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Experimental manipulation of egg quality in chickens: influence of albumen and yolk on the size and body composition of near-term embryos in a precocial bird

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Abstract The importance of avian egg components in the determination of hatchling size and quality has yet to be fully evaluated. In the first experiment, 20% of the albumen and/or the yolk was removed from chicken eggs to determine the impact of each egg component on metabolism and various size measures in near-term embryos. Results show that metabolic rate, dry body mass, and internal organ mass are largely independent of egg composition. Removal of albumen resulted in a decrease in wet body mass corresponding to decreases in water content in the body and the yolk sac, and decreased tibiotarsus length. Removal of yolk resulted in no change in body mass, but decreases in both wet and dry yolk sac mass. In a second experiment, removal of 15% of either egg component led to reductions in hatchling mass similar to those observed in whole near-term embryos. Albumen, as the primary source of water in the egg, is the primary determinant of hatchling size and may influence hatchling success through size-related limiting factors. Differences in yolk content may influence neonatal quality as a nutritional supplement, but seem not to result in greater tissue formation during embryonic development.

Key words Birds · Egg composition · Egg size · Embryo composition · Embryo size

Introduction

The avian egg serves as a microhabitat in which the embryo develops in preparation for the next phase of its life in the external environment. The contents and composition of an egg can thus greatly influence the development of the embryo contained within, as well as its success as a hatchling. Variation in the composition of avian eggs occurs among species (Carey et al. 1980; Warham 1983; Sotherland and Rahn 1987) and within species (reviewed in Carey 1996). The differences in egg composition among species have been correlated with varying levels of hatchling precocity among taxa (Carey et al. 1980; Sotherland and Rahn 1987). Eggs from species that are more precocial at hatching tend to have a higher proportion of yolk in the egg and a corresponding lower proportion of albumen, a composition which is perhaps necessary to ensure that eggs are provisioned with enough nutrients for the prolonged incubation and development of the embryos of precocial species (Sotherland and Rahn 1987). Although the scaling of egg composition with egg mass within species varies considerably among different taxa (Carey 1996), intraspecific variation in the mass of many avian eggs is primarily attributable to variation in albumen mass (Sotherland et al. 1990; Hill 1995) and as egg mass increases, the mass of the albumen increases disproportionately whereas the proportion of yolk decreases (Romanoff and Romanoff 1949; Ricklefs 1984).

The mass and composition of an egg have considerable impact on the successful development of the embryo and may influence the subsequent survival of the neonate. Growth rate of the embryo during incubation varies in direct proportion to egg mass (Byerly 1932). Studies have also shown that with increasing egg mass (Nisbet 1978; Stokland and Amundsen 1988; Amundsen and Stokland 1990) and volume (Parsons 1970; Lundberg and Vaisanen 1979) there is an increase in hatchling mass and a corresponding increase in survivability (Nisbet 1978), although egg size may only appreciably

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impact survival during the first few days posthatching (Williams et al. 1993; Williams 1994).

Several suggestions have been made as to the relative importance of each egg component in the determination of hatchling quality. Nisbet (1978) suggested that the dry component of albumen may be the crucial factor. Albumen, which contains approximately 67% of the protein content of the egg (Romanoff and Romanoff 1949), increases in mass more acutely than does the total mass of the egg, providing more protein for assimilation into tissue during embryonic development. Nutritional enhancement during embryonic development might provide chicks from large eggs with increased success during direct competition with their siblings for food. Kear (1965) suggested that, in addition to providing nutrients to the developing embryo, the contents of the yolk sac remaining at the end of incubation might serve as a nutritional source during the first few days post-hatching. Parsons (1970) noted that a substantial portion of variation in hatchling mass can be attributed to differing amounts of yolk reserve in the hatchlings, and suggested that larger yolk reserves in chicks from larger eggs assist the survival and activity of the hatchling.

In contrast to the studies above, which have suggested that the key to variation in hatchling size and quality lies in variation in the amount of nutrients invested in the eggs, some studies have suggested that the water content of eggs may be a major determinant of hatchling size and subsequent success. Though some authors have dismissed the notion that modification of the water content of eggs by itself can lead to differential hatchling size, composition and/or performance (e.g. Arnold et al. 1991), the effects of variation in the amount of water available to the embryo on these parameters in the hatchlings have been well documented in reptiles (reviewed in Packard 1991). Moreover, several studies suggest that variation in the water content of eggs may also influence hatchling size and quality in birds. Simkiss (1980a) demonstrated that hatchling mass could be decreased with the removal of water by removing allantoic fluid. Increased water loss by evaporation has also been shown to result in decreased hatchling mass (Simkiss 1980a; Tullett and Burton 1982; Davis and Ackerman 1987). Sotherland et al. (1990) noted that intraspecific increases in hatchling mass occur at a rate similar to the increase in albumen in the egg, which is 90% water (Sotherland and Rahn 1987).

