YOLK:ALBUMEN RATIO IN EXPERIMENTAL HYBRID LAYERS WITH DIFFERENT PATERNAL GENOTYPE

RELACIÓN YEMA:ALBUMEN EN HÍBRIDOS EXPERIMENTALES DE GALLINAS PONEDORAS CON DIFERENTE GENOTIPO PATERNO

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PALABRAS CLAVE ADICIONALES

Componentes del huevo. Fayoumi. Análisis multivariado. Gallinas ponedoras.

SUMMARY

Egg weight and major egg components, with special emphasis on yolk:albumen ratio, were studied in three experimental hybrids of laying hens with Barred Plymouth Rock maternal genotype and either Fayoumi (F), White Leghorn (L) or Rhode Island Red (R) sires. Data were collected from eggs laid in the first (37 weeks of age) and in the second (85 weeks of age) laying cycle. Asymptotic body weight ranked following the same order as paternal genotypes (F < L < R) and, at both ages, mean egg weight ranked following the same order as hens' average body weight. F birds, with the lightest eggs, showed the highest shell and yolk proportions, the lowest albumen percentage and the highest yolk to albumen ratio, in both laying cycles. Significant genotype and laying cycle effects and a non-significant interaction between them were observed for all traits. The joint variation of the three major egg components - yolk, albumen and shell - expressed as a percentage of egg weight was analysed using a multivariate principal component technique. The two first principal components explained almost all the generalized variance (99.93 percent) grouping hens by the paternal genotype. The first principal component discriminated birds for their yolk and albumen percentages while the second one was correlated with percent shell values. Fayoumi genes diminished body weight, food intake and egg weight and augmented yolk to albumen ratio. These modifications could be considered suitable in terms of the human target population with unsatisfied nutritional requirements to whom these hens are provided. They are also remarkable because the introgression of Fayoumi genes in commercial populations would be useful to enhance yolk production.

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RESUMEN

Los pesos del huevo y sus componentes mayores, con especial énfasis en la relación yema:albumen, se estudiaron en tres híbridos experimentales de gallinas ponedoras resultantes del cruzamiento del genotipo materno Plymouth Rock Barrado con tres genotipos paternos: Fayoumi (F), White Leghorn (L) y Rhode Island Red. Los datos se obtuvieron de huevos puestos en el primer (37 semanas de edad) y en el segundo (85 semanas de edad) ciclo de postura. En ambas edades, el peso corporal asintótico de cada grupo se ordenó de la misma manera que el genotipo paterno (F<L<R), al igual que el peso promedio de los huevos de cada híbrido. Los híbridos con padre F mostraron las proporciones más elevadas de cáscara y yema, el porcentaje de albumen más bajo y la relación yema:albumen más elevada, en ambos ciclos. El genotipo paterno y el ciclo de postura fueron efectos estadísticamente significativos, mientras que la interacción entre ambos caracteres no fue significativa. La variación conjunta de los tres componente mayores –yema, albumen, cáscara- expresados como porcentajes del peso del huevo se analizaron con la técnica multivariada de componentes principales. Los dos primeros componentes principales explicaron casi toda la variación (99,3 p.100) agrupando a las aves por el genotipo paterno. El primer componente principal discriminó a las aves por los porcentajes de yema y de albumen de sus huevos, mientras que el segundo estuvo correlacionado con el porcentaje de cáscara de los mismos. Los genes Fayoumi disminuyeron el peso corporal, el consumo de alimento y el peso del huevo y aumentaron la relación yema:albumen. Estas modificaciones se consideran deseables en relación con la población humana con requerimientos nutricionales insatisfechos a los que se destinan estas aves. También resultan de importancia en términos de la posibilidad de introgresión de genes Fayoumi en las poblaciones comerciales y de la identificación de QTLs para el carácter y su utilización en programas de selección asistida por marcadores.

INTRODUCTION

The hen's egg has been traditionally considered an important source of nutrients for humans. Nowadays it is widely recognized that eggs are more than a source of dietary nutrients and extensive studies identifying and characterizing their biologically active components have been carried on (Mine and Kovacs-Nolan, 2004). The egg size as well as the weight of its major components is influenced by genetic and nongenetic factors (Sáinz *et al.*, 1983). The egg yolk contains several substances of nutritional and therapeutic relevance (Hartmann and Wilhelmson, 2001). The relatively high level of cholesterol in the avian egg has become of increasing interest because of concern about the effect of dietary cholesterol on health (Washburn, 1990). This statement could be relativized both in terms of new information available on this issue (Stadelman, 1999) and also regarding the nutritional status of the human target population.

