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Effect of pipping rate and hatching nature on the development of ostrich chicks

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Abstract

High embryonic mortality is concerning because of the effect on commercial ostrich farming. The number of viable chicks can be improved by appropriate interventions in the hatching chicks. Data from 2 683 fertile eggs were collected from the commercial, pair-bred ostrich flock on the Oudtshoorn Research Farm, South Africa, with 169 chicks being reported. Fertile eggs were randomly divided into three groups on days 41, 42, and 43 of incubation. There were four treatments: hatchlings that reached climax and broke free from the eggshell themselves (T1), hatchlings that were assisted to reach climax at the first signs of external pipping (T2), hatchlings that were removed from the eggshell at the first sign of external pipping (T3), and eggs that pipped internally after 43 d but failed to pip externally were cracked (T4). Clinical measurements (heart rate, body temperature, and oedema) were taken at hatch. The chicks were weighed for 7 d and then on days 28, 84, 147, 227, 300 and 365. Chicks that were assisted after internal pipping took longer to hatch. The heart rate of 115 beats per minute (bpm) for chicks hatching on their own was lower than the 132 bpm recorded for the other treatment groups. Up to day two after hatching, a decline in chick weight of ~4% was found. Chick weight increased from 0.85 kg to 1.11 kg in the first week. At 147 d, body weights between treatments were 12.6% higher for the chicks hatching on their own compared with the chicks where the eggshell was cracked, and 24.6% higher for chicks where the eggshell was removed after external pipping. Chicks benefitted by climaxing themselves, but for chicks struggling to hatch, this study provides guidance to hatchery operators on the specific stages where monitoring and assistance is important to improve hatchability.

Keywords: Artificial incubation, chick mortality, chick weight, hatchery management, hatchability, heart rate, oedema
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Introduction

The success of ostrich farming depends largely on the production of fertile eggs, but scientific reports on fertility and hatchability of artificially-incubated ostrich eggs show that hatching results are highly variable (Deeming & Ar, 1999). A substantial amount of embryonic mortalities occur in the last few days of incubation, and it is evident that there is still considerable room for improvement in this area of commercial ostrich production (Brand *et al.*, 2008). Several factors do affect hatchability, including storage conditions of eggs prior to setting, water loss (WL) during incubation, season, and female age (Blood *et al*., 1998; van Schalkwyk *et al*., 1999; Brand *et al*., 2007). The optimal WL in artificially-incubated ostrich eggs is 13–15%, whereas too low (<10%) and too high (>18%) WL at 35 d of incubation leads to sharp increases in embryonic mortality (Deeming, 1995; Ar, 1996; Blood *et al*., 1998; Deeming & Ar, 1999).

Genetic make-up influences the performance of individuals (Petitte & Davis, 1999), with van Schalkwyk *et al*. (1996) and Cloete *et al*. (1998) publishing the first reports of estimates for repeatability

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and phenotypic correlations among reproductive traits in ostriches. Subsequent estimates of genetic parameters for egg, chick, and reproductive traits followed (Cloete *et al*., 2008; Brand *et al*., 2012). Egg quality also have a substantial genetic component (Stewart, 1995).

Among studies determining the impact of malpositioning on near-term embryonic deaths, Brand *et al.* (2017) reported that most near-term embryos that fail to hatch are in the correct position; chicks that pipped in the correct position had a greater probability of surviving hatching than those that pipped in the incorrect position. A positive relationship was established between eggshell quality, WL, and late embryonic deaths (Brand *et al.*, 2012). Gonzalez *et al.* (1999) reported that a substantial proportion of late embryonic deaths due to suffocation could be related to poor eggshell quality.

In investigating the hatching sequence of ostrich embryos, Deeming (1995) defined six stages in the hatching process. In contrast to the domestic chicken, the ostrich embryo lacks an egg tooth and has to create a tear in the membrane by rubbing its beak against the shell. An additional difference is that during internal pipping, the air space is pulled toward the beak of ostrich chicks, whereas in chicken embryos, the beak moves to the air space (Cooper, 2016). When the inner membrane is ripped open, the ostrich chick pushes its body towards the air cell. To achieve external pipping, the chick must throw its head backwards against the shell to crack it (Bond *et al*., 1988; Deeming, 1995). The right foot is also used in unison with the beak and head to break the shell, a unique adaptation to an egg with a hard, brittle shell (Cooper, 2016). After cracking the eggshell, hatching proceeds by a combination of head and foot movements while the embryo rotates within the egg in a diagonal direction towards the equator of the egg. The opening in the eggshell before climax aids the chick in aerating its lungs to achieve the proper ventilation and oxygenation needed for the final kick-out. Hatching climax can only commence once the transition to pulmonary respiration is completed, the chorioallantois has been depleted of blood, and the yolk has been retracted into the body cavity (Kovach, 1970; Vince, 1980; Burger & Bertram, 1981; Bond *et al*., 1988). Cloete *et al*. (2012) found that assisted ostrich chicks had greater rates of early mortality if compared to those hatched normally, whereas the mortality rates were greater for the chicks that pipped first and last, if compared to the survival of median hatchlings.

There are different ways to assess post-hatch development and health status. In chickens, healthy development is indicated by both the initial days of body weight decrease and the subsequent weight gain (Lourens *et al*., 2005). Heart rate is used as a gauge for stress imposed by both external stimuli and activity (Nevarez, 2005; Michael *et al*., 2016). The degree of oedema (Snyder & Birchard, 1982; Badley, 1997) and body temperature (Nevarez, 2005; Jerem et al., 2015) are used as metrics to assess the chick's activity and metabolic processing levels.

This study investigated the effects of human interference during hatching on the physiological development and growth potential of the ostrich chick. Information guiding the correct methods and timing to support ostrich chicks suffering from hatching complications can putatively increase the number of viable day-old chicks available for commercial production.

Materials and Methods

Eggs used during this study were collected from the commercial, pair-bred ostrich flock at the Oudtshoorn Research Farm, South Africa during 2019 and 2020. The flock consisted of 151 breeding pairs aged between 2 and 10 y, mostly consisting of birds of the South African Black genotype. Birds from Zimbabwean Blue and Kenyan Redneck strains were also introduced for crossbreeding between genotypes. However, the present analysis was confined to birds of the South African Black (*Struthio camelus* var*. domesticus*) strain to constrain the number of variables. The climate at the experimental site was arid, with temperatures exceeding 40 °C during summer and an average annual precipitation of 330 mm. Females were pair-bred annually from mid-May to mid-December. Eggs were collected in the late afternoon (~16h00) and early morning (~08h00), disinfected in an ultra-violet machine for 20 min, weighed, and identified by date and paddock of origin. All eggs collected were stored for no more than 6 d at a temperature of 17 °C and relative humidity (RH) of 75% prior to setting in the incubator. The methods of egg collection, sanitation, and storage on the research farm followed procedures described previously (Van Schalkwyk, 1998; Van Schalkwyk *et al*., 1999; Bunter & Cloete, 2004; Brand *et al*., 2007).

