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USING STEPWISE REGRESSION ANALYSIS TO DETERMINE THE FACTORS AFFECTING CHICK WEIGHT AT HATCH IN OSTRICH (STRUTHIO CAMELUS)

By

S.A. El-Safty

Fac. of Agric., Poultry Production Dept., Ain Shams Univ., Cairo, Egypt

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Abstract: *This study aims on one hand to describe and determine the relationship between the characteristics of hatching traits with each other and their relationship to ostrich chick weight at hatching. The main factors affecting chick weight at hatching were sorted using stepwise regression analysis. One thousand and two hundred eggs were collected from African Black Neck ostrich flock located at Bia Valley project, north of Libya. The traits studied in the current trial were fertility and hatchability percent, egg weight, egg weight loss, eggshell characteristics (weight, thickness, porosity and area) and chick weight at hatch. The main results of the current study could be summarized as follows: The average of chick weight and its percent of initial egg weight were 845.4 g. and 63.4%, respectively. The maximum egg weight loss was existed in both third and fourth periods of incubation intervals. The highly positive correlation was realized between chick weight and periodical egg weight during the incubation interval (from at set to at 40th day of incubation, r = 0.82). There was a significantly negative relationship between chick weight and the total egg weight loss (from at set of eggs to 40th day of incubation). The gradually factors affecting chick weight upon hatching were egg weight, eggshell weight and egg weight loss, respectively, while the pores count of eggshell surface had no effect on newly hatched chicks weight.*

Key words: ostrich, hatching eggs, chick's weight, egg weight loss.

INTRODUCTION

Ostrich (*struthio camelus*) is the longest and largest of the flightless birds around the world, and have been domesticated for more than 100 year (Stewart, 1993). The dearth of information pertaining to optimum conditions of artificial egg incubation, factors affecting fertility and hatchability percentages, healthy chicks at hatching could be the factors hampering growth of the ostrich industry. It is striking to note that both fertility and hatchability percentages of ostrich eggs have a wide rang, where, the fertility trait ranged from 37- 90% (Deeming, 1995; Dzama et al., 1995; Park et al., 2001) and the hatchability counterpart ranged from 39.4- 83.6% (Deeming, 1996; More, 1996; Badley, 1997; Mushi et al., 2008 and Dzoma and Motshegwa, 2009). Factors associated with low hatchability percentage include prolonged pre-incubation storage (up to 2 weeks), season, poor breeder nutrition, breeder age, improper egg handling, contamination, incubator or hatcher malfunctions and humidity or temperature problems (Nahm, 2001; Cabassi et al., 2004; Hassan et al., 2004; Ipek and Sahan, 2004 and Malecki et al., 2005). The relationship between temperature and humidity of egg incubation and their effects on egg weight loss have never been successfully standardized to consistently yield healthy ostrich chicks as in the chicken, turkey and duck industries (Ar, 1991). At various temperatures and humidities, the literatures reported that the ostrich egg weight losses ranging from 11.4 to 19.6% (Deeming, 1993). Actually, achieving the correct water loss (WL) during artificial incubation is a challenge as it is influenced by both incubator conditions, the physical properties of the eggshell, and internal factors as the embryo develops (Ar 1991).Wilson and Eldred (1995) stated that the average ostrich chick weight as a percentage of initial egg weight was 63.6% and ranged from 56 to 69%. However, definitive information on the relationship among egg hatching traits of ostrich is lacking. The aim of this study was, therefore to investigate the relationships among main hatching traits such as egg weight, egg weight loss, eggshell area, shell thickness, porosity and chick weight at hatching time. The main factors affecting chick weight at hatching were sorted using stepwise regression analysis.

