Effects of time of egg collection and pre-incubation treatment on blastoderm development and embryonic mortality in ostrich embryos

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The hatching success of artificially incubated ostrich eggs can be influenced by egg management prior to setting the eggs in the incubator. This can include the length of time and conditions to which eggs are exposed in nests prior to collection and the conditions under which eggs are stored prior to incubation. We investigated the effects of time of egg collection, storage position and temperature, and pre-storage conditions on blastoderm development and embryonic mortality during incubation in ostrich eggs. Eggs collected soon after laying tended to have lower levels of embryonic mortality (16.6%) than eggs left overnight in nests and collected the following morning (22.9%). Embryonic mortality was not, however, significantly affected by storing eggs in either the vertical (with the air cell at the top or the air cell at the bottom) or horizontal position. The development of the blastoderm was studied in eggs stored for seven days at 20, 25, 26 and 27°C. Storage at 25, 26, and 27°C resulted in significant increases in blastoderm size after seven days compared to eggs stored at 20°C (12.1–42.1 mm vs 6.0 mm). The effect of storage temperature was tested by subjecting batches of ostrich eggs to five treatments; (1) Stored for <7 days at 17°C immediately after collection (control); (2) Stored for <7 days at 25°C immediately after collection; (3) Incubated at 36°C for 12 h prior to storage at 25°C; (4) Incubated at 36°C for 12 h prior to storage at 17°C; (5) Incubated at 36°C for 48 h prior to storage at 25°C. The latter three treatments simulated eggs that were subjected to high summer temperatures in their nests for varying periods prior to collection. Embryonic mortality was lowest in batches of eggs stored at 17°C immediately after collection (26.7%) and when incubated at 36°C for 12 h before storage at 17°C (31.8%). The embryonic mortality of eggs stored at 25°C immediately after collection averaged 45% but increased significantly to exceed 50% in batches of eggs exposed to 36°C for 12 or 48 h before being stored at 25°C. Pre-heating batches of ostrich eggs at 36°C for 4 h after collection and prior to storage at 17°C resulted in a significant reduction in embryonic mortality compared to eggs not subjected to pre-heating (17.1 vs. 26.2%). Embryonic mortality was not affected by pre-warming ostrich eggs from storage temperature to incubation temperature prior to setting in the incubator.

Keywords: Ostrich eggs, storage, collection, artificial incubation, embryo mortality

Introduction

Commercially farmed ostriches in South Africa are mostly flock-mated in large (20–100 ha) breeding paddocks (Van Schalkwyk *et al.*, 1996). Eggs are collected from the paddocks only two or three times a week on some farms or daily in more intensive systems. Infrequent collection of eggs potentially exposes them to the vagaries of the weather and to bacterial contamination. Baxter-Jones (1991), for example, indicated that the frequent collection of nest-laid chicken eggs is essential for the production of eggs with a low bacterial count and Deeming (1996) showed that microbial contamination was a major cause of failure of ostrich eggs to hatch in the United Kingdom. Furthermore, in the Klein Karoo region of South Africa, where most ostriches are farmed, daytime temperatures are usually high and can even exceed 40°C during summer. Eggs not collected frequently can consequently be exposed to such conditions for extended periods prior to collection, particularly when nests in large paddocks are overlooked. Moreover, eggs are generally set only once a week, and may therefore be stored for up to six days before setting in the incubators. Storage temperatures vary from 17°C on farms with temperature-controlled facilities to room temperature, which can exceed 30°C on farms without such facilities. For most birds (including commercially incubated species), the critical temperature for the initiation of embryonic development appears to be about 25–27°C (Funk & Biellier, 1944; Lundy, 1969; Drent, 1975), although lower storage temperatures of 16–17°C are normally recommended for chicken eggs (Mayes & Takeballi, 1984). Although presumably similar to other birds, there is no information on the temperature required to initiate blastoderm development in ostrich eggs and although storage periods exceeding seven days are known to depress hatchability (Wilson *et al.*, 1997), optimum storage temperatures are not known.