Recently developed techniques for manipulating the mass of hatchlings through the removal of egg contents have provided a means of experimentally examining the relationship between egg content and hatchling mass without restriction to natural levels of variation in relative egg content. Removal of yolk from the eggs of iguanid lizards (reviewed in Sinervo 1993) had a direct effect on the mass and locomotor performance of the hatchlings. Similar techniques were employed by Hill (1993) to modify the size of hatchling chickens via removal of albumen from the eggs. The latter study focused heavily on the removal of albumen protein as an

explanation of the observed reductions in hatchling size and performance, and essentially ignored the possibility that modification of the water content of the eggs may have also accounted for these observations, even though albumen removal would result in a greater modification of the relative water content of the egg than of the relative protein content of the egg. In the present study, we used similar techniques with avian eggs to examine the effects of egg mass and the albumen and yolk contents of the eggs on the size and composition of near-term embryos and hatchlings. We sought to determine which of two possible egg content manipulations, modification of the albumen content (primarily the water content of the egg) or modification of the yolk content (primarily the nutrient content of the egg), had a greater influence on neonatal size and quality.

Materials and methods

Near-term embryos

Fertilized leghorn chicken eggs ($n = 210$) were procured from Townline Poultry (Zeeland, Mich.) 1 day after laying. Eggs were numbered and weighed to obtain initial mass. A sample of 60 eggs representative of the total range in mass for our population was selected and used to determine egg composition. The remaining eggs were assigned randomly to one of five groups of 30 eggs each. Three groups served as test groups while the other two functioned as controls.

In order to remove the appropriate amount of yolk and/or albumen from the test eggs (see below), estimates of the amount of each component within each egg needed to be obtained. To obtain these estimates, regression equations were developed to predict the mass of each component from whole egg mass. Each of the 60 eggs used to determine egg composition was opened in such a way as to allow the albumen to flow through the fingers of the investigator and into a funnel over a pre-weighed jar. All albumen was removed from the shell and the intact yolk with the fingers, with small amounts of deionized water used to facilitate albumen removal from the shell. The intact yolk was then weighed to the nearest milligram on a Mettler PM 400 digital balance to determine wet yolk mass. The yolk, along with the shell and the albumen, was dried to a constant mass in a drying oven at 60 °C. Wet albumen mass was obtained by subtracting the sum of wet yolk mass and dry shell mass from initial egg mass. From the wet and dry mass data collected for each of these components, linear regression equations were computed to predict the amount of each component present in an egg given its initial mass (Fig. 1). Power function regressions ($y = ax^b$) were also calculated to demonstrate proportional scaling of egg component mass with initial egg mass.

The predicted mass of wet albumen and yolk in the remaining eggs was determined with the regression equations for each respective component. The predicted mass of each component (yolk and albumen) was then converted to volume using the specific densities for each component: 1.04 g/ml for albumen and 1.03 g/ml for yolk (Needham 1963). Under sterile conditions in a laminar flow hood, eggs from four of the five groups were swabbed with 95% ethanol and a hole 3–5 mm in diameter was drilled with a dental drill into the shells near the pointed end of each egg. The egg was held horizontally with the hole on the side of the egg perpendicular to the counter surface. A sterile 16-gauge hypodermic needle attached to a 10 ml syringe was inserted into the egg. From the yolk treatment group, 20% of the predicted yolk content of each egg was removed. Similarly, 20% of the albumen content of each egg was removed from eggs in the albumen treatment, and 20% of both contents were removed from the yolk + albumen treatment. In a sham-treated control group (sham), the needle was

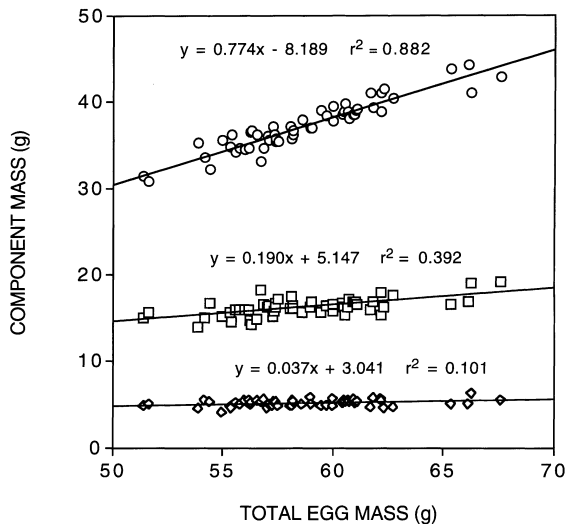


Fig. 1 Mass (g) of egg components plotted as a function of total egg mass. Sample size: $n = 60$. Circles show wet albumen mass, squares show wet yolk mass, diamonds show dry shell mass

inserted far enough to pierce the vitellin membrane, but no egg content was removed. The eggs were again swabbed with 95% ethanol and the holes sealed with wax. The last group (normal) served as an untreated control. The mass of the test eggs after treatment was estimated by subtracting the predicted mass of albumen or yolk extracted from pre-extraction egg mass. Post-extraction egg mass for the control eggs was considered to be the same as pre-extraction egg mass.

