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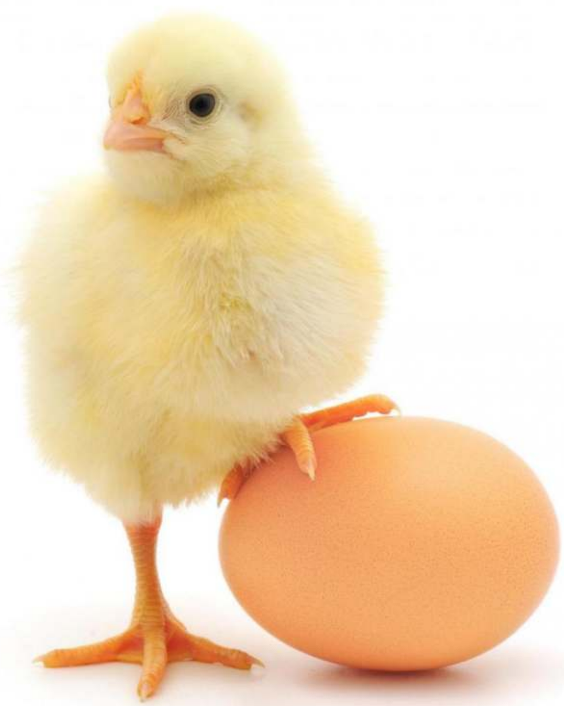

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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Double and Single Yolked Duck Eggs: Their Contents and Dimensions Compared and the Mechanical Stimulation Hypothesis for Albumen Secretion Is Supported

Attila Salamon[1,2] and John P. Kent[2]
[1]School of Biology and Environmental Science, University College Dublin, Science Center West, Belfield, Dublin 4, Ireland
[2]Ballyrichard House, Arklow, Co. Wicklow, Ireland

Abstract: The external dimensions, weight and contents (yolk, albumen, shell) of Double Yolked (DY) and Single Yolked (SY) eggs of the duck (*Anas platyrhynchos domesticus*) were measured and compared. The yolks in DY eggs did not differ in weight ($p = 0.144$), although each double yolk was significantly lighter than the yolk of a SY egg ($p<0.001$). DY albumen weight was below that expected of a SY egg of comparable weight. However, DY eggs had 24.5% more albumen on the basis of SY yolk weight. In DY eggs, the yolk closer to the airspace (Yolk 1) was heavier in 62.5% of the eggs and heavier yolks are regarded as the first in the ovulation sequence. Thus, DY eggs were divided into two groups based on yolk weight. In Group A, Yolk 1 was heavier. In Group B, Yolk 2 was heavier. Significantly more albumen was found in Group B (Group A: 51.37% vs. Group B: 53.29% albumen; $p = 0.031$). This supports the mechanical stimulation hypothesis, with the larger Yolk 2 stimulating the secretion of additional albumen by the magnum wall. It is suggested that work with DY eggs could provide a useful non-invasive tool to examine the mechanisms underlying albumen secretion.

Key words: Albumen, duck, double yolked egg, egg content, egg yolk, mechanical stimulation

INTRODUCTION

Double Yolked (DY) eggs are formed when two yolks ovulated within three hours of each other are enclosed in one egg (Warren and Scott, 1935; Conrad and Warren, 1940). If two follicles are at the same developmental stage and are ovulated at or about the same time, little variation in yolk size is expected (Warren and Scott, 1935). However, difference in yolk weight should occur if one yolk is released prematurely. Zelenka *et al.* (1986) found with pullets using dying techniques, that 58% of DY yolks were at the same stage of development at ovulation, whereas 34% of DY yolks differed by one developmental day.

