Condor 84:338–339

OBSERVATIONS ON HANDLING PROCEDURES AND COMPOSITION OF EUROPEAN STARLING EGGS

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Procedures for analyzing the biochemical composition of avian eggs differ among studies. In some cases, eggs are hard-boiled to facilitate separation of the yolk and albumen (e.g., Jones, Auk 96:407–408, 1979). When these components of fresh (rather than boiled) eggs are separated, some albumen inevitably sticks to the surfaces of the yolk and the shell membrane. Eggs also may be stored for varying periods between collection and processing. Differences in these handling procedures may affect the comparability of results among studies. Here I report observations on the effects of hard-boiling and variation in the period of storage on the composition of eggs of the European Starling (*Sturnus vulgaris*). The data were gathered incidentally to a study on intrapopulation variation in egg composition. The species was chosen because it is readily available in numbers.

Eggs were collected between zero and four days after laying in April and May 1976 from boxes at the Stroud Water Research Center near Kennett Square, Pennsylvania. The eggs were refrigerated in airtight plastic containers for between 0 and 18 days before they were processed. Seven of the refrigerated eggs, weighed at intervals of one or two days, lost an average of 6.8 ($\pm$0.24 SD) mg day^{-1}, or approximately 0.1% day^{-1}. The remaining eggs were not weighed precisely (0.0001 g) until they were processed. An analysis of variance revealed that decreases in an index of egg density (mass divided by length $\times$ breadth^2) during the periods between laying and collecting and between collecting and processing were negligible compared to variation among eggs ($F(2,114) = 0.05$, $P > 0.50$).

Ten eggs were placed in boiling water for periods of 1, 2, 4, 5, or 7 min to determine the time needed for hard-boiling. Both of the 1-min eggs and one of the 2-min eggs were soft-boiled. The 4- and 5-min eggs were hard-boiled, but in three of these four eggs the yolks and albumen did not separate easily. By 7 min, the yolk separated cleanly from the albumen, which in turn peeled easily from the shell membrane. All the hard-boiled eggs analyzed in this study were boiled for 7–10 min after being allowed to warm at room temperature for one hour. After boiling, the eggs were removed from the water and allowed to cool at room temperature. The 10 eggs in the initial boiling experiment were weighed before immersion and 45 min after removal from the water. They lost between 17 and 175 mg (initial masses 6.7–8.0 g); the losses were not correlated with the boiling period.

Fresh and hard-boiled eggs ($n = 88$ and $n = 21$, respectively) were measured (length and breadth) to the nearest 0.01 cm with dial calipers and weighed to the nearest 0.0001 g on a Sartorius analytical balance. They were then separated into shell (including associated membranes), albumen, and yolk components and placed in preweighed aluminum pans. The components were air-dried at 65°C to constant mass (<24 h). The yolks were then extracted for 24 h in each of two baths of a 5:1 mixture of petroleum ether (30–60°C b.p.) and chloroform to remove lipids, and air-dried to constant mass. In the following discussion, water content = wet mass minus dry mass; lipid content of yolks = dry mass minus nonlipid dry mass.

Because eggs were not weighed before they were boiled, comparisons between treatments were based upon an index of volume, length $\times$ breadth^2 (LB2). I determined by one-way analysis of variance that the average size of eggs in the two samples (fresh LB2 = 13.493 cm^3, boiled LB2 = 13.476 cm^3) did not differ significantly ($F[1,107] = 0.01$, $P = 0.94$, error mean square = 0.880 cm^6). The slopes of the regressions relating the log of mass (M) to the log of LB2 did not differ between the fresh and boiled samples of eggs. Because the common slope (0.977 $\pm$ 0.022 SE) did not differ significantly from 1.0, the relationship appears to be essentially linear. A similar ANCOVA relating M to LB2 revealed a common slope of 0.521 ($\pm$0.012 SE) g cm^{-3} ($F[2,106] = 982$, $R^2 = 0.949$, $P < 0.0001$). The intercepts for fresh and boiled eggs differed significantly ($F[1,106] = 91$, $P < 0.0001$) and the adjusted least squares means (7.185 and 6.914 [$\pm$0.014 SD] g, respectively) indicated that fresh and boiled eggs differed by an average of 0.27 g (3.8%).

In order to determine which components were responsible for the difference in the masses of fresh and boiled eggs, I compared the adjusted least squares means of each of the components, which were calculated in separate ANCOVAs with LB2 as the covariate (independent variable) and assuming a common slope (Table 1). The totals of the processed components (fresh = 7.1489 g and boiled = 6.8716 g) were 0.0381 and 0.0432 g (about 0.5%) less than the preprocessing masses, most likely owing to evaporation of water. Losses of mass during processing from fresh and boiled eggs did not differ significantly (t[adjusted $n = 49.7$] = 1.3, $P > 0.10$, variances unequal). From Table 1, it can be seen that the "shell" and "albumen" components of boiled eggs contained less water than did those of fresh eggs. The shell component may contain less water because boiled albumen did not stick to the shell membranes. The percent of water in whatever the shell component "loses" upon boiling (89.9%) was nearly identical to that of the albumen (89.8%). The yolks of boiled eggs contained somewhat more water than did those of fresh eggs, but this difference was balanced by a difference in ether-extractible material in such a way that masses of the yolks of fresh and boiled eggs did not differ significantly. In the egg as a whole, boiling was associated with a difference of −4.8% in total water and −5.5% in total ether-extractible material.

