Changes in Some Egg Components and Analytical Values Due to Hen Age[1]

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ABSTRACT The influence of hen age on some egg characteristics was studied. Two commercial breeds of brown hens, namely Warren and Hy-Line, were considered at seven different ages. The variables analyzed were the weights of yolk and thick and thin albumen, pH, and the concentrations of glucose, uridine, and uric and pyroglutamic acids of separated yolk and albumen. Albumen and yolk average weights and the proportion of yolk in the edible part of egg increased with hen age, whereas the average ratio of thick to thin albumen was not influenced by the progress of the laying cycle. Glucose, uridine, and uric acid were also not influenced by hen age. Pyroglutamic acid, which is detectable in yolk and not in the albumen of a fresh egg, showed a characteristic trend in yolk. Its concentration dramatically increased in the middle of the laying cycle and then decreased to values close to those observed in eggs of the young layers.

(*Key words*: hen age, egg components, egg composition, high-performance liquid chromatography, brown egg layer)

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INTRODUCTION

Numerous factors affect the composition and structure of eggs. These are genetic characteristics (Washburn, 1978), such as egg weight, hen age, type of breeding (e.g., in cages, free on the ground), type of feeding, season, and room temperature (although these last two factors have lost most of their importance as a source of variation because light and temperature conditions are carefully controlled and kept constant in modern farms) (Sauveur and de Reviers, 1988). At equal strain, feeding, and breeding conditions, variability of fresh egg characteristics is due to the biological cycle of the layers covering a period of time between the 20th and 70 to 75th wk of life.

During the aging of the layers, some typical phenomena observed are the increase in egg weight and in the proportion of yolk (Burley and Vadehra, 1989; Kaminska, 1990; Kaminska and Skraba, 1991), the decrease in the percentage of thick albumen (Monsey *et al.*, 1977; Hill and Hall, 1980), the decrease in the solid content of albumen (Marion *et al.*, 1964; Fletcher *et al.*, 1983), and the decrease in total lipid and cholesterol of yolk (Sauveur and de Reviers, 1988). Fletcher *et al.* (1983) reported that, at equal hen age, some variations in composition are observed depending on egg weight.

Some of the variables stated above are often correlated to the quality of shell egg. These variables include the proportion of thick albumen, which gives a gel-like appearance to a fresh egg white, or the yolk to albumen weight ratio. Actually, the criteria chosen for assessing quality change according to the needs or idea of quality of producers, consumers, and manufacturers. For producers, quality is related mainly to egg weight, shell strength, and lack of defects such as external dirt, cracked shells, and blood spots. For consumers, quality is related

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mainly to freshness and sensory characteristics, such as shell and yolk colors, even though consumers can judge these only empirically. For egg processors and food manufacturers, quality is based on ease of shelling and ease of separation of yolk from albumen, functional properties, and yolk color, especially for bakery and noodle manufacturers. For all of the above, microbiological safety represents an unavoidable criteria of quality.

Some of the factors cited above are evaluated by specific but semi-quantitative tests, such as Haugh units for albumen quality, the Roche scale for yolk color, and instrumental strength needed to break eggshell. Unfortunately, criteria for judging freshness are not yet supported by objective and quantitative methods. The European Economic Community (EEC) regulations (1991) grade eggs as first quality (grade A) when they satisfy conditions such as the height of the inner air cell must be lower than 6 mm and the albumen must look gel-like.

The research reported here is part of a wider study designed to assess quality standards for egg and egg products. Some of the variables quantified in the present study are commonly measured in eggs, such as the weight of egg components (yolk and thick and thin albumen), pH, and glucose; others are variables not yet considered as indicators of egg freshness, such as the content of uridine and pyroglutamic and uric acids. Uridine and pyroglutamic acid are particularly relevant for the definition of egg freshness, as their concentration changes during egg storage depending on temperature (Rossi and Pompei, unpublished data). Morris *et al.* (1989) have already reported the presence of the nucleoside uridine in eggs and stressed its role as precursor of uracile, an interesting indicator of egg product spoilage. These authors also observed the presence in eggs of pyroglutamic acid that, following Van der Werf *et al.* (1971), originates in animal tissue from glutathione via enzymatic reactions (the γ-glutamyl cycle). The present paper is a preliminary study intended to evaluate the variability of some parameters in fresh eggs laid by Warren and Hy-Line brown hens at different times during their laying cycle.