The eggs were then placed in automatic setters to incubate at 36.2 ºC and 24% RH. The setters were configured to turn eggs automatically through 90° hourly for the first 5 w of incubation. Incubated eggs were candled and weighed on days 21 and 35 of incubation. Together with initial egg weight, these weights were used to derive water loss. On day 35 of incubation, 1992 fertile eggs were moved from the setters to a hatcher operating at 36 °C and 24% RH.

On days 41, 42, and 43 of incubation, the fertile eggs in the hatcher were monitored hourly to follow the hatching sequence. The six definite stages in the hatching sequence of the ostrich embryo, as reported by Deeming (1995), were used as a guideline, i.e., stage A, pre-internal pipping; stage B,

internal pipping; stage C, external pipping; stage D, consolidation of the pipping hole; stage E, emergence; and stage F, drying off. Eggs showing signs of internal pipping (Fig. 1), were randomly divided into the three treatment groups. Group 1 included eggs/hatchlings that reached climax and broke free from the eggshell by themselves, serving as the control group; Group 2 included eggs/hatchlings that were assisted to reach climax as soon as the first signs of external pipping (Fig. 2) were observed ("assistance" consisted of the slight cracking of the eggshell to allow the hatchling to break free from the shell with more ease); Group 3 included eggs/hatchlings that were "artificially climaxed" by breaking and removing the eggshell as soon as the first signs of external pipping were observed to allow the hatchling to be totally freed from the shell. An additional treatment, Group 4, was included for eggs/hatchlings that pipped internally after 43 d of incubation but failed external pipping. These eggs were then opened at the air sac area, the inner membrane was opened around the head of the hatchling to allow it to breathe freely, and the shell cracked to assist the hatchling to break free.

Figure 1. Internal pipping - chick breaking through internal membrane

Figure 2. External pipping – chick breaking through the eggshell

The day and time of external pipping, together with the pipping and chick position, were recorded. The pipping hole dimensions (i.e., length and width) were measured with the onset of external pipping (stage C; Fig. 2) with a digital calliper to an accuracy of 10 µm. With external pipping, a digital stethoscope was placed on the left side of the thoracic cavity for 3 s to obtain a mean heart rate reading of the chick. Body temperature readings of the hatchling were done using a digital thermometer inserted into the cloaca and measurements were taken the moment the chick escaped from the egg. At this time the oedema thickness in the thigh and neck were measured with a digital calliper. The digital calliper was placed dorso-medial to the neck to ensure to enfold only the posterior, oedematous muscle (*complexis*) of the neck to minimize discomfort for the hatchling. The thigh was measured by placing the digital calliper anterior-medial to the *M. fibularis longus* muscle to ensure only enfoldment of the anterior, oedematous muscle of the thigh.

On the 43rd day of incubation, a penlight torch was used to inspect remaining eggs in the hatcher for signs of life before assistance to hatch. Dead-in-shell eggs were weighed and the embryo position, as well as the developmental stage were documented.

After being allowed to dry-off, the chicks were weighed on a gram-sensitive scale, sexed, the navel disinfected with a prescribed disinfectant, and the chick was supplied with an identity tag (Bonato *et al.*, 2009). Sex determination on the hatched chicks was done through cloacal examination using the method described by Gandini & Keffen (1985). The examiner everted the tail portion of the ventral wall of the cloaca by manipulation and rotation of the lower area of the vent. The dorsal lips of the vent were pulled back and down to examine the organ. The presence of a phallus with a sulcus and visible blood vessels were used to distinguish males from females.

The chicks were then moved from the hatchers to the chick room in the hatchery. The temperature of the chick room was kept constant at 26 °C for the chicks to acclimatise. The chicks were given a balanced pre-starter diet and clean municipal water *ad libitum*. Within 36 h, the chicks were transferred to intensive chick-rearing facilities.

During their first 2 w in the chick-rearing facility, a constant temperature of 25 °C was maintained. Thereafter, the temperature in the chick rearing facility was decreased by 2 °C per week, until heating was suspended at 2 months of age. Light was provided from 08:00 to 17:00; food and fresh water were supplied *ad libitum*. The body weight of 169 chicks was recorded daily up to seven days of age and

then at days 28, 84, 147, 227, 300 and 365 of age. These weights were used to evaluate both live weight loss and weight gained during the post hatch-growing phase. Chick mortalities and mortality causes were noted. The project ran over two consecutive breeding seasons (2019 and 2020). Ethical clearance to conduct this study was granted by the Departmental Ethical Committee for Research on Animals of the Western Cape Department of Agriculture (Ref No.: R19/129) and Unisa-CAES Animal Research Ethics Committee (2019/CAES_AREC/130).

Data were subjected to analysis to least-squares analysis to determine if early post-hatching growth was affected by assistance at hatch, subsequent mortality, or sex. Differences between comparable means were discerned using least significant differences (LSD), provided that it was protected by a significant F-value in the ANOVA (Snedecor & Cochran, 1967). The fixed effect model fitted to the post-hatching chick weight data included the fixed effects of age (0, 1, 2, 3, 4, 5, 6 and 7 days for the early data and 7, 28, 84, and 147 days for the later data), chick mortality (died or survived), and sex (male or female). Although it is uncommon to have subsequent mortality status in a fixed effect in an analysis involving weight to 7 d since dead chicks do not have any weight to contribute, it is also important to know whether chicks that succumbed were already lagging earlier. The inclusion of mortality status on weights up to 7 d is thereby warranted.

The interaction of age, as defined above, with the other fixed effects was used to establish whether age trends were different for chicks assisted at death, for those that subsequently died, and those belonging to the different sexes. As the data on heart rate, body temperature, oedema, and body weight were based on repeated records of individuals that were sampled repeatedly, random animal permanent environmental effects were added to the fixed effect models in ASReml (Gilmour *et al*., 2015). While accounting for the variance associated with the repeated sampling of the same individual, the between-animal effects also allowed for the derivation of repeatability estimates for live weight across the two growth periods.

Chi-square procedures were used to assess the proportion of chicks that survived and succumbed for the different treatments (Van Ark, 1990). The Chi^2 statistic is calculated by finding the difference between each observed and theoretical frequency for each possible outcome, squaring them, dividing each by the theoretical frequency, and by computing the sum of the results. Outcomes in this instance involved a chick being either dead or alive at a specific stage. To present the data in an orderly fashion, the data were presented in proportions rather than frequencies, as is suggested by Snedecor & Cochran (1967). The Bonferroni correction (Napierala, 2012) was applied to all analyses, since all analyses involved multiple comparisons.