Apart from length of time and temperature of storage, there are several other aspects of egg storage that can influence subsequent successful incubation. The position of eggs during storage, for example, may affect hatchability. Hatchability in chicken eggs is improved when eggs are stored with their pointed end up for >10 days (Proudfoot & Hulan, 1983; Butler, 1991) but we could find no information in the literature to indicate that this might apply to ostrich eggs. Deeming (1997) also suggested that ostrich eggs stored at relatively low temperatures should not be placed directly into incubators because of possible condensation of moisture on the eggs and lowering of incubator temperature. Fasenko *et al.* (1994) suggested that longer nest-holding times improved post-laying pre-incubation embryonic growth in turkeys. Deeming (1996) and Van Schalkwyk (1998) acknowledge the possible advantages of nest holding time, but because ostriches do not begin incubation until all eggs are laid (Bertram, 1980), they suggested that it should be balanced against collection soon after lay to reduce the probability of microbial spoilage. Against this background, we investigated embryonic mortality of artificially incubated ostrich embryos in relation to the collection and handling of eggs prior to setting in the incubator.

Materials and methods

Eggs used in the study were obtained from the commercial ostrich breeding flock at the Klein Karoo Agricultural Development Centre near Oudtshoorn. The management of the breeding flock and the treatment of eggs were described by Van Schalkwyk *et al.* (1996). Five separate trials were conducted on batches of eggs subsequently incubated in electronic incubators:

Trial 1: time of egg collection

Batches of ostrich eggs were collected according to two treatments; namely late afternoon between 16:00 and 18:00 h (2–3 h after being laid) or in the morning between 09:00 to 11:00 (16–18 h after

being laid). Eggs were stored for a maximum of six days at 17°C and 75% RH before being set. Each treatment was replicated eight times with 13–63 eggs constituting a replication, depending on numbers available. Eggs were subsequently incubated at 36°C and a relative humidity of 28% in an electronic Buckeye® incubator (Buckeye Poultry Equipment, P.O. Box 1749, Krugersdorp, 1749, South Africa). Eggs were incubated in the horizontal position for two weeks, before being placed in the vertical position (Van Schalkwyk, 1998). Eggs were rotated hourly through 60° throughout. The eggs were candled at 21 days, using a 150 W candling lamp. Eggs not fitting the developmental stage of ostriches at that time (Van Schalkwyk *et al.*, 1994) were opened and inspected for embryonic development. Eggs not showing any development were regarded as infertile, and those with embryonic development that had ceased as embryonic mortality. Subsequent shell deaths (between 22 and 42 days) were also classified as embryonic mortality. Infertility, embryonic deaths and the hatching of live chicks were recorded individually, and totalled for batches of eggs. Infertility was expressed as a proportion of eggs set, and embryonic mortality as percentage of fertile eggs within batches.

Trial 2: storage position

Batches of ostrich eggs were stored at a constant temperature of 17°C and a relative humidity of 75% in a storeroom for a maximum of six days. The eggs were placed into Buckeye® trolley trays which were turned once a day through an angle of 90°. Before storage, the position of the air cell was determined by candling the egg with a 3 V flashlight with a head circumference of 2.5 cm. By placing the light source tightly against the shell surface on either side the air cell of approximately 3 cm could clearly be discerned. On each egg, the position of the air cell was marked by pencil. Batches of 30– 68 eggs were subsequently allocated to one of the following three treatments at random:

- vertical with air cell at the top,
- vertical with air cell at the bottom, or
- horizontal.

Eggs collected on day 7 were set as fresh eggs 3–5 hours after collection as a fourth treatment, together with eggs from the other three treatments.

After storage for 2–6 days these batches of eggs were set in Buckeye® electronic incubators. Incubation procedures as well as the recording of infertility and embryonic deaths were as described above. Each treatment was replicated four times during consecutive weeks.