MATERIALS AND METHODS

Birds, Husbandry and Egg Incubation

The current study was carried out on ostrich eggs (throughout February and March, 2009) which were collected from African Black Neck ostrich flock (9 to 11 yr of age) located at Bia Valley project, north of Libya. Birds were fed about 1.5 kg daily of a pelleted ratite breeder ration (17.5% CP, 2650 kcal, ME/kg, 2.7% Ca, 0.95% av. P of feed). Water was supplied for *ad libitum* consumption. Each trio (one male and two females) was housed in a fenced pen (25X15m.). Scrapes of floor sand were routinely checked two to three times daily for the presence of eggs. Eggs were washed and disinfected for seconds in warm water containing CHEM-50 solution (Iodine family). After sanitation, eggs were stored for 4 days at 17°C and 80% relative humidity (RH) in vertical position up to incubation. The incubator and hatcher used was Victoria type (1200 eggs capacity for incubator and 576 eggs for the hatcher) machine. The temperature of the incubator ranged from 36.1- 36.3°C and 23- 30% RH. whereas the hatcher was operated at 35°C and 50% RH. Eggs were put with air sac upside (vertically) in the incubator racks and turned by 45° up and down from the horizontal position 6 times a day during the first 39 days of incubation. The eggs were then transferred to the hatching unit up to hatching. All eggs were candled three times during the incubation using 150-watt candling lamp; the first time at 10 days of incubation to eliminate the checked eggshell or eggs which have fungi, the second time at 21 days to estimate eggs fertility, and then the fertilized eggs were chosen for the current experiment. The last candling was at 39 days to follow up the growth of the embryos.

Fertility and Hatchability Percentages

One thousand and two hundred eggs were set in the incubator, which were collected from 1500 ostrich females. Both the fertility and hatchability were computed as follow:

Fertility%= Total number of fertile eggs/Total number of eggs laid*100

Hatchability%= Total number of eggs that hatched successfully/ Total number of eggs laid*100

Eggshell Porosity, Eggshell Thickness and Eggshell Surface Area

An estimate of an individual egg's pores (small and large) was determined by averaging pore counts obtained from discretionary sampling at five independent 1 cm^2 sites on an egg's surface. Four sites were chosen approximately equidistant along the equator and one site was chosen that approximated the center of the air-cell. To better visualize and facilitate a more accurate counting of egg's pores, each selected site was dyed with $KMnO_4$ dye. The counting operation was using a magnifying lens. Total pores number on egg surface was calculated according to the average numbers of pores in cm^2 and the egg surface area. An estimate of overall shell thickness was obtained by averaging thickness measurements made at the same five shell sites used to determine eggshell porosity. A digital micrometer was used to make individual thickness estimates to the nearest 0.01 mm. Eggshell surface area was also determined according to the following equation by Paganelli *et al.* (1974):

Egg surface area (cm^2) = $4.735W^{0.662}$, where W is the weight of egg.

Egg Weight, Egg Weight Loss and Chick Weight

One hundred fertilized eggs and 100 chicks of African Black Neck ostrich were used in the current trial. Eggs were individually identified and weighed (±0.01 g) at set, 10^{th}, 20^{th}, 30^{th}, 40^{th} day of incubation. Likewise, egg weight loss (EWL), in grams and percent was determined throughout four intervals (each 10 days from the egg set), where EWL % = (egg weight $_{at\ first}$ − egg weight $_{at\ second}$)/ egg weight $_{at\ first}$ X 100. Chick weights were determined upon hatching using an electronic pan balance that was accurate to 0.01 g.

Statistical Analysis

Data were analyzed using (PROC MEANS) procedure of SAS (SAS Institute, 1998) to compute means and their standard errors for all the studied traits. Percentage data were transformed to arc sine and reanalyzed. In order to statistical trends were similar for both transformed and untransformed data, the untransformed results will be presented. Correlation coefficients between chick weight and egg traits were computed using the PROC CORR procedure. Stepwise regression analysis was used to verify and sort the main factors affecting ostrich chick weight at hatching time. The following model was used;

$$Y = a + b1X1 + b2X2 + b3X3 + e$$

Where;

Y = Dependent variable (Chick weight at hatch) and the independent variables (X) are as follow:

X1 = egg weight,

X2 = shell weight,

X3 = egg weight loss,

a = the regression intercept,

b = regression coefficient (1, 2, 3),

e = error term.