Results and Discussion

Descriptive statistics for the data are presented in Table 1, including the overall means of the duration of stages B and C.

Table 1 Descriptive statistics for the overall means of the duration of stages B and C, heart rate, chick body temperature, pipping hole dimensions, as well as the oedema thickness in the thigh and neck

Trait	Records	Mean ± SD	Range
Interval (h)	186	33.0 ± 18.9	1.8–94.7
Heart rate (bpm)	127	123 ± 39	57–221
Temperature (˚C)	127	38.3 ± 2.6	32.2–41.2
Pipping hole length (mm)	133	7.7 ± 3.1	1.0–20.1
Pipping hole width (mm)	133	7.6 ± 3.8	0.6–21.2
Thigh - oedema thickness (mm)	79	120 ± 4	117–132
Neck - oedema thickness (mm)	79	121 ± 3	111–133
Chick weight (week 1) (kg)	1314	0.914 ± 0.141	0.650–1.470
Chick weight (later) (kg)	588	6.7 ± 6.1	0.67–42.6

There were marked variations between all the different pipping stages (Figure 3), starting with internal pipping and proceeding throughout the entire break-out process. Results from this study show there was marked variation, as reflected by standard deviations commonly exceeding the mean of time lapses (Fig. 3) on when internal pipping started and ended. These results correspond with reports by Oppenheim (1972), Vince (1980), and Bond *et al.* (1988) that time between pipping cycles varies (within and) among species. It was difficult to accurately quantify and detect the start of stage A of the hatching

sequence using a candling lamp and not opening the egg. Therefore, no duration results for this stage were included in the results.

The extreme variation in pipping interval between embryos can be seen in the minimum and maximum values during stages B and C (11.8–48.4 h and 1.8–94.7 h, respectively). On average (± SE), stage C took longer to complete compared to stage B (37.2 ± 2.2 vs. 27.3 ± 3.2 h; P <0.05; Fig. 3) and overall, the earlier stages (B and C) had the longest duration. The intervals of stages D, E, and F were completed faster (2.79 ± 1.42; 9.47 ± 4.42 and 1.38 ± 0.53 h, respectively). However, the transitions were difficult to detect, resulting in small record numbers and high SE's. Chicks from different species benefit from parental support to reduce the duration of climax, thereby limiting the extent of brain cell damage that occurs during an extended climax (Bond *et al.*, 1988).

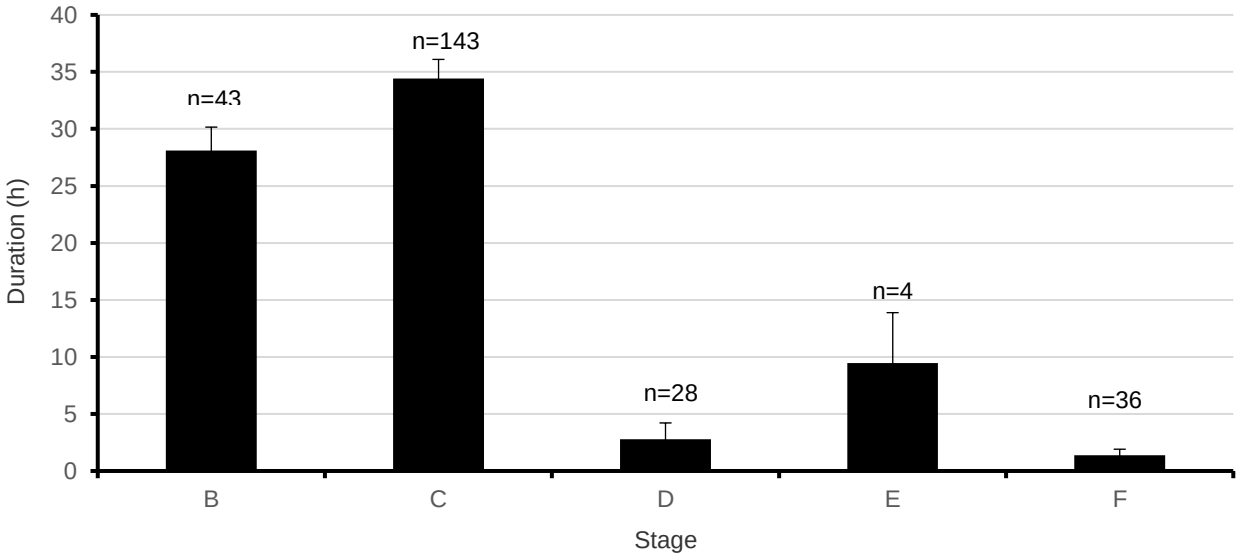

Figure 3 Predicted means (± SE) depicting the duration of the different hatching stages

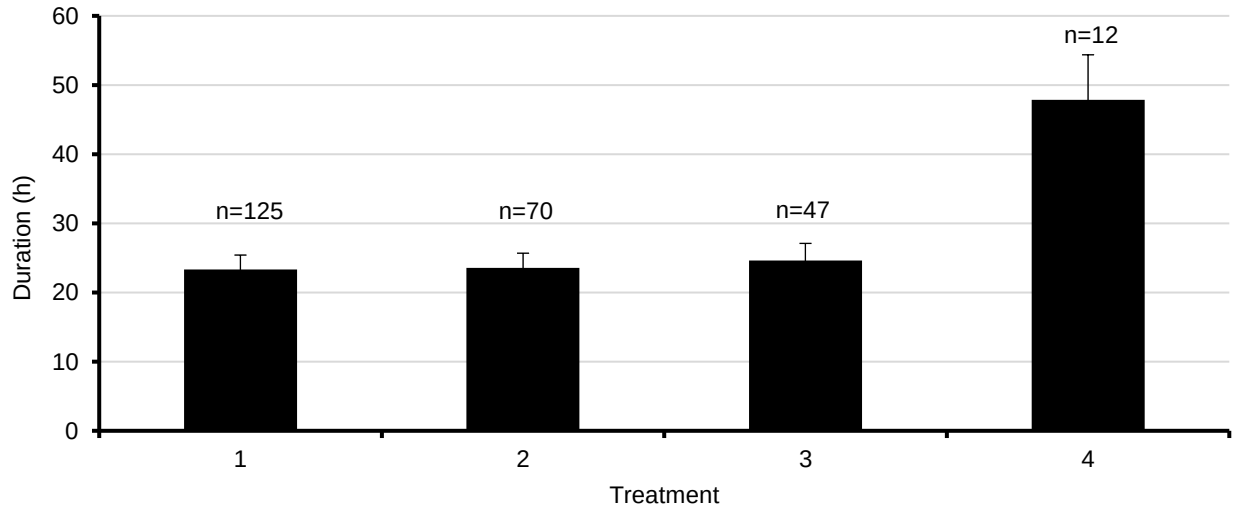

Figure 4 Predicted means (± SE) depicting the duration of the different hatching treatments

When assessing the duration of the different treatments, no significant differences were found between Treatments 1, 2 and 3 (23.3 ± 2.11; 23.6 ± 2.13; 24.6 ± 2.46 h, respectively; Fig. 4).

