STUDIES ON EMBRYONIC DEVELOPMENT AND HATCHABILITY OF OSTRICH EGGS

by

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own original work, that I am the sole author thereof and that I have not previously in its entirety or in part been submitted it for obtaining any qualification.

March 2012

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Abstract

The ostrich industry experiences high rates of embryonic mortalities during artificial incubation of eggs. Studies have been carried out to investigate factors influencing hatchability, as well as determining genotypic effects for commercial production. Eggs from the combination of South African Black (SAB) male ostriches crossed with Zimbabwean Blue (ZB) female ostriches had embryonic losses of 45.7%. The embryonic mortality of eggs produced by pure bred SAB or ZB breeding birds subjected to pure breeding was similar at around 33 - 34%, but embryonic mortality was improved in eggs produced by ZB males and SAB female crosses (27%). Female age had a significant effect on the proportion of chicks pipped, as well as on early and late the embryonic mortalities. Chicks from eggs stored for intermediate periods, i.e. 3, 4 and 6 days prior to being set, were more likely to pip than chicks from those eggs set directly after collection without storage. Embryonic mortality was increased in eggs that were set directly (32.0%) or subjected to longer than 6 days of storage (43.5%). Chicks that pipped in the correct position had a higher probability of successfully hatching than those pipping in the incorrect position. Transfer of eggs between setters (i.e. disturbance of eggs) during incubation reduced the number of ostrich chicks pipping in the correct position. Incubated ostrich eggs with intermediate levels of water loss, i.e. between 9.0 and 19% of fresh egg weight, were more likely to pip in the correct position than those with higher or lower levels of water loss. Such eggs were also less likely to sustain early, late or overall embryonic mortalities.

To optimise hatching success it is important to understand embryonic development. After 2 days of incubation the blastoderm area in eggs from the SAB x ZB crosses (104.5 mm) was lower ($P < 0.05$) compared to the pure SAB (141.0 mm), pure ZB (161.7 mm) and ZB x SAB crosses (166.1 mm). For embryos incubated for 7 to 42 days, both embryonic and leg growth during the 42 days of incubation was similar and approximately linear, more or less doubling in size up to 35 days of incubation. The embryo eye size increased more rapidly than beak length and reached full size of approximately 16.2 mm by 28 days of incubation, whereas the beak length continued to increase until the chick hatched at 42 days. Incubation position, vertical or horizontal, did not affect any of the measurements of the developing embryo throughout the 42-day incubation period. Air cell volume at 29 day of incubation for infertile eggs (19.3%) was significantly ($P < 0.05$) higher when compared to dead-in-shell eggs (14.3%) and eggs that hatched successfully (13.8%). Air cell volume was largely independent of strain (SAB or ZB) and whether chicks were assisted to hatch or not. After 41 days of incubation there was a significantly greater ($P < 0.05$) air cell volume in eggs that hatched normally compared to dead-in-shell eggs (28.3% vs. 21.7%, respectively, suggesting that insufficient water loss contributed to reduced survival. This study provides an insight into the complexity of embryo development and all the factors playing a role in successful hatching of ostrich eggs.

Data from a pair-mated ostrich flock were used to estimate genetic parameters for egg weight (EWT), weight of day-old chicks (CWT), water loss to 21 (WL21) and 35 (WL35) days of incubation, and pipping time (PT). Single-trait estimates of heritability (h^2) were high and significant ($P < 0.05$) at 0.46 for EWT, 0.34 for CWT, 0.34 for WL21, 0.27 for WL35 and 0.16 for pipping time. Genetic correlations with EWT amounted to -0.21

for WL21 and to -0.12 for WL35. Corresponding correlations of CWT with WL were highly significant ($P < 0.05$) at -0.43 and -0.54.

Physical characteristics of the eggshell were found to affect water loss and hatchability. Estimates of genetic parameters of 14 146 ostrich eggs for eggshell traits showed that heritability was 0.42 for pore count (PC), 0.33 for shell thickness (ST) and 0.22 for permeability (PERM). PC was negatively correlated with average pore diameter (-0.58) and ST (-0.23), while PC was positively correlated with total pore area (0.58), WL21 (0.24) and WL35 (0.34). The correlations of PC with total pore area and PERM were high and significant. ST was negatively correlated to WL21 and WL35. Additive genetic parameters strongly indicate that it should be possible to alter evaporative water loss and eggshell quality of ostrich eggs through genetic selection.

When assessed as a trait of the individual egg or chick, embryonic mortalities exhibited moderate levels of genetic variation both on the normal scale ($h^2 = 0.16 - 0.22$) and the underlying liability scale ($h^2 = 0.21 - 0.31$). Early embryonic survival and late embryonic survival was governed mostly by the same genes ($r_g = 0.78$). Late embryonic survival was genetically correlated to WL35, at -0.22. It was concluded that embryonic survival could be improved by using husbandry measures, a knowledge of the stage when incubation mortalities occur, and by genetic selection, using an integrated approach.

Findings from this study will help to understand the mechanisms involved in hatching from artificial incubation better to improve hatchability and also implement selective breeding programs.

Opsomming

Die volstruisbedryf ondervind tans ‘n baie hoë voorkoms van embrionale mortaliteite tydens die kunsmatige uitbroei van eiers. Studies is uitgevoer om die faktore wat uitbroeibaarheid beinvloed te ondersoek en om genotipiese effekte te bepaal vir kommersiële produsente. Eiers van die kombinasie van Suid-Afrikaanse swart (SAB) mannetjie volstruise, met Zimbabwean blou (ZB) wyfies, het embrionale mortaliteite van 45.7% gehad. Embrionale mortaliteite van eiers gelê deur suiwer SAB of ZB volstruise was dieselfde op omtrent 33 - 34%, maar embrionale mortaliteite was laer vir eiers geproduseer deur SAB wyfies wat gekruis was met ZB mannetjies (27%). Wyfie ouderdom het ‘n betekenisvolle effek gehad op die proporsie van kuikens wat gepik het, asook die aantal vroeë- en laat embrionale mortaliteite. Kuikens vanuit eiers wat vir die periode 3, 4 dae en 6 dae voor pak in die broeikaste gestoor is, was meer geneig om te pik as kuikens vanaf eiers wat direk na kolleksie gepak is. Embrionale mortalitiete het verhoog vir eiers wat direk na kolleksie gepak was (32.0%) of vir eiers wat langer as 6 dae gestoor was (43.5%). Kuikens wat in die korrekte posisie pik het ‘n hoër kans op uitbroei gehad as kuikens wat in die verkeerde posisie gepik het. Die skuif van eiers tussen verskillende broeikaste (of enige steurnisse) gedurende die broeiproses het ‘n verlaging in die aantal kuikens wat in die korrekte posisie pik, gehad. Volstruiseiers met ‘n gemiddelde vogverlies van tussen 9.0 en 19% van die vars eier massa, was meer geneig om in die korrekte posisie te pik as eiers met laer of hoër vlakke van vogverlies. Sulke eiers was ook minder geneig tot vroeë, laat en totale embrionale mortaliteite.

Vir optimale uitbroeisukses is dit belangrik om die ontwikkeling van die embrio te verstaan. Na 2 dae van broei was die blastoderm area in eiers van SAB x ZB kruisings (104.5 mm) kleiner ($P < 0.05$) as die blastoderm area van suiwer SAB (141.0 mm), suiwer ZB (161.7 mm) en ZB x SAB kruise (166.1 mm). Beide embrionale- en beengroei tydens die 42 dae broeiproses was dieselfde en nagenoeg lineêr, met ‘n verdubbeling in grootte tot en met 35 dae broei. Die embrio se oog vergroot vinniger as wat die snawel verleng en bereik reeds volle grootte van ongeveer 16.2 mm op 28 dae van broei, terwyl die snawel aanhou groei tot uitbroei van die kuiken op 42 dae. Nie die vertikale of horisontale broeiposisie het enige invloed op die metings van die ontwikkelende embrio tot op 42 dae gehad nie. Lugsakvolume vir geil eiers (19.3%) op 29 dae van broei was groter ($P < 0.05$) as beide die lugsakke van eiers wat dood-in-dop (14.3%) en eiers wat suksesvol uitgebroei het (13.8%). Die lugsakvolume was onafhanklik van beide genotype en of die kuiken met of sonder hulp uitgebroei het. Na 41 dae broei was lugsakvolume groter ($P < 0.05$) vir eiers wat uitgebroei het teenoor eiers wat dood-in-dop was (28.3% vs. 21.7%, onderskeidelik), wat impliseer dat onvoldoende vogverlies moontlik kan bydrae tot ‘n verlaging in embrionale oorlewing. Hierdie studie gee ‘n insig in die kompleksiteit van embrionale ontwikkeling en al die faktore wat ‘n rol speel in die suksesvolle uitbroei van volstruiseiers.

Tydens die bepaling van genetiese parameters vir spesifieke uitbroei-eienskappe in volstruise, is data gebruik afkomend van ‘n teelkudde in ‘n enkelparing stelsel om genetiese waardes vir eiermassa (EWT), dagoud kuikenmassa (CWT), vogverlies tot 21 dae broei (WL21), vogverlies tot 35 dae broei (WL35) en piktyd (PT) gebruik. Enkeleienskap-beraming vir oorerflikheid (h^2) was hoog en betekenisvol teen 0.46 vir EWT, 0.34 vir CWT, 0.34 vir WL21, 0.27 vir WL35 en 0.16 vir piktyd. Genetiese korrelasies met EWT was -

0.21 vir WL21 en -0.12 vir WL35. Ooreenkomstig was korrelasies van CWT met WL21 en WL35 hoog ($P < 0.05$) met -0.43 en -0.54 onderskeidelik.

Fisiese eienskappe van die eiers het beide vogverlies en uitbroeibaarheid beinvloed. Beramings van genetiese parameters vir 14 146 volstruiseiers se dopeienskappe het gewys dat oorerflikehid 0.42 was vir die aantal porieë (PC), 0.33 vir dopdikte (ST) en 0.22 vir deurlaatbaarheid (PERM). PC was negatief gekorreleerd met gemiddelde porieë deursnee (-0.58) en ST (-0.23), terwyl PC positief gekorreleerd was met totale porieë area (0.58), WL21 (0.24) en WL35 (0.34). Die korrelasie van PC met totale porieë area en deurlaatbaarheid was hoog en betekenisvol. ST was negatief gekorreleerd met WL21 en WL35. Additiewe genetiese parameters het sterk daarop gedui dat dit moontlik sou wees om vogverlies en eierkwaliteit (bv. dopkwaliteit en poreusiteit) van volstruiseiers te verander deur genetiese seleksie.

Indien embrionale mortaliteit geevalueer word as ‘n kenmerk van die eier of kuiken, toon dit matige vlakke van genetiese variasie op beide die normale ($h^2 = 0.16 - 0.22$) en die onderliggende skale ($h^2 = 0.21 - 0.31$). Beide vroeë- en laat embrionale oorlewing word deur dieselfde stel gene beheer ($r_g = 0.78$). Laat embrionale oorlewing was geneties gekorreleerd met WL35 teen -0.22. Die gevolgtrekking was dat embrionale oorlewing verbeter kan word deur verbeterde broeikamerpraktyke, kennis van op watter stadium van ontwikkelings embrionale mortaliteite plaasvind en deur genetiese seleksie.

Bevindinge vanuit hierdie studies sal help om die meganismes betrokke by die kunsmatige uitbroei van volstruiskuikens beter te verstaan om sodoende uitbroeibaarheid te verbeter en ook suksesvolle seleksie programme te implementeer.

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CHAPTER 1

General introduction

INTRODUCTION

Artificial incubation has become an essential part of the commercial ostrich farming in South Africa, but our understanding of artificial incubation in ostriches is still poor when compared to domesticated poultry. Problems with artificial methods of incubation and chick rearing are thus still among the most important constraints on the development of the ostrich industry. High levels of reproductive failure, specifically during the artificial incubation phase and the subsequent chick rearing phase, are impacting on the economic viability of the commercial ostrich production industry.

In an attempt to understand the high levels of incubation failure, the present study aims to report upon the stage-specific levels of hatching failure, and the systemic factors associated with these losses. Apart from this, a good understanding of the growth and development of the embryo during incubation is necessary to identify incubation problem areas and to provide an insight into issues that need to be addressed before better results can be expected. The importance and effect of systemic factors like female age, incubators and season on the hatchability of ostrich eggs, as well as factors associated with incubation, are to a large extent unknown. In previous studies, the importance of the appropriate levels of water loss has been highlighted, but environmental factors affecting water loss are still largely unknown. The same argument also applies to eggshell quality and the part it may play in the successful hatching of a healthy ostrich chick.

The performance of livestock is not only determined by environmental factors, but also by the contribution of genetics to the observed performance of individuals. Little is known in the ostrich industry about either genetic parameters or responses to selection for specific traits and it is generally accepted that genetic progress in the broader industry is unlikely. Egg weight for ostrich females can range from 825 g to 1 827 g, with great variation also occurring in egg production of female ostriches during a breeding season, ranging from 0 - 121 eggs produced. Genetic make-up is one of the factors influencing the performance of individuals and genetic improvement may be achieved by selection for certain traits. Estimates of genetic parameters for female traits like egg production, chick production and hatchability are limited to only a few studies, while no information is available on genetic parameters for ostrich incubation traits. The general lack of genetic parameters for incubation traits, essential for a meaningfull selection programs, places a limitation on efficient commercial ostrich production. To require a better understanding of genetic factors influencing the hatchability of ostrich eggs is thus crucial.

Against this background, the objectives of this study were thus:

1. to investigate factors related to embryonic mortalities and the influence of incubation management on hatchability and embryonic survival;
2. to study and describe the stages of development of the embryo from fresh, fertile eggs to 42 days of incubation;
3. to establish the environmental or systematic affects impacting on incubation traits and hatching failure, and to estimate genetic parameters of importance during the incubation process of ostrich eggs.

It was reasoned that a better understanding of these factors may contribute to an improved hatching success, and would aid selective breeding programs.

PART I

CHAPTER 2

Hatching failure of ostrich chicks, statistical analyses and aims of the study

1. Introduction

Ostriches are indigenous to Africa and ostrich farming originally commenced in South Africa during the early 1860's. The increasing demand for ostrich feathers led to the creation of a domestic industry and this progressed further with the invention of an artificial incubator for ostrich eggs in 1869 by Arthur Douglass (Deeming & Ar, 1999). World War I brought an end to the demand for ostrich feathers, which led to a collapse of ostrich farming. The development of a leather market in the 1950's revived the industry, with a further boost in the 1960's by the use of the meat. The initial confinement of the ostrich industry to South Africa contributed to a lack of research into production of farmed ostriches. As a consequence, relatively little is known about many aspects of ostrich farming and several production challenges have remained largely unaddressed.

Different structured breeding systems are currently used on commercial ostrich farms, e.g. quads (one male with three females), trios (one male with two females) and pairs (one male and one female). However, almost 80% of the national ostrich breeding population is maintained in breeding colonies, ranging from 50 - 100 birds at a ratio of 5 - 6 males for every 10 females. This makes identification of non-producing females, as well as poorly reproducing females impossible, especially with females laying in more than one nest and multiple females laying in a particular nest (Lambrechts, 2004). Literature also indicates that the range in egg production of pair-bred females is extremely large, with some females literally producing an egg every second day, while others fail to produce a single egg during an 8 - month breeding season (Van Schalkwyk *et al*., 1996; Cloete *et al*., 1998). If these facts are considered, it is clear that colony breeding structures are very uneconomic, especially if considering that feeding cost contributes almost 83% of the total input costs of a breeding system (Van Zyl, 2001).

The success of ostrich farming depends largely on the production of fertile eggs, but scientific reports of fertility and hatchability of artificially incubated ostrich eggs in different countries show that hatchability results are highly variable (Deeming & Ar, 1999). Although hatchability of artificially incubated ostrich eggs can be as high as 80%, it is typically between 30% to approximately 60% (Deeming & Ar, 1999; Van Schalkwyk *et al.,* 2000). Deeming & Ar (1999) have reported hatchability figures of fertile eggs as low as 11% in extreme situations.

The ostrich egg is unusual for its large size, ranging between 1 - 2 kg, averaging at approximately 1.5 kg (Deeming *et al*., 1993). Although an ostrich egg is the largest of the living birds, it is also the smallest (1.5%) in proportion to the adult body weight (Badley, 1997). This large egg size in not only interesting in general, but it also has an important impact on commercial artificial incubation (Deeming & Ar, 1999). Compared with other birds, the duration of incubation (42 days) is considerably shorter than the predicted interval of 58.8 days, as calculated from egg weight (Deeming & Ar, 1999).

2. Genotype

Zimbabwean Blues have been introduced into commercial breeding programmes to produce offspring with an improved live weight (Essa & Cloete, 2006) and an improved mature weight (Brand *et al.,* 2005). However, the effect of crossbreeding on egg production and fertility has not been considered. Embryonic mortality as a result of genetic problems can negatively influence hatchability, but this has not yet been demonstrated in ostriches (Badley, 1997). In the comparison between purebreeds and crosses, it should also be considered that SA Black females overall had a markedly higher overall egg and chick production than Zimbabwean Blue females irrespective of the genotype of the sire (Brand *et al.,* 2005; Davids, 2011). At this stage, it is not clear how incubation failure may contribute to chick production of the pure breeds and their crosses. This study will report the effect of breed upon hatching failure at different stages of incubation.

3. Female age

Ostrich females have a markedly longer productive life compared to all the other domestic poultry species (Ipek & Sahan, 2004) with females starting egg production at 2 - 2.5 years of age, while peak egg and chick production are achieved at 8 - 9 years of age (Smith *et al*., 1995; Cloete *et al.*, 2006). The age of the female appears to be one of many factors influencing the number of eggs produced as well as the hatchability of eggs. The egg production potential per breeding season of breeders in South Africa increased from an initial around 22 eggs at 2 year of age to a peak of around 40 eggs at 9 years of age, but showed a slow decline thereafter to around 45 - 50% at 17 years of age (Bunter *et al*., 2001; Cloete *et al*., 2006). Cloete *et al*. (2006) reported that reduced performance of older females was more pronounced for chick production and females > 10 years did not produce more chicks than 2-year-old females. Female age also affected egg and chick weights, peaking in 4 - 5 year-old females, while females > 11 years produced lighter chicks than 2-year-olds (Bunter *et al*., 2001). In both broiler breeders and quail, young females had a higher proportion of early embryonic mortalities than mature females (Reis *et al.,* 1997; Hocking & Bernard, 2000; Yildirim, 2005). For ostriches the opposite seems to be true in the sense that embryonic survival decreases over subsequent laying seasons (Badley, 1997). This trend also was hinted at by Bunter *et al*. (2001) and Cloete *et al*. (2006). This study seeks to quantify the effect of female age on embryonic mortalities at different stages of incubation.

4. Season and/or year of production

Ostriches are generally regarded as seasonal breeders, with breeding season coinciding with an increase in photoperiod (fall) (Smith *et al*., 1995; Horbaňczuk & Sales, 1999; Ipek & Sahan, 2004). Timing and duration of the ostrich breeding season can also vary with latitude and altitude (Bertram, 1979). In Israel, the bulk of eggs are produced between mid-February and September (Ar, 1996), thus from an early spring (northern hemisphere). In contrast, the peak of the breeding season was over a much longer period between July and March in Queensland, Australia (More, 1996). The peak period for egg production was between mid - April and mid - September (i.e. spring to autumn) in Britain (Deeming, 1996a). In southern Africa, ostrich breeding seasons are normally from June (mid-winter) to January (summer) of the following year (Jarvis *et al*., 1985; Lambrechts, 2004). Wilson *et al.* (1997) reported that the hatchability for the number of eggs set decreased

linearly as the breeding season progressed. Egg output was found to taper off in the period leading to and after the summer solstice (Lambrechts, 2004; Fair *et al.,* 2005). Environmental stress such as sudden rain or sudden heat spells may reduce egg laying temporarily (Deeming & Ar, 1999). However, such effects may be transient and unpredictable, making it difficult to plan for. Several authors have reported significant season or production year effects on the reproductive performance of ostriches, e.g. egg- and chick prodution (Van Schalkwyk *et al*., 1996; Bunter *et al*., 2001). This study will report on the year/season effects upon artificial incubation failure.

5. Storage time

The importance of storage of ostrich eggs cannot be underestimated (Deeming & Ar, 1999). Storage of eggs is an integral part of normal hatchery practice. When performed properly, cold storage should not be detrimental to hatchability. Pre-incubation storage leads to morphological changes in the blastoderm and to a lower growth rate of the embryo in small domestic poultry species (Meijerhof, 1992; Fasenko *et al.,* 1992). The threshold temperature for avian embryonic growth is 20 °C and ostrich eggs can be stored at 17 - 20 °C for up to 6 days without a significant reduction in viability, while a significant decrease in hatchability occurred in eggs stored for longer periods (Swart *et al*., 1987; Van Schalkwyk *et al*., 1999a). Sahan *et al.* (2004) found that late embryonic mortalities increased from 14.3% in eggs stored for one day to 18% in eggs stored for 10 days. After also finding a decreased hatchability in eggs stored for both 1 day and 6 - 7 days, Ar & Gefen (1998) suggested that ostrich eggs may benefit from a storage period of only 3 - 4 days. Ostrich eggs stored at 25 °C show a significant increase in late embryonic mortality (Van Schalkwyk *et al*., 1999a). Relative humidity near 75% is recommended to prevent excess evaporative water loss from the eggs during storage (Stewart, 1995). Albumen protects the embryo from infection, therefore storage conditions and duration need to be adjusted to consider the quality of albumen (Badley, 1997). Ar & Gefen (1998) also reported a significant increase in rotten eggs after 7 days of storage. Current practices of ostrich egg storage vary, but eggs are generally stored at temperatures ranging from 15 - 30 °C and turned once a day by hand. This study seeks to establish the effect of different storage periods upon embryonic losses at different stages of incubation.

6. Incubators

Artificial incubation has become an integral part of any commercial poultry enterprise (Badley, 1997). Despite substantial advances in incubator design and incubation techniques since the ostrich industry began in South Africa during the 1800s, problems with embryonic mortality during artificial incubation is still one of the main constraints to the development of the ostrich industry world-wide (Brown *et al.,* 1996; Deeming & Ar, 1999). As with other species of birds, the physiological requirements of the developing ostrich embryo can be met during artificial incubation by providing an appropriate temperature (Van Schalkwyk *et al.,* 1999a), humidity (Swart *et al*., 1987), the correct gaseous environment (Van Schalkwyk *et al.,* 2002) and the proper turning of eggs (Van Schalkwyk *et al.*, 2000) in automatic incubators. Ideally, variation in size, etc. between eggs set in specific incubators should not be extreme so that single settings for temperature and humidity would produce a suitable incubation environment for most eggs in multi-stage incubators (e.g.

batches of eggs containing several stages of development within the same machine) (Deeming & Ar, 1999). A wide range in egg weight in the ostrich poses a problem for incubation. The different surface area:volume ratio of the different sized eggs mean that temperature profiles of the eggs may differ, with eggs of higher metabolic rate producing more heat (Deeming & Ar, 1999). An average of 15% evaporative water loss of the initial weight for a 1.5 kg egg will be achieved for one humidity setting, but it is likely that this setting may be unsuitable for smaller and larger eggs differing in their shell conductance. This may result in excessive or inadequate water loss percentages, respectively (Deeming & Ar, 1999). Ar (1996) showed that the hatchability of large and small eggs were 28 and 14% respectively lower than that of eggs of average weight. Commercial incubation of ostrich eggs is unlikely to improve unless there is greater uniformity in egg size and shell quality hence similar rate of water loss from eggs incubated (Deeming & Ar, 1999). So far this is achieved by utilizing different incubator humidity settings for eggs with different shell conductance or size characteristics during single-stage incubation. Alternatively, the range in egg weight and shell water vapour conductance may be restricted by discarding eggs with extreme values for weight and eggshell conductance and retaining those with limited variation (Meir *et al*., 1987; Ar, 1996).

Exposure to heat stress can impair hatchability (Ande & Wilson, 1981) and high temperatures can lead to an increase in early- and late embryo mortalities (French, 1997; Badley, 1997; Hassan *et al.,* 2005). The same detrimental effect on hatchability was also shown in ostrich eggs, where severe temperature gradients were shown to exist in forced-draught wooden incubators (Van Schalkwyk *et al.,* 1999b). Against this background, this study will compare the hatching success of ostrich eggs that were incubated in different incubators.

7. Malpositioning

Embryonic mortality patterns in avian species appears to follow a pattern typified by the chicken embryo, with one mortality peak during the first few days of development and a second, larger peak during the last few days of incubation (Deeming & Ar, 1999). Results from work done by both Deeming (1996a;b) and Ar (1996) correspond with this pattern. Malpositioning of embryos with respect to the air cell generally results in failure to hatch (Brown *et al.*, 1996). Brown *et al.* (1996), Deeming (1997) and Ipek & Sahan (2004) reported that embryonic mortalities in ostrich eggs due to malpositions of the embryo occur commonly, and may contribute up to 55% of all hatching failures. Developing ostrich chicks start to turn in the egg to assume the correct pipping position from day 35 of incubation and have usually assumed the correct pipping position by day 42 of incubation (Deeming, 1995). At this stage, the neck of the normally presented chick lays from left to right with the right foot next to the beak and the left foot positioned in the nape of the neck. This pipping position differs from that of domestic fowl (Deeming, 1994). Most of the malpositioning could be related to problems with rotation, where the embryo was incorrectly positioned at the end of incubation, with the head and beak turned away from the air cell (Button *et al*., 1994; Brown *et al*., 1996; Deeming, 1997). This study will consider the positions of 2 675 near-term normally developed chicks that succumbed during the final week of incubation, as well as possible causes for malposition during artificial incubation.

8. Water loss

A significant proportion of overall chick and embryo mortalities during artificial incubation can be related to incorrect water loss and possibly inadequate gas exchange during incubation. Weight loss is entirely due to a loss of water as the volume of oxygen absorbed by the egg is balanced by the release of carbon dioxide (Badley, 1997). On average 15% of the initial egg weight is lost during incubation and an additional 6% is lost between pipping and hatching (Ar & Rahn, 1980). Swart *et al.* (1987) determined that the total water loss from ostrich eggs incubated in natural nests amounts to around 13% of the initial egg weight, while studies by Deeming (1995), Ar (1996) and Blood *et al.* (1998) showed that the optimal water loss in artificially incubated ostrich eggs is between 13 and 15%. Although there is some variation in the percentage water loss at which ostrich eggs will still successfully hatch, there is, however, a sharp increase in embryonic mortality below 10% and above and 18% water loss at 35 days of incubation (Deeming & Ar, 1999; Blood *et al*., 1998). Excessive water loss during incubation causes early depletion of allantoic fluids, which results in subsequent dehydration of the embryo and extends the period of osmotic stress (Davis *et al*., 1988). It can result from high permeability of the eggshell to water vapour and other gases or an incubator set at a too low humidity level. In contrast, insufficient water loss from the egg can result in water retention by the chick, potentially causing embryonic mortality through respiratory insufficiency (Musara *et al.,* 1999) and a high proportion of chicks that are malpositioned at the point of hatch or may have unabsorbed yolk sacs (Horbaňczuk *et al.,* 1999). Insufficient water loss indicates a low permeability of the eggshell to water vapour and other gases, or an elevated incubator humidity level.

In artificially incubated eggs, there are then two main factors that affect water loss from the eggs; relative humidity (RH) in the incubator and the porosity of the eggshell itself to water vapour. While incubator humidity can usually be controlled, the large variation in water loss (and subsequent hatching success) in ostrich eggs under controlled incubator conditions suggests a wide variation in shell porosity, possibly indicating a genetic variability for this trait among ostrich females. Wilson (1996) suggested that hatchability of ostrich eggs could be substantially improved by selecting females that lay eggs with good shell qualities and with an adequate, uniform shell porosity. This study will attempt to quantify the impact of evaporative water loss on embryonic mortality levels of ostrich eggs during artificial incubation.

9. Statistical analysis

The main statistical tool used for this section is the Chi^2-statistic, which is briefly descriped in this paragraph. Pearson's Chi^2 tests a null hypothesis stating that the frequency distribution of certain events observed in a sample is consistent with a particular theoretical distribution (Pearson, 1990). It is used to assess two types of comparisons, namely: tests of goodness of fit and tests of independence. In the present analyses the test of independence which assessed whether paired observations differed among defined causative effects were used (i.e. days stored, genotypes, incubators, etc.). 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 mostly involved a chick or embryo 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). Since Chi²-statistics cannot be calculated if only proportions or percentages are known, the Yates correction for continuity was applied to ensure a robust outcome by not reporting any false positive results (Snedecor & Cochran, 1967). The effect of the Yates correction is considered to be relatively small when analyses involve substantial numbers of records (as in the present study). Multiple comparisons are often made when larger experimental data sets are analysed by Chi²-procedures in n x n contingency tables. In such cases the level of significance needs to be adjusted to account for the fact that multiple comparisons are involved. The Bonferoni correction is indicated to ensure that the statistical level of detection is harmonised with the number of comparisons involved (Van Ark, 1990).

It should be noted that internet resources are readily available to analyse frequencies following a Chi² distribution (see http://www.quantpsy.org/chisq/chisq.htm for instance). Data of up to a 10 x 10 table can be analysed in the latter application, with the choice to obtain Chi²-statistics with and without the Yates correction for continuity.

10. Aims of this section of the study

With the exception of a few papers on production and reproduction, factors influencing fertility, hatchability and incubation have not yet been investigated in ostriches. The high levels of hatching failure of ostrich eggs need to be addressed for ostrich farming to become more profitable. Identification of such non-genetic factors affecting reproduction and incubation traits may be useful for improving flock productivity by management and sound hatchery practices. Thus the main objective of the study was to investigate non-infectious factors that potentially influence embryonic mortality in ostrich eggs, specifically genotype, female age, year and season of production, storage time prior to the setting of eggs as well as the incubator used. This part of the dissertation therefore focuses on the following aspects:

- Factors related to shell deaths during artificial incubation of ostrich eggs
- The influence of incubation management on pipping position, hatching ability and survival of near-term ostrich chicks
- The dead-in-shell positions of near-term ostrich embryos

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CHAPTER 3

Factors related to shell deaths during artificial incubation of ostrich eggs

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Abstract

The ostrich industry experiences a high rate of embryonic mortalities during artificial incubation of eggs. Embryonic deaths were studied from data recorded on 37 740 fertile eggs incubated artificially during the 1998 - 2005 breeding seasons. Roughly 10 000 eggs that sustained embryonic mortalities were classified according to the stage and nature of death, i.e. before 21 days of incubation, after 21 days of incubation, deaths after pipping, and rotten eggs. Although infection may have played a role in 1 300 rotten eggs, no detailed knowledge of the pathogens involved was available. The remainder of deaths could not be related to pathogens, and the deaths were thus generally referred to as non-infectious. The overall level of embryonic mortality in all the eggs studied was 28.5%. Overall embryonic mortality was affected by incubator, with higher levels (57.0%) found in eggs incubated in an African Incubator® and also in eggs that were transferred between incubators during incubation (38.1%). Overall embryonic mortality also increased in eggs produced by older females. Eggs produced in the autumn had the highest level of embryonic mortality at 53.6%, whereas eggs produced in the winter had a marginally higher level of embryonic mortalities of 29.2% compared to eggs produced during summer (27.4%). Eggs produced by South African (SA) Black males crossed to Zimbabwean Blue females had high levels of embryonic losses of 45.7%. The embryonic mortality of eggs produced by SA Blacks or Zimbabwean Blue breeding birds subjected to pure breeding was similar at around 33 - 34%, but embryonic mortality was improved in eggs produced by Zimbabwean Blue males crossed to SA Black females (27%). Embryonic mortality was increased in eggs that were set directly (32.0%) or subjected to longer than 6 days of storage (43.5%). Embryonic mortality was affected by year. These results will assists to determine non infectious factors that have a negative effect on hatching success. Steps can thus be taken to eliminate such factors that may compromise hatching success.

1. Introduction

Artificial incubation has become an essential part of any commercial poultry enterprise (Badley, 1997). Ideally, every fertile egg should produce a healthy hatchling. In reality this situation is never achieved in a commercial hatchery. Despite substantial advances in incubator design and incubation techniques since the ostrich industry in South Africa began in the 1800s, problems with embryonic mortality during artificial incubation is still one of the main constraints to the development of the ostrich industry world-wide (Brown *et al.*, 1996; Deeming & Ar, 1999). Optimum incubator temperature used in automatic incubators is normally defined as that required to achieve maximum hatchability (French, 1997). The physiological requirements of the developing ostrich embryo are met by the control of temperature (Van Schalkwyk *et al.*, 1999b), humidity (Swart *et al.*, 1987), gaseous environment (Van Schalkwyk *et al.,* 2002) and the turning of eggs (Van Schalkwyk *et al.*, 2000). Although hatchability of artificially incubated ostrich eggs can reach 80%, it is typically between 30% to approximately 60% (Deeming & Ar, 1999; Van Schalkwyk *et al.*, 2000). Deeming & Ar (1999) have reported hatchability of fertile eggs as low as 11% in extreme situations. Shell-deaths thus contribute to a large extent to the low hatching rates in the ostrich industry. Embryonic mortality in ostrich eggs usually occurs either early or late in the incubation period, with relative few deaths in mid-term (Badley, 1998).

The age of the female appears to be one of many factors influencing the number of eggs produced as well as hatchability. Females start egg production at an age of 2 - 2.5 years and peak egg and chick production are achieved at 8 - 9 years of age. This peak was followed with a general decline in reproductive performance at greater ages, which was more pronounced for chick production than for egg production (Cloete *et al.*, 2006). Ostrich females have a longer economic life compared with the other poultry species (Ipek & Sahan, 2004), therefore making it difficult to compare ostrich breeders to the small domestic poultry species traditionally used for egg production. In both broiler breeders and quail, young females had a higher proportion of early embryonic deaths than mature females (Hocking & Bernard, 2000; Reis *et al.,* 1997; Yildirim, 2005). For ostriches the opposite seems to be true in the sense that embryonic survival decreases over subsequent laying seasons (Badley, 1997).

Ostriches are generally regarded as seasonal breeders, with the commencement of the breeding season coinciding with an increase in photoperiod (Ipek & Sahan, 2004). According to Lambrechts (2004), peak production for ostriches in the southern hemisphere occurs between winter (July) and summer (January). Genetic make-up is one of the factors influencing the performance of individuals and by selecting for certain traits, genetic improvement may be achieved (Petitte & Davis, 1999). Egg quality is also reported to have significant genetic components (Stewart, 1995). Fertility in turkeys is influenced genetically, with strain and variety differences that are apparent (Badley, 1997).

Pre-incubation storage leads to morphological changes in the blastoderm and to a lower growth rate of the embryo in small domestic poultry (Meijerhof, 1992; Fasenko *et al*., 1992). Albumen quality is compromised by prolonged storage time (Badley, 1997). A proportionate increase in early embryonic mortality occurs with an increased storage time of duck and quail eggs (Narahari *et al*., 1991; Yildirim, 2005). This coincides with

results of Deeming & Ar (1999), reporting a lower hatchability in ostrich eggs that could be attributed to an increase in early mortalities for eggs stored between 12 - 14 days. Ar & Gefen (1998), Badley (1997), Sahan *et al.* (2004) and Hassan *et al.* (2005) also reported an increase in early embryonic mortalities for eggs stored for extended periods up to 10 days and longer.

The main objective of the present study was to investigate non infectious factors that potentially influence embryonic mortality in ostrich eggs, specifically genotype, female age, year and season of production, storage time prior to setting eggs in the incubator and the incubator used.

2. Material and Methods

2.1. Animals

Eggs were derived from the commercial ostrich breeding flock maintained at the Oudtshoorn Research Farm in the Klein Karoo region of South Africa. The origin of the ostrich flock and the general management procedures implemented has been described previously (Van Schalkwyk *et al.*, 1996; Bunter & Cloete, 2004). Data for this study were collected from the 1998 - 2006 breeding seasons. In total, 48 027 eggs were produced by the flock during this period. The data were edited to exclude infertile eggs (n = 10 173), eggs not set because of breakages and cracks in the shell (n = 1 495), eggs with defects, i.e. too small, soft or chalky shells, etc. (n = 805) and eggs not set for various other reasons (n = 1 178). The latter classification included eggs left as nest eggs, eggs left with breeding pairs that were used for chick rearing, as well as eggs used in other experiments. Details with regard to the genotype, female age, date of lay, year and season of lay, storage time, and specific incubator used were known for individual eggs and were evaluated for the remaining 37 740 eggs in an attempt to derive robust trends involving the influence of various non-infectious factors on embryonic mortality.

Unless specified otherwise, each breeding bird received a ration of 2.5 kg dry matter per day throughout the breeding season, which lasted from the beginning of June until the end of January for most years. The exceptions to this were the 1999 breeding season (when the birds were also retained in the breeding paddocks for February) and 2002 (when some breeding pairs were left in the mating paddocks throughout the year, as part of a separate experiment described by Lambrechts (2004)).

During 2003 Zimbabwean Blue (ZB) breeders were introduced to the flock during 2003 and mated in various combinations with South African Black (SAB) males and SAB females (Brand *et al*., 2005). Data that were recorded for 2003 - 2006 thus involved various combinations of the two purebred bloodlines as well as the reciprocal crosses between them. Because ZB birds were heavier than their SAB contemporaries (Brand *et al*., 2005), they were assumed to have a larger maintenance requirement and therefore received 2.8 kg feed/breeding bird per day.

2.2. Egg storage and Incubation

Methods for collection, sanitation and storage at the experimental site are well documented (Van Schalkwyk *et al.,* 1998; Van Schalkwyk *et al*., 1999a). Briefly, eggs were collected daily, weighed and identified by date and paddock (female) of origin. The surface of each egg was sterilized by 20 min of ultraviolet exposure and labelled with a permanent marker. During the breeding season, eggs were stored for no more than 6 days at a temperature of 17 ºC and relative humidity (RH) of 75%. At the beginning and end of the season, however, there were insufficient eggs to occupy the available incubator space optimally. These eggs were consequently stored for periods not exceeding 20 days. Eggs were artificially incubated at 36 ºC and 24% RH in Buckeye®, Prohatch®, Natureform® or African International® incubators. A practice of moving eggs between incubators during the incubation process was necessary because of limited space within incubators and the large numbers of eggs being produced each week during peak production. Space in individual incubators dictated the placement of eggs. The Buckeye® and Prohatch® incubators were preferred as the primary incubators throughout the laying season. The other incubators were primarily used during peak-laying in winter and spring to accommodate the overflow. This resulted in the occasional use of a combination of incubators for a single setting of eggs. The capacity and operation of the incubators, with the exception of the African Incubator® (which was more recently acquired) have been described by Cloete *et al*., 2001. The African Incubator® had a capacity of 1 000 eggs and operated at 36 °C and a RH of 24 %. During incubation, the eggs were turned through angle of 60° hourly and eggs were treated as described by Van Schalkwyk *et al.* (1999b).

2.3. Statistical methods

Overall embryonic deaths were classified as embryonic deaths from 0 to day 21 of incubation (1st half), after 21 days of incubation (2nd half), post-pipping and rotten eggs. Eggs not showing any macroscopic development (blastoderm size < 3mm) were regarded as infertile and those with embryonic development that had ceased (blastoderm size > 3mm) as 1st-half embryonic deaths (Van Schalkwyk *et al*., 2000).

It is conceded that infection may have played a role in around 1 300 rotten eggs. However, no detailed knowledge of the pathogens was available. The remainder of deaths could not be related to pathogens, and the deaths were thus referred to as non-infectious.

Chi square procedures were used to assess the effects of genotype, female age, year and season of lay, storage time, as well as of the incubator used (Van Ark, 1990). The Bonferoni correction was applied to all analyses, since all analyses involved multiple comparisons. Embryonic deaths during the 2nd half of the incubation period and deaths post-pipping were expressed relative to all fertile eggs, or relative to the eggs still being incubated after candling at 21 days (i.e. after infertile eggs and eggs with embryonic mortalities during the 1st half of the incubation period were removed). Both sets of figures are presented but it needs to be stressed that the conclusions derived from this study were largely independent of this classification.

3. Results

3.1. Genotype

Since the ZB birds were introduced to the Research Farm during 2003, only the production seasons of 2003 - 2006 were considered. Eggs produced by SAB females mated to ZB males had the lowest proportion of embryonic mortality (0.270; Table 1). The latter proportion is significantly lower than those derived for purebred SAB eggs (0.338) and from an uncharacteristically high proportion of overall embryonic mortalities in the SAB male x ZB female combination (0.457) ($P < 0.05$). Embryonic mortalities during the 1st half and 2nd half of incubation, as well as rotten eggs were the main contributors to the poor performance of eggs produced by the latter breed combination compared to those eggs produced by ZB males mated to SAB females in particular. Overall embryonic mortalities were fairly consistent across seasons for SAB (ranging from 0.331 in summer to 0.341 in the winter), and the two crossbred combinations (ranges of 0.267 - 0.277 in the ZB x SAB combination and 0.439 - 0.472 in the SAB x ZB combination). By contrast, embryonic mortalities of pure ZB eggs were affected by season ($\chi^2 = 6.431$; degrees of freedom = 3; $P < 0.05$). Closer scrutiny of the data revealed that embryonic mortalities tended to be lower during summer (0.239) than in winter (0.365) and in spring (0.302). However, no significant differences between seasons were detected after the Bonferoni correction was applied to the data, owing to the low number of eggs produced in summer (n = 61).

Table 1 Proportions of overall and classified embryonic mortalities in relation to genotype for the 2003 - 2005 breeding seasons.

Genotype	**Category**							
	Number of eggs	Embryonic mortalities 1st half	Embryonic mortalities 2nd half	Second half (live)*	Death post-pipping	Death post-pipping (live)*	Rotten	Overall
Overall	19 925	0.073	0.207	0.223	0.012	0.016	0.042	0.333
SAB ♂ x SAB ♀	15 102	0.072^{a}	0.211^{b}	0.227^{b}	0.011	0.015	0.044^{b}	0.338^{b}
ZB ♂ x ZB ♀	894	0.097^{b}	$0.178^{a,b}$	$0.197^{a,b}$	0.020	0.028	$0.034^{a,b}$	0.329^{b}
SAB ♂ x ZB ♀	976	0.122^{c}	0.255^{c}	0.291^{c}	0.015	0.025	0.065^{c}	0.457^{c}
ZB ♂ x SAB ♀	2 953	$0.059^{b,c}$	0.177^{a}	0.188^{a}	0.012	0.015	0.022^{a}	0.270^{a}
χ^2		51.735	35.446	46.134	7.458	9.035	44.854	122.028

^{a,b,c} Denote significant ($P < 0.05$) differences in columns

Critical χ^2 ($P = 0.05$) for 3 degrees of freedom = 7.815

*Expressed relative to eggs still being incubated after candling at 21 days

SAB = South African Black; ZB = Zimbabwean Blue

3.2. Female age

The proportion of overall embryonic mortality increased with female age ($P < 0.05$; Table 2). Both embryonic mortalities during the 1st half and 2nd half of incubation were proportionally increased in older females, the effect being more pronounced for deaths during the 2nd half of incubation ($P < 0.05$). Embryonic mortalities

belonging to other classifications were less clearly related to female age, although a small number of significant differences were also found for those eggs for which a cause of mortality could not be ascribed (Table 2). Embryonic mortality post-pipping, and rotten eggs, were largely independent of female age ($P > 0.05$). However, the eggs produced by the oldest female age category of > 10 years appeared to sustain higher levels of spoilage than those produced by some younger age groups ($P < 0.05$).

Table 2 Proportions of overall and classified embryonic mortalities in relation to female age from 2 to > 10 years.