The yolk passes, over a 2-3 hour period, through the magnum (Warren and Scott, 1935; Aitken, 1971; Etches, 1996; Williams, 2012), where the synthesis and process of albumen secretion takes place (Gilbert, 1971; Edwards *et al.*, 1976; Palmer and Guillette, 1991; Etches, 1996). The mechanisms that trigger albumen secretion are not fully understood but mechanical stimulation of the magnum wall by the descending yolk is regarded as an important factor (Aitken, 1971; Gilbert, 1971; Palmer and Guillette, 1991; Etches, 1996). However, neuronal or endocrine stimulations may play a role (Palmer and Guillette, 1991; Deeming, 2011). There is evidence that the magnum has sufficient water soluble protein for two eggs (Aitken, 1971; Gilbert, 1971) but Edwards *et al.* (1976) found with SY eggs that the synthesis of albumen proteins takes 20-23 hours and suggested that sufficient protein was available for only one egg. However, in DY pheasant eggs, 33.7% additional albumen was secreted as compared with the albumen weight of a single yolk (Deeming, 2011), showing that protein is available for more than one egg. Thus it is not as yet clear if the magnum is able to secrete sufficient albumen for two eggs / yolks at a given time period.

A Single Yolked (SY) duck egg contains approximately 31% yolk, 59% albumen and 10% shell (Romanoff and Romanoff, 1949; Etches, 1996; Kokoszynski *et al.*, 2007), although over the laying period (January-July) of domestic ducks the proportion of yolk increases by 4.6%, while albumen and shell decreases by 4.1 and 0.5%, respectively (Kokoszynski *et al.*, 2007).

In DY eggs, the positioning of the embryos is of key importance, especially towards the end of the incubation period, as such eggs have low hatchability (Monkman, 1963). This may be related to yolk positioning which, in turn, may be related to albumen content of the egg. Recently, the relative contents of SY and DY pheasant eggs were compared with emphasis on the mechanism of albumen secretion (Deeming, 2011). Deeming (2011) considered the heavier yolk as Yolk 1, as it would have formed the basis of the SY egg and would have been the

Corresponding Author: Attila Salamon, School of Biology and Environmental Science, University College Dublin, Science Center West, Belfield, Dublin 4, Ireland, E-mail: attila.salamon@ucdconnect.ie

next in the laying sequence; however the bigger yolk need not necessarily be the next in the laying sequence (Conrad and Warren, 1940; Badyaev *et al*., 2005; but see Williams, 2012).

Here, with domestic ducks, we compared the dimensions and contents (yolk, albumen, shell) of DY and SY eggs and examine possible mechanisms governing albumen secretion. Yolk size and positioning were recorded and their possible role in albumen secretion examined.

MATERIALS AND METHODS

A commercial flock of Aylesbury ducks (n = 254) was maintained at Ballyrichard, Arklow, Ireland (52.83°N, 6.13°W) from which eggs were collected daily between December 2008 and February 2009 for use in this study. From three batches of eggs that were washed and stored overnight at room temperature, 55 DY were identified by individual candling and 55 SY control eggs were selected (Batch 1:13 eggs, Batch 2:12 eggs and Batch 3:30 eggs from each type).

Eggs were weighed on a digital scale to the nearest 0.1 g, length and width measured using a digital caliper to the nearest 0.01 mm. Eggs were opened and the yolks separated from the albumen using a separating spoon in accordance with the method of Burke *et al.* (1997). Prior to weighing, the yolks were rolled on a dry paper towel to remove residual albumen and the chalazae. Egg shells were rinsed with water and allowed to air dry for approximately 72 hours before being weighed. Albumen weight was calculated by subtracting yolk and shell weights from the egg weight. In DY eggs, yolk order was recorded and the yolk closer to the airspace was termed Yolk 1. If yolks burst when separating egg contents, the egg was discarded. This resulted in a complete data set of 40 DY eggs (Batch 1:9 eggs, Batch 2: 7 eggs and Batch 3:24 eggs) and 51 SY control eggs (Batch 1:11 eggs, Batch 2:11 eggs and Batch 3:29 eggs). Data were analyzed using SPSS 20 following the procedures used by Deeming (2011). Independent t-tests were used to compare the traits of SY and DY eggs. Linear regression analysis was used to determine the relationship between egg weight and contents and the slopes of regression estimates of SY and DY eggs were compared using the method of Bailey (1995). To examine the importance of yolk size in albumen secretion DY eggs were divided into two groups based on yolk weight. Group A: Yolk 1 heavier. Group B: Yolk 2 heavier. The albumen proportions of the two groups were compared using one-tailed t-test. Shell proportions were also compared between the two groups. The proportion of yolk, albumen and shell were used when statistically comparing the same in DY vs. SY eggs (Deeming, 2011) but these measures are presented here as percentages for ease of understanding.