There was marked variation in duration of hatch within different treatments, ranging from 0.10 to 92.7 h. Treatment 4 took longer to complete compared to the mean of Treatments 1–3 (47.8 ± 6.53 vs. 23.8 ± 2.23 h; P <0.05; Fig. 4).

Since not all the stages (A, D, E, and F) were represented in all the treatments, only the interval of stages B and C are presented here. The interaction between stage and treatments was not significant (P = 0.175) and only the main effects are therefore presented. On average, chicks in Treatment 4 took

longer (41.8 ± 5.6 h) to complete stages B and C than those in Treatments 2 and 3 (27.7 ± 2.9 h and 26.0 ± 3.5 h, respectively; P <0.05; Table 2). These results can indicate which eggs/chicks will need assistance in the form of cracking of eggshell after internal pipping at an early stage of the hatching sequence. Hatching success for all hatcheries is determined by the hatching percentage realised by the hatchery manager. Hatching percentage can not only be improved by monitoring the eggs from day 41 of incubation, but it will also have a positive effect on the profit margin with the prices of day-old chicks currently being exceedingly high (ZAR 700–800 per day-old chick).

Chicks usually start pipping from days 40/41 of incubation, thus while removing the hatched chicks, the remaining eggs can be checked and sorted according to internal activity. After ~24 h, the group of eggs where internal pipping occurred, but not showing any signs of external pipping, can be assisted. Burger & Bertram (1981) observed that chicks taking longer to hatch are often weak and will need assistance. It is standard practice in hatcheries to monitor hatching progress and to inspect (using a penlight torch) for signs of internal pipping after day 43 of incubation. Thus, starting to monitor the eggs two days earlier should be achievable to implement as part as hatchery management practices with minimal cost and maximum return.

The mean embryonic heart rate of ostrich chicks was 115–132 bpm (Table 2). The mean heart rate of chicks in Treatment 1 was slower by ~15 % when compared to the mean for Treatment 2 (P <0.05), with Treatment 3 intermediate and not different (P >0.05) from either of the other groups. Results from this study are in line with Tazawa *et al.* (1998), who reported an average heart rate of between 146 bpm for embryos that were incubated for 33 d and 108 bpm for embryos that were incubated for 37 d. Rezakhani *et al.* (2007) also reported that the average heart rate of ostrich chicks (below 3 months old) was 171.47 ± 9.03 bmp, with a range of 107 to 250 bpm. The slower heart rate for chicks in Treatment 1 might be attributed to the lower degree of stress since they experienced no human interference during the hatching process (Duncan *et al.*, 1986). Alternatively, the elevated heartbeat of the hatching could be due to the stress of not being able to hatch on its own accord. The effect of stress via hatching assistance needs further investigation, but it will ultimately be the hatchery manager who will determine the need for assistance of the hatchling. The mean chick body temperature was independent of treatment (P >0.80) and constant for all groups at 38.3 ± 2.6 °C (Table 2). The result on body temperature recorded in this study corresponds with findings by Elsayed (2016).

Table 2 Predicted means (± SE) depicting the effect of treatment on the average duration of stages B and C, heart rate, chick cloacal temperature, pipping hole dimensions and skin thickness

Trait	Treatment			
	1	2	3	4
Interval (h)	33.5 ± 2.7^{a,b}	27.7 ± 2.9^{a}	26.0 ± 3.5^{a}	41.8 ± 5.6^{b}
Heart rate (bpm)	115 ± 5.0^{a}	132 ± 6.0^{b}	131 ± 7.5^{a,b}	NA
Temperature (˚C)	38.6 ± 0.3	38.5 ± 0.3	38.4 ± 0.3	NA
Pipping hole length (mm)	9.43 ± 0.42^{b}	6.75 ± 0.41^{a}	6.68 ± 0.45^{a}	NA
Pipping hole width (mm)	10.24 ± 0.48^{b}	6.54 ± 0.47^{a}	5.66 ± 0.52^{a}	NA
Skin thickness thigh (mm)	119 ± 1.0	120 ± 1.0	121 ± 1.0	NA
Skin thickness neck (mm)	118 ± 1.0^{a}	120 ± 1.0^{b}	121 ±1.0^{b}	NA

^{a,b} Means with different superscripts are different in rows (P <0.05); NA: not applicable as Treatment 4 only recorded 1 chick or no chicks for these traits

The mean size of the pipping hole in this study (5.66 ± 0.52 mm and 10.24 ± 0.48 mm) was smaller than the mean of 20–60 mm reported by Deeming (1995). The pipping hole dimensions for chicks in Treatment 1 were larger than in the other two treatments (P <0.05) where the eggshell was either cracked or removed after external pipping. A stronger, more viable chick should be capable of executing a more forceful kicking action, thereby causing a larger hole in the eggshell (Bond *et al.*, 1988). Differences between treatments approached significance for skin thickness at the thigh (P <0.06), but chicks in Treatment 1 had thinner neck skins that those in the other two treatments (P <0.05; Table 2). This result could be attributed to the fact that chicks in Treatment 1 had to make use of more of their stored metabolic energy reserves during hatch (De Oliveira *et al.*, 2008). The embryo's metabolism alters substantially while hatching. The specific signals that induce metabolic and hormone alterations during hatching remain unknown (De Oliveira *et al.*, 2008).

Chick live weight up to 7 d was highly repeatable (± SE) at 0.77 ± 0.02.The repeatability of subsequent chick weight was lower at 0.17 ± 0.04 (Table 3). This value is a bit lower than the corresponding value of 0.30 (the sum of a direct heritability estimates (h^2) of 0.21 ± 0.03 and a female permanent environment estimate (c^2) of 0.09 ± 0.04) reported by Bunter & Cloete (2004) for 6-month-old ostrich chicks. The latter authors noted that live weight ~3 months had a particularly low repeatability of ~0.10 (the sum of a h^2 estimate of 0.02 ± 0.06 and a c^2 estimate of 0.08 ± 0.04).