Age	Category							
	Number of eggs	Embryonic mortalities 1st half	Embryonic mortalities 2nd half	Second half (live)*	Death post-pipping	Death post-pipping (live)*	Rotten	Overall
Overall	37740	0.060	0.178	0.189	0.011	0.014	0.036	0.285
2 years	2100	0.044^{a}	0.119^{a}	0.125^{a}	0.013	0.016	$0.038^{a,b}$	0.214^{a}
3 years	5477	0.051^{a}	0.158^{b}	0.167^{b}	0.010	0.013	0.034^{a}	0.253^{b}
4 years	5921	0.051^{a}	$0.171^{b,c}$	$0.180^{c,b}$	0.012	0.016	0.032^{a}	$0.266^{b,c}$
5 years	5732	$0.060^{a,b}$	0.165^{b}	$0.175^{c,b}$	0.012	0.016	$0.041^{a,b}$	$0.277^{b,c}$
6 years	5092	0.064^{a}	$0.179^{b,c}$	0.192^{c}	0.007	0.009	0.033^{a}	0.283^{c}
7 years	4529	0.070^{b}	$0.181^{b,c}$	0.195^{c}	0.011	0.014	0.038^{a}	$0.299^{c,d}$
8 years	3485	0.062^{a}	0.219^{d}	$0.233^{e,c}$	0.011	0.016	0.034^{a}	$0.326^{d,e}$
9 years	2402	0.078^{b}	0.230^{d}	$0.249^{e,d}$	0.009	0.013	0.031^{a}	0.348^{e}
10 years	1599	0.058^{a}	$0.187^{b,d}$	$0.198^{d,c}$	0.015	0.020	$0.036^{a,b}$	$0.295^{c,d}$
>10 years	1403	0.076^{b}	$0.205^{c,d}$	0.222^{d}	0.014	0.020	0.059^{b}	$0.355^{d,e}$
χ^2		56.047	166.296	186.891	15.101	14.162	33.154	205.679

^{a,b,c,d,e} Denote significant ($P < 0.05$) differences in columns

Critical χ^2 ($P = 0.05$) for 9 degrees of freedom = 16.919

*Expressed relative to eggs still being incubated after candling at 21 days

3.3. Year

Significant variation was found for the overall embryonic survival of eggs produced in different years. Significant ($P < 0.05$) variation between years was also found for the classified causes of embryonic mortality. These results were not tabled, for reasons that are outlined in the Discussion.

3.4. Season

Overall, embryonic mortalities was particularly high in a small number of eggs produced during autumn ($P < 0.05$; Table 3). The overall proportion of embryonic mortalities was marginally lower ($P < 0.05$) in eggs produced during summer (December - February) than in eggs produced during winter (June – August), at 0.274 and 0.292 respectively. Compared to the eggs produced out of season during autumn, however, the latter difference is of a small magnitude. Season had no effect on embryonic mortalities that occurred post-pipping (Table 3).

Table 3 Proportions of overall and classified embryonic mortalities in relation to the season of production.

Season	Category							
	Number of eggs	Embryonic mortalities 1st half	Embryonic mortalities 2nd half	Second half (live)*	Death post-pipping	Death post-pipping (live)*	Rotten	Overall
Overall	37740	0.060	0.178	0.189	0.011	0.014	0.036	0.285
Winter	13766	$0.062^{b,c}$	0.171^{a}	0.183^{a}	0.012	0.015	0.047^{b}	0.292^{b}
Spring	16425	0.059^{b}	0.183^{a}	0.194^{a}	0.011	0.015	0.026^{a}	$0.279^{a,b}$
Summer	7260	$0.053^{a,b}$	0.174^{a}	0.184^{a}	0.010	0.013	0.037^{b}	0.274^{a}
Autumn	289	0.159^{d}	0.294^{b}	0.350^{b}	0.007	0.013	0.076^{c}	0.536^{c}
χ^2		58.289	33.695	47.980	1.525	1.236	108.969	100.051

^{a,b,c,d} Denote significant ($P < 0.05$) differences in columns

Critical χ^2 ($P = 0.05$) for 3 degrees of freedom = 7.815

*Expressed relative to eggs still being incubated after candling at 21 days

3.5. Storage time

The overall proportions of embryonic mortalities were higher ($P < 0.05$) at 32.0% in freshly laid eggs that were set directly without storage and in those eggs stored for > 6 days (43.5%; Table 4).

Table 4 Proportions of overall and classified embryonic mortalities in relation to storage time from collection to setting.

Store time	Category							
	Number of eggs	Embryonic mortalities 1st half	Embryonic mortalities 2nd half	Second half (live)*	Death post-pipping	Death post-pipping (live)*	Rotten	Overall
Overall	34289	0.060	0.177	0.189	0.010	0.013	0.036	0.283
1 day	4956	$0.057^{a,b}$	0.218^{d}	0.231^{c}	0.013	0.018^{b}	0.032^{a}	0.320^{d}
2 days	4808	$0.058^{a,b}$	$0.179^{b,c}$	0.191^{b}	0.011	$0.015^{a,b}$	0.035^{a}	$0.284^{b,c}$
3 days	4791	0.049^{a}	$0.170^{a,b,c}$	$0.179^{a,b}$	0.010	$0.013^{a,b}$	0.033^{a}	$0.263^{a,b,c}$
4 days	4512	$0.058^{a,b}$	0.152^{a}	$0.169^{a,b}$	0.012	$0.015^{a,b}$	0.039^{a}	0.261^{a}
5 days	4950	$0.058^{a,b}$	0.150^{a}	0.160^{a}	0.007	0.009^{a}	0.037^{a}	0.253^{a}
6 days	4575	0.065^{b}	$0.181^{b,c}$	0.193^{b}	0.007	0.009^{a}	0.038^{a}	$0.291^{c,d}$
7 days	4808	0.066^{b}	$0.174^{a,b,c}$	0.186^{b}	0.010	$0.013^{a,b}$	0.033^{a}	0.282^{c}
> 7 days	889	0.107^{c}	0.243^{d}	0.272^{c}	0.004	$0.007^{a,b}$	0.081^{b}	0.435^{d}
χ^2		51.390	129.631	129.798	18.867	20.850	58.288	179.548

^{a,b,c,d} Denote significant ($P < 0.05$) differences in columns

Critical χ^2 ($P = 0.05$) for 7 degrees of freedom = 14.067

*Expressed relative to eggs still being incubated after candling at 21 days

The deleterious effect of a short storage time was confined to the late embryonic mortalities. Embryonic survival in general (except for deaths post-pipping) was adversely affected by prolonged storage ($P < 0.05$).

3.6. Incubators

Information on the incubator used to incubate specific eggs was not recorded during the 1998 breeding season. This influence of the incubator on embryonic mortalities could consequently only be considered for eggs produced subsequently (Table 5). Overall, embryonic mortalities were higher (57.0%) for eggs incubated in the African Incubator® and in those incubated in combinations (38.1%) ($P < 0.05$; Table 5). The overall embryonic mortality of eggs incubated in the Prohatch® incubator were also somewhat higher than those incubated in the Buckeye® or Natureform® incubators. Embryonic mortalities during the 1st half of incubation were particularly high in the African Incubator®, whereas embryonic mortalities during the 2nd half of the incubation period were higher in both the African Incubator® and in eggs set in combinations of incubators ($P < 0.05$). Although other significant ($P < 0.05$) differences between designations were also found for other categories of embryonic mortalities, the magnitude of these differences were modest compared to those above. The only category that was not affected by incubator was those deaths that occurred post-pipping, after the eggs had been transferred to the hatcher.

Table 5 Proportions of overall and classified embryonic mortalities in relation to the incubator used during the 1999 - 2005 breeding seasons.

Incubator	**Category**							
	Number of eggs	Embryonic mortalities 1st half	Embryonic mortalities 2nd half	Second half (live)*	Death post-pipping	Death post-pipping (live)*	Rotten	Overall
Overall	34221	0.060	0.177	0.188	0.001	0.013	0.036	0.283
Buckeye®	23196	0.053^{a}	0.161^{a}	0.169^{a}	0.009	0.012	0.036^{b}	0.259^{a}
Prohatch®	7368	0.061^{b}	0.200^{b}	0.213^{b}	0.011	0.015	0.033^{b}	0.305^{b}
African Incubator®	718	0.273^{c}	0.258^{c}	0.354^{d}	0.007	0.015	0.032^{a,b}	0.570^{d}
Natureform®	473	0.076^{a,b}	0.127^{a}	0.137^{a}	0.013	0.016	0.015^{b}	0.230^{a}
Combinations	2466	0.062^{a,b}	0.251^{c,d}	0.267^{c}	0.011	0.015	0.058^{a}	0.381^{c}
χ^2		599.733	200.959	273.667	2.949	4.624	41.312	497.835

^{a,b,c,d} Denote significant ($P < 0.05$) differences in columns

Critical χ^2 ($P = 0.05$) for 4 degrees of freedom = 9.488

*Expressed relative to eggs still being incubated after candling at 21 days

4. Discussion

4.1. Genotype

In our breeding programme, ZB have been introduced to produce offspring with an improved live weight (Essa & Cloete, 2006) and an improved carcass weight (Brand *et al.,* 2005). However, the effect of crossbreeding on egg production and fertility also needs to be considered. Embryonic mortality as a result of genetic problems can negatively influence hatchability, but this has not yet been recorded in ostriches (Badley, 1997). The unexpectedly high level of embryonic mortalities in the SAB male x ZB female combination is a cause of concern, especially since the best hatchability results in absolute terms were achieved in the reciprocal cross. Further research is required to enable a better understanding of this

phenomenon, since it cannot be readily explained at present. Differences between the purebred genotypes and the ZB male X SAB female cross were of a smaller magnitude, albeit also significant ($P < 0.05$). In the comparison with the breeds and crosses, it should also be considered that SAB females overall had a markedly higher overall egg and chick production than ZB females irrespective of the genotype of the sire (Brand *et al.,* 2005).

4.2. Female age

In the resource population used, overall hatchability was lower in 2-year-old females, most likely due to lower levels of fertility (Cloete *et al.*, 2006). Although the oldest age groups were still capable of good egg production, chick production declined owing to higher levels of embryonic mortality (Bunter, 2002; Cloete *et al.*, 2006). The latter trend coincides with our findings in that young females had the lowest percentage of embryonic mortalities during the 1st half and 2nd half of incubation. There was also an increase in overall embryonic maortlaities. The data suggest that females older than 8 - 10 years should be culled in breeding operators. The higher embryonic mortalities can be related to changes in egg weight and shell quality with hen age, which is presumed to ultimately influence the hatchability of eggs (Bunter, 2002; Cloete *et al.,* 2006).

4.3. Year

Even though year affected the hatchability of ostrich eggs, year effects are generally transient and unpredictable. Year effects may depend on typical climatic conditions; variation in the chemical composition of the raw materials used to compose diets and managerial regimes occurring for that specific year. Although eggs being incubated are shielded from changes in atmospheric climate conditions by a climate controlled environment, it is conceivable that ambient climatic conditions may affect eggs prior to incubation. However, such effects are poorly understood at present and are still being investigated. It would suffice to state that year effects are not repeatable, and average performance in a given year cannot be predicted with any reasonable accuracy. Such effects have, therefore, little practical application, except for the identification of possible long-term trends.

4.4. Season

The highest overall embryonic mortalities of 53.6% in autumn are, in the context of ostrich production, probably irrelevant because autumn usually falls in the normal rest period of the breeders. Furthermore, most of the relatively small number of eggs designated as autumn eggs was acquired only over a single season. Moreover, since only part of the flock was involved, eggs had to be stored for slightly longer periods than usual to utilise incubator space optimally. The other seasons were represented throughout the experimental period, and results are probably more representative. In the other seasons, embryonic mortalities were highest during winter at 29.2%. From this level, embryonic mortalities declined by 1.8% towards summer (Table 3). Our results differ from those of Wilson *et al.* (1997) that hatchability for the set number of eggs decreased linearly as the breeding season progressed. In this study the hatchability of

fertile eggs increased as breeding season progressed from winter to summer. Whereas the summer season resulted in the lowest proportion of embryonic mortalities for ostrich eggs in the present study, the winter season resulted in the best hatching results for duck eggs (Chowdhury *et al.*, 2004). The slight increase in embryonic mortality early in the breeding season should be balanced against the higher overall egg production in the winter and spring seasons compared with summer. Egg output was found to taper off in the period leading to and after the summer solstice (Lambrechts, 2004; Fair *et al.,* 2005). This effect is clearly discernable in the egg numbers provided in Table 3.

4.5. Storage time

Embryonic mortalities in the 1st half of the incubation period increased nearly 2-fold in eggs stored for > 7 days. Sahan *et al.* (2004) correspondingly found that late embryonic mortalities increased from 14.3% in eggs stored for one day to 18% in eggs stored for 10 days. In this study, embryonic mortalities in the 2nd part of incubation increased by 4.1% after 6 days of storage, corresponding to the proportion of mortalities in eggs that were set directly. Ar & Gefen (1998) also reported a significant increase in rotten eggs after 7 days of storage. In this study, rotten eggs increased by about 5% for eggs stored for more than 6 days. The results suggested that fresh eggs have higher embryonic mortalities in the 2nd half of the incubation period compared to eggs stored between 1 and 6 days. Understandably this was also reflected in higher overall embryonic mortalities in freshly-packed eggs. These results are in accordance with findings by Ar & Gefen (1998), Narahari *et al.* (1991), Fasenko *et al.* (1992), Deeming (1996), Wilson *et al.* (1997) and Horbańczuk (2000) who reported that hatchability decreased as the duration of pre-incubation storage time for ostrich eggs increased.

4.6. Incubators

As with other species of birds, the physiological requirements of the developing ostrich embryo can be met during artificial incubation by provision of appropriate temperature (Van Schalkwyk *et al*., 1999a), humidity (Swart *et al*., 1987), the correct gaseous environment (Van Schalkwyk *et al*., 2002) and the proper turning of eggs (Van Schalkwyk *et al.*, 2000) in automatic incubators. There are currently a number of commercially available ostrich incubators on the market, ranging from wooden incubators which provide only temperature control and air circulation to those that allow electronic control of all variables. In our study, all the incubators were of the latter types. All the incubators were set to provide the same conditions with regard to temperature, 36 °C and RH, 24%. Despite this, there was differential embryonic mortality. The African Incubator® had the highest proportion of embryonic mortalities in the 1st half of the incubation period and also the highest overall embryonic mortalities. Because this incubator was mainly used during winter and spring, when egg production outstripped the capacity of the other incubators, this trend could not be attributed to usage during seasons when high levels of embryonic mortalities were expected. Conversely, its undesirable performance could rather be attributed to temperature gradients caused by suboptimal incubator design, which was evident in the fluctuation in temperature readings taken in its compartments throughout the breeding season. Exposure to heat stress can have a significant influence on hatchability of eggs (Ande & Wilson, 1981) and high temperatures can lead an increase in early- and late embryo mortalities (French, 1997; Badley, 1997; Hassan *et al*., 2004). The same detrimental effect on hatchability was also shown in

ostrich eggs, where severe temperature gradients were shown to exist in forced-draught wooden incubators (Van Schalkwyk *et al.*, 1999b). The use of a combination of incubators resulted in a significantly higher level of late embryonic mortalities (25.1%). We did not find any corresponding literature in other avian species, but it seems reasonable to postulate that the increased handling of these eggs may have contributed to this result.

5. Conclusions

This study did not attempt to clarify of infectious causes of embryonic mortality of ostrich eggs, since the pathogens involved in a minority of rotten eggs were not identified. It has to be conceded that embryonic mortalities probably originate from numerous multi-factorial causes, which cannot be dealt with exhaustively in a single paper. However, a number of non infectious effects related to embryonic mortality of ostrich eggs could be identified. Some have direct and immediate application, such as the culling of females older than 8 - 10 years from the breeding flock, the storage of eggs for only 1 - 6 days where at all practicable, and to assure that all incubators are set optimally for maximised hatching success. It also seems to be a good management practice to minimize the transfer of eggs being incubated between incubators. Furthermore, an extended research effort is indicated as far as the observed bloodline effects are concerned. Such studies may play a major role in the better understanding and eventual resolution of the problem of production losses in commercially incubated ostrich eggs.

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CHAPTER 4

The influence of incubation management on pipping position, hatching ability and survival of ostrich chicks

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Abstract

Despite numerous studies, the effect of artificial incubation on the hatchability and survival of near-term ostrich chicks is still not well understood. Records from 13 975 eggs with embryos of 35 days and older, artificially incubated between 2006 and 2008, were analysed to determine the potential effect of pipping position upon the hatchability, and subsequent survival of ostrich chicks. A total of 864 near-term chicks (6.9%) failed to pip. Chicks that pipped in the correct position had a higher probability of surviving hatch than those pipping in the incorrect position. Genotype did not affect the proportion of chicks pipping in the correct position, or the survival of hatching ostrich chicks pipping in either the correct or incorrect positions. Although female age had a significant effect on the proportion of chicks pipping, survival of hatch was independent of female age. Chicks hatching during winter were more likely to pip than chicks hatching in spring, whereas chicks hatching in summer were more likely to pip in the correct position. In winter the proportion of chicks pipping in incorrect positions were significantly higher than in either summer or autumn. The survival rate of chicks hatching during winter was generally higher than those hatching in the other seasons. Transfer of eggs between setters during incubation had a negative influence on the ability of ostrich chicks to pip in the correct position. Incubated ostrich eggs with intermediate levels of water loss, i.e. between 9.0 and 18.9% of fresh egg weight, were more likely to pip in the correct position overall than those with higher or lower levels of water loss. Chicks from eggs stored for intermediate periods, i.e. 3, 4 and 6 days prior to being set, were more likely to pip than chicks from those eggs set directly after collection without storage. Storage time also affected pipping position, with chicks from eggs stored for 5 days being more likely to pip in the correct position than chicks from those eggs set directly after collection. These results emphasize the need that ostrich incubation facilities need to avoid transfer of eggs between setters during artificial incubation, strive to achieve an optimal level of water loss, and apply a protocol of not setting eggs immediately after collection to maximize the hatchability of chicks pipping in the correct position and post-hatch survival.

1. Introduction

Despite being a well established livestock industry, the production of ostriches does not compare well with more conventional domesticated poultry species. Artificial incubation of ostrich eggs is poorly understood when compared with poultry. Low hatchability of artificially incubated eggs is considered to be one of the constraints in the production efficiency of commercial ostrich production systems worldwide (Deeming, 1995a). According to Deeming *et al.* (1993), a lack of understanding of the pattern of embryonic development, especially factors affecting the pipping position just prior to hatching, contributes to the high incidence of embryonic mortalities during this period.

Malposition of embryos with respect to the air cell generally results in failure to hatch (Brown *et al.*, 1996). Developing ostrich chicks start to turn in the egg to assume the correct pipping position from day 35 of incubation and have usually assumed the correct pipping position by day 42 of incubation (Deeming, 1995b). At this stage, the neck of the chick lays from left to right with the right foot next to the beak and the left foot positioned in the nape of the neck. This pipping position differs from that of domestic fowl (Deeming, 1994). The most common malposition for ostrich embryos is with the head at the opposite end to the air cell (malposition 2 for chickens). Chicks presented in this position die because they are unable to penetrate the air cell, but a small percentage of embryos may pip in the bottom of the egg and still survive. The embryo may also be positioned with its head to the left side instead of to the right side. Other abnormalities observed include having the foot positioned under the head, while some chicks get their head stuck across their right leg or the right foot gets stuck over the head or in the beak (Deeming, 1995b).

Van Schalkwyk *et al.* (1996) found that more than 70% of all dead-in-shell cases occur during the pipping stage, mainly caused by inadequate incubation equipment, which results in high relative humidity, overheating and inadequate hygiene management. Brown *et al.* (1996) reported that more than 55% of shell deaths in ostrich eggs are due to malpositions of the embryo. This observation was confirmed in a study by Ipek & Sahan (2004). Successful artificial incubation is also affected by a number of factors including female age, season, and storage conditions of eggs prior to setting in the incubator, as well as the type of incubator (Blood *et al.*, 1998; Van Schalkwyk, 1999; Brand *et al.*, 2007; 2008a). Egg production of ostrich females starts at 2 - 2.5 years of age and peak egg and chick production occurs at 8 - 9 years. Female age, however, is known to influence the number of eggs laid as well as egg weight and, consequently, chick weight at hatching (Bunter & Cloete, 2004; Ipek & Sahan, 2004; Lambrechts, 2004; Cloete *et al.*, 2006a; Brand *et al.*, 2007). Both embryonic mortalities during the first half and second half of incubation were proportionally increased in older females, the effect being more pronounced for deaths during the second half of incubation (Brand *et al.*, 2007).

Hassan *et al.* (2005) reported that storage period affected egg weight loss, while Deeming *et al.* (1993) determined that an increase of storage time resulted in a reduction in embryo vitality. Storage of ostrich eggs for periods longer than 7 days results in an increase in embryonic mortality (Wilson *et al*., 1997; Brand *et al*., 2007). Results from studies by Deeming (1995a), Ar (1996) and Blood *et al.* (1998) showed that the optimal water loss for artificially incubated ostrich eggs amount to approximately 15% but, like other birds,

ostriches show some latitude in the amount of water loss at which eggs will still hatch successfully. Eggs which lost less than 10% or more than 20% of their initial weight were less likely to hatch. Excessive water loss during incubation causes early depletion of allantoic fluids, which results in subsequent dehydration of the embryo and extends the period of osmotic stress (Davis *et al*., 1988). On the other hand, an insufficient water loss from the egg results in water retention by the chick, potentially causing embryonic mortality through respiratory insufficiency (Musara *et al.*, 1999). It also results in a high proportion of chicks that are malpositioned at the point of hatch or have unabsorbed yolk sacs (Horbańczuk *et al.*, 1999). Malpositioning generally resulted from incorrect turning, and oedema was significantly related to the quantity of water lost (Brown *et al.*, 1996).

When introducing different genotypes into an ostrich breeding flock, the effect of crossbreeding on egg production and fertility needs to be considered. Embryonic mortality as a result of genetic problems can compromise hatchability, but such an effect has not yet been recorded in ostriches (Badley, 1997). In a study involving the South African Black and Zimbabwean Blue breeds and their crosses, there was some evidence supporting the existence of genotypic differences (Brand *et al*., 2007).

A better understanding of how systematic factors influence the successful artificial incubation of ostrich eggs is essential (Cloete *et al*., 2002), especially during the crucial last few days of incubation when chicks move into the correct position for hatching. The aim of this study was thus to investigate the effects of environmental factors such as production year, season, female age, genotype, water loss, the incubator type used as well as storage time on the proportions of eggs pipping, the pipping of eggs in the correct position, as well as survival of chicks, both in eggs pipped in the correct and incorrect positions.

2. Material and Methods

Eggs were obtained from the commercial ostrich breeding flock maintained at the Oudtshoorn Research Farm in the Klein Karoo region of South Africa. The origin of the ostrich flock and general husbandry of the breeding flock has been well described by Van Schalkwyk *et al*. (1996), and Bunter & Cloete (2004). Data for this study were collected during the 2006, 2007, and 2008 breeding seasons. Unless specified otherwise, each breeding bird received a ration of 2.5 - 3 kg DM/bird/day throughout the breeding season, which commenced from the beginning of June, and lasted till the end of January for 2006 and 2007. The exception was in 2008, when the breeding season started mid-May and ended mid-December.

Eggs were collected daily, weighed and identified by date and paddock of origin. Methods for collection, sanitation and storage at the experimental site are well documented (Van Schalkwyk *et al*., 1998; Van Schalkwyk *et al*., 1999; Bunter & Cloete, 2004; Brand *et al*., 2007). At the beginning and end of the season, however, there were insufficient eggs to occupy the available incubator space optimally. These eggs were consequently stored for periods not exceeding 20 days. Eggs were artificially incubated at 36 ºC and 24% relative humidity (RH) in Buckeye®, Prohatch® or African International® incubators and all incubators were set to turn eggs automatically through an angle of 60 - 90° on an hourly basis. The capacity and operation of the Buckeye® and Prohatch® incubators are described by Cloete *et al*. (2001) and the African Incubator® are

described by Brand *et al. (*2007). On day 35 of incubation, eggs were transferred from the setters to a Prohatch® hatcher, which also operated at 36 °C and a RH of 24%. Eggs were set vertically with their air cells positioned upwards in the hatcher and from this stage the eggs were not turned anymore. Eggs were checked twice daily to see whether external pipping had occurred. Eggs with signs of external pipping were transferred to a second hatcher, a Buckeye®, to facilitate identification of the chicks. The external pipping position of each egg was also recorded to assess whether chicks pipped in the correct position. All eggs where external pipping occurred around the air sac area were classified as pipped in the correct position, whereas eggs where pipping occurred towards the middle or bottom of the egg were classified as having been pipped in the incorrect position. On day 44 of incubation, eggs that did not hatch were candled to see if any movement could be detected, thus indicating whether internal pipping did/did not occur. These eggs were manually opened at the air sac area, and the position of the embryo and point of internal pipping noted.

During 2003 Zimbabwean Blue (ZB) breeders were introduced to the flock and mated in various combinations with South African Black (SAB) males and SAB females (Brand *et al*., 2005). During 2007 Kenyan Red Neck (KAR) breeding birds were introduced to the flock and mated with SAB females. Data that were recorded in 2006 thus involved various combinations of the two purebred bloodlines (SAB and ZB) as well as the reciprocal crosses between them, while data recorded for 2007 included combinations of the third purebred bloodline (KAR) as well as KAR males mated to SAB females.

A total of 23 709 eggs with pedigrees were collected during the three breeding seasons. Eggs were excluded from analyses if they had a defect, i.e. holes in the shell or dull shells that prevented them from being set (1 314); rotten eggs (840), infertile eggs (5 629), were used in other experiments (830), or had embryos that died before 21 days of incubation (1 342). A further 1 101 eggs were excluded because subsequent inspection of the dead-in-shell chicks showed they died between 21 and 35 days of incubation, which is prior to the stage where embryos are expected to begin orientating into the correct position for pipping and when malpositioning becomes evident. Only records from eggs with chicks of 35 days and older were thus used. A further 12 records with uncertain pipping data were also excluded. The final number of eggs analysed was thus 12 659, of which 2 675 died after 35 days of incubation (21.1%). A further 864 eggs (6.9%) did not pip externally and were excluded from all analyses involving pipped eggs. Some analyses contained slightly fewer eggs, e.g. in assessing the effect of genotype because genotypes represented by very low numbers were excluded from the analysis.

Data was classified into three categories, i.e. chicks that pipped successfully; chicks that pipped in the correct position and chicks that pipped in the incorrect position. The latter two categories were further divided into chicks that survived after pipping and those that succumbed in the period after pipping. Chi-square procedures (Van Ark, 1990) were used to assess the effects of genotype, female age, season, incubator, year, water loss and storage time on the incidence of pipping of ostrich chicks, the number of chicks pipping in the correct or incorrect position, as well as the subsequent survival of hatch in chicks pipping in the correct or incorrect positions.

3. Results and Discussion

The position of chicks from eggs that failed to hatch will be dealt with in a separate study. Chicks hatching from eggs pipped in the correct position (9 841/10 526 = 0.935) had a significantly higher survival rate than chicks that hatched from eggs pipped in the incorrect position (436/1 254 = 0.348; $P < 0.01$).

Results of the present study indicated that genotype had no significant effect on the proportion of chicks that pipped, the proportion of chicks that pipped in the correct position or the survival of chicks pipping in the correct position (Table 1). These results suggest that crossing of different strains of breeders can be done without compromising hatchability of late-term eggs (≥ 35 days of incubation). An impaired hatchability owing to embryonic mortality as a result of genetic problems may compromise chick production in poultry, but such an effect has not yet been reported in ostriches (Badley, 1997). Brown *et al.* (1996) suggested that malpositioning of ostrich embryos with respect to the air cell could possibly be related to genetic factors. Brand *et al*. (2007) reported an unexpectedly high level of overall embryonic mortalities in the progeny of SAB males mated to ZB females. This was surprising, especially since the best hatchability results in absolute terms were achieved in the reciprocal cross.

Table 1 The influence of genotype on the pipping success, pipping position and subsequent survival of chicks hatched from eggs produced during the 2006, 2007, and 2008 breeding seasons (frequency in brackets).

Genotype	Category				
	Number of eggs	**Eggs pipped**	**Pipped in correct position**	**Survival of chicks pipped in correct position**	**Survival of chicks pipped in incorrect position**
Overall	12270	11433 (0.932)	10225 (0.894)	9560 (0.935)	424 (0.351)
SAB ♂ x SAB ♀	8184	7601 (0.932)	6772 (0.891)	6325 (0.934)	274 (0.331)
ZB ♂ x ZB ♀	456	419 (0.919)	376 (0.897)	357 (0.949)	21 (0.488)
ZB ♂ x SAB ♀	1238	1174 (0.948)	1045 (0.890)	985 (0.943)	45 (0.349)
SAB ♂ x ZB ♀	407	371 (0.912)	346 (0.933)	323 (0.934)	11 (0.440)
SAB ♂ x ZBSAB ♀	1078	1005 (0.932)	902 (0.898)	834 (0.925)	39 (0.379)
SAB ♂ x SABZB ♀	490	466 (0.951)	434 (0.931)	418 (0.963)	12 (0.375)
KAR ♂ x KAR ♀	160	147 (0.919)	129 (0.878)	115 (0.891)	5 (0.278)
KAR ♂ x SAB ♀	257	250 (0.973)	221 (0.884)	207 (0.937)	17 (0.586)
Chi^2		20.385	14.522	13.827	13.854

Critical Chi^2 ($P = 0.05$) for 6 degrees of freedom = 14.067

SAB = South African Black; ZB = Zimbabwean Blue; ZBSAB = Zimbabwean Blue male x South African Black female cross; SABZB = South African Black male x Zimbabwean Blue female cross; KAR = Kenyan Red Necks

The influence of season on the pipping frequency of eggs is shown in Table 2. During winter and summer the proportion of chicks that pipped (0.950 and 0.942, respectively), was higher ($P < 0.05$) than in eggs

hatched during the spring (0.915). A possible contributing factor is changes in egg-shell structure, but more research is required to determine whether seasonal changes in eggshell structure (as determined by the female) contributed to a lower pipping proportion during spring. Previous research suggested the possibility that eggshell characteristics of females may compensate for climatic conditions to ensure a relative constant water loss (Cloete *et al*., 2006b).

Table 2 Influence of season on the pipping success, pipping position, and survival of chicks hatched from eggs produced in the Southern hemisphere.

Season	Category				
	Number of eggs	**Eggs pipped**	**Pipped in correct position**	**Survival of chicks pipped in correct position**	**Survival of chicks pipped in incorrect position**
Overall	12659	11780 (0.931)	10526 (0.894)	9842 (0.935)	818 (0.652)
Winter	4286	4071 (0.950[a])	3634 (0.893[a])	3441 (0.947[a])	187 (0.428[a])
Spring	6530	5972 (0.915[b])	5292 (0.886[a])	4922 (0.930[b])	223 (0.328[b])
Summer	1843	1737 (0.942[a])	1600 (0.921[b])	1480 (0.925[b])	26 (0.190[c])
Chi²		54.613	17.375	13.512	28.633

Critical Chi² (P = 0.05) for 6 degrees of freedom = 5.991

[a,b,c] Denote significant (P < 0.05) differences in columns between frequencies in brackets

The proportion of chicks pipping in the correct position was higher during summer (0.921), while survival of chicks that pipped in the correct position was higher during winter (0.947). Survival of chicks from eggs pipped in the incorrect position was significantly higher for the winter at 42.2%, followed by spring and then by summer at 32.8% and 19.0%, respectively. The seasonal differences in the pipping performance of near-term ostrich chicks seemed to be conflicting in some instances, e.g. the poor survival of chicks pipped during summer seems to be in conflict with the generally better pipping performance in this season. No comparable literature in other avian species could be found and it is too early to speculate on a possible underlying cause for this phenomenon without further investigation.

With respect to overall embryonic mortalities, Brand *et al.* (2007) found that chicks hatched from eggs produced in the Southern hemisphere at the beginning of the breeding season, namely during winter, were more likely to succumb prior to hatching, this proportion reduce towards the end of the breeding season during summer. The latter results differed from those of Wilson *et al.* (1997) in that hatchability for set eggs decreased linearly as the breeding season progressed. His study was conducted in the Northern hemisphere in Florida. The winter season also seems to generate the best hatching results for duck eggs, with low rainfall and suitable room temperatures as the main contributing factors for this result (Chowdhury *et al.,* 2004). It could be speculated that the cold weather in winter had an influence on hatchability, due to the marked decrease in temperature during night time eggs collected in the mornings were quite wet from dew forming on the outer eggshell.

The traits investigated in this study were mostly independent of female age, with the only significant difference observed for eggs produced by 3-year old females that were more likely to pip than eggs produced by females at nine years of age (Table 3) ($P < 0.05$). Bunter (2002) and Cloete *et al*. (2006a) reported that, although older ostrich females are still capable of good egg production, chick production declined overall due to higher levels of embryonic mortality. The findings of this study failed to support the findings of Brand *et al*. (2007), who reported that fertile eggs produced by older females are less likely to hatch than eggs produced by younger females. It was postulated by Brand *et al*. (2007) that higher embryonic mortalities in older females were possibly related to changes in egg weight and shell quality with hen age, which presumably influence the hatchability of eggs through other factors such as water loss, with a more distinct impact on embryonic mortalities earlier in incubation. The present study suggests that the survival of hatch of near-term ostrich chicks probably not as dependent on female age than embryonic mortalities occurring at earlier ages.

Table 3 Influence of female age on the pipping success, pipping position, and survival of chicks hatched from eggs produced in the Southern hemisphere.

	Category				
Age	**Number of eggs**	**Eggs pipped**	**Pipped in correct position**	**Survival of chicks pipped in correct position**	**Survival of chicks pipped in incorrect position**
Overall	12049	11231 (0.932)	10027 (0.893)	9375 (0.935)	787 (0.654)
2 years	1268	1195 (0.942^{ab})	1079 (0.903)	1026 (0.951)	75 (0.647)
3 years	2421	2278 (0.941^{b})	2055 (0.902)	1940 (0.944)	129 (0.578)
4 years	1733	1608 (0.928^{ab})	1442 (0.897)	1350 (0.936)	105 (0.633)
5 years	1781	1670 (0.938^{ab})	1475 (0.883)	1369 (0.928)	134 (0.687)
6 years	1073	997 (0.929^{ab})	891 (0.894)	825 (0.926)	96 (0.651)
7 years	1079	994 (0.921^{ab})	888 (0.893)	834 (0.939)	72 (0.679)
8 years	806	753 (0.934^{ab})	658 (0.874)	617 (0.938)	62 (0.653)
9 years	644	581 (0.902^{a})	520 (0.895)	474 (0.912)	42 (0.689)
10+ years	1244	1155 (0.928^{ab})	1019 (0.882)	946 (0.928)	99 (0.728)
Chi^2		18.084	9.416	15.149	10.842

Critical Chi^2 ($P = 0.05$) for 6 degrees of freedom = 15.507

^{a,b} Denote significant ($P < 0.05$) differences in columns between frequencies in brackets

Despite the fact that all incubators were adjusted to provide the same incubation and hatching conditions, the frequency of chicks that pipped in the correct position differed ($P < 0.05$) between incubators, owing to a lower pipping frequency of chicks in those eggs transferred between incubators compared to chicks from eggs incubated throughout in a single incubator (Table 4). Survival of chicks pipping in the correct position was higher in the Buckeye® incubator, compared to chicks hatching from eggs transferred between incubators ($P < 0.05$), although the observed effect was quite small (1.5%). A contradictory effect was

observed when the survival of small numbers of chicks pipping in the incorrect position was considered, which was higher in those chicks from eggs incubated in more than one incubator compared to the Buckeye® incubator. It also seems that arguments in favour of an impaired pipping ability due to more frequent handling of transferred eggs do not seem to be valid, for such transfers are usually performed during routine husbandry procedures like candling. Eggs returned to the same incubator are thus also subjected to the same set of procedures as those returned to other incubators. No apparent explanations can be provided for these results.

Table 4 The influence of incubator on the pipping success, pipping position, and survival of chicks hatched from eggs produced during three consecutive breeding seasons.

	Category				
Incubator	**Number of eggs**	**Eggs pipped**	**Pipped in correct position**	**Survival of chicks pipped in correct position**	**Survival of chicks pipped in incorrect position**
Overall	12651	11778 (0.931)	10524 (0.894)	9840 (0.935)	436 (0.348)
Buckeye®	6584	6137 (0.932)	5544 (0.903[a])	5217 (0.941[a])	165 (0.278[a])
Prohatch®	1181	1096 (0.928)	1012 (0.923[a])	941 (0.930[ab])	27 (0.321[ab])
African Incubator®	195	190 (0.974)	177 (0.932[ab])	169 (0.955[ab])	4 (0.308[ab])
Combinations	4691	4355 (0.928)	3791 (0.870[b])	3510 (0.926[b])	240 (0.426[b])
Chi^2		6.498	43.684	9.971	28.024

Critical Chi^2 (P = 0.05) for 6 degrees of freedom = 7.815

[a,b] Denote significant (P < 0.05) differences in columns between frequencies in brackets

A significant effect of production year on malpositioning and survival of ostrich chicks was observed, with the overall proportion of chicks pipping in 2007 (0.940) and 2008 (0.944) being higher than in those eggs that pipped in 2006 (0.913; Table 5). The proportion of chicks pipping in the correct position, as well as the survival of chicks that pipped in the incorrect position, was higher during the 2006 and 2007 breeding seasons, 0.897 and 0.909 respectively, when compared to the 2008 breeding season at 0.880 (P < 0.05). There was no difference between years for the survival of hatch in chicks pipping in the correct position.

Table 5 Influence of production year on the pipping success, pipping position, and survival of chicks hatched form eggs produced during three consecutive breeding seasons.

Year	Category				
	Number of eggs	Eggs pipped	Pipped in correct position	Survival of chicks pipped in correct position	Survival of chicks pipped in incorrect position
Overall	12659	11780 (0.931)	10526 (0.894)	9842 (0.935)	429 (0.342)
2006	5086	4644 (0.913[a])	4165 (0.897[a])	3890 (0.934)	126 (0.263[a])
2007	3007	2826 (0.940[b])	2568 (0.909[a])	2411 (0.939)	51 (0.198[a])
2008	4566	4310 (0.944[b])	3793 (0.880[b])	3539 (0.933)	259 (0.501[b])
Chi^2		40.627	15.620	0.881	94.286

Critical Chi^2 (P = 0.05) for 6 degrees of freedom = 5.991

[a,b] Denote significant (P < 0.05) differences in columns between frequencies in brackets

Even though year affected the hatchability of ostrich eggs, year effects are generally inconsistent and unpredictable, and unlikely to be repeated during consecutive years (Brand *et al.,* 2007). Factors such as climatic conditions; variation in the chemical composition of the raw materials used to formulate diets and changes in husbandry practices, may all contribute to potential variation between years. Although eggs in the process of incubation are shielded against changes in e.g. atmospheric climatic conditions by controlling the incubation environment, it is possible that exposure of eggs to the elements prior to incubation may influence hatchability. However, the impact of the mentioned effects on the hatchability of ostrich eggs is poorly understood and need to be investigated on a larger scale and using a longer time frame (Malecki *et al*., 2005).

There were significant differences in pipping position and survival of hatched chicks for different levels of water loss (WL) to 35 days of incubation (Table 6). Pipping frequency of incubated eggs, as well as survival of chicks, both from chicks pipping in the correct or incorrect positions were lowest for those eggs where moisture loss was either below 9% or above 19% over the first 35 days of incubation, i.e. these traits had an intermediate optimum. These results coincide with findings by Deeming (1995a) that patterns of survival beyond day 35 of incubation were closely linked with variation in the quantity of weight lost by eggs. Brown *et al.* (1996) and Badley (1997) hypothesized that malpositioning of embryos may be caused by insufficient water loss. Insufficient water loss results in oedema which usually causes impaired oxygen diffusion across the moist shell membranes (Brown *et al.*, 1996). In contrast, excessive water loss results in dehydration of the embryos and the drying out of shell membranes (Brown *et al.*, 1996). Deeming (1995a) found that the pattern of mortality in chicks surviving beyond day 36 of incubation was closely linked to the degree of variation in the amount of water lost from the egg. Deeming (1995b) reported a significant relationship between percentage weight loss and the location of the pipping hole. As water loss increased, the pipping hole was more likely to be situated closer to the equator of the egg.

Table 6 Influence of percentage water loss up to day 35 of artificial incubation on the pipping success, pipping position, and survival of chicks hatched form eggs produced during three consecutive breeding seasons.

Water loss to 35 days (% fresh egg weight)	Category				
	Number of eggs	Eggs pipped	Pipped in correct position	Survival of chicks pipped in correct position	Survival of chicks pipped in incorrect position
Overall	12659	11780 (0.931)	10526 (0.894)	9842 (0.935)	436 (0.348)
<7	105	72 (0.686^{a})	52 (0.722^{a})	48 (0.673^{a})	1 (0.050 ^{ab})
7-8.9	1070	948 (0.886^{b})	807 (0.851^{ab})	806 (0.850^{b})	32 (0.230 ^{a})
9-10.9	2698	2512 (0.931^{d})	2255 (0.898^{cd})	2379 (0.947^{c})	90 (0.350 ^{ab})
11-12.9	3473	3291 (0.948^{de})	3024 (0.919^{d})	3123 (0.949^{c})	98 (0.367 ^{ab})
13-14.9	2878	2740 (0.952^{e})	2440 (0.891^{c})	258 (0.954^{c})	127 (0.423 ^{b})
15-16.9	1542	1430 (0.927^{d})	1276 (0.892^{bcd})	1194 (0.936^{c})	60 (0.390 ^{ab})
17-18.9	541	498 (0.921^{cde})	438 (0.880 ^{bcd})	403 (0.920^{c})	16 (0.267 ^{ab})
>19	352	289 (0.821^{ab})	234 (0.810 ^{ab})	(0.816^{ab})	14 (0.236 ^{ab})
Chi²		232.956	85.359	239.118	31.926

Critical Chi² (P = 0.05) for 6 degrees of freedom = 14.067

^{a,b,c,d} Denote significant (P < 0.05) differences in columns between frequencies in brackets

Storage time prior to setting had a significant influence on all traits reported in Table 7. The proportion of chicks pipped was the highest for eggs stored for between 2 and 5 days, while it was reduced for chicks that hatched from eggs that were not stored before setting (P < 0.05). The best performance in terms of chicks pipping in the correct position, as well as survival of chicks pipping in the correct position, was found for eggs stored for 4 days. Apart from a reduced pipping percentage, chicks from eggs that were not stored before setting also had a poorer (P < 0.05) survival of hatch when pipping in the correct position. Survival of chicks pipping in the incorrect position was independent of the number of days the eggs were stored.

Prolonged storage of eggs before setting leads to malformation in the embryo and to a reduced growth rate of the embryos of the domestic fowl (Fasenko *et al.*, 1992; Meijerhof, 1992) and the ostrich (Malecki *et al.*, 2005). Fasenko (2007) also reported that the embryonic output rate of CO_2 from eggs that were stored for a prolonged period was slower than the output of embryos from eggs stored for shorter periods. Previous studies have shown that the embryonic survival of ostrich chicks was impaired in eggs that were stored for seven days and longer (Brand *et al*., 2007), which was consistent with the findings of Ar & Gefen (1998), Deeming (1996), Wilson *et al.* (1997) and Horbańczuk (2000). According to Table 7, these limitations were not as evident in near-term chicks over the interval from 35 days of incubation to hatching.

Table 7 Influence of duration of pre-incubation storage on the pipping success, pipping position, and survival of chicks hatched form eggs produced in the Southern hemisphere.