In Argentine, a national program of social assistance that includes the distribution of layers to low resources families has been developed. These hens are bred in a public institution (EEA INTA Pergamino) by crossing local strains of Rhode Island Red as sire line and Barred Plymouth Rock as dam line, thus generating an autosexing hybrid. One-day-old female chickens are allotted to these families jointly with technical and educational assistance. As a genetic alternative to reduce the feeding costs of the program a new set of experimental crosses was proposed using Fayoumi (ultralight) or White Leghorn (light) breeds with the aim of diminishing maintenance costs by reducing the mean body weight of the progeny (Dottavio *et al*., 2001). Among other traits, the Egyptian Fayoumi breed is characterized by a low body weight and eggs of high shell strength and high yolk to albumen ratio (Amer, 1972).

The purpose of this study was to compare the major components of eggs laid by experimental hybrids layers obtained by crossing Barred Plymouth Rock hens to sires belonging to three breeds with different adult body weight (Fayoumi, White Leghorn and Rhode Island Red), in two laying cycles. The body weight-egg weight relationship and the effect of introducing Fayoumi genes as an alternative to improve yolk:albumen ratio was particularly emphazied.

MATERIAL AND METHODS

BIRDS

Data were collected on hybrid layers sampled from the respective populations produced at the Facultad de Ciencias Veterinarias, Universidad Nacional de Rosario, Argentina. Three experimental F1 crosses with Barred Plymouth Rock as maternal genotype and either Fayoumi (F), White Leghorn (L) or Rhode Island Red (R) as paternal genotypes were produced via artificial insemination. Chicks from the three genetic groups were obtained from only one hatch in Spring (October) and were reared intermingled on the floor. Birds were fed a standard starter ration (21 percent crude protein CP) from hatch to 56 days of age, a standard developing ration (18-19 percent CP) from 57 to 140 days of age, and a standard laying ration (16 percent CP) from 141 days of age on. At 18 weeks of age, 30 birds randomly sampled from each genetic group were moved to the laying house and allocated to individual laying cages (40 cm high, 25 cm wide, and 35 cm deep). The lighting program used was one with an evenly decreasing day length from 24 hours the first week to 13 hours at 18 weeks of age. Day length was then increased by 30 minutes per week up to the limit of 17 hours per day.

MAJOR EGG COMPONENTS

Three eggs of each of twenty hens of each genetic group were chosen at random at 37 weeks of age (first laying cycle) and at 85 weeks of age (second laying cycle). A third group of eggs was studied at 124 weeks of age to determine the mature values of all traits. Eggs were gathered early in the morning and kept at room temperature until evaluation on the afternoon of the same day. Individual eggs were weighed, then broken and whites and yolks carefully separated manually. Most of the albumen was discarded upon initial separation from the yolk. Further separation of the adhering albumen was made by carefully rolling the yolk several times on a paper towel. The egg shells, including membranes, and yolks were weighed separately with an accuracy to the nearest 0.1 g. Albumen weight was determined by subtracting fresh yolk weight plus fresh shell weight from the original egg weight. Using the individual weight of each egg and its components, yolk percentage [(yolk weight / egg weight) 100], albumen percentage [albumen weight / egg weight) 100], shell

***Table I.** Body weight and voluntary feed intake (mean ± standard error) in three experimental hybrids of laying hens with Barred Plymouth Rock maternal genotype and different paternal genotype.* (Peso corporal y consumo voluntario de alimento (media ± error estándar) de tres híbridos experimentales de gallinas ponedoras con genotipo materno Plymouth Rock Barrado y diferente genotipo paterno).

	Sire Genotype		
	Fayoumi	White Leghorn	Rhode Island Red
Body weight (g)	1772 ± 27.5[a]	1937 ± 55.1[b]	2508 ± 61.8[c]
Daily intake (g)	107.7 ± 1.64[a]	103.2 ± 1.65[a]	135.2 ± 2.10[b]

Asymptotic body weight . Sample size: 20 hens per genotype. [abc]Values with different superscript differ at least at p<0.05.

percentage [(shell weight / egg weight) 100], and yolk: albumen (Y:A) ratio (yolk weight / albumen weight) were calculated.