Between breeding-pair variances were <0.10 for most traits, smaller or similar to the corresponding standards errors, and not significant (P >0.05; Table 3). In fact, the between-breeding pair variance went to the boundary of parameter space (zero) for the pip hole dimensions that were recorded and these traits are thus not tabulated. The exceptions to the rule were the two skin thickness measurements. Oedema thickness at the thigh was moderately repeatable at 0.28 (P <0.05), whereas the estimate of 0.19 for oedema thickness at the neck exceeded the corresponding standard error of 0.13 (P >0.05; Table 3). No literature of previous studies on the repeatability of any of the traits presented in Table 3 could be found.

Table 3 Between-breeding pair and residual variance components and repeatability estimates for the chick traits considered

Trait	Between breeding pairs	Residual	Repeatability ± SE
Hatching interval (h)	29.37	189.9	0.09 ± 0.06
Heart rate (bpm)	32.48	1437.1	0.02 ± 0.06
Temperature (˚C)	0.17	4.52	0.04 ± 0.04
Skin thickness thigh (mm)	4.19	10.57	0.28 ± 0.13
Skin thickness neck (mm)	2.03	8.58	0.19 ± 0.13
Chick weight (week 1) (kg)		0.003	0.77 ± 0.02
Chick weight (later) (kg)		7.12	0.17 ± 0.04

The body weight of an ostrich chick reflects in both size and condition (Wang, 2012). Table 4 shows that neither hatching sequence, subsequent mortality, nor sex had any effect on the overall mean chick weight over the first seven days post-hatch (P ≥0.16). The overall mean chick weight during this period was 0.92 kg. Findings by Horbańczuk& Sales (2000) and Wang *et al.* (2012) concur that chick weight was independent of assistance at hatch or sex. The higher standard error (SE) value in hatching sequence (0.91 ± 0.03) observed for group 4 (n = 12) in comparison to the other groups (n = 41 for Group 3 to 75 for Group 1) can be attributed to the small number (n = 20) of eggs that pipped internally by day 43 of incubation that needed assistance.

Table 4 Predicted means (± SE) showing overall chick weight (kg) across ages from hatching to 7 d in relation to hatching sequence, subsequent mortality, and sex

Effect and description	Records	Chick weight ± SE
Hatching sequence		0.28*
Group 1 - Control	408	0.92 ± 0.01
Group 2 – Slight cracking of eggshell at external pipping	448	0.92 ± 0.01
Group 3 – Remove eggshell at external pipping	380	0.92 ± 0.01
Group 4 – Cracking of eggshell at internal pipping	78	0.91 ± 0.03
Subsequent mortality		0.17*
Survived	966	0.94 ± 0.02
Died	348	0.89 ± 0.01
Sex		0.16*
Male		0.92 ± 0.01
Female		0.91 ± 0.01

*Actual significance for P >0.05

Figures 5, 6, and 7 illustrate the interaction of hatching sequence, survival, and sex on chick body weight with age. As expected, increased chick age had a marked effect on the recorded chick weights (P <0.05). Although there was no evidence that the observed age effects differed according to hatching sequence (the interaction involving this main effect with age was not significant, P >0.05) this interaction is presented in Figure 5. There was an initial decline from hatching to the second day after hatching in the weight of ostrich chicks amounting to 3–4% for the different treatment groups (Fig. 5). These results correspond with previous findings by Deeming *et al.* (1993), Lambert *et al.* (1995) and Horbańczuk & Sales (2000). Deeming & Ayers (1994) reported that excess moisture not lost in the hatching process was stored in the skeletal muscles and under the skin of the hatchling. The initial body weight lost could be attributed to the loss of this excess moisture through defaecation and exhalation during the first few days after hatch.

After this initial decline, the recorded chick weights for the different treatment groups increased from 0.85 kg to 1.11 kg with an increased chick age to 7 d (P <0.05). Figure 6 shows the same trend and the decline in weight was ~3% and ~5% for chicks that survived and chicks that died, respectively. With an increased chick age, the recorded chick weights (P <0.05) for chicks that survived increased from 0.87 kg to 1.13 kg, whereas chicks that did not survive increased from 0.85 kg to 1.07 kg. Prior to 5 d of age, there were no statistical differences between the average live weights of chicks that survived or subsequently died. However, the average live weights of chicks that survived after 7 d of age exceeded those of the chicks that succumbed by 4.7% at 5 d and by 5.3% at 7 d, resulting in an interaction of mortality status with chick age (P <0.05; Cloete *et al.*, 2001).

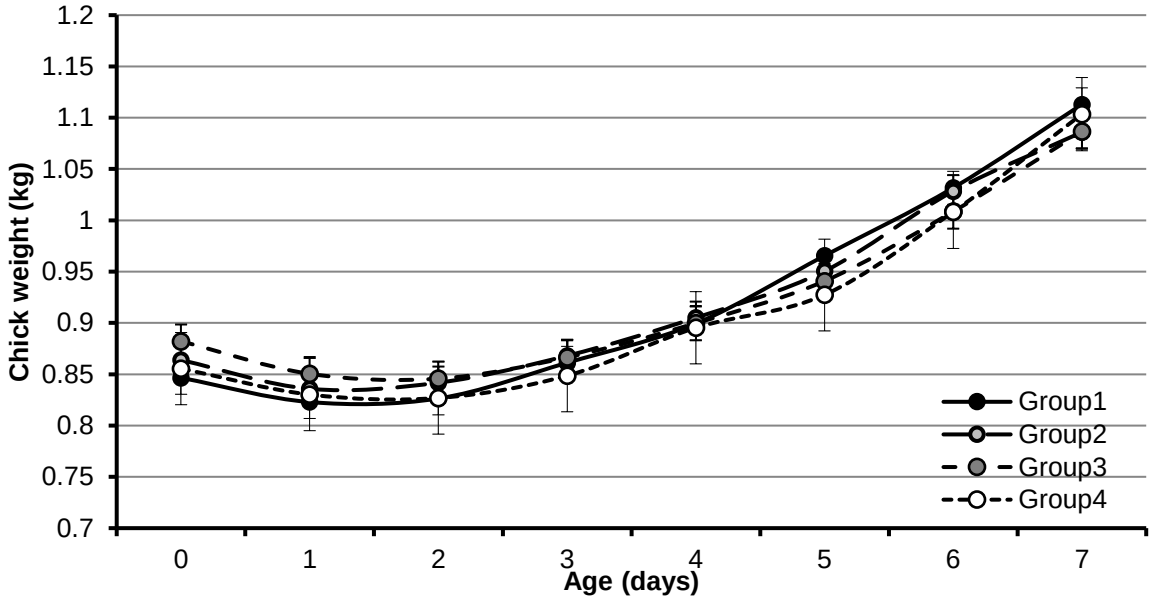

Figure 5 Predicted means (± SE) depicting the interaction of the hatching sequence with chick age, as recorded from hatching (day 0) to 7 d of age. Individual means were based on 49–53 chicks for Treatment 1, 50–56 chicks for Treatment 2, 47–50 chicks for Treatment 3, and 9–10 chicks for Treatment 4