Trial 3: critical zero temperature

Batches of eggs were stored at constant temperatures of 20 (n = 5; control), 25, 26 and 27°C (n = 10 each) in La Nationale® incubators. After storage for seven days, the eggs were opened and the diameter of the blastoderm of each egg was measured to the nearest 0.1 mm with a calliper.

Trial 4: storage temperature conditions

Depending on numbers of eggs available, batches of 8–17 eggs were allocated to one of the following five treatments at random:

- stored at 17°C immediately after collection (control),
- stored at 25°C immediately after collection,
- incubated at 36°C for 12 h before being stored at 25°C,
- incubated at 36°C for 12 h before being stored at 17°C, or
- incubated at 36°C for 48 h before being stored at 25°C.

The latter three treatments simulated eggs that were subjected to high summer temperatures in paddocks for varying periods prior to collection. Each treatment was replicated six times during consecutive weeks. Subsequent incubation procedures and the recording of infertility and embryonic deaths were as described previously.

Trial 5: pre-storage and pre-incubation heating

Batches of 25–55 eggs collected in the afternoon were allocated to four treatments in a 2 (heated prior to storage or not) × 2 (heated prior to incubation or not) factorial design. The first factor included the pre-heating of eggs for 4 hours after collection at 36°C before storage for 2–6 days at 17°C. The second factor included the pre-heating of eggs overnight in the incubation room at 25°C for 16 hours before setting. The respective control treatments were eggs stored for 2–6 days at 17°C immediately after collection, and the immediate transfer of eggs stored at 17°C to an incubator operating at 36°C. Each treatment was replicated four times during consecutive weeks. Subsequent incubation procedures and the recording of infertility and embryonic deaths were as described.

Statistical analysis

All means are presented ± Standard Error. The effects of collection time, storage position and temperature were assessed in completely randomized statistical designs. Preliminary analyses had the data blocked by week of incubation. In the absence of significant ($P > 0.05$) block effects, it was decided to pool the degrees of freedom for blocks with that of remainder. The effect of pre-storage and pre-incubation heating of ostrich eggs was assessed in a 2 × 2 factorial design (Snedecor & Cochran, 1967). A number of the percentages analysed were outside the 30–70% range. These data were transformed to angles, using the arcsine transformation (Snedecor & Cochran, 1967). Conclusions derived from the transformed data were, however, similar to those made on untransformed data. Consequently, for ease of comprehension, only untransformed percentage data are presented.

Results

Trial 1: time of egg collection

Infertility averaged 10.9 ± 1.7 %. Embryonic mortality of eggs collected between 16:30 and 18:30 in the afternoon averaged 16.6 ± 2.9% and tended to be slightly, but not significantly ($P = 0.15$) lower than eggs collected in the morning between 09:00 and 11:00 (22.9 ± 2.9%).

Trial 2: storage position

The mean infertility of eggs in this trial was similar between treatments and averaged 25.3 ± 1.8%. Embryonic deaths were not significantly affected by storage position ($P < 0.05$). Embryonic mortality averaged 21.9% for eggs stored with the air cell at the top, 21.1% for eggs stored with the air cell at the bottom, 22.9% for eggs stored in the horizontal position and 25.3% for eggs that were set immediately after collection. The SE for the comparison of the means was 2.7%.

Trial 3: critical zero temperature

No development of ostrich embryos was expected to take place in eggs kept at 20°C and this treatment was regarded as the control. Blastoderm size of eggs stored at 20°C for a week averaged 6.0 ± 0.9 mm. Eggs incubated at 25°C for a week had blastoderms that averaged 12.1 ± 0.6 mm, significantly larger ($P < 0.01$) than those of the control eggs. Increasing the storage temperature to 26 and 27°C resulted in further significant increases in blastoderm size to 20.2 ± 0.6 and 42.1 ± 0.6 mm, respectively ($P's < 0.01$).

