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Short Communication

A preliminary study on the application of image analysis for the measurement of ostrich eggshell traits

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Abstract

Embryonic mortality in developing ostrich embryos has been linked to water loss in eggs during artificial incubation. Traits possibly related to water loss were assessed on shell samples obtained from 512 hatched ostrich eggs. Moderate to high repeatability estimates (0.43 to 0.86) were derived from significant between female variance components for all traits. Two-trait between female correlations were unity or near unity when the same trait was assessed at either the side or the top of individual eggshells. Recordings at either site can thus be considered as the same on the level of the individual female, implying that in future studies only one measurement is needed during the evaluation of the traits investigated in this study.

Keywords: Artificial incubation, pore number, pore size, repeatability, shell thickness, water loss

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Hatchability of ostrich eggs is poor when compared to that obtained for domestic poultry. In general, approximately 50% of ostrich eggs that are set, hatch (Deeming & Ar, 1999; Bunter & Cloete, 2004), while chicken eggs achieve a hatchability of 80% and higher (Tullet, 1995). Research on ostrich hatchability encompasses a wide variety of topics, i.e. the microclimate in ostrich nests (Swart *et al.*, 1987; Swart & Rahn, 1988), disinfectant protocols (Van Schalkwyk *et al.*, 1998), pre-incubation treatment (Van Schalkwyk *et al.*, 1999a), temperature and spatial position in the incubator (Van Schalkwyk *et al.*, 1999b) and egg turning (Van Schalkwyk *et al.*, 2000). Water loss during artificial incubation is a contributing factor to the hatchability of ostrich eggs (Blood *et al.*, 1998). Eggshell structure is related to evaporative water loss by Fick's law of diffusion (Paganelli, 1980). Fick's law states that mass flow of a given fluid through a permeable barrier is directly proportional to the area available to diffusion and the concentration gradient of the fluid across the barrier. In contrast, mass flow rate is indirectly proportional to the thickness of the barrier. For application to the conductance of water across eggshells, some reasonable assumptions have to be made, namely: 1) Diffusion of water vapour only takes place through the pores in the shell, allowing the area available for diffusion to be considered as the total pore area of the shell; 2) Respiratory gas exchanges of O_2 and CO_2 play an insignificantly small role in the weight loss of the egg, leaving water vapour as the only contributing element; 3) The concentration difference across the shell is accurately described by the difference in water vapour pressure. The rate of mass flow out of the egg can thus be expressed in Equation 1, slightly adapted from Ar *et al*. (1974):

$$M_{H_2O} = D_{H_2O} \cdot \frac{A_p}{L} \cdot c \cdot \Delta P \qquad [g/s] \qquad \text{Equation 1}$$

In this equation, D_{H_2O} is the temperature dependent diffusion coefficient of water; A_p the total pore area; and L shell thickness. A conversion factor c is needed to transform concentration difference to vapour pressure difference. The vapour pressure difference across the shell is denoted by ΔP. It should be possible to calculate the rate of mass transfer from the egg having acquired measured values of A_p, L and ΔP.

The objectives of this study were to automate the acquisition of values for A_p and L (thereby enabling the processing of a higher number of eggs) and to determine if these values are repeatable for individual hens.

A total of 132 breeding pairs from the Oudtshoorn Experimental Farm produced the eggs used for this experiment during November–December 1997, as well as during January 1998. The management and husbandry of the birds were described by Van Schalkwyk *et al*. (2000) and Bunter & Cloete (2004). Eggs were identified by the date and paddock of origin. A maximum of six days storage at 17 ºC and 75% relative humidity was followed by incubation at 36 ºC and 28% relative humidity. Methods for collection, sanitation, storage and incubation are well documented (Van Schalkwyk *et al*., 1999a; b; 2000). The software package, AnalySIS® (Soft Imaging System, 1999), was used to perform image analysis on the fractured shells from hatched eggs. Images were obtained by a specialized video camera with a photographic lens under strong lighting. Small representative samples of the eggshells (exceeding 2 cm^2 in size) were used. The outside eggshell surfaces were dyed with standard grade blue food colorant (Gonzalez *et al*., 1999; Sahan *et al*., 2003; Amer, 2005), rendering the pore clusters on the outside of the shell discernable. A random 2 cm^2 area from the side (taken in an area near the equator) as well as from the top of each egg (situated over the air-cell) was considered. A total of 512 eggs was processed over a 4-week period. From these images, the software was used to calculate average pore diameter, the number of pores and pore area (A_p). Shell thickness (L) was measured after the inner membranes were removed, using a digital calliper accurate to 10 µm. These recordings were used to derive the permeability of the barrier (defined as the ratio of pore area relative to shell thickness) as described in Equation 1.