RESULTS

DY eggs were 21.5% heavier, than SY eggs ($p<0.001$) and differed significantly from SY eggs in all measurements (SY n = 51, DY n = 40; Table 1). In DY eggs the two yolks differed on average by 0.3 g (range = 0.2-5.2 g) which was not significant when compared on the basis of yolk position ($p = 0.144$). When yolks were sorted by size (see Deeming, 2011), a significant difference in weight was found ($p<0.001$).

Table 1: Dimensions, weight and contents of SY (n = 51) and DY (n = 40) eggs. Comparisons of means in any row were by independent t-tests unless indicated

	Single Yolked (SY)		Double Yolked (DY)		Comparison	
Trait	Mean (±SD)	Range	Mean (±SD)	Range	t	P
Length (mm)	70.16 (±2.46)	66.42-76.31	78.37 (±5.15)	69.15-94.67	-10.03	<0.001
Width (mm)	50.31 (±1.32)	47.96-54.31	52.34 (±2.83)	47.54-58.8	-4.55	<0.001
Length/Width	1.395 (±0.068)	1.23-1.52	1.499 (±0.07)	1.35-1.71	-6.89	<0.001
Egg weight (g)	98.2 (±5.24)	89-113.3	119.31 (±18.74)	92.2-159.8	-7.68	<0.001
Shell weight (g)	9.47 (±0.69)	8-10.8	10.64 (±1.28)	8.4-12.8	-5.61	<0.001
Shell proportion (%)	9.7 (±0.8)	8.3-11.3	9 (±0.7)	7.3-10.5	4.35	<0.001
Albumen weight (g)	55.16 (±3.55)	49.6-63.7	62.05 (±9.75)	43.7-94.1	-4.67	<0.001
Albumen proportion (%)	56.2 (±1.8)	52.5-60.2	52.1 (±3.2)	45.4-59	7.69	<0.001
SY yolk weight (g)	33.58 (±2.58)	28.6-41.2	-	-	-	-
DY Yolk 1 weight (g)	-	-	23.46 (±5.3)	17.1-39.9	11.96[1]	<0.001
DY Yolk 2 weight (g)	-	-	23.16 (±4.62)	16.4-35.1	-	-
DY Total yolk weight (g)	-	-	46.62 (±9.86)	33.5-74.6	-9.07[2]	<0.001
SY yolk proportion (%)	34.2 (±1.6)	31.4-38.6	-	-	-	-
DY Total yolk proportion (%)	-	-	38.9 (±3.5)	32.9 - 47.2	-8.63[3]	<0.001

[1]Two-sample t-test compared SY yolk weight and DY yolk 1 weight.
[2]Two-sample t-test compared SY yolk weight and DY total yolk weight.
[3]Two-sample t-test compared SY yolk proportion and DY total yolk proportion.

