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Full Length Article

Effect of Laying Month and Storage Length on the Hatchability of Ostrich (*Struthio camelus*) Eggs

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Abstract

The effects of laying month and length of the storage period on hatchability of ostrich (*Struthio camelus*) eggs were studied. A total of 1,740 hatching eggs gathered between April and May from a farm located in the province of Córdoba, Southern Spain, were stored at 17–18.5°C and 30% RH for periods varying from 1 to 28 days, and subsequently incubated at 36.4°C and 22% RH. It was found that egg weight increased as the laying season progressed, with the eggs laid in April showing lower weights than the eggs laid from May to August ($P < 0.05$). There was also a seasonal effect on hatchability ($P < 0.001$), that peaked in May. It was found an effect of the length of storage period on hatchability ($P < 0.001$). Storage of ostrich eggs up to 12 d did not affect subsequent hatchability under profitable conditions, although a significant decline in hatchability occurred when the eggs were stored for longer periods. This finding has practical implications for successful hatchery management because allows prolonged storage of eggs, while being gathered sufficient-sized batches to be incubated and shipment of long shelf-life hatching eggs at ostrich farms, while maintaining optimum hatchability until further incubation. © 2014 Friends Science Publishers

Keywords: *Struthio camelus*; Egg storage; Artificial incubation; Egg weight loss; Embryonic mortality

Introduction

Ostrich farming has become an alternative livestock system in many countries across the world during last decades (Carbajo *et al.*, 1995; Anderloni, 2000). Semi-intensive ostrich production system that optimises reproductive performance requires the artificial incubation of the eggs as a technique to produce viable chicks (Burger and Bertram, 1981; Deeming, 1995a; Cooper, 2001). Until now most of the incubation centres are managed according to procedures based on daily collection of eggs from nests, and subsequent storage of these eggs until their incubation (Carbajo *et al.*, 1995). The length of this storage period determines the workload of each incubation centre, mainly due to the marked seasonality of the laying pattern of the ostriches which leads to an increased laying frequency in the middle of the reproductive season (Carbajo *et al.*, 1995; Dzoma, 2010). Moreover, factors as the pre-storage treatments, storage temperature and a too long storage period are also known that influence the viability of the eggs in several poultry species (González-Redondo, 2010; Lotfi *et al.*, 2011) including ostriches (Szabo-Willin and Tavas, 1999; Sahan *et al.*, 2003b; 2004; Malecki *et al.*, 2005). This leads to a variation in hatchability and could worsen the results of the incubation process. The balance between obtaining acceptable hatching rates and optimisation of volume of

eggs to be stored and subsequently incubated in each incubation centre is crucial for the profitability of one of the processes that make production costs high in ostrich farming, and is highly related to the eggs storage time. The maximum duration of time for storing ostrich eggs, while maintaining optimum hatchability, however, has not been clearly established. Thus, several authors (Krawinkel, 1994; Wilson *et al.*, 1997; González *et al.*, 1999; Schalkwyk *et al.*, 1999; Szabo-Willin and Tavas, 1999; Nahm, 2001; Sahan *et al.*, 2003b; Malecki *et al.*, 2005) have investigated the effects of heterogeneous storage periods lasting from one to three weeks, although do not unequivocally establish the lifespan of ostrich hatching eggs before incubation. In consequence, a more consistent research on long-term storage of ostrich eggs under farming conditions is lacking. In this context, the present study investigated the effects of the laying month and of 1 to 28-days storage periods on weight loss during the incubation, embryonic mortality and hatchability of ostrich (*Struthio camelus*) eggs under farming conditions.

