



Improving the Accuracy and Precision of Egg Volume Measurement and Comparing Hoyt's Equation and Troscianko's Egg Volume Estimation for Gallinaceous Bird Species

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Abstract

The recognition that egg volume variation has widespread implications in avian biology led us to test the accuracy and precision of the most commonly used egg volume determination methods. As a benchmark for the tests, we used real egg volume values determined by water submersion. We identified some evident limitations of this approach and attempted to improve the method by using distilled water and accurate temperature correction. Starting from the assumption that our methodological proposals can effectively improve the accuracy of egg volume measurements, we compared the outcomes with two widely used volume estimation methods based on Hoyt's equation and Troscianko software estimate for five gallinaceous bird species (forty eggs from each species). We found that Hoyt's and Troscianko's egg volume estimation methods strongly correlate with our volume measurements. Despite the highly significant and relatively high values of coefficients of determination, further analyses reveal some important differences among the methods.

Introduction

Former studies have provided strong evidence that larger egg volume may result in heavier chicks and ultimately an enhanced survival rate of fledglings (Reid and Boersma, 1990; Williams, 1994; Amat *et al.* 2001; Narushin *et al.* 2002). Using egg parameters, the fundamental hypotheses of life-history theory, i.e. different types of trade-offs between the allocation of energy for "fitness-maximizing," can be tested. Egg size may vary with laying order; within a clutch; and between first and replacement clutches. As egg size correlates with hatching success, chick growth rate, and fledging success; the parental quality can be expressed as the relative size of produced eggs (Reid and Boersma, 1990; Williams, 1994; Amat *et al.* 2001; Williams, 2001; Fernández and Reboreda, 2008; Dolenc, 2016). Moreover, strategies in poultry breeding programs aim to increase egg volumes, feed efficiency, growth rate, and body weight, but to

decrease abdominal fat and production costs (Mohammadabadi *et al.*, 2010; Mohammadifar *et al.*, 2013; Mohammadifar and Mohammadabadi, 2017).

Due to the wide applicability of egg size data, methodological aspects related to egg dimension are undeniably important. Most egg volume determination methods rely on immersing an egg in water and measuring the volume or weight of the displaced water (Hoyt, 1976; Alberico, 1995; Kern and Cowie, 1996; Rush *et al.* 2009; Boersma and Rebstock, 2010). Previous concerns about the negative impact of submerging the eggs into the water have not been confirmed when the eggs had more developed embryos (Alberico, 1995; Rush *et al.* 2009). A newer approach incorporates comparing egg weight in the air with egg weight submerged in water (Troscianko, 2014).

Some basic and axiomatic rules were discovered during the first egg volume estimation attempts. Rahn and Ar (1974) stated that bird eggs begin to lose

weight as soon as they are laid, and Hoyt (1979) mentioned that the volume and linear dimensions of eggs do not change during incubation. Therefore, in contrast to mass, the most reliable parameters to use as reference variables in breeding biology studies seem to be linear dimensions and volume (Ruiz *et al.* 1992). The linear dimensions used for volume and fresh weight estimations were egg length and breadth (Preston, 1968; Hoyt, 1976; 1979). Hoyt (1979) found that the volumes of most bird eggs can be determined within a 2% error margin using length, maximum breadth, and a specific shape index (K_V) of 0.51. The relationship known as Hoyt's (1979) equation was the following: $V = 0.51LB^2$; where V = volume, 0.51 = the shape index (K_V), L = the maximum length and B = the maximum breadth. The egg shape index or volume coefficient developed by Hoyt (1979) derives from the following relationship: $K_V = V/LB^2$; where V = the egg's measured volume.

Nevertheless, problems related to the inaccuracy of the volume determination methods, the inherent field use difficulties, and concerns about the negative effects of these methods on embryos (Hoyt, 1979; Rush *et al.* 2009) necessitate further refinements leading to better field practicability while maintaining accuracy and precision of present methods. The 2% error level is too high in some cases. Moreover, the universal index of 0.51 is not precise enough (Rush *et al.* 2009), and the species-specific indexes cannot be used reliably beyond the original test populations (Troscianko, 2014). Fortunately, the volume coefficients can be recalculated for every tested species and population.