TABLE 1. Month and hen age at sampling

Hen age[1] (wk)	Month of sampling
23	September
30	June[2]/November[3]
40	June
47	March
53	June
66	September
70	June

[1]Weeks after hatch.

[2]Warren.

[3]Hy-Line.

MATERIALS AND METHODS

Eggs analyzed in the present study were laid by brown hens of the commercial breeds Warren and Hy-Line, which are layers widely used in large Italian poultry farms. Eggs of both breeds were collected from hens of seven different ages, as reported in Table 1, where the age of hens is expressed in terms of weeks after hatch. Sampling was performed in March, June, September, and November. The laying cycle of the hens started at 20 wk after hatch. Groups of hens of different ages and breeds were simultaneously present at the farm, each group being housed in separated houses containing about 50,000 birds each. Hens were bred in battery cages under a nonconditioned environment at a house temperature of about 15 to 20 C in the cold months (November and March) and of 25 to 28 C in the warm months (June and September). Other breeding conditions were a light program of 16 h of uninterrupted light, and an all-vegetable feeding made of a mix of corn and soybean (17% CP; 2,820 kcal ME/kg feed).

Samples of 30 newly laid eggs were collected from each group of hens, homogeneous for breed and age. They were randomly collected directly on the conveyor belt at the house, choosing only warm eggs to guarantee freshness. After cooling for about 1 h at room temperature, eggs were candled (only sound eggs were used) and packed in cardboard trays that

were put into a cardboard box suitable for transport. Packed eggs were stored at 18 C until loading on an insulated truck. Transport lasted about 3 h. Eggs reached our laboratory less than 24 h after being laid and were analyzed immediately. Ten eggs out of each sample were randomly chosen at our laboratory for analysis. These 10 eggs were used for weight evaluation of separated yolk and albumen fractions and, after homogenization of all albumens and yolks combined in pooled samples, for HPLC analysis and pH evaluation.

Weight Evaluation

The eggs were manually shelled and the yolk and albumen were carefully separated and weighed. Before weight evaluation, the thin film of albumen adhering to yolk was removed using blotting paper. Each albumen was then poured, for the separation of thick from thin albumen, into a filtration apparatus made up of a 3-mm mesh nylon sieve assembled over a 7.5-cm diameter funnel. The filtrate (the thin albumen: TNA) was then collected in a 25-mL graduated cylinder and weighed. Filtration stopped after 60 s. What remained on the sieve (the thick albumen: TKA) was accurately transferred into another 25-mL cylinder and weighed. Results of weight evaluation were reported as the average of 10 measurements (± one standard deviation).

All TNA and TKA of the same sample were then combined and homogenized to perform pH measurement[2] and HPLC analysis. Yolks of the same sample were similarly combined and homogenized before analysis. Homogenization was carried out using a Sörvall Omni Mixer[3] at 4,000 rpm for 30 s.

High-Performance Liquid Chromatography Analysis

Extraction of egg samples for HPLC analysis was performed following the method of Morris (1987), using four times the sample size and the reagent volumes he suggested. The extract thus prepared was filtered on a .22-μm GS Millipore membrane and injected in the chromatograph (injection volume, 50 μL).

Operative conditions for HPLC analysis of organic acids, glucose, and uridine were as follows: column, Aminex HPX-87H[4] (300 $\times$7.8 mm); column temperature, 45 C; guard column, Cation H cartridge;[4] mobile phase, .01 N H_2SO_4; flow rate, .6 mL/min. Two detectors connected in series were used: 1) a Waters Photodiode Array Detector[5] set in the 200 to 285 nm wavelength range; and 2) a HP Refractive Index Detector[6] maintained at 40 C. The refractive index detector was used for glucose detection. Organic acids were detected at 210 nm, except that uric acid was detected at 284 nm. Uridine was detected at 260 nm. Peaks in the chromatograms were identified by both comparison of peak elution times and spectra with those of pure compounds and examining the chromatograms of spiked samples.