Materials and Methods

Birds and Husbandry

A total of 1,740 hatching eggs were gathered from 15 trios

(one male and two females) of breeding ostriches (crossbred type with predominant involvement of South African Black ostrich, *S. camelus* var. *domesticus*) aged five years old. The breeding ostriches were housed in outdoor pens, subjected to natural lighting, in a commercial farm located in Alcaracejos (province of Córdoba; Southern Spain) with geographic coordinates 38° 25' N and 4° 67' W at an altitude of 602 meters above sea level. Eggs laying commenced during February. However, sampling was carried out in an intermediate period of the reproductive season comprised between April and August, in order to avoid low fertility of eggs laid at the beginning and at the end of the laying period, given the reproductive seasonality of this species (Madeiros, 1994; Deeming, 1995a, c; Wilson *et al.*, 1997). During the sampling period, average daily temperatures increased from 16.2 to 28.2°C and average relative humidity decreased from 53 to 34%. The eggs were collected twice daily from the nests, then being immediately disinfected by fumigation with standard quantities of 45 mL formalin (40% formaldehyde) and 30 potassium permanganate per 1 m³ of disinfection chamber. Before their incubation, eggs were kept small end down in a storage room maintained at 17 to 18.5°C and 30% RH, being turned four times a day at regular intervals. The storage periods of the eggs batches ranged from 1 to 28 days before being loaded into the incubator. The eggs were incubated at 36.4°C and 22% RH during the first 39 days in an incubator (Masa-Proavic, S.A., Vilanova i la Geltrú, Spain), being turned four times per day at regular intervals, and thereafter, were incubated in a hatcher (Masa-Proavic, S.A., Vilanova i la Geltrú, Spain) at 35°C and 30 to 40% RH without turning them until the hatch.

Data Recorded

The laying month and the length of the storage period (in days) before incubation were registered for each single egg. All eggs were weighed at the beginning and after 39 days of incubation. Weight loss after 39 d of incubation was calculated for each individual egg, as a percentage of the initial weight. In order to determine their fertility and embryonic mortality, the eggs were analyzed by candling and subsequent breakout examination of the clear eggs carried out on days 13 and 39 of incubation (Deeming, 1995b; Badley, 1996). According to its embryonic viability, the eggs were classified into five categories:

- 1). Normal hatched eggs: produced a normal chick that was viable at least 12 h after hatching.
- 2). Unfertilized eggs: showed no appreciable embryonic development in the candling carried out on day 13 of incubation.
- 3). Embryonic death: eggs that had no clearly differentiated the air cell, nor the embryo showed any movement in the candling carried out on day 39 of incubation.

4). Chick death: chicks that, between 42 and 44 days of incubation, had needed assistance at hatch and that did not survive after the first 12 h of life.

5). Infected eggs: failed to hatch because of infection with bacteria, that were identified by their bad odour or because suppurred a purulent fluid.

In some analyses, eggs from the categories 2 to 5 were grouped into a single category of unhatched eggs in order to compare it with hatched eggs. Hatchability was calculated as the percentage of successfully hatched eggs with respect to the incubated eggs.

Statistical Analysis

Statistical differences in the initial and final weights, as well as weight losses of the eggs during the incubation, as a function of the laying month, were analyzed by one-way analysis of variance. When differences among laying months were significant, means were separated using Tukey's multiple range tests at the 5% level of significance. Statistical differences in the hatchability of the incubated eggs as a function of the laying month were analyzed using contingency tables on which Pearson's χ^2 tests were performed. When differences among laying months were significant, hatchabilities were separated by calculating the standardised residuals. In the interpretation of the standardised residuals 1.96 was considered to be the discriminant value for a confidence level of 95%. Regression equations were calculated for predicting hatchability, incidence of dead embryos and incidence of dead chicks at hatch as a function of storage time of the eggs. A piecewise linear regression (a discontinuous regression in which the nature of the relationship between dependent and independent variables changes over the range of the independent variable, being able to differentiate two or more phases with linear relationship) was performed to predict the hatching probability for the eggs as a function of the storage time. Because it is broadly recommended not to store ostrich eggs longer than a week (Carbajo *et al.*, 1995; Anderloni, 2000; Cooper, 2001), Fisher's exact tests were calculated to compare, by pairs, the average hatchability for the eggs stored up to 7 days and the eggs stored for longer periods and to estimate the expected frequency of 60% of hatching eggs ($n = 1,000$ observations) needed to reach a minimum profitability in an ostrich hatchery (Adams and Revell, 1998; Leitón, 2006; Kontecka *et al.*, 2011). A multiple general linear separate-slope model was calculated to predict and compare hatchability, for each single laying month comprised between April and August, as a function of the initial weight of the eggs and its storage time. The relative components of the variance for the effects of laying month, storage time of the eggs, initial egg weight, and egg weight loss after 39 days of incubation on the variability of hatchability were also calculated. The analyses were carried out using Statistica v.6.0 software (Statsoft Inc[®]).