Another divergent approach in egg volume estimation methodologies is digital photography and automated or analytical image analysis (Hoyt, 1976; Mänd *et al.* 1986; Mónus and Barta, 2005; Bridge *et al.* 2007; Severa *et al.* 2013; Troscianko, 2014). Using digital photography to calculate egg metrics has several advantages. Digital cameras are cheap, widely available, and easy to use. Digital photography also shortens the handling time thereby reducing the risk of egg damage. Nevertheless, it is not without disadvantages. Image thresholding, camera distance and angle controlling, standardization of background contrast and lighting, further user programming, image software processing or model fitting, and a large number of egg width measurements are the most prominent disadvantages of this method (Mónus and Barta, 2005; Bridge *et al.* 2007; Troscianko, 2014). In addition, methods based on digital photography are time-consuming. Despite its aforementioned disadvantages, digital photography can be useful for volume estimations when eggs are not fresh and more difficult to handle them with water submersion.

From the perspectives of poultry science in general and the wide applicability of egg volumes as

reference data in avian studies, we attempted to improve the direct measurement of egg volumes in 5 captive-bred gallinaceous bird species., then compared the accuracy and precision of such method with two generally used egg volume estimation methods, the Hoyt's (1979) equation and the Troscianko's software estimations based on digital photography,

Materials and methods

The eggs

A total of 200 freshly laid eggs from five captive-bred bird species (Grey partridge – *Perdix perdix* L.; Japanese quail – *Coturnix japonica*; Common pheasant – *Phasianus colchicus*; Guinea fowl - *Numida meleagris*; and the domestic hen – *Gallus gallus domesticus*) were collected in Sopron city, Hungary.

Linear dimensions and volume measurements

Linear dimensions, such as the greatest length and width of eggs, were measured in millimeters by digital calipers within two digits of accuracy. For the egg volume measurements, we used a system consisting of two sub-components. The first component was an Orma BC 250 precision balance (Orma S.R.L., Italy), with 500 g capacity, 110 mm pan size, 0.001 g resolution, ± 0.002 g linearity, ≤ 2 seconds response time, and, external calibration. The second sub-component was a liquid density meter. Before every measuring session, calibration of the balance together with the liquid density meter was performed using 100 and 200-gram weights.

Instead of measuring the volume by water displacement (Hoyt, 1976; Alberico, 1995; Kern and Cowie, 1996; Rush *et al.* 2009; Boersma and Rebstock, 2010), the comparisons of egg weight in the air was compared with egg weight submerged in water as described by Troscianko (2014). One obvious concern about Troscianko's egg volume measurement method relates to the physical properties of the water used. As rediscovered by green chemistry, water is one of the best and most abundant solvents, but in nature, it possesses impurities in every available form (Sheldon, 2005; Wilk, 2006; Hailes, 2007). However, this error was eliminated by using distilled water to measure egg volume. This way we also sought to alleviate the controversies of unspecified water sources (Hoyt, 1976; Kern and Cowie, 1996; Boersma and Rebstock, 2010) and those of similarly unclear fresh water (Rush *et al.* 2009).

The temperature-dependent density of the water was the second concern. In order to tackle this issue, all volume measurements were done in an air-conditioned laboratory with the temperature set at 20 °C. Since the standard density of water at 20 °C is 0.9982063 g /

1cm³ (Lide, 2006), we used this coefficient for temperature corrections.

Calculations based on measured egg parameters

Newly developed egg volume estimations are regularly tested against Hoyt's (1979) method (Bridge et al. 2007; Rush et al. 2009; Troscianko, 2014). Therefore, we also performed the calculations of Hoyt's (1979) volume coefficients and the shape indexes (K_v) of the five sets of eggs from the different species. Considering the species and population specific shape indexes, egg volume was estimated using the following relationship: $K_v = V/LB^2$; where V = measured egg volume, L = the maximum length, and B = the maximum breadth. The population-specific shape indexes were calculated as average values for the whole samples.

Digital photography and software estimations

Troscianko's (2014) method was used to estimate egg volume. An EOS 1000D digital camera with an 18 – 55 mm EFS lens (Canon Inc, Japan) was used to photograph the eggs. The camera was fixed to a stand at an angle of 90° to the eggs' long axis, and an artificial light source was used. The camera-to-object distance was 40 cm. To standardize the contrast, the eggs were laid on dark background. We also used a ruler in the images as a scale bar; however, to ensure higher accuracy, the known (measured) lengths of the eggs were used in the calculations. 14 – 18 anchor points, the rule being the quality of the fit between the software calculated shape and real eggshell

contour. During anchor point fittings, the tip and the base of eggs were precisely selected.