Some of the identified compounds were also quantified using the external standard method. Calibration curves were prepared using six different standard concentrations per each: uridine[7] from 0 to 10 ppm; uric acid[8] from 0 to 10 ppm; L-pyroglutamic acid[9] from 0 to 100 ppm; glucose[7] from 0 to 2 g/L.

Based on the calibration curves, the limit of detection, for each compound, was calculated as the value of the intercept of the regression line plus three times the standard error of estimate (Miller and Miller, 1988). Two extractions were performed on each sample and the extracts were analyzed. The results were the average of the two analyses.

Statistical Analysis

The weights of yolk, albumen, TKA, TNA, the TKA to TNA ratio, and yolk

[2]PHM82 Standard pH Meter, Radiometer Analytical A/S, DK-2880 Bagsvaerd, Denmark.
[3]Model 17106, DuPont De Nemours and Co., Newton, CO 06470.
[4]Bio-Rad Laboratories, Hercules, CA 94547.
[5]Model 990, Millipore Waters Division, Milford, MA 01757.
[6]Model 1037A, Hewlett Packard Co., Palo Alto, CA 94303.
[7]Sigma Chemical Co., St. Louis, MO 63103.
[8]Merck, D-6100 Darmstadt 1, Germany.
[9]Fluka AG, CH-9470 Buchs, Switzerland.

