South African Journal of Animal Science 2020, 50 (No. 4)

Short communication

Hatch traits of artificially incubated ostrich eggs as affected by setting position, angle of rotation and season

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(Submitted 10 July 2018; Accepted 2 August 2020; Published 27 September 2020)

Abstract

High levels of hatching failure in artificially incubated ostrich eggs cause considerable loss of income for the industry. In the 2015 - 2016 breeding seasons, between 846 and 1 549 egg records were used to determine the effect of various setting positions during artificial incubation. Fresh eggs were placed in trolleys in the setter that were turned automatically hourly through 60 degrees or 90 degrees. The additional treatments as factorial design included eggs set horizontally for five weeks in the setter; in horizontal position for three weeks and vertically for two weeks; and vertically for five weeks. These treatments were repeated over two production years to represent the seasons, namely winter (June to August), spring (September to November), and summer (December). Late embryonic mortalities improved significantly in eggs that were set to turn through 90 degrees (0.16 ± 0.02) compared with those set to turn through 60 degrees (0.28 ± 0.02), regardless of season and setting position. The preferred way of setting ostrich eggs would therefore be vertically in a trolley that turns hourly through 90 degrees with the air cell upwards to utilize incubator space optimally.

Keywords: chick weight, embryonic mortalities, ostrich, pipping time, moisture loss
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The artificial incubation of ostrich eggs has become a major part of the ostrich industry. But the success rate of artificially incubated ostrich eggs is low at only 50 - 60% (Brown *et al.*, 1996; Deeming & Ar, 1999; Van Schalkwyk, 2000; Brand *et al*., 2007), compared with commercially reared chickens (90 - 95%), turkeys (75 - 77%), and ducks (65 - 82%) (Hodgetts, 1990; Deeming & Ar, 1999). More than 70% of all embryonic mortalities occur in the second half of incubation (Brand *et al*., 2007; 2012) and it is evident that there is considerable room for improvement.

Numerous factors affect the successful hatching of artificially incubated eggs, including evaporative water loss, age of female, season, storage conditions before setting, and genotype (Blood *et al*., 1998; Van Schalkwyk *et al*., 1999; Brand *et al*., 2007; 2008). However, almost all avian eggs need to be turned throughout incubation for correct embryonic development to take place (Tullett & Deeming, 1985; Deeming, 1991). More than 55% of shell deaths in ostrich eggs are caused by malpositioning of the embryo (Brown *et al.*, 1996). Although most embryos adopt the correct pipping position (Deeming, 1995), Brand *et al*. (2017a) found that a substantial proportion of dead-in-shell chicks that pipped internally were positioned with their heads oriented towards the middle of the egg instead of the air cell. Embryos with pipping positions away from the air cell were more likely to succumb to asphyxia as they would be unable to penetrate it in the final stages of incubation (Ley *et al*. 1986; Brand *et al*., 2017a).

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v50i4.12

Badley (1997) reported that hatchability was improved in ostrich eggs when set vertically and turned through a 180 degree angle along the long axis, compared with a 90 degree angle, although this caused a significant increase in late embryonic mortalities in chicken eggs. These results suggest that ostrich eggs respond differently to hatchery practices that are used for chicken eggs. Vertical hatching is not the normal position of eggs incubated naturally in ostrich nests (Wilson, 2003). Takeshita & McDaniel (1982) reported that early embryonic development of poultry embryos was improved in eggs that were incubated horizontally. In contrast, Van Schalkwyk *et al*. (2000) found that the hatchability of fertile ostrich eggs was relatively low, but similar when set in vertical or horizontal positions for six weeks. These contradictory findings indicated scope for a more detailed study. This study sought to establish the effects of setting positions on the overall hatchability of ostrich eggs.

Eggs were obtained from the commercial ostrich breeding flock that is maintained at Oudtshoorn Research Farm in the region of Klein Karoo, South Africa, during the 2015 - 2016 breeding seasons. The flock consisted of 155 breeding pairs and the breeders ranged from two to ten years old. The flock included mostly the South African Black (SAB) genotype, but birds from the Zimbabwean Blue and Kenyan Redneck strains had been introduced from 2003 to study crossbreeding of the SAB with these genotypes. The climate at the experimental site is arid with an average annual precipitation of 330 mm. The breeding season started in mid May and concluded in mid December in each year.