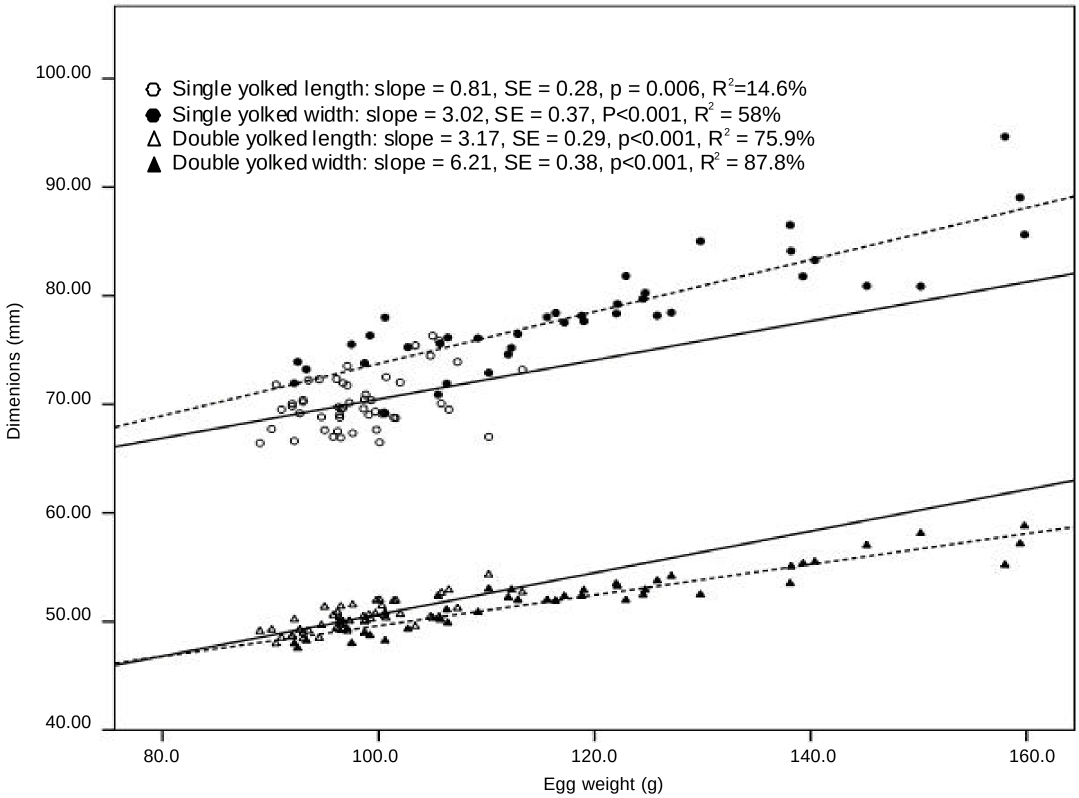

Fig. 1: Egg dimensions, i.e., length and width (mm) as a function of egg weight (g) for SY (n = 51, solid regression lines) and DY (n = 40, dashed regression lines) duck eggs. The slope and SE of the regression lines are shown with significance levels and R^2 values

Table 2: Regression estimates of the slope of the relationships between egg weight (g) and egg content weight (g) as shown in Fig. 2-4

	Single yolked (SY)		Double yolked (DY)		Comparison of slopes	
Component	Slope (SE)	R^2	Slope (SE)	R^2	d	p
Albumen weight	1.27 (0.11)	0.75	1.74 (0.13)	0.82	-2.77	<0.01
SY yolk weight	1.58 (0.18)	0.61	-	-	-	-
DY Yolk 1 weight	-	-	3.13 (0.27)	0.78	-4.82[4]	<0.001
DY Total yolk weight	-	-	1.7 (0.14)	0.8	-0.53[5]	ns
Shell weight	1.71 (1.06)	0.05	12.56 (1.24)	0.73	-6.66	<0.001

[4]The slopes of SY yolk weight and DY yolk 1 weight were compared here.
[5]The slopes of SY yolk weight and DY total yolk weight were compared here.

DY eggs were 11.7% longer ($p<0.001$) and 4% wider ($p<0.001$), than SY eggs. This meant that the shape index (length/width) was also significantly greater in DY eggs ($p<0.001$; Table 1). Extrapolation of regression analyses for the relationship between linear dimensions and egg weight showed that DY eggs were longer but narrower, than predicted on the basis of a SY egg of the same weight (Fig. 1).

DY eggs had significantly more albumen compared to SY eggs (62.05±9.75 vs. 55.16±3.55 g, respectively; $p<0.001$) but it formed a significantly smaller proportion of their weight (52.1±3.2 vs. 56.2±1.8%, respectively; $p<0.001$; Table 1). Albumen weight in DY eggs was significantly less, than is predicted on the basis of a SY egg of equivalent weight (Fig. 2, Table 2). For the relationship between egg weight and albumen weight the slope of DY eggs was significantly flatter, than of the SY eggs ($p<0.01$, Fig. 2, Table 2).

The combined weight of two yolks in a DY egg was significantly heavier (46.62±9.86 g; $p<0.001$), while the weight of Yolk 1 in a DY egg was significantly lighter (23.46±5.3 g; $p<0.001$), than a yolk in SY eggs (33.58±2.58 g) (Table 1). For a given egg weight, the slope of SY yolk weight was significantly different from the slope of DY Yolk 1 weight ($p<0.001$) but was not significantly different from the total yolk weight in DY eggs (Fig. 3; Table 2).