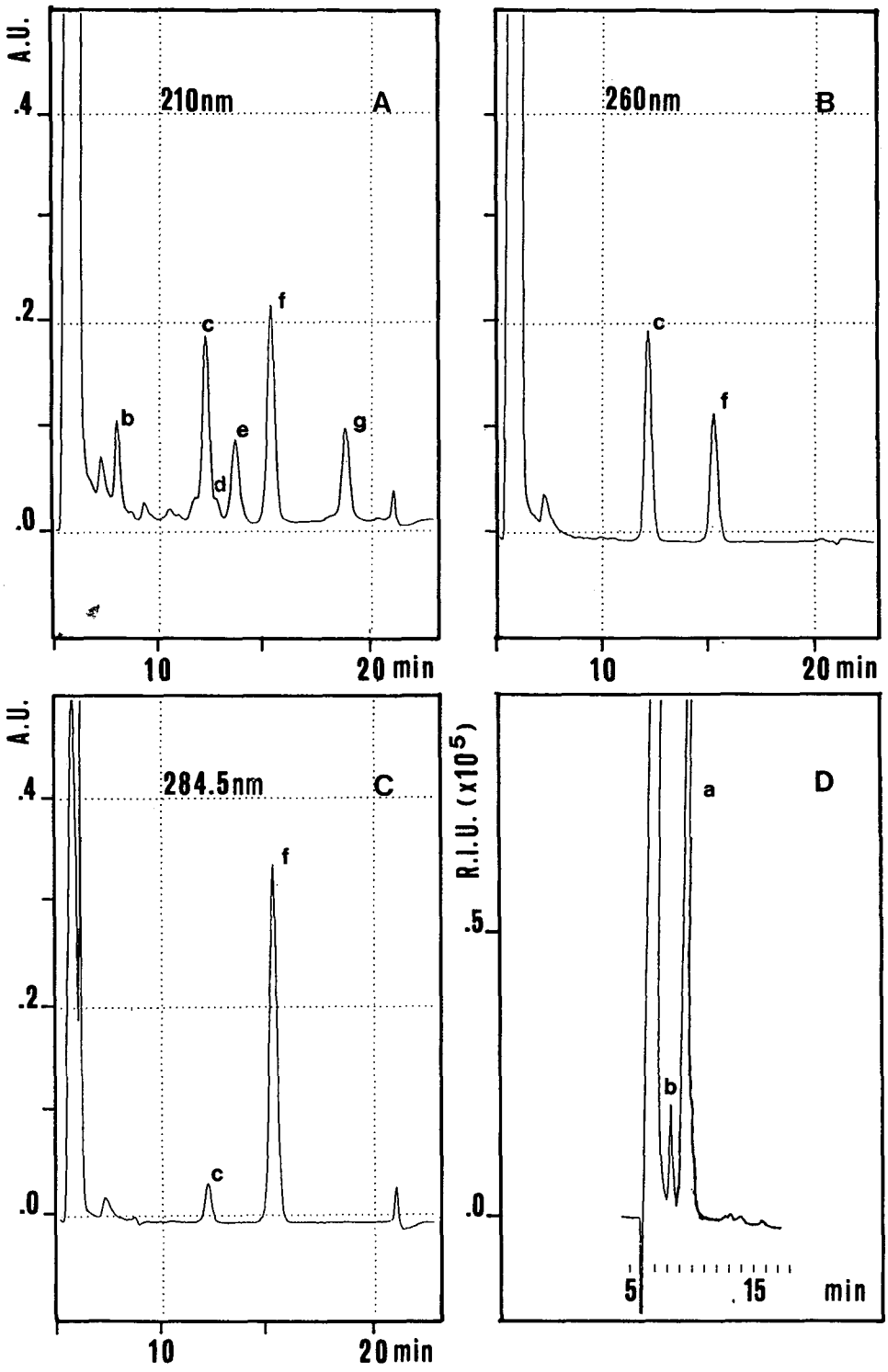

FIGURE 1. High-performance liquid chromatography chromatograms of a whole egg sample. Patterns A, B, and C were obtained through a photodiode array detector (A.U. = absorbance units) and pattern D through a refractive index detector (R.I.U. = refractive index units). Peaks identification: a) glucose; b) citric acid; c) uridine; d) lactic acid; e) formic acid; f) uric acid; g) pyroglutamic acid.

TABLE 2. Some characteristics of the regression lines for the compounds measured by HPLC

Compound	Linearity range[1]	r^2	Detection limit in the sample
	(ppm)		(ppm)
Glucose	0 to 2,000	.999	14.6
Uridine	0 to 10	.995	3.35
Pyroglutamic acid	0 to 100	.999	5.95
Uric acid	0 to 10	.998	2.02

[1]In the injected standard solution.

percentage at equal breed and age were statistically elaborated for each variable to calculate the variation around the mean in terms of standard deviation. One-way analysis of variance was also performed on these variables to study the differences between the two breeds at equal age, and the differences among ages at equal breed, at a confidence level of 95%. The pH values and the results of HPLC analysis were elaborated to calculate the mean value, the standard deviation, and the relative standard deviation per each breed and for both breeds combined over the laying cycle.

RESULTS AND DISCUSSION

Optimization of the High-Performance Liquid Chromatography Analysis

Some preliminary chromatographic runs were carried out to study the effect of column temperature and acid concentration in the eluant on separation efficiency of the peaks. The chromatographic conditions reported in the Materials and Methods section were based on this preliminary study. By using a photodiode array detector connected in series with a refractive index detector, all the compounds of interest were detected simultaneously.

Figure 1 represents, as an example, the chromatographic patterns obtained for a whole egg sample. Table 2 reports the limits of detection and the determination coefficients (r^2) calculated for the calibration curves of the measured compounds.

In order to check the extraction yields of the analytical method, a whole egg was also spiked with different amounts of pure compounds before proceeding to extraction. By expressing the egg spiking in terms of concentration in the injected sample, the following extraction yields were obtained: 100% for uridine additions in the range 2 to 15 ppm; average 95% for pyroglutamic acid additions in the range 10 to 96 ppm;

TABLE 3. Representation of the statistical differences between groups of eggs laid by hens of the same breed, Warren (W) or Hy-Line (H), of different ages

	Yolk weight		Albumen weight		TKA weight		Yolk %	
Hen age[1]	W[2]	H	W	H	W	H	W	H
(wk)								
23	a	a	a,b	a	a,b,c	a	a	a
30	b	b	a	a	a,b	a	b	b,c
40	c	c	c,d	b	d	a	b	b
47	c	c	b	a	c,d	a	b	d
53	d	d	b,c	b	b,c,d	b	c	b,c,d
66	e	e	e	c	e	c	d	e
70	d	d	d,e	b	a	a,b	b	c,d

[1]Weeks after hatch.