Results

Egg Weight

Table 1 shows egg weights at the beginning and after 39 days of incubation, as well as egg weight loss after 39 days of incubation, according to the laying month, for the laying period comprised between April and August. Average weight of the eggs at the beginning of the incubation was 1,526.5 g, and increased as the laying season progressed, with the eggs laid in April showing lower weights than the eggs laid from May onwards ($P<0.05$). On the other hand, as a consequence of the differences in the initial weights, egg weights at 39 days of incubation (1,308.8 g) also differed ($P<0.01$) with the laying month (Table 1). Egg weight loss after the first 39 days of incubation averaged 14.3% and showed differences according to the laying month (Table 1).

Hatchability

Average hatchability was 54.2% (Table 2 and 3). There was a seasonal effect on hatchability ($P<0.001$; Table 2), which increased from April to May, when it peaked. Moreover, hatchability showed a spurious, high fall by July (Table 2).

Hatchability of ostrich eggs was affected by the length of the storage period before incubation (Table 3), it being markedly reduced for longer periods. The regression equation describing hatchability as a function of storage time of eggs was (Fig. 1; $R^2=0.937$;

Table 1: Egg weight and egg weight loss during incubation in ostrich eggs according to the laying month¹

Laying month	n	Egg weight at the beginning of incubation (g)	Egg weight after 39 days of incubation (g)	Egg weight loss after 39 days of incubation (%)
April	187	1,498.9±10.68 ^b	1,275.0±9.66 ^b	14.9±0.26 ^a
May	330	1,532.0±8.04 ^a	1,319.6±7.27 ^a	13.8±0.20 ^b
June	343	1,539.8±7.89 ^a	1,312.1±7.13 ^a	14.8±0.20 ^a
July	121	1,532.5±13.28 ^a	1,312.2±12.01 ^a	14.4±0.33 ^a
August	217	1,520.5±9.91 ^a	1,315.2±8.97 ^a	13.5±0.25 ^b
Total	1,198	1,526.5±9.56	1,308.8±8.62	14.3±0.24
P		0.032	0.004	<0.001

¹Mean±SEM

^{a-c}Means in the same column with different superscripts are significantly different

Table 2: Hatchability in ostrich eggs according to the laying month

Laying month	Number of eggs		Hatchability (%)
	Incubated	Hatched	
April	213	126	59.2 ^b
May	479	351	73.3 ^a
June	407	253	62.2 ^a
July	401	43	10.7 ^c
August	240	170	70.8 ^a
Total	1,740	943	54.2
P			<0.001

^{a-c}Values with different superscripts are significantly different

Table 3: Hatchability and embryonic mortality in ostrich eggs according to the storage time of eggs before incubation

Storage time (days)	Eggs stage (n)					Hatched	Hatchability (%)
	Total	Unfertilized	Dead embryo	Dead chick	Infected egg		
1	5	1	0	0	0	4	80.0
2	10	2	0	0	0	8	80.0
3	78	6	10	7	0	55	70.5
4	129	9	12	5	1	102	79.1
5	118	14	9	6	0	89	75.4
6	157	24	30	11	0	92	58.6
7	183	31	21	17	1	113	61.8
8	147	13	12	8	0	114	77.6
9	115	13	14	4	1	83	72.2
10	98	11	7	9	1	70	71.4
11	74	11	7	5	0	51	68.9
12	54	6	8	8	0	32	59.3
13	66	18	8	4	0	36	54.6
14	72	23	5	7	1	36	50.0
15	41	6	13	1	0	21	51.2
16	37	8	15	0	0	14	37.8
17	41	9	18	4	0	10	24.4
18	34	14	16	0	0	4	11.8
19	55	28	20	3	0	4	7.3
20	36	26	6	3	0	1	2.8
21	31	24	5	1	0	1	3.2
22	34	26	6	0	0	2	5.9
23	26	22	4	0	0	0	0.0
24	36	34	1	0	0	1	2.8
25	24	22	2	0	0	0	0.0
26	16	14	2	0	0	0	0.0
27	19	19	0	0	0	0	0.0
28	4	4	0	0	0	0	0.0
Total	1,740	438	251	103	5	943	54.2

$P<0.001$):

$$H = 1.012 - 0.204 \times ST + 0.046 \times ST^2 - 0.004 \times ST^3 + 1.533 \times 10^{-4} \times ST^4 - 1.965 \times 10^{-6} \times ST^5$$

Where, H: hatchability (%), and ST: storage time of the egg (days).