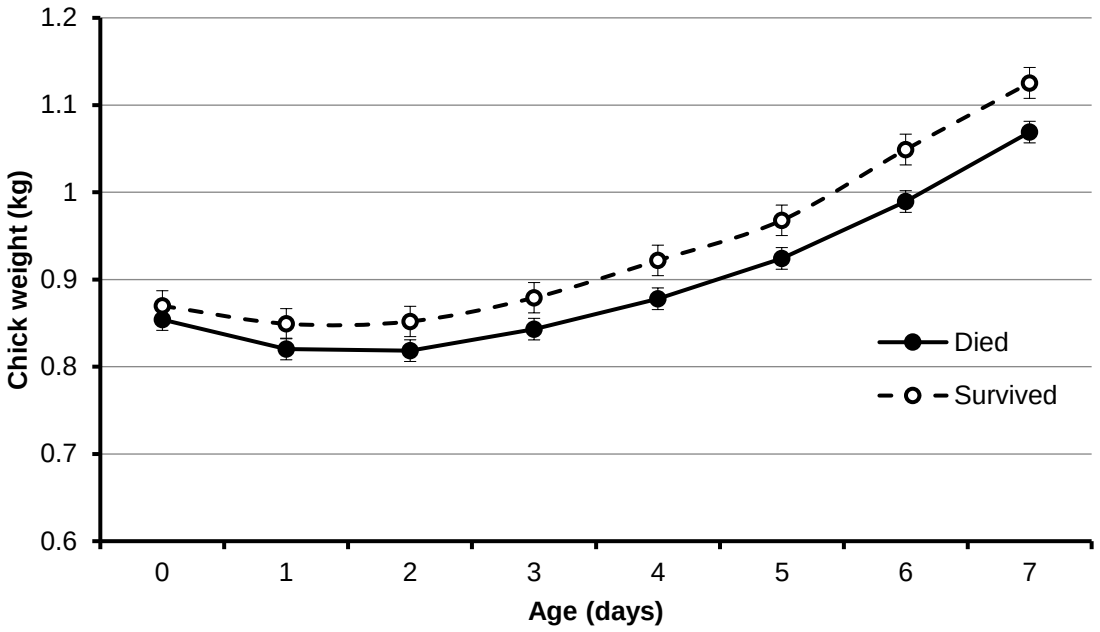

Figure 6 Predicted means (± SE) depicting the interaction of survival status (died or survived) with chick age, as recorded from hatching (day 0) to 7 d of age. Individual means were based on 120–122 chicks for survivors and 41–47 chicks for those that succumbed.

A similar age trend could be observed between Figure 6 and Figure 7 when the interaction of sex with chick age was considered. Male and female chicks showed a decline in chick weight from hatch to day 1 and day 2 respectively. The fact that sex had no effect on early chick survival concurs with findings of Lambert *et al.* (1995), Cloete *et al.* (2001), and Wang *et al.* (2012). Prior to 6 d of age, there were no marked differences between the average live weights of male or female chicks. This result corresponds with the chick weight of 0.85 kg recorded for male and female chicks by Cooper & Mahroze (2004). The live weight of male chicks was 3.1% higher than those of female chicks on days 6 and 7 of age (P <0.05, although it does not seem to be so, based on SE).

Carstens *et al*. (2014) reported that growth was relatively linear from hatch until ~250 d of age, which corresponds with the finding in this study that growth rate from 7 d onwards (~5 months of age) was linear (Fig. 8). There was no conclusive evidence that chick weight after 7 d was unaffected either by the hatching sequence or sex of the chick (Table 5; P >0.05). The mean chick weight for the different hatching sequences was 6.65–7.73 kg. No interactions of age with any of these fixed effects was found (P >0.05). The SE value observed for group 4 for chick weight was very high due to the small number of chicks that required assistance during hatching. For this reason, Group 4 was excluded in the report on body weight gain to ~5 months.

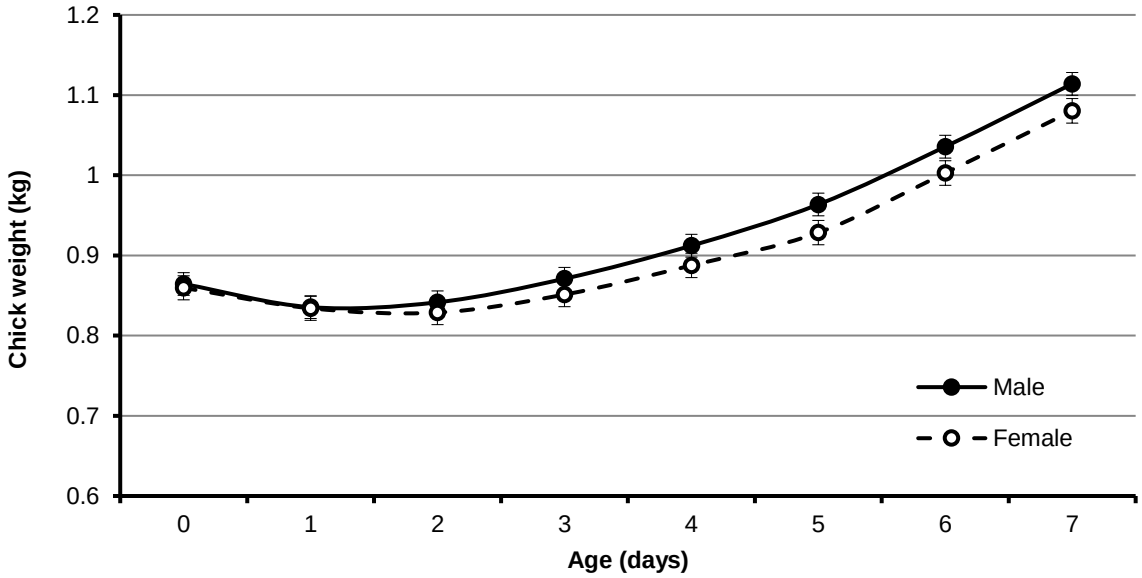

Figure 7 Predicted means (± SE) depicting the interaction of sex with chick age, as recorded from hatching (day 0) to 7 d of age. Individual means were based on 95–96 chicks for males and 67–71 chicks for females

Table 5 Overall means (± SE) of chick weight (kg) across ages from 1 w to approximately 5 months as affected by hatching sequence, subsequent mortality and sex

Effect and description	Records	Chick weight ± SE
Hatching sequence		0.56
Group 1 - Control	145	7.72 ± 0.27
Group 2 – Slight cracking of eggshell with external pipping	159	7.23 ± 0.25
Group 3 – Remove eggshell with external pipping	138	6.65 ± 0.28
Group 4 – Cracking of eggshell with internal pipping	25	7.56 ± 0.64
Sex		0.30
Male	272	7.43 ± 0.24
Female	195	7.15 ± 0.25

Actual significance for $P > 0.05$

Chick growth increased linearly from 1.08–15.2 kg with chick age from 8–147 days (Fig. 8). Although the chick weights recorded in this study were lower than weights reported by Bunter & Cloete (2004), the growth curve corresponded with that reported by Cooper & Mahroze (2004).