Data were analysed by a repeatability model, using ASREML (Gilmour *et al*., 1999). The software allows for the fitting of various random effects to animal breeding data, while also enabling the prediction of least square means for desired fixed effects. In initial one-trait analyses, the identity of the female producing the eggs was fitted as a random effect, while female age, month of lay and the gender of the hatched chick were fitted as fixed effects. Interactions between fixed effects were not significant, and were excluded from final analyses. Repeatability of eggshell traits was derived from the calculated variance components (Turner & Young, 1969). The one-trait analyses were followed by two-trait analyses, where a specific trait measured on the side of the eggs was analysed with the same trait measured on the top. These analyses enabled the estimation of between female, environmental and phenotypic correlations between traits measured on the side or the top of eggs. Climate statistics on the Oudtshoorn Experimental Farm were also related to differences in monthly shell traits. These data were assessed by one-way analysis of variance procedures (Snedecor & Cochran, 1967).

The fact that only eggs that hatched were assessed could be seen as a limitation. No consensus exists in the literature on whether shell traits differ between hatched and non-hatched eggs (Gonzalez *et al*., 1999; Sahan *et al*., 2003; Amer, 2005), although previous studies included fewer than 100 eggs. Since the main object of this preliminary investigation was to determine whether eggshell traits derived from image analysis were repeatable, the fact that only hatched eggs were studied is not seen as a constraint. Since all eggs with fragments hatched, it was impossible to obtain a relationship of shell deaths with water loss. Water loss was thus not considered.

The observed trait distributions did not deviate markedly from normality (Table 1). Positive kurtosis values for all traits, especially pore area, indicated a steeper than normal spread. Significant deviations from normality could be ascribed to kurtosis rather than skewness. Interpretation of results was thus continued (Glass *et al*., 1972). Coefficients of variation (CV) were reasonably high, i.e. more than 10% for most traits. The only trait with a narrow range was shell thickness, with CV's of below 10%. The number of pores per cm^2 accorded with means of 17.7 pores per cm^2 to 20.2 pores per cm^2 reported by Christensen *et al*. (1996) for seven ostrich eggs. However, the means for pore number from the present study were slightly higher than most reported previously (Gonzalez *et al*., 1999; Sahan *et al*., 2003; Amer, 2005). The differences between the present study and previous results are ascribed to the fact that previous studies focused on what was termed as the large pores, while it was possible to consider all pores present in the current study. Average eggshell thickness accorded with values of 1.70 mm to 1.94 mm (Christensen *et al*., 1996; Gonzalez *et al*., 1999; Sahan *et al*., 2003; Amer, 2005).

Table 1 Descriptive statistics for the different shell traits measured on the side or top of individual eggs

Location and trait	Mean ± s.d.	CV	Skewness	Kurtosis	Minimum	Maximum
Side:						
Pore count (pores/cm^2)	22.0 ± 6.1	27.6	0.77	1.43	7.0	47.0
Average pore diameter (mm)	0.59 ± 0.10	16.9	0.74	2.28	0.19	1.05
Pore area (mm^2) (A_p)	9.84 ± 3.80	38.6	1.26	4.08	3.03	34.8
Thickness (mm) (L)	1.83 ± 0.13	7.1	-0.65	1.41	1.28	2.12
Pore area/thickness (mm²/mm)	5.37 ± 2.06	38.4	1.37	4.30	1.67	18.7
Top:						
Pore count (pores/cm^2)	21.7 ± 6.5	30.2	0.74	1.23	5.0	49.0
Average pore diameter (mm)	0.58 ± 0.10	17.2	0.81	1.43	0.38	1.00
Pore area (mm^2) (A_p)	9.63 ± 3.97	41.2	1.34	3.33	1.87	32.2
Thickness (mm) (L)	1.92 ± 0.13	6.8	-0.88	2.22	1.31	2.21
Pore area/thickness (mm²/mm)	5.01 ± 2.01	40.1	1.49	3.65	1.29	16.1

Table 2 Means (± s.e.) for average ambient temperature and water evaporation rate, as measured according to standard procedures using evaporation pans, during the different months of the study

Period	Average temperature (ºC)	Evaporation rate
November	20.7 ± 0.4^a	7.0 ± 0.7
December	22.2 ± 0.4^b	7.8 ± 0.7
January	23.3 ± 0.4^c	7.6 ± 0.7

^{a,b,c} - Means with different superscripts differ in columns ($P < 0.05$)

Shell traits were independent of female age, the gender of the hatched chick and interactions ($P > 0.05$). Month of lay influenced pore area, shell thickness as well as pore area per unit shell thickness ($P < 0.05$) in fragments obtained from the side or the top of individual eggs. Average ambient temperature was higher during December and increased further in January, compared to that in November ($P < 0.05$; Table 2). A corresponding trend was observed in evaporation rate, although seasonal differences failed to reach statistical significance.