Storage time	Category				
	Number of eggs	Eggs pipped	Pipped in correct position	Survival of chicks pipped in correct position	Survival of chicks pipped in incorrect position
Overall	12659	11780 (0.931)	10526 (0.894)	9842 (0.935)	436 (0.348)
0	1779	1608 (0.904 [a])	1395 (0.868[a])	1271 (0.911[a])	72 (0.338)
1	1796	1669 (0.929 [ab])	1479 (0.886[ab])	1378 (0.932[ab])	52 (0.274)
2	1946	1818 (0.934 [b])	1629 (0.896[ab])	1518 (0.932[ab])	72 (0.381)
3	1663	1572 (0.945[b])	1419 (0.903[ab])	1332 (0.939[ab])	53 (0.346)
4	1808	1684 (0.931 [ab])	1544 (0.917[b])	1470 (0.952[b])	49 (0.350)
5	1671	1577 (0.944 [b])	1413 (0.896[ab])	1335 (0.945[ab])	65 (0.396)
6	1797	1664 (0.926 [ab])	1483 (0.89 [ab])	1382 (0.932[ab])	66 (0.365)
>6	199	188 (0.945 [ab])	164 (0.872[ab])	154 (0.939[ab])	7 (0.292)
Chi^2		31.342	24.5988	23.740	7.875

Critical Chi^2 (P = 0.05) for 6 degrees of freedom = 14.067

[a,b] Denote significant (P < 0.05) differences in columns between frequencies in brackets

4. Conclusion

The present study showed that the frequency of pipping, pipping in the correct position, as well as survival for ostrich chicks pipped in either the correct or incorrect position, were affected by a number of environmental factors. The influence of genotype and female age ranged from absent to very small for the traits analysed, while the effects of water loss, incubator and storage time prior to setting were of greater practical significance. Although season had an effect on the pipping performance of near-term ostrich chicks, conflicting results were reported in some instances. For both year and season, the present study clearly shows that data needs to be recorded over more years to assess the possible long term trends on the pipping performance in near-term ostrich eggs. Some of the factors noted have direct and immediate application, such as a preference not to move eggs between incubators and the setting eggs after being stored for a short period (2 - 3 days). Incubators should also be set to optimally control water loss within the ranges required for optimal hatching success. Above mentioned factors should all be considered when planning commercial ostrich husbandry and artificial incubation operations.

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CHAPTER 5

Dead-in-shell positions of near-term ostrich embryos

Abstract

The patterns of embryonic development, especially for the last stage of hatching, are still not well understood in ostriches. This study examined between 3 468 and 3 484 dead-in-shell eggs with chicks that died between day 35 and day 42 of artificial incubation. Most dead-in-shell (DIS) chicks were positioned correctly with their heads towards the air cell (52.6%). DIS chicks positioned their heads near to the equator of the egg amounted to 46.5%, while a small percentage (0.9%) were positioned with their upper body towards the bottom. More DIS chicks tend to pip internally near to the equator of the egg (37.6%) than DIS chicks that pipped internally through the membranes into the air cell (34.4%). Most of the DIS chicks either had their head turned in the correct position from left to right (54.4%), whereas their beaks were mostly positioned towards the air cell (52.9%). The highest proportion of DIS chicks had their feet in the upwards position (52.4%), while 46% had their feet either across or below the head. The wings of all DIS chicks were positioned next to the body. Results from the study showed that most of the dead-in-shell chicks were in the correct position, but were still unable to hatch. This warrant future research to investigate the reasons preventing correctly positioned chicks from hatching.

1. Introduction

Ostriches are an important commercial species in South Africa. Whereas problems of egg quality such as shell thickness, hatching and embryonic development of the economically most important domesticated birds are relatively well understood and described, very little information exists on embryogenesis and problems specifically related to ostriches. Artificially incubated ostrich eggs generally have a lower hatching success than most domesticated poultry species with high chick mortality, especially in the period close to pipping and hatching. An especially poor understanding of patterns of embryonic development around this period contributes to the high incidence of embryonic mortality (Deeming *et al.,* 1993). Developing ostrich chicks start to turn themselves in the egg to assume the correct pipping position from day 35 of incubation onwards (Deeming, 1994; 1997). Deeming (1995) reported that the correct pipping position for ostrich embryos is reached by day 42 of incubation, with the neck of the chick laying from left to right with the right foot next to the beak and the left foot positioned in the nape of the neck.

Brown *et al.* (1996), Deeming (1997) and Ipek & Sahan (2004) reported malpositioning of embryos with regard to positioning next to the air cell generally results in more than 55% of embryonic mortality in ostrich eggs, with more than 70% of all dead-in-shell cases occurring during the pipping stage. It has been suggested that these losses are mainly caused by inadequate incubation equipment, which results in high relative humidity, overheating and often inadequate hygiene management (Brown *et al.*, 1996; Van Schalkwyk *et al.,* 1996). This observation has been confirmed in studies by Sahan (2003) and Ipek & Sahan (2004). About 25% of all fertile eggs that failed to hatch contain chicks in one or more of several malpositions (Byerly & Olsen, 1936). Successful artificial incubation is also affected by a number of factors including female age, season, and storage conditions of eggs prior to setting in the incubator, as well as the type of incubator (Blood *et al*., 1998; Van Schalkwyk, 1999; Brand *et al*., 2007; 2008). A better understanding of the positions in which chicks succumb may contribute to a better insight into the development of the chick in the period immediately before hatching. The aim of this study was thus to investigate the positions of the dead-in-shell chick and its body parts in order to improve our understanding of factors preventing the hatchability of healthy ostrich chicks.

2. Material and Methods

Eggs were obtained from the commercial ostrich breeding flock maintained at the Oudtshoorn Research Farm in the Klein Karoo region of South Africa during the 2006 - 2008 breeding seasons. Specifics about the flock, management and feeding program are described by Van Schalkwyk *et al*. (1996), Bunter & Cloete (2004) and Brand *et al*. (2007). Eggs used in this study included the three genotypes, South African Blacks (SAB), Zimbabwean Blues (ZB), Kenyan Red Necks (KAR) and various combinations between the breeds. Between 3 472 and 3 484 records from eggs with dead-in shell embryos 35 days and older were used. The normal position of the embryo before pipping was used as a reference to identify embryos in malposition (Deeming, 1995). Eggs were collected daily, weighed and identified by date and paddock (female) of origin. Methods for collection, sanitation and storage at the experimental site are well documented (Van Schalkwyk

et al., 1998; 1999; Brand *et al*., 2007). The detail on the incubators used and incubation procedures are described in detail by Cloete *et al*. (2001) and Brand *et al.* (2007; 2011).

On day 44 of incubation, eggs that did not hatch were candled to see if any movement could be detected, which would indicate that internal pipping occurred. These eggs were then manually opened at the air sac area and the position of the embryo and point of internal pipping, if any, noted. If internal pipping had taken place, the location of the pipping was noted. The positions of DIS chicks of all eggs were noted and described, as well as the position of the head, legs, beak and wings. Where appropriate, closely related positions were pooled to ensure that fewer positions were considered, and that positions were represented by as many eggs as possible.

The frequencies of embryos positioned with their heads near to the top (near the air cell), equator, or bottom of the eggs were compared using the one-sample Chi²-test (Van Ark, 1990). The frequencies of DIS embryos conforming to specific positions of the head, beak, feet and wing were similarly compared using one-sample Chi²-tests. In these tests, the observed frequencies were assessed against the hypothesis that all the frequencies were even, as described by Van Ark (1990).

3. Results

The large number of permutation describing the different positions for each of the dead chicks, its head, feet and beak complicated analysis. The different positions for each body part was categorised to ensure adequate numbers for the simplified permutations (see Figure 1). Frequencies for the different positions of dead-in-shell chicks are shown in Table 1. The frequencies of DIS chicks positioned correctly with their heads directed towards the air cell (52.6%), as well as those turned with their heads midway towards the air cell (46.5%) were higher ($P < 0.05$) compared to the group with their heads positioned towards the bottom of the egg (0.9%). In 26.8% of DIS eggs, no signs of internal pipping were observed. More DIS chicks tended to pip internally at the equator of the egg (37.6%) than DIS chicks that pipped internally through the membranes into the air cell (34.4%). Only a very small percentage of chicks (1.2%) managed to pip internally at the bottom of the egg.

The highest percentage of DIS chicks either had their head turned from left to right (54.4%; considered the correct position) or from right to left (44.6%). A very small number of DIS chicks had their head in a host of alternate positions, such as on either of the thighs, on different locations on the body, or turned down and lying on either of the wings. The most common positions for the beak was turned towards the air cell (52.9%; considered to be the correct position) and across either of the wings (44.1%). Positions occurring at lower frequencies included the beak lying across or below the feet, across or below either of the thighs or pointed downwards.

The highest percentage of DIS chicks did have their feet in the upwards position (52.4%), while 46% had their feet either across or below the head. A very small number had their feet across or below the neck or placed elsewhere. The wings were positioned next to the body for all of the DIS chicks.

Table 1 The different positions of dead-in-shell ostrich chicks for the 2006 - 2008 breeding seasons

Description	Number of records	Embryo orientation	Proportion (%)
Chick position	3482	Top by air sac (normal)	52.6
		Middle	46.5
		Bottom	0.9
Chi² (df* = 2)			1671.8
Pipping position	3484	None	26.8
		Top by air sac	34.4
		Intermediate of egg	37.6
		Bottom	1.2
Chi² (df* = 3)			1138.8
Head position	3474	Normal (left to right)	54.4
		Right to left	44.6
		On thigh	0.2
		On body	0.7
		On wing	0.1
Chi² (df* = 4)			5130.3
Beak position	3468	Turned upwards (normal)	52.9
		Across or below feet	1.3
		Across wing	44.1
		Across or below thighs	1.0
		Pointed downwards	0.6
Chi² (df* = 4)			4772.4
Feet position	3484	Turned upwards (normal)	52.4
		Across or below head	46.0
		Across or below neck	1.6
		Other	0.1
Chi² (df* = 3)			3285.7
Wing position	3472	Next to body (normal)	100

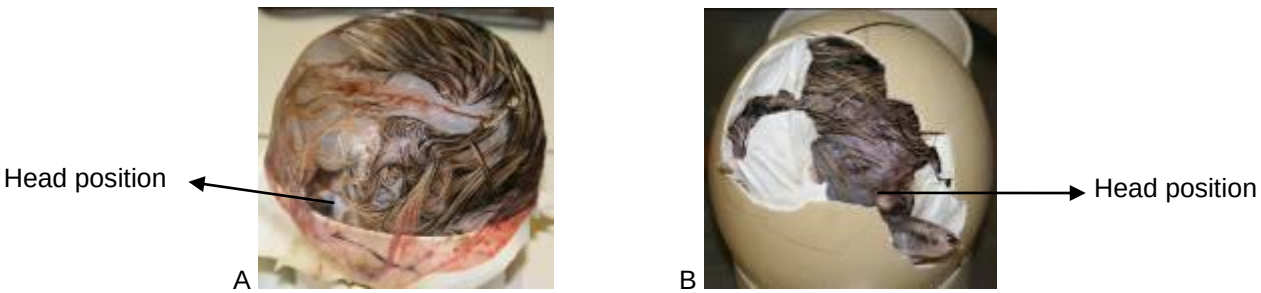

Figure 1 Malposition of dead-in-shell chick with head positioned towards the bottom of the egg (A) and malposition of a dead-in-shell chick with head midway towards the air cell (B). The air cell is denoted with a cross on the egg-shell surface in Figure 1B.

4. Discussion

A definite pattern was evident for the position of each of the different limbs. Most chicks tended to assume the correct pipping position as described by Deeming (1995). Although a high proportion of DIS chicks in the present study were in the correct position, a substantial proportion of DIS chicks that pipped internally, were positioned with their heads in the equator of the egg. Only a very small number (0.9%) was presented with their heads towards the bottom of the egg, away from the air cell, while only 1.2% pipped internally at the bottom of the egg. This finding differs from those of Deeming (1995) who reported that the most common malposition for ostrich embryos is with the head at the opposite end to the air cell (malposition II). Our findings do, however, correspond with Ley *et al*. (1986), who stated that chicks with pipping positions away from the air cell were more likely to succumb due to suffocation as they would be unable to penetrate the air cell. If external pipping did occur in these positions, it is possible that up to 65% of chicks would survive to hatching (Brand *et al*., 2011).

Our observations on the head positions correspond with findings by Deeming (1995) who reported that the chick may also be positioned with their heads to the left side instead of to the right side, while some get their heads stuck across their right leg or the right foot gets stuck over the head or in the beak. Although pipping would still be possible even though the heads were positioned towards the right side, chicks would not be able to pip if the head or beak were obstructed in any way. Results from our study could also be compared to observations by Brown *et al*. (1996) that common malpositions were legs facing upwards, but the head facing the opposite pole and chicks on their sides with their legs at the middle of the egg, with the head either turned towards the air cell or towards the bottom of the egg.

The ideal position for the feet would be the upwards position, because the feet are used during the hatching process when kicking against the eggshell is required to hatch (Deeming, 1994). Deeming (1995) also identified the foot positioned on the underside of the head as a problem preventing hatching of some chicks. The wing position of ostrich chicks during hatching differs from that of chickens, where the beak is positioned under the wing at the time of hatching (Deeming, 1994). This study did not attempt to compare malpositions of dead-in-shell ostrich chicks with the different positions found in chickens.

Results from our study showed that approximately 50% of the dead-in-shell chicks were correctly positioned, thus making suffocation because of a malposition unlikely. Some contributing factors to the inability of the chicks to hatch could be a too thick shell making it impossible to break the shell, a low eggshell porosity limiting oxygen supply, insufficient water loss causing some oedema or excess water loss causing dehydration (Deeming *et al*., 1993; Brown *et al*., 1996).

5. Conclusion

Deviations from the normal hatching position vary greatly in ostrich chicks. Further work is needed to refine the different dead-in-shell positions before trying to classifying them. At this point it seems that about 50% of the chicks that failed to hatch could not complete rotation and therefore suffocated in the egg. Although the

rest of the dead-in-shell chicks were positioned correctly, they still failed to hatch and the reasons causing this situation need to be studied further.

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PART II

CHAPTER 6

Developmental aspects of the ostrich embryo and aims of this study

1. Introduction

Currently the low hatching rate of artificially incubated ostrich eggs is of great concern for the ostrich industry. It is thus important to correctly diagnose problems during embryonic development. Brown *et al.* (1996), Deeming & Ar (1999) and Van Schalkwyk (2000) reported that hatching success of ostrich eggs is poor compared to poultry with hatchability rates of around 50 - 60%. A poor understanding of the pattern of embryonic development in ostriches may contribute to the observed poor hatching results. To alleviate problems associated with a poor hatchability of fertile eggs (see Part 1), it is important to correctly diagnose problems during embryonic development. This however requires good description and understanding of developmental stages of the ostrich embryo to ensure that the underlying biological processes and normal development occur under optimal conditions. An important tool for identifying incubation problems that cause low hatchability is the knowledge of the age and degree of development of the embryo at the time of death (Ar & Gefen, 1998).

2. Embryo development

The incubation period for ostrich eggs is widely reported to be 42 days, but is highly dependent upon the incubation temperature (Deeming *et al.,* 1993). Temperature readings of ostrich eggs in the nest indicate that there is a 4 - 6 °C temperature gradient across the 12 cm horizontal hight (short axis) of the egg (Swart & Rahn, 1988). Ostriches require a lower incubation temperature than poultry species, namely 36 - 36.5 °C, compared to 37.5 - 37.8 °C (Deeming *et al.,* 1993). Throughout incubation the developing embryo draws on the yolk and the albumen for its nutrients and water (Deeming *et al.,* 1993). The membranes are responsible for supplying the embryo with oxygen, which diffuses from outside the egg through the pores in the shell across the albumen. Albumen is used up during embryo development. From the start of incubation, water moves out of the albumen either to the embryo or by evaporation out of the egg. As water is removed, the volume of albumen diminishes and the air cell at one end of the egg increases in size with the entry of air. The remainder of the albumen flows into the amniotic cavity with the yolk where it is ingested by the embryo during the fifth week of incubation.

The ostrich embryo undergoes a complex pattern of growth and differentiation in a series of developmental steps during the 42 - day incubation period. The timing of specific developmental stages, however, remains largely unknown (Reiner *&* Dzapo, 1994; Deeming, 1995). Until recently, descriptions of embryonic development of the ostrich were generally photographic representations of changes observed during candling, with pictures of embryos at different stages of development (Van Schalkwyk *et al*., 1994). Studies by Malecki *et al*. (2005) described for the first time the morphology of the ostrich blastoderm and demonstrated that its development depends on the pre-incubation storage temperature and storage time. It suggests that those early stages could have an effect on later stages of development and hatching. However, the progression of the ostrich embryo to stages beyond blastoderm and how development in those early stages relates to hatching have not yet been studied. The embryo goes through two distinct phases of development. The first is the differentiation stage, which takes place during the first half of incubation (Deeming, 1997). The formation of new structures and is characteristic of the first half of embryonic development, as was also found in the chicken embryo (Ar & Gefen, 1998; Gefen & Ar, 2001). However, in

relation to the chicken, there are differences in the second half of incubation, which is characterized mainly by growth, specifically changes in the beak, wing, and leg length, as well as the wet weight of the embryo (Gefen & Ar, 2001). On the basis of this, Gefen & Ar (2001) suggested that embryonic age estimation of one species couldn't be inferred from relative changes in linear dimensions of another species. These differences between embryonic developments of the two species made it necessary to re-examine the development of the ostrich embryo.

Brown *et al.* (1996) reported that, while the highest percentage of embryonic mortalities occurs during the last 7 - 14 days before hatching, a proportion of eggs discarded as infertile could rather be classified as early embryonic mortalities. The ability of hatchery technicians to differentiate an early dead embryo from an infertile germinal disc is questionable (Sellier *et al.*, 2006), especially with ostrich eggs where candling of eggs is commonly used to determine fertility. It is difficult to determine if an egg is fertile before 7 days of incubation using candling, because a shadowed area indicative of a developing embryo is yet very poorly defined (Deeming, 1995). The correct classification of such eggs could only be done through egg breakout and examination the germinal disc area (Malecki et al 2005), the only objective method to determine true fertility of eggs. In the turkey for example, using the egg break out method the magnitude of a substantial number of early embryonic mortalities could be revealed (Sellier *et al.*, 2006).

3. Collection time and genotype

It is preferable that eggs are collected as soon as possible after lay, to avoid over-exposure to the sun or risk of microbial contamination in cool, wet conditions (Badley, 1997). It is a common practice to collect ostrich eggs in late afternoon, after most eggs are laid, but depending on the time of the year such eggs may be exposed to very low or very high ambient temperatures that can affect blastoderm size (Van Schalkwyk *et al*., 1999). This could contribute to embryo mortalities during incubation. Romanoff & Romanoff (1960) and Eyal-Giladi & Kochav (1976) reported that the avian embryo begins its development in the upper oviduct and this continues throughout egg formation. The embryonic development becomes "arrested" after oviposition as the egg cools down and awaits incubation (Malecki *et al*., 2005). Whether changes in blastoderm size, as affected by the ambient temperature the eggs are exposed to after oviposition, can affect early embryo development has not been studied in the ostrich.

Shafey (2004) suggested that growth and hatchability performance of chicken embryos may depend upon their genetic make-up and the incubation environment provided for their growth.

4. Setting and hatching position

In nature, the eggs lay on their sides in the nest and the parent birds turn it several times throughout the day by rolling it around the long axis (Badley, 1997). The setting and subsequent hatching position of the ostrich egg is one aspect of artificial incubation that may in part explain poor hatching success. The preferred way of setting ostrich eggs is in the vertical position with the air cell up and rotated through a 90° angle every hour (Van Schalkwyk *et al*., 2000), the reason being that more incubator space is made available by setting

eggs vertically. Van Schalkwyk also reported that for best hatchability eggs must be set with the air cell to the top. In wooden incubators or incubators only turning through an angle of 60°, eggs may be set horizontally for the first two weeks and then turned vertically with the air cell up for the rest of the incubation period. This is not a natural position of the egg incubated naturally in the nest (Wilson, 2003). With the exception of Van Schalkwyk *et al*. (2000), no literature results could be found on different setting positions for avian species, and ostriches in particular.

5. Candling and water loss

Egg candling is commonly used on commercial ostrich farms during artificial incubation to determine fertility and to monitor the progress of the developing embryo, but in most cases the latter is not very effective. Badley (1998) found detection of infertile eggs by candling to be reasonably reliable on day 14, but it became increasingly difficult to distinguish between live and dead embryos as incubation progressed. There are few studies reporting observations of ostrich eggs with developing embryos by using candling, other than to report an increase in dark shadows (Deeming, 1995). One feature, however, that is usually easily distinguishable is the air cell at the blunt end of the egg. The air cell is initially formed between the two shell membranes as the egg cools after hatching. It subsequently increases in size during incubation as water is lost from the egg. A measure of the air cell volume on specific days of incubation may provide hatchery managers with a simple tool to estimate whether development is proceeding according to expectations, the age of the developing embryo or the age of embryonic mortality. To date there has been no study of the air cell volume changes during embryonic development, thus warrant further investigation.

Studies by Swart *et al.* (1987), Deeming (1995), Ar (1996) and Blood *et al.* (1998) showed that the optimal water loss (WL) in artificially incubated ostrich eggs is between 13 and 15%. Although there is some variation in the percentage WL at which ostrich eggs will still successfully hatch, there is, however, a sharp increase in embryonic mortality below 10% and above and 18% WL at 35 days of incubation (Blood *et al*., 1998). Excessive WL results in dehydration of the embryo (Davis *et al*., 1988) while insufficient WL from the egg, can result in water retention by the chick, potentially causing embryonic mortality through respiratory insufficiency (Musara *et al.*, 1999) and a high proportion of chicks that are malpositioned at the point of hatch or have unabsorbed yolk sacs (Horbaňczuk *et al.*, 1999).

6. Aims of this study

An important tool for solving incubation problems is the knowledge of the age and degree of development of the embryo at the time of death (Ar & Gefen, 1998). In this study the focus has been on factors effecting hatchability of eggs. Factors such as collection time, water loss and genotype influencing the development of the ostrich embryo in the first 7 days and between 7 and 42 days of incubation and on the size of the air cell volume were studied. The aim of this part of the thesis was to determine the following aspects:

- Description of embryo development for fresh fertile eggs to 7 days of incubation

- Description of embryo development for 7 days to 42 days of incubation as well as embryo positions for eggs hatched in the horizontal or vertical positions
- Changes in the air cell volume of artificially incubated ostrich eggs.

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CHAPTER 7

Embryonic development in artificially incubated ostrich (*Struthio camelus*) eggs in the first 7 days

Abstract

The study was undertaken to examine the early development of the ostrich embryo as affected by time of egg collection and genotype. A total of 321 ostrich eggs were collected during the 2008 and 2009 breeding seasons and the development of the embryo for up to 7 days of incubation were described and analysed. A sample of the incubated eggs were weighed and opened daily to investigate developmental changes that took place. In fresh eggs, the blastoderm contained a round, translucent dark area pellucida (AP) in the center, with a surrounding thin white ring, likely to be the beginning of the area opaca (AO). The blastoderm area for fresh eggs was 15.8 mm^2 and 143.3 mm^2 after 2 days of incubation. By 3 days of incubation the area vasculosa (AV) was discernable in the posterior half of the blastoderm. Embryo length was 5.01mm at 3 days of incubation and 14.5mm after 7 days of incubation. At 2 days of incubation the blastoderm area in eggs from the South African Blacks (SAB) x Zimbabwean Blue (ZB) crosses (104.5 ± 18.6 mm^2) was significantly lower ($P < 0.05$) than the pure SAB (141.0 ± 10.5 mm^2), ZB (161.7 ± 13.5 mm^2) and ZB x SAB crosses (166.1 ± 14.2 mm^2). At 7 days of incubation AV length for both ZB x SAB (53.2 mm) and SAB x ZB crosses (54.1 mm) were significantly longer than embryos from the pure breeds. Results from our study can be put to practical use when determining if eggs are infertile or fertile and also investigating the age of early embryonic mortalities.

1. Introduction

Ostrich farming is a major agricultural enterprise in South Africa. The common commercial practice is usually to keep breeding birds in large groups or as pairs in small enclosures. Eggs laid are subsequently collected and incubated artificially. Hatching success of ostrich eggs is relatively poor compared to that of commercial poultry species, with hatchability figures of around 50 to 60% (Brown *et al.*, 1996; Deeming & Ar, 1999; Van Schalkwyk, 2000). This low hatching success represents a considerable loss of production and is a cause of concern in the ostrich industry. Although the highest proportion of embryonic mortalities occurs during the last 7 - 14 days before hatching (Brown *et al.*, 1996; Brand *et al*., 2007), a proportion of eggs discarded as infertile could be early embryonic mortalties. The ability of hatchery technicians to differentiate an early dead embryo from an infertile germinal disc is questionable (Sellier *et al.*, 2006), especially with ostrich eggs where candling of eggs is commonly used to determine fertility. Before 7 days of incubation, it is difficult to determine if an egg is fertile using candling because a shadowed area of an embryo is still very poorly defined (Deeming, 1995). Only by opening a clear egg can it be assessed if it was infertile or fertile and had an early embryonic mortality. By using egg breakout the magnitude of early embryonic mortalities was revealed in some turkey hatcheries, indicating that early embryonic mortalities can be a major problem (Sellier *et al.*, 2006). Embryonic development, as described by Hamilton & Hamburger (1951), is a series of consecutive rather than chronological stages, which account for the variation between embryos of the same chronological age (Gefen & Ar, 2001). Gefen & Ar (2001) suggested that this variation might result from factors such as differences in physical conditions of incubators, embryonic stage when incubation commence and genetic variation among embryos. These changes are most pronounced during the early stages of development (Hamilton, 1952).

Malecki *et al*. (2005) described the morphology of the ostrich blastoderm and demonstrated that its development depends on the pre-incubation storage temperature and storage time. It is a common practice to collect ostrich eggs in the late afternoon, the time most eggs are laid but, depending on the time of the year, such eggs may be exposed to very low or very high ambient temperatures that can affect blastoderm size (Van Schalkwyk *et al*., 1999), which could contribute to embryo mortalities during incubation. Whether changes in blastoderm size, as affected by the ambient temperature to which the eggs are exposed after oviposition, can affect early embryonic development has not been studied in the ostrich.

The aim of the present study was thus to describe the early embrynic development for up to the first 7 days of incubation and determine the effect of collection time and the genotype on the embryonic changes that take place. This could aid in an early diagnosis of embryo mortality and enable the hatchery manager to minimize incubation losses and more accurately identify incubation problems.

2. Material and Methods

Eggs used for this study were from the commercial pair-bred ostrich flock maintained at the Oudtshoorn Research Farm, South Africa, in the 2008 and 2009 breeding seasons. The husbandry and management of the flock have been described previously by Cloete *et al.* (1998) and Bunter & Cloete (2004). The flock consisted of the South African Black (SAB) genotype, the Zimbabwean Blue (ZB), and the crosses (SAB x

ZB) between these genotypes. Eggs were collected in the afternoon and early morning, disinfected in an Ultra-violet machine, weighed, and identified by date and paddock (female) of origin. Details on the methods of egg collection, sanitation and storage on the research farm have been previously documented (Van Schalkwyk *et al.*, 1999; Brand *et al*., 2007).

For the purpose of the trial, all eggs were stored for 3 days, prior to opening or setting into the incubator, at a temperature of 17 °C and relative humidity (RH) of 75%. The period of 3-day for storage time was chosen in accordance with findings by Brand *et al.* (2007), which showed that best hatching results are obtained from eggs stored for 3 - 4 days. Eggs were artificially incubated at 36 ºC and 24% RH in Buckeye® incubator, set to turn eggs automatically through 90° angle hourly. Between 37 and 45 eggs were collected for each of the developmental stages: fresh and 1 - 7 days of incubation. On the pre-selected day, eggs were weighed, measured and opened to investigate developmental changes. The eggs were opened by breaking the eggshell at the region of the air cell and by removing the membranes covering the embryo. Excess albumin was removed to get a clearer image of the embryo. On opening, the fresh eggs were considered fertile if the germinal disc region contained a blastoderm and infertile if the blastoderm was absent (Malecki *et al.*, 2005). Infertile or rotten eggs were discarded. The opened eggs were then placed under an Olympus SZ-61 microscope with a LG-PS2 light guide illumination system for a clear image. Digital images of all the developing embryos (fresh eggs up to 7 days of incubation) were taken with a Color View camera, by Soft Imaging System, which was mounted on the microscope. The AnalySIS program (Soft Imaging System, 1999) was used for the different measurements of the embryo. The traits measured were the blastoderm area, area pellucida (AP), area opaca (AO), area vasculosa (AV), embryo area and the embryo length. Due to considerable changes in embryo development over the 7 days and for comparable analysis, the measurements taken in fresh eggs, eggs incubated for 1 day and for 2 days were grouped into stage A, while measurements of embryos incubated for 3 - 7 days were grouped into stage B. Embryo length from 4 - 7 days incubated eggs was done by running a line from the tip of the tail along the spinal cord and the blood line in the brain area to the defined point on the head edge. The measurement unit was millimeters and measurements of all eggs per age group were pooled for a mean value. The AO area included the AP area as well as the embryo area, whilst the AP area included the embryo area. The data were then subjected to least-squares analysis, using ASREML (Gilmour *et al.*, 1999). Fixed effects considered were year of lay, collection time and genotype. Differences between comparable means were discerned with the least significant difference (LSD) method, provided that it was protected by a significant F-value in the ANOVA (Snedecor & Cochran, 1967).

3. Results

Descriptive statistics for stage A and images of developing blastoderms are presented, respectivly in Table 1 and in Figures 1 (A - C). In the fresh eggs (Figure 1A), the blastoderm contained a round, translucent dark area pellucida (AP) in the center. The AP was surrounded by a thin white ring, likely to be the beginning of the area opaca (AO). The outermost translucent ring, the periblast, was clearly visible. A lot of variation was evident in the area of the blastoderm between eggs in the same treatment, ranging from 9.24 mm to 31.1 mm, 18.4 mm to 144.2 mm and 42.7 mm to 294.9 mm, for fresh eggs, eggs incubated for 1 day and eggs

incubated for 2 days, respectively. The area of the blastoderm area for the fresh eggs was 15.8 mm^2, with an AO and AP area of 13.2 mm^2 and 2.7 mm^2, respectively. The length of the stage A embryos was measured from the posterior to anterior ends of the AP to be comparable to embryonic length of stage B embryos. At 1 day of incubation (Fig 1B), the hypoblast stage was already gone and a pear shape of the area pellucida had begun to form. The blastoderm area was 52.6 mm^2, the AO was 40.2 mm^2 and the AP was 9.0 mm^2. The embryo length and width were 2.3 mm and 1.9 mm respectively. At 2 days of incubation (Fig 1C) the blastoderm area was 143.3 mm^2 and the AP had become pear-shaped. The embryo area, length and width were, respectively 5.6 mm^2, 3.3 mm and 2.2 mm. The surrounding AO area had expanded to 128.5 mm^2.

Table 1 Means and standards errors (SE) for developing embryos measured from fresh eggs to eggs incubated for 2 days (stage A).

Measured traits	Mean ± SE		
	Fresh egg	1 day	2 days
Blastoderm area (mm^2)	15.8 ± 8.2	52.6 ± 9.93	143.3 ± 7.05
AO area (mm^2)	13.2 ± 7.6	40.2 ± 9.34	128.5 ± 6.63
AP area (mm^2)	2.69 ± 1.48	9.03 ± 1.81	9.88 ± 1.34
Embryo width (mm)	1.8 0± 0.13	1.83 ± 0.16	2.19 ± 0.12
Embryo length (mm)	2.09 ± 0.19	2.14 ± 0.24	3.31 ± 0.17
Embryo area (mm^2)	2.98 ± 0.88	3.36 ± 1.09	5.57 ± 0.77

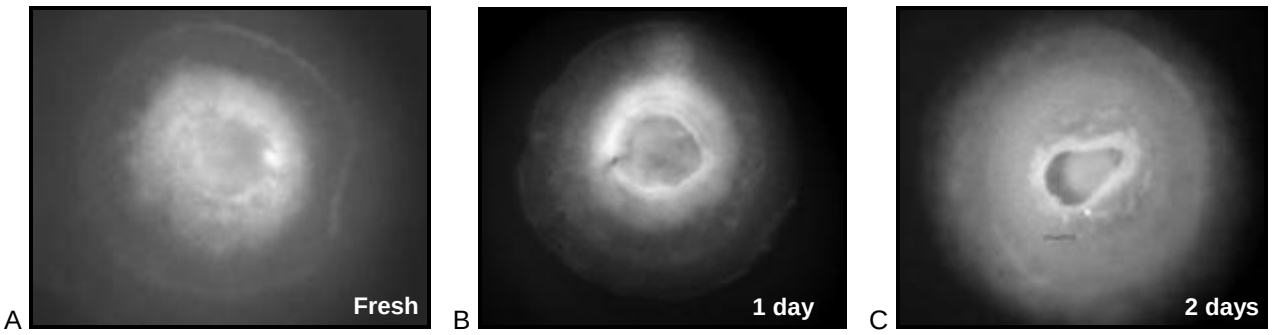

Figure 1 Surface view of the blastoderm of a fresh ostrich egg (A), at 1 day of incubation (B) and at 2 days of incubation (C).

Descriptive statistics for stage B and images of developing blastoderm are presented respectively in Table 2 and in Figure 2 (A - C). The AP and AO were difficult to distinguish from this stage onwards, thus measurements were focused on the embryo and area vasculosa (AV). After 3 days of incubation, the embryo appeared to be in the late primitive steak stage and blood islands within the AV were observed in the posterior half of the blastoderm in some eggs (Figure 2A). The widest area of the pear-shape AP was identified as the anterior end where development of the head folds started and this was measured to

determine the embryo head width (2.8 mm). At 4 days of incubation a wide network of blood vessels surrounded the developing embryo while the heart was visible.

After 5 days of incubation the body of the developing embryo had rotated to the right with the tail bud bent ventrally. The heart was S-shaped and in some instance the non-pigmented eye was discernable. The lower measurements of the head width at 5 days if compared to 4 days could be ascribed to a change in embryo position and shape (Figures 2B and C). The rotation of the body had been completed by 6 days of incubation and the embryo length was 9.9 mm (Figures 3A). The tail bud started turning to the right, whilst the eyes were not yet pigmented. At 7 days of incubation the allantois was vesicular and variable in size, whilst the eyes were faintly grey in color (Figures 3B).

Table 2 Means and standards errors (SE) for developing ostrich embryo traits measured in eggs incubated from 3 - 7 days (stage B).

Embryo traits	Mean ± SE				
	3 days	4 days	5 days	6 days	7 days
Head width (mm)	2.82 ± 0.12	3.91 ± 0.12	2.78 ± 0.15	3.99 ± 0.14	4.95 ± 0.12
Length (mm)	5.01 ± 0.17	7.88 ± 0.18	9.92 ± 0.21	12.6 ± 0.21	14.5 ± 0.18
Total area (mm^2)	11.3 ± 0.80	22.9 ± 0.81	14.9 ± 0.98	20.7 ± 0.95	31.9 ± 0.83
AV length (mm)	6.54 ± 0.72	11.9 ± 0.73	22.7 ±.0.87	32.3 ± 0.88	51.0 ± 0.78
AV width (mm)	5.13 ± 0.60	10.6 ± 0.61	19.7 ± 0.73	28.9 ± 0.73	46.0 ± 0.65
AV area (mm^2)	29.7 ± 8.27	109 ± 8.34	268 ± 24.2	337 ± 48.4	297 ± 54.5

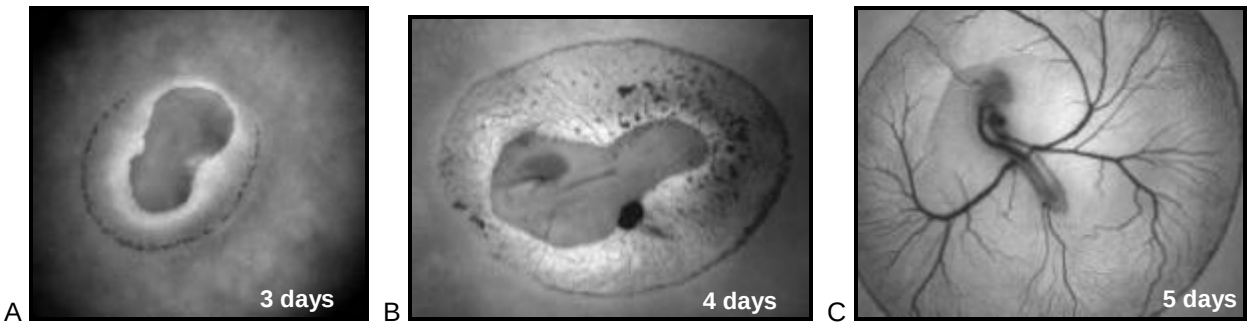

Figure 2 Surface view of the developing embryo of ostrich egg incubated for 3 days (A), 4 days (B) and 5 days (C).

Table 3 contains the main values over the total incubation period for the respective measurements. For the blastoderm area, AO area or AP area it is the means of the measurements taken over 2 days of incubation and for the embryonic and AV measurements it is the means of the measurements taken over 7 days of incubation. Neither year nor collection time affected the blastoderm area, AO area or AP area. Measurements for embryo length, embryo area and embryo head width were significantly higher for 2009 than 2008. Collection time also affected embryo length, embryo area and embryo head width, with higher (P

< 0.05) measurements for eggs collected in the afternoon. The AV development from 3 days to 7 days of incubation was not affected by either year or collection time.

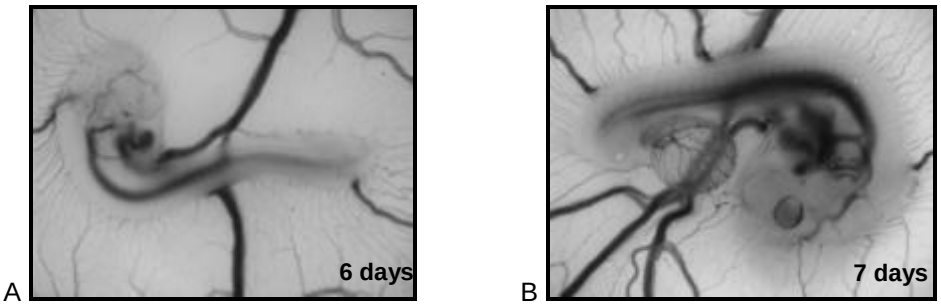

Figure 3 Surface view of the developing embryo of ostrich eggs incubated for 6 days (A) and 7 days (B).

Table 3 Means and standards errors (SE) depicting the effect of year and collection time on embryonic development.

Measured traits	Year		Collection time	
	2008 (n = 126)	2009 (n = 195)	Afternoon (n = 194)	Morning (n = 127)
Blastoderm area (mm^2)	67.5 ± 7.18	73.6 ± 5.72	74.4 ± 6.29	66.7 ± 7.06
AO area (mm^2)	8.09 ± 1.31	6.30 ± 1.07	8.15 ± 1.16	6.24 ± 1.30
AP area (mm^2)	56.6 ± 6.75	64.7 ± 5.38	63.2 ± 5.92	58.1 ± 6.64
Embryo length (mm)	7.00 ± 0.11^a	7.36 ± 0.08^b	7.31 ± 0.09^a	7.05 ± 0.11b
Embryo area (mm^2)	13.9 ± 0.49^a	14.6 ± 0.37^b	14.8 ± 0.40^a	13.72 ± 0.19^b
Embryo head width (mm)	2.83 ± 0.07^a	3.23 ± 0.05^b	3.16 ± 0.06^a	2.91 ± 0.07^b
Area vasculosa length (mm)	24.5 ± 0.59	25.3 ± 0.40	25.4 ± 0.44	24.4 ± 0.58
Area vasculosa width (mm)	22.0 ± 0.50	22.2 ± 0.34	22.4 ± 0.37	21.7 ± 0.49
Area vasculosa area (mm^2)	152.6 ± 8.10	156.6 ± 7.28	152.2 ± 6.44	157.0 ± 8.78

^{a,b}- Means in the same row with different superscripts are significantly different (P < 0.05)

The effect of genotype on blastoderm growth for the first 2 days incubation period is shown in Figure 4. There was no difference in blastoderm area between genotypes for fresh eggs and, while the blastoderm area of the reciprocal crosses was slightly bigger for the crosses (59 mm^2 - ZB x SAB and 66 mm^2 - SAB x ZB) for at 1 day of incubation than the two pure bred lines (46 mm^2 - SAB and 40 mm^2 - ZB), the difference was not significant. At 2 days of incubation the blastoderm area of the SAB x ZB crosses (n = 44; 104.5 mm^2) was significantly smaller (P < 0.05) than the pure ZB (n = 73; 161.7 mm^2) and ZB x SAB crosses (n = 81; 166.1 mm^2). The blastoderm area of the ZB x SAB crosses was also significantly larger than of the pure SAB line (n = 123). The effect of genotype on the AO area size corresponds with the effect of genotype on blastoderm growth. The same trend was true for the AP area for the fresh eggs, but the AP areas of the pure breeds were inclined to be higher than the reciprocal crosses after 1 day of incubation, although not significant. Again, the ZB x SAB cross had a significantly larger AP area (14.5 mm^2) at 2 days of incubation

when compared to the pure breeds (9.6 mm^2 for the SAB), with the AP area of the pure ZB the smallest at 5.3 mm^2.

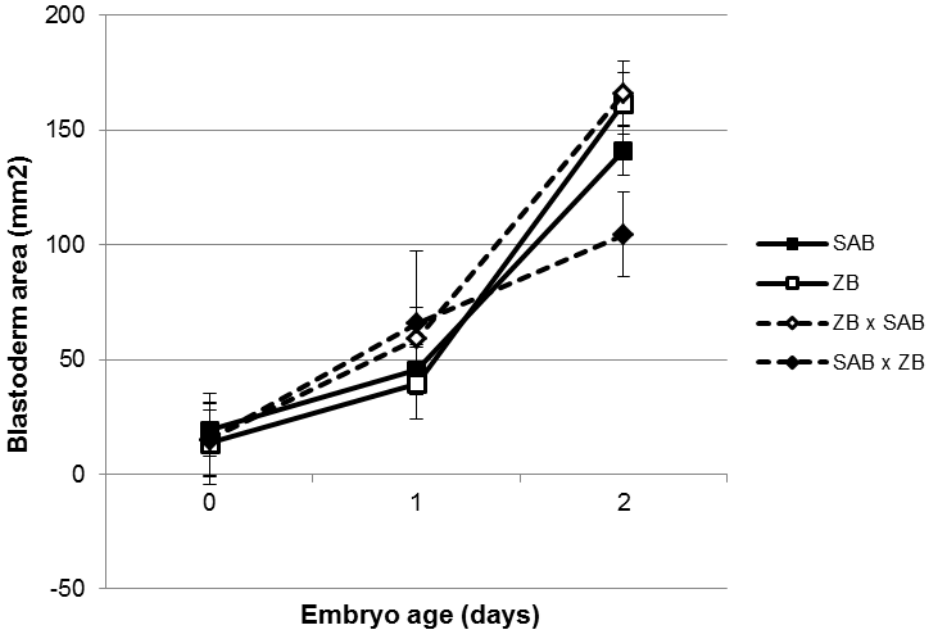

Figure 4 Effect of genotype on blastoderm area of fresh ostrich eggs, eggs incubated for 1 day and eggs incubated for 2 days.

Figure 5 illustrates the effect of genotype on embryo length over a 7 day incubation period. Genotype did not affect the embryo of the freshly laid egg, with embryo lengths of the different genotypes ranging between 1.8 mm and 2.3 mm and embryo head width between 1.5 mm and 2.1 mm. At 3 days of incubation the pure ZB embryos had a significantly ($P < 0.05$) smaller area (8.8 mm^2) than the other genotypes (ranging from 11.5 mm^2 to 12.8 mm^2). At days 4 of incubation, the SAB embryo was significantly shorter 7.2 mm in length than the pure ZB and the two cross combinations (7.8 mm to 8.3 mm). Embryos from both pure ZB (25.2 mm^2) and crosses of SAB x ZB (25.2 mm^2) had a bigger area if compared to embryos from pure SAB (20.5 mm^2) and ZB x SAB crosses (20.7 mm^2). Both embryo length (15.1 mm) and area (35.1 mm^2) of the ZB x SAB cross were significant bigger at 7 days of incubation when compared to the other genotypes (14.1 mm to 14.5 mm for length and 30.1 mm^2 to 31 mm^2 for area respectively).