STATISTICAL ANALYSIS

Univariate data were analyzed by a two-way analysis of variance using the following linear model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where Y_{ijk} is the k^{th} observation in the ij^{th} genetic group, μ is the overall mean,

***Table II.** Egg weight and absolute and relative major egg components (mean ± standard error) in three experimental hybrids of laying hens with Barred Plymouth Rock maternal genotype and different paternal genotype, in their first laying cycle.* (Peso del huevo y peso absoluto y relativo de los componentes mayores del huevo (media ± error estándar) de tres híbridos experimentales de gallinas ponedoras con genotipo materno Plymouth Rock Barrado y diferente genotipo paterno, en su primer ciclo de postura).

	Sire Genotype		
	Fayoumi	White Leghorn	Rhode Island Red
Egg weight (g)	51.5 ± 0.60[a]	56.8 ± 0.83[b]	63.6 ± 0.88[c]
Shell weight (g)	5.72 ± 0.09[a]	6.13 ± 0.13[b]	6.57 ± 0.11[c]
Yolk weight (g)	15.0 ± 0.20[a]	15.0 ± 0.25[a]	16.9 ± 0.23[b]
Albumen weight (g)	30.8 ± 0.51[a]	35.7 ± 0.56[b]	40.1± 0.72[c]
Shell (percent)	11.1 ± 0.11[a]	10.8 ± 0.13[a]	10.3 ± 0.12[b]
Yolk (percent)	29.1 ± 0.44[a]	26.4 ± 0.29[b]	26.7 ± 0.35[b]
Albumen (percent)	59.8 ± 0.38[a]	62.8 ± 0.27[b]	63.0 ± 0.90[b]
Y : A ratio	0.489 ± 0.0104[a]	0.421 ± 0.0062[b]	0.424 ± 0.0078[b]

Sample size: 20 hens per genotype and three eggs per hen.[abc]Values with different superscript differ at least at p<0.05.

***Table III.** Egg weight and absolute and relative major egg components (mean ± standard error) in three experimental hybrids of laying hens with Barred Plymouth Rock maternal genotype and different paternal genotype, in their second laying cycle.* (Peso del huevo y peso absoluto y relativo de los componentes mayores del huevo (media ± error estándar) de tres híbridos experimentales de gallinas ponedoras con genotipo materno Plymouth Rock Barrado y diferente genotipo paterno, en su segundo ciclo de postura).

	Sire Genotype		
	Fayoumi	White Leghorn	Rhode Island Red
Egg weight (g)	57.5 ± 0.88^a	60.8 ± 1.09^b	69.1± 1.18^c
Shell weight (g)	6.24 ± 0.14^a	6.46 ± 0.15^a	7.02 ± 0.16^b
Yolk weight (g)	18.1 ± 0.30^a	17.8 ± 0.25^a	19.8 ± 0.27^b
Albumen weight (g)	33.1 ± 0.70^a	36.6 ± 0.86^b	42.3 ± 1.05^c
Shell (percent)	10.9 ± 0.18^a	10.6 ± 0.15^a	10.2 ± 0.19^b
Yolk (percent)	31.6 ± 0.52^a	29.3 ± 0.54^b	28.7± 0.49^b
Albumen (percent)	57.8 ± 0.51^a	60.1 ± 0.53^b	61.1 ± 0.78^b
Y : A ratio	0.553 ± 0.0145^a	0.490 ± 0.0133^b	0.472 ± 0.0120^b

Sample size: 20 hens per genotype and three eggs per hen. ^{abc}Values with different superscript differ at least at p<0.05.

α_i is the fixed effect of the ith genotype (i= 1,... 3), β_j is the fixed effect of the jth laying cycle (j= 1,2), $(\alpha\beta)_{ij}$ is the interaction of the ith genotype with the jth laying cycle and e_{ijk} is the random error (Sokal and Rohlf, 1969).

The joint variation of the three major egg components was multivariately characterized by means of a principal component analysis (Tatsuoka, 1971) using yolk, albumen and shell percentage values standardized into zero means and unity standard deviations (Mc Cune and Mefford, 1999). This procedure classifies phenotypic variation into independent systems of correlated traits reducing the *m* original variables into *n* components, namely PC1, PC2,....., PCn, which account for most of the generalized variance. In addition, as each component represents an independent entity resulting from the linear combination of the original variables, some interpretation of the new dimensions of the data not evident by themselves in the univariate approach could be done.

RESULTS

Genotype differences in body weight and feed consumption are presented in **table I**. Asymptotic body weight of hybrids ranked in the same order as that of paternal genotypes (F < L < R). Hybrids hens with Fayoumi or White Leghorn sires did not differ in their average daily intake but both of them eat less than Rhode Island Red x Barred Plymouth Rock hens.