Embryo Mortality

Table 3 shows embryonic mortality in ostrich eggs according to the storage time of eggs before incubation. Part of the loss in hatchability was due to an increase in dead embryos as storage time of the eggs was prolonged (Table 3; Fig. 2). In fact, incidence of dead embryos clearly peaked between 15 to 19 days of storage before incubation. The regression equation describing incidence of dead embryos as a function of storage time of eggs was ($R^2=0.723$; $P<0.01$):

$$DE = -0.199 + 0.21 \times ST - 0.048 \times ST^2 + 0.005 \times ST^3 - 1.861 \times 10^{-4} \times ST^4 + 2.618 \times 10^{-6} \times ST^5$$

Where, DE: dead embryos (%), and ST: storage time of the egg (days).

In the same vein, reduction in hatchability was also due to chicks failing at hatch (Table 3; Fig. 3). Thus, the

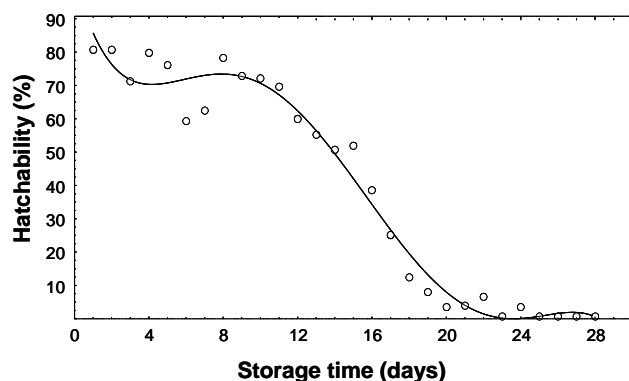


Fig. 1: Fifth-degree polynomial fit for hatchability as a function of the storage time of the eggs

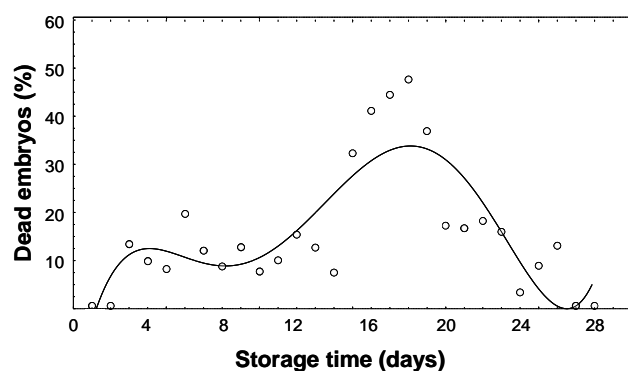


Fig. 2: Fifth-degree polynomial fit for dead embryos as a function of the storage time of the eggs

regression equation describing incidence of dead chicks at hatch as a function of storage time of eggs was ($R^2=0.494$; $P<0.05$):

$$DC = -0.008 + 0.021 \times ST - 0.002 \times ST^2 + 8.206 \times 10^{-5} \times ST^3 - 2.831 \times 10^{-6} \times ST^4 + 4.601 \times 10^{-8} \times ST^5$$

Where, DC: dead chicks at hatch (%), and ST: storage time of the egg (days).

Incidence of infected (suppurated) eggs in the present trial was very low (Table 3).

Maximum Viable Eggs Storage Period before Incubation

Due to the fact that hatchability of ostrich eggs declined with the length of the storage period before their incubation (Table 3 and Fig. 1), it will be useful for the incubation centres to propose the maximum time the eggs can be held while maintaining optimum hatchability. Below, this duration is determined by the combination of three criteria: by identifying the breakpoint in a piecewise regression, by comparing hatchability between the first week of storage and longer periods, and by considering the minimum hatchability needed for ensuring profitability.