After manipulation, the eggs were placed in an incubator at $37.2 \pm 1^\circ\text{C}$. Humidity in the incubator was at a level such that eggs lost ca. 11% of their initial mass by the 20th day of incubation. Each egg was weighed every 2 days throughout the incubation period. On the 12th day of incubation all eggs that showed either no development or clear signs of mortality were removed.

In order to determine whether manipulation of egg content influenced embryonic metabolism, the rate of oxygen consumption of each egg was measured on the 18th day of incubation using an apparatus similar to that described by Tullett and Deeming (1982) and modified by Booth and Sotherland (1990). Each subject was sealed in a chamber made from a ca 1l paint can which was then lowered into a 37°C water bath. After being allowed to equilibrate for 15 min, the chamber was connected via Tygon tubing to a graduated cylinder filled with O_2 , which in turn was connected by tubing to a water reservoir. As the embryos consumed O_2 in the chamber it was replaced by O_2 from the graduated cylinder which was replaced in turn by an equivalent volume of water from the reservoir. Volumetric measurements of the amount of water accumulated in the graduated cylinder were taken at 0, 15, 30, and 45 min. Absolute metabolic rate was determined by the volume of oxygen consumed (V_{O_2}) divided by the time over which the measurements were made [$\dot{V}_{\text{O}_2} \times \text{day}^{-1}$]. Metabolic measurements were corrected to standard temperature and pressure dry.

Because it was unclear whether embryos from eggs from which such large amounts of content had been removed could successfully complete incubation and escape from the eggs (Hill 1993), the experiment was terminated on the 20th day of incubation (1 day before hatching), before embryos pipped internally. Eggs were weighed, then opened, and the embryos were removed, decapitated, and dissected. The yolk sac, heart, liver, and gizzard-stomach (with its contents removed) were removed and weighed, and the length of the tibiotarsus was measured. The heart, liver, and gizzard-stomach were then returned to the body and the yolk sac and body were each dried to a constant mass in a drying oven at 60°C .

A one-way analysis of variance (ANOVA) was conducted for different measures of egg mass to assess differences among the five

groups, with a significance level of $\alpha = 0.05$. Tukey-Kramer pairwise comparisons were conducted to examine pairwise differences between the groups where appropriate. Preliminary comparisons of measures obtained from embryos in the two control groups were conducted using unpaired Student *t*-tests. As these preliminary comparisons revealed differences in embryonic measures likely owing to the perturbation of the egg by needle insertion (see below), subsequent analyses included only the sham treatment as the control group. Embryonic oxygen consumption and various mass measures obtained from the embryos were analyzed using a two-way ANOVA which tested for the effects of yolk removal and albumen removal (and possible interaction between the two) on embryonic parameters. In situations where a significant interaction between the effects of yolk removal and albumen removal was detected, least-squares means tests were conducted to determine significant pairwise differences between treatments. Pairwise error rates were Bonferroni-adjusted to $\alpha = 0.017$ (for six such comparisons) to maintain an experiment-wide error rate of $\alpha = 0.05$.

Hatchlings

In order to verify that observed differences in day 20 embryo mass resulting from content extraction carry over to the hatchlings, we conducted similar content manipulations on a second set of eggs. In this experiment, 15% of the yolk or albumen was removed from the eggs in the two treatment groups ($n = 60$ each). A control group made up of sham-treated eggs ($n = 30$) was also included. Eggs in this experiment were incubated under the conditions above until hatching. Day of hatching was recorded, as well as the mass of the hatchling as soon as its down had dried. A one-way ANOVA was conducted for each response variable to assess differences among the three groups, with a significance level of $\alpha = 0.05$. Tukey-Kramer pairwise comparisons were conducted to examine pairwise differences between the groups where appropriate.

Results

Egg contents and extraction

Separation of fresh eggs into their three components allowed for the generation of linear regression equations with which to predict the mass of albumen, yolk, and shell for an egg given its initial mass (Fig. 1). With an increase in egg mass, the most notable increase in component mass was that of albumen, constituting approximately 77% of the increase in initial egg mass. Yolk and shell constituted roughly 19% and 4% of the initial egg mass increase respectively. The relative composition of the eggs also varied with initial egg mass, with power function regressions demonstrating a greater than proportional increase in wet albumen mass with increasing egg mass ($b = 1.23$, $r^2 = 0.880$), but less than proportional increases in wet yolk mass ($b = 0.68$, $r^2 = 0.383$) and dry shell ($b = 0.42$, $r^2 = 0.097$).

To determine whether the masses of the eggs in the five treatments were equal, a comparison of the mean initial egg mass was made (Table 1). A one-way ANOVA of these data showed no significant difference among the five groups. The predicted mass of eggs following extraction (Table 1), however, demonstrated a significant difference among the five treatments owing to the substantial reduction of mass in the test groups, with

content removals of 3.255 ± 0.195 g, 7.385 ± 0.407 g, and 10.357 ± 1.000 g (mean \pm SD) from the yolk, albumen, and yolk + albumen treatments, respectively.