Genotype did not affect AV measurements for eggs incubated for 3 days, but AV area (84.0 mm^2) were throughout significantly smaller at 4 days of incubation for the pure SAB when compared to those of the other genotypes, ranging between 111.6 mm^2 and 120.7 mm^2 (Figure 6). At 4 days of incubation the AV area of embryos of SAB and ZB crosses (348.7 mm^2) was significantly larger than the AV area of the other genotypes (240. 4 mm^2 to 243.3 mm^2). At 7 days of incubation AV length for both the reciprocal crosses between SAB and ZB (53.2 mm and 54.1 mm respectively for ZB x SAB and SAB x ZB crosses) were significantly longer than embryos from the pure breeds (47.7 mm and 49.1 mm for SAB and ZB respectively).

AV width of embryos from eggs incubation for 7 days of was significantly narrower (42.3 mm) for pure SAB when compared to areas of other genotypes (46.6 mm to 48.5 mm).

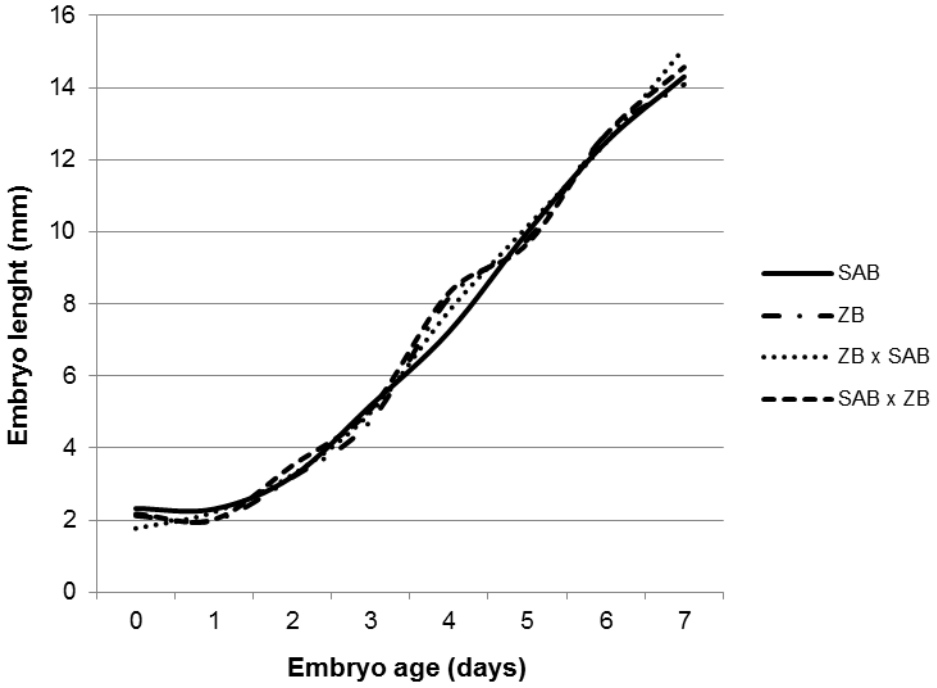

Figure 5 Effect of genotype on embryo length from the fresh egg till eggs incubated for 7 days.

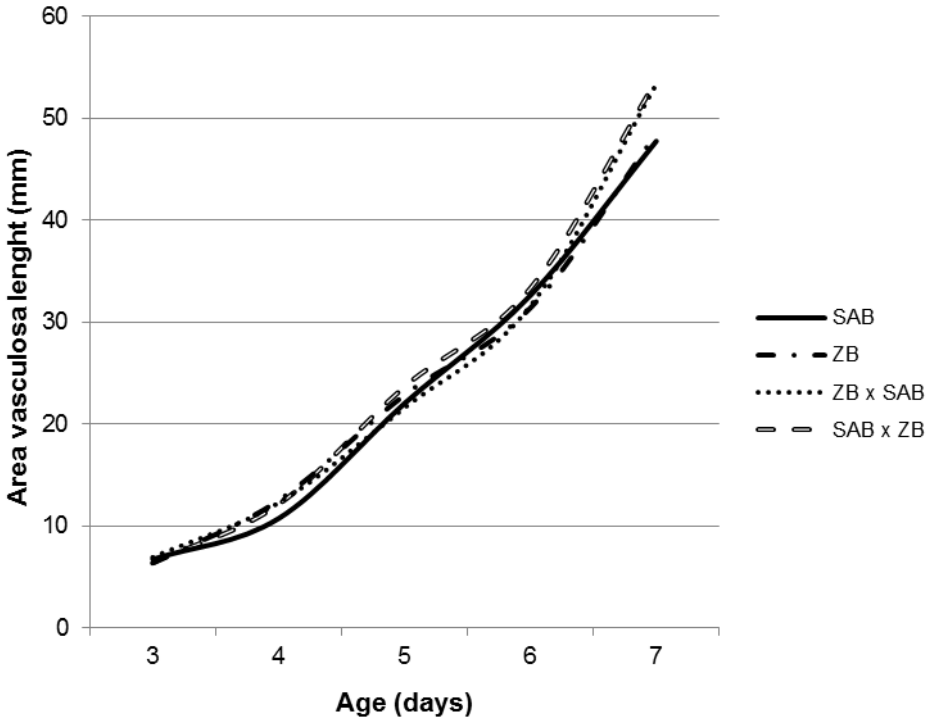

Figure 6 Effect of genotype on area vasculosa length from eggs incubated for 3 days up till eggs incubated for 7 days.

4. Discussion

Previous work had been done on the development of older ostrich embryos, but no comparitive literature could be found on very early embryonic development. The appearance of the blastoderm in our study corresponds with the description in eggs soon after oviposition provided by Malecki *et al.* (2005). The general morphology of the blastoderm at this stage was comparable to chicken Stage X (frechly laid egg), turkey Stage VII or duck Stage 9, because of the well-developed AP and the early differentiation of the AO (Eyal-Giladi & Kochav, 1976; Bakst *et al.*, 1998; Dupuy *et al.*, 2002; Bellairs & Osmond, 2005; Malecki *et al.*, 2005). The pear-shape form of the blastoderm at 2 days of incubation in our study resembled the late primitive steak phase (stage 4 - 5; 18 - 22 hours) in the chicken (Hamburger & Hamilton, 1951) and also stage 17 in the duck (Dupuy *et al.*, 2002). Our results differ from Gefen & AR (2001), who observed blood islands in ostrich eggs only from 4 days of incubation. After 3 days of incubation the ostrich embryo is consistent with stage HH8 (26 - 29 hours) for chickens (Hamburger & Hamilton, 1951) and stage 21 for ducks (Dupuy *et al.*, 2002). The mean length (11.9 mm) of the AV at 4 days of incubation in our study corresponded with the 10 mm reported by Gefen & Ar (2001). The rotation of the embryo at 5 days of incubation corresponded with stage HH16-17 (51 - 64 hours) of the chicken (Hamburger & Hamilton, 1951). Embryos from eggs incubated for 7 days were similar to stage HH20 (70 - 72 hours) in chickens (Hamburger & Hamilton, 1951) and stage 33 for ducks (Dupuy *et al.*, 2002), with the tail bud curved, tip pointing forward towards the anterior end. The variable sizes of the allantois and the greying of the eye color, observed at 7 days of incubation were consistent with findings reported by Ar & Gefen (1998).

Even though year affected the hatchability of ostrich eggs, year effects are generally transient and unpredictable. Even though eggs being artificially incubated are shielded from changes in atmospheric climatic conditions in the controlled incubators, it is possible that ambient climatic conditions may still affect eggs prior to incubation. It is highly likely that year effects are not repeatable, and average performance in a given year cannot be predicted with any reasonable accuracy. Results for our study showed that the measurements of embryo length, embryo area and embryo head width were larger for eggs collected in the afternoon compared to eggs collected in the morning. Romanoff & Romanoff (1960) and Eyal-Giladi & Kochav (1976) reported that the avian embryo begins its development in the upper oviduct and this continues throughout egg formation. The embryonic development becomes “arrested” after oviposition as the egg cools down and awaits incubation (Malecki *et al*., 2005). It could be speculated that, because of the practise of collecting eggs in the afternoon and the immediate placement of the collected eggs into an Ultra violet machine to be disinfected, the cooling down process of the egg is delayed. This might cause continuous growth of the developing embryo, whereas eggs collected in the morning were subjected to immediate cooling down, because of the lower night time temperatures.

Shafey (2004) suggested that in the chicken growth and hatchability performance of embryos may depend upon their genetic make-up and the incubation environment provided for their growth. Results from our study showed a significantly smaller blastoderm area for the SAB x ZB crosses, which was unexpected since SAB x ZB crosses produced, together with the pure ZB line, the heaviest eggs (Brand *et al.*, 2008). This difference in blastoderm area could be attributed to the theory that the rate of embryonic growth in larger

eggs is slowed, thus the need for a longer incubation time. Our results correspond with findings by Shafey (2004) that the genetic make-up of birds influenced the physical dimensions and the eggshell characteristics of their eggs and these affected embryonic growth.

5. Conclusions

The stages of development of the ostrich embryo in the present study were closely related to stages in chickens, as described by Hamburger & Hamilton (1951). Although hatchability of ostrich eggs was affected by year, the effects are generally transient and unpredictable. It would suffice to state that year effects are not repeatable, and such effects have therefore little practical application, except for the identification of possible long-term trends. Genotype had a clear effect on the growth of the embryo and needs to be considered in the developmental staging of embryos. Due to an increased in length for embyos in eggs collected during the afternoons, collection time also needs to be considered in determining the age of developing embryos. Information stemming from these observations can be used for the identification of incubation problems that result in low hatchability.

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CHAPTER 8

Ostrich (*Struthio camelus*) embryonic development and relative changes in the egg components from 7 to 42 days of incubation for eggs set horizontally or vertically

Abstract

It is important to understand embryonic development for successful artificial incubation. Growth of both the embryo and legs during 42 days of incubation was similar and about linear, with a more or less doubling in size up to 35 days of incubation. The embryo eye size increased more rapidly than the beak length and stabilised at around 16.2 mm by 28 days of incubation, while beak length increased continuously until hatching at 42 days. Although growth of the upper wing was initially slower than that of the lower wing, it compensated after 21 days of incubation, whereafter it grew significantly faster than the lower wing. At day 42 of incubation (when hatching is due) there was no difference in length between the upper and lower wings. Incubation position of eggs (vertical vs. horizontal) mostly did not affect the measurements of the developing embryo throughout the 42-day incubation period. The exception was acceleration in the growth of the upper wing of embryos for eggs set vertically after 28 days of incubation, if compared to embryos from eggs set horizontally. This resulted in the upper wings of embryos in vertically set eggs being significantly longer than those of embryos in eggs set horizontally by 42 days of incubation (45.3 mm vs. 41.4 mm). Information stemming from these results can be used to estimate the age of dead-in-shell embryos in an attempt to identify incubation problems resulting in low hatchability of fertile eggs.

1. Introduction

The success of the artificial incubation of ostrich eggs plays a major role in the financial success of the ostrich industry. If compared to poultry, hatching success of ostrich eggs is poor with hatchability figures of around 50 - 60% of egg set (Brown *et al.*, 1996; Deeming & Ar, 1999; Van Schalkwyk, 2000). Modern commercial farming strives for maximal yield, which would require optimal incubation conditions. Currently the low chick production is of great concern to the industry. It is thus important to correctly diagnose problems during embryonic development, but the developmental stages of the ostrich embryo need to be well described to enable this. During the 42-day incubation period, the ostrich embryo undergoes a complex pattern of growth and differentiation in a series of developmental steps. Progress of this development and the timing of specific developmental stages of the ostrich embryo, however, remain largely unknown (Reiner & Dzapo, 1994; Deeming, 1995). Ostriches are precocial birds (Brown & Prior, 1999), which undergo a longer phase of tissue maturation than altricial species (Ricklefts & Starck, 1998). The first 42 normal stages in chickens, as described by Hamburger & Hamilton (1951), can be applied equally well to altricial and precocial development. As a result, embryonic stages of chickens previously served as a reference for other understudied avian species. However work done by Richardson *et al.* (1998) showed differences in embryonic development between species. Limited observations on ostrich embryos also suggested that the basic pattern of embryonic development differs little from that of the domestic fowl (Deeming *et al.*, 1996; Ar & Gefen, 1998). The incubation period of ostriches is exactly double that of chickens and a “rule of thumb” is that any particular stage of embryonic ostrich development can be obtained by reference to the corresponding stage of development in the chicken. The embryo undergoes two distinct phases of development. The first is the differentiation stage, which takes place during the first half of development (Deeming, 1997). This period is characterized by the formation of new structures and is similar to experience in the chicken (Gefen & Ar, 2001). However, there are differences in the second half with the rest of the development characterized mainly by growth, specifically changes in the beak, wing, and leg length, as well as the wet weight of the embryo (Gefen & Ar, 2001). On the basis of this, they suggested that embryonic age estimation of one species cannot be inferred from relative changes in linear dimensions of another species. These differences between embryonic development of the chicken and the ostrich necessitate the examination of the development of the ostrich embryo.

The setting and subsequent hatching position of the ostrich egg is one aspect of incubation that may affect hatching success. The preferred way of setting ostrich eggs are in the vertical position with the air cell up and rotated over 90° angle every hour (Van Schalkwyk *et al*., 2000), the reason being that more incubator space is made available by setting eggs vertically. Wooden incubators or incubators only turning egg through an angle of 60°, would require eggs to set horizontally for the first two weeks and then turned vertically with the air cell up for the rest of the incubation period (Van Schalkwyk *et al*., 2000). This is not a natural position of the egg incubated naturally in the nest (Wilson, 2003). Many reports indicated that natural incubation yields better hatch results than artificial incubation, but the reason for that is yet unclear. With the exception of Van Schalkwyk *et al*. (2000), no literature results could be found on different setting positions for avian species.

An important tool for identifying incubation problems that causes low hatchability is knowledge of the age and degree of development of the embryo at the time of death (Ar & Gefen, 1998). In this study, we describe the stages of the development of ostrich embryos and related them to the separate components of ostrich eggs during the incubation period in an attempt to identify predictive variables for accurate age determination.

2. Material and Methods

Eggs that originated from the commercial ostrich flock at the Oudtshoorn Research Farm, South Africa, during 2009 were used for the study. Cloete *et al.* (1998) and Bunter & Cloete (2004) described the origin of flock, the management of the breeders, and incubation practices for the eggs have been described previously (Van Schalkwyk *et al.*, 1998; 1999; Brand *et al*., 2007). For this study, only eggs of the South African Black (SAB) genotype were used. The methods of egg collection, sanitation and storage on the research farm followed that described previously (Van Schalkwyk *et al*., 1998; 1999; Brand *et al*., 2007). All the eggs were stored prior to setting into the incubator for 3 days at a temperature of 17 °C and relative humidity (RH) of 75 %. The 3-day storage time was chosen in accordance with findings by Brand *et al.* (2007), which suggested that the best hatching results are from eggs stored for 3 to 4 days. The eggs were randomly divided into two groups, one group was set horizontally and the other group was set vertically, with the air cell towards the top. Placing the groups of eggs set horizontal into the incubator, a sticker was placed on the egg-shell to indicate the topside of the egg. This was done to ensure that upon opening the eggs, all eggs were consistently opened at the same location to be able to describe the position of the embryo. For the group of eggs hatched vertically, a sticker was put on the egg-shell facing forward. Eggs were artificially incubated at 36.2 ºC and 24% RH in a Buckeye® incubator, set to turn eggs automatically through 90° angle hourly. Eggs hatched horizontally turned through the 90° angle on its long axis, while the top of the eggs (containing the air cell) turned through 90° for eggs set vertically.

Between 21 and 34 eggs per incubation time were processed to investigate developmental changes that took place on days 7, 21, 28, 35 and 42 of incubation. All the eggs were weighed, then opened by breaking the egg-shell at the region of the air cell and removing the membranes covering the embryo. Only eggs containing live embryos were considered for data collection. The stage of embryonic development was noted and after separation, the shell, embryo, yolk and albumen were weighed to the nearest gram. The embryos from eggs incubated for 7 days were too small to measure manually and were placed under an Olympus SZ-61 microscope with a LG-PS2 light guide illumination system for a clear image. Digital images of the developing embryos were taken with a ColorView camera mounted on the microscope. After the developing embryos were photographed, the AnalySIS program (Soft Imaging System, 1999) was used to measure embryo length and eye size. The embryos of eggs incubated for ≥ 14 days were removed from the eggshell to measure the eye, beak (from the feather line where the beak begin to the tip of the upper beak), leg (from the body-leg joint to the tip of the claw), upper wing (from the body joint to the elbow), lower wing (from the elbow to the tip of the second digit) and body length. Only the extremities on the left side of the embryo were measured. The measurements were carried out with a digital caliper to 10 µm. Photos were taken to examine the morphological changes that took place. Separation of the yolk and amniotic fluid at this

stage was not possible, because at removal of the embryo from the egg, the amnion sac had to be opened and spillage of the amniotic fluid occurred. The amniotic fluid was thus weighed together with the yolk. Since ostrich eggs normally start to hatch between day 41 and day 42 of incubation, measurements were taken on the live chicks during the standard practicing procedures, when the chick is weighted and tagged. On opening the eggs, the head position of the embryo was noted by dividing the opened area into four sectors relative to the air cell and the placement of the sticker attached upon setting. The data were then analysed to depict trends associated with incubation period (7, 14, 21, 28, 35 and 42 days), using least squares analyses in ASREML (Gilmour *et al.*, 1999). The position of the egg during incubation (vertical or horizontal) were also included in the analysis and interacted with stage of incubation. Differences in egg size were accounted for by the inclusion of the length, width and weight of the egg as linear covariates in the analyses. Differences between comparable means were discerned with the least significant difference (LSD) method on the provision that it was protected by a significant F-value in the ANOVA (Snedecor & Cochran, 1967). Chi-square procedures (Van Ark, 1990) were used to assess the effects of hatching position on hatchability.

3. Results

3.1. General embryonic development

After 7 days of incubation (Figure 1A) the tail bud of the embryo started to curve, tip pointing forward towards the anterior end. The embryo had reoriented from laying ventral on the yolk surface to turning onto its side and floating within the fluid filled amniotic sac above the yolk surface. The allantois grows out on the right side of the embryos gut and was vesicular and variable in size, while the eyes were faintly grey in color. On day 14, the blood vessel network and the amniotic sack enclosing the embryo covered the upper surface of the yolk (Figure 1B). The beak was distinct at this stage with the maxillary about twice the length of the mandible. The distinct grooves between the two toes and the three digits of the legs and the wings respectively, were clearly visible at this stage. An increase of ± 40% in the mean weight of the yolk occurred between day 7 and day 14 of incubation and the consistency of the albumen was jelly-like, decreasing 37.5% in weight. Rudimentary feathers were evident after 21 days of incubation (Figure 1C). The eyeballs had been covered with eyelids to an oval opening. The embryo had sunken into a depression in the yolk surface.

Figure 1 Surface view of the developing embryo of ostrich eggs incubated for 7 days (A), 14 days (B) and 21 days (C).

On day 28, nails appeared on the toes of the embryo. The embryo was turned with its spine parallel to the long axis of the egg, with the head bend towards the breast and tucked between the legs. Embryo length increased by 81% from 21 to 28 days of incubation, while leg length doubled (Table 1). Embryo weight increased more than 7-fold during the same period. At 35 days of incubation, the beak was orientated towards the right and the legs were pulled closer to the body, with the feet positioned adjacent to the neck. The yolk sac had been retracted about half-way into the abdominal cavity. The embryo was covered with a thick coat of feathers. About 74% of the albumen had been used by now. The first chicks started to pip on day 40, with most pipping on days 41 and 42. At this stage the ostrich embryo was fully grown with the beak pointing right over the wing, the right foot next to the beak and the left foot behind the head. The yolk sac had been fully retracted into the body cavity and all the albumen had been used. Internal pipping occurred when the chick breaked the membranes next to the air cell with its beak, followed by external pipping. A combination of pecking, kicking and body extension movements, then allows chicks to hatch. It subsequently took up to 12 h for the chick to break free from the shell.

Table 1 Least squares means (± SE) for weights and measurements of the developing embryo and egg components for eggs incubated for 7 to 42 days. Egg weight at setting was included as a linear covariate in the analysis, to account for differences in egg size.

Measured traits	Mean ± SE					
	7 days	**14 days**	**21 days**	**28 days**	**35 days**	**42 days**
Embryo length (mm)	14.9 ± 2.56	36.9 ± 2.56	91.4 ± 2.76	166 ± 2.55	235 ± 2.56	267 ± 2.94
Eye size (mm)	0.77 ± 0.32	8.60 ± 0.24	15.5 ± 0.26	16.2 ± 024	16.3 ± 0.24	15.9 ± 0.29
Beak length (mm)	-	3.37 ± 0.44	10.2 ± 0.36	16.5 ± 0.33	20.8 ± 0.33	21.7 ± 0.38
Leg length (mm)	-	12.4 ± 1.63	47.3 ± 1.75	95.8 ± 1.62	139 ± 1.63	181.3 ± 1.92
Upper wing (mm)	-	5.05 ± 0.89	16.3 ± 0.73	29.0 ± 0.66	37.7 ± 0.67	43.3 ± 0.77
Lower wing (mm)	-	6.32 ± 0.61	16.2 ± 0.50	26.7 ± 0.45	35.6 ± 0.47	43.4 ± 0.53
Embryo weight (g) log	0.17 ± 0.15	2.78 ± 0.23	21.0 ± 0.83	156 ± 4.73	399 ± 11.9	910 ± 31.1
Shell weight (g)	269 ± 5.47	273 ± 4.57	273 ± 5.05	279 ± 4.77	282 ± 4.83	259 ± 6.01
Albumen weight (g)	691 ± 18.8	432 ± 16.4	481 ± 18.7	273 ± 19.8	181 ± 38.2	-
Yolk weight (g)	366 ± 21.9	614 ± 18.6	575 ± 20.5	634 ± 20.2	550 ± 20.9	-

-Data not availible

3.2. Embryo size, weight and egg components

The measurements for all components of the developing embryo are presented in Table 1. Initial egg weights for each stage of opening ranged between 1 405 g and 1 466 g. The lenght growth of the embryo and the leg during the 42 days of incubation was similar and about linear (Figure 1). Both the embryo length and its leg length increased nearly doubled for each week of incubation up to 35 days of incubation (respectively 235 and 139 mm), whereafter smaller length increases occurred for the last week of incubation (respectively 267 and 181 mm). Embryonic weight increased from 29% to 64% of initial egg weight during the last week of incubation. The embryo eye size increased more rapidly than the beak length and stabilised at 6.2 mm from 28 days of incubation, while the beak length continuously increased until chicks hatched at

42 days (Figure 2). Although the growth of the upper wing was initially slower than that of the lower wing (Figure 3), it compensated after 21 days of incubation, whereafter it grew significantly ($P < 0.05$) faster than the lower wing. By hatching on day 42 of incubation, there again was no difference in length between the upper (43.3 mm) and lower wings (43.4 mm).

Shell weight remained mostly constant at just below 20% of initial egg weight throughout incubation (Figure 4). Albumen weight decreased linearly (except for a slight discrepancy between 14 and 21 days of incubation) from 7 days of incubation until it was depleted at 42 days of incubation. After an initial increase between 7 days of incubation and 14 days of incubation, the yolk weight stayed relatively constant until absorption between 35 and 42 days of incubation.

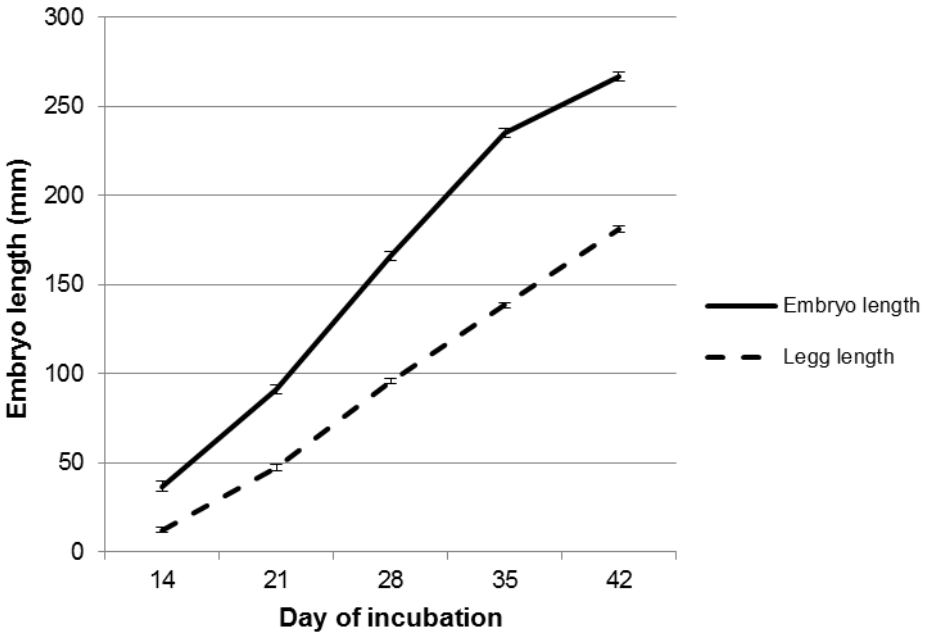

Figure 1 Growth of the ostrich embryo and the leg between 14 to 42 days of incubation.

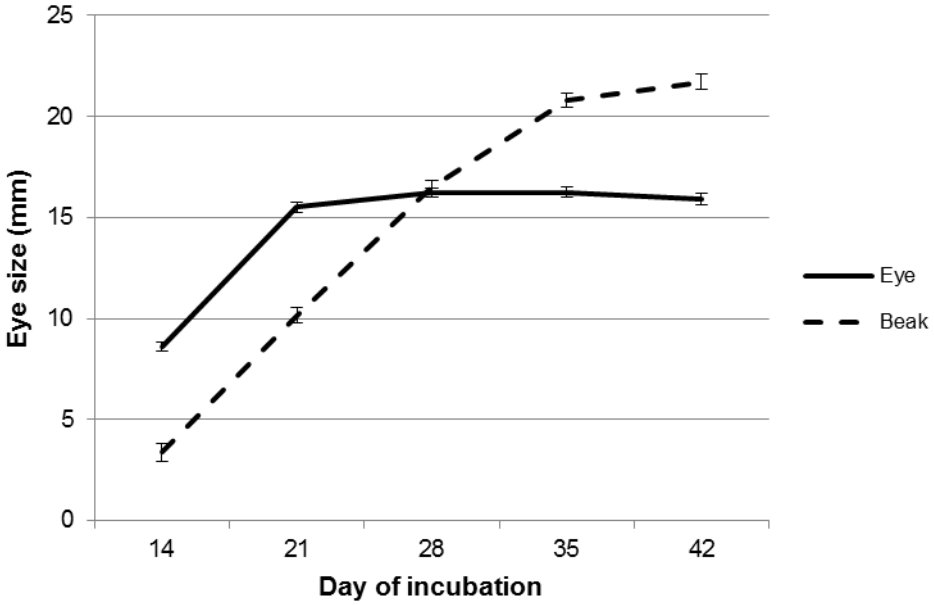

Figure 2 Growth of the eye and beak of ostrich embryos for eggs incubated 14 to 42 days.

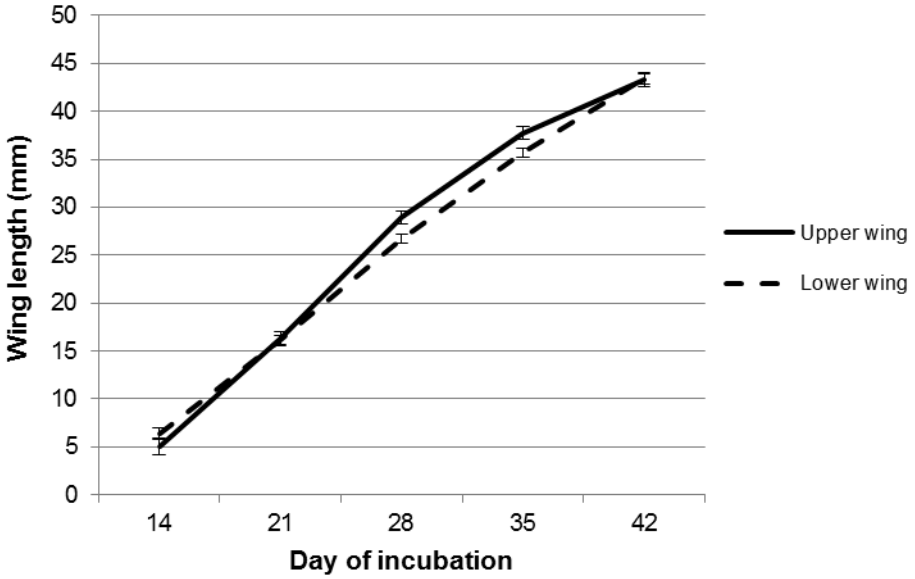

Figure 3 Growth of the upper and lower wings of ostrich embryos for eggs incubated 14 to 42 days.

Figure 4 demonstrates the changes in weight for the different egg components. The weight of the embryo increased exponentially in the 42 days incubation period, with a slow rate of increase between day 7 and day 21 of incubation followed by a rapid rate of increase thereafter.

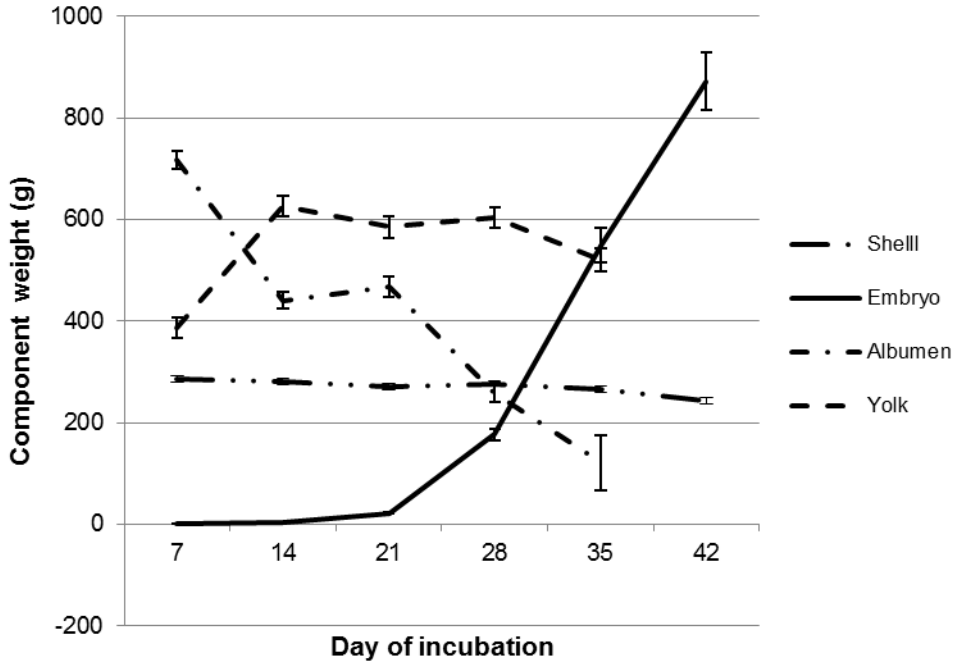

Figure 4 The relationships of egg weight, shell weight, embryo weight, yolk weight and albumen weight with stage of incubation in ostrich eggs (7 to 42 days of incubation).

3.3. Setting and incubation position

Embryos in eggs incubated horizontally or vertically both started to turn with its spine parallel to the long axis of the egg on 28-day of incubation. Results showed no difference in hatchability between eggs hatched in the horizontal position (13/18 = 0.72) and eggs hatched in the vertical position (11/18 = 0.61) (Chi^2 = 0.13; df = 1; P = 0.72). The development of the eye and beak was independent of setting position throughout the 42-day incubation period. Embryo length during the 42-day incubation period was accordingly not affected by the vertical or horizontal position. The setting position also did not affect the growth of the leg throughout incubation (P > 0.05).

The length of the upper wing of embryos form vertically set eggs started to increase at a faster rate after 28 days of incubation compared to embryos from eggs set horizontally (Figure 5). As a result, the upper wing of embryos in eggs set vertically was significantly (P < 0.05) longer than those of embryos in horizontally set eggs on day 42 days (45.3 vs. 41.4 mm). The length of the lower wing for embryos incubated vertically were not significantly longer than lower wing length of horizontally hatched embryos. The only exception was on 35 days of incubation when the vertically set embryo's lower wing (36.4 mm) was significantly longer (P < 0.05) than the lower wing (34.7 mm) of the horizontally set embryo. On opening, most embryos in the horizontally incubated eggs were positioned with their heads in the direction of the air cell. All the embryos in the vertically incubated eggs were orientated with their heads towards the top (air cell) part of the egg and the distribution for the 4 quadrants was about equally. The orientation of embryos relative to the 4 quadrants was about equally distributed.

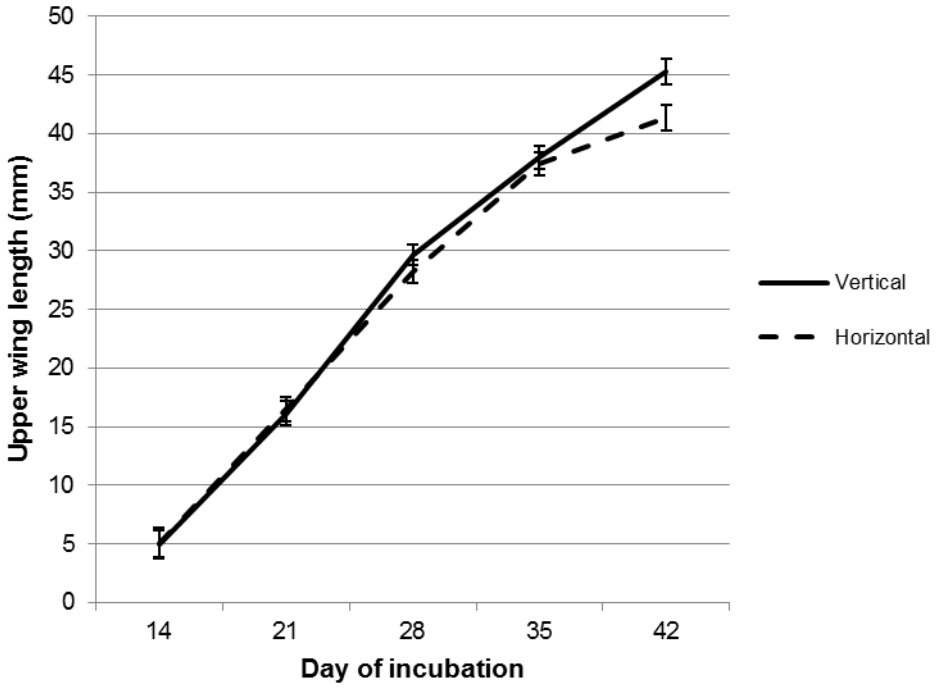

Figure 5 Effect of hatching position on upper wing length of ostrich eggs for eggs incubated 14 to 42 days.

4. Discussion

4.1. General embryonic development

The general appearance of the embryo at 7 days of incubation in our sturdy corresponds with the report by Gefen & Ar (2001). The appearance of the ostrich embryo on day 7 was also similar to stage HH20 (70 - 72 hours) in chickens (Hamburger & Hamilton, 1951) and stage 33 for ducks (Dupuy *et al.*, 2002), while the positioning of the ostrich embryo corresponded with reports on the position of chicken embryos (Buhr & Rowland, 1997). In the present study, as well as in previous studies by Ar & Gefen (1998) and Gefen & Ar (2001), the appearance of eye pigment began after 7 - 8 days of incubation for the ostrich embryos. As in the chicken egg, the amnion enfolds the embryo closely at the time of its formation, but soon after, fluid begins to accumulate within the amniotic cavity (Hamilton, 1952). The latter gradually enlarge so that the embryo lies within a considerable fluid-filled space, which increases gradually up to the latter part of the incubation and then diminishes again, so that the embryo finally occupies most of the cavity. The function of the yolk sac is to provide a large surface area, which is achieved by foldings of the wall projecting into the yolk (Hamilton, 1952). At the height of its development the inner surface of the yolk sac is covered with numerous folds or septa projecting into the yolk, which are highest at the equator and decrease in both directions away from the equator. In accordance with results presented by Ar & Gefen (1998) and Gefen & Ar (2001), the groove between the two toes was visible on day 14 of incubation, when the eye lids also started to cover the eyeballs. The development of the blood vessel network and the size of the amniotic sack on day 14 of incubation resemble the eighth day in the development of the chicken embryo (Hamilton, 1952).

4.2. Embryo size, weight and egg components

The mean embryo weight of 2.78 g and mean embryo length of 36.9 mm in the present study at 14 days of incubation corresponded with results from Gefen & Ar (2001). The mean weight of 21 g after 21 days of incubation for ostrich embryos was consistent with the results reported by Gefen & Ar (2001). Lower wing length of 16.2 mm also relates with results from Gefen & Ar (2001). Although observations on the development of the embryo up to 28 days of incubation match results from Gefen & Ar (2001), the mean for the beak and leg length in the present study was much higher. This discrepancy could be due to a different approach to taking the measurements. Hamilton (1952) observed that chicken embryos started to turn lengthwise between day 12 and day 16 of incubation. Both Buhr & Rowland (1997) and Gefen & Ar (2001) described the turning of ostrich embryos at day 28 of incubation, as well the appearance of toe nails and fine feathers. The latter results are consistent with results from the present study. The latter study (Gefen & Ar, 2001) also reported an average embryo weight of 145 g at 28 days of incubation, which is compatible with the 156 g of the present study at a similar stage of incubation. The increased yolk weight at 14 days of incubation could be due to the fact that upon removing the embryo from the egg for weighing, the amnion sac broke and the fluids were released into the egg. This was then weighed together with the yolk. Initially, albumen comprised approximately 51% of the total egg weight, which is within the range of 44 - 76% of initial egg weight for precocial birds in general (Tullet, 1984). The rapid reduction in albumen weight at 14 days of incubation are in line with findings by Hamilton (1952) in the chicken, who reported that during the first days of incubation the albumen losses water rapidly and becomes more viscious, settling as this takes place, towards the yolk-sac umbilicus.

Gefen & Ar (2001) reported a mean embryo weight of 359 g (n = 3) at 34 days of incubation. The corresponding mean at 36 days was 439 g (n = 2). Our mean of 399 g after 35 days of incubation was intermediate, thus in agreement with those previous results. In contrast with embryo weight at earlier stages, the mean embryo weight of 910 g after 42 days of incubation in our study was substantially higher than the weight of 680 g at 40 days of incubation reported by Gefen & Ar (2001). It is conceded that embryo weight in our study is based on a wet embryo, since several sources give the average day-old weight of Oudtshoorn ostrich chicks at 855 - 862 g (Bunter & Cloete, 2004; Cloete *et al*., 2004; 2005). The description and the position of the embryo at both 35 and 42 days of incubation, as well as the pipping sequence, correspond with reports of Deeming (1995). While chicks of the domestic fowl rotates 270° within the egg during hatching, ostrich chicks and rhea chicks rotates less than 90° (Deeming, 1997). Unlike the chicken, the ostrich chick does not have an egg tooth to make a hole in the shell and it primarily uses its legs to break open the shell. This was enabled by a combination of kicking, pipping and body movements. These actions were also observed by Deeming (1994). The slight reduction observed in shell weight at 42 days of incubation can be attributed to the fact that some chicks pipped before day 42 and some of the smaller bits of shell and membranes could not be retrieved to weigh.

4.3. Setting and incubation position

No literature regarding the effect of incubation position on embryonic development or orientation in egg could be found for ostriches. However, Van Schalkwyk *et al*. (2000) found that the hatchability of fertile eggs were

relatively low, but unaffected by setting in either the vertical or horizontal position for six weeks. This result is consistent with the present finding. The hatchability of eggs incubated horizontally accordingly did not differ from that of eggs incubated vertically in poultry (Van de Ven *et al*., 2011). However, Takeshita & McDaniel (1982) reported that early embryonic development of poultry embryos was improved in those eggs that were incubated horizontally. These results are not consistent with the present findings, but indicate scope for a more detailed study in future.

5. Conclusions

The stages of development of the ostrich embryo in the present study were closely related to similar results reported by Gefen & Ar (2001), even though the latter authors used a slightly higher incubation temperature than in our study (36.5 ºC vs. 36.2 ºC). These results thus appear to be quite robust for ostrich eggs in general. Information stemming from observations regarding age of dead-in-shell embryos can be used to identify the stage of incubation during which problems resulting in a low hatchability occurred.

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CHAPTER 9

Changes in the air cell volume of artificially incubated ostrich eggs

Abstract

A total of 2 160 images of candled, incubated ostrich eggs were digitized to determine the percentage of the egg volume occupied by the air cell at different stages of incubation. The air cell occupied 2.46% of the volume of fresh eggs. For eggs that hatched successfully, this increased to an average of 9.31% at 15 days of incubation, 11.2% at 22 days of incubation, 13.8% at 29 days of incubation, 15.18% at 36 days of incubation and 24.4% at 41 days of incubation, just prior to hatching. Air cell volume at 29 day of incubation for infertile eggs (19.3%) was significantly ($P < 0.05$) higher when compared to dead-in-shell eggs (14.3%) and eggs that hatched (13.8%). Air cell volume was largely independent of strain (SAB or ZB) and whether chicks were assisted to hatch or not. At 41 days of incubation there was a significantly greater ($P < 0.05$) air cell volume in eggs that hatched normally compared to dead-in-shell (DIS) eggs (28.3% vs. 21.7%, respectively). No significant differences in air cell volume were observed up to day 20 of incubation between eggs that exhibited high, average or low rates of water loss. However, for the dead-in-shell eggs, air cell volume was consistently higher in eggs that exhibited high rates of water loss. Although some subtle differences were detected between hatched and DIS chicks during this study, it is unlikely to find application in the broader industry.

1. Introduction

Ostrich farming is a major agricultural enterprise in South Africa. The usual commercial practice is to keep breeding birds in large groups or as pairs in small enclosures. To maximize production, eggs laid are collected and incubated artificially. With rates of only 50 - 60% (Brown *et al.*, 1996; Deeming & Ar, 1999; Van Schalkwyk, 2000), the hatching success of artificially incubated ostrich eggs is low compared to those of commercially reared chickens (90 - 95%), turkeys (75 - 77%) and ducks (65 - 82%) (Hodgetts,1990; Deeming, 1999). This low hatching success represents a considerable loss of production and is cause for concern in the industry. A poor understanding of the pattern of embryonic development in ostriches may contribute to the poor hatching results. An important tool for identifying incubation problems that cause low hatchability is knowledge of the age and degree of development of the embryo at the time of death (Ar & Gefen, 1998). Egg candling is commonly used on commercial ostrich farms during artificial incubation to determine fertility and monitor progress of the developing embryo, but in most cases the latter is not very effective. Badley (1998) found detection of infertile eggs by candling to be reasonably reliable on day 14, but it became increasingly difficult to distinguish between live and dead embryos as incubation progressed. There are few studies reporting observations of developing ostrich eggs using candling other than to report an increase in dark shadows (Deeming, 1995). One feature, however, that is usually easily distinguishable is the air cell at the blunt end of the egg. The air cell is initially formed between the two shell membranes as the egg cools after hatching. It subsequently increases in size during incubation as water is lost from the egg. A measure of the air cell volume on specific days of incubation may provide farmers with a simple approach to determine whether development is proceeding, the age of the developing embryo or the age of embryonic mortality. In this study, we report on aspects of water loss of artificially incubated ostrich eggs, by assessing changes in air cell volume and factors influencing it during the incubation period.