On the one hand, the piecewise linear regression

Table 4: Fisher's exact tests comparing the average hatchability of eggs stored up to seven days before incubation and eggs stored for longer periods

Storage time	Number of eggs	Hatchability (%)	Fisher's exact P ¹
Up to one week	680	68.1	-
8 day	147	77.6	0.022
9 days	115	72.2	0.292
10 days	98	71.4	0.427
11 days	74	68.9	1.000
12 days	54	59.3	0.183
13 days	66	54.5	0.030

¹Exact probability of the Fisher's test performed between hatchability of eggs stored a number of days and average hatchability of eggs stored up to 7 days

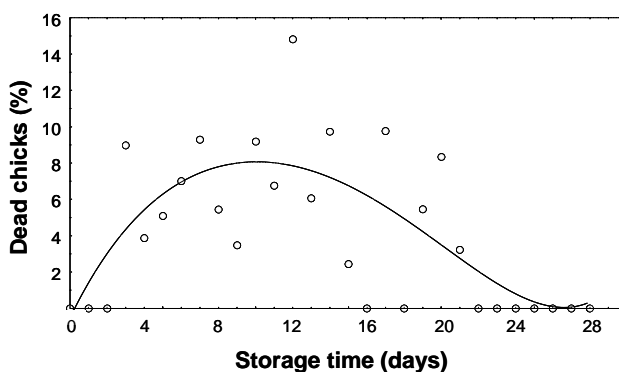


Fig. 3: Fifth-degree polynomial fit for dead chicks as a function of the storage time of the eggs

carried out to predict the hatching probability for the eggs as a function of the storage time (Fig. 4) yielded a breakpoint that corresponds to a mean value of hatching probability loss of 0.3815, that it is reached on day 14 of storage with a $R^2 = 92.9\%$ ($P<0.001$). This piecewise regression consisted of two phases:

$$HP = 0.823340 - 0.018713 \times ST, \text{ for } PH > 0.38151$$

$$HP = 0.155982 - 0.004281 \times ST, \text{ for } PH \leq 0.38151$$

Where, HP: hatching probability (range: [0, 1]), and ST: storage time of the egg (days).

The second criterion was based on the broad recommendation to avoid storing ostrich eggs longer than a week. Thus, the Fisher's exact tests carried out to compare, by pairs, the average hatchability for the eggs stored up to 7 days and the eggs stored for longer periods (Table 4) showed that when the eggs were stored for 13 days the hatchability was significantly reduced in comparison to the average one showed by the eggs stored up to one week. Thus, under this criterion the eggs could be stored up to 12 days without losing viability.

The third criterion implies that, in order to maintain a minimum profitability, in an ostrich hatchery it is necessary to reach at least 60% in hatchability. Thus, by performing a Fisher's exact test for an expected frequency of 60% of hatching eggs for $P < 0.05$ ($n=1,000$ observations), this implies that the observed frequency of hatched eggs should

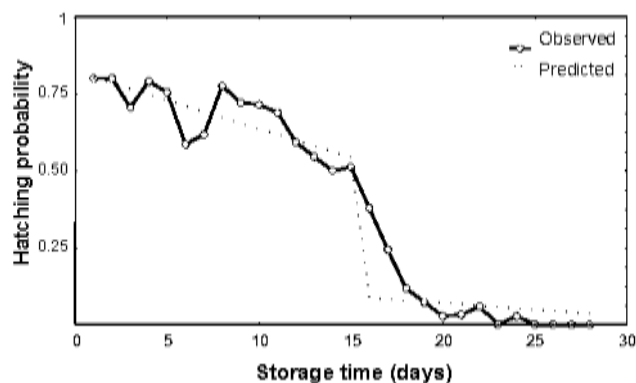


Fig. 4: Piecewise regression fit for hatching probability as a function of the storage time of the eggs

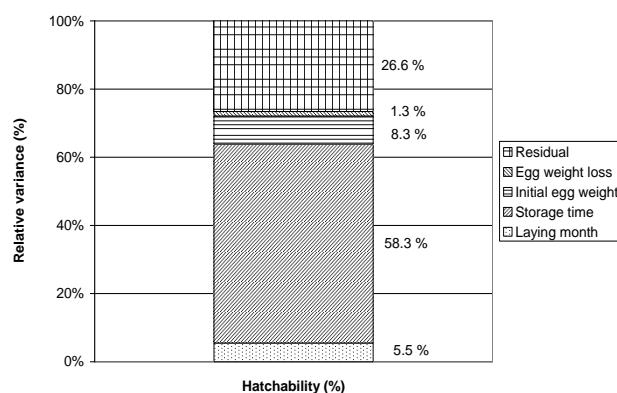


Fig. 5: Relative components of the variance for the effects of laying month, storage time, initial egg weight, egg weight loss after 39 days of incubation on the variability of hatchability (%)

be at least 56%. This corresponds to eggs stored for a maximum of 12 days, because if storage period is prolonged up to 13 days, hatchability is then reduced to 54.6% (Table 3 and 4).