Sample sizes shown in Tables 1, 2, and 5 vary among the treatments because most of the eggs in the unmanipulated (normal) and sham treatments survived to day 20, whereas a large percentage of those eggs from which albumen and/or yolk were removed did not survive. There were no noticeable microbial infections in the eggs, and those embryos that did survive to day 20 or to hatching appeared normal except for the measurable differences noted below.

Egg mass on day 20 of incubation (Table 1) was significantly lower in the test eggs than in the control eggs, reflecting a pattern among the five treatments similar to that of predicted post-extraction egg mass. A comparison of the rates of mass loss (by water evaporation) among the five treatments revealed no difference in the rate of mass lost during incubation (Table 1).

A preliminary comparison of the various embryonic measures (Table 2) between the two control groups revealed significant differences in all embryonic measures except for wet and dry yolk-sac masses (Table 3). Even with Bonferroni adjustment of the error rate for pairwise comparisons ($\alpha = 0.005$) to maintain an experiment error of $\alpha = 0.05$, six of the ten parameters measured, including metabolic rate, were significantly lower in sham embryos than in the normal embryos. This suggests that differences between normal embryos and other treatments might be confounded by the manipulation procedure on metabolism, and not the removal of egg content. The normal group was therefore removed from subsequent analyses of the effects of egg content alteration on embryo size and composition, with the sham group acting as the sole control group.

Metabolism (Table 2) was not significantly affected by removal of either yolk or albumen (Table 4).

Mass of day-20 embryos (Table 2) was significantly reduced by both albumen removal and by yolk removal (Table 4) in a manner similar to that of day-20 egg mass (Table 1). Moreover, there was a significant correlation between day-20 whole embryo mass and the mass of the eggs on day 0 following content extraction (Fig. 2), with a near isometric scaling of embryo mass with post-extraction egg mass in the combined manipulated treatments, which is similar to that observed in normal eggs.

Measures of yolk-free embryo mass and yolk-sac mass among the various treatments (Table 2) demonstrated different influences of yolk and albumen on hatchling composition (Table 4). Wet yolk-free embryo mass was significantly reduced in eggs from which albumen was removed, but was not affected by yolk removal. Dry yolk-free embryo mass was not affected by either albumen or yolk removal. Wet yolk-sac mass was significantly reduced by removal of either egg content, but dry yolk-sac mass was only reduced in treatments where yolk was removed.

Tibiotarsus length and internal organ mass (Table 2) also demonstrated different influences of yolk and albumen on body composition (Table 4). Albumen removal led to a significant reduction in tibiotarsus length, whereas yolk removal had no effect. Heart mass was greater in the yolk group than in the sham group, whereas the yolk + albumen treatment had significantly lower heart mass than the other three groups ($P < 0.017$, least-squares means test). Neither gizzard mass nor liver mass were significantly affected by content removal.

All hatchlings hatched on day 21 of incubation, with no apparent difference among the treatments with regard to hatching time. As in the case of the embryos,

Table 1 Comparison of characteristics of eggs among the five experimental groups

Parameter	Treatment ^a	Mean \pm SD	Tukey ^b	$F_{3,70}$	P
Initial egg mass (g)	Normal	59.033 \pm 2.157	–	0.541	0.656
	Sham	59.745 \pm 3.361	–		
	Albumen	58.589 \pm 2.646	–		
	Yolk	58.550 \pm 5.124	–		
	Yolk + albumen	57.107 \pm 5.209	–		
Post-extraction egg mass (g)	Normal	59.033 \pm 2.157	A	25.378	< 0.001
	Sham	59.745 \pm 3.361	A		
	Albumen	50.909 \pm 2.222	B		
	Yolk	55.197 \pm 4.924	C		
	Yolk + albumen	46.751 \pm 4.209	D		
Final egg mass (g)	Normal	51.998 \pm 2.339	A	21.415	< 0.001
	Sham	52.238 \pm 3.489	A		
	Albumen	43.819 \pm 2.707	B		
	Yolk	48.166 \pm 5.055	C		
	Yolk + albumen	39.747 \pm 4.710	D		
Rate of mass loss (g \times day ⁻¹)	Normal	0.304 \pm 0.048	–	0.992	0.402
	Sham	0.326 \pm 0.055	–		
	Albumen	0.317 \pm 0.071	–		
	Yolk	0.300 \pm 0.033	–		
	Yolk + Albumen	0.296 \pm 0.015	–		

^a Sample sizes: normal, $n = 28$; sham, $n = 25$; albumen, $n = 11$; Yolk, $n = 10$; yolk + albumen, $n = 6$

^b Groups with *like letters* are not significantly different from one another ($q < 3.728$, $P > 0.05$, Tukey-Kramer Multiple comparisons test). Post hoc tests were not performed in the absence of significant differences

Table 2 Comparison of characteristics of near-term embryos in control and content-manipulated treatment groups