The trend for eggshell structure mirrored that of evaporation rate (Figure 1), suggesting that shell structure becomes more resistant to diffusive water transfer during months having climates more conducive to water loss, as reflected by the evaporation rate. It can thus be hypothesized that the characteristics of the eggshell compensate for climatic conditions to ensure a relatively constant rate of water loss. However, this outcome is based on data of only a few months, and cannot be regarded as conclusive. Further research is required to determine whether such trends are robust within seasons and across years.

Between female variance components were significant for all traits (Table 3), leading to medium to high repeatability estimates (Turner & Young, 1969). A measure of correspondence in shell structure between eggs of the same female is thus evident. No corresponding results were found in the literature, but Wilson (1996) suggested that the marked variation in shell porosity implies genetic variation in ostriches. The observed medium to high repeatability coefficients between hens is consistent with this assessment, but the data structure did not allow the partitioning of genetic and permanent environmental effects in the present study. Research on a broader sample of hatched and non-hatched shells across production years is presently being conducted.

The relationships between the same traits measured on the top and the side of the same egg were partitioned in the between female, environmental (residual) and phenotypic components in two-trait analyses. The between female correlations approached or reached unity for all traits considered, ranging from 0.97 ± 0.01 for shell thickness to 1.00 ± 0.01 for average pore diameter, total pore area and pore area/thickness. This indicates that, on the level of the individual female, measurements made on poth site are essentially the same trait. Therefore, in future studies measurements can be taken only at one of these sites, and thus facilitating the assessment of a larger number of eggs. Derived environmental correlations were substantially lower, ranging from 0.34 ± 0.04 for average pore diameter to 0.66 ± 0.03 for shell thickness. The corresponding estimates for phenotypic correlations ranged from 0.65 ± 0.04 for pore number to 0.88 ± 0.02 for shell thickness. These results suggest that variation does occur on the level of the individual eggs analysed.

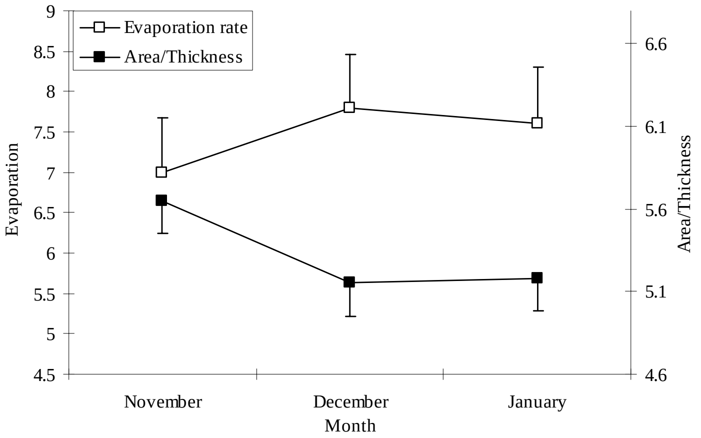

Figure 1 Trends for pore area/shell thickness and the evaporation rate induced by the climate as derived from the available data. The vertical bars on or below derived means represent standard errors

Table 3 Variance components and ratios for the respective shell traits on the top and the side of ostrich eggs

Location and trait	σ^2_{female}	σ^2_e	σ^2_p	Repeatability	s.e.
Side:					
Pore count (pores/cm^2)	68.32	90.65	158.97	0.430	0.063
Average pore diameter (mm)	0.00599	0.00492	0.0109	0.549	0.059
Pore area (mm^2) (A_p)	7.833	6.539	14.372	0.545	0.059
Thickness (mm) (L)	0.0109	0.0045	0.0154	0.708	0.047
Pore area/thickness (mm²/mm)	2.240	1.956	4.196	0.534	0.060
Top:					
Pore count (pores/cm^2)	78.71	106.11	184.82	0.426	0.063
Average pore diameter (mm)	0.0055	0.0045	0.010	0.552	0.059
Pore area (mm^2) (A_p)	9.045	8.062	17.11	0.529	0.060
Thickness (mm) (L)	0.0116	0.0045	0.0161	0.722	0.045
Pore area/thickness (mm²/mm)	2.575	2.251	4.826	0.534	0.060

σ^2_{female} – Between female variance; σ^2_e – Environmental variance; σ^2_p - Phenotypic variance

In conclusion, it is clear that image analysis facilitated the measurement of a considerable number of eggs in a relatively short period. Data obtained in this way allowed the estimation of significant between female variance components for eggshell traits. Medium to high repeatability estimates for eggshell traits were derived from these components. These variance ratios should be partitioned further, assessing the possible genetic basis for differentiation between females on eggshell traits. Data recorded across production seasons are needed for such an analysis. Further research on the relationship between shell structure and monthly climate is also indicated, to confirm or refute the results obtained in the present study.

Traits measured on the top and side of the eggshell were effectively the same on the between female level. Measurements can thus only be taken at one of these sites, to facilitate the assessment of larger numbers of eggs within the shortest possible period.

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