RESULTS AND DISCUSSION

As illustrated in Figure 1, the fertility percent of ostrich egg was extremely high value (89.25%). This result was in agreement with the finding of Malecki et al. (2004). Who reported that fertilization rate in ostrich eggs is high because most eggs contain excessive numbers of sperm yet very low numbers of sperm appear sufficient to achieve fertilization. Dzoma and Motshegwa (2009) stated that the fertility percent of ostrich eggs ranged from 63.5- 89% with an average percent of 76.3%. With respect to hatchability trait, the corresponding figure was low (48.5%). As mentioned in the study of Gonzalez et al. (1999), ostrich eggs often have low hatchability percent because they don't lose sufficient weight throughout incubation period. As mentioned in literatures, the hatchability percentage ranged from 39.4- 83.6% and 27- 67% (Deeming, 1996; More, 1996; Bradly, 1997; Mushi et al., 2008). Presented in Table 1 are the mean values obtained for ostrich chick weight and egg characteristics. The mean of egg weight was in the normal range reported for egg ostriches as noted in the literatures. Regarding the egg weight loss (%), the mean value obtained was 15% and it was in accordance with the results of (Ar, 1991; Deeming, 1993; More, 1996). The chick weight and its percent of initial egg weight were also determined, the corresponding mean values were 845.4 g. and 63.4%, respectively. Consistent with these observations, Wilson et al. (1997) and Gonzalez et al. (1999) reported that the ostrich chick weight as a percent of initial egg weight ranged from 53 to 70%. In accordance with the results of Di Meo et al. (2003) and Mahrose, (2007), both shell weight percentage and shell thickness were 19.3 % and 1.9 mm, respectively. With respect to egg surface area measure, the result of the current study was in good agreement with Superchi et al. (2002). Eggshell porosity recorded by counting both large and small pores in cm^2 of the shell, and the average pores count was 26.6. By knowing the eggshell surface, it could be supposed that the total count of eggshell pores is almost 14 thousands. According to our knowledge, little is known about the concise count of the ostrich eggshell pores (large and small). Gonzalez et al. (1999) estimated the number of large pores per cm^2 of eggshell surface, and it was ranged from 8.9 to 11.2. Alongside, Cloete Jr et al. (2006) recorded the all pores count (large and small) on the surface of 1 cm^2 eggshell, the mean value was 22.0.

The results in Table 2 revealed that the egg weight gradually decreased from at set of hatching eggs in the incubator until 40^{th} day of incubation interval (at the end of incubation period). The opposite trend was realized for egg weight loss (in gram or %), however the maximum egg weight loss was existed in both third and fourth periods of egg incubation. In consistent with the observations of the authors, the average egg weight loss throughout the incubation stage was around 15%. Accordingly, it can worthy be stated that the justification of relative humidity inside the setter is very important to keep the weight of chick in the normal range, especially during the third and fourth stages of incubation (20^{th} to 40^{th} day of incubation period) which have more egg weight loss. It is of interest to note that there was a negative relationship between egg weight loss and chick body weight at hatch (Hegab, 2006 ; Mahrose et al., 2009).

In domestic fowl, the literatures reported the strong positive correlation between the weight of an egg and the weight of the hatched chick (Wilson, 1991). Table 3 clearly demonstrated that, this relationship was also existed in egg of ostriches, where the highly positive correlation was realized between chick weight and periodical egg weight during the incubation intervals (from at set to at 40^{th} day of incubation, $r = 0.82$). These results are in good agreement with conclusions drawn by Mahrose (2007); Cooper (2008); Brand et al. (2009); Mahrose et al. (2009). Who indicated that laying egg weight is the most important factor affecting chick weight at hatch, however the correlation between them was significantly high and positive ($r = 0.86$- 0.91). Respecting the periodicals egg weight loss during egg incubation, the results of Table (4) revealed that there was slightly negative correlation between chick weight upon hatching and the studied stages of egg loss, but this relationship was significantly negative with the total egg weight loss during incubation period. Therefore, the results indicate that there is no critical period of egg weight loss may be influenced hatching weight of ostrich chicks. Congruent to these findings, Deeming and Ayres (1994); Ali (2004) ; Mahrose et al. (2009) stated that there was a significant relationship between the percentage egg weight loss on day 40 of incubation and the weight of the hatched chicks as a percentage of initial egg weight. In this concern, Brand et al. (2009) reported negative correlation between chick weight and egg weight loss at both 21 and 35 days of incubation.

In an attempt to describe the relationship between chick weight of ostrich at hatching time and eggshell characteristics, Table 5 showed that there was a highly positive correlation between chick weight and eggshell area. In this latter respect, it could be reported that eggshell area beside egg volume plays an important role as effectors on chick weight upon hatching. On the other hand, the highly negative correlation was observed between chick weight and eggshell percent, where the corresponding value was moderate (- 0.37). The last observation may be attributed to the ostrich embryos use eggshell contents (Ca and P) to build up their bones during the embryonic development. Likewise, very weak relationships were realized among chick weight, eggshell thickness, porosity per cm^2 of eggshell (tend to weak negative correlation) and total count of pores (tend to weak positive correlation). There is a lack in the references dealing with the effects of eggshell characteristics on the productive performance of the ostriches. Therefore, further investigations on the relationships between ostrich chick weight and eggshell traits are suggested to ensure their effects on chick weight at hatching and also at latter weights. Table 6 explained the relationships among hatching egg weights of ostrich and some external egg traits. However, there was significantly positive correlation between eggs weight and both eggshell area (r = 0.99) and eggshell weight (r = 0.49). Contrary was observed with both eggshell thickness and pores count per cm^2, where the negative relationship was realized.