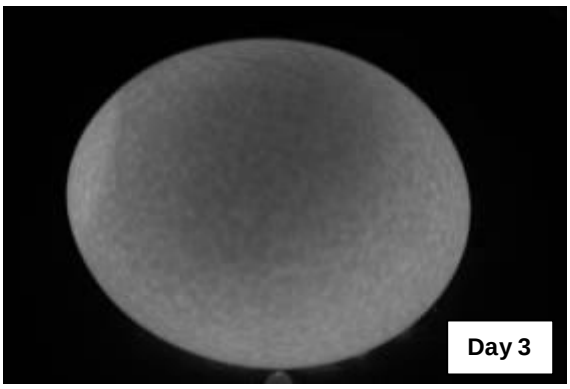
2. Material and Methods

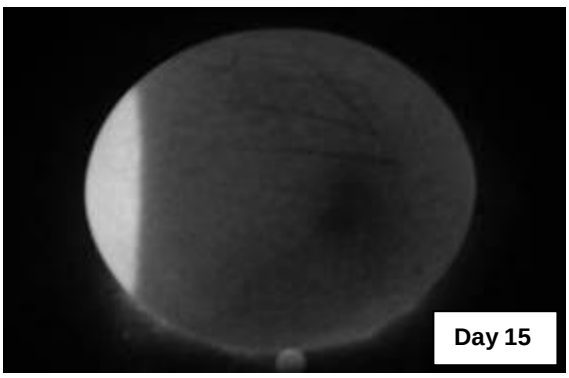
Data obtained for this study were derived during the 2007 breeding season from eggs that originated from the commercial ostrich flock at the Oudtshoorn Research Farm in the Klein Karoo region of South Africa. The origin of birds, their management, and incubation practices for the eggs have been described previously (Van Schalkwyk *et al*., 1996; Van Schalkwyk, 1998; Bunter & Cloete, 2004). The eggs used were weighed and identified by date and paddock (female) of origin. Methods for collection, sanitation and storage at the experimental site are well documented (Van Schalkwyk, 1998; Van Schalkwyk *et al*., 1999; Brand *et al*., 2007). Data involved various combinations of the two purebred bloodlines, South African Black (SAB) and Zimbabwean Blues (ZB), as well as ZB male x SAB female crosses.

It is common practice in the South African ostrich industry to candle eggs mostly on day 21 of incubation to establish whether they are fertile, whereas the transition from the incubator to the hatcher usually takes place on the 35th day. One hundred and twenty fresh eggs (chosen at random to represent eggs at that stage) were weighed and candled with a 100 Watt candling light directly under the egg to visualize detail of the internal egg. This allowed as clear a view of the interior of the egg as was possible through the eggshell. The eggs were then photographed using a Pentax P30T 35 mm single lens reflex camera equipped with a 35 - 80 mm zoom lens mounted on a tripod. Images were captured using a shutter speed of one second at

an aperture of f4 on ISO 100 film. These eggs were set and incubated in an electronic Buckeye® incubator at a temperature of 36 °C and relative humidity of 24% to investigate the changes in air cell volume and the development of the embryo. The eggs were candled and photographed every 2 - 3 days throughout the 42 day incubation period, to monitor air cell size. A total of 2 160 images were digitized from the photographs and assessed using the software package AnalySIS® (Soft Imaging System, 1999). The percentage of the egg volume occupied by the air cell was determined. The volume occupied by the air cell (measured in pixels as determined with AnalySIS®) was expressed as a percentage of the volume occupied by the entire egg (also expressed in pixels). Examples of images acquired in this way for embryos of 3 to 42 days of age, are depicted in Figure 1. In practice, infertile eggs would be removed during candling at 21 days of incubation but we left all eggs in the incubators for the entire 42-day incubation period to investigate the dynamics of changes in the air cell volume. Eggs were weighed on day 21 and on day 35 of incubation to determine weight loss of the eggs at that stage. This was done by subtracting the egg weight at 21 and 35 days of incubation respectively from the initial fresh egg weight. On day 44 of incubation, eggs that did not hatch were candled to see if any movement could be detected, thus indicating whether internal pipping had or had not occurred. These eggs were manually opened at the air cell region, and the position of the embryo and point of internal pipping (if any) noted. The rest of the eggs that did not hatch were opened and the causes of mortality were recorded. This includes infertile eggs, as well as eggs with early and late embryo mortalities.

The data were subjected to analysis, using ASREML software (Gilmour *et al.*, 1999). The software is suitable for fitting a wide range of fixed and random effects, while least-squares means for selected systematic effects are predicted simultaneously. Fixed effects were tested for significance, using an F-test in the analysis of variance table. Fixed effects that were considered included the designation of the egg (hatched, infertile or dead-in-shell), as well as bloodline (SAB, ZB or ZB male x SAB female crosses). These effects were interacted with the appropriate number of days incubated. Traits that were considered were percentage water loss up to 21 days of incubation (WL21), percentage water loss up to 35 days of incubation (WL35), percentage of egg occupied by the air cell. The random effect of egg was included in the analysis to account for the variation accounted for by the repeated sampling of specific eggs.

A 

B 

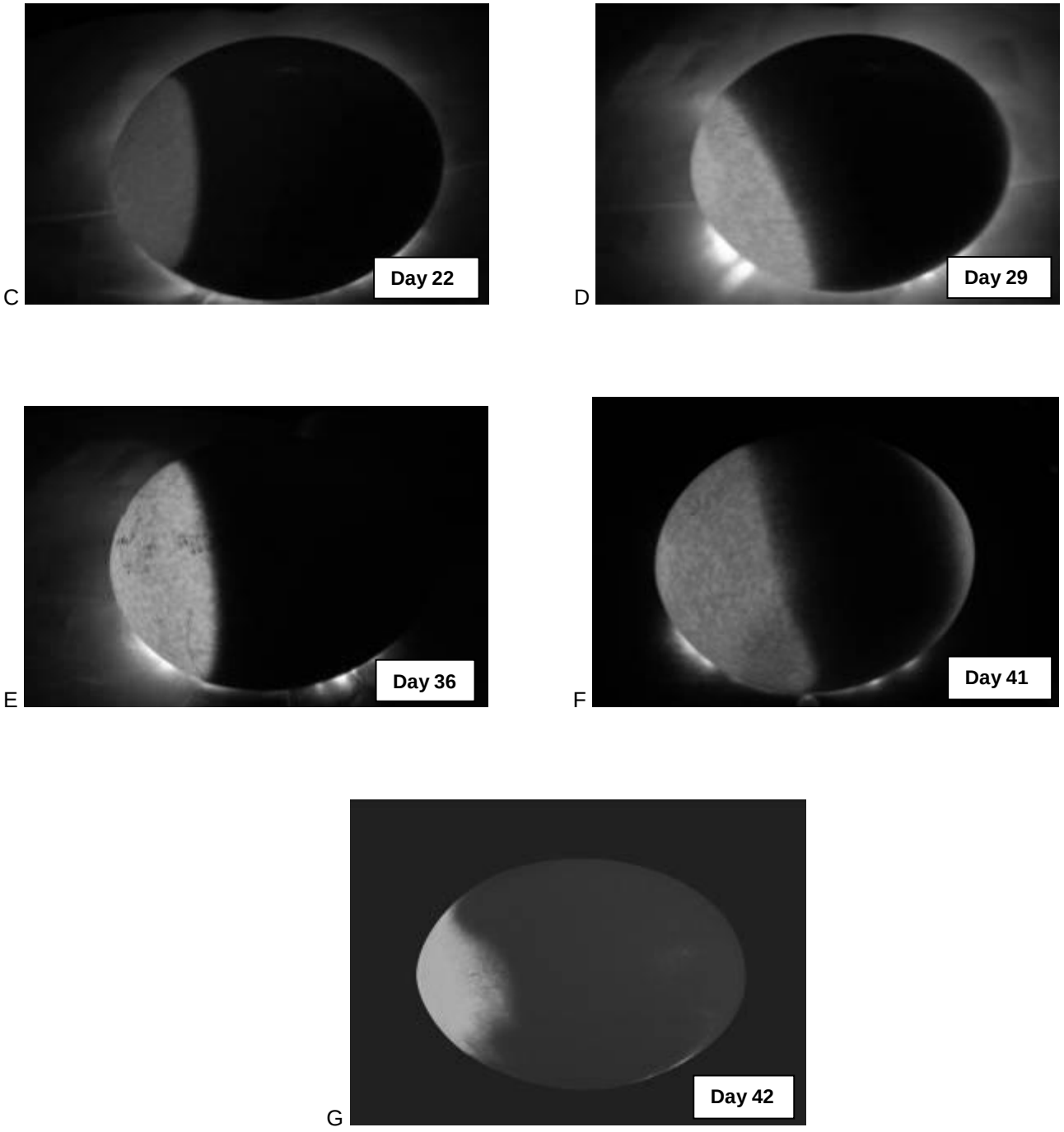


Figure 1 Images of the ostrich egg used to derive the percentage of the candled eggs occupied by the air cell from 3 days - 42 days of incubation (A - G).

3. Results and Discussion

The average weight of the 120 eggs used in this study was 1 389 ± 112 g with a coefficient of variation (CV) of 8.11% (Table 1). This mean was slightly lower than previous weights reported by Cloete *et al.* (2004), Bunter & Cloete (2004) and Brand *et al.* (2008a; b) for eggs at the same site. Water loss ranged from 4.6 - 13% of fresh egg weight at 21 days of incubation and 7 - 21% at 35 days of incubation, with higher CV's of 23.9 and 24.1%, respectively, similar to previous results reported by Brand *et al.* (2008a; b) for eggs

collected at the same site. The percentage of the egg occupied by the air cell varied considerably between eggs candled throughout incubation. Air cell volume averaged 11% for eggs incubated for 21 days and 19% for eggs incubated for 35 days respectively, with high corresponding CV's of 31.5 and 31.9%, respectively. No comparable estimates could be found in the literature.

Table 1 Traits recorded from eggs of breeding ostrich females for the 2007 production year.

Traits	Mean ± SD	CV (%)	Min	Max
EWT (g)	1389 ± 112	8.11	1121	1765
WL21 (% fresh egg weight)	7.66 ± 1.83	23.9	4.57	12.6
AC 21(%)	10.9 ± 3.43	31.5	4.45	20.0
WL35 (% fresh egg weight)	12.4 ± 2.99	24.1	7.37	20.2
AC 35(%)	18.5 ± 5.90	31.9	9.21	39.7

SD = standard deviation, CV% = coefficient of variation, EWT = egg weight at time of lay, WL21 = water loss at day 21of incubation, AC 21% = Air cell as % of egg volume at 21 days of incubation, WL35 = water loss at day 35 of incubation, AC 35% = Air cell as % of egg volume at 35 days of incubation

The prercentage of the egg occupied by the air cell increased through incubation (Figure 2). In fertile eggs, the regressions of the percentage of the egg occupied by the air cell on water loss to 21 days of age of incubation (b ± SE = 0.292 ± 0.045 %, r = 0.54; n = 96) and to 35 days of incubation (b ± SE = 0.368 ± 0.068 %, r = 0.49; n = 96) were highly significant (P< 0.01). The average percentage of the egg occupied by the air cell for fertile eggs that hatched successfully was 2.5% for fresh eggs, 9.3% at 15 days, 11.2% at 22 days, 13.8% at 29 days, 15.18% at 36 days and 24.4% at 41 days of incubation. By day 28 of incubation, the dark, shadowed area of the developing embryo covered almost the entire eggshell except for the air cell region in fertile eggs. From day 29 of incubation onwards, air cell volume for infertile eggs (19.3%) was significantly higher (P < 0.05) if compared to dead-in-shell eggs (14.3%) and eggs that hatched successfully (13.8%). Similar to findings by Deeming *et al.* (1993), there appeared to be a plateau in the expansion of the air cell volume from day 17 to day 27 of incubation. The occurrence of this plateau is curious for the embryo increase substantially in both on weight and length during this incubation period (Deeming & Ar, 1999). A rapid increase in air cell size towards the end of incubation was similarly observed by Jarvis *et al.* (1985) and Deeming (1995) when manually measuring the air cell. This increase in air cell volume from day 38 of incubation could be attributed to an increase in embryonic heat production, which in turn leaded to an increase in egg temperature, contributing to increased water loss. The steepest increase in O_2 consumption of ostrich eggs occurs between 26 and 31 days of incubation, as reported by Van Schalkwyk *et al.* (2002), together with a similar increase in embryonic metabolism at day 25 of incubation (Reiner & Dzapo, 1994) could attribute for this increase in air cell volume towards the end of incubation. The first indication of the commencement of hatching took place at about 42 days of incubation with the intrusion into one side of the air cell of a shadow as the chick pipped internally (Figure 1g). Movement of the chick could be observed throughout internal pipping up to external pipping when the chick broke through the eggshell.

Air cell volume was generally independent of genotype, with eggs with the SAB, ZB and ZB x SAB genotypes generally adhering to the same basic pattern. Occasional significant ($P < 0.05$) differences between genotypes could be ascribed to sampling, as a small number of eggs were included for the ZB strain in particular. Air cell volume of those eggs hatching naturally and those with chicks that were assisted to hatch were accordingly similar, without any significant ($P < 0.05$) differences throughout the period of incubation.

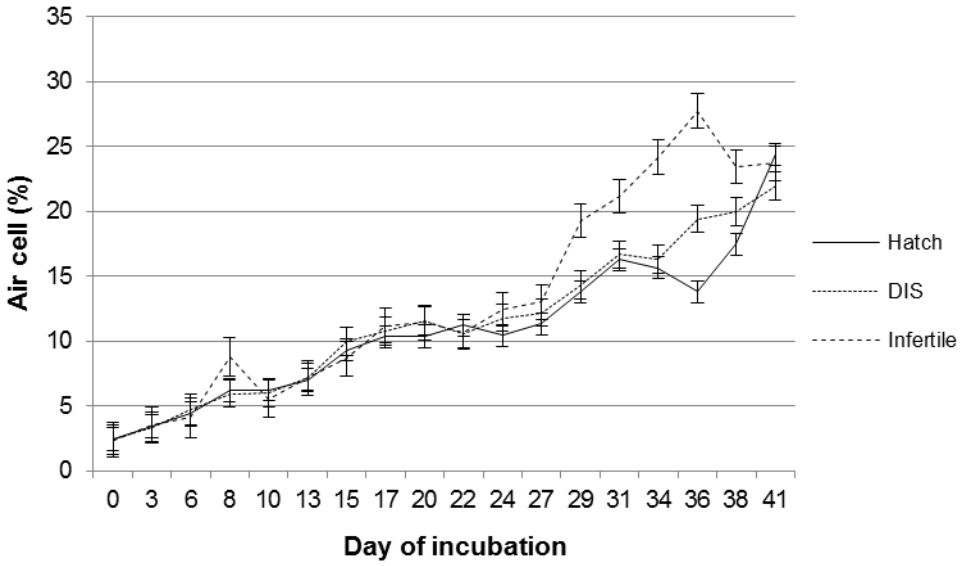


Figure 2 The percentage of the egg volume (± S.E.) occupied by the air cell in eggs that hatched (Hatch), infertile (Infertile) and dead-in-shell (DIS) ostrich eggs during the 41 day incubation period.

Air cell volume of dead-in-shell eggs with a low WL (4.37 - 14.8%) followed the same trend as that of hatched eggs (3.36 - 16.3%) from the start of the incubation proses up to 38 days of incubation (Figure 3a). At 41 days of incubation a sharp significant ($P < 0.05$) increase in air cell volume occurred for eggs that hatched normally (21.7% and 28.3%, respectively, for DIS and hatched eggs). This increase in air cell volume could be attributed to an increase in water loss following internal pipping and the resultant tearing of the shell membranes. No differences in air cell volume were found between DIS and hatched eggs for the medium WL (2.49 - 22.3% and 2.6 - 23.0% respectively) group for the entire the incubation period, the only exception being for 38 days of incubation (Figure 3b). Air cell volume for the high WL group (1.41 - 22.6%) was generally higher throughout for the dead-in-shell eggs, significantly so ($P < 0.05$) on days 15, 24 and 38 of incubation. At 41 days of incubation, means switched around, with the air cell volume of the hatched eggs increasing relative to their DIS contemporaries (22.6% and 26.9%; Figure 3c).

As previously mentioned, increased WL happened as soon as internal pipping started and in some instances internal pipping also occurred for dead-in-shell chicks. There were no significant differences between the volume of air cell in eggs that hatched from eggs with high, average and low levels of WL up to day 20 of incubation. From day 20 of incubation there are a trend in the high WL group to also have a bigger air cell volume, while from 21 days of incubation the air cell volume for the eggs with low WL was significantly

smaller if compared to the average and high WL eggs. Air cell volume for the low WL group were 14 - 16% for days 34 and 36 of incubation respectively and did not differ significantly from the average WL group (15.5 - 17%). These results were consistent with findings published by Swart & Rahn (1988), Blood *et al*. (1998) and Brand *et al.* (2008a, b) of a water loss of around 13% in ostrich eggs up to 35 days of incubation. At 16.8 - 22% for days 34 and 36 of incubation respectively, air cell volumes for the high WL group were significantly higher ($P < 0.05$) if compared to the lower and medium WL groups. From day 38 of incubation the air cell volume of the low WL group that hatched increased rapidly and, together with the high WL group, were significantly higher at 41 days of incubation than the average WL group. Average WL for the DIS group was around 2% lower and around 3% higher respectively for the low and high water loss groups if compared to eggs that hatched. Deeming (1995) was not able to establish a significant difference in air cell volume between hatched and dead-in-shell eggs. The air cell volume of the low WL dead-in-shell group accelerated at day 39 of incubation and no significant difference was found between the air cell volume of the different levels of WL at 41 days of incubation. When candled at 43 to 44 days of incubation, air cell volume for DIS eggs remained round in appearance, with no movement detected.

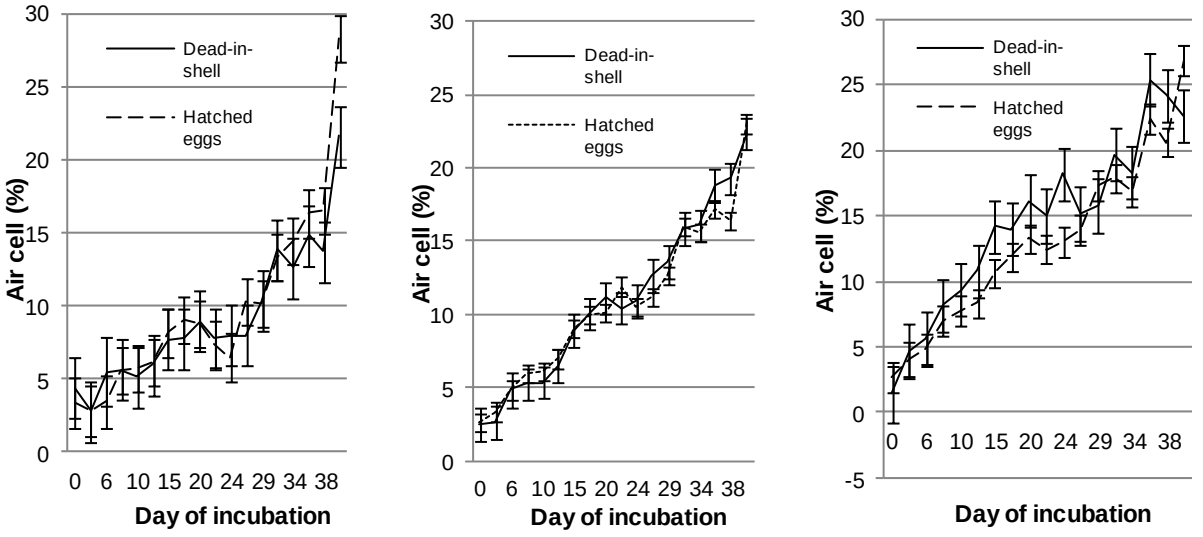


Figure 3a, b, c The percentage of the egg volume occupied by the air cell in dead-in-shell and hatched ostrich eggs during the 41 day incubation period for eggs with low (a), medium (b) and high (c) water loss.

4. Conclusions

Egg candling remains a useful tool to assess egg development, and to identify infertile eggs, as well as eggs sustaining early embryonic mortalities. In this study, it was attempted to expand this utility to also assist with determining embryonic mortality at later stages. However, while changes in the air cell volume conformed to expectations, differences between hatched eggs and those with dead-in-shell chicks were not marked. Even when significant, such differences were unlikely to be observed outside a rigorous experiment, such as this.

Fairly large levels of variation within classes involving hatched, infertile and dead-in-shell chicks also makes it difficult to draw up robust guidelines that would be of value at the level of the individual hatchery, where similar records are highly unlikely to be noted. It can thus be concluded that, although this study was able to detect subtle differences between hatched and DIS chicks, it is unlikely to find application in the broader industry.

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PART III

CHAPTER 10

Genetic and environmental effects on the artificial incubation of ostrich eggs, statistical analyses and aims of the study

1. Introduction

Ostriches farmed in South Africa are considered as domesticated livestock, represented predominantly by the *Struthio camelus domesticus* strain bred originally in the Oudtshoorn locality and selected for feather quality (Smit, 1963). However, if compared to other livestock species, the time period over which domestication occurred was relative short (around 150 years). Emphasis has moved from feather production to leather and meat production in recent years. The observation that ostriches should be considered as a relatively unadapted to and unimproved in the farming environment is supported by generally poor production statistics of ostriches relative to other domestic birds used for egg and meat production (Bunter, 2002).

Reproduction is considered the cornerstone of production in virtually all farmed livestock species. Genetic make-up is one of the factors influencing the performance of individuals, while genetic improvement can be achieved by selection for certain traits (Van Schalkwyk *et al*., 1996; Petitte & Davis, 1999). Meat and skins are important sources of income for the ostrich producers and contribute to around 90% of the total income from a slaughter bird (Cloete *et al*., 1998). High egg production, together with the production of good quality chicks is thus vital for profitability. Heritability estimates are of value because they are estimates of the proportion of phenotypic variance that is controlled by genes, and thus serves as an indication of the rate of improvement that is expected to stem from selection (Kinney, 1969). On the other hand, genetic correlations are based on the covariance and the respective variances of two traits and are subject to the same sources of bias with regard to variance component estimation as heritability estimates (Kinney, 1969). They serve as an indication of what the effects of selection for a specific trait would be upon other traits of biological or economic importance. Extensive research has been carried out on selective breeding to improve production traits in species of common domestic livestock over the past few decades. Genetic and crossbreeding parameters, as well as line and breed differences for these mainstream livestock species are thus readily available. Access to this information ensures structured breeding programmes where optimal levels of additive genetic gains are achieved, while adequate information involving line- and cross-breeding and exploiting sexual dimorphism and heterosis are available for commercial success.

When the success of improving egg production in chickens (Gowe & Fairfull, 1985) and turkeys (Nestor *et al*., 1969) by means of structured breeding is considered, it is clear that this aspect should receive serious attention in ostriches. Unfortunately little is known about either genetic parameters or responses to selection for specific traits in ostriches and little has been achieved as far as genetic progress is concerned in the broader industry (Van Schalkwyk *et al*., 1996; Cloete *et al*., 2004). By selecting for certain desirable traits, genetic improvement can be achieved in breeding stock (Petitte & Davis, 1999). Genetic selection and highly developed management practices have greatly improved the efficiency of meat and egg production in modern broilers (Boerjan, 2004).

2. Breeding parameters

Kinney (1969) stated that heritability estimates are needed to make decisions regarding the type of mating system that will allow the most rapid improvements in chickens. Definite breeding objectives and industry breeding structures are largely absent in ostriches (Van Schalkwyk, 1998; Cloete *et al.*, 2002; 2008b), as past selection was largely based on feather characteristics. Moreover, typical ostrich production systems, such as flock-mating, communal nesting systems and a very narrow male to female ratio, also present challenges for implementing selective breeding programmes aimed at improving some specific traits (Cloete *et al.*, 1998; Bunter, 2002). Ostrich females also have a markedly longer productive life compared to other poultry species, making the average layer age a major constraint in achieving genetic progress (Smith *et al*., 1995; Ipek & Sahan, 2004).

The repeatability of some of the production parameters in ostriches as reported by Smith *et al*. (1995) are presented in Table 1.

Table 1 The repeatability (%) for some production parameters in ostriches (Smith *et al*., 1995).

Trait	Repeatability
Egg production (per year)	20
Egg weight	74
% Infertile eggs	27
% Embryonic mortalities	10
% Eggs that hatch	14

Van Schalkwyk *et al*. (1996) and Cloete *et al*. (1998) were the first to report estimates for repeatability of, and phenotypic correlations among, reproductive traits in the ostrich. Contradictory to poultry, that achieves production levels much closer to their potential, great variation exists both in egg production and egg size of ostriches, ranging from 0 - 121 eggs produced during a breeding season and from 825 g to 1 827 g for egg size (Cloete *et al*., 2004). Subsequent estimates of genetic parameters for egg and chick production, as well as other reproductive traits are still limited to only a few studies (Bunter *et al*., 1999; 2001; Bunter & Cloete, 2004; Cloete *et al*., 2004; 2005; Lambrechts, 2004), all modelled as traits of the female. According to Turner & Young (1969) heritability (h^2) estimates are considered as low for a value < 10%, medium estimates range between 10% and 20%, while estimates > 20% are regarded as high. The h^2 for early and mature egg weight for poultry ranges from 0.34 and 0.89 respectively (Kinney, 1969), while h^2 estimates for fertility in poultry ranged between 0.01 and 0.14. Estimates for hatchability ranged between 0.02 and 0.10, while embryonic mortalities had an h^2 estimate of 0.01 (Kinney, 1969).

Estimates of heritability for quantitative reproductive traits of ostriches in earlier analysis were low (h^2 = 0.10), but moderately repeatable. With the expansion of the data set, Cloete *et al*. (2005; 2006) reported higher h^2 estimates of between 0.2 – 0.3. The increase in the number of males and females, as well as a deeper pedigree (pedigreed animals represented in more back generations) facilitated a repartitioning of repeatable between-female variances in h^2 and animal permanent environmental (pe^2) effects (Cloete *et al*., 2008b).

Repeatable animal effects, representing the correlation between reproduction records of females across years, can be partitioned in h^2 and pe^2 effects by the usage of pedigree information. The heritable portion of the variation is transferred across generations, while the pe^2 effect is only present in the current flock (i.e. animals of the current generation recorded for traits across years). Recent analysis showed that both h^2 was fairly high (Table 2) which creates the opportunity for selection for improved reproduction traits in future generations. The qualitative reproduction traits (i.e. average egg weight and average chick weight) had much higher h^2 estimates (0.43 - 0.74), while pe^2 accounted for between 21 – 32% of the overall phenotypic variance (Bunter *et al*., 2001; Cloete *et al*., 2004; 2005).

Table 2 Heritability estimates (h^2) and female permanent environmental variance ratios (pe^2) for some reproductive of ostrich females (reviewed by Cloete *et al*., 2008b).

Trait	**Variance ratios**	
	h^2 range	**pe^2 range**
Egg production	0.12 - 0.29	0.16 – 0.32
Hatchability	0.05 - 0.10	0.21 – 0.24
Chick production	0.11 - 0.26	0.18 – 0.33
Average egg weight	0.43 - 0.72	0.25 – 0.32
Average chick weight	0.51 - 0.74	0.21 – 0.25

From Table 2 it is clear that estimates of between female variances, as partitioned into genetic and female permanent environmental variances, are available for quantitative and qualitative reproduction traits of ostrich females. However, information as pertaining to those traits accociated with artificial incubation is scant, thus motivating the need for studies of the nature of the present thesis.

3. Statistical methods

Analyses of normally distributed data: The statistical procedures employed in this section are typical of those used during routine genetic analysis that rely heavily on the concept of mixed model methodology, as introduced by Henderson in 1949 (Mrode, 2005). At the time of the theoretical derivation of mixed model methodology, the available computing power did not allow the full exploitation of the power of the concept. Genetic evaluation therefore evolved from paternal half-sib analyses (Harvey, 1982a) to the sire-model and eventually to the animal model (Mrode, 2005). The methods basically involved the partitioning of fixed environmental or systematic effects (i.e. herd-year-season, sex, age of dam) from random effects involving animals sampled from the population, using matrix-algebra methods. Relationships between animals, as determined from common ancestry or pedigree information, facilitated this process. The paternal half-sib analysis made use of relationships between animals with a common sire. Expansion to the sire-model involved obtaining additional information from the maternal side by also including on the maternal grandsire in the mixed model equations (MME). As computing power increased it was logical to expand these methods to the animal model, involving all relationships between all animals present in the pedigree. The

linear mixed model can estimate fixed effects and random effects simultaneously and can be presented as follows:

$$y_{ij} = \mu + c_i + a_j + e_{ij}$$

Where -

y_{ij} = vector of observations for the trait under consideration

μ = overall mean of the trait in the population under consideration

c_i = fixed effects, as defined previously

a_j = random effects based on relationships between animals in the animal model

e_{ij} = random residual error term

This model can be rewritten in matrix notation (Mrode, 2005) as:

$$y = Xb + Za + e$$

Where -

y = n × 1vector of observations; n = number of records

b = p × 1vector of fixed effects; p = number of levels for fixed effects

a = q × 1 vector of random animal effects; q = number of levels for random effects

e = n × 1vector of random residual effects

X = design matrix of order n × p, which relates records to fixed effects

Z = design matrix of order n × q, which relates records to random animal effects

The MME to estimate fixed and random effects thus look like this (Mrode, 2005):

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + A^{-1} \end{bmatrix} \begin{bmatrix} c \\ a \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

With -

$X'X$ = incidence matrix relating the fixed effects to the data

$Z'Z$ = incidence matrix relating all animals to data records

$X'Z$ and $Z'X$ = incidence matrices relating elements in $X'X$ to $Z'Z$

A^{-1} = Wright's numerator relationship matrix

Advances in computer hardware and software resulted in the rapid expansion of the basic model to include more random terms than only the direct genetic effect, such as maternal genetic and maternal permanent environmental effects (Lewis & Beatson, 1999). Maternal variation refers to the relationship between records from dams that accrue across production years. In ostriches, egg and chick weights are typically maternally-influenced traits (Bunter & Cloete, 2004). It is possible to partition the variation among dams into maternal genetic and permanent environmental components, where the former is transmitted across generations and the latter not. In this case, efficient pertitioning depends on the presence of dams, grand dams and great grand dams with their own records for the specific trait in the pedigree, and not only records from their progeny.

Many efficient algorithms for the solution of the magnitude of equations in the mixed-model analysis were developed over the past two decades. One such application is average information Residual Maximun Likelihood (REML), which allows the efficient solving of many MME in animal breeding (Gilmour *et al.*, 1995). This methodology is embodied in the ASREML program that was used for the estimation of fixed effects, and subsequently to derive variance components in this manuscript (Gilmour *et al.*, 1999). The average information algorithm employed by ASREML concurrently provides estimates of standard errors for parameters derived from the random effects in the MME. Analyses initially involved only one trait at a time (i.e. single-trait analyses). Single-trait analyses were followed by series of two-trait analyses making it possible to estimate direct genetic, maternal genetic, maternal permanent environmental and environmental correlations between traits. This approach is consistent with standard procedures for the evaluation of all species of farmed livestock.

Analyses on binomially distributed embryo survival data: In the past general linear model theory (as described under the previous heading) was the most frequently used for the analysis of both continuous and discontinuous animal production data (Henderson, 1973). This approach was also followed in Chapter 16 of this thesis. However, this method does not take the binomial character of survival as well as the nature of threshold traits into consideration. It is generally accepted that threshold traits are affected by an underlying normal distribution. However, the trait is only expressed on the observed scale when a specific threshold is reached. Analyses involving binomial threshold traits are affected by the incidence of a specific outcome (i.e. the survival of a specific embryo). In general, linear models serve as a good approximation, particularly at incidences of 0.3 - 0.7 (Harvey, 1982b). Larger discrepancies may occur at very high or very low incidences.

Bayesian inference, using Gibbs sampling (a numerical integration technique) was introduced to describe the uncertainty about the true value of some parameters involving binomial threshold traits, using probabilities as a measurement of this uncertainty (Blasco, 2001). The Bayesian way of approaching an estimation problem is always the same: to derive the posterior distribution of the parameters to be estimated, given the data. If the parameter of interest is (for example) heritability, the aim of Bayesian inference is to find a probability density of the heritability given the information that is available in the data. The density function is called a posterior distribution, and could be equated as follows:

$$f(h^2/y)$$

Where -

- f = the density function
- y = vector of observations
- h^2 = heritability

Posterior distributions for direct and maternal genetic components of a threshold trait (lamb survival) and a continuous trait (birth weight) are given in Figure 1a and Figure 1b respectively, as adapted from Cloete *et al*. (2009). The skew distribution of the threshold trait relative to the closer to normal distribution of birth weight is evident.

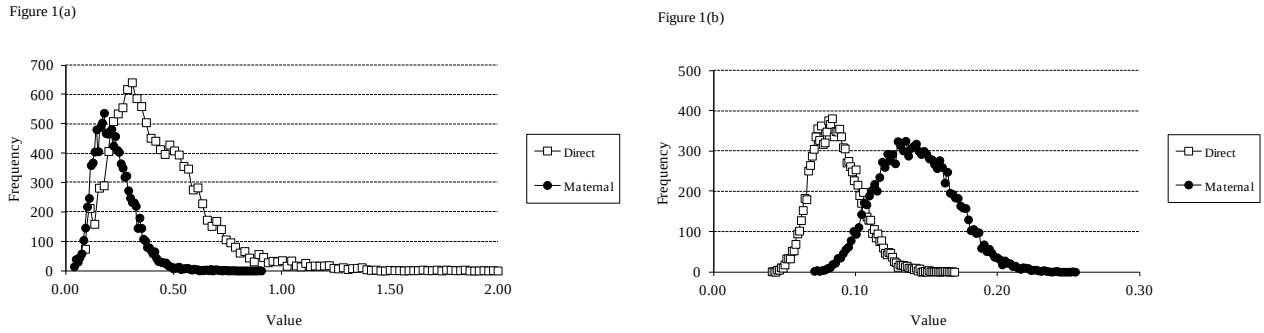

Figure 1 The posterior distribution of direct and maternal variance components of lamb survival, as a threshold trait (a) and birth weight, as a normally distributed trait (b). The figures were adapted from Cloete *et al*. (2009).

At present, there is software available for the routine analysis of threshold traits in animals breeding, using Monte Carlo Markov Chain algorithms in Gibbs Sampling, using (for instance) THRGIBBSF90 software (Misztal, 2008). Post Gibbs analyses are also available by using the POSTGIBBSF90 program for the determination of highest posterior density intervals, point estimates for genetic parameters and posterior distributions. Threshold traits depend on fixed effects and random variance parameters (to derive variance ratios and breeding values), and these effects depend on the variance components to be estimated (Blasco, 2001).

Estimation of variance components and ratios: Variance components derived from genetic analyses are used for the computation of variance ratios of interest, irrespective of the approach used to derive such components (mixed linear models or Bayesian inference) in Chapters 12, 13 and 15 - 17. The first and foremost parameter mostly to be estimated in animal breeding is the heritability (h^2), which is defined as the proportion of phenotypic variation in a population that is due to genetic variation between individuals (as derived from pedigree information through the numerator relationship matrix). Phenotypic variation among individuals may be due to genetic, environmental factors, and/or random chance (Turner & Young, 1969). Heritability expresses the fraction of the overall phenotypic variation (σ^2_p) that can be attributed to direct genetic variation or additive genetic effects (σ^2_a), as follows:

$$h^2 = \sigma^2_a / \sigma^2_p$$

The additional random effects of maternal origin can accordingly be derived as:

$$m^2 = \sigma^2_m / \sigma^2_p$$

for the maternal genetic effects, and as:

$$c^2 = \sigma^2_c / \sigma^2_p$$

for dam permanent environmental effects.

The phenotypic variation depends on the residual (σ^2_e) variation, as well as the other souces of variation that were included in the model of analysis. For a model that includes direct direct genetic effects, maternal genetic effects as well as maternal permanent environmental effects, the phenotypic variation can thus be described as:

$$\sigma^2_p = \sigma^2_a + \sigma^2_m + \sigma^2_c + \sigma^2_e$$

Animal solution depicting estimated breeding values can be derived from linear models, using normally distributed data, as well as from threshold models involving binomially distributed data. These estimated breeding values can be used for the selection of superior animals as the parents of the next generation, or for the construction of genetic trends, as was done in Chapter 16.

4. Embryonic mortalities

Little information is available on specific underlying traits that contribute to reproduction success of females, however, embryonic mortalities was conclusively related to differences in evaporative water loss of egg during incubation (Blood *et al*., 1998). Optimal water loss (WL) both in natural nests and in artificial incubation for optimal results is around 13 to 15% of the initial egg weight (Swart *et al.*, 1987; Deeming, 1995; Ar, 1996; Blood *et al.*, 1998). Higher rates of embryonic mortalities were found in eggs showing very little or excessive water loss to 35 days of incubation and water loss is repeatable for females (Blood *et al*., 1998). This provided evidence of the importance of eggshell quality traits in determining reproductive outcomes. Although there is some variation in the percentage WL at which ostrich eggs will still successfully hatch, there is, however, a sharp increase in embryonic mortality below 10% and above and 18% WL at 35 days of incubation (Blood *et al*., 1998). Excessive WL during incubation causes early depletion of allantoic fluids (Davis *et al*., 1988), while insufficient WL from the egg, can result in water retention by the chick, potentially causing embryonic mortality through respiratory insufficiency (Musara *et al.*, 1999).

In artificially incubated eggs, there are then two main factors that affect WL from the eggs; relative humidity (RH) in the incubator and the porosity of the eggshell itself to conduct water vapour. While incubator humidity can usually be controlled, the large variation in WL (and subsequent hatching success) in ostrich eggs under controlled incubator conditions suggests a wide variation in shell porosity, possibly indicating a potential genetic variation for this trait among ostrich females. Although no information is available on genetic parameters for ostrich incubation traits, within-season evaporative water loss has also been shown to be repeatable in ostrich females (Blood *et al*., 1998). Wilson (1996) suggested that hatchability of ostrich eggs could be substantially improved by selecting females that lay eggs with good shell qualities and with an adequate, uniform shell porosity. However, no genetic parameters for incubation traits of ostrich eggs were determined.

Embryonic mortalities as a result of genetic mutations can negatively affect hatchability particularly where the level of inbreeding is high, but specific lethal genes have not yet been recorded in ostriches (Badley, 1997).

5. Eggshell characteristics

As in any avian species, the function of the eggshell is to protect the embryo and contents. The eggshell also allows the passage of water, vapor and respiratory gasses in and out of the egg and prevents the penetration of bacteria into the egg. The eggshell is thus important for overall overall egg quality, and problems with shell thickness, composition, porosity and integrity can greatly influence the outcome of

incubation (Badley, 1997; Deeming & Ar, 1999). The shell itself has many tiny openings or pores which allow gas exchange across the shell. The majority of birds produce eggshells with simple, funnel-shape pores (Bowsher, 1992). The ostrich eggshell, because of its thickness and size, has the most extensive pore system of any avian egg consisting of unbranched and multiple pores which open in pits on the outer surface of the shell (Tullett, 1984). The eggs from the ratite group have a reticulate pore system and the pore numbers vary greatly between eggs, averaging around 15 pores cm^2 (Keffen & Jarvis, 1984; Tullett, 1984). Rhea eggs have a similar system of branched and unbranched pores, differing from ostrich in that the branching occurs in one plane only (Browsher, 1992). Browsher (1992) reported that developing ostrich embryos obtain their oxygen entirely by diffusion through pores in the shell, making it impossible for the ostrich embryo to increase its respiration rate to match its metabolic needs as adult birds do. Necropsy results reveal that a significant proportion of late embryonic mortality appears to stem from suffocation in oedematous embryos (Gonzalez *et al*., 1999). The movement of respiratory gases is restricted and the late embryo suffers a shortage of oxygen at the time of its greatest need and dies of hypoxia. This is more important than an excess in carbon dioxide (hypercapnia), which the embryo seems to be able to tolerate. Eggshell porosity therefore will influence hatchability of the near-term embryo, as it determines the potential for an egg to exchange respiratory gases and water during incubation. Very porous shells will lose excessive amounts of water and result in dehydration of the embryo, whereas eggs with a reduced porosity lose too little water (Ar, 1991). This may cause oedema (water retention) in ostrich embryos, which makes hatching more difficult (Brown *et al*., 1996; Badley, 1997). Swart *et al*. (1987) reported an increase in water loss of 20% during the latter half of incubation and Ar (1991) suggested that the change in water loss can be due to metabolic heat production that increases egg temperature and water vapour pressure inside the egg, thus altering the diffusion gradient.

Chick mortality during artificial incubation represents a major loss of productivity to the industry which is largely related to incorrect water loss and possibly inadequate gas exchange during incubation. Gonzalez *et al*. (1999) reported that there are indications that water loss is closely related to some measurable eggshell parameters and that eggshell conductance is greatly influenced by porosity and shell thickness. The pores determine the potential for respiratory gas exchange and water vapour conductance across the shell. The number of pores in the eggshell, the average diameter of these pores, the total area of pores on the eggshell and eggshell thickness were identified as possible influential factors in the successful hatchability of ostrich eggs. Poor eggshells quality is common in immature hens and in hens under any stress or suffering from oviduct infections (Badley, 1997). Badley (1997) reported that defects include shells which have either high or low porosity (thin or thick shells), excessive ridging or other deformities that interfere with gas exchange).

Egg quality is reported to have a significant genetic component in ostriches, while shell traits directly affect these through their effect on water loss and gas exchange (Button *et al*., 1994; Stewart, 1995). Ar (1996) could not find any correlation between egg weight and shell conductance, but Satteneri & Satterlee (1994) found that either high and low pore numbers in ostrich eggshells were associated with a reduced hatchability. Kinney (1969) reported h^2 estimates for shell thickness of between 0.27 and 0.38 for poultry. Chickens bred for several generations in a dry desert environment and at high altitudes tend to lay eggs with

a lower shell conductance (Badley, 1997). In this respect, it has been demonstrated that some measures of eggshell quality in ostriches was moderately to highly repeatable within a season (Cloete *et al*., 2006b), but the data analysed lacked the genetic structure to partition additive genetic and female permanent environmental effects. Further study in this respect is thus warranted.

6. Environmental and systemic effects

The success of artificial incubation is affected by a number of environmental and systemic factors which include season, female age, storage conditions of eggs prior to setting in the incubator, water loss from the egg during incubation and different types of incubators (Blood *et al*., 1998; Van Schalkwyk *et al*., 1999). Lambrechts (2004) reported that peak production for ostriches in the southern hemisphere occurs between winter (July) and summer (January). Ostrich females begin laying at 2 - 2.5 years old and peak egg and chick production are achieved at 8 - 9 years of age. Female age, however, is known to influence the number of eggs laid as well as egg weight and, consequently, chick weight at hatching (Bunter & Cloete, 2004; Ipek & Sahan, 2004; Lambrechts, 2004; Cloete *et al.*, 2006a). Pre-incubation storage leads to morphological changes in the blastoderm and to a reduced growth rate of the embryos of chickens (Meijerhof, 1992; Fasenko *et al.*, 1992) and ostriches (Malecki *et al.*, 2005). Storage of ostrich eggs for periods longer than 7 days results in an increase in embryonic mortality (Badley, 1997; Ar & Gefen, 1998; Deeming & Ar, 1999).

The amount of water lost during incubation is an important factor in the successful hatching of a healthy chick. Achieving the appropriate water loss during artificial incubation is one of the problems regularly encountered because water loss is influenced by a number of factors including physical characteristics of the eggshell, incubator conditions and heat production of the developing embryo (Ar, 1991). Results from studies by Deeming (1995), Ar (1996) and Blood *et al. (*1998) showed that the optimal water loss for artificially incubated ostrich eggs amount to approximately 15%. Eggs losing less than 10% or more than 20% of their initial weight were less likely to hatch.