Trial 4: storage temperature conditions

Infertility of eggs used in this experiment averaged 17.6 ± 2.5%. Early embryonic mortality was independent of treatment (Table 1). Late embryonic mortality of the treatments subjected to storage at 25°C was elevated ($P < 0.05$) relative to the Control treatment. The late embryonic mortality of the treatment stored at 17°C after incubation at 36°C for 12 hours (Treatment 3) was lower ($P < 0.05$) than that of the treatments involving storage at 25°C after incubation (Treatments 2 and 4). Overall embryonic mortality was lowest in the Control treatment, stored at 17°C immediately after collection (Table 1). Embryonic mortality of eggs incubated for 12 h prior to storage at 17°C (Treatment 3) did not differ ($P > 0.05$) from the Control treatment. In contrast, embryonic mortality was significantly elevated to 53.8 and 59.0% respectively, in eggs exposed to high temperatures for 12 and 48 h before storage at 25°C (Treatments 2 and 4 respectively). Eggs stored at 25°C throughout (Treatment 1) were intermediate for embryonic mortality, not differing ($P > 0.05$) from those stored at 17°C (Control treatment and Treatment 3), or those stored at 25°C after prior incubation (Treatments 2 and 4).

Table 1 Effect of different storage conditions on embryonic mortality of ostrich eggs

Treatment*	Incubation period	Storage	Embryonic mortality (%)		
	(h at 36°C)	temperature (°C)	Early	Late	Overall
Control	–	17	5.6	21.1[a]	26.7[a]
Treatment 1	–	25	2.1	42.7[b,c]	44.8[a,b,c,]
Treatment 2	12	25	7.0	46.8[b,c]	53.8[b,c]
Treatment 3	12	17	2.4	29.4[a,b]	31.8[a]
Treatment 4	48	25	5.2	53.8[c]	59.0[c]
SE mean	–	–	3.3	6.9	6.0*

* Means are based on 6 replications each

[a,b,c] Means followed by different superscripts are significantly ($P < 0.05$) different

Trial 5: pre-storage and pre-incubation heating

Because no significant interactions were observed, the main effects are presented separately. Infertility of eggs used in this trial were similar between treatments and averaged 31% (Table 2). Early embryonic mortality was not affected by treatment ($P > 0.05$) but late embryonic mortality was significantly reduced by nearly 10% when eggs were pre-heated before storage ($P < 0.01$). This was also reflected in an overall reduction in embryonic mortality ($P < 0.05$).

Infertility of eggs used in this trial were similar between treatments and averaged 31% (Table 3). Pre-heating ostrich eggs overnight prior to incubation did not affect the incidence of early, late or overall embryonic mortality when compared to those eggs that were not pre-heated ($P > 0.05$).

Table 2 Effect on embryonic mortality of ostrich eggs of pre-storage heating of eggs for 4 h after collection immediately prior to storage

	Pre-heating prior to storage[#]		
Parameters	No	Yes	SE mean
In relation to eggs incubated			
Infertile eggs (%)	32.3	29.0	2.3^{ns}
In relation to fertile eggs			
Early embryonic mortality (%)	5.95	6.15	1.81^{ns}
Late embryonic mortality (%)	20.2	11.0	2.1**
Embryonic mortality (%)	26.2	17.1	2.3*

[#] Means are based on eight replications of 25 to 55 eggs each

ns = not significant ($P > 0.05$)

* significant ($P \leq 0.05$)

** significant ($P \leq 0.01$)

Table 3 Effect on embryonic mortality of ostrich eggs of pre-storage heating of eggs overnight