Parameter	Treatment ^a	Mean \pm SD
Absolute metabolic rate (ml O ₂ \times day ⁻¹)	Normal ^b	448 \pm 42
	Sham	393 \pm 91
	Albumen	377 \pm 59
	Yolk	385 \pm 65
	Yolk + albumen	384 \pm 53
Embryo wet mass (g)	Normal	39.8 \pm 2.2
	Sham	37.6 \pm 4.3
	Albumen	31.3 \pm 2.4
	Yolk	34.0 \pm 2.9
	Yolk + albumen	28.4 \pm 3.8
Embryo yolk-free wet mass (g)	Normal	28.8 \pm 2.2
	Sham	26.3 \pm 2.8
	Albumen	22.7 \pm 3.0
	Yolk	25.7 \pm 2.6
	Yolk + albumen	21.6 \pm 2.3
Embryo yolk-free dry mass (g)	Normal	5.46 \pm 0.49
	Sham	4.84 \pm 0.61
	Albumen	4.75 \pm 0.55
	Yolk	4.78 \pm 0.56
	Yolk + albumen	4.19 \pm 0.38
Yolk-sac wet mass (g)	Normal	11.0 \pm 1.9
	Sham	11.2 \pm 2.7
	Albumen	8.60 \pm 1.6
	Yolk	8.31 \pm 1.4
	Yolk + albumen	6.81 \pm 2.3
Yolk-sac dry mass (g)	Normal	5.37 \pm 0.84
	Sham	5.30 \pm 1.27
	Albumen	4.87 \pm 1.01
	Yolk	3.92 \pm 0.70
	Yolk + albumen	3.52 \pm 1.22
Tibiotarsus length (cm)	Normal	3.33 \pm 0.12
	Sham	3.17 \pm 0.18
	Albumen	3.03 \pm 0.18
	Yolk	3.13 \pm 0.14
	Yolk + albumen	2.93 \pm 0.28
Heart mass (g)	Normal	0.177 \pm 0.017
	Sham	0.162 \pm 0.017
	Albumen	0.164 \pm 0.017
	Yolk	0.181 \pm 0.025
	Yolk + albumen	0.138 \pm 0.011
Gizzard mass (g)	Normal	1.58 \pm 0.28
	Sham	1.39 \pm 0.33
	Albumen	1.31 \pm 0.37
	Yolk	1.39 \pm 0.35
	Yolk + albumen	1.14 \pm 0.12
Liver mass (g)	Normal	0.549 \pm 0.055
	Sham	0.484 \pm 0.076
	Albumen	0.476 \pm 0.082
	Yolk	0.502 \pm 0.071
	Yolk + albumen	0.441 \pm 0.068

^a Sample sizes: normal, $n = 28$; sham, $n = 25$; albumen, $n = 11$; yolk, $n = 10$; yolk + albumen, $n = 6$

^b One data point inadvertently not recorded; $n = 27$

reduction of egg content resulted in reductions in hatchling mass reflecting the amount of egg content removed (Table 5). Hatchling mass scaled nearly proportional to post-extraction egg mass among the three treatments ($y = 0.992x^{0.928}$, $r^2 = 0.648$).

Discussion

Modifications of embryo or hatchling mass and composition by variation in egg components occur naturally in eggs of avian species. Romanoff and Romanoff (1949)

list several factors that account for the variation in egg mass. Variation exists among different breeds and among individuals of the same breed owing to factors such as parental age, heredity and seasonal changes. Some variation occurs in the mass of eggs even when produced by the same individual during the same laying period. Variation also appears in the amounts of egg components. With an increase in the mass of chicken eggs there is an increase in the absolute amount of resources available within the egg's closed environment for the embryo to use during development, yet albumen mass increases at a greater rate than yolk mass (Fig. 1).

Table 3 Comparisons of embryonic metabolism and mass measures between normal and sham control groups

Parameter	t^a	P
Absolute metabolic rate (ml O ₂ × day ⁻¹)	2.833 ^b	0.0033
Embryo wet mass (g)	2.382	0.0105
Embryo yolk-free wet mass (g)	3.634	0.0003
Embryo yolk-free dry mass (g)	4.099	< 0.0001
Yolk-sac wet mass (g)	0.315	0.3772
Yolk-sac dry mass (g)	0.888	0.1894
Tibiotarsus length (cm)	3.845	0.0002
Heart mass (g)	3.067	0.0012
Gizzard mass (g)	2.267	0.0138
Liver mass (g)	3.594	0.0007

^a $df = 51$

^b $df = 50$

These data corroborate those presented by others (Romanoff and Romanoff 1949; Nisbet 1978; Warham 1983; Rohwer 1986), who showed that with increasing egg mass there is a disproportionate increase in the amount of albumen and a decrease in the proportion of yolk. Such correlations may confound tests of the relative influence of each egg component on the mass and composition of the embryo. Our experimental procedures allowed for modification of yolk and albumen contents in a manner independent of initial egg size or composition so as to determine which egg component has greater influence on the size of the hatchlings.