In order to ensure the hatching of a healthy chick the needs of the developing ostrich embryo must be met during artificial incubation by provision of appropriate temperature (Van Schalkwyk *et al*., 1999), humidity (Swart *et al*., 1987), the correct gaseous environment (Van Schalkwyk *et al.,* 2002) and the proper turning of eggs (Van Schalkwyk *et al.*, 2000) in automatic incubators. There are currently a number of commercially available ostrich incubators on the market, ranging from wooden incubators which provide only temperature control and air circulation to those that allow electronic control of all variables.

7. Genotype

The genetic make-up is one of the factors influencing the performance of individuals and by selecting for certain traits, genetic improvement may be achieved (Petitte & Davis, 1999). Zimbabwean Blue ostriches (ZB) and Kenyan Red Neck ostriches (KAR) have been introduced in the production system to produce offspring with an improved live weight and an improved carcass weight (Brand *et al.,* 2005; Essa & Cloete, 2006; Davids, 2011). However, the effect of crossbreeding on egg production, fertility and embryonic

mortalities also needs to be considered. Embryonic mortality as a result of genetic problems can negatively influence hatchability, but this has not yet been recorded in ostriches (Badley, 1997). In the comparison with the breeds and crosses, it should also be considered that South African Black (SAB) females overall had a markedly higher overall egg and chick production than ZB and KAR females (Brand *et al.,* 2005; Davids, 2011). Davids (2011) reported that in contrast to the pure ZB, the crossbred females (ZB x SAB, SAB x ZB) mostly resembled the SAB breed for reproduction performance. The prospects of sustainable genetic improvement in the reproduction of ostriches appear to be good from previous work done, with few undesirable correlations (Cloete *et al*., 2008a). According to the latter reference, genetic gains in ostrich chick production amounted to > 3% of the phenotypic mean, which compares favourably to comparable estimates of genetic improvement in other species.

8. Aims of the study

Genetic research on ostrich reproduction so far mostly centred around the estimation of genetic parameters for quantitative traits, like egg production and chick production (Cloete *et al*., 2008b). Although hatchability has received some attention, component traits like the fertility of eggs and the survival of embryos have largely be ignored, as acknowledged by Cloete *et al*. (2008b). This part of the thesis seeks to address this shortcoming, by focusing on the following aspects:

- Systematic factors that affect ostrich egg incubation traits
- To determine genetic parameters for ostrich incubation traits in South Africa
- The genetic relationships between water loss and shell deaths in ostrich eggs, assessed as traits of the dam
- Heritability of shell-deaths in ostrich eggs and factors affecting hatching failure of fertile eggs during artificial incubation
- Genetic (co)variances between water loss and shell-deaths in ostrich eggs
- Genetic parameters for eggshell traits in ostriches
- Effect of female age and genotype on eggshell quality in eggs of females ostriches

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CHAPTER 11

Systematic factors that affect ostrich egg incubation traits

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Abstract

Data obtained from a pair-mated ostrich flock maintained at Oudtshoorn, South Africa, were used to estimate environmental and genetic parameters for egg weight (EWT), water loss of incubated eggs up to 21 days of incubation (WL21), water loss up to 35 days of incubation (WL35), pipping time (PT) and weight of day-old chicks (CWT). Between 13 806 and 19 913 artificially incubated ostrich eggs during the 2003 - 2006 production years were used. Systematic factors affecting these traits such as production year, breeding season, female age, incubator type, storage time and ostrich breed, were initially assessed in single-trait-analyses, using ASREML. Eggs and chicks produced by Zimbabwean Blue (ZB) females were 5 and 7% heavier, respectively, than those produced by South African Black (SAB) females. WL21 and WL35 were not significantly different between ZB and SAB birds. There were trends for within-season effects on EWT and CWT, but no general, robust trend applicable to all years could be discerned. Season had an effect on WL21, WL35 and PT. An increase was apparent in EWT, CWT and PT with an increase in female age. There was a linear increase in pipping time as egg storage time prior to incubation increased. Incubator type had an effect on WL21 and WL35. Systematic factors affect traits like WL21, WL35 and PT should be accounted for before the estimation of genetic parameters. These factors should be considered when planning commercial ostrich husbandry and artificial incubation operations.

1. Introduction

Artificial incubation has become an integral part of any commercial ostrich enterprise. Successful artificial incubation is, however, affected by a number of factors including storage conditions of eggs prior to setting in the incubator, water loss from the egg during incubation, season, female age and genetic make-up (Blood *et al*., 1998; Brand *et al*., 2007; Van Schalkwyk *et al*., 1999). Peak production for ostriches in the southern hemisphere occurs between winter (July) and summer (January) (Lambrechts, 2004). Ostrich females begin laying at 2 - 2.5 years old and peak egg and chick production are achieved at 8 - 9 years of age. Female age, however, is known to influence the number of eggs laid as well as egg weight and, consequently, chick weight at hatching (Bunter & Cloete, 2004; Ipek & Sahan, 2004; Lambrechts, 2004; Cloete *et al.*, 2006).

Pre-incubation storage leads to morphological changes in the blastoderm and to a reduced growth rate of the embryos of chickens (Fasenko *et al.*, 1992; Meijerhof, 1992) and ostriches (Malecki *et al.*, 2005). Albumen quality is compromised by a prolonged storage time (Badley, 1997), which can lead to a proportionate increase in early embryonic mortality in duck and quail eggs (Narahari *et al.*, 1991; Yildirim, 2005). Storage of ostrich eggs for periods longer than 7 days results in an increase in embryonic mortality (Brand *et al*., 2007). Because the storage time and storage temperature of ostrich eggs are usually variables that can easily be managed, it is important to get an indication of possible factors predisposing ostrich eggs that were stored for longer periods to a higher incidence of embryonic mortalities.

Avian eggs lose water during incubation and the amount of water lost is important for successful hatching. Achieving the appropriate water loss during artificial incubation is one of the problems regularly encountered because water loss is influenced by a number of factors including physical characteristics of the eggshell, incubator conditions and heat production of the developing embryo (Ar, 1991). Swart *et al.* (1987) determined that the total water loss from eggs in natural ostrich nests amounts to about 13% of the initial egg weight; the main driving force behind incubation water loss being the gradient in water vapour pressure across the incubating eggshell. Results from studies by Deeming (1995), Ar (1996) and Blood *et al. (*1998) showed that the optimal water loss for artificially incubated ostrich eggs amount to approximately 15% but, like other birds, ostriches show some latitude in the amount of water loss at which eggs will still hatch successfully. Eggs which lost less than 10% or more than 20% of their initial weight were less likely to hatch. Horbańczuk *et al*. (1999) found a higher incidence of malpositioned chicks and chicks with unabsorbed yolk sacs if incubator humidity was too high. Relative humidity and thus vapour pressure inside the incubator can, to a large extent, be controlled during artificial incubation.

Genetic make-up is one of the factors influencing the performance of individuals (Petitte & Davis, 1999). Egg quality is reported to have significant genetic components (Stewart, 1995). Shell deaths were accordingly influenced by breed combination in the study of Brand *et al*. (2007), involving the South African Black and Zimbabwean Blue breeds. At present there is no indication of how these differences are related to evaporative water loss of the eggs produced by the different breed combinations. No quantitative information is available for the water loss from eggs of different ostrich lines or strains.

A better understanding of how systematic factors influence the successful artificial incubation of ostrich eggs is essential (Cloete *et al*., 2002). The aim of this study was thus to quantify the effects of environmental factors such as production year, season, female age, the incubator type used and storage time on egg weight, chick weight, water loss and pipping time of ostrich eggs. The effect of breed combination was also assessed as a systematic effect.

2. Material and Methods

The experimental population used for the study (2003 - 2006) was the commercial, pair-bred ostrich flock at the Oudtshoorn Research Farm in the Klein Karoo region of South Africa. The origin of the flock and the general management procedures implemented has been described previously (Van Schalkwyk *et al.*, 1998; Bunter & Cloete, 2004). During 2003, Zimbabwean Blue (ZB) breeders (21 females; 33 males) were introduced to the flock and mated in various combinations with South African Black (SAB) males and females (Brand *et al.*, 2005). Data that were recorded for 2003 - 2006 thus involved various combinations of the two purebred bloodlines (SAB and ZB), as well as the reciprocal crosses between them. The flock consisted of 188 breeding pairs. Birds in the flock ranged between 2 and 11 years of age, and the annual breeding season usually lasted for about eight months followed by a four month rest period. A total of 20 740 eggs were available for analyses. After excluding eggs with broken or cracked shells and eggs used in other experiments, a total of 19 913 eggs were analysed. The number of records analysed ranged from 13 248 for chick weight to 19 913 for egg weight. Methods of collection, sanitation and storage of eggs at the research farm are well documented (Van Schalkwyk *et al.*, 1998; Van Schalkwyk *et al.*, 1999; Bunter & Cloete, 2004). Briefly, eggs were collected daily, weighed and identified by date and paddock (female) of origin. The surface of each egg was sterilized by 20 min of ultraviolet exposure and labelled with a permanent marker. With the exception of the first two weeks of the breeding season, eggs were stored for no more than 6 days at a temperature of 17 ºC and relative humidity (RH) of 75%. During the first two weeks of the breeding season, egg production is still very low and eggs were stored for 14 days to accumulate enough eggs to put in setters. Eggs were then artificially incubated at 36 ºC and 24% RH in Buckeye®, Prohatch®, African International® or Natureform® incubators. The capacity and operation of the incubators, with the exception of the African International® incubator are described by Cloete *et al.* (2001). Information regarding the African International® incubator can be obtained from the paper by Brand *et al*. (2007). Details of the genotype, female age (only known explicitly for all individuals in the SAB breeds), year, season, and specific incubator used were known for individual eggs. Traits that were considered were egg weight at collection (EWT) and at candling after 21 or 35 days of incubation. These weights were used to derive water loss (% of fresh egg weight) up to 21(WL21) and 35 days (WL35) of incubation. Eggs were transferred to the hatcher at day 35 of incubation and were inspected twice daily (at 08:00 and 16:00) for external pipping from day 39 of incubation. The pipping time of the eggs was recorded. These data were used to derive the number of days from the commencement of incubation to the recorded external pipping time (PT). Day-old chick weight was recorded after the chicks were allowed to dry off for 24 hours.

The data were subjected to a genetic analysis, using ASREML software (Gilmour *et al.*, 1999). The software is suitable for fitting a wide range of random effects in animal breeding, while least-squares means for

selected systematic effects are predicted simultaneously. Such effects are tested for significance, using an F-test in the analysis of variance table. Fixed effects that were considered included sire line (SAB or ZB), dam line (SAB or ZB), year of production (2003 - 2006), laying season (winter, spring or summer), female age (2 - 11 years), incubator (as defined above) and storage time (0 days - 7+ days). Various two-factor interactions were considered initially, but only the year x season interaction was significant and retained in the final analyses. The sire line x dam line interaction was also estimated, although it only approached significance in the analysis on WL35 (P = 0.09). This paper only includes information on the fixed effects that were considered. Random effects and genetic parameters are reported in a subsequent paper (Brand *et al*., 2008).

3. Results and Discussion

The average weight of over 19 000 ostrich eggs was 1 424 g, with a coefficient of variation (CV) of 9.4% and average weight of day-old chicks was 854 g with a coefficient of variation of 12.2% (Table 1). These are consistent with previous results from the same breeding population (Cloete *et al.*, 1998; Bunter *et al.*, 1999; Bunter & Cloete, 2004). The average chick weight represented about 60% of fresh egg weight, which corresponds with other avian species (Wilson, 1991a). Incubation time to external pipping averaged 42 days and had a very low coefficient of variation of 3.3% (Table 1). No comparable estimate could be found in the literature. In contrast, CV's of WL21 and WL35 were higher at 25.5% and 24.2% respectively. The change in egg weight at 35 days, expressed as a percentage of initial egg weight, is an indication of an evaporative water loss of approximately 13% of fresh egg weight during incubation, which is characteristic of ostriches (Blood *et al*., 1998; Swart & Rahn, 1988). Deviations from normality for WL21 and WL35 involved kurtosis rather than skewness, and the interpretation of the results was thus continued without attempting to transform the data to obtain a better distribution (Glass *et al*., 1972).

Table 1 Descriptive statistics for traits recorded from eggs of breeding ostrich females for the 2003 - 2006 production years.

Traits	Number of records	Mean ± SE	CV (%)	Kurtosis	Skewness
EWT (g)	19 913	1424 ± 0.134	9.40	0.10	0.26
CWT (g	13 248	854 ± 0.104	12.2	0.17	0.24
WL21 (% egg weight)	19 800	7.90 ± 2.00	25.3	5.50	1.22
WL35 (% egg weight)	19 561	12.8 ± 3.10	24.2	3.76	1.09
PT (days)	13 806	41.8 ± 1.40	3.30	0.41	0.32

S.E. = standard error, CV% = coefficient of variation, EWT = egg weight at time of lay, CWT = chick weight at one-day-old, WL21 = water loss at day 21of incubation, WL35 = water loss at day 35 of incubation, PT = incubation time to external pipping.

Table 2 Least squares means (± SE) depicting the effect of genotype (SAB or ZB and the reciprocal cross between them) on EWT, CWT, WL21, WL35 and PT.

	GENOTYPE						
Genotype of the sire (GS)	**SAB**		**ZB**		**GS**	**GD**	**GSxGD**
Genotype of the dam (GD)	**SAB**	**ZB**	**SAB**	**ZB**			
EWT (g)	1 412 ± 0.01[a]	1481 ± 0.02[b,c]	1 440 ± 0.02[a,b]	1 504 ± 0.02[c]	0.002	<0.001	0.73
CWT (g)	849 ± 0.01[a]	917 ± 0.01[b]	863 ± 0.01[a]	928 ± 0.01[b]	0.03	<0.001	0.59
WL21 (%)	7.90 ± 0.17	7.61 ± 0.26	7.51 ± 0.24	7.52 ± 0.26	0.26	0.48	0.30
WL35 (%)	12.8 ± 0.25	12.4 ± 0.40	12.0 ± 0.36	12.3 ± 0.40	0.31	0.73	0.09
PT (days)	41.9 ± 0.08	42.1 ± 0.12	41.7 ± 0.10	41.9 ± 0.12	0.06	0.15	0.80

[a,b] Means in the same row with different superscripts are significantly different ($P < 0.05$).

EWT = egg weight at time of lay, CWT = chick weight at one-day-old, WL21 = water loss at day 21of incubation, WL35 = water loss at day 35 of incubation, PT = incubation time to external pipping.

Zimbabwean Blue females laid significantly heavier eggs (5%), which resulted in their chicks being 7% heavier than those of SAB females ($P < 0.05$; Table 2). Crossing ZB males with SAB females also resulted in heavier eggs being laid by their SAB mates compared to pure-bred SAB birds, although the difference only approached significance ($P < 0.10$). Eggs produced by the reciprocal cross (SAB males x ZB females) were substantially heavier than those produced by SAB females subjected to pure breeding. On average, ZB breeding birds are approximately 10% heavier than the SAB (Jarvis, 1998; Brand *et al*., 2005), which can be a contributing factor to the heavier eggs produced by ZB birds. Water loss over the periods up to 21 and 35 days of incubation were independent of sire line, dam line or the sire line x dam line interaction.

Incubation time to external pipping (PT) was not different ($P > 0.05$) between the different breeds and breed combinations (Table 2). Incubation water loss was generally independent of sire bloodline and/or dam bloodline, although there was a tendency for an interaction between these main effects for WL35 ($P = 0.09$).

All traits were affected by an interaction between year of production and season (winter, spring or summer) of production ($P < 0.05$). Trends within seasons could be discerned, but no general, robust trend applicable to all years was observed. The effects of the year x season interaction are thus detailed in Figures 1 - 3 for CWT, WL35 and PT. There appear to be a more or less linear decline in CWT as the season progressed during 2003 and 2005. Day-old weight of chicks hatched during spring resembled winter values during 2004 and 2006, with a subsequent decline towards the summer.

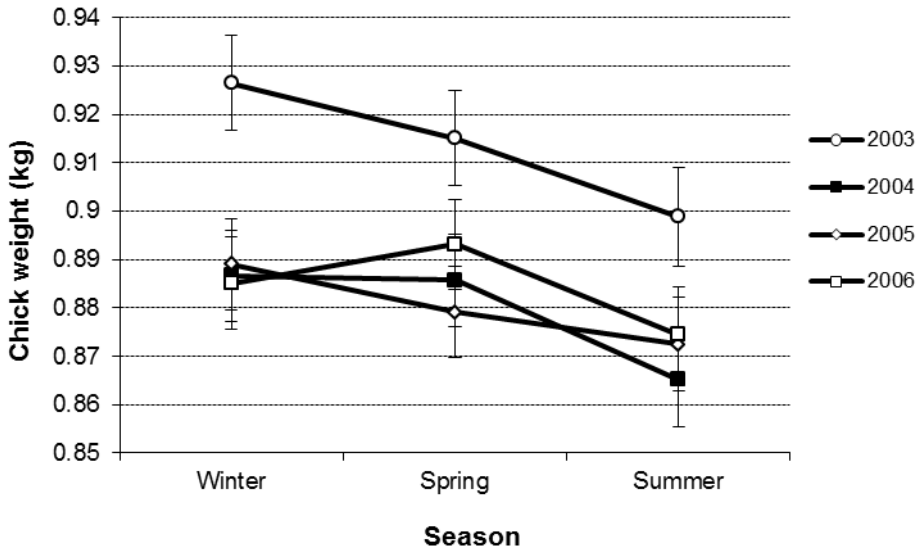

Figure 1 Least-squares means depicting the interaction between season and year for chick weight (kg). Vertical lines about the means denote standard errors.

During 2004, WL35 increased more or less linearly as the season progressed from winter to summer (Figure 2). Seasonal effects were not as obvious during 2003 and 2005, although the absolute values tended to increase between winter and summer. Conversely, WL35 increased from winter to spring in 2005, with no further change to summer. The reasons for these interactions are unclear, but slight differences or changes between year-season ambient climate and between incubators in the micro environment of the incubators could contribute. Although the incubators are set at 24% RH and 36 °C, the absolute humidity or vapour pressure and temperature surrounding the eggshell could differ from these settings because of incubator design and ambient conditions inherent to incubators and year-season combinations. It is generally accepted that year-season effect are transient and unpredictable and unlikely to be repeated. All these factors could contribute to the observed findings. Another possible contributing factor is changes in eggshell structure or egg composition between year-season combinations. More research is required to determine whether seasonal changes in eggshell structure (as determined by the female) contributed to this variation between year and season for water loss. Previous research suggested that eggshell characteristics of females were adapted to mirror changes in the ambient climate (Cloete *et al*., 2006). However, the data used by Cloete *et al*. (2006) were obtained in a single production year, and should be subjected to further investigation. The hatching of lighter chicks from eggs with a higher water loss can be expected. The overall outcome of the trends for CWT, i.e. general decline from winter to summer, and for WL35, i.e. general increase from winter to summer, is therefore reasonable.

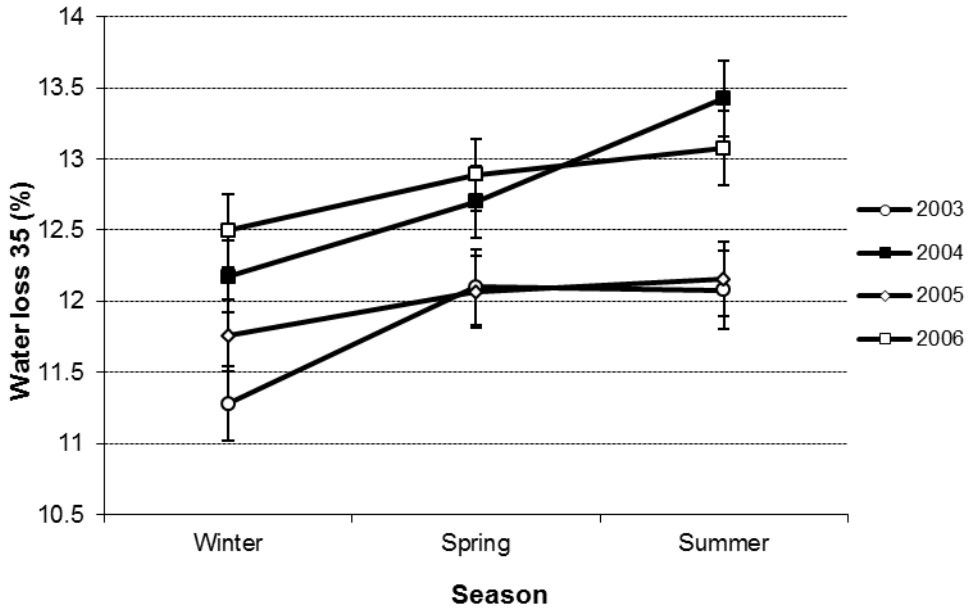

Figure 2 Least-squares means depicting the interaction between season and year with water loss at day 35 of incubation. Vertical lines about the means denote standard errors.

In general, ostrich chicks hatching from eggs laid during the summer had a shorter PT compared to those hatching from eggs laid in winter (Figure 3). During spring, average PT more closely resembled means for the winter in 2004 - 2006. The opposite was true during 2003, when the PT of chicks hatching during spring was significantly lower than those hatching in the winter, and was closer to summer pipping times. We did not find comparable results in the literature to relate these findings to.

Ostrich females have a markedly longer productive life compared to other poultry species (Ipek & Sahan, 2004), making it difficult to compare ostriches to the smaller domestic poultry species traditionally used for egg and chick production. In this study, female age significantly influenced all the traits under consideration. EWT and CWT increased by about 7% between the second and third years of production of individual ostrich females ($P < 0.05$). These traits reached their peak at five years of age ($P < 0.05$; Figure 4), as previously noted by Ipek & Sahan (2004) and Bunter & Cloete (2004). Production was then effectively constant until 11 years-old. Bunter & Cloete (2004) reported that both EWT and CWT decreased at older ages, but this decrease was only evident in the eggs or chicks of females of 11 - 12 years and older. These age groups were not represented in the data set used for the present study, as the population was allowed to become substantially younger (Cloete *et al*., 2006).

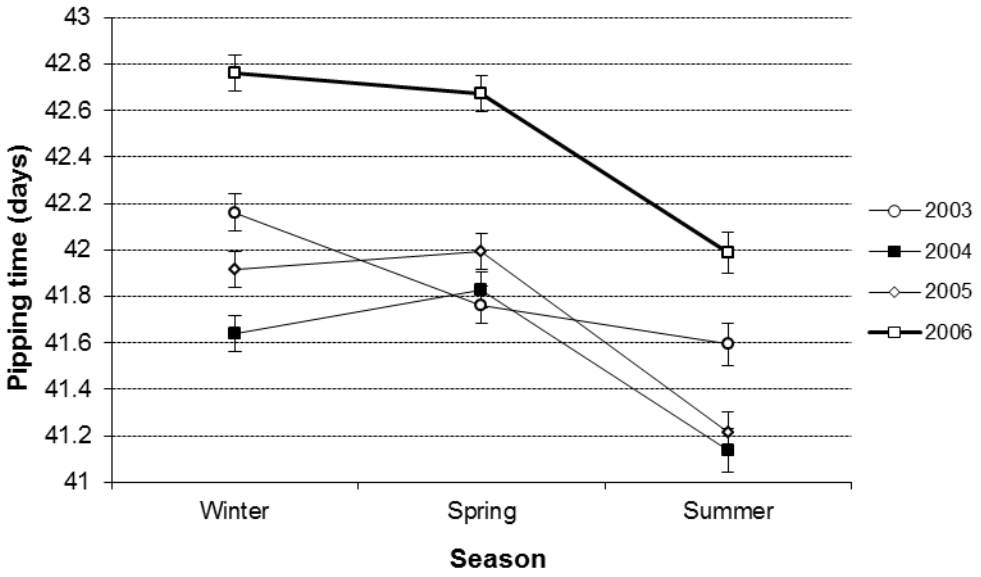

Figure 3 Least-squares means depicting the interaction between season and year with pipping time. Vertical lines about the means denote standard errors.

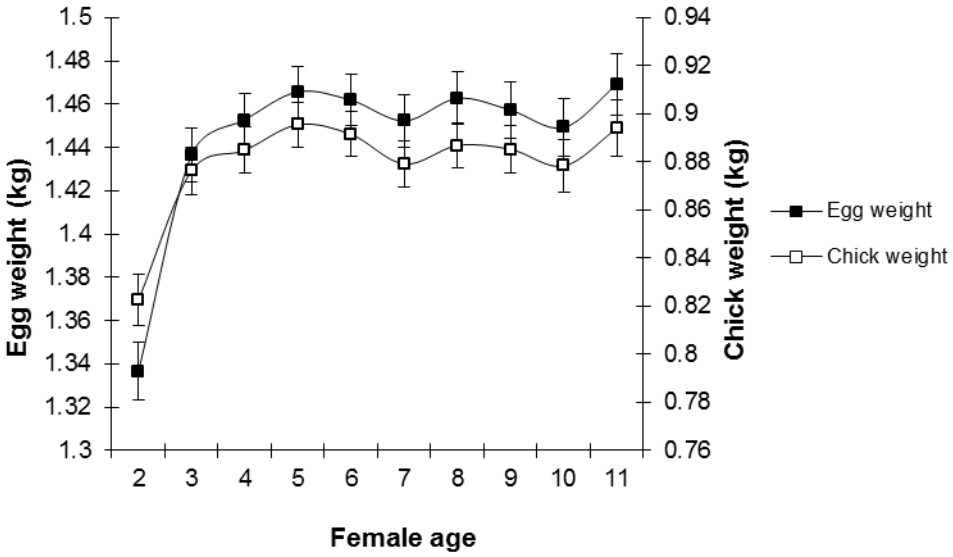

Figure 4 Least-squares means depicting the effect of female age on egg- and chick weight, respectively. Vertical lines about the means denote standard errors.

Water loss to 21 and 35 days of incubation was largely independent of female age (Figure 5), although some evidence of a linear incline was present (P = 0.16 for WL21 and P = 0.06 for WL35). There is strong evidence to suggest that embryonic mortality of ostrich chicks increases with age of ostrich females (Brand *et al*., 2007). The present results, however, are inconclusive whether this increase could be attributed to age-related changes in water loss. More research is required to better understand the mechanism involved in a reduced embryonic survival of eggs produced by older females. Although speculative, it could possibly

be attributed to a decrease in albumin quality with age. In a study by Benton & Brake (1996) it was found that the albumen quality at oviposition in breeder broilers decreased with an increase in flock age.

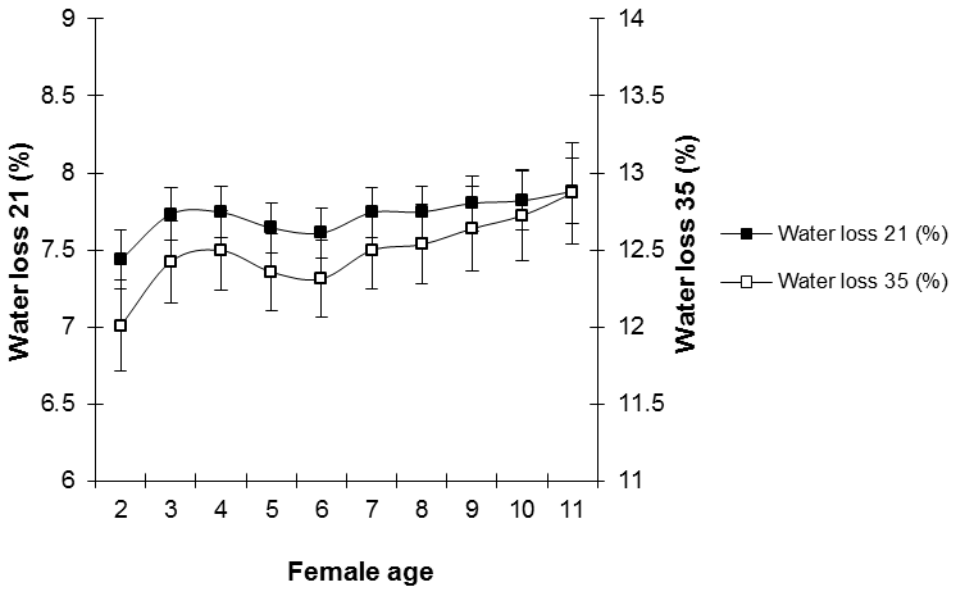

Figure 5 Least-squares means depicting the effect of female age on water loss at days 21 and 35 of artificial incubation, respectively. Vertical lines about the means denote standard errors.

Pipping time of ostrich chicks gradually increased as females got older ($P < 0.05$), and reached a peak for eggs laid by females 8 - 9 years (Figure 6). The overall magnitude of this increase is very small (0.3 days) and it is uncertain whether this would be of any biological relevance. It is, however, worthwhile noting that our findings in this study differ from reports for both broilers and quails, in that the eggs from older hens were found to require less time to hatch than eggs of younger flock mates (Shanawany, 1984; Suarez *et al.*, 1997; Yildirim, 2005). Butler (1991) found that the embryos of eggs laid by older broiler hens were more developed and at a more advanced stage compared with from eggs produced by younger birds. For ostriches, there is only an indication that incubation time may decrease for eggs laid by females 10 year and older. For females of four years and younger, this can possibly be attributed to the fact that egg weight increases with female age, possibly resulting in an increased incubation time (Figure 4), since incubation time is positively correlated with egg weight (Wilson, 1991a).

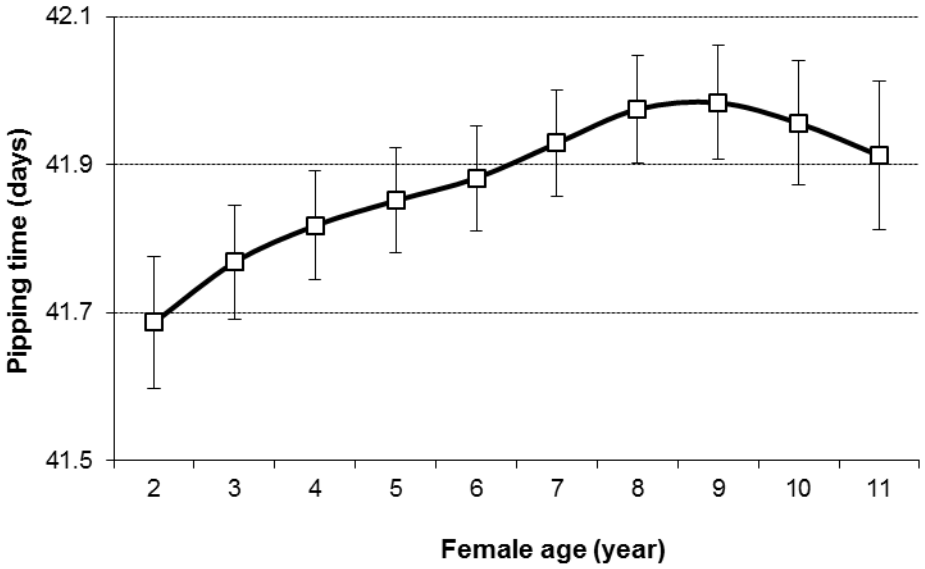

Figure 6 Least-squares means depicting the effect of female age on pipping time. Vertical lines about the means denote standard errors.

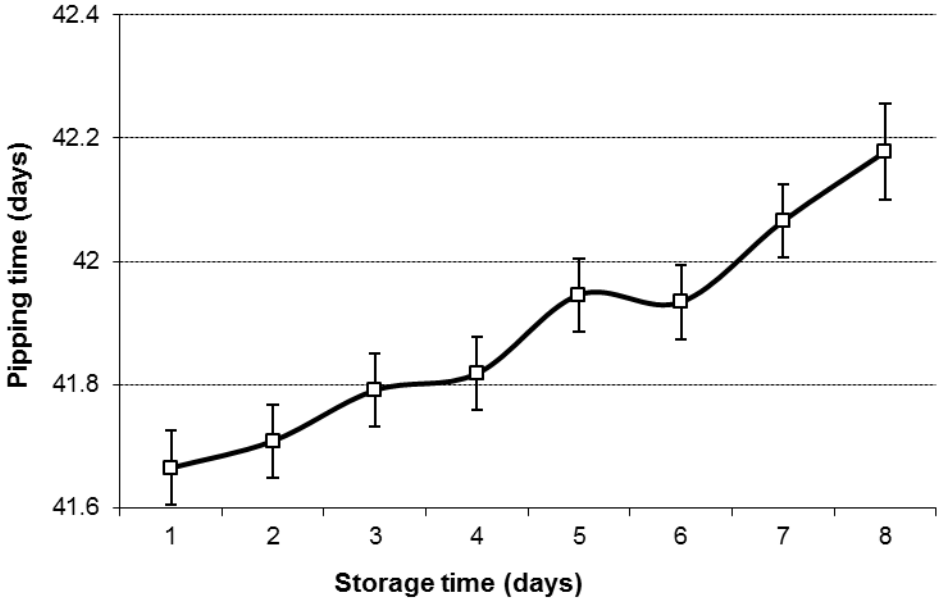

Figure 7 Least-squares means depicting the effect of storage time on pipping time. Vertical lines about the means denote standard errors.

A linear increase ($P < 0.05$, Figure 7) occurred in pipping time from eggs that were set immediately (fresh eggs - 41.7 ± 0.1 days) to eggs stored for longer than 7 days (42.2 ± 0.1 days). Wilson (1991b) and Yildirim (2005) also reported that ostrich eggs set on the day of laying required less time to incubate than eggs stored for longer periods. Accordingly, Tona *et al.* (2003) found that 18 days pre-incubation storage

prolonged the incubation of broiler eggs by at least 15 h compared to eggs stored for only 3 days. In previous research it was shown that the embryonic survival of ostrich chicks was impaired in those eggs that were stored for seven days and longer (Brand *et al*., 2007). Thus prolonged egg storage appears to affect embryonic development that either can result in embryonic mortality or a delayed pipping time. A previous study by Brand *et al*. (2007) indicated that embryonic survival was compromised both in eggs set directly and in those stored for > 7 days.

Ostrich eggs lost between 7.5 and 7.8% of their fresh eggs weight up to 21 days of incubation (Table 3). By day 35 of incubation the average egg had lost between 12.2 and 12.7% of their fresh egg weight. Water loss at day 21 of incubation did not differ ($P > 0.05$) for the different incubators. At 35 days of incubation a significant difference was found between the Africa International® incubator (12.7 ± 0.3% water loss) and the Natureform® incubator (12.0 ± 0.3%; $P < 0.05$). Previous research suggested that eggs set in the former incubator were less likely to hatch than those incubated in the other incubators (Brand *et al*., 2007). Embryonic survival in the Africa International® incubator was compromised throughout incubation, although eggs set in this incubator were particularly vulnerable in the period from setting to 21 days. The less than optimal performance of the Africa International® incubator pertaining to hatching performance was attributed to a design that resulted in excessive temperature gradients within the incubator. It seems reasonable to assume that the same flaws that predispose eggs in this incubator to higher levels of embryonic mortalities could be involved in the excessive water loss experienced. According to Brand *et al*. (2007), the overall hatching performance of the other incubators was markedly better than that of the African International® incubator, and all within acceptable bounds. It is not sure whether this relatively small difference could contribute to the higher overall levels of embryonic mortality recorded for the former incubator by Brand *et al.* (2007). The Buckeye® and Prohatch® incubators did not differ significantly from the other incubators. The make of the incubator affected PT, with eggs incubated in the Buckeye® incubator pipping earlier than those incubated in the other incubators. It has to be stated that the magnitude of the difference between the Buckeye® and the other incubators were still very small.

Table 3 Least squares means (± SE) depicting the effect of incubator on CWT, WL21, WL35 and PT.

Incubator	CWT (kg)	WL21 (% fresh egg weight)	WL35 (% fresh egg weight)	PT (days)
Buckeye	0.89 ± 0.01	7.55 ± 0.16	12.2 ± 0.25[a,b]	41.7 ± 0.07[a]
Prohatch	0.89 ± 0.01	7.66 ± 0.16	12.5 ± 0.25 [a,b]	42.0 ± 0.07 [b]
Africa International	0.89 ± 0.01	7.80 ± 0.17	12.7 ± 0.26[b]	42.0 ± 0.10 [b]
Natureform	0.89 ± 0.01	7.53 ± 0.16	12.0 ± 0.25 [a]	42.0 ± 0.08 [b]

[a,b]Denote significant ($P < 0.05$) differences in columns.

EWT = egg weight at time of lay, CWT = chick weight at one-day-old, WL21 = water loss at day 21of incubation, WL35 = water loss at day 35 of incubation, PT = incubation time to external pipping.

4. Conclusion

Ostrich incubation traits were affected by a number of environmental factors, female age and genotype. These factors need to be considered during routine genetic evaluation. Failure to consider these factors will

lead to biased genetic parameters and inaccurate breeding values. Year and season effects are often transient and unpredictable, as it may depend on ambient climate during a specific period. Yet cognisance should be taken of these effects, even if it is only to include it in genetic analyses to get rid of 'nuisance' variation. It is however, important to consider the effects of genotype, female age, incubator type and setting, as well as storage time, as it has been shown that these effects have a marked influence on shell deaths in artificially incubated ostrich eggs (Brand *et al*., 2007). Combinations that are compatible with commercial ostrich production (i.e. the keeping of younger females, setting of eggs in functional incubators and the prevention of storage periods exceeding 7 days) have the potential to improve commercial chick production. These factors should thus be considered when planning commercial ostrich husbandry and artificial incubation operations.

5. Acknowledgements

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CHAPTER 12

Genetic parameters for ostrich incubation traits in South Africa

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(S. Afr. J. Anim. Sci. 2009, 39, 253-259, *published*)

Abstract

Data obtained from a pair-mated ostrich flock located at Oudtshoorn, South Africa, were used to estimate genetic parameters for egg weight (EWT), weight of day-old chicks (CWT), water loss to 21 (WL21) and 35 (WL35) days of incubation, and pipping time (PT) for between 13 806 and 19 913 artificially incubated ostrich eggs during the 2003 - 2006 production years. Data were initially analysed as single traits using ASREML. Covariance components and ratios were subsequently derived from two-trait analyses. Single-trait estimates of heritability (h^2) were 0.46 ± 0.08 for EWT, 0.34 ± 0.07 for CWT, 0.34 ± 0.07 for WL21, 0.27 ± 0.06 for WL35 and 0.16 ± 0.04 for pipping time. Estimates of maternal genetic effects (m^2) were 0.23 ± 0.12 for EWT and 0.29 ± 0.10 for CWT. A maternal permanent environmental effect amounted to 0.25 ± 0.10 for EWT, 0.12 ± 0.09 for CWT, 0.25 ± 0.04 for WL21 and 0.30 ± 0.04 for WL35. Genetic correlations with EWT amounted to -0.21 ± 0.13 for WL21 and to -0.12 ± 0.14 for WL35. Corresponding correlations with CWT were -0.43 ± 0.07 and -0.54 ± 0.11. Parameters indicate that it should be possible to alter evaporative water loss of ostrich eggs by genetic selection. A feasible selection strategy, however, needs to be devised as it is challenging to effect genetic change in a trait with an intermediate optimum.

1. Introduction

Genetic make-up is one of the factors influencing the performance of individuals and genetic improvement may be achieved by selection for certain traits (Petitte & Davis, 1999). During the past few decades extensive research has been carried out on selective breeding to improve production traits in species of common domestic livestock. Genetic and crossbreeding parameters, as well as line and breed differences for these livestock species are thus readily available. Access to this information ensures structured breeding programmes, involving line- and cross-breeding and exploiting sexual dimorphism and heterosis.

In the ostrich industry, however, little is known about either genetic parameters or responses to selection for specific traits. Definite breeding objectives and industry breeding structures are largely absent (Cloete *et al.*, 2002; 2008), as past selection was largely based on feather characteristics. Moreover, typical ostrich production systems, such as flock-mating, communal nesting systems and a very narrow male to female ratio, also present challenges for implementing selective breeding programmes aimed at improving some specific traits (Cloete *et al.*, 1998). Van Schalkwyk *et al.* (1996) and Cloete *et al.* (1998) were the first to report estimates for repeatability of, and phenotypic correlations among, reproductive traits in the ostrich. Subsequent estimates of genetic parameters for egg, chick and reproductive traits are still limited to only a few studies (Bunter *et al.*, 1999; 2001; Bunter & Cloete, 2004; Cloete *et al.*, 2004; 2005; Lambrechts, 2004). Egg quality is also reported to have a significant genetic component (Stewart, 1995). No information is available on genetic parameters for ostrich incubation traits, although systematic factors affecting ostrich incubation traits were recently estimated by Brand *et al*. (2008). These results showed that evaporative water loss from artificially incubated ostrich eggs to 35 days of incubation depended on the interaction between year and season and the incubator used. The time interval from the commencement of incubation to external pipping depended on the interaction between year and season, the storage period of eggs prior to incubation, and the incubator used. These systematic effects thus need to be considered during the genetic analysis of incubation traits in ostriches. Within-season evaporative water loss has also been shown to be repeatable in ostrich females (Blood *et al*., 1998). No other reports of inter-individual variation were found for incubation traits in ostriches.

Evaporative water loss is known to be curvilinear related to embryonic mortality (Blood *et al*., 1998), and is therefore of importance for artificial incubation. The general lack of genetic parameters for incubation traits has previously been highlighted as a limitation to efficient commercial ostrich production (Cloete *et al*., 2002) and it is therefore evident that a better understanding of genetic factors influencing the hatchability of ostrich eggs is essential (Cloete *et al*., 2002). The aim of this study therefore was to estimate genetic parameters for egg weight, chick weight, water loss and pipping time of ostrich eggs.

2. Material and Methods

The study was carried out over the 2003 - 2006 breeding seasons. The experimental population used for the study was the commercial, pair-bred ostrich flock at the Oudtshoorn Research Farm in the Klein Karoo region of South Africa. The origin of the flock and the general management procedures implemented has been described previously (Van Schalkwyk *et al.*, 1998; Bunter & Cloete, 2004). Brand *et al*. (2008) recently

reported the effects of a number of systematic factors on egg weight after collection (EWT), the percentage of water loss from eggs up to 21 and 35 days of incubation (respectively WL21 and WL35), as well as chick weight at one-day-old (CWT) and the interval from the commencement of incubation to external pipping (PT). These traits and the effects of the relevant systematic factors were defined by latter reference, as well as in Chapter 11. The present investigation estimated genetic parameters for the traits considered. The same analyses used for the estimation of systematic effects were used to estimate variance components for the traits set out above. An animal model was used, which attributed each EWT, WL21, WL35, CWT and PT record to an individual female. Maternal effects of the female were modelled by fitting female permanent environmental (c^2) and maternal genetic (m^2) effects. Initial analyses also included the effect of the breeding paddock occupied by the parents producing a specific egg/chick, but this effect was not significant for the egg and incubation traits that were considered and was consequently omitted from subsequent analyses. Random terms were then added to the operational model including only fixed and interaction effects (Model 1), resulting in the following models for analyses (in matrix notation):

$y = Xb + Z_1a + e$ (Model 2)
$y = Xb + Z_1a + Z_2c + e$ (Model 3)
$y = Xb + Z_1a + Z_3m + e$ (Model 4)
$y = Xb + Z_1a + Z_3m + Z_2c + e$ (Model 5)

In these analyses, y was a vector of observations for EWT, WL21, WL35, CWT and PT records, and b, a, m and c vectors of fixed effects, direct genetic variances, maternal genetic variances and maternal permanent environmental variances respectively. X, Z_1, Z_2 and Z_3 were the corresponding incidence matrices relating the respective effects to y, while e was the vector of residuals.
It was assumed that:
$V(a) = A\sigma^2_a$; $V(m) = A\sigma^2_m$; $V(c) = I\sigma^2_c$; $V(e) = I\sigma^2_e$,

With A being the matrix describing relationships between animals (the numerator relationship matrix), I being identity matrices with the order corresponding to the number of dams for dam permanent environment and the number of records for the residual; σ^2_a, σ^2_m, σ^2_c and σ^2_e the direct genetic variance, maternal genetic variance and the maternal permanent environmental variance and environmental (residual) variance respectively. These analyses yielded estimates of genetic and permanent environmental variances. Ratios for direct additive genetic, maternal genetic as well as maternal permanent environmental variances were computed from these estimates. These variances were expressed relative to the total phenotypic variance.