After collection, the eggs were sterilized by exposure to ultraviolet light for 20 minutes and weighed. The collection time, origin (paddock number) and date of collection were recorded. The eggs were divided randomly into three groups, of which two were stored horizontally and the third was stored vertically. All eggs were stored in their position at setting under controlled conditions (temperature of 17 °C; relative humidity of 75%) and rotated twice daily through 180 ° until they were set. Eggs were set to turn automatically through a 60 ° or a 90 ° angle (i.e. 30 ° and 45 ° either side of the setting position) at hourly intervals. They were turned around the long axis when set horizontally and around the short axis when set vertically. The treatments per tray, which represented each turning angle, consisted of i) eggs set horizontally for five weeks before being transferred to the hatcher; ii) eggs set horizontally for three weeks and vertically for two weeks; and iii) eggs set vertically for five weeks. These treatments were repeated over two years to include the seasons, namely winter (June to August), spring (September to November), and summer (December). Set eggs were candled and weighed on days 21 and 35 of incubation. Together with initial egg weight, these weights were used to derive moisture loss on day 21 of incubation (ML21) and day 35 of incubation (ML35). On day 35, the eggs were moved from the setters to a hatcher, which also operated at 36 °C and a relative humidity of 24%. Eggs were retained in their setting positions in the hatcher. Eggs that did not show signs of continuing development at candling at 21 and 35 days of incubation (about 20% and 10%) were opened and assessed for early embryonic development or infertility (Brand *et al*., 2012). Eggs with clear evidence of embryonic development that had subsequently ceased were regarded as embryonic mortalities during the first half of incubation (early embryonic mortalities (EEM)). Subsequent embryonic mortalities were classified as late embryonic mortalities (LEM), that is, occurring after 21 days of incubation. Overall embryonic mortality (OEM) was calculated and included EEM and LEM, and embryos that died during and after pipping (about 10% of eggs). Embryonic mortality was calculated on a setting batch level as proportions of set eggs.

Data for 846 to 1549 egg records at setting were used to derive averages for groups of eggs that were treated similarly. Batch data were then subjected to factorial analysis in a 2 (years) x 3 (seasons) x 3 (setting positions) x 2 (angles of rotation) design. Each unit represented between 24 and 43 eggs in the analysis of variance (ANOVA). The data were subjected to standard factorial analyses (Snedecor & Cochran, 1967). Least significant differences were derived to compare treatments, provided that they were protected by a significant F-value in the ANOVA.

The significance of the fixed effects and their interactions is presented in Table 1. Late embryonic mortalities and OEM were affected (P <0.01) by year, setting position and turning angle, whereas year and season affected moisture loss, pipping time and day-old chick weight (P <0.01). Only main effects are presented because there were no significant interactions between fixed effects for the traits.

Table 1 Significance of fixed effects and interactions affecting embryos and eggs of ostriches

Trait	Year	Setting position	Year * Setting position	Season	Season * Setting position	Turning angle	Turning angle * Setting position
EEM	0.10	0.25	0.48	0.71	0.24	0.69	0.79
LEM	<0.01	<0.05	0.48	0.18	0.80	<0.01	0.15
OEM	<0.01	0.09	0.85	0.40	0.45	<0.01	0.25
EWT (g)	<0.05	0.72	0.26	<0.01	0.26	0.49	0.39
ML21 (%)	0.85	0.27	0.39	<0.01	0.36	0.40	0.86
ML35 (%)	0.69	0.10	0.49	<0.01	0.55	0.25	1.00
Piptime (days)	<0.01	0.08	0.09	<0.01	0.72	<0.05	0.95
CWT (g)	<0.01	0.97	0.81	<0.01	0.53	0.91	0.41

EEM: early embryonic mortality; LEM: late embryonic mortality; OEM: overall embryonic mortality; EWT: egg weight at time of lay; ML21: moisture loss at 21 days of incubation; ML35: moisture loss at 35 days of incubation; piptime: incubation time until external pipping; CWT: chick weight at one day old

Moisture loss, pipping time and one-day-old chick weight were largely independent of setting position (Table 2). The incubation positions of the eggs generally did not affect the measurements of the developing embryo throughout the 42-day period (Brand *et al*., 2012; Brand *et al*., 2017b). However, the findings in this study were not consistent with those of Van Schalkwyk *et al*. (2000), who reported improved hatchability of up to 36% in batches of eggs that were incubated horizontally for two or three weeks and then vertically. The present results contrast with literature from other avian species. Funk and Forward (1960) obtained better hatchability when chicken eggs were incubated vertically with the blunt end up as opposed to a horizontal setting. Wilson (1991a,b) obtained significantly better hatchability when eggs from chickens and waterfowl were turned in a horizontal position. In this study, LEM was improved by about 10% in eggs set vertically compared with eggs set horizontally for three weeks and vertically for two weeks.

Table 2 Effects of setting position during incubation on mean value for incubation traits of ostriches

Trait	Horizontal	Horizontal/ Vertical	Vertical	SE	*P*-value
EEM	0.06	0.03	0.04	0.01	0.25
LEM	0.22	0.27	0.17	0.03	<0.05
OEM	0.28	0.31	0.21	0.03	0.09
EWT (g)	1482	1484	1492	9	0.72
ML21 %	8.05	8.31	8.10	0.11	0.27
ML35 %	12.19	12.37	12.01	0.11	0.10
Piptime (days)	42.68	42.59	42.53	0.04	0.08
CWT (g)	906	905	908	11	0.97