	Pre-heating prior to incubation[#]		
Parameters	No	Yes	SE mean
In relation to eggs incubated			
Infertile eggs (%)	31.8	29.5	2.3^{ns}
In relation to fertile eggs			
Early embryonic mortality (%)	6.14	5.96	1.81^{ns}
Late embryonic mortality (%)	14.8	16.4	2.1^{ns}
Embryonic mortality (%)	20.9	22.4	2.3^{ns}

[#] Means are based on eight replications of 25 to 55 eggs each

ns = not significant ($P > 0.05$)

Discussion and conclusions

Trial 1: time of egg collection

The female ostriches used in the present study start laying from 12:00 until 17:00 (H. Lambrechts, 1998, pers. comm.). This pattern is consistent with that reported by Deeming (1996) for ostriches in Britain. Although embryonic mortality did tend to be lower in eggs collected in the afternoon soon after laying, we found no conclusive evidence to suggest that leaving eggs in nests overnight had any substantial impact on hatchability. Prolonged exposure to nest conditions in poultry houses does, however, increase the chances of subsequent microbial infection and North (1984) recommends that chicken eggs should be collected at least four times daily for optimum embryonic viability and hatchability. In ostriches in Britain, microbial spoilage is regarded as the biggest single problem in commercial chick production (Deeming, 1996). The absence of an organic cuticle on the external shell surface makes them especially prone to microbial contamination if not collected soon after laying (Deeming, 1996). Deeming (1996) also observed that microbial contamination increased significantly as the breeding season progressed. The fact that ostriches in small breeding camps use the same nesting site throughout the year may contribute to such increased microbial contamination. The birds used in the present study not only used the same nesting site throughout the breeding season but a large proportion of the pairs used the same nest every year (Van Schalkwyk *et al.*, 1996). Because ostrich nests are situated in open paddocks, it is impossible to implement nest hygiene measures as recommended for the poultry industry. Furthermore, the eggs are also subject to prevailing weather, which can include high temperatures (see below) and rain that can saturate nests. Deeming (1996) accordingly suggested that ostrich eggs should be collected soon after lay to reduce microbial spoilage, a recommendation that we support.

Trial 2: storage position

The position of chicken eggs during storage has been reviewed extensively. Storing eggs small end up without turning resulted in an increased hatch, which was not further improved by turning (Proudfoot & Hulan, 1983; Mayes & Takeballi, 1984; Butler, 1991). It was suggested that storing eggs small end up may result in the central placement of the yolk in the albumen, giving the embryo greater protection from dehydration and adhesions (Mayes & Takeballi, 1984). In contrast to chickens, no significant difference was found in the hatchability of ostrich eggs stored small end up compared to horizontal storage. The possibility of storing eggs either vertically or horizontally without adversely affecting subsequent incubation may have implications for farmers whose storage space is limited.

Trials 3 and 4: critical zero temperature and storage temperature conditions

Although there was some development of the blastoderm after seven days at 20°C, the size of the blastoderm of ostrich eggs more than tripled when eggs were stored between 25 and 27°C. This suggested that the critical zero temperature for ostrich eggs is broadly similar to that of chicken embryos as reviewed by Lundy (1969). It is also in agreement with findings of Miller & Wilson (1975) who concluded that the temperature required to initiate blastoderm development of Bobwhite quail embryos was between 24.4 and 25.6°C.

Wilson (1991) suggested that fertile chicken eggs could be stored for several days under optimal storage conditions without a major loss in hatchability. Johnson & MacIlraith (1967), however, observed that cooling turkey eggs to 13°C during the first day after collection depressed hatchability compared to storage at 22.0–26.5°C. In contrast to this, acceptable hatching performance was found in ostrich eggs stored below 20°C for 7 days immediately after collection. Hatching