Predicted albumen weight of DY eggs was calculated on the basis of SY yolk weight ($AW = 0.525YW+37.531$; where, AW = albumen weight, YW = yolk weight) which

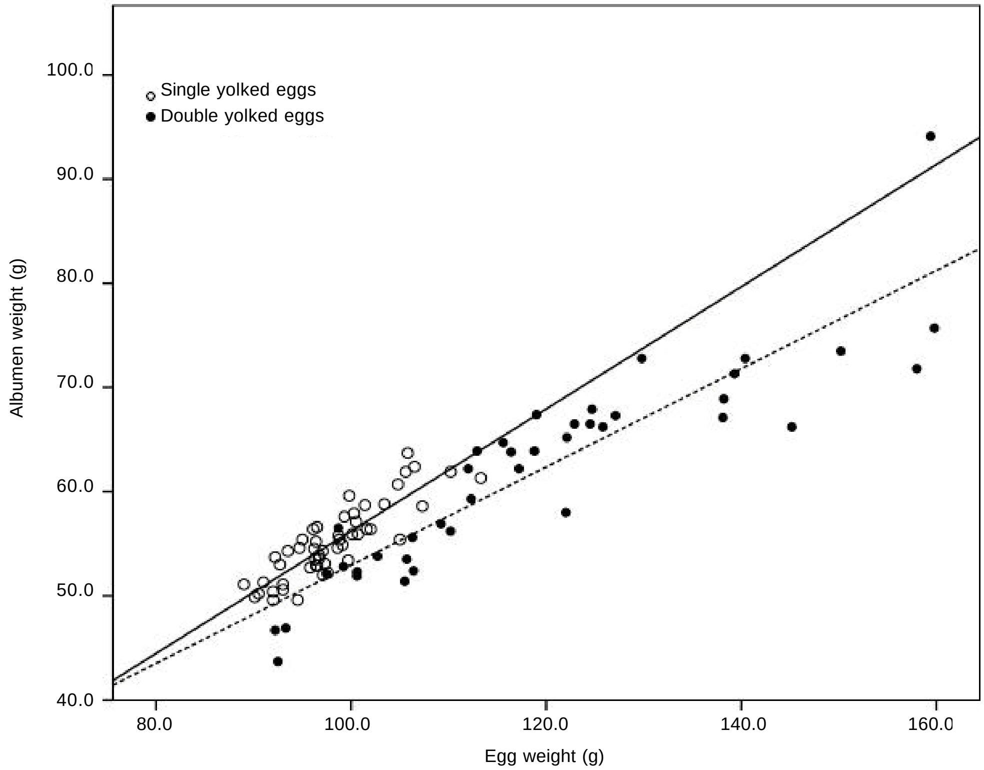

Fig. 2: Albumen weight (g) as a function of egg weight (g) for SY (n = 51, solid regression line) and DY (n = 40, dashed regression line) eggs (Table 2)

was subtracted from the observed albumen weight (Deeming, 2011). On average, the additional albumen for Yolk 2 in DY eggs weighed 12.2 g (SE = 8.34) and was 24.5% of the albumen weight predicted on the basis of SY yolk size.

Based on yolk weight the DY eggs were divided into two groups. Group A included all DY eggs where Yolk 1 was heavier; while in Group B Yolk 2 was heavier. The albumen proportion between Group A (51.37±0.04%) and Group B (53.29±0.02%) was significantly different (p = 0.031) showing that the heavier Yolk 2 in Group B stimulated the secretion of additional albumen.

DY eggs had significantly more shell, than SY eggs (10.64±1.28 vs. 9.47±0.69 g, respectively; $p<0.001$), however, it formed a significantly smaller proportion of their weight (9±0.7 vs. 9.7±0.8%, respectively; $p<0.001$; Table 1). For eggs lighter than 100 g, DY eggs had lighter eggshells than would be predicted from a SY egg of the same weight. However, for eggs heavier than 100 g, DY eggs had heavier eggshells than would be predicted from a SY egg of equivalent weight (Fig. 4; Table 2). The slope of the relationship between egg weight and shell weight for SY eggs was significantly flatter, than that of the DY eggs ($p<0.001$; Fig. 4; Table 2). When the Surface Area (SA) of the eggs was calculated ($SA = 4.835EW^{0.662}$, where EW = egg weight; Paganelli *et al*., 1974), there was no difference in the shell weight per cm^2 between DY and SY eggs of comparable weight (0.093±0.006 g/cm^2 vs. 0.094±0.007 g/cm^2, respectively; p = 0.461). The shell proportion between Group A (8.82±0.01%) and Group B (9.25±0.01%) was significantly different (p = 0.031).