Prediction of Hatchability Across the Laying Season

A regression analysis ($R^2=0.675$; $P<0.001$) enabled to predict hatchability, for each single laying month comprised between April and August, as a function of the variables that can be controlled and measured before the eggs are incubated (the initial weight of the eggs and its storage time):

$$\text{April: } H = 8.779 + 0.0479 \times IW - 2.442 \times ST$$

$$\text{May: } H = 8.779 + 0.0479 \times IW - 4.225 \times ST$$

$$\text{June: } H = 8.779 + 0.0479 \times IW - 6.993 \times ST$$

$$\text{July: } H = 8.779 + 0.0479 \times IW - 7.526 \times ST$$

$$\text{August: } H = 8.779 + 0.0479 \times IW + 18.098 \times ST.$$

Where, H: hatchability (%), IW: initial weight of the egg (g), and ST: storage time of the egg (days).

Suitability of the Model Studied

The relative components of the variance for the effects of laying month, storage time of the eggs, initial egg weight, and egg weight loss after 39 days of incubation on the variability of hatchability is shown in Fig. 5. The most relevant variable to explain hatchability was storage time of the eggs, which amounted nearly 60% of the variance. The second variable explaining hatchability was the initial egg weight, contributing 8.3% of the variance. The laying month and the egg weight loss at the end of the incubation period explained only 5.5 and 1.3% of the variance in hatchability, respectively.

Discussion

Average weight of the eggs at the beginning of the incubation was in keeping agreement with that described in the literature for this species under farming conditions. Specifically, the initial weight of the eggs found in the present research coincided with 1,522 g found by Hassan *et al.* (2004) and was within the range of 1,500 to 1,600 g described by Deeming (1997). However, we found egg weights higher than 1,453 g reported by Brown *et al.* (1996), something that can be attributed to differences in the subspecies of ostriches involved in each case (Cloete *et al.*, 2006).

As observed in this trial, several authors also describe a seasonal effect in the egg size as a function of the laying month (Rizzi *et al.*, 2002; Cloete *et al.*, 2006; Elsayed, 2009), that is, in part, due to the improvement of the reproductive performance as the reproductive season progresses, markedly influenced by the environmental conditions, mainly photoperiod and temperature (Shanawany and Dingle, 1999). It is also known that the increase in egg weight towards the end of the breeding season can be explained by the fact that it takes some time for the body condition of the females to restore after the improvement in nutrition at the start of the breeding season, especially when little effort is made to maintain the breeder's condition during the off season (Madzingira *et al.*, 2000).

Egg weight loss after the first 39 days of incubation found in this study was comparable to 15% reported by Deeming (1997) for ostrich eggs weighing 1,500 g at the beginning of the incubation, and 13-15% reported by Ar *et al.* (1996) as the optimum weight loss to achieve a successful incubation. In the same vein, Hassan *et al.* (2004) find variations in the range of 13 to 15% of the initial weight when incubation temperature is changed from 36.5 to 37.5°C and relative humidity is changed from 20 to 30%, respectively. Brown *et al.* (1996) find 13.3% of weight loss after incubating eggs at 36°C and 37% RH, a humidity level that is substantially higher than 22% set in our incubation.

The absence of a definite pattern in the variation of the egg weight loss during the incubation according to the

laying month in the present trial agrees with other authors that do not find differences in egg weight loss during incubation nor within the same breeding season (Rizzi *et al.*, 2002; Elsayed, 2009) or among different breeding seasons (İpek and Şahan, 2004; Zoccarato *et al.*, 2004).