[2]Different letters in the same column identify groups significantly different at a confidence level of 95%.

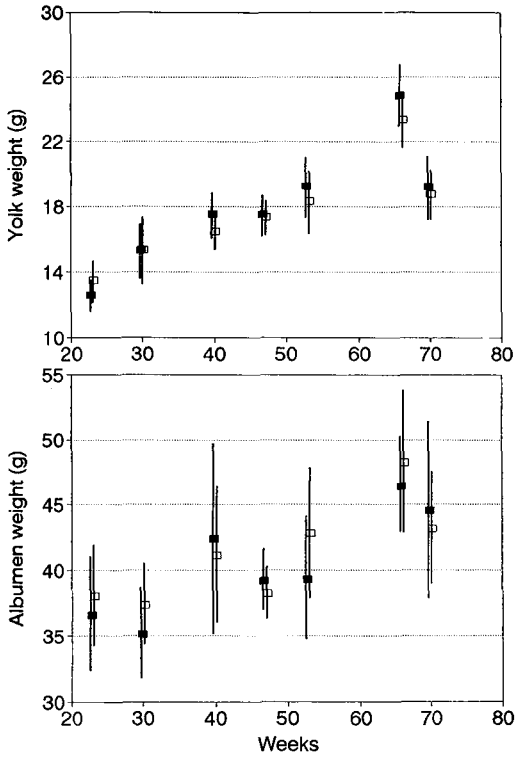

FIGURE 2. Yolk and albumen weights as a function of hen age. Solid squares and empty squares represent the breeds Warren and Hy-Line, respectively. Vertical bars represent the variation around the mean (the mean ± one standard deviation.

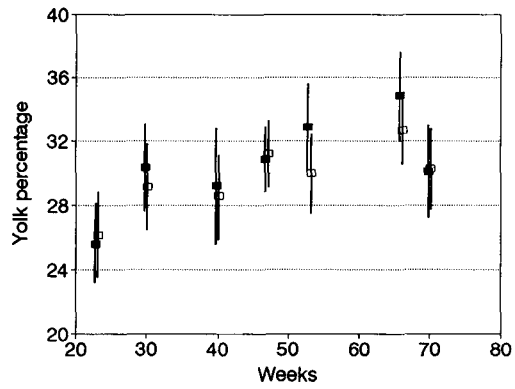

FIGURE 3. Yolk percentage in the edible part of egg, as a function of hen age. Solid squares and empty squares represent the breeds Warren and Hy-Line, respectively. Vertical bars represent the variation around the mean (the mean ± one standard deviation).

increasing yields between 93 and 98% for glucose additions in the range between 10 to 100 ppm, respectively.

Variability during the Laying Cycle

Figure 2 represents the average values, at each age and for both breeds of hens, of yolk and albumen weights, Figure 3 represents the percentage of yolk on the edible part of the egg (yolk plus albumen), Figure 4 represents TKA and TNA weights, and Figure 5 the ratios between them.

At equal hen age, the two breeds show no difference at a confidence level of 95%, except for yolk percentage in the eggs of hens of 53 and 66 wk ($P < .05$) (Figure 3). One-way analysis of variance did not show differences ($P > .05$) between the groups of eggs laid by hens of the same breed but of different ages as regards TNA weight (Figure 4) and TKA to TNA weight ratio (Figure 5). Table 3 is a representation of the statistical differences ($P < .05$) observed for the other variables.