DISCUSSION

DY eggs were significantly heavier, longer and wider than SY eggs and the egg contents apart from yolk weight were heavier in DY eggs (Table 1), in accord with previous studies with hens (Curtis, 1914; Burke *et al*., 1997; Fasenko *et al*., 2000), pheasants (Deeming, 2011) and domestic ducks (Salamon and Kent, 2011). The egg contents in the SY eggs (34.2% yolk, 56.2% albumen, 9.7% shell) are consistent with previous findings with domestic ducks (Romanoff and Romanoff, 1949; Etches, 1996; Kokoszynski *et al*., 2007).

A DY egg's individual yolks weighed approximately 70% of those in SY eggs. However, when the weights of the two yolks in DY eggs were combined there was proportionately more yolk in a DY egg (approximately 38.8% more; $p<0.001$; Table 1). This is similar to findings with broiler breeder hens (Burke *et al*., 1997) and pheasants (Deeming, 2011).

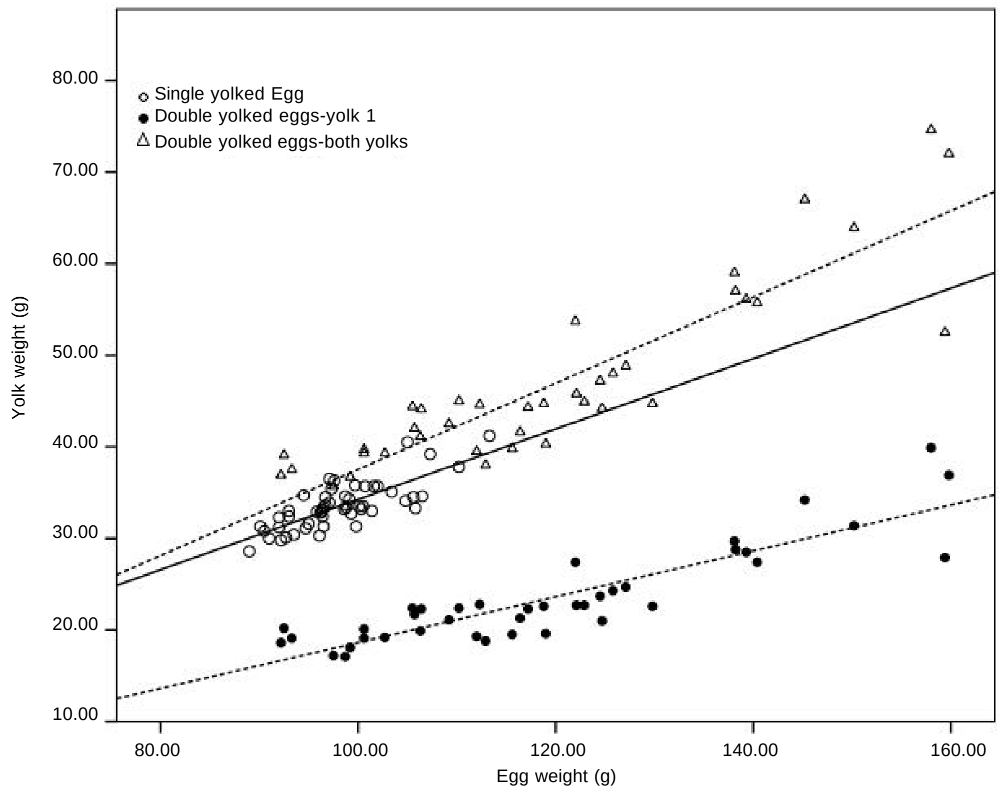

Fig. 3: Yolk weight (g) as a function of egg weight (g) for SY (n = 51, solid regression line) and DY (n = 40, dashed regression lines) eggs (Table 2). For DY eggs, Yolk 1 (closer to airspace) and Yolk 1 and 2 combined are shown