Random terms were added to analytical models sequentially. Likelihood Ratio Tests (LRT) was used to assess the significance of the contribution of each random term to improvements in the model. The LRT is based on testing the increase in Log-likelihood resulting from adding an additional random term to the model of analysis (starting with model 1) as a Chi^2 statistic. When two models included the same number of random terms, the model with the higher value for the Log-likelihood was preferred (i.e. when Model 3 was compared to Model 4). The ASREML program was used for estimations of fixed effects and subsequently to

derive variance components for each trait in single-trait analyses (Gilmour *et al.*, 1999). ASREML estimates variance components for mixed models by residual maximum likelihood, employing an average information algorithm that concurrently provides estimates of standard errors for parameters (Gilmour *et al.*, 1995). A series of two-trait analyses were then performed to estimate direct genetic, maternal genetic, maternal permanent environmental and environmental correlations between traits.

3. Results and Discussion

Log-likelihood values under alternative random effects models are presented in Table 1. The addition of the direct additive effect as a single random effects resulted in significant improvements in the LRT throughout. When further random effects were added, the LRT indicated that the best models (indicated in bold italics in Table 1) for WL21, WL35 and PT should include direct additive and dam permanent environmental effects as random sources of variation. Models including direct additive, maternal additive and permanent environmental effects were used for EWT and CWT.

Table 1 Log-likelihood values for the respective traits under different random effects models, with the best model for each trait represented in bold italic figures.

Trait	Model 1	Model 2	Model 3	Model 4	Model 5
EWT	1115.21	1144.36	1568.28	1730.93	***1734.98***
CWT	3984.6	8714.84	8835.6	8839.48	***8840.67***
WL21	-3046.62	-9054.17	***-9022.69***	-9024.34	-9021.74
WL35	-1650.57	-7168.01	***-7108.23***	-7128.71	-7107.61
PT	-10525.5	10043.9	***-10038.8***	-10043.1	-10038.8

EWT = egg weight at time of lay, CWT = chick weight at one-day-old, WL21 = water loss at 21 days of incubation, WL35 = water loss at 35 days of incubation, PT = incubation time to external pipping.

Initially, variance components and ratios were estimated for the five traits (EWT, CWT, WL21, WL35 and PT) in single-trait analyses (Table 2). Estimated heritabilities (h^2) for EWT and CWT were high at 0.46 and 0.34, respectively. These estimates were somewhat higher than those previously reported by Bunter *et al.* (1999) (EWT = 0.21; CWT = 0.13) and Bunter & Cloete (2004) (EWT = 0.19; CWT = 0.16). The higher estimates of h^2 could be associated with an improved data structure for the partitioning of sire, dam and paddock effects, caused by the intentional re-allocation of specific females to different mates and breeding paddocks, as recommended by Bunter (2002). Estimates of h^2 were also high for evaporative water loss at 0.34 for WL21 and 0.27 for WL35. The heritability for PT was moderate at 0.16. No previous heritability estimates were found for the latter three traits in ostriches, but Blood *et al*. (1998) reported that water loss from ostrich eggs were highly repeatable within a production season. The h^2 estimate for PT is somewhat lower than a comparable estimate of 0.49 for incubation time in female breeder broilers (Suarez *et al.*, 1997). All h^2 estimates were significant. The maternal genetic effect (m^2) was only significant for the egg traits and was moderate to high both for EWT and CWT at 0.19 ± 0.11 and 0.28 ± 0.11, respectively. The estimate for EWT

was slightly lower than a comparable estimate of 0.31 published by Bunter & Cloete (2004). The present estimate for CWT, however, was consistent with an estimate of 0.26 reported by Bunter & Cloete (2004). Hen permanent environment variation (c^2) accounted for < 15% of the phenotypic variation in CWT and PT. In contrast, it contributed between 20 and 30% of the phenotypic variation to EWT, WL21 and WL35.

Table 2 Estimates of variance components and ratios (± S.E.) for EWT, CWT, WL21, WL35 and PT from single-trait analysis.

Effect	EWT	CWT	WL21	WL35	PT
Components					
σ^2_a	0.008	0.003	1.41	2.65	0.27
σ^2_m	0.003	0.003	-	-	-
σ^2_c	0.010	0.001	1.01	2.89	0.08
σ^2_p	0.020	0.010	4.09	9.80	1.71
σ^2_e	0.001	0.003	1.68	4.26	1.37
Ratios					
h^2	0.46 ± 0.08	0.34 ± 0.07	0.34 ± 0.07	0.27 ± 0.06	0.16 ± 0.04
m^2	0.19 ± 0.11	0.28 ± 0.11	-	-	-
c^2	0.28 ± 0.10	0.13 ± 0.09	0.25 ± 0.04	0.29 ± 0.04	0.04 ± 0.01

σ^2_e = environmental (residual) variance component, σ^2_a = direct additive genetic variance, σ^2_m = maternal additive genetic variance, σ^2_c = dam permanent environmental variance, σ^2_p = phenotypic variance, h^2 = direct heritability, m^2 = maternal heritability, c^2 = ratio of permanent environmental variance to phenotypic variance.

Estimates of genetic parameters from a series of two-trait analyses are presented in Table 3. Estimates of h^2 for EWT and CWT were slightly higher than those reported from single-trait analysis, whereas estimates for WL21 and WL35 were slightly lower. Heritability estimates for PT were consistent with those obtained from single-trait analysis. In broilers, the genetic correlation between egg weight and embryo weight is near zero at the beginning of incubation, but it increases to a maximum at hatch (Suarez *et al.*, 1997). In the present study, egg weight and day-old chick weight were highly correlated on the genetic level (0.97 ± 0.01), which corresponds with the previous estimate of 0.95 reported by Bunter & Cloete (2004). This high genetic correlation indicates that egg weight and chick weight are essentially the same trait, that is, they are governed by a largely similar set of genes. The strong positive correlation between egg weight and chick weight at hatch is fairly constant across species (Wilson, 1991a) with chick weight being primarily determined by initial egg weight, although it is also affected by evaporative water loss during incubation.

Table 3 Estimates of heritability (h^2), maternal genetic effects (m^2), maternal permanent environmental effects (c^2), and correlations between these effects, residual and phenotypic variance and correlations between egg weight (EWT), chick weight (CWT), water loss to 21 days (WL21), water loss to 35 days (WL35) and pipping time (PT) from multi-trait analyses.

Traits	EWT	CWT	WL21	WL35	PT
Additive genetic correlations (h^2 in bold)					
EWT	**0.49 ± 0.08**	0.97 ± 0.01	-0.21 ± 0.13	-0.12 ± 0.14	0.22 ± 0.15
CWT		**0.37 ± 0.07**	-0.73 ± 0.07	-0.54 ± 0.11	0.12 ± 0.16
WL21			**0.34 ± 0.07**	1.00 ± 0.00	0.07 ± 0.17
WL35				**0.26 ± 0.06**	-0.04 ± 0.17
PT					**0.16 ± 0.04**
Maternal genetic correlations (m^2 in bold)					
EWT	**0.23 ± 0.12**	0.99 ± 0.03	-	-	-
CWT		**0.29 ± 0.10**	-	-	-
WL21			-	-	-
WL35				-	-
PT					**-**
Permanent environmental correlations (c^2 in bold)					
EWT	**0.25 ± 0.10**	0.97 ± 0.35	0.11 ± 0.14	0.02 ± 0.13	0.60 ± 0.17
CWT		**0.11 ± 0.09**	-0.47 ± 0.12	-0.54 ± 0.11	0.45 ± 0.27
WL21			**0.25 ± 0.04**	1.00 ± 0.00	0.14 ± 0.16
WL35				**0.30 ± 0.04**	0.155 ± 0.15
PT					**0.04 ± 0.01**
Residual correlation (σ^2_e in bold)					
EWT	**0.0008**	0.31 ± 0.03	-0.01 ± 0.21	-0.19 ± 0.21	-0.07 ± 0.12
CWT		**0.002**	-0.33 ± 0.11	-0.53 ± 0.06	-0.16 ± 0.05
WL21			**1.68**	0.86 ± 0.01	0.04 ± 0.04
WL35				**4.30**	0.07 ± 0.03
PT					**1.37**
Phenotypical correlation (σ^2_p in bold)					
EWT	**0.02**	0.86 ± 0.01	-0.06 ± 0.04	-0.06 ± 0.04	0.11 ± 0.02
CWT		**0.01**	-0.51 ± 0.03	-0.48 ± 0.03	-0.01 ± 0.02
WL21			**4.12**	0.95 ± 0.002	0.05 ± 0.02
WL35				**9.81**	0.05 ± 0.02
PT					**1.71**

Genetic correlations of WL with EWT were negative, but not significantly different from zero. A negative correlation was expected due to the surface area : volume ratio, which results in heavier eggs, at least in theory, being expected to lose less water than light eggs with a wider surface area : volume ratio. It is conceded that this relationship is not a simple one as other factors such as eggshell structure and porosity also contribute to water loss. Direct genetic correlations of WL with CWT were negative and high at -0.73

and -0.54 respectively for WL21 and WL35. This result was expected since eggs with high levels of water loss will produce lighter chicks as a result of dehydration. This principle is well illustrated in small domestic poultry species, as demonstrated by Tullett & Burton (1982) and Davis *et al.*, (1988). The direct genetic correlation between WL21 and WL35 amounted to 1.00 ± 0.0003, suggesting that these traits are essentially the same. Pipping time has no significant genetic correlation with any of the other traits with estimated correlations ranging from -0.04 ± 0.17 with WL35 to 0.22 ± 0.15 with EWT. This result corresponds with findings by Suarez *et al.* (1997) that incubation time and egg weight of broiler breeders were not highly correlated. The maternal additive correlation was only significant for EWT and CWT with a very high correlation of 0.99. The corresponding correlation reported by Bunter & Cloete (2004) amounted to 0.96.

The hen permanent environmental correlation between EWT and CWT was high at 0.97 ± 0.35. This value is slightly higher than the value of 0.89 reported by Bunter & Cloete (2004). Hen permanent environmental correlations of EWT with WL were low and not significant. The corresponding maternal permanent environmental correlations of CWT with WL were negative and in the order of -0.50 in magnitude. On the maternal permanent environmental level, EWT was positively related to PT, the estimate approaching 0.60 (Table 3). This result seems to imply that larger eggs are likely to hatch later on the dam permanent environmental level. Dam permanent environmental correlations of PT with other traits were not significant and ranged from 0.14 ± 0.16 with WL21 to 0.45 ± 0.27 for CWT.

The residual correlation between EWT and CWT were substantially lower than unity at 0.31 ± 0.03. The corresponding correlation reported by Bunter & Cloete (2004) amounted to 0.57. The estimated phenotypic variances for the respective traits were largely similar to the estimates from single-trait analyses. There was also a strong phenotypic correlation (r_p) between egg and chick weight (0.86 ± 0.01), while the phenotypic correlations between EWT and WL21, Wl35 and PT were low and not significant.

4. Conclusion

Estimates of h^2 indicate that it is possible to alter evaporative water loss of ostrich eggs by genetic selection. The only previous reference suggesting that selection for water loss may result in current flock gains was the finding of Blood *et al*. (1998) that the within-season water loss of eggs from individual females was highly repeatable. A feasible selection strategy, however, needs to be devised, as it may be challenging to effect genetic change in a trait with an intermediate optimum. Egg and day-old chick weights, as well as pipping time, were also demonstrated to be heritable, and should respond to selection, should change be desired. The potential role of these traits in an integrated breeding scheme, however, needs further study before being applied in the industry. It is conceded that the standardisation of egg weight to reduce the variation in evaporative water loss during artificial incubation may assist in alleviating the present high level of embryonic mortalities. However, larger chicks are generally favoured by commercial chick raisers. Grade 1 chicks in South Africa are expected to be heavier than 750g (Verwoerd *et al*., 1999), exerting a direct influence on the price realised for day-old chicks. If it is considered that day-old chick weight should be about 60% of egg weight (Wilson, 1991b), this implies that eggs to be set should be at least 1 250 g in weight. The lower 95% confidence limit for eggs used in the present study was 1 156 g, indicating that a substantial number of the

eggs used were below this recommended weight. An additional advantage of producing heavier day-old chicks is an improved chick survival to one month of age (Cloete *et al*., 2001), provided that the chicks do not suffer from other complications such as oedema owing to insufficient water loss.

Direct and maternal genetic correlations among traits were either high to very high in the desired direction, or small in magnitude. Given the high levels of incubation failure, further research is urgently needed to gain a better understanding of the mechanisms involved and strategies with the potential to improve incubation success.

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CHAPTER 13

The genetic relationships between water loss and shell deaths in ostrich eggs, assessed as traits of the dam

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Abstract

The ostrich industry suffers from a high rate of embryonic mortality during artificial incubation of ostrich eggs. Data from 34 285 eggs were used to derive 969 female-year records for evaporative water loss (WL), treated as a trait of the female. Heritability was significant for WL at a level of 0.40 - 0.41 (both after 21 and 35 days of incubation). WL at 21 and 35 days was negatively correlated on the genetic level with chick weight at hatching (-0.84 and -0.81 respectively). Shell deaths did not exhibit high levels of genetic variation (0.06), but was affected by the permanent environment of the female (0.33). Shell deaths were correlated with WL on a genetic level (-0.34 to -0.41), but the estimated genetic correlations were associated with high standard errors and are, therefore not very robust. Further research is indicated to obtain more accurate genetic relationships between traits influencing incubation.

1. Introduction

Compared to other commercial avian species, the hatchability of artificially incubated ostrich eggs is relatively low and highly variable, ranging, on average, from less than 30% to around 60% (Van Schalkwyk *et al.*, 2000). Approximately 20% of the ostrich eggs that failed to hatch under artificial incubation were the result of embryonic mortalities (Brand *et al*., 2007), making it an important contributor to overall poor hatching results. Bird eggs lose water during incubation and the amount of water lost is important for successful hatching. Achieving the correct water loss (WL) during artificial incubation is a challenge as it is influenced by both incubator conditions, the physical properties of the eggshell, and internal factors as the embryo develops (Ar, 1991).

Swart *et al.* (1987) determined that the total WL from ostrich eggs incubated in natural nests amounts to around13% of the initial egg weight. Studies by Deeming (1995), Ar (1996) and Blood *et al.* (1998) showed that the optimal WL in artificially incubated ostrich eggs is around 13 - 15%. Although there is some variation in the percentage WL at which ostrich eggs will still successfully hatch, there is, however, a sharp increase in embryonic mortality below 10% and above and 18% WL at 35 days of incubation (Blood *et al*., 1998). Excessive WL during incubation causes early depletion of allantoic fluids, which results in subsequent dehydration of the embryo and extends the period of osmotic stress (Davis *et al*., 1988). It can result from high permeability of the eggshell to water vapour and other gases or inadequate incubator humidity. However, insufficient WL from the egg can result in water retention by the chick, potentially causing embryonic mortality through respiratory insufficiency (Musara *et al.*, 1999) and a high proportion of chicks that are malpositioned at the point of hatch or have unabsorbed yolk sacs (Horbaňczuk *et al.*, 1999). Insufficient WL indicates a low permeability of the eggshell to water vapour and other gases or elevated incubator humidity.

In artificially incubated eggs, there are then two main factors that affect WL from the eggs; relative humidity (RH) in the incubator and the porosity of the eggshell itself to water vapour. While incubator humidity can usually be controlled, the large variation in WL (and subsequent hatching success) in ostrich eggs under controlled incubator conditions suggests a wide variation in shell porosity, possibly indicating a wide genetic variability for this trait among ostrich females. Wilson (1996) suggested that hatchability of ostrich eggs could be substantially improved by selecting females that lay eggs with good shell qualities and with an adequate, uniform shell porosity. However, no genetic parameters for incubation traits of ostrich eggs were determined.

The aim of this study was to estimate environmental and genetic parameters for WL and shell deaths of ostrich eggs, modelled as traits of the ostrich female. It was reasoned that a better understanding of these factors may contribute to an improved hatching success and aid selective breeding programs.

2. Material and Methods

The experimental population used during the period of the study (1998 - 2006) was a commercial, pair-bred ostrich flock at the Oudtshoorn Research Farm, South Africa. The flock consisted of 188 breeding pairs of ostriches at the end of the study. The age of the breeding birds in the flock ranged between 2 and 11 years of age. The husbandry and management of the flock has been described previously by Cloete *et al.* (1998) and Bunter & Cloete (2004). The flock mostly included birds of the South African Black genotype, but birds from the Zimbabwean Blue and Kenyan Redneck strains were also introduced recently to study crossbreeding between these genotypes (Engelbrecht *et al.*, 2008). However, the present analysis was confined to birds of the South African Black strain as all genotypes were not represented throughout the experimental period. Unless otherwise specified, each breeding bird received a production diet of 2.5 kg dry matter/day throughout the breeding season.

The breeding season started at the beginning of June and lasted until the end of January the following year. Exceptions to this were for the 1999 and 2002 breeding seasons, when the birds remained in the breeding paddocks until February 2000 or throughout the year as described by Lambrechts (2004). Outside the breeding season (February - May), male and female birds were kept in separate flocks for resting.

The methods of egg collection, sanitation and storage on the research farm are well documented (Van Schalkwyk *et al.*, 1998; 1999). The fate of each individual egg was recorded as described by Bunter (2002). Weights of individual eggs were recorded, eggshells were sterilized by exposure to ultraviolet light for 20 min and labelled with a permanent marker. Eggs were then stored for no more than 6 days at a temperature of 17°C and RH of 75% before being artificially incubated at 36 °C and 24% RH in Buckeye (Hatchery Equipment Suppliers, Krugersdorp, South Africa), Prohatch (Prohatch Ostrich Incubation System, Somerset-Wes, South Africa), Natureform (NatureForm Hatchery Systems, FL, USA) or Africana (Africana Ostrich International Rustenburg, South Africa) incubators (Brand *et al.*, 2007).

Eggs were candled and weighed on days 21 and 35 of incubation. Together with initial egg weight, these weights were used to derive WL, expressed as percentages of the initial egg weight, up to 21 and 35 days. Eggs not showing any macroscopic embryonic development after 21 days of incubation were regarded as infertile and were not considered in any analysis. Those with clear evidence of embryonic development that had subsequently ceased were considered as shell deaths during the first half of incubation (early embryonic deaths). Subsequent shell deaths were classified as late embryonic mortalites (> 21 days of incubation). However, no distinction was made between early and late embryonic mortalites for the purpose of this study. Such a distinction would have reduced the number of usable records (already at a minimum for a genetic analysis) even further. Chicks from those eggs that hatched successfully were weighed at 1 day old.

Data of 34 285 eggs were averaged to obtain 1 029 female-year records for females pertaining to initial egg weight, WL (as proportion of the initial egg weight) up to 21 days of incubation, WL up to 35 days of incubation, day-old chick weight (ACW) and overall shell deaths. These data were screened to exclude female-year records of females producing fewer than five fertile eggs that were incubated and classified as

fertile by candling and egg break-out at 21 days. A total of 969 hen-year records were available for analysis after editing. These records represented 284 individual females, mated to 311 males to form 410 unique breeding pair combinations. The pedigree file included all the animals that were available for selection during the course of the study, as well as the base animals that were present since recording started in 1990. In total, the pedigree file included 2 371 individuals, the progeny of 340 sires and 322 dams. In the pairbred situation, a roughly similar number of sires and dams are usually expected. However, sires paired of with dams producing substantial numbers of unfertilised eggs are expected to be replaced earlier than the corresponsing female, hence the slightly higher number of sires in the pedigree file.

The data were then subjected to genetic analysis, using ASREML software (Gilmour *et al.*, 1999). The random effects that were fitted included the direct, additive effect of animal (h^2; fitted as default for all traits) and the animal permanent environment (pe^2). Previous research indicated that traits like average egg weight (AEW) and (ACW) may also depend on the service sire (Cloete *et al*., 2004), although variance ratios were generally below 0.10. It is difficult to perceive how service sire effects could influence evaporative WL and shell deaths, and this effect was therefore not considered in the present study. It also needs to be conceded that service sires was partially confounded with females, which could complicate the partitioning of variances. ASREML was used to fit fixed effects (year of production and the age of the female) while simultaneously deriving variance components for each trait in single-trait analyses. ASREML estimates variance components for mixed models by REML, employing an average information algorithm that concurrently provides estimates of standard errors (S.E.) or parameters (Gilmour *et al.*, 1995). The likelihood ratio test was used for the identification of the most suitable random effects model. However, animal pe^2 effects were significant for all traits, and these results are not presented.

A series of two-trait analyses were then performed to estimate correlations between traits. Traits considered were AEW, percentage WL up to 21 days of incubation (WL21), WL up to 35 days of incubation (WL35), ACW and percentage shell deaths (DIS%) of individual females.

3. Results

The average weights for ostrich eggs of individual females were around 1 430 g, with a coefficient of variation (CV) of 7.5% (Table 1). ACW was around 860 g with a CV of 9.1%. The change in egg weight by 35 days, expressed as a percentage of initial egg weight, was around 13%. Corresponding estimates of CV were 17.5% for WL21 and 16.8% for WL35. Corresponding ranges were 5 - 14% and 8 - 23% for WL21 and WL35 respectively. The average for DIS% was 29.9% with a CV of 62.9%, and a range from 0% to 100%. All traits were normally distributed (Table 1), and no need for data transformation was evident.

Table 1 Descriptive statistics for traits recorded from eggs of breeding ostrich females for the 2003 - 2006 production years.

Traits	Abbreviations	Number of records	Mean ± SD	Skewness	Kurtosis
Average egg weight (g)	AEW	969	1 425 ± 107	0.195	-0.026
Water loss to 21 days (%)	WL21	969	7.95 ± 1.39	0.526	0.736
Water loss to 35 days (%)	WL35	969	13.10 ± 2.20	0.577	0.752
Average chick weight (g)	ACW	963	858 ± 78	0.194	-0.011
Shell deaths (%)	DIS	969	29.90 ± 18.80	1.006	1.138

SD = standard deviation

The results of single-trait analyses on AEW, WL21, WL35, ACW and DIS% are provided in Table 2. Heritability estimates for AEW, WL21, WL35 and ACW were all high, ranging between 0.41 ± 0.13 and 0.56 ± 0.11. All these h^2 estimates were significant (at least double the corresponding S.E.). In contrast, the h^2 estimate of shell deaths was not significant at 0.06 ± 0.06. Animal pe^2 accounted for between 21 and 39% of the phenotypic variation associated with the respective traits.

Table 2 Estimated variance components and ratios (± S.E.) of average egg weight (AEW), water loss to 21 days (WL21), water loss to 35 days (WL35), average chick weight at 1 day old (ACW) and percentage shell deaths (DIS%) as derived from single-trait analyses.

Effect	AEW	WL21	WL35	ACW	DIS%
Components					
σ^2_e	2723	0.36	0.93	1404	194.10
σ^2_a	4935	0.67	1.87	3487	18.60
σ^2_{pe}	3669	0.61	1.78	1291	106.90
Ratios					
σ^2_{pe}	0.44 ± 0.12	0.41 ± 0.13	0.41 ± 0.13	0.56 ± 0.11	0.06 ± 0.06
pe^2	0.32 ± 0.11	0.37 ± 0.12	0.39 ± 0.12	0.21 ± 0.10	0.33 ± 0.06

σ^2_e = environmental (residual) variance component; σ^2_a = direct additive variance component; σ^2_{pe} = female permanent environmental variation component; pe^2 = hen permanent environment

Estimates of genetic parameters from the two-trait analyses are presented in Table 3. Derived variance ratios for all traits were generally similar to those estimated from single-trait analyses. Genetic correlations (r_g) of AEW with WL21 and WL35 were negative in sign and high at -0.72 ± 0.19 and -0.69 ± 0.20 respectively. The r_g of WL21 and WL35 with ACW were negative and high at -0.83 ± 0.11 and -0.81 ± 0.12, respectively. AEW was highly positive correlated with ACW at 0.97 ± 0.02. The r_g of WL21 with WL35 was unity, suggestion that it was essentially the same trait. The r_g of WL21 and WL35 with DIS% were negative at -0.34 ± 0.43 and -0.41 ± 0.42, respectively.

The female permanent environment correlations (r_{pe}) of AEW with WL21and WL35 amounted to 0.40 ± 0.19 and 0.37 ± 0.19, respectively. A high r_{pe} of 0.82 ± 0.07 was found between AEW and ACW. Animal *pe* correlations involving DIS% were variable in sign, but low in magnitude (Table 3). The residual correlation (r_e) of AEW with ACW was highly significant at 0.85 ± 0.01. WL21 was also highly correlated with WL35 on the environmental level (0.95 ± 0.01). Estimates of r_e were negative for ACW with WL21 and WL35 at -0.34 ± 0.03 and -0.33 ± 0.03, respectively. Phenotypic correlations were mostly similar in sign to r_e, but they were somewhat larger in magnitude in most cases.

Table 3 Estimates ±S.E. of heritability (h^2), female permanent environmental effects (pe^2), residual and phenotypic variances and correlations between average egg weight (AEW), water loss to 21 days (WL21), water loss to 35 days (WL35), average chick weight at 1 day of age (ACW) and percentage shell deaths (DIS%) as derived from a series of two-trait analyses.

Traits	AEW	WL21	WL35	ACW	DIS%
Additive genetic correlations (h^2 in bold)					
AEW	**0.41 ± 0.10**	-0.72 ± 0.19	-0.69 ± 0.20	0.97 ± 0.02	0.27 ± 0.42
WL21		**0.40 ± 0.13**	1.00 ± 0.01	-0.83 ± 0.11	-0.34 ± 0.43
WL35			**0.40 ± 0.13**	-0.81 ± 0.12	-0.41 ± 0.42
ACW				**0.56 ± 0.11**	0.18 ± 0.40
DIS%					**0.06 ± 0.06**
Permanent environmental correlations (pe^2 in bold)					
AEW	**0.35 ± 0.10**	0.40 ± 0.19	0.37 ± 0.19	0.82 ± 0.07	-0.05 ± 0.18
WL21		**0.38 ± 0.12**	1.00 ± 0.01	-0.13 ± 0.22	0.03 ± 0.18
WL35			**0.39 ± 0.12**	-0.16 ± 0.21	0.05 ± 0.18
ACW				**0.22 ± 0.10**	-0.07 ± 0.23
DIS%					**0.33 ± 0.06**
Phenotypic correlations (σ^2_p in bold)					
AEW	**11 250**	-0.14 ± 0.06	-0.13 ± 0.06	0.89 ± 0.01	0.03 ± 0.04
WL21		**1.65**	0.99 ± 0.01	-0.49 ± 0.04	-0.04 ± 0.05
WL35			**4.49**	-0.48 ± 0.04	-0.04 ± 0.05
ACW				**6185**	-0.01 ± 0.05
DIS%					**319.6**
Residual correlations (σ^2_e in bold)					
AEW	**2724**	-0.07 ± 0.04	-0.06 ± 0.04	0.85 ± 0.01	0.01 ± 0.04
WL21		**0.36**	0.95 ± 0.01	-0.34 ± 0.03	0.02 ± 0.04
WL35			**0.93**	-0.33 ± 0.03	0.02 ± 0.04
ACW				**1408**	-0.07 ± 0.04
DIS%					**194.1**

σ^2_p = environmental (residual) variance component phenotypical variances; σ^2_e = environmental (residual) variance component. See Table 1 for trait names, units and definitions.

4. Discussion

Problems with eggs losing insufficient water are associated with over hydration and inadequate respiratory gas exchange (Bowsher, 1992). The most visible effect of low WL in the egg is the smaller air cell formed by an insufficient diffusive WL. Ostrich eggs (and those of other species of birds) with high WL are generally associated with shells that have higher than normal porosity (Bowsher, 1992). Owing to the increased amount of water vapour escaping through the pores, such eggs may dehydrate too quickly. Blood *et al*. (1998) clearly indicated that the lowest embryonic losses were sustained in eggs exhibiting intermediate levels of WL.

The AEW and ACW found in the present study are consistent with previous results from the same breeding population (Bunter *et al.*, 2001; Cloete *et al.*, 2004; 2005). The ACW represented around 60% of fresh egg weight, which corresponds with findings by Wilson *et al.* (1997). Results from our study showed that evaporative WL to 35 days of incubation amounted to around 13% of fresh egg weight during incubation, which is characteristic of ostriches (Swart & Rahn, 1988; Blood *et al*., 1998). The value of 29.9% for shell deaths corresponds with findings by Deeming *et al.* (1993), but is slightly higher than the value of 21.1 ± 13.4% mortality reported by Van Schalkwyk *et al.* (1996). The latter estimate, however, was based on all the eggs that were incubated and did not include only fertile eggs.

High h^2 estimates for W21 and WL35 in the single-trait analyses and in two-trait analyses (Table 2 and 3) suggest that it should be feasible to alter evaporative WL of ostrich eggs by genetic selection of females based on average performance. It has to be conceded that the CV for WL is considerably lower than for ostrich reproduction traits (Cloete *et al*., 2004; 2005). The lower inherent variation in this trait may impede genetic response to selection to an extent. Previous studies suggest that WL is affected by egg traits, which include egg weight, egg size, shell thickness, shell strength, shell weight and shell porosity (Soliman *et al.*, 1994; Sahan *et al.*, 2003; Cloete *et al*., 2006). The study by Cloete *et al*. (2006) suggested that at least some of these characters are repeatable for females within a season. Results from studies on chickens showed that the rate of decline in weight experienced by eggs during incubation is at least partly a heritable character associated with the hen producing those eggs (Sotherland *et al*., 1979).

The h^2 of AEW was previously estimated at 0.43 ± 0.16 by Bunter *et al*. (2001), at 0.72 ± 0.04 by Cloete *et al*. (2004) and at 0.59 ± 0.04 by Fair *et al*. (2005). The former estimate is consistent with the present estimate (Tables 2 and 3), but are somewhat lower. The h^2 estimates of 0.56 ± 0.11 for ACW in our study (Tables 2 and 3) corresponds with estimates of 0.56 ± 0.05 (Fair *et al*., 2005) and 0.43 ± 0.16 (Bunter *et al.*, 2001), but it is lower than the estimates of 0.74 ± 0.03 and 0.71 ± 0.03 reported by Cloete *et al.* (2004; 2005). It needs to be stated that the animal variances were entirely attributed to genetic differences between animals in the studies by Cloete *et al*. (2004; 2005), as the pe^2 variances were not significant. The latter studies included generally larger data sets and spanned more generations. It is reasonable to assume that the larger and more informative data sets could facilitate the partitioning of genetic and pe^2 variances.

The negative r_g between AEW and WL (Table 3) were expected, owing to the surface area : volume ratio, resulting in heavier eggs, at least in theory, being expected to have lower rates of WL. Egg size (weight and /or volume) varies considerable within species, with a large component of this variation being attributed to additive genetic variance (Martin & Arnold, 1991).

The negative r_g between ACW and WL are not unexpected, since females producing eggs with higher levels of WL will produce lighter chicks (Tullett & Burton, 1982), owing to dehydration. The highly positive correlation between AEW and ACW corresponds with the previous estimates by Cloete *et al.* (2004) and Fair *et al*. (2005) on average weights, analysed as traits of the female. Bunter & Cloete (2004) accordingly found a high r_g of 0.95 when individual egg and chick weights were analysed. These high r_g indicate that egg weight and chick weight are essentially the same trait, which is governed by a largely similar set of genes. The same reasoning applied to WL to 21 or 35 days, where the genetic and female pe^2 correlations did not differ from unity. The strong positive correlation between egg weight and chick weight at hatch is fairly constant across species (Wilson, 1991b). Both Wilson (1991a) and Deeming & Birchard (2007) stated that chick weight is primarily determined by initial egg weight, while it is also affected by weight loss during incubation. Although the correlations between WL and DIS% are reasonably large in magnitude, they were associated with very high S.E., and therefore not very robust. Doubtlessly, the low levels of genetic variation in DIS% contribute to this lack of accuracy. A negative r_g between WL and DIS% would indicate that an increase in the amount of WL leads to a reduction of shell deaths. These results coincide with findings of Deeming (1995) in that the patterns of mortality in eggs which survive beyond day 36 of incubation were closely linked with the degree of variation in amount of WL from the egg. Eggs which lost less than 10%, or more than 20%, of their initial weight were less likely to hatch. Results from studies by Snyder & Birchard (1982) and Badley (1997) on domestic chickens showed that early and mid-term embryonic mortalities increase with an increase in WL and that this relationship was the principal cause of poor hatchability of eggs. The result of incorrect WL during incubation is primarily exhibited during the last phase of incubation, when the changes associated with hatching are about to take place (Bowsher, 1992).

There are no comparable studies pertaining to the genetic relationship of evaporative WL with shell deaths in either ratites or small domestic poultry species. It needs to be stated that the very low phenotypic correlations of WL with shell deaths did not support the existence of a useful relationship between these traits. Further studies of this nature should thus be encouraged to fill in this gap in incubation knowledge.

5. Conclusions

It is conceded that the study involved barely sufficient records for a genetic analysis, as with many preliminary studies on new traits. There is, however, some evidence to suggest that the average WL of eggs produced by individual females will respond to selection. The non-linear relationship between embryo survival and WL, relatively low CV for WL, low levels of genetic variation for shell deaths and a lack of a robust $\boldsymbol{r_g}$ between these traits complicates the practical application of these results in ostrich breeding flocks. The lack of genetic variation for shell deaths may indicate that this trait should possibly be improved by management rather than by selection. Alternatively, the lack of genetic variation may be attributed to few

generations being represented in the 9 years over which data were recorded. With a generation interval of > 5 years (Cloete *et al*., 2008) in the experimental population, thus the experiment spanned fewer than two generations. Further research to incorporate selection for an improved hatching success in ostrich breeding programs should continue.

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CHAPTER 14

Effect of female age and genotype on eggshell quality in ostrich females

Abstract

Eggshell characteristics of birds affect water loss and gas exchange during incubation. It is consequently important to understand factors that potentially affect eggshell quality and characteristics in artificially incubated ostrich eggs to ensure successful hatching of healthy chicks. For this study around 14 000 eggshells from the pair-bred ostrich flock maintained at Oudtshoorn, South Africa, were measured and analysed. Systemic factors affecting eggshell quality included female age and genotype. Both pore count and permeability of eggs increased significantly in eggs of females older than 10 years. A significant increase in shell thickness was evident for eggs from females aged 2-year-old compared to females of 3 years and older and there was a marked reduction in shell thickness of eggs from females older than 10 years. South African Black (SAB) female ostriches had more pores per 2 cm^2 (4.73 ± 0.03) than did the Zimbabwe Black (ZB) phenotype (4.62 ± 0.04), different combinations of SAB and ZB (4.60 ± 0.05 and 4.58 ± 0.05 for ZB♂ x SAB♀ and for SAB♂ x ZB♀ respectively), as well as Kenyan Red (KAR) (4.57 ± 0.11). From these results, there seem to be a number of environmental factors to consider when assessing ostrich eggshell characteristics and hatchery practice.

1. Introduction

The avian eggshell must meet two conflicting demands; mechanical protection for the egg's contents and the developing embryo (Broad & Sparks, 1991) on the one hand and on the other hand the exchange of oxygen, carbon dioxide and water across it at a rate commensurate with the embryo's needs (Tullet & Burton, 1985). It also acts as a barrier to the hatching of the ostrich chick. Weak, watery embryos trapped in a low porosity egg may not be able to break through the shell to pip or hatch (Bowsher, 1992). The moment the egg is laid, eggshell porosity is fixed yet it must accommodate changes in blood-gas and acid-base status as incubation progresses with embryo growth and an increased oxygen demand and carbon dioxide production (Tullet & Burton, 1985). Because of diffusive respiration, the ostrich embryo is unable to increase its respiration rate to match its metabolic needs as adult birds do, resulting in eggshell porosity having a significant effect on hatchability. Three factors determine eggshell porosity; the number of pores, their individual cross-sectional area and their length (the thickness of the eggshell) (Tullet, 1984). The ostrich eggshell differs from other avian species in exhibiting the most elaborate pore structure of any species, with a mixture of simple unbranched pores and multiple branching pores (Tullet, 1984).

Problems related to egg quality and embryonic development are relatively well understood and described for most of the economically important domestic species of birds. Although some research has been done on factors affecting hatchability of artificially incubated ostrich eggs, there is still relatively little known about their eggshell characteristics and factors affecting eggshell quality (e.g. number of pores, pore density and shell thickness). Poor eggshell quality in laying hens has resulted in significant economic losses to the poultry industry in many countries (Roberts & Balnave, 1992). Good egg quality is thus very important to ensure successful embryonic development and the hatching of a healthy chick. Factors defining egg quality includes egg size, quality of the eggshell and the internal proportions of the albumin, yolk and air sac (Stadelman & Owen, 1995).

Rodriguez-Navarro (2007) stated that quantification of micro-structural parameters is important to understand changes in the eggshell because of factors such as hen age, diet pollution and moulting. It is well documented that eggshell quality declines and incubation problems increase as laying years progress for poultry (Meir & Ar, 1988; Gunaratne & Boorman, 1996). Tullet & Smith (1983) also reported that flock age brought about changes in egg weight and shell porosity, which alters the humidity necessary to achieve the recommended evaporative water loss during artificial incubation, while heat stress effects reproduction efficiency of female poultry by reducing egg production and eggshell quality (Oguntunji & Alabi, 2010).

Eggshell characteristics are maternally specific and a sample of eggs from one hen may be much more uniform in shell thickness and porosity than eggs from other hens (Tullet, 1984). Petitte & Davis (1999) noted that genetic make-up is one of the factors influencing the performance of individuals, while Stewart (1995) reported that egg quality has a significant genetic component. Embryonic mortality has been shown to be influenced by genotype combination involving the South African Black (SAB) and Zimbabwean Blue (ZB) strains (Brand *et al*., 2007). Although ZB females and crosses of ZB males with SAB females laid

significantly heavier eggs than purebred SAB females, at present it is not clear whether, or how, this difference may be related to shell quality of the eggs produced by the different breed combinations.

The aim of this study was to investigate the effect of environmental and genetic factors such as female age and genotype on pore count, average pore diameter, total pore area, permeability and eggshell thickness of ostrich eggs. As two operators did eggshell measurements, it was also tested whether the records they accumulated were dependent on the operator.

2. Material and Methods

Eggs were collected from the commercial, pair-bred ostrich flock at the Oudtshoorn Research Farm in the Klein Karoo region of South Africa during the 2005 - 2008 breeding seasons. Van Schalkwyk *et al*. (1996) and Bunter & Cloete (2004) previously described the origin of the ostrich flock and the general management procedures implemented. The flock consists of Zimbabwean Blue (ZB) breeding birds mated in various combinations with South African Black (SAB) males and SAB females (Brand *et al*., 2005). During 2007 Kenyan Red Necks (KAR) breeding birds were introduced to the flock and mated with SAB females. Data that were recorded from 2005 thus involved various combinations of the two purebred bloodlines (SAB and ZB) as well as the reciprocal crosses between them, whereas data recorded from 2007 included combinations of a third purebred strain (KAR). Unless specified otherwise, each breeding bird received a ration of 2.5 - 3 kg dry matter/day throughout the breeding season, which lasted from the beginning of June until the end of January for all the years. The exception was the 2008 breeding season when the breeding season started mid-May and ended mid-December.

Eggs were collected daily, weighed and identified by date and paddock (female) of origin. Methods for collection, sanitation and storage at the experimental site are well documented (Van Schalkwyk, 1998; Van Schalkwyk *et al*., 1999; Brand *et al*., 2007). Eggs were artificially incubated at 36 ºC and 24% RH and all incubators were set to turn eggs automatically through 60 - 90° hourly. The capacity and operation of the incubators are described by Cloete *et al*. (2001) and Brand *et al.* (2007).

In a preliminary study Cloete *et al*. (2006b) described the procedure of staining shell fragments and the determination of a number of shell traits on selected shell fragments. In our study similar samples of eggshells were also collected from infertile and dead-in-shell eggs. Four properties of the eggshell were recorded; the number of pores (pore count, PC), the average pore diameter (APD), the total pore area of all the pore clusters in a given area (TPA) and shell thickness (ST). The outside of the eggshell surfaces were dyed with standard grade blue food colorant, rendering the pore clusters on the outside of the shell discernable. The software package, AnalySIS® (Soft Imaging Systems, 1999) was used to perform image analysis on the fractured shells. Images were taken using a digital camera with a standard photographic lens under strong lighting. Shell thickness was measured after the membranes were removed using a digital calliper accurate to 10 µm. These recordings were used to derive the permeability of the barrier (defined as the ratio of pore area relative to shell thickness - PERM) as described by Cloete *et al.* (2006b). Eggshells were stained and measured over a period of four years by two operators.

A total of 14 146 eggs were processed, including infertile eggs, hatched eggs and dead-in-shell eggs. The data were then subjected to least-squares analysis, using ASREML (Gilmour *et al.*, 1999). The software is suitable for fitting a wide range of random effects in animal breeding, while least-squares means for selected systematic effects are predicted simultaneously. Such effects are tested for significance, using an F-test in the analysis of variance table. Fixed effects that were considered included strain (SAB, ZB or KAR), female age (2 - 11 years), assistance during hatching (assisted or not) and the operator measuring the eggshell. Other fixed effects that were considered were year and season. Such effects depend on typical climatic and managerial influences and are often transient and unpredictable, and unlikely to be repeated. These results are thus not presented.

3. Results and Discussion

Ostrich females have a considerably longer productive life than other poultry species, making comparison to traditional domestic avian species for shell quality difficult. The current study showed no significant difference in PC or PERM for eggs produced by females from 2 - 9 years of age, but a significant increase in both PC and PERM occur for females of 10 years and older (Figure 1). These results are consistent with findings by Rahn *et al.* (1981) who reported a significant increase of 20% in pore number and a corresponding 19% in permeability as the laying cycle progressed. An increase in shell porosity was also observed for ducks as the breeding period progressed and the females aged. Eggs from young ducks, in their first laying year, had low shell porosity and showed a reduced hatchability reflecting the inability of embryos to achieve a normal respiratory gas exchange across the low porosity eggshell (Tullet & Smith, 1983).

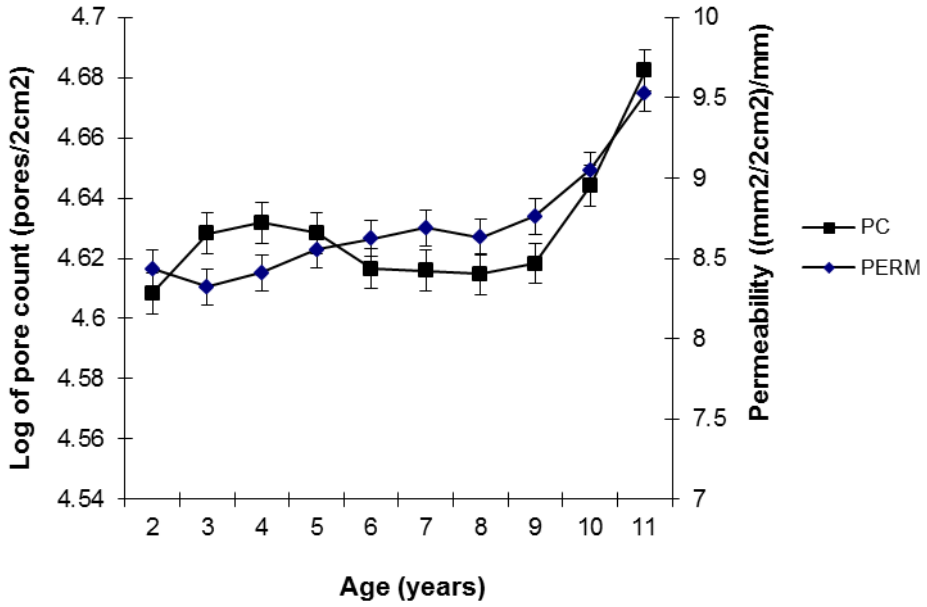

Figure 1 Least-squares means depicting the effect of female age on the log of pore count (PC - pores/2cm^2) and permeability (PERM - mm^2/2cm^2.mm). The figure is based on between 876 records for 9-year-old females to 2651 records for 3-year-old females.