performance of ostrich eggs was, however, impaired if they were stored at temperatures approaching the critical zero temperature (25°C) after exposure to elevated temperatures prior to storage. Scott *et al.* (1993) also reported higher levels of early and late embryonic deaths when chicken eggs were incubated at 30°C for 24 h prior to storage at 20°C for 7 days. Kaufman (1948) and Lundy (1969) reported that cooling of eggs previously exposed to temperatures above the physiological zero should be avoided, as embryonic development still proceeds. Cooling to a temperature that does not produce a complete cessation of development could be more harmful than cooling to a temperature that brings about complete stoppage. This finding was in accordance with earlier work by Romanoff *et al.* (1938). Taylor *et al.* (1933) and Romanoff (1960) suggested that the sensitivity of the embryo to cooling from just below the physiological zero to above freezing point increased with embryonic age. The effect of cooling on the viability of embryos beyond the 48 h developmental stage was not investigated in this study. Older ostrich embryos may suffer even more from cooling to temperatures below the physiological zero. Landauer (1967) suggested that chicken embryos had a higher resilience to cooling during the first two weeks than during the last two weeks of incubation.

Trial 5: pre-storage and pre-incubation heating

Pre-storage heating in our study simulated a longer period for nest-holding time and exposure to elevated ambient temperatures. Late embryonic mortality was significantly reduced by pre-heating ostrich eggs for a period of 4 h prior to storage at 17°C. Conversely, pre-heating for 12 h prior to storage resulted in no change in embryonic mortality (Table 1). These results are consistent with previous studies on other species. Jones (1986) and McDaniel (1990) suggested that exposure to temperatures above the physiological zero after collection allowed the embryo to continue growing and resulted in better hatchability compared to eggs cooled immediately after collection. Both Kosin (1956) and Becker & Bearse (1958) found that warming of chicken eggs for 5 h at 37.8°C prior to storage had a beneficial effect on hatchability. Lancaster & Jones (1986) found that, if pre-warming periods prior to storage exceeded 5 h, hatchability of chicken eggs stored for long periods was impaired. Fasenko *et al.* (1994) suggested that eggs collected 6 h after lay had significantly larger blastoderm diameters than those collected 1 h after lay.

Abnormalities in chicken embryos are more prevalent when development is arrested prior to or during early gastrulation or one day or more after gastrulation was completed (Hutt & Pilkey, 1930; Hays & Nicolaides, 1934). Arora & Kosin (1966) postulated that the extent of gastrulation at oviposition could influence the capability of the chick blastoderm to survive storage. In our study, blastoderm size was not measured after the pre-heating period of 4 h at 36°C. One can, however, assume that such an increase would have occurred. This could have increased the ability of embryos to survive particularly during the second half of incubation. The contamination of eggs, however, also poses a serious threat as reflected by the tendency towards a higher proportion of embryonic deaths in eggs left in the nests for any length of time. This study suggests that heating of eggs for 4 h before storage may serve as a substitute for a longer nest holding time.

Deeming (1997) suggested that the condensation of water on the shell surface should be prevented during incubation. Egg temperature may be lower than dew point for the humidity of the air in the incubator, especially when eggs were stored at temperatures <20°C. Moisture may serve as a growth medium for microbes when temperature is elevated at the commencement of incubation. This did not appear to be a problem in our study. Eggs placed directly into the incubator without pre-heating after storage showed no difference in hatchability compared to eggs that were pre-heated.

In conclusion, the results of our study suggest that overnight delays in collecting eggs have a

slight, but not significant, impact on subsequent embryo mortality. Collection of eggs soon after laying potentially reduces the risk of bacterial contamination. Eggs are best stored below 20°C after a pre-storage heating to incubation temperature for about 4h. Eggs exposed to high ambient temperatures in the nest prior to collection are generally not adversely affected provided they are stored at temperatures below that at which embryonic development takes place. Farmers who leave eggs in the nest on warm days and who subsequently store eggs at room temperatures will, however, likely experience an increase in late embryonic mortality. It does not appear necessary to pre-warm eggs prior to setting in the incubator, although doing so does not affect subsequent incubation success and will minimise cooling in the incubator if large numbers of eggs are set.

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