DY eggs contained proportionately less albumen (Table 1) in accordance with findings in broiler breeder hens (Burke *et al*., 1997) and pheasants (Deeming, 2011). On the basis of SY egg's yolk weight, 24.5% additional albumen was secreted in the DY eggs here. This is comparable to the 33.7% additional albumen secreted in DY pheasant eggs (Deeming, 2011). Conrad and Scott (1942) using a recently dispatched hen found two yolks in the oviduct (one in the uterus and one in the isthmus, thus released within three hours of each other) with the yolk in the isthmus acquired only 10.7% of the albumen of the preceding yolk. Thus the question arises as to the mechanism of albumen secretion.

Deeming (2011) with DY pheasant eggs considered the heavier yolk as the first to be ovulated (Yolk 1) and the smaller yolk (Yolk 2) in this study was on average 95.88% of the weight of the heavier yolk. It was suggested that the smaller yolk was released from the ovary prematurely and entered the infundibulum closely behind Yolk 1 (Deeming, 2011). This is consistent with one of the yolks being ovulated at the normal time and the other one day prematurely which according to Conrad and Warren (1940) occurs in 25% of DY hen eggs. This reasoning is based on evidence of a pause in egg laying the day after a DY had been laid (Conrad and Warren, 1940). Also, Zelenka *et al*. (1986) found in pullets that 34% of DY egg yolks differed in rapid growth by one day.

However in this study, yolk order was recorded during measurement with the yolk closer to the airspace being termed Yolk 1 on the assumption that it was the first yolk released. Yolk 1 was the heavier in 62.5% of DY eggs consistent with being first ovulated. When two follicles are in the same developmental stage little variation in yolk size is expected (Warren and Scott, 1935). Badyaev *et al*. (2005) using house finches suggested that the largest yolk may not be the first ovulated and such may explain differences in yolk weight relative to position found here. This is as far as we are aware the first study to record yolk weight relative to yolk position in the egg which is made easier in this species due to the size and shape of the egg.

The size and positioning of yolks in DY eggs is important, as mechanical stimulation by the yolk is necessary for the secretion of albumen (Aitken, 1971; Gilbert, 1971; Palmer and Guillette, 1991; Williams, 2012). To examine the importance of yolk size in albumen secretion DY eggs were divided into two groups, Group A (Yolk 1 heavier) and Group B (Yolk 2 heavier). Significantly more albumen was produced in