Statistical analyses

Comparisons of different egg volume estimation methods and those between measured and estimated values were tested with linear regression analyses between pairs of values mentioned in Table 2 using the method of least squares. Coefficients of determination (R-squared) were calculated to quantify the proportion of total variation of outcomes explained by the fitted linear trend equations. For statistical individual comparison of egg volume measurement and estimation outcomes, T-test was used for dependent samples. Aiming to find explanations for different outcomes of volume measurements and estimations, the coefficients of variation (%) for measured and estimated egg shape parameters were compared in the studied bird species. All variables were checked for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Statistical significance for R-squared tests (analysis of variance – ANOVA) was inferred at $\alpha = 0.01$, while for Student's T-test tests, $\alpha = 0.05$ was inferred. Statistical analyses were carried out using STATISTICA software version 13.1 (Dell Inc., Tusla, USA).

Results

We found Hoyt's shape indexes (K_v) for the test species as presented in Table 1.

Table 1. Descriptive statistics of Hoyt's shape indexes (K_v) for the test species

Variables	n	Mean	Minimum	Maximum	Std.Dev.
K _v quail	40	0.5101	0.4989	0.5262	0.0062
K _v guinea fowl	40	0.5036	0.4894	0.5178	0.0078
K _v pheasant	40	0.5038	0.4906	0.5313	0.0072
K _v hen	40	0.5143	0.4924	0.5271	0.0066
K _v partridge	40	0.5023	0.4903	0.5111	0.0047

Using calculated Hoyt's shape indexes and Troscianko's egg shape modeling software, we performed basic comparisons among the results of

egg volume estimation methods and the egg volume measurement (Table 2).

Table 2. R-squared values of correlations between results of egg volume measurement and different estimation methods

Variables	Quail	Guinea fowl	Pheasant	Hen	Partridge
Measured vs. software	0.9935*	0.9943	0.9857	0.9950	0.9963
Measured vs. Hoyt's K _v	0.9892	0.9713	0.9578	0.9739	0.9933
Software vs. Hoyt's K _v	0.9859	0.9694	0.9730	0.9741	0.9868

* All R-squared values are significant at $\alpha = 0.01$ level.

Despite the highly significant and relatively high values of coefficients of determination (Table 2), further analyses revealed some important differences among the methods (Tables 3-5). First, comparisons among the volume measurement and the two estimation methods led us to conclude that Troscianko's software generally overestimates the

egg volumes. Hoyt's estimates based on species-specific indexes of test populations also overestimate the volumes except for the partridge eggs, which were underestimated by Troscianko's software.

In addition, results comparisons between Troscianko's software estimations and effective volume measurements show similar patterns to those between

Hoyt's equation and Troscianko's software estimations (Table 3).

Table 3. Differences in percent between measured and estimated average egg volumes

Variables	Quail	Guinea fowl	Pheasant	Hen	Partridge
Measured vs. software	-0.378%*	-0.846%	-0.862%	-0.752%	0.898%
Measured vs. Hoyt's KV	-0.015%	-0.029%	-0.011%	-0.028%	-0.003%
Software vs. Hoyt's KV	0.364%	0.824%	0.859%	0.729%	-0.893%

* average value with the first method against the average value of the second method

The outcome of Troscianko's software estimation method acts similarly against effective volume measurement and Hoyt's equation as well, regardless of the studied species.

Our results indicated that Troscianko's software overestimated the volumes by 0.38% - 0.86% against actual volume measurements, and by 0.36% - 0.86% against Hoyt's estimates (Table 3). Both tested

methods underestimated partridge egg volumes by about 0.90%. The results of Hoyt's method in comparison with those of volume measurements also suggest a slight overestimation, with minute differences (0.003 - 0.029%). However, the overall relatively low differences (below 1%) cannot be neglected because most are statistically significant (Table 4).