Stepwise regression equations, which weighted gradually by factors affecting chick weight upon hatching are given in Table 7. The current results would indicate that the egg weight had a major effect on hatched chick weight. Likewise, the eggshell weight has found to be second factor affecting chick weight. Whereas, the egg weight loss was a limiting factor affecting chick weight. It is useful to know that the maximum accuracy of prediction (R= 0.86) could be obtained using the third equation, in turn it is possible to predict chick weight at hatch using egg weight, shell weight and egg weight loss during incubation period. Concerning the pores count as a factor in stepwise regression analysis, the analysis steps demonstrated that the effect of pores was neglected and excluded throughout the steps of analysis. Therefore, the egg weight and its components will remain the main and only factors affecting chick weight at hatch and may be by early later time of age. In full agreement, both Cooper (2008); Brand et al. (2009) stated that the egg weight is the most important factor affecting chick weight at hatch.

CONCLUSION

The role of physical characteristics of ostrich eggs in hatching performance should be taking into account to help us in understanding how to obtain ideal eggs for hatching, in turn ideal chicks for breeding. There is no doubt that further research is needed to ensure the relationships among hatching egg traits influencing ostrich chick weight at hatch, especially with different breeding systems of parent stocks which are using and also with different incubation conditions of ostrich eggs which are applied. From the statistical point of view in the present study, it could be reported that the egg weight and its components will remain the main and only factors affecting chick weight at hatch and may be also by early later days of age.

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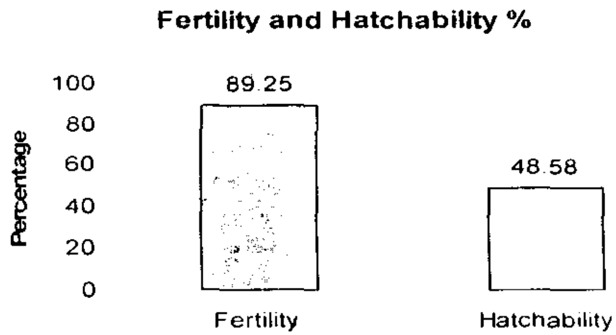

Figure 1. Fertility and hatchability percentages of ostrich eggs.

Table (1): Means ± SE for chick weight and egg characteristics in ostrich.

Trait	Mean ± SE
Egg weight, g	1334.55 ± 7.42
Egg weight loss, g	200.4 ± 1.21
Egg weight loss, %	15.02 ± 0.05
Chick weight, g	845.40 ± 5.39
Chick weight, %	63.44 ± 0.24
Shell weight, g	257.07 ±3.13
Shell weight, %	19.25 ± 0.20
Shell thickness, mm	1.91 ± 0.012
Egg surface area, cm^2	554.81 ± 2.03
Pores count in cm^2	26.67 ± 0.54
Total pores count on egg surface	14796.4 ± 303.73

Table (2): Means ± SE for egg weights (g) and egg weights loss (%) in ostrich during different periods of incubation.

Trait	Mean ± SE	
Egg weight, g		
Egg weight at set	1334.55 ± 7.42	
Egg weight at 10^{th} day	1286.55 ± 7.09	
Egg weight at 20^{th} day	1236.50 ± 6.85	
Egg weight at 30^{th} day	1185.25 ± 6.58	
Egg weight at 40^{th} day	1134.15 ± 6.40	
Egg weight loss (EWL), g		EWL%
Egg weight loss1 (0- 10 day)	48.0 ± 0.48	3.6
Egg weight loss 2 (10- 20 day)	50.1 ± 0.44	3.8
Egg weight loss 3 (20- 30 day)	51.3 ± 0.45	4.1
Egg weight loss 4 (30- 40 day)	51.1 ± 0.39	4.3
Egg weight loss (0- 40 day)	200.5 ± 1.22	15.0

Table (3): Pearson correlation coefficients between chick weight and egg weights in ostrich during different periods of incubation.