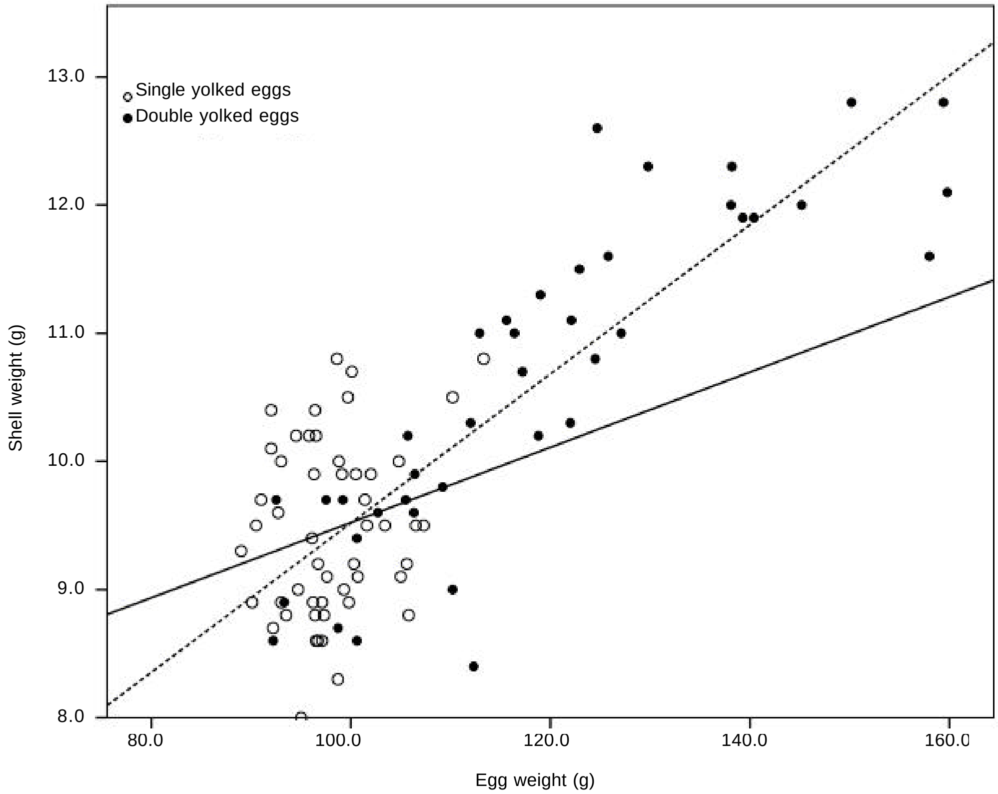

Fig. 4: Shell weight (g) as a function of total egg weight (g) for SY (n = 51, solid regression line) and DY (n = 40, dashed regression line) eggs (Table 2)

Group B with the heavier Yolk 2 (Group A: 51.37% vs. Group B: 53.29%; p = 0.031) suggesting that the larger Yolk 2 stimulated the secretion of more albumen in accord with the mechanical stimulation hypothesis. Deeming (2011) suggested that Yolk 2 may not provide a comparable mechanical stimulus to Yolk 1. This study demonstrates that when Yolk 2 is smaller less albumen is produced. However, either way it is clear that in DY eggs proportionately less albumen is produced than in a SY egg and we found here that yolk size plays a significant role in albumen secretion.

Further, as pointed out above, individual yolks in DY eggs were approximately 70% of the weight of a SY yolk and thus are not the optimal sizes as one would expect to stimulate albumen secretion. This may explain the lower proportion of albumen found in DY eggs.

Other factors such as time spent in the magnum may also play a role in albumen secretion (Deeming, 2011). In laying hens the use of ahemeral lighting programme (14L:13D) increased the weight of albumen and shell significantly but had no significant effect on yolk weight (Morris, 1973). This can be explained by the prolonged period of egg formation under ahemeral lighting programmes, as the yolk spent 15 minutes more in the magnum and isthmus (Melek *et al.*, 1973) supporting the role of time as a determining factor in albumen secretion (Deeming, 2011). Longer egg formation time is associated with increased egg contents (reviewed in Shanawany, 1990).

Here, DY eggs below 100 g (n = 6) had lighter eggshells than would be predicted from a SY egg of the same weight. However, DY eggs over 100 g (n = 34) had heavier eggshells than would be predicted from a SY egg of equivalent weight (Fig. 4). This result differs from findings in laying hens, broiler breeder hens and pheasants, where the DY egg shell was heavier than SY egg shell but proportionately DY eggs contained less shell (Harms and Abdallah, 1995; Burke *et al.*, 1997; Deeming, 2011). Small SY and DY eggs are commonly produced at the onset of laying (Christmas and Harms, 1982; Salamon and Kent, 2011) and the DY eggs below 100 g (n = 6) possibly belong to that group of small eggs. This might explain the difference in the slopes of SY and DY egg shell weight. There was no difference between the amount of shell per cm^2 between DY and SY eggs of comparable weight (0.093±0.006 g/cm^2 versus 0.094±0.007 g/cm^2, respectively; p = 0.461), similar to the finding in pheasants (Deeming, 2011) showing that shell thickness might be independent of egg size. However, the shell proportion between Group

A (8.82±0.01%) and Group B (9.25±0.01%) was significantly different ($p = 0.031$) showing that the larger Yolk 2 may also positively influence the amount of shell deposited by mechanisms unknown.

In conclusion, here we provide evidence that in DY eggs the presence of Yolk 2 stimulates the secretion of additional albumen, though the proportion of albumen in DY eggs is less than in SY eggs. The mechanical stimulation hypothesis is supported by the finding that a larger Yolk 2 stimulated the secretion of more albumen than a smaller Yolk 2. It would be instructive to compare the constituents in the albumen of DY and SY eggs to test these hypotheses further. The comparative method and the use of DY eggs should provide a useful non-invasive model to examine the mechanisms of albumen secretion.

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