expressed as mean ± standard deviation (SD). Means within a row with different superscripts (a, b) differ, $P < 0.05$. BR: brown-shell egg; WH: white shell egg; DO: domestic farm egg.

Table 7 shows the fatty acid composition of yolks from Isa Brown, Lohman and Araucana eggs. Palmitic acid (C16:0) was the most abundant among saturated fatty acids (SFA), showing a value near to 27 % in all samples tested. It was followed by stearic acid (C18:0) which reached a content of 9%. Both values were similar to those found by Cobos *et al.* [19] in Leghorn hens with different diets, but other authors reported lower palmitic (22%) and similar stearic acid (9%) contents in Lohman birds [24]. Oleic acid (C18:1), from the n-9 series, was the main fatty acid in all samples, reaching a lower value (38-40 %) in eggs laid by balanced diet fed hens than in those laid by free range hens (44 %), although the difference was not statistically significant.

Table 7. Fatty acid profiles of yolk lipids from different eggs.

Fatty acid	Balanced diet		Free range
	Isa Brown (BR)	Lohman (WH)	Araucana (DO)
C14:0	0.20 ± 0.03 ^a	0.22 ± 0.03 ^a	0.19 ± 0.04 ^a
C16:0	26.63 ± 3.07 ^a	27.19 ± 2.12 ^a	27.11 ± 3.94 ^a
C16:1	1.79 ± 0.77 ^a	2.25 ± 0.66 ^a	2.05 ± 0.41 ^a
C18:0	9.02 ± 2.73 ^a	8.95 ± 1.47 ^a	9.07 ± 1.89 ^a
C18:1 (n-9)	39.69 ± 7.31 ^a	37.74 ± 2.80 ^a	44.04 ± 41 ^a
C18:2 (n-6)	19.71 ± 2.40 ^a	20.38 ± 2.08 ^a	14.01 ± 2.56 ^b
C18:3 (n-3)	0.23 ± 0.04 ^a	0.27 ± 0.06 ^a	0.32 ± 0.06 ^a
C20:4 (n-6)	1.53 ± 0.31 ^a	1.87 ± 0.07 ^a	2.03 ± 0.48 ^a
C22:6 (n-3)	1.20 ± 0.47 ^a	1.13 ± 0.19 ^a	1.18 ± 0.25 ^a
Σ Saturated	35.85 ± 2.06 ^a	36.36 ± 1.65 ^a	36.37 ± 3.45 ^a
Σ Monounsaturated	41.48 ± 5.45 ^a	39.99 ± 3.45 ^a	46.09 ± 5.12 ^a
Σ Polyunsaturated	22.67 ± 3.34 ^a	23.65 ± 1.46 ^a	17.54 ± 2.80 ^b
PUFA/SFA	0.63 ± 0.10 ^a	0.59 ± 0.07 ^a	0.48 ± 0.08 ^b
n-6	21.24 ± 2.45 ^a	22.25 ± 1.72 ^a	16.04 ± 2.56 ^b
n-3	1.43 ± 0.34 ^a	1.40 ± 0.14 ^a	1.50 ± 0.54 ^a
n-6/n-3	14.85 ± 3.83 ^a	15.89 ± 1.20 ^a	10.69 ± 3.44 ^b

Results are expressed as percentage of the total fatty acids. Data represents mean ± SD. Averages followed by different letters in the same line are significantly different ($P < 0.05$). BR: brown-shell egg; WH: white shell egg; DO: domestic farm egg; PUFA/SFA: ratio of polyunsaturated to saturated fatty acids; n-6: total omega-6 fatty acids; n-3: total omega-3 fatty acids; n-6/n-3: ratio of omega-6 to omega-3 fatty acids.

Linoleic acid (C18:2) content, of the n-6 series, was significantly different with values of 19.71, 20.38 and 14.01 % in Isa Brown, Lohman and Araucana eggs respectively, while arachidonic acid (C20:4, n-6), with values between 1.53 and 2.03 %, showed the highest concentration in Araucana eggs. Therefore, fatty acids of n-6 series were in significant lower amount in Araucana eggs (16.04 %) than in the other kind of eggs (21.24 and 22.25 %).

Diet produces the main changes in the n-3 fatty acids content of yolk [25]-[29]. However, in the present study, the percentage of n-3 acids (α -linolenic acid, C18:3 plus docosahexaenoic acid, C22:6) of Araucana eggs was similar to that of brown and white eggs: 1.50, 1.43 and 1.40 %, respectively.

In general, the nutritional quality of foods is assessed by the ratio of n-6/n-3 fatty acids and PUFA/SFA. The n-6/n-3 ratio observed in this study was similar in eggs laid by hens fed a balanced diet (14.85 and 15.89 respectively), but it was significantly different from that of free range eggs where a value of 10.69 was found. Values found in supermarket eggs were in agreement with reports of other authors who calculated n-6/n-3 ratios of 16 for Lohman eggs [24] and 13 for those of Hy-line W-98 layers [3]. A lesser value was determined for Araucana eggs in the present study and it was directly related to their linoleic content.

Linoleic acid (LA) is the major n-6 fatty acid, and alpha-linolenic acid (ALA) is the major n-3 fatty acid provided by the diet. They are defined as “essential” fatty acids since they are not synthesized in the animal’s body, where LA is metabolized to arachidonic acid (AA), and ALA is metabolized to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Clinical studies point out that the ingested ratio n-6 to n-3 fatty acids is a key determinant for the maintenance of cardiovascular health. Therefore, caution must be taken to avoid the intake of the disproportionate n-6/n-3 ratio associated to increased risk of cardiovascular disease. A balanced n-6/n-3 ratio in the diet is also essential for normal growth and development and should lead to decreases in some chronic diseases and improve mental health. Healthy ratios of n-6/n-3 in a range of 1/1 to 4/1 [30] and adequate intakes (AI) of 6/1 based on published literature describing practical dietary intakes [31] are reported. In turn, the World Health Organization (WHO) [32] suggests the daily consumption of diets with a ratio n-6/n-3 of no more than 10/1. These ratios are much lower than those really found nowadays in our diets [29]. Various types of food contain different proportions of fatty acids belonging to the series n-6 and n-3 and the impact of eggs consumption must be analyzed in the context of the whole diet. However, it is important to note that Araucana eggs have a ratio n-6/n-3 close to the value suggested by the WHO.

Previous studies reported PUFA/SFA ratios from 0.45 to 0.77 in eggs laid by birds receiving a different diet [24]. In the present study, the PUFA/SFA ratios were statistically different with values of 0.63, 0.59 and 0.48 for Isa Brown, Lohman and Araucana eggs respectively. Even when Araucana eggs presented the lowest value, this was within the range informed by other authors and suggested as safe.

Conclusions

A comparison among Isa Brown, Lohman and Araucana eggs was made taking into account the different race and feeding of laying hens. The main differences among the three kinds of eggs were found in dry matter, lipids content and cholesterol that were higher in Araucana eggs, but within accepted values for human daily consumption. That was attributed to the consumption of food with higher lipid amount provided by corn and grass and to genetic differences among hens. Lower amount of linoleic acid, better n-6/n-3 ratio and an appropriated PUFA/SAT ratio were also found in Araucana eggs. The results obtained in this study allow to infer that free range fed Araucana hen is a domestic farm resource that provides eggs that may be qualified as nutritious and healthy.

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