Egg weight	Correlation coefficient
Egg weight at set	0.81***
Egg weight at 10th day	0.82***
Egg weight at 20th day	0.81***
Egg weight at 30th day	0.81***
Egg weight at 40th day	0.82***

***P≤0.001

Table (4): Pearson correlation coefficients between chick weight and egg weights loss% in ostrich during different periods of incubation.

Egg weight loss %	Correlation coefficient
Egg weight loss1 (0- 10 day)	0.06
Egg weight loss 2 (10- 20 day)	-0.05
Egg weight loss 3 (20- 30 day)	-0.01
Egg weight loss 4 (30- 40 day)	-0.05
Total egg weight loss (0- 40 day)	-0.12*

*P≤0.05

Table (5): Pearson correlation coefficients between chick weight and eggshell characteristics in ostrich.

Trait	Correlation coefficient
Egg shell area	0.82***
Shell %	- 0.37***
Shell thickness	- 0.02
Pores count in cm^2	- 0.03
Total pores count	0.11

***P≤0.001

Table (6): Pearson correlation coefficients between egg weight eggshell characteristics in ostrich.

Trait	Correlation coefficient
Egg shell area	0.99***
Shell weight	0.49***
Shell thickness	- 0.06
Pores count in cm^2	- 0.03
Total pores count	0.15

***P≤0.001

Table (7): Prediction equations to predict chick weight using egg weight (EW), shell weight (SW) and egg weight loss (EWL).

Equation (E)	Intercept	Regression coefficient			R^2
		EW	SW	EWL	
E1= 51.95 + 0.60EW	51.95	0.60**	-	-	0.67
E2= 35.28 + 0.77EW- 0.83 SW	35.28	0.77**	-0.83**	-	0.84
E3= 51.11+ 0.94 EW- 0.85 SW-1.20EWL	51.11	0.94**	-0.85**	1.20	0.86

**$P \leq 0.01$

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الملخص العربي

استخدام تحليل الانحدار التدريجي لتقدير العوامل المؤثرة على وزن كتكوت النعام عند الفقس

صلاح الدين عبد الرحمن الصفتي

كلية الزراعة، قسم إنتاج الدواجن، جامعة عين شمس، القاهرة ، مصر

تهدف هذه الدراسة بشكل أساسي إلى وصف وتقدير العلاقة بين صفات الفقس فيما بينها وعلاقتها بوزن كتكوت النعام عند الفقس، بالإضافة إلى تقدير العوامل الرئيسية المؤثرة على وزن الكتكوت الفاقس والتي تم ترتيبها باستخدام تحليل الانحدار التدريجي. تم جمع عدد (١٢٠٠) بيضة من قطيع النعام الافريقي أسود الرقبة بمشروع "وادي بيا" بشمال ليبيا. وكانت الصفات المدروسة خلال التجربة هي نسب الخصوبة والفقس ووزن البيض والفقد في وزن البيض خلال مراحل التفريخ المُختلفة وصفات قشرة البيضة (الوزن والسمك والمسامية ومساحة السطح) ووزن الكتكوت عند الفقس. وكانت اهم النتائج المتحصل عليها من التجربة هي: سجل متوسط وزن الكتكوت عند الفقس ونسبته من وزن البيضة الأبتدائي ٨٤٥.٤ جرام و٦٣.٤% على الترتيب. سجلت كل من الفترة الثالثة والرابعة أعلى نسب فقد في وزن البيضة خلال مراحل التفريخ المُختلفة. تم تسجيل مُعاملات ارتباط عالية وإيجابية بين وزن الكتكوت ووزن البيض المُسجل عند فترات التفريخ المُختلفة (منذ وضع البيض حتى اليوم الأربعون من التفريخ، بمُعامل ارتباط بلغ تقريباً ٠.٨٢). تم تسجيل ارتباط سلبي وبدرجة معنوية بين وزن الكتكوت وإجمالي الفقد في وزن البيضة خلال مرحلة التفريخ (حتى اليوم الاربعون من التفريخ). كانت العوامل الرئيسية المؤثرة على وزن الكتكوت الفاقس على الترتيب هي وزن البيضة ووزن القشرة والفقد الحادث في وزن البيضة، بينما لم يلاحظ أي تأثير لعدد ثغور قشرة البيضة على أوزان الكتاكيت الفاقسة.