The metabolism of chicken embryos increases greatly and then plateaus during the last 7 days (i.e. the last third) of incubation, corresponding to an increase in the rate of growth (Vleck et al. 1980; Vleck and Vleck 1987). Our data demonstrate that content extraction does not affect metabolic rate, supporting the assertion of Davis and Ackerman (1987) that egg content does not affect metabolism. These data also show that the sham-manipulated treatment serves as the valid control for comparisons among manipulated groups, as perturbation of the egg in this manner, for reasons yet unclear, appears to cause some reduction of metabolism, which likely accounts for differences in body measures such as wet and dry yolk-free embryo masses and tibiotarsus length between the two controls.

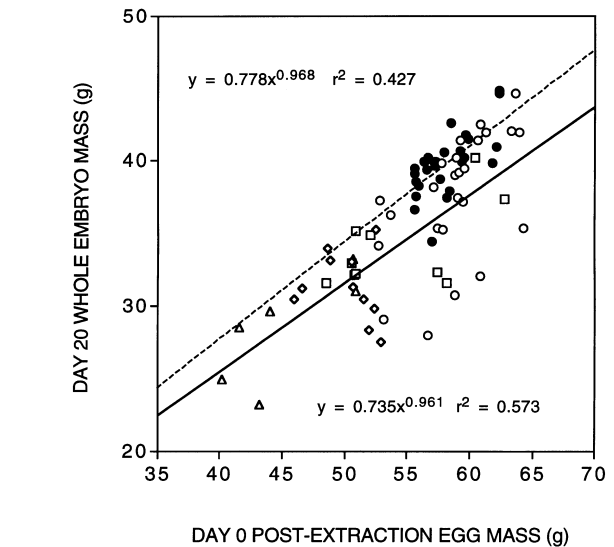


Fig. 2 Mass (g) of embryos plotted as a function of “post-extraction” egg mass (g). Filled circles show normal, open circles show sham, open squares show yolk, open diamonds show albumen, open triangles show yolk + albumen. The dashed line represents the power function regression for normal eggs, whereas the solid line represents the power function regression for the combined manipulated treatments

Manipulated treatment serves as the valid control for comparisons among manipulated groups, as perturbation of the egg in this manner, for reasons yet unclear, appears to cause some reduction of metabolism, which likely accounts for differences in body measures such as wet and dry yolk-free embryo masses and tibiotarsus length between the two controls.

Mortality was high within the experimental groups in both the day-20 embryos and in the hatchlings. Hill (1993) also encountered high mortality rates (> 80%) with the removal of 8% or more of the albumen content

Table 4 Statistical analysis of the effects of yolk and albumen removal on metabolism and various mass measures of the embryos

Parameter	Factor ^a	$F_{1,49}$	P
Absolute metabolic rate (ml O ₂ × day ⁻¹)	Yolk	0.01	0.919
	Albumen	0.25	0.616
Embryo wet mass (g)	Yolk	9.12	0.004
	Albumen	31.25	< 0.001
Embryo yolk-free wet mass (g)	Yolk	0.98	0.326
	Albumen	22.25	< 0.001
Embryo yolk-free dry mass (g)	Yolk	1.91	0.174
	Albumen	2.24	0.141
Yolk-sac wet mass (g)	Yolk	13.75	< 0.001
	Albumen	11.46	0.014
Yolk-sac dry mass (g)	Yolk	16.57	< 0.001
	Albumen	1.57	0.216
Tibiotarsus length (cm)	Yolk	1.15	0.288
	Albumen	8.16	0.006
Heart mass (g)	Yolk	8.12 ^b	0.006
	Albumen	0.09 ^b	0.776
	Yolk × albumen	15.51 ^b	< 0.001
Gizzard mass (g)	Yolk	0.40	0.529
	Albumen	1.87	0.178
Liver mass (g)	Yolk	0.00	0.978
	Albumen	1.34	0.254

^a Yolk = 20% yolk content removal; albumen = 20% albumen content removal

^b $df = 1, 48$

Table 5 Comparison of post-extraction egg mass and hatchling mass in three treatment groups

Parameter	Treatment ^a	Mean \pm SD	$F_{2,48}$ ^b	<i>P</i>
Post-extraction egg mass (g)	Sham	55.750 \pm 2.248	32.655	<0.0001
	Albumen	49.278 \pm 1.756		
	Yolk	53.586 \pm 2.149		
Hatchling mass (g)	Sham	39.119 \pm 1.663	40.566	<0.0001
	Albumen	33.884 \pm 1.128		
	Yolk	36.421 \pm 1.739		

^a Sample sizes: sham, $n = 24$; albumen, $n = 10$; yolk, $n = 17$

^b All groups are significantly different from one another ($q > 3.425$, $P < 0.05$, Tukey-Kramer Multiple comparisons test)

of the hatchlings, but higher hatching success in groups where smaller proportions of albumen were removed. Lerner and Cruden (1951) suggested that hatching success in chickens is maximized at an optimal ratio of albumen content to yolk content. However, as high mortality was observed in the yolk + albumen treatment, it appears that the mortality observed in the other two treatments is not due to an upsetting of the relative amounts of yolk and albumen present in the egg beyond natural levels. Moreover, as high levels of mortality were observed in both present experiments and by Hill (1993), it is likely that the high mortality is due directly to the removal of egg content, and not an unrelated pathology.