Figures 8 to 10 presents the same trends and with increased chick age, the recorded chick weights ($P < 0.05$) increased for the different hatching sequences, subsequent mortality, and different sexes. At 147 d, a marked difference can be seen in the trend lines of the different hatching methods. The control group that hatched naturally (Group 1), had the highest chick live weight at 16.9 kg, followed by Group 2 (15.1 kg) where the eggshell was cracked slightly at external pipping (Fig. 8). Chicks from Group 3, where the eggshell was removed at external pipping, showed the slowest live weight gain, resulting in a mean of 13.6 kg at 147 d. Chick live weight was 12.6% higher for the group hatching on their own, compared to the group where the eggshell was cracked and 24.6% higher for chicks from eggs where the eggshell was removed after external pipping ($P < 0.05$). Sex nor whether the chick survived or succumbed affected chick weight up to 147 d (Figs 9 and 10; Deeming *et al.,* 1993; Horbanczuk & Sales, 2000; Wang, 2012). In contrast, Mushi *et al*. (1998) and Deeming *et al*. (1993) reported a greater mean weekly live weight gain for female ostrich chicks compared to male chicks.

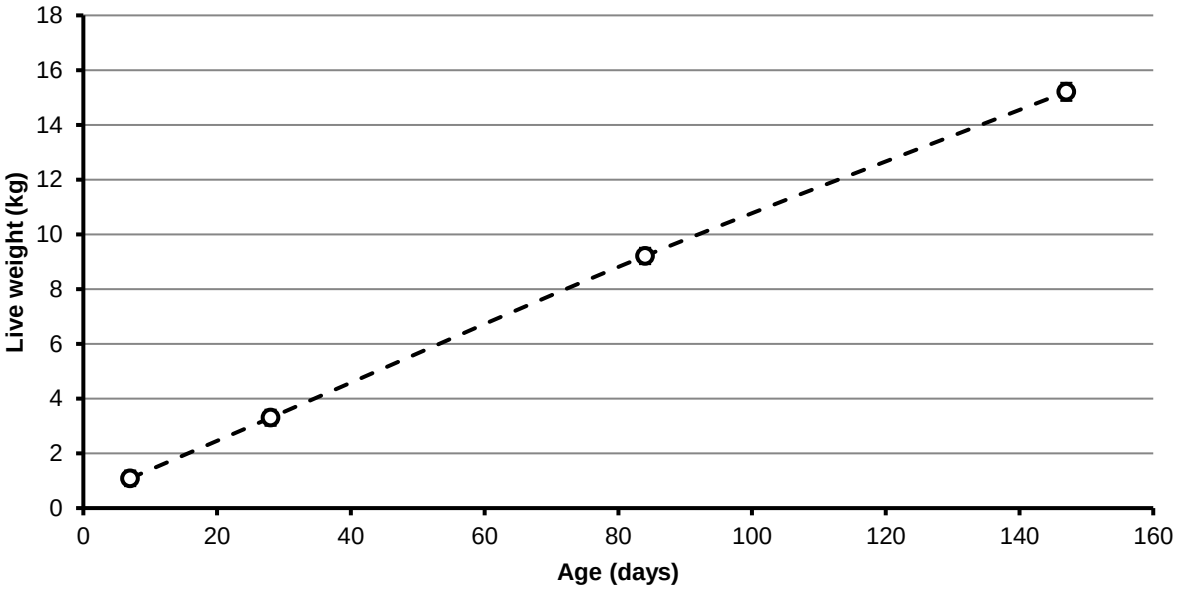

Figure 8 Predicted means (± SE) depicting the effect of age of chicks, as recorded from a week old to approximately 5 months of age. Individual means were based on between 162 chicks for an age of 7 d and 146 chicks for an age of 150 d

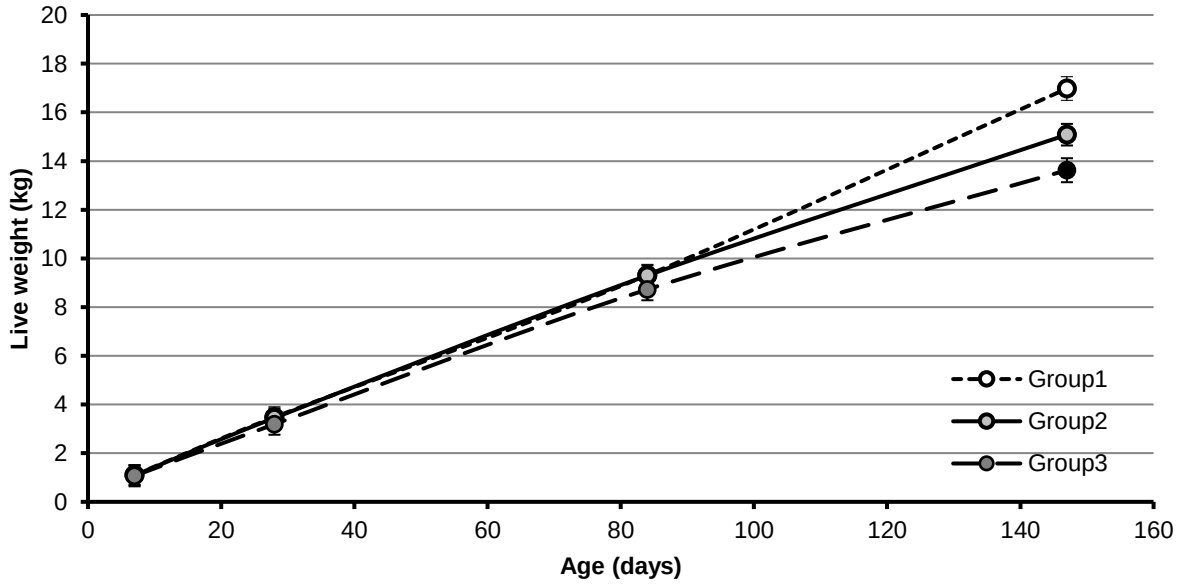

Figure 9 Predicted means (± SE) depicting the interaction of different methods of hatching with age of chicks, as recorded from 7 d of age to 147 d of age (P >0.05). Individual means were based on 45–51 chicks for Treatment 1, 49–55 chicks for Treatment 2, and 44–47 chicks for Treatment 3.