Both the albumen and yolk weights increase with hen age (Figure 2), but as the relative increase of weight is larger for yolk than for albumen, the proportion of yolk in the edible part increases as the laying cycle progresses (Figure 3). The trends observed are consistent with the results obtained in similar studies (Fletcher *et al.*, 1983; Kaminska and Skraba, 1991). When the hens are near the end of their laying cycle, between 66 and 70 wk after hatch, a decrease in the amount of yolk is observed (Figure 2).

The relative standard deviation (RSD) of the results obtained for samples of the same breed and age was less than 10 to 12% for the yolk weight (Figure 2). Variability was greater for the two albumen fractions: values up to 26% RSD were observed for TKA and up to 44% for TNA (Figure 4).

Table 4 reports the values of pH, and glucose, uridine, and uric and pyroglutamic acid contents measured in the egg samples laid by the two hen breeds during their laying cycle. Pyroglutamic acid, though present in yolk, is undetectable in the albumen of eggs aged less than 24 h. The variability of pH values, expressed by RSD

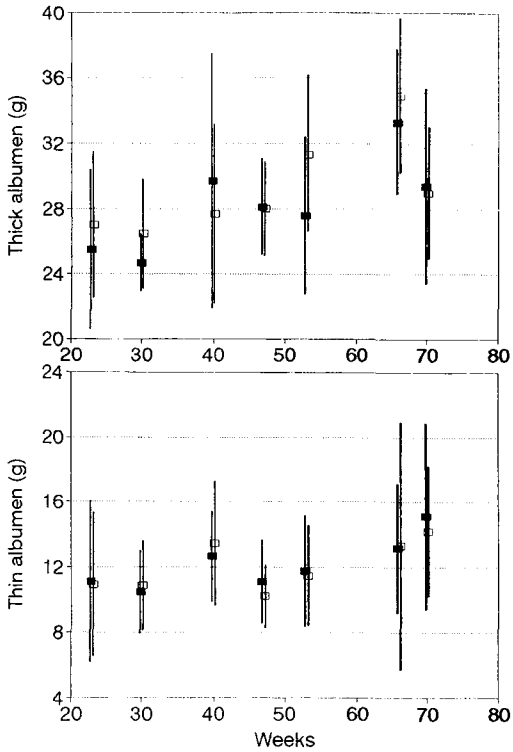

FIGURE 4. Thick and thin albumen weights as a function of hen age. Solid squares and empty squares represent the breeds Warren and Hy-Line, respectively. Vertical bars represent the variation around the mean (the mean ± one standard deviation).

for the entire laying cycle, is quite low, being close to 2 to 3%. Relatively low RSD is also obtained for glucose and uric acid. Higher RSD is observed for uridine. However some of the analytical values for this compound in yolk are close to or slightly below detection limit.

The high variability of pyroglutamic acid in yolk is explained by the characteristic trend observed for this acid during hen aging (Figure 6). The concentration of this acid dramatically increased in the middle of the laying cycle for both hen breeds and does not seem to be related with molt or feeding factors: as a matter of fact, feeding never changed during the time of trials and molt was not induced.

In conclusion, this study demonstrates that the variables analyzed had similar values and trends for the eggs laid by the two breeds. Some variables remained relatively constant during the laying cycle, such as TNA and the TKA to TNA ratio. On the basis of RSD, also pH and glucose showed low variability during the laying cycle. Other variables, such as albumen and yolk weight and yolk percentage increased with hen age. Pyroglutamic acid, which is not detected in the albumen of fresh egg, shows a characteristic trend in yolk, increasing its value in the middle of the laying cycle and then decreasing to values close to those observed in eggs of the young hens.