Tables II and **III** show the average values for egg weight, absolute and relative egg components and Y:A ratio

Table IV. *Mature egg weight and absolute and relative major egg components (mean ± standard error) in three experimental hybrids of laying hens with Barred Plymouth Rock maternal genotype and different paternal genotype.* (Peso maduro del huevo y peso absoluto y relativo de los componentes mayores del huevo (media ± error estándar) de tres híbridos experimentales de gallinas ponedoras con genotipo materno Plymouth Rock Barrado y diferente genotipo paterno).

		Sire Genotype	
	Fayoumi	White Leghorn	Rhode Island Red
Egg weight (g)	59.6 ± 1.48^{a}	60.1 ± 1.31^{a}	71.1 ± 1.27^{b}
Shell weight (g)	8.31 ± 0.33^{a}	8.04 ± 0.41^{a}	8.73 ± 0.17^{a}
Yolk weight (g)	19.8 ± 0.44^{a}	18.1 ± 0.35^{b}	20.2 ± 0.41^{ac}
Albumen weight (g)	31.6 ± 0.73^{a}	33.9 ± 0.31^{a}	42.1± 1.20^{b}
Shell (percent)	13.9 ± 0.47^{a}	13.4 ± 0.65^{a}	12.3 ± 0.24^{a}
Yolk (percent)	33.3 ± 0.69^{a}	30.2 ± 0.38^{b}	28.6 ± 0.70^{b}
Albumen (percent)	52.8 ± 0.56^{a}	56.4 ± 0.83^{b}	59.1 ± 0.78^{b}
Y : A ratio	0.623 ± 0.0181^{a}	0.538 ± 0.0138^{b}	0.485 ± 0.0174^{b}

Sample size: 20 hens per genotype and three eggs per hen. ^{abc}Values with different superscript differ at least at $p<0.05$.

in the first and in the second laying cycles, respectively. At both ages the mean egg weight ranked following the same order as hens' average body weight. F birds, with the lightest eggs, showed the highest shell and yolk proportion, the lowest albumen percentage and the highest Y:A ratio in both laying cycles. In contrast, R hens laid the heaviest eggs, with the lowest shell and yolk percentages, the highest albumen proportion and the lowest Y:A ratio. L birds showed, in general, intermediate values with regard to the other two hybrids. At maturity **(table IV)**, hens with Fayoumi sire equalized L hens in terms of egg weight and showed the lowest albumen proportion and the highest yolk percentage and Y:A ratio.

The principal component analysis showed that the two first principal components explained almost all the generalized variance (99.93 percent). The proportion of the total variance accounted for by the first principal component (PC1) was 67.42 percent. This component showed a positive and significant correlation with yolk percentage (r = 0.956) and a negative correlation with albumen percentage (r = -0.999). The second principal component (PC2) explained 32.51 percent of the total variance and correlated negatively (r = - 0.943) with percent shell values. **Table V** shows the mean values for both principal components in the three genotypes and in both laying cycles. As expected from the univariate analysis, F hens were located in the right size of the scatterplot **(figure 1)** because of the high yolk proportion of their eggs. F and L birds showed very similar PC2 values at both ages, reflecting their similar figures for shell relative

Table V. *First (PC1) and second (PC2) principal components (mean ± standard error) for egg traits in three experimental hybrids of laying hens with Barred Plymouth Rock maternal genotype and different paternal genotype in two laying cycles.* (Primera (PC1) y segunda (PC2) componente principal (media ± error estándar) para los componentes del huevo de tres híbridos experimentales de gallinas ponedoras con genotipo materno Plymouth Rock Barrado y diferente genotipo paterno en dos ciclos de postura).

Cycle	First laying cycle			Second laying cycle		
Sire genotype	Fayoumi	White Leghorn	Rhode Island Red	Fayoumi	White Leghorn	Rhode Island Red
PC1	0.5057[a] ± 0.1940	- 0.9278[b] ± 0.1697	- 0.9901[b] ± 0.2501	1.6661[a] ± 0.2662	0.3311[b] ± 0.2825	- 0.2195[b] ± 0.3065
PC2	- 0.4857[a] ± 0.1815	- 0.4574[a] ± 0.1877	0.1616[b] ± 0.1599	0.1116[a] ± 0.2743	0.1380[a] ± 0.2064	0.6347[a] ± 0.2425

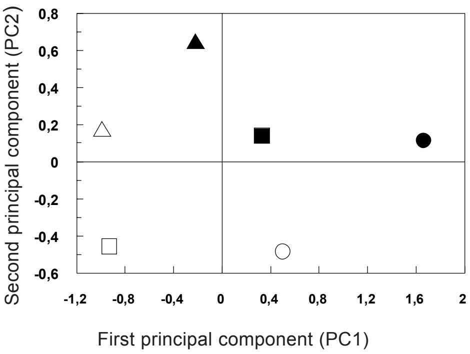
Sample size: 20 hens per genotype in each laying cycle. [abc]Values with different superscript differ at least at p<0.05, for comparisons among genotypes within laying cycle.

content. R birds, with roughly the same mean Y:A ratio as L hens had higher PC2 values as a consequence of their lower shell percentage.