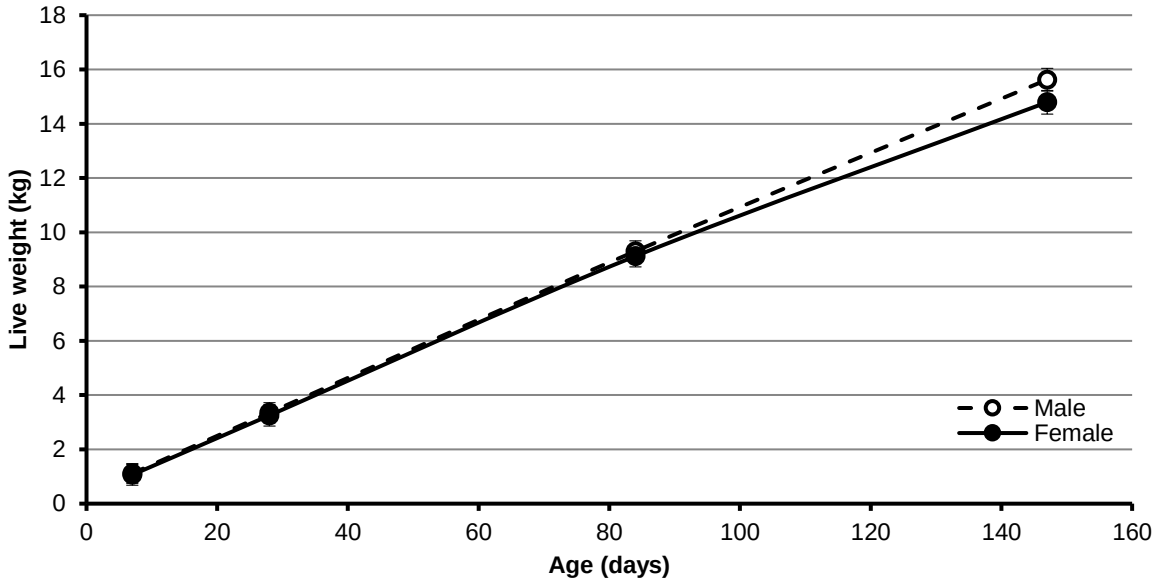

Figure 10 Predicted means (± SE) depicting the interaction of sex with age of chicks, as recorded from 7 d of age to 147 d of age (P >0.05). Individual means were based on 84–95 chicks for males and 62–67 chicks for females

Of 169 chicks recorded, a total of 50 (29.6%) died during the study. In total, eight causes of chick mortality were identified, namely injuries, prolapse, poor condition (not eating), intestinal infections, sticks/stones in gut, abnormalities, poor quality chicks, and yolk sac infection. A total of 21 (42%) of chick deaths was associated with sticks/stones in gut, 12 (24%) with injuries, 5 (10%) with intestinal infections, 4 (8%) with abnormalities, 3 (6%) with poor chick quality and yolk sac infection, and 1 (2%) with prolapse and poor condition. Burger & Bertram (1981) and Micheal *et al*. (2016) stated that early intervention in the case of weakened chicks is crucial for survival, whereas Deeming & Ayers (1994) and Cooper (2016) countered that chicks should not be assisted during hatching, because this only results in introducing poor quality chicks into the industry. In the current study, chick mortality was independent of the treatment supplied to individual chicks and in Table 6, the results of a Chi^2-test on the proportion of chicks that survived and succumbed is shown. Kingston (1979) reported that mortality rates among poultry chicks up to 10 d of age were greater for the chicks that pipped first compared to chicks that pipped last. Cloete *et al*. (2001), however, did note a marked difference in the mortality rates of ostrich chicks that hatched at the beginning and end of the breeding season when compared to those that hatched during the peak of the season. The proportion of survival between the treatments was between 0.60 and 0.72, with $Chi^2 = 0.62$ (degrees of freedom = 3). Like the hatching treatments, sex also had no effect of the survival rate of chicks (male: 31/98 = 0.316 vs. female: 19/71 = 0.267; $Chi^2 = 0.26$; P = 0.61; degrees of freedom = 1). These findings are consistent with previous reports by Horbanczuk & Sales (2000) and Cloete *et al*. (2001).

Table 6 Frequencies of chicks that succumbed and survived according to the hatching treatment they received tested using a Chi^2-test with 3 degrees of freedom

Treatment	Proportion	Chick numbers		
	Survival	Survived	Succumbed	Total
Group 1 - Control	0.698	37	16	53
Group 2 – Slight cracking of eggshell with external pipping	0.714	40	16	56
Group 3 – Remove eggshell with external pipping	0.720	36	14	50
Group 4 – Cracking of eggshell with internal pipping	0.600	6	4	10
Calculated Chi^2	0.618			
Critical Chi^2 for 3 degrees of freedom	7.815			

Conclusion

The timing and duration of internal pipping vary considerably. The minimum and maximum pipping intervals changed considerably between stages B and C, with the earlier stages also requiring the most time to complete. Chicks from eggs where the eggshells were cracked with internal pipping needed the most time to complete stages B and C. Additional research into the duration of phases B and C, as well as its impact, is necessary to determine the best possible moment to assist the chick with its emergence from the shell. Chicks that take longer in these stages may benefit from assistance in the form of fracturing the eggshell at the airspace region, which would allow the lungs to fill with oxygen to provide the energy needed to emerge from the shell in a more expedient manner. The physiological development of the chicks after hatching also differed considerably for the different hatching treatments. The chicks that hatched naturally and without any assistance showed an improved growth rate towards the end of the study when compared to chicks that had hatched either with minimal interference or those with complete assistance to climax. From a welfare aspect, the slower heartbeat found for unassisted chicks can be an indicator that less interference during the hatching process can supply a calmer environment for the hatchlings. In the future, emphasis should be placed on the time intervals of, and between Stages B and C, and interventions should occur somewhere within this time interval. Furthermore, it is evident that the chick benefits from the struggle to emerge, and that artificial climax should be a last resort.

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Authors' contributions

Concept, design, data collection, drafting of paper and submitting the manuscript, ZB. Critically analysed, SWPC. All the authors made substantial contributions to the original conception and design, acquisition of data, analysis and interpretation of data. All the authors have approved the manuscript being submitted.

Conflict of Interest

There is no conflict of interest.

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