Mass of the embryo at day 20 of incubation and of the hatchling reflects the amount of material present in the egg at oviposition. Both embryo mass (Fig. 2) and hatchling mass scale nearly isometric to day-0 egg mass; thus, larger eggs, which contain more materials at oviposition, yield larger hatchlings.

Because the majority of the yolk sac is composed of non-cellular material, removal of the yolk sac allows a more accurate analysis of the incorporation of egg components into live embryonic tissue. The significant reduction of wet yolk-free mass but not dry yolk-free mass in the albumen treatment indicates that body mass is largely dictated by the amount of water available to the embryo and perhaps the incorporation of that water into the cells or extracellular fluid (including blood).

The remaining contents of the yolk sac at the end of incubation function as an energy reserve during the first few days posthatching (Kear 1965; Peach and Thomas 1986) and provide nutrients for the rapid growth of the digestive system (Peach and Thomas 1986). Although the yolk content reduction performed here resulted in a decrease in wet yolk-sac mass in both albumen and yolk treatments, the reduction in albumen-treated yolk sacs might reflect a decrease in the amount of water present in the yolk as shown by a comparison of dry yolk-sac masses. This suggests that embryos developing in reduced-water eggs use water present in the yolk to counteract the reduction of water in the tissue, effectively regulating water content with the resources available (Simkiss 1980b).

A noteworthy observation is that albumen contributes most to variation in egg mass (Fig. 1), as seen in many species of birds (Sotherland et al. 1990; Hill 1995), and variation in, or manipulation of, this component of

avian eggs leads to differences in hatchling mass (whole and yolk-free) as noted by Ricklefs et al. (1978). These data complement those of Sinervo (1990) and Sinervo and Huey (1990) by providing further insights into the effects of egg size and composition on hatchling size in oviparous vertebrates. An important difference, however, between avian eggs and lizard eggs is that almost all the variation in lizard initial egg mass is due to variation in yolk mass (Tracy and Snell 1985), whereas albumen plays a major role in determining egg (and probably hatchling) mass in most birds (Sotherland et al. 1990). Therefore, because water is the major component of albumen (ca. 90% in all bird eggs; Sotherland and Rahn 1987) variation in yolk-free hatchling mass of many birds will be due, to a large extent, to the water content of the hatchlings. Access to water during embryonic development also plays a major role in determining hatchling size in the more ectohydric (Tracy and Snell 1985) eggs of reptiles (Packard 1991).

Measurement of tibiotarsus length and the mass of the heart, gizzard, and liver allowed us to examine any relationship between egg composition and the size of individual organs, and to consider the impact that modifications in organ size may have on hatchling success. Leg length, here expressed as tibiotarsus length, was of particular interest because its role in movement may in turn affect phenomena such as food procurement and predator evasion for this precocial and largely cursorial bird (see Sinervo 1990; Sinervo and Huey 1990). Our data suggest a relationship between tibiotarsus length and the albumen content of the egg, through differences in protein content and/or differences in the availability of dissolved calcium for bone construction.

The masses of the three internal organs examined (heart, gizzard and liver) likewise appear to be largely independent of egg content. Alteration of egg content does not appear to affect the mass of the gizzard or liver, suggesting a high degree of regulation of water and solid ratios in the development of these organs (see Simkiss 1980a,b). One notable anomaly was observed in heart mass, where the removal of yolk seemed to increase heart mass but the removal of both yolk and albumen seemed to lower heart mass. Two embryos in the yolk treatment had particularly large heart masses, thereby elevating the mean heart mass of this group. As one would not expect yolk removal to induce ventricular hypertrophy when albumen removal (which would likely

have a greater impact on blood volume) did not lead to compensatory increases in heart mass, it is unclear why large heart masses were observed in these two individuals. Reduction of heart mass in the double-extracted treatment may have resulted from substantial decreases in protein available to the embryos.

Our results indicate that albumen, and primarily the water content of albumen, is the major determining factor of near-term embryo size and hatchling size in these precocial birds. Modification of the amount of yolk in the eggs did not influence the degree to which that yolk was assimilated into tissue, but simply modified the amount of residual yolk remaining in the yolk sac at the end of incubation. Moreover, there was little evidence that reduction of the protein content of the egg through albumen removal resulted in differential tissue formation in the hatchlings. Thus, suppositions that increased investment of solid materials into eggs is necessary for producing larger and more developed offspring (e.g., Nisbet 1978; Arnold et al. 1991; Hill 1993) seem unfounded. Almost all appreciable differences in body mass were due to differences in the water content of the animals. While the nutritional supplementation of additional yolk posthatching may certainly benefit the hatchling, increasing the nutrient content of the egg should not imply that more nutrients will be incorporated into the developing embryo during incubation.