The two-way analysis of variance showed significant genotype ($p<0.001$) and laying cycle ($p<0.001$) effects and a non-significant ($p>0.05$) genotype x laying cycle interaction effect for all traits. Birds in their second laying cycle laid heavier eggs with more yolk, less albumen and less shell than in the first one. The coordinates of the points depicting second laying cycle mean values for the three genetic groups showed a very similar displacement towards the upper right quadrant in comparison to first cycle averages.

DISCUSSION

In accordance with the well known positive genetic correlation between body weight and egg weight, genotypes ranked in the same order for both variables. Hens with Rhode Island Red paternal genotype were the heaviest, laid the heaviest eggs and showed the highest voluntary intake. Meanwhile, birds with Fayoumi paternal genotype were the lightest, laid the lightest eggs and showed a lower food consumption than R. Hybrid layers with White Leghorn paternal genotype showed intermediate values for body and egg weight and similar food intake values as F hens in both laying cycles. L and R hens with significant different egg size did not differ in yolk and albumen percentage, either in the first or in the second laying cycle; therefore, they did not differ in their Y:A ratio. On the other hand, F hens laid smaller eggs with more yolk, less albumen and more Y:A ratio. Although the latter rela-



First laying cycle: open symbols; Second laying cycle: solid symbols.
Paternal genotype: Fayoumi, ○/●*; White Leghorn,*□/■*; Rhode Island Red,* △/▲.

***Figure 1.** Scatterplot for the two first principal components. Only mean values are shown.* (Diagrama de dispersión para las dos primeras componentes principales. Sólo se indican los valores promedio).

tionships are in agreement with the results of Ahn *et al.* (1997) and Suk and Park (2001) which showed that the proportion of yolk and the Y:A ratio tended to be greater in smaller eggs than in larger eggs if compared at the same age, this did not hold good for the former. Therefore, a different response was observed when Fayoumi genes or White Leghorn genes were introduced in the one way hybrid instead of Rhode Island Red genes. This could be explained in terms of a particular effect ascribed to Fayoumi genes and some of the previously established particularities of this breed could be confirmed (Mérat *et al.*, 1983; Benabdeljelil and Mérat, 1995; Dottavio *et al.*, 2001). This particular effect was also evident in the scatterplot resulting from the multivariate analysis where hens with Fayoumi paternal genotype were located at the right of the plot and were isolated from the other two hybrid combinations **(figure 1)**.

The incorporation of Fayoumi genes diminished body weight and, as a consequence, these hens ate less food and laid lighter eggs. These eggs have a high proportion of yolk and a high Y:A ratio and though they do not differ in the levels of cholesterol on a per-gram-of-yolk-basis, they are likely to contain more total cholesterol. Although it could be considered a negative feature in some countries or in some social strata with freely access to, and frequently excessive intake of all kind of nutrients, the nutritious quality of the egg makes its consumption highly commendable for people with unsatisfied nutritional requirements. This could be the case of the human target population to whom these layers are provided. In this context, hens with a low intake because of their low body weight, which lay a relatively light egg for the same reason but with a high yolk content could ensure the provision of a complement of nutrients capable of sustaining life. In the future, this approach could be extended to more sophisticated interventions modifying the composition of table eggs by feeding hens with modified foods and using them as carriers of special nutrients or as a source of specific chemicals or pharmaceuticals, all of them included in the enriched medium of the egg yolk as has been suggested (Stadelman, 1999; Hartmann and Wilhelmson, 2001). Finally, regarding the potential of several biologically active substances present in the yolk of avian eggs, the introgression of Fayoumi genes in commercial populations of layers could be an alternative to improve yolk production by breeding besides the direct exploitation of additive genetic variation by artificial selection (Hartmann *et al.*, 2000) and also in terms of generating pedigrees suitable for QTLs mapping and their potential utilization in breeding programs using marker-assisted-selection (Burt, 2002).

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