Table 4. Comparisons of the outcome of egg volume measurement and different volume estimate methods (T-test for dependent samples)

Species	Mean var. 1 (cc)	Mean var. 2 (cc)	T-value	df	P-value	CI -95.00%	CI +95.00%
Measured (var. 1) vs. Troscianko's software (var. 2)							
Quail	12.4899	12.5373	-2.3577	39	0.02350	-0.0881	-0.0067
Guinea fowl	34.7309	35.0274	-8.1398	39	<0.00001	-0.3701	-0.2228
Pheasant	27.6213	27.8616	-6.3718	39	<0.00001	-0.3165	-0.1640
Hen	55.5160	55.9365	-8.2289	39	<0.00001	-0.5238	-0.3171
Partridge	12.5078	12.3965	7.9934	39	<0.00001	0.0832	0.1396
Measured (var. 1) vs. Hoyt's equation (var. 2)							
Quail	12.4899	12.4918	-0.0755	39	0.94018	-0.0520	0.0482
Guinea fowl	34.7309	34.7411	-0.1230	39	0.90275	-0.1776	0.1573
Pheasant	27.6213	27.6243	-0.0461	39	0.96343	-0.1341	0.1282
Hen	55.5160	55.5315	-0.1348	39	0.89347	-0.2478	0.2168
Partridge	12.5078	12.5082	-0.0191	39	0.98483	-0.0388	0.0381
Troscianko's software (var. 1) vs. Hoyt's equation (var. 2)							
Quail	12.5373	12.4918	1.58529	39	0.12098	-0.0126	0.1036
Guinea fowl	35.0274	34.7411	3.35394	39	0.00178	0.1136	0.4589
Pheasant	27.8616	27.6243	4.56638	39	0.00005	0.1322	0.3423
Hen	55.9365	55.5315	3.54125	39	0.00105	0.1737	0.6363
Partridge	12.3965	12.5082	-4.21448	39	0.00014	-0.1654	-0.0581

Bold indicates the statistical significance at $\alpha=0.05$. cc: cubic centimeter, df: degree of freedom, CI \pm 95.00%: confidence intervals on the mean, p-value: levels of statistical significance between egg volumes defined by different methods

Significant differences between egg volume measurement and Troscianko's software estimations as well as between the tested egg volume estimation methods were noted. However, there was an exception in the case of quail. In this species, there were no significant differences between the two volume estimation methods ($t = 1.58529$, $df = 39$, $P = 0.12098$).

Another important finding of our comparative analysis was that there were no significant differences between the measured and estimated egg volumes using Hoyt's equation based on species-specific indexes of test populations in any of the studied species.

The coefficients of variation for the measured and the software-calculated shape variables ranged from 0.94% to 1.54%, while for the length, breadth, and egg index it ranged from 2.64% to 4.89%, respectively. The variation of volume results for measurement and estimation methods ranged between 7.16% and 12.17% (Table 5).

Egg volume measurement results showed the lowest variations, while Hoyt's volume estimates showed the highest. The coefficients of variations of Troscianko's software estimates showed medium values on most species.

Table 5. Coefficients of variation (%) for measured and estimated shape parameters of eggs of studied bird species

Variable	Quail	Guinea fowl	Pheasant	Hen	Partridge
Length (mm) (L)	4.81260	4.26824	3.39138	3.60096	4.56720
Breadth (mm) (B)	4.05397	2.98084	2.64558	3.56573	4.22838
Egg index (BxL/100)	3.26594	3.65937	3.58105	4.89066	2.84514
Volume (cc) measured	11.93368	8.67376	7.17258	7.91030	11.52244
Volume (cc) software	12.17062	8.68134	7.16555	7.99894	11.63944
Shape variable measured (K _v)	1.21028	1.54213	1.42862	1.28475	0.94138
Shape variable from values calculated by the software	1.40026	1.53968	1.14376	1.28713	1.40082
Volume with an average shape index	12.03685	8.88733	7.19922	8.08743	11.65543
Surface (cm ²) by software	8.15393	5.90769	5.19404	5.22387	8.05835

Discussion

The liquid density measurement equipment proved to be appropriate for egg volume measurements with their limitations being restricted only to fluid properties. We solved these limitations by using distilled water and temperature-related density correction. In addition to increased accuracy and precision, the use of this equipment requires a shorter handling time compared to water displacement methods (Rush *et al.* 2009), or methods based on digital photography (Troscianko, 2014). The lack of limitations, the high level of precision, and the short time required for measurements make our method especially suitable for the determination of egg volumes under real conditions.

From the available egg volume estimation methods, we tested the precision of Hoyt’s equation (Hoyt, 1979) and that of Troscianko’s software (Troscianko, 2014). We found highly significant ($P < 0.01$ at $\alpha=0.01$) coefficients of determination ($r^2 = 0.9578 - 0.9963$), which suggests that both methods provide similar sets of values for egg volumes. This indicates that the ranking or other volume-based classification of eggs of a gallinaceous bird species is possible with any of the tested methods because of the strong relationship between the measured and estimated volumes. Similarly strong relationships ($r^2 = 0.85$; $n = 346$) were previously found by Kern and Cowie (1996) on Pied Flycatcher (*Ficedula hypoleuca*) in egg volumes based on the water displacement and Hoyt’s estimates performed with an arbitrarily selected shape index of 0.500.