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References

- Amundsen T, Stokland JN (1990) Egg size and parental quality influence in nestling growth in the Shag. *Auk* 107: 410–413
- Arnold TW, Alisauskas RT, Ankney CD (1991) Egg composition of American Coots in relation to habitat, year, laying date, clutch size, and supplemental feeding. *Auk* 108: 532–547
- Booth DT, Sotherland PR (1990) Oxygen consumption, air-cell gas tensions, and incubation parameters of mute swan eggs. *Physiol Zool* 64: 473–484
- Byerly TC (1932) Growth of the chick embryo in relation to its food supply. *J Exp Zool* 9: 15–44
- Carey C (1996) Female reproductive energetics. In: Carey C (ed) *Avian energetics and nutritional ecology*. Chapman and Hall, New York, pp 324–374
- Carey C, Rahn H, Parisi P (1980) Calories, water, lipid and yolk in avian eggs. *Condor* 82: 335–343
- Davis TA, Ackerman RA (1987) Effects of increased water loss on growth and water content of the chick embryo. *J Exp Zool Suppl* 1: 357–396
- Hill WL (1993) Importance of prenatal nutrition to the development of a precocial chick. *Dev Psychobiol* 26: 237–249
- Hill WL (1995) Intraspecific variation in egg composition. *Wilson Bull* 107: 382–387
- Kear J (1965) The internal food reserves of hatching mallard ducklings. *J Wildl Manage* 29: 523–528
- Lerner IM, Cruden D (1951) The heritability of egg weight: the advantages of mass selection and of early measurements. *Poult Sci* 30: 34–41
- Lundberg C, Vaisanen RA (1979) Selective correlation of egg size with chick mortality on the Black-headed Gull (*Larus ridibundus*). *Condor* 81: 146–156
- Needham J (1963) *Chemical embryology*. Hafner, New York
- Nisbet ICT (1978) Dependence of fledgling success on egg-size, parental performance and egg-composition among Common and Roseate Terns, *Sterna hirundo* and *S. dougallii*. *Ibis* 120: 207–215
- Packard GC (1991) Physiological and ecological importance of water to embryos of oviparous reptiles. In: Deeming DC, Ferguson MJW (eds) *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge University Press, New York, pp 213–228
- Parsons J (1970) Relationship between egg size and post-hatching chick mortality in the Herring Gull (*Larus argentatus*). *Nature* 228: 1221–1222
- Peach HC, Thomas VG (1986) Nutrient composition of yolk in relation to early growth of Canada Geese. *Physiol Zool* 59: 344–356
- Ricklefs RE (1984) Variation in the size and composition of eggs of the European Starling. *Condor* 86: 1–6
- Ricklefs RE, Hahn DC, Montevecchi WA (1978) The relationship between egg size and chick size in the Laughing Gull and Japanese Quail. *Auk* 95: 135–144
- Rohwer FC (1986) Composition of Blue-winged Teal eggs in relation to egg size, clutch size and time of laying. *Condor* 88: 513–519
- Romanoff AL, Romanoff AJ (1949) *The avian egg*. Wiley, New York
- Simkiss K (1980a) Eggshell porosity and the water metabolism of the chick embryo. *J Zool Lond* 192: 1–8
- Simkiss K (1980b) Water and ion fluxes inside the egg. *Am Zool* 20: 385–393
- Sinervo B (1990) The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution* 44: 279–294
- Sinervo B (1993) The effect of offspring size on physiology and life history. *Bioscience* 43: 210–218
- Sinervo B, Huey RB (1990) Allometric engineering: an experimental test of the causes of interpopulational differences in performance. *Science* 248: 1106–1109
- Sotherland PR, Rahn H (1987) On the composition of bird eggs. *Condor* 89: 48–65
- Sotherland PR, Wilson JA, Carney KM (1990) Naturally occurring allometric engineering experiments in avian eggs. *Am Zool* 30: 86A
- Stokland JN, Amundsen T (1988) Initial size hierarchy in broods of the Shag: relative significance of egg size and hatchling asynchrony. *Auk* 105: 308–315
- Tracy CR, Snell HL (1985) Interrelations among water and energy relations of reptilian eggs, embryos, and hatchlings. *Am Zool* 25: 999–1008
- Tullett SG, Burton FG (1982) Factors affecting the weight and water status of the chick at hatch. *Br Poult Sci* 23: 361–369
- Tullett SG, Deeming DC (1982) The relationship between eggshell porosity and oxygen consumption of the embryo in the domestic fowl. *Comp Biochem Physiol A* 72: 529–533
- Vleck CM, Vleck D (1987) Metabolism and energetics of avian embryos. *J Exp Zool Suppl* 1: 111–125
- Vleck CM, Vleck D, Hoyt DF (1980) Patterns of metabolism and growth in avian embryos. *Am Zool* 20: 405–416
- Warham J (1983) The composition of petrel eggs. *Condor* 85: 194–199
- Williams TD (1994) Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. *Biol Rev* 68: 35–59
- Williams TD, Lank DB, Cooke F, Rockwell RF (1993) Fitness consequences of egg-size variation in the lesser snow goose. *Oecologia* 96: 331–338

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