Average hatchability found in this trial could be considered acceptable taking into account that, in general terms, the artificial incubation of ostrich eggs is characterised by low hatchability and is a major source of loss in the ostrich farming (Van Zyl, 1997; Cooper, 2001). This was a higher value than 14.5-25.9% found by Hassan *et al.* (2004), than that found by Deeming (1995a) in eggs from farms in Zimbabwe whose hatchability was only 37.2% and the result of high rates of infertility and contamination. However, the hatchability found in this research showed a lower value than 63.3% found by Nahm (2001) and 64 to 74% described by Sahan *et al.* (2003a). The hatchability found in the present study agrees with the values found for eggs submitted to a similar turning pattern during incubation (Schalkwyk *et al.*, 2000).

The seasonal effect on hatchability observed in this research is consistent with the marked reproductive seasonality characterising this species (Dzoma, 2010). Thus, Wilson *et al.* (1997) describe a linear reduction in hatchability as the reproductive season progress, and Deeming (1995a, c) highlights the importance of the laying time within the laying season in influencing this parameter. The spurious, high fall in hatchability observed by July (Table 2), probably due to a variation in microclimatic conditions those are known to influence hatchability (Deeming, 1995c).

As discussed below, and in keeping agreement with that described in the literature (Wilson *et al.*, 1997; Szabo-Willin and Tavas, 1999; Cooper, 2001; Sahan *et al.*, 2004), hatchability of ostrich eggs was affected by the length of the storage period before incubation.

Most part of the loss in hatchability observed in this research was attributable to an increase in dead embryos as storage time of the eggs was prolonged (Deeming, 1995a; Deeming, 1996). In the same vein and as reviewed by Cooper (2001), reduction in hatchability was also due to chicks failing at hatch. Contrarily, incidence of infected eggs in the present trial was very low compared to the findings of other authors (Cooper, 2001), something that indicates a good eggs handling.

For a joint consideration of the three criteria analysed for egg storage period, it is proposed that, under the experimental conditions described, ostrich eggs may be stored while maintaining their viability up to 12 days. This storage time without losing hatchability would be longer than that found in ostriches by many other authors (7 days by Krawinkel, 1994, Szabo-Willin and Tavas, 1999 and less than 7-8 days by Sahan *et al.*, 2004). Our results confirm previous research stating that, if necessary, ostrich eggs may be stored for long periods without relevant loss in hatchability (10 days by González *et al.*, 1999; 12 days by

Horbaniczuk, 2000; 14 days by Nahm 2001). As suggested by González *et al.* (1999), the nesting behaviour of the ostrich in the wild may help to explain the longevity of eggs from this species, which has a long laying interval and often brood a clutch of eggs the first of which have been left up to 3 weeks before brooding starts (Bertram and Burger, 1981).

The equations fit to predict hatchability for each single laying month are useful because enables to predict the expected hatchability for eggs produced in each time of the laying season and as a function of storage time and its initial weight, a factor that it is also known that affects hatchability (Cooper, 2001).

Analysis of the relative components of the variance confirms the strong influence of the storage time in the viability of the hatching eggs of ostrich, as discussed previously. The importance of other studied variables in determining hatchability, as the initial egg weight, laying month and egg weight loss, was lower. On the other hand, the remaining 26.6% of unexplained variance is likely due to other factors, as service sire (Cloete *et al.*, 2006), genetics, or nutritional ones (Dzoma, 2010), not considered in this study.

In conclusion, long-term storage of ostrich eggs before incubation may be feasible up to 12 days without relevant loss in hatchability when these are kept at 17-18.5°C, 30% RH, and turned four times a day. After this initial period however, hatchability is liable to decline rapidly. In addition to storage time, hatchability of each single egg is also modulated by its weight and by the laying month. The length of time ostrich eggs can be held without impairment to the potential embryo has practical implications, particularly for two reasons. Firstly, to hatch in small farms useful sized batches of chicks at the beginning and at the end of the laying season, because in these periods the eggs are stored for many days to accumulate greater numbers. This reduces the need to incubate small batches or to throw away the earliest and latest eggs, that are laid at low rates because the eggs production increases and decreases slowly in these periods of the laying season, given the marked reproductive seasonality of the ostrich. Secondly, for shipment of long shelf-life hatching eggs, while maintaining optimum hatchability until further incubation, because these are one of the commercial products of the ostrich farms.

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