Eggs analyzed in fresh condition were either collected during the period of first clutches (22–28 April) and analyzed within seven days (average = 3.5 days) or they were collected from replacement clutches (3–12 May) and analyzed between 14 and 18 days later (average = 16.8 days). Hard-boiled eggs were collected on 26 April 1976 and processed after nine days of refrigeration. Because eggs subjected to the different methods of analysis were collected from different samples and received different preanalysis treatment, differences in their composition may have resulted from these factors instead. To examine possible heterogeneity among samples, I compared the composition of eggs from initial and replacement clutches (Table 2). Eggs from replacement clutches differed little from eggs laid earlier, only having relatively less shell and more nonlipid dry matter and water in the yolk. Yolks themselves did not differ significantly in size nor did the total amounts of water, lipid, and nonlipid dry matter differ between the samples.

Although it is not possible to separate the effects of time of season, preanalysis treatment, and method of analysis in this study, the results do suggest certain conclusions.

TABLE 1. Adjusted least squares means of components of fresh (n = 89) and boiled (n = 21) Starling eggs.

Component	Adjusted means (g) Fresh	Boiled	Difference (g)	Significance[a]
Shell				
Dry	0.4657	0.4446	−0.0211	***
Water	0.2931	0.1058	−0.1873	***
Total	0.7588	0.5501	−0.2087	***
Albumen				
Dry	0.5218	0.5501	+0.0283	**
Water	4.6050	4.4803	−0.1247	***
Total	5.1268	5.0303	−0.0965	***
Yolk				
Nonlipid dry	0.1828	0.1862	+0.0034	NS
Lipid	0.3771	0.3563	−0.0208	*
Water	0.7035	0.7486	+0.0451	**
Total	1.2633	1.2911	+0.0278	NS
Whole egg[b]				
Nonlipid dry	1.1703	1.1810	+0.0107	NS
Lipid	0.3771	0.3563	−0.0208	*
Water	5.6015	5.3343	−0.2672	***
Total	7.1489	6.8716	−0.2773	***

[a] Significance: NS, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[b] Sum of shell, albumen, and yolk components.

TABLE 2. Adjusted least squares means of components of fresh eggs from first clutches (n = 69) and replacement clutches (n = 20).

Component	Adjusted means (g) First[a]	Replacement[a]	Difference (g)	Significance[b]
Shell				
Dry	0.4708	0.4487	+0.0221	***
Water	0.2890	0.3075	−0.0185	NS
Total	0.7598	0.7562	+0.0036	NS
Albumen				
Dry	0.5211	0.5251	−0.0040	NS
Water	4.6191	4.5624	+0.0567	NS
Total	5.1401	5.0875	+0.0526	NS
Yolk				
Nonlipid dry	0.1801	0.1924	−0.0123	**
Lipid	0.3798	0.3680	+0.0118	NS
Water	0.6955	0.7314	−0.0359	*
Total	1.2554	1.2917	−0.0363	NS
Whole egg[c]				
Nonlipid dry	1.1719	1.1662	+0.0059	NS
Lipid	0.3798	0.3680	+0.0118	NS
Water	5.6036	5.6012	+0.0024	NS
Total	7.1553	7.1354	+0.0199	NS

[a] First clutches, 22–28 April 1976; replacement clutches, 3–12 May 1976.
[b] Significance: NS, not significant; *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$.
[c] Sum of shell, albumen, and yolk components.

Storage in closed containers in a refrigerator for periods up to two weeks does not appear to affect gross composition. Loss of mass was about 0.1% per day in Starling eggs and this loss, presumably of water, was small compared to the differences in water content among a sample of eggs treated identically (CV about 2%). In addition, eggs stored for longer periods contained no less water than those processed soon after collecting. But significant differences in the composition of the yolk of these eggs, amounting to 6.6% of the average content of nonlipid dry matter and 5.0% of the average content of water, suggest effects related either to season or egg replacement.

The differences observed between fresh and boiled eggs were substantially different from those associated with time of season and preanalysis treatment, suggesting that the differences resulted from method of analysis.

Separating the shell, albumen, and yolk of both fresh and boiled eggs resulted in a loss of about 0.5% of the initial mass. Hard-boiling, which required 7–10 min for the 7-gram Starling egg, resulted in the additional loss of about 4% of the initial mass of the egg, primarily as water from the albumen. Boiling does allow one to separate shell, yolk, and albumen more cleanly than with fresh eggs. Compared to fresh eggs, the shells of boiled eggs had 0.021 g less dry matter, and the albumen 0.28 g more, indicating that in separated fresh eggs about 4.5% of the albumen remained with the shell component. With respect to ether-extractible material, boiling may either increase the volatilization of some lipid fractions upon drying or bind some of the lipids to proteins making them difficult to extract.

I thank Carol Vleck, Paul Sotherland, Lloyd Kiff, and several anonymous reviewers for helpful comments on the manuscript. The study was supported by National Science Foundation grants GB 42661 and DEB77-27071.

Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104. Received 19 October 1981. Final acceptance 18 March 1982.

Condor 84:339–341

ESTIMATING THE INITIAL DENSITY OF BIRDS' EGGS

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Three basic physical characteristics of birds' eggs are their volume and initial mass and density at the time of laying. However, the initial values of mass and density change with incubation, because of the large loss of water vapor, and have rarely been reported. A simple field method for determining initial egg mass at any stage of incubation was recently described by Grant et al. (1982). In this study we report an equation for predicting initial egg density based upon egg dimensions published by Schönwetter (1960–1981). Our predictions of initial egg density are compared with direct measurements in 44 species.

METHODS

To derive a predictive equation for initial egg density it was first necessary to establish a common value for the density of egg content. For this purpose we collected fresh eggs in the field (Alaska, Marshall Islands, Gulf of California) and at the Zoological Garden in Buffalo, New York,