Other studies involving bird egg volume determinations focused on the difference in outcome instead of on the strength of the relationship between measured and calculated values (Rush *et al.* 2009; Troscianko, 2014) as neither the outcomes of Troscianko’s photograph-based volume estimates nor Hoyt-based volume estimates (using population-specific shape coefficients) differed significantly from real egg volumes. Therefore, comparison of methods could only be made based on precision, and Troscianko’s software and Rush *et al.*’s water displacement methods seem to be more precise than Hoyt’s estimates. Nevertheless, we should mention

that Rush *et al.* (2009) performed the Hoyt estimates with the universal 0.51 (Hoyt, 1979), and with an arbitrarily selected 0.49 shape index. In addition, none of the egg volume measurements considered the effect of the temperature and the impurities-dependent density of water.

The significant differences in our study between real egg volumes and those of Hoyt’s estimates could derive from methodological issues. It is possible that the significant differences lay within the errors of the new testing method when the accuracy of some models was tested in comparison with the outcomes of Hoyt’s estimates (Bridge *et al.* 2007; Rush *et al.* 2009), but these errors could be inherent in the methodologies of Hoyt’s estimates as well. The latter presumption seems to be plausible because Bridge *et al.* (2007) used a shape coefficient of another species (*Turdus migratorius* for estimating the volume of *Aphelocoma coerulescens*) established in another previous study (Hoyt, 1979), while Rush *et al.* (2009) used universal and arbitrary selected shape indexes, as mentioned above. Our study shows partially similar results in that we found no significant differences between real egg volumes and the outcomes of Hoyt’s estimates. Yet, Troscianko’s estimates differed significantly from measured egg volumes in all studied species and from the outcome of Hoyt’s estimates except for quail eggs.

In general, Hoyt’s method overestimates real egg volume (Székely *et al.* 1994; Kern and Cowie, 1996; Rush *et al.* 2009; present study), but in case of significant differences, there are methodological concerns (sample size, shape indexes selected independently to original test populations, etc.). It is also plausible that Hoyt’s (1979) equation is less robust at the extreme ends of the shape index range (Bridge *et al.* 2007), but not in the gallinaceous bird species studied. With an elongation (Length / Breadth ratio) of between 1.27 and 1.32, none of the eggs of the species studied can be considered unusually round or pointed.

Relatively small (below 5%) variations in the studied species’ egg shape parameters led to significant intraspecific differences in measured and estimated volumes. Similarly, small variations in

length, breadth, and egg shape index were found also by Mónus and Barta (2005) on Tree Sparrow (*Passer montanus*); Amat *et al.* (2001) on Kentish Plover (*Charadrius alexandrinus*), and by Boersma and Rebstock (2010) on Magellanic Penguin eggs (*Spheniscus magellanicus*). Despite egg-laying order-dependent volume differences, and regardless of the gallinaceous bird species studied, it seems that the intraspecific variances are small but statistically significant. Due to its lack of limitations, our fresh egg volume measurement method seems to be the most appropriate to accurately measure those variances (at least on gallinaceous bird species). If we are interested only in egg volume via easy measurements in the field, we can agree with Székely *et al.* (1994) that “it is more accurate to measure volume than to estimate it from linear measurements”, as cited by Kern and Cowie (1996). Nevertheless, we found a strong relationship between the outcomes of our improved egg volume measurements and Hoyt’s estimates based on population-specific shape indexes. Moreover, the differences between measurements and estimations are statistically irrelevant ($P = 0.893 - 0.984$; $\alpha = 0.05$; 5 species; $n = 200$). Therefore, Hoyt’s estimates could be extremely useful and provide some advantages for measurements, such as data about the shape variations and effectiveness in volume estimations of older eggs that have positive buoyancy and do not sink in water. In these latter cases, risks associated with accidental breakage during handling arise, both in eggs with developed embryos and egg collections. In this context, methods based on digital photography could be more feasible, despite their lower accuracy. However, in these cases, the sample sizes are much smaller than in artificial hatchings at an industrial level, so disadvantages such as technical or time concerns are not as important. Moreover, digital methods like Troscianko’s are the most practical in cases requiring photographic archives.

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Conflicts of Interest

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