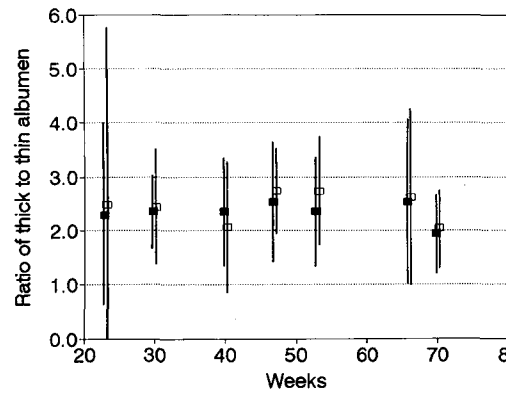

FIGURE 5. Ratio of thick to thin albumen as a function of hen age. Solid squares and empty squares represent the breeds Warren and Hy-Line, respectively. Vertical bars represent the variation around the mean (the mean ± one standard deviation).

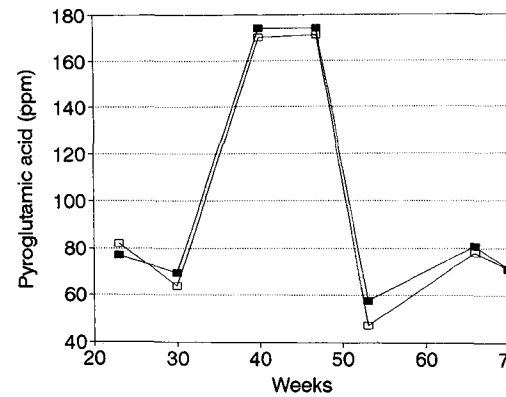

FIGURE 6. Pyroglutamic acid in yolk as a function of hen age. Solid squares and empty squares represent the breeds Warren and Hy-Line, respectively.

TABLE 4. Analytical variables measured in fresh egg samples laid by hens of the breeds Warren (W) and Hy-Line (H) and of different ages (expressed as weeks after hatch)

Age of hens	pH				Glucose				Uric acid				Pyroglutamic acid				Uridine			
	Albumen		Yolk		Albumen		Yolk		Albumen		Yolk		Albumen		Yolk		Albumen		Yolk	
	W	H	W	H	W	H	W	H	W	H	W	H	W	H	W	H	W	H	W	H
(wk)													(ppm)							
23	8.75	8.75	6.47	6.63	3,138	3,824	1,938	2,006	12.3	13.5	9.2	8.7	ND	ND	77	82	22.1	20.1	5.0	4.8
30	8.52	8.61	6.08	6.2	3,197	3,602	2,552	1,973	15.2	12.2	8.9	8.3	ND	ND	69	64	15.1	15.9	4.0	3.1
40	8.57	8.61	6.13	6.11	3,645	3,700	2,198	2,227	11.6	11.7	8.7	8.3	ND	ND	174	170	15.7	13.1	4.9	3.1
47	8.76	8.27	6.15	6.14	3,780	4,064	2,143	2,276	12.1	11.2	10.5	8.6	ND	ND	174	170	12.6	12.1	2.2	2.4
53	8.36	8.43	6.09	6.12	3,517	3,468	2,010	2,344	15.0	17.8	9.4	6.1	ND	ND	58	47	14.4	14	3.7	2.3
66	8.69	8.66	6.43	6.44	3,904	3,930	2,135	1,901	13.4	16.6	10.1	11.0	ND	ND	81	78	12.8	19.7	4.0	4.2
70	8.44	8.45	6.08	6.09	3,627	4,167	2,055	2,312	16.0	15.4	10.5	10.3	ND	ND	71	71	9.6	11.1	2.7	2.6
$\bar{x}$	8.58	8.54	6.20	6.25	3,544	3,822	2,147	2,148	13.7	14.1	9.6	8.8	. . .	. . .	101	97	14.6	15.1	3.8	3.2
RSD, %[1]	1.8	1.9	2.7	3.3	8.1	6.6	9.2	8.5	12.8	18.3	7.7	17.8	. . .	. . .	50.5	52.4	26.5	23.7	28.4	29.7
$\bar{x}^2$	8.56		6.23		3,683		2,148		13.9		9.2		. . .		99		14.9		3.5	
RSD, %[1,2]	1.8		2.9		8.0		8.5		15.3		13.7		. . .		49.4		24.2		29.1	

[1]Relative standard deviation.

[2]With reference to the combined values of the two breeds.

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