EEM: early embryonic mortality; LEM: late embryonic mortality; OEM: overall embryonic mortality; EWT: egg weight at time of lay; ML21: moisture loss at 21 days of incubation; ML35: moisture loss at 35 days of incubation; piptime: incubation time until external pipping; CWT: chick weight at one day old

There was an improvement of up to 50% in LEM for eggs set in trolleys that turned through 90 degrees compared with a 60 degree angle (Table 3), regardless of season and setting position. The effect of turning angle on LEM was carried over to OEM. This was consistent with the findings of Van Schalkwyk *et al*. (2000), who found the lowest hatchability (26.5%) was recorded in eggs that were not turned at all and that the highest hatchability was found for eggs turned 90°. Not turning eggs results in retarded development of the vasculosa area and the extra-embryonic membranes, retarded embryonic growth, reduced oxygen uptake and reduced albumen absorption in other avian species (Tullett & Deeming, 1987; Deeming, 1989a; 1989b; 1989c). However, Brand *et al.* (2012; 2017b) found that position of the egg generally did not affect the measurements of the developing ostrich embryo throughout the 42-day incubation period, and the hatching of fertile eggs was also independent of setting position.

Table 3 Effects of turning angle during incubation of ostrich eggs on mean values of incubation traits

Trait[1]	60 ° angle	90 ° angle	SE	Significance
EEM	0.05	0.04	0.01	0.69
LEM	0.28	0.16	0.02	**
OEM	0.32	0.21	0.03	**
EWT (g)	1490	1483	7	0.49
ML21 %	8.10	8.21	0.09	0.40
ML35 %	12.11	12.26	0.09	0.25
Piptime (days)	42.66	42.53	0.04	*
CWT (g)	907	906	9	0.91

**P* <0.05; ***P* <0.01; actual significance for *P* >0.05
[1] EEM: early embryonic mortality; LEM: late embryonic mortality; OEM: overall embryonic mortality; EWT: egg weight at time of lay; ML21: moisture loss at 21 days of incubation; ML35: moisture loss at 35 days of incubation; piptime: incubation time until external pipping; CWT: chick weight at one day old

Season did not affect EEM or LEM (Table 4). These results contradicted previous findings of Brand *et* al. (2007) that chicks from eggs that were produced at the beginning of the breeding season, namely in winter, were more likely to succumb prior to hatching. However, egg weight, moisture loss, pipping time and day-old chick weight were affected by season, with moisture loss decreasing from winter to spring to summer. In contrast, pipping time became later and day-old chick weight became heavier from winter to autumn to summer. In the current study, ML21 and ML35 decreased towards the summer, which could explain the later pipping time and increase in chick weight. Brand *et al*. (2008) reported that chick weight and pipping time in spring resembled winter values, but a subsequent decline took place towards summer. Moisture loss also increased from winter to summer. The anomaly in these results could be attributed to the relatively small number of eggs that were used in the current trial, and to seasonal differences. Ostrich nests are situated in open paddocks and the freshly laid eggs are subject to extreme weather fluctuations before they are collected and brought into the controlled environment at a hatchery.

Table 4 Effects of season during incubation of ostrich eggs on mean values of incubation traits

Trait[1]	Winter	Spring	Summer	SE	Significance
EEM	0.049	0.050	0.0369	0.014	0.71
LEM	0.189	0.212	0.263	0.028	0.18
OEM	0.238	0.262	0.298	0.031	0.40
EWT (g)	1457	1493	1510	9	**
ML21 (%)	8.81	7.98	7.67	0.11	**
ML35 (%)	13.1	12.1	11.4	0.11	**
Piptime (days)	42.61	42.72	43.67	0.04	**
CWT (g)	875	903	941	11	**

**P* <0.05; ***P* <0.01; actual significance for *P* >0.05
[1] EEM: early embryonic mortality; LEM: late embryonic mortality; OEM: overall embryonic mortality; EWT: egg weight at time of lay; ML21: moisture loss at 21 days of incubation; ML35: moisture loss at 35 days of incubation; piptime: incubation time until external pipping; CWT: chick weight at one day old

Because ostrich farming is more extensive than most other avian farming enterprises, a better understanding of environmental impacts on the success of artificial hatching is important. Data from this study suggested that season affected moisture loss, pipping time and day-old chick weight more than setting position and angle of rotation. The preferred way of setting ostrich eggs would be vertically in a trolley that turns hourly through 90 degrees with the air cell upwards to utilize incubator space optimally.

Acknowledgements

The authors are grateful to the Western Cape Department of Agriculture and Oudtshoorn Research Farm for the use of the resource flock and facilities. They thank I. Janse and U. Izaks for opening all the dead-in-shell eggs to record the data on the positions of the chicks for this study.

Authors' Contributions

Concept, design, data collection, drafting of the paper and submitting the manuscript, ZB. Critical analyses, SWPC. All authors made substantial contributions to the original conception and design, acquisition of data, analyses and interpretation of data. All the authors approved the manuscript.

Conflict of Interest Declaration

There are no conflicts of interest.

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