Average pore diameter was independent of female age in this study, but here was a linear increase in TPA in eggs for females from 2 - 11 years (Figure 2). These findings contrast with those of Cloete *et al.* (2006b) who reported that female age had no effect on any of the eggshell traits, based on the analysis of a much smaller sample of hatched ostrich eggs.

Shell thickness increased significantly ($P < 0.05$) from 2-year-old females to females of 3 years and older (Figure 3). ST then remained relatively constant in eggs produced by 3-year to 10-year-old females, where after a marked decrease occurred in ST. Both poultry and turkeys it were found to follow the same pattern in that older hens produce eggs with thinner eggshells, greater conductance, higher water vapour loss and altered oxygen/carbon dioxide exchange (Christensen, 1978; Morris, 1985; Bennett, 1992). Selection for improved eggshell thickness in pullets was hampered, as it was generally done part-way through the first laying year, which was too early to discriminate against birds with a poor shell quality (Morris, 1985). Thin-shelled eggs generally showed a reduced hatchability, with a higher number of embryos that died throughout incubation compared to eggs with thicker shells (Bennett, 1992). At no time did the eggshell appear to be too thick to affect successful hatching and overly thick shells consequently did not appear to be a concern (Bennett, 1992). Previous studies on ostriches indicated that chick production decrease for females older than 10 years, whereas egg production is largely unaffected (Bunter, 2002; Cloete *et al*., 2006a). Brand *et al*. (2007) also reported that embryonic mortality increased significantly for older females. The problem of age-related physiological processes controlling shell formation needs much more attention (Morris, 1985).

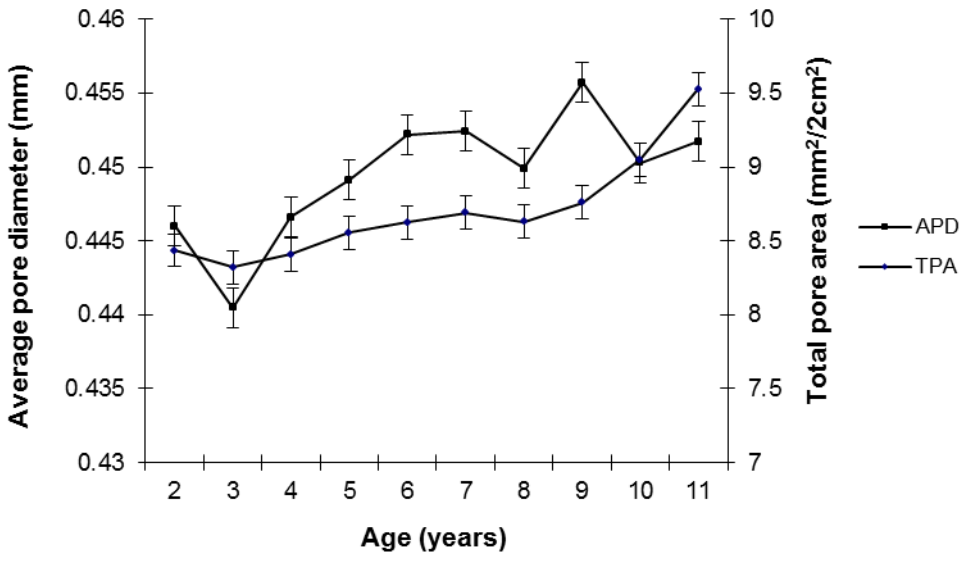

Figure 2 Least-squares means (± S.E.) depicting the effect of female age on the average pore diameter (APD - mm) and total pore area (TPA - $mm^2/2cm^2$). The figure is based on between 876 records for 9-year-old females to 2651 records for 3-year-old females.

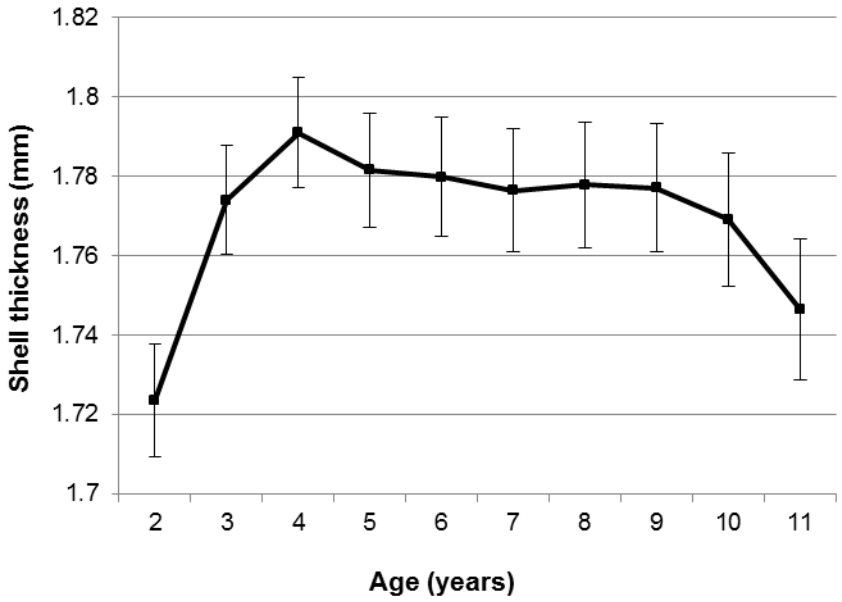

Figure 3 Least-squares means depicting the effect of female age on the shell thickness (mm). Vertical lines about the means denote standard errors. The figure is based on between 876 records for 9-year-old females to 2651 records for 3-year-old females.

Overall, genotype did not affect any of the eggshell traits, except for the pore count (Table 1). SAB females had more pores per 2 cm^2 than ZB females, as well as the different combinations of SAB and ZB and KAR females. Heavier ZB and ZB cross females have previously been shown to produce heavier eggs than their SAB contemporaries (Brand *et al*., 2008), but this does not appear to be reflected in different eggshell characteristics. These results are consistent with Tullet & Board (1977) that indicated that there were fewer pores per cm^2 as egg size increased for avian. Rahn *et al.* (1981) reported an increased egg weight in turkey eggs with progression of the laying cycle linked to a significant increase of 20% in pore number, but this was applicable to only one turkey genotype.

Table 1 Least-squares means (±S.E.) depicting the effect of genotype on eggshell characteristics of ostriches.

Trait	Genotype				
	SAB	**ZB**	**ZB ♂ x SAB ♀**	**SAB ♂ x ZB ♀**	**KAR**
PC (pores/2cm^2)	4.73 ± 0.032[a]	4.62 ± 0.044 [b]	4.60 ± 0.046 [b]	4.58 ± 0.053 [b]	4.57 ± 0.105 [b]
APD (mm)	0.439 ± 0.007	0.447 ± 0.010	0.462 ± 0.010	0.455 ± 0.012	0.454 ± 0.023
TPA (mm^2/2cm^2)	15.6 ±0.469	14.9 ± 0.665	15.5 ± 0.692	14.9 ± 0.816	14.8 ± 1.60
ST (mm)	1.78 ± 0.013	1.74 ± 0.019	1.77 ± 0.020	1.80 ± 0.021	1.81 ± 0.048
PERM (mm^2/2cm^2.mm)	8.78 ± 0.295	8.79 ± 0.412	8.86 ± 0.433	8.42 ± 0.506	8.21 ± 0.965

[a,b]Denote significant ($P < 0.05$) differences in rows

PC = pore count; APD = average pore diameter; TPA = total pore area of all the pore clusters in a given area; ST = shell thickness; SAB = South African Black; ZB = Zimbabwean Blue; KAR = Kenyan Red Necks.

No significant difference could be found between eggshell traits for eggs with chicks that required assistance to hatch at 43 days of incubation and chicks that hatched without outside assistance. In contrast, Bowsher (1992) reported that ostrich eggshells for chicks requiring assistance were significantly thicker (1.91mm) than shells of the unassisted group (1.81 mm). In support of the present study, Khalifa *et al.* (2006) found no significant difference in mean eggshell thickness or mean eggshell porosity when comparing eggs that hatched or failed to hatch. The only measurement affected significantly by operator was ST (1.76 $\pm$ 0.02 mm for operator 1 vs. 1.80 $\pm$ 0.01 mm for operator 2; $P < 0.05$).

As one operator processed most samples towards the later part of the study, this effect may have been a function of any of a host of environmental factors more prevalent recently.

4. Conclusion

Pore count, permeability as well as shell thickness were negatively affected in eggs of older females. These results suggest culling of older females from the breeding flock may be beneficial to eggshell quality. It seems that different genotypes may have a small but significant influence on pore count of ostrich eggs. It is unsure if this would compromise the standard practice of incubating eggs from different genotypes in the same incubator. It is thus important to consider all these aspects when planning breeding flock structure and incubation procedures.

5. Acknowledgements

We want to thank Schalk Cloete (Jr) for developing the technique for measuring traits in ostrich eggshells and Dr Ansie Scholtz for the maintenance of the image analyses infrastructure. Further, we want to thank Henri Cloete and Reinhardt Biermann for measuring all the eggshell traits for this study.

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CHAPTER 15

Genetic parameters for eggshell traits in ostriches

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(Brit. Poultry Sci. *accepted for publication*)

Abstract.

This study was conducted on around 14 000 ostrich eggs to estimate genetic parameters for eggshell traits that could potentially benefit the hatchability of ostrich eggs. Traits considered were the number of pores on the eggshell, the average diameter of these pores, the total area of pores on the eggshell, permeability (pore area/shell thickness) and eggshell thickness. Heritability estimates ranged from 0.16 for total pore area to 0.41 for the natural logarithm of pore count. The heritability estimates for water loss (WL) on 21 and 35 days of incubation was high at 0.23 and 0.24, respectively. On a genetic level, pore count was negatively correlated with average pore diameter (-0.73) and shell thickness (-0.28), whereas it was positively correlated with total pore area (0.58), WL21 (0.24) and WL35 (0.34). The direct and maternal genetic correlations of pore count with total pore area (0.58) and permeability (0.59) were high and significant. Permeability was positively correlated to WL21 and WL35, both on the direct and maternal genetic levels. Parameters indicate that it should be possible to select for the various eggshell traits in ostrich eggs, or for permeability and water loss. As a trait with an intermediate optimum, permeability would need complicate selection strategies. The possible application of these results to improve hatchability of ostrich eggs in the future needs consideration.

1. Introduction

The genetic make-up of individual animals is one of the factors influencing the production performance in commercially bred species. By selecting for certain desirable traits, genetic improvement can be achieved in breeding stock (Petitte & Davis, 1999). Extensive research has been carried out on selective breeding to improve production traits in species of common domestic livestock, thus making genetic parameters, as well as line and breed differences for these livestock species, readily available. Genetic selection and highly developed management practices have greatly improved the efficiency of meat and egg production in modern broilers (Boerjan, 2004), for example the rearing period decreased from 84 day to 42 days, while selection also resulted in larger eggs with a decreased yolk volume. In contrast, little is known about either genetic parameters or responses to selection for specific traits in ostriches. According to Cloete *et al.* (2002; 2008) definite breeding objectives and industry breeding structures are largely absent because previous selection was largely based on feather traits at a time in the early 1900's when this feathers were the main commercial product. Since, no concerted efforts were made towards genetic improvement in ostriches, and Van Schalkwyk *et al.* (1996) and Cloete *et al.* (1998) were the first to report estimates for repeatability of, and phenotypic correlations among, reproductive traits in the species. Estimates of genetic parameters for egg, chick and reproductive traits are still limited to a few studies (Bunter *et al.*, 1999; 2001; Bunter & Cloete, 2004; Cloete *et al.*, 2004; 2005; Lambrechts, 2004) and genetic parameters for ostrich incubation traits have only recently been estimated by Brand *et al*. (2008a; 2009).

Egg quality is also reported to have a significant genetic component in ostriches, while shell traits directly affect these through their effect on water loss and gas exchange (Button *et al*., 1994; Stewart, 1995). Embryonic mortality during artificial incubation represents a major loss of productivity to the industry and much of this is related to incorrect water loss and possibly gas exchange during incubation. The eggshell allows for the conduction of respiratory gases and heat to the developing embryo and also protects the embryo from dehydration by conserving water resources within the shell (Bowsher, 1992). Eggshell conductance is greatly influenced by porosity and shell thickness (Gonzalez *et al*., 1999). The pores, minute openings in the shell, determine the potential for respiratory gas exchange and water vapour conductance across the shell.

The majority of birds produce eggshells with simple, funnel-shaped single pores (Ar & Rahn, 1985). In ratite eggs, however, pores are branched to accomplish this function and to provide additional ventilation for the significantly larger embryo. Developing ostrich embryos obtain their oxygen entirely by diffusion through pores in the shell. Because of diffusive respiration, the ostrich embryo is unable to increase its respiration rate to match its metabolic needs as adult birds do. Necropsy results reveal that a significant proportion of late embryonic mortality appears to stem from suffocation in oedematous embryos (Gonzalez *et al*., 1999). The movement of respiratory gases is restricted and the late embryo suffers a shortage of oxygen at the time of its greatest need and dies of hypoxia. This is more important than excess carbon dioxide (hypercapnia), which the embryo seems to be able to tolerate. Eggshell porosity therefore will influence hatchability of the near-term developed embryo, as it determines the potential for an egg to exchange respiratory gases and

water during incubation. Very porous shells will lose excessive amounts of water and result in dehydration of the embryo, whereas eggs with a reduced porosity lose too little water (Ar, 1991). This may cause oedema (water retention) in ostrich embryos, which makes hatching more difficult (Brown *et al*., 1996; Badley, 1997). Swart *et al.* (1987) reported an increase in water loss of 20% during the latter half of incubation and Ar (1991) suggested that the change in water loss can be due to metabolic heat production that increases egg temperature and water vapour pressure inside the egg, thus altering the diffusion gradient. The oedematous embryos trapped in a low porosity egg may not be able to break through the shell to pip or to hatch (Bowsher, 1992).

Results from a number of studies of artificially incubated ostrich eggs indicate that water loss during incubation is an important contributing factor to the successful hatching of chicks (Swart *et al.*, 1987; Swart & Rhan, 1988; Blood *et al.*, 1998; Brand *et al*., 2008b). There are also indications that water loss is closely related to some measurable eggshell parameters (Gonzalez *et al*., 1999). The number of pores on the eggshell, the average diameter of these pores, the total area of pores on the eggshell and eggshell thickness were identified as possible influential factors in the successful hatchability of ostrich eggs. The aim of this study therefore was to estimate genetic parameters for traits associated with eggshell porosity.

2. Material and Methods

Eggs were collected from the commercial ostrich breeding flock maintained at the Oudtshoorn Research Farm in the Klein Karoo region of South Africa. The origins of the ostrich flock and general management procedures implemented have been described previously (Van Schalkwyk *et al*., 1996; Bunter & Cloete, 2004). Eggs for this study were collected during the 2005 - 2008 breeding seasons. Unless specified otherwise, each breeding bird received a ration of 2.5 to 3 kg dry matter/day throughout the breeding season, which lasted from the beginning of June until the end of January for the first three years. The exception to this was the 2008 breeding season when the season started mid-May and ended mid-December.

Eggs were collected daily, weighed and identified by date and paddock (female) of origin. Methods for collection, sanitation and storage at the experimental site are well documented (Van Schalkwyk *et al*., 1998; Van Schalkwyk *et al*., 1999; Brand *et al*., 2007). Eggs were artificially incubated at 36 ºC and 24% RH in Buckeye®, Prohatch® or African International® incubators and all incubators were set to turn eggs automatically through 60 - 90° hourly. The capacity and operation of the incubators are described by Cloete *et al*. (2001) and Brand *et al.* (2007). Eggs were candled and weighed on days 21 and 35 of incubation. Together with initial egg weight, these weights were used to derive rate of water loss, expressed as percentages of the initial egg weight, up to 21 and 35 days of incubation. On day 35 of incubation, the eggs were moved from the setters to a hatcher, which also operated at 36 °C and a RH of 24%. Eggs were set vertically in the hatcher with their air sacs positioned upwards and were not subsequently turned.

Bowsher (1992) found no statistical difference in pore density for the top (air cell side) and side of ostrich eggshells. These results were verified by Cloete *et al*. (2006) on a larger sample of eggs (> 500). Cloete *et*

al. (2006) also found that measurements for the traits average pore diameter, total pore area and shell thickness were effectively the same trait on the female level irrespective of where the measurement was taken. Since it is not possible to obtain whole fresh eggs to measure eggshell conductance, Portugal *et al.* (2010) conducted a test on egg fragments and concluded that using egg fragments is an efficient way of measuring interspecific water vapor conductance. As a result, a 2 cm^2 area from a selected piece of eggshell from the air cell area of each egg was collected after hatching, stored and used for the measurements. Similar samples of eggshells were also collected from infertile and dead-in-shell eggs. Measurements were converted to a 1 cm^2 area to facilitate comparison with previous studies. In the preliminary study by Cloete *et al*. (2006), the procedures of staining the eggshell sections to make the pores visible, as well as the method of analysis, were described.

Briefly, the outside of the eggshell surface was dyed with standard grade blue food colorant, rendering the pore clusters on the outside of the shell easily discernable (Gonzalez *et al.*, 1999; Sahan *et al*., 2003; Amer, 2005). The food colorant made the pore clusters on the outside of the shell clearly discernable for the image analysing software, as can be observed in Figure 1. The software package AnalySIS® (Soft Imaging System, 1999) was used to do image analysis on the fractured eggshells. Images were obtained by using a specialized digital camera with a standard photographic lens under strong illumination. From these images, the software was used to calculate the parameters: pore count (PC), average pore diameter (APD) and the total pore area of all the clusters in a given area (TPA). The shell thickness (ST) was measured using a digital caliper accurately to 10 *μm* after first removing the membranes. These measurements were used to derive the permeability of the shell barrier (defined as the ratio of pore area to shell thickness) as described by Cloete *et al.* (2006).

A total of 14 146 eggs were processed, including infertile eggs, hatched eggs and dead-in-shell eggs. The data were then subjected to genetic analysis, using ASREML (Gilmour *et al*., 1999). Fixed effects that was considered included parent lines (South African Black (SAB), Zimbabwean Blue (ZB) or Kenyan Red Necks (KAR)), year-season effect (winter, spring or summer), female age (2 to 11 years), chicks assisted during hatching by cracking eggshell and operator measuring the eggshell. The random effects that were fitted included the direct genetic effect of the animal (h^2; fitted as default for all traits), maternal genetic effects (m^2) and the female permanent environment (c^2). Random terms were added to analytical models sequentially. Likelihood Ratio Tests (LRT) was used to assess the significance of the contribution of each random term to improvements in the model. The LRT is based on testing the increase in double the Log-likelihood resulting from adding an additional random term to the model of analysis as a Chi^2 statistic. When two models included the same number of random terms, the model with the higher value for the Log-likelihood was preferred. Initially, variance components for each trait were estimated in single-trait analyses. ASREML estimates variance components for mixed models by residual maximum likelihood, employing an average information algorithm that concurrently provides estimates of standard errors for parameters (Gilmour *et al*., 1995). A series of two-trait analyses was then performed to estimate correlations among traits. Traits considered were: the pore count for the clusters (PC), the average diameter of the pore opening on the surface of the eggshell (APD), the total pore area of all the clusters in a given area (TPA), the shell thickness

(ST) and permeability (PERM), percentage water loss up to 21 days of incubation (WL21%) and percentage water loss up to 35 days of incubation (WL35%) percentage.

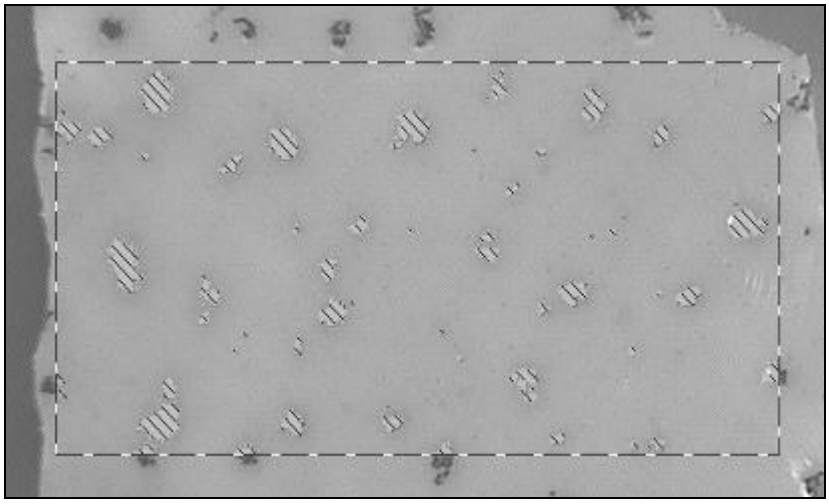

Figure 1 The porous area on an eggshell as identified with AnalySIS®.

3. Results

Descriptive statistics for the eggshell parameters for individual eggs are presented in Table 1. The pore number ranged between 55.0 and 57.8 pores per square centimeter (cm^2) with an average of 57.1. Average pore diameter ranged between 0.10 and 0.46 mm^2 and the coefficient of variation (CV) amounted to 18.2%. The shell thickness ranged between 1.02 and 2.35 mm, with the lowest CV of 7.95%. The mean permeability of the eggshell was 4.20 (mm^2/cm^2)/mm, with high CV of 38.0%. Means for percentage water loss at 21 days and at 35 days of incubation were 7.69% and 12.57% respectively. Corresponding CV's were 25.31% for WL21 and 24.55% for WL35. A transformation to natural logs partially normalised the distribution for the number of pores.

Log-likelihood values under alternative random effects models are presented in Table 2. The addition of the direct additive effect as a single random source of variation resulted in significant improvements in the LRT throughout. When further random effects were added, the LRT indicated that the model for all the traits, PC, APD, TPA, ST, PERM, WL21 and WL35, should include only direct additive and maternal additive effects as random sources of variation.

Table 1 Descriptive statistics for the different shell traits measured of the individual eggs.

Location and trait	Number of traits	Mean ± SD	CV (%)	Skewness	Kurtosis	Min	Max
PC (pores/cm^2)	14146	57.1 ± 27.0	0.470	5.29	58.26	5.50	578
APD (mm^2)	14146	0.44 ± 0.08	18.18	0.25	0.22	0.19	0.92
TPA (mm^2/cm^2)	14145	7.38 ± 2.75	37.26	1.17	3.52	0.05	32.04
ST (mm)	14142	1.76 ± 0.14	7.95	0.001	0.25	1.02	2.35
PERM ((mm^2/cm^2)/mm)	14141	4.20 ± 1.60	37.98	1.38	4.62	0.03	18.44
WL21 (% egg weight)	13833	7.69 ± 1.94	25.31	1.25	5.63	2.00	31.20
WL35 (% egg weight)	12474	12.57 ± 3.01	24.55	1.09	3.94	2.70	42.25

S.D. = standard deviation, CV% = coefficient of variation, PC = pore count, APD = average pore diameter, TPA = total pore area, ST = shell thickness, PERM = permeability, WL21 = water loss at 21 days of incubation, WL35 = water loss at 35 days of incubation.

Table 2 Log-likelihood values for the respective traits under different random effects models, with the best model for each trait represented in bold italic figures.

Trait	Fixed only	+ h^2 (Model 1)	+ h^2 + m^2 (Model 2)	+ h^2 + c^2 (Model 3)	+ h^2 + m^2 + c^2 (Model 4)
PC	8372.41	10982.71	**11016.2**	11009.8	11016.30
APD	939.20	2179.04	**2274.68**	2262.77	2274.99
TPA	-1046.82	-9022.40	**-8954.68**	-8956.53	-8953.01
ST	1921.18	6628.44	**6826.49**	6814.75	6826.90
PERM	-3248.78	-1103.91	**-1041.83**	-1040.50	-1040.65
WL21	-5498.51	-3149.84	**-3069.30**	-3072.28	-3067.61
WL35	-9590.47	-7197.99	**-7110.44**	-7112.42	-7108.56

PC = pore count, APD = average pore diameter, TPA = total pore area, ST = shell thickness, PERM = permeability, WL21 = water loss at 21 days of incubation, WL35 = water loss at 35 days of incubation

Variance components and ratios were initially estimated for all the traits in single-trait analyses (Table 3). Derived two-trait variance ratios for all traits were consistent, with differences between duplicate estimates seldom exceeding 0.01, and estimates being generally similar to those estimated from single-trait analyses (Table 4). Heritability (h^2) estimates were high for most eggshell traits and ranged between 0.22 for total pore area per unit shell thickness to 0.42 for pore count. The exception was TPA with a somewhat lower h^2 of 0.16 ± 0.04. The h^2 estimates for WL21 and WL 35 was high at 0.23 ± 0.04 and 0.24 ± 0.05 respectively. All heritability estimates were significant (at least double the corresponding S.E.). The maternal genetic effect (m^2) was highly significant for all traits, ranging between 0.25 ± 0.04 for PC and 0.48 ± 0.03 for ST.

Table 3 Estimates of variance components and ratios (S.E.) for traits as derived from single-trait analyses.

Effect	PC	APD	TPA	ST	PERM	WL21	WL35
Components							
σ^2_a	0.055	0.00143	5.470	0.00662	2.55	0.859	2.389
σ^2_m	0.033	0.00202	11.381	0.00969	3.83	1.31	3.702
σ^2_p	0.131	0.00621	34.620	0.02000	11.77	3.98	10.12
σ^2_e	0.043	0.00276	17.770	0.00374	5.39	1.81	4.03
Ratios							
h^2	0.42 ± 0.07	0.23 ± 0.05	0.16 ±0.04	0.33 ± 0.05	0.22 ± 0.05	0.23 ± 0.04	0.24 ± 0.05
m^2	0.25 ± 0.04	0.33 ± 0.03	0.33 ± 0.03	0.48 ± 0.03	0.33 ± 0.03	0.33 ± 0.03	0.37 ± 0.03

σ^2_a = direct additive genetic variance, σ^2_m = maternal additive genetic variance, σ^2_p = phenotypic variance, σ^2_e = environmental (residual) variance component, h^2 = direct heritability, m^2 = maternal heritability; PC = pore count, APD = average pore diameter, TPA = total pore area, ST = shell thickness, PERM = permeability, WL21 = water loss at 21 days of incubation, WL35 = water loss at 35 days of incubation.

The negative genetic correlation (r_g) of pore count with pore diameter was high and significant (-0.58 ± 0.10), indicating that eggs with more pores will generally have smaller pores. Pore count was positively and significantly correlated on a genetic level with TPA and PERM at 0.58 ± 0.10 and 0.59 ± 0.09 respectively. Increased PC will lead to a larger pore area with an increased permeability. Although the r_g of PC with shell thickness was also negative, it did not quite reach a magnitude of double the corresponding standard error at -0.23 ± 0.12. The r_g of average pore diameter was positive with all traits. TPA was highly correlated with PERM (0.95 ± 0.01) suggesting a very close relationship between the two traits. The genetic correlations of shell thickness with both PERM and water loss indicates that with thinner shells, increased permeability occurred, resulting in higher water loss. This is also confirmed by the high r_g of PERM with WL21 and WL35. The r_g of WL21 with WL35 was unity, suggesting that it was essentially the same trait.

The maternal genetic correlations (r_m) were mostly similar to genetic correlations in sign and magnitude. The correlation of pore count with pore diameter was high and negative at -0.40 ± 0.08, confirming that shells with many pores would have smaller pores on the maternal genetic level. The r_m for pore count was moderate to high and positive among TPA, WL21 and WL35, ranging between 0.36 ± 0.09 and 0.54 ± 0.07. With the exception of shell thickness, the correlation on the maternal level between APD and all the other traits was moderate to high and ranged between 0.27 ± 0.09 and 0.50 ± 0.07. The r_m estimates of TPA and PERM with WL21 and WL35 were both high and the identical at 0.51 ± 0.07 and 0.48 ± 0.07 respectively, demonstrating the link between permeability and water loss. The maternal genetic correlation of WL21 and WL35 was unity, again pointing to these traits being controlled by the same genes on the maternal genetic level.

Table 4 Estimates of heritability (h^2 ± S.E.), maternal genetic effects (m^2 ± S.E.) and correlations between these effects, residual and phenotypic variance and correlations between the eggshell traits, water loss to 21 days and water loss to 35 days from multi-trait analyses.

Traits	PC	APD	TPA	ST	PERM	WL21	WL35
Additive genetic correlations (h^2 in bold)							
PC	**0.41 ± 0.07**	-0.58 ± 0.10	0.58 ± 0.10	-0.23 ± 0.12	0.59 ± 0.09	0.24 ± 0.12	0.34 ± 0.12
APD		**0.24 ± 0.05**	0.30 ± 0.15	0.30 ± 0.12	0.16 ± 0.15	0.10 ± 0.14	0.06 ± 0.14
TPA			**0.16 ± 0.04**	-0.01 ± 0.15	0.95 ±0.01	0.34 ± 0.14	0.42 ± 0.13
ST				**0.33 ± 0.05**	-0.31 ± 0.13	-0.34 ± 0.12	-0.28 ± 0.12
PERM					**0.23 ± 0.05**	0.47 ± 0.12	0.54 ± 0.01
WL21						**0.25 ± 0.05**	1.00 ± 0.00
WL35							**0.26 ± 0.05**
Maternal genetic correlations (m^2 in bold)							
PC	**0.25 ± 0.04**	-0.40 ± 0.08	0.54 ± 0.07	0.05 ± 0.09	0.53 ± 0.07	0.38 ± 0.09	0.36 ± 0.09
APD		**0.32 ± 0.03**	0.50 ± 0.07	0.15 ± 0.08	0.47 ± 0.07	0.27 ± 0.09	0.29 ± 0.08
TPA			**0.32 ± 0.03**	0.11 ± 0.08	0.97 ± 0.01	0.51 ± 0.07	0.48 ± 0.07
ST				**0.48 ± 0.09**	-0.13 ± 0.08	0.02 ± 0.08	0.02 ± 0.08
PERM					**0.32 ± 0.03**	0.51 ± 0.07	0.48 ± 0.07
WL21						**0.33 ± 0.03**	1.00 ± 0.00
WL35							**0.36 ± 0.03**
Residual correlation (σ^2_e in bold)							
PC	**0.05**	-0.33 ± 0.04	0.53 ± 0.03	0.34 ± 0.08	0.50 ± 0.04	0.18 ± 0.05	0.15 ± 0.06
APD		**0.0027**	0.51 ± 0.03	-0.05 ± 0.06	0.54 ± 0.04	-0.03 ± 0.04	-0.01 ± 0.05
TPA			**17.54**	0.19 ± 0.06	0.99 ± 0.01	0.15 ± 0.03	0.16 ± 0.04
ST				**0.0038**	0.09 ± 0.07	0.07 ± 0.07	0.05 ± 0.07
PERM					**5.32**	0.13 ± 0.04	0.14 ± 0.04
WL21						**1.82**	0.90 ± 0.01
WL35							**4.00**
Phenotypical correlation (σ^2_p in bold)							
PC	**0.13**	-0.42 ± 0.02	0.53 ± 0.02	0.03 ± 0.03	0.53 ± 0.02	0.25 ± 0.02	0.27 ± 0.03
APD		**0.0063**	0.46 ± 0.02	0.13 ± 0.03	0.43 ± 0.02	0.10 ± 0.03	0.11 ± 0.03
TPA			**34.82**	0.10 ± 0.03	0.97 ± 0.01	0.30 ± 0.02	0.32 ± 0.02
ST				**0.0203**	-0.11 ± 0.03	-0.07 ± 0.03	-0.06 ± 0.03
PERM					**11.52**	0.33 ± 0.02	0.35 ± 0.02
WL21						**3.97**	0.96 ± 0.01
WL35							**10.07**

PC = pore count, APD = average pore diameter, TPA = total pore area, ST = shell thickness, PERM = permeability, WL21 = water loss at 21 days of incubation, WL35 = water loss at 35 days of incubation.

With the exception of APD, the residual correlations (r_e) between PC and the rest of the traits was positive and ranged between 0.15 ± 0.06 and 0.53 ± 0.03. The r_e of APD was also small in magnitude and negative

with ST, WL21 and WL35%. The r_e correlation between TPA and PERM was again high at 0.99 ± 0.01. All residual correlations with shell thickness were low and positive, ranging between 0.05 ± 0.07 and 0.09 ± 0.04. WL21 was highly correlated with WL35 on the residual level (0.90 ± 0.01). The phenotypic correlation (r_p) between PC and APD was high and negative at -0.42 ± 0.02, while PC was positively correlated to TPA at 0.53 ± 0.02. For the rest of the traits, the r_p were mostly similar in sign to r_e, but in most cases they were somewhat larger in magnitude.

4. Discussion

With the exception of PC, the deviations of the distribution were because of kurtosis and not resulting from skewness. The distribution for PC was normalised by transforming data to natural logs. After this transformation, analyses and the interpretation of the results was continued without attempting further transformations to improve the properties of the distribution (Glass *et al.*, 1972). The mean large pore number from previous studies by Gonzalez *et al*. (1999) and Sahan *et al*. (2003) was between 8.9 and 11.2 large pores per cm^2, while Bowsher (1992), Christensen *et al.* (1996), and Cloete *et al*. (2006) reported mean pore counts of 21.89, 17.7 and 22.02 pores per cm^2 respectively. Results from the present study showed a much higher mean pore counts of 57.1 ± 27.0 pores per cm^2. The large difference in results can be atributed to the fact that most previous studies focused only on large pores on a cm^2 area, whereas all pores were considered in the present study, irrespective of size. The minimum pore size was 100 µm in the present study. Pore diameter and pore area are in line with previous results from Cloete *et al*. (2006). The shell thickness in our study corresponds with those reported prevoiusly at 1.92 mm (Brown *et al*., 1996); 1.8 - 1.88 mm (Gonzalez *et al*., 1999); 1.64 - 2.10 mm (Sahan *et al*., 2003); 1.28 - 2.12 (Cloete *et al*., 2006). The mean permeability of the eggshell was slightly lower at 4.2 (mm^2/cm^2)/mm compared to the 5.37 (mm^2/cm^2)/mm derived from the study by Cloete *et al*. (2006). Results from our study showed a water loss of around 13% to 35 days of incubation, which is consistent with previous studies on ostriches (Swart & Rahn, 1988; Blood *et al*., 1998; Brand *et al.,* 2008a; b).

Variance components were significant for all parameters assessed, leading to medium to high h^2 and m^2 estimates, indicating that significant genetic variation (direct and maternal) existed for all traits. Tullet (1984) stated that shell characteristics are maternally specific and that a sample of eggs from one hen will be much more uniform in shell thickness and porosity than eggs collected from different hens. No previous results involving genetic parameters for ratites or other avian species could be sourced from the literature for comparison with the present study. However, when these estimates were totalled to get an indication of repeatability, estimates ranging from 0.49 for total pore area to 0.71 for shell thickness were derived. Cloete *et al*. (2006) observed comparable medium to high repeatability coefficients for eggshell parameters (ranging from 0.43 for pore count to 0.72 - 0.73 for shell thickness, but their study did not support the partitioning of animal effects into direct and maternal genetic effects. Farzin *et al.* (2006) reported a heritability estimate for shell thickness of 0.68 in laying hens. This coincides with the results from our study, but both estimates are higher than the heritability of 0.33 reported by Johansson *et al.* (1996). The h^2 estimates for WL21 and WL 35 corresponded with findings from Brand *et al*. (2009). For understandable reasons, these estimates were somewhat lower than those of Brand *et al*. (2008a), where these traits were assessed as traits of the female.

When estimates for water loss were combined as repeatability estimates, these estimates also confirmed reports from Blood *et al.* (1998) that water loss from ostrich eggs was highly repeatable within a production season. Previous studies suggest that WL is affected by egg traits which include shell thickness and shell porosity (Soliman *et al*., 1994; Sahan *et al*., 2003; Cloete *et al*., 2006).

The negative genetic correlation between pore count and average pore diameter corresponds with a between-female correlation reported by Cloete *et al*. (2006), where it was impossible to partition the between female covariance into direct and maternal components. This correlation confirms that pore diameter has to decrease as the total number of pores increases if permeability to water vapour is to remain the same. There were a lack of significant direct and maternal genetic correlations between pore number and shell thickness. It is possible that this phenomenon could be related to findings by Satteneni & Satterlee (1994), who suggest that these two traits could be affecting hatchability independently. The part-whole relationship shown by Cloete *et al*. (2006) is confirmed by high positive direct and maternal genetic correlations between TPA and APD. As expected, an increase in either pore size or pore counts would result in an increase of the total pore area. Adequate gas exchange is especially important for embryos during the last days of incubation when the oxygen requirement increases (Van Schalkwyk *et al*., 2002).

Although we found a positive r_g between PC and water loss in our study, it was not as high as a corresponding phenotypic correlation reported by Gonzalez *et al*. (1999), and only significant for WL35 days of incubation. During incubation, water is lost across the eggshell at a rate that depends on the number and area of pores, the shell thickness and the water vapor pressure gradient between the inside of the egg and the environment (Brown *et al*., 1996). Tullet (1984) observed that functional eggshell porosity is precisely matched to the size of the egg, the time required for incubation and the metabolic rate of the embryo. This precise matching is important for the overall water budget during incubation. The transfer of gases across the eggshell is best described by Fick's Law, which implies that diffusion through a pore is proportional to the surface area of the aperture, but inversely proportional to the length of the pore (Rahn *et al.*, 1987). If inadequate water loss in the egg is due to a shell with a low porosity, the embryo may also be subject to suboptimal exchange of respiratory gases. This has been suggested to be the cause of many embryonic deaths in turkeys with retarded development, especially late in the incubation period (Bagley *et al*., 1988). High water loss is usually associated with eggs with shells that have higher than normal levels of porosity. Gas exchange is usually not a problem but, due to the increased amount of water vapor escaping through the pores, the egg may dehydrate too quickly (Bowsher, 1992). The positive r_g between pore density and water loss in our study corresponds with earlier findings by Satteneni & Satterlee (1994); Gonzalez *et al*. (1999) and Sahan *et al*. (2003), indicating that water loss (on the phenotypic level) during incubation, increases as pore density increases.

In accordance with previous reports (Brown *et al.*, 1996; Gonzalez *et al*., 1999; Sahan *et al*. 2003) shell thickness was negatively correlated on both the direct and maternal genetic level with water loss. Ostrich eggs with thick shells have longer pore lengths. A low number of pores in such eggs, and thus a reduction in total pore area, will result in poor hatchability, because such eggs are likely to suffer from a reduced eggshell

conductance (Gonzalez *et al*., 1999). This observation may explain why ostrich embryos experience a high incidence of death during the last few days of incubation. Corresponding with our findings, Cloete *et al*. (2006) found that shell thickness had no strong correlation between females with any of the other eggshell traits. Sahan *et al*. (2003) reported that low water vapour shell conductance was associated with both a low rate of water loss and insufficient diffusion of respiratory gases. This contention is supported by the high and significant r_g and r_m between permeability and water loss to 21 and 35 days of incubation in the present study. The lowest embryonic losses were sustained in eggs exhibiting intermediate levels of water loss (Blood *et al*., 1998). Ostrich egg losing between 11 - 14% of their initial weight should produced day-old chicks weighing approximately 60% of the initial egg weight (Bunter & Cloete, 2004; Brand *et al*., 2008b). These results are similar to the day-old chick weighing 68.7% of fresh egg weight in hatchlings from chicken eggs with water loss of 12% (Tullet & Burton, 1982). Christensen *et al.* (1996) suggested that incubator humidity for ostrich eggs should be less than 25% to allow for a 15% loss of initial egg weight during a 42 day incubation period. Matching the humidity of the incubator to eggshell conductance may be a useful way to optimise water loss and to maximise embryo survival (Sahan *et al*., 2003). It is conceded that the variation observed in eggshell traits will make it exceedingly difficult to apply this principle in practice, as incubators set to deliver a range of moisture loss regimes should be available. The mismanagement of water available to the embryo will have a cumulative effect that will manifest itself mainly towards the end of incubation when changes associated with hatching are about to take place. These changes include a high metabolic rate, embryonic activity as the chick moves into the correct hatching position and starts to pip, absorption of the yolk sac and a series of anatomical and physiological changes associated with the transition from diffusive to convective respiration (Meir *et al*., 1984).

5. Conclusion

The medium to high repeatability estimates for eggshell traits in the present study were partitioned into direct and maternal genetic components. Medium to high h^2 estimates indicate that it will be possible to select females for an improved eggshell quality, with the intention of improving overall hatchability of ostrich eggs. With traits like permeability and water loss having an intermediate optimum, selection strategies would, however, be complicated and the method of selection will need to be refined in further studies.

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CHAPTER 16

Heritability of embryonic mortalities in ostrich eggs and factors affecting hatchability failure of fertile eggs during artificial incubation

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Abstract

The high rate of embryonic mortality during artificial incubation of ostrich eggs is a major concern in the ostrich industry. Data from 48 126 individual egg records were available to derive genetic parameters for embryonic mortalities, modelled as a trait of the individual egg. Embryonic mortality was classified according to stage of death, i.e. early embryonic mortality that occurred before 21 days of incubation (EEM), late embryonic mortality that occurred after 21 days of incubation (LEM) and overall embryonic mortalities (OEM). LEM increased significantly for eggs laid by females > 10 years old. Transfer of eggs between incubators during incubation also impaired hatchability. An increase in OEM occurred for eggs freshly set (43%) as well as for eggs stored for more than 6 days (50%). Medium to high heritability estimates were derived for all the embryonic death traits and ranged between 0.16 ± 0.02 for LEM and 0.22 ± 0.03 for EEM. The dam permanent environmental effect was low but estimated with low standard errors and was thus significant for all traits, ranging between 0.021 ± 0.005 for LEM and 0.046 ± 0.008 for EEM. Hatchability of fertile ostrich eggs may consequently be improved by removing older females from breeding flocks, setting of eggs between 2 and 6 days after collection, and by refraining to transfer of eggs between incubators during incubation. Moderate h^2 estimates indicate that breeding may be used as a tool to enhance chick production in ostriches. This contention is supported by the fact that selected breeding for chick production and live weight appeared to result in genetic changes in embryonic mortality rates.

1. Introduction

In commercially reared domesticated avian species, the ideal scenario is for every fertile egg incubated to produce a healthy chick. Hatchability of artificially incubated ostrich eggs is, however, low at between 30 - 60% (Deeming, 1995; Brown *et al.*, 1996), with losses of chicks because of embryonic mortality may exceed 30% of fertile eggs set (Brand *et al*., 2007). Embryonic mortality, usually during the early and late incubation periods (Badley, 1998), thus contributes substantially to hatching failure, and it is evident that there is still considerable room for improvement in the ostrich industry.

Successful artificial incubation depends upon good egg quality and proper egg management during the pre-incubation and incubation periods, whilst optimal conditions for incubation and hatching need to be adhered to (Badley, 1997). Even small improvements in the hatchability of the ostrich eggs can potentially result in important economic gains. Optimal conditions during artificial incubation, like the correct incubator temperature (French 1997; Van Schalkwyk *et al.*, 1999a), humidity (Swart *et al.*, 1987; Blood *et al.*, 1998), gaseous environment (Van Schalkwyk *et al.*, 2002) and the turning of eggs (Van Schalkwyk *et al.*, 2000) are all crucial for maximum hatchability.

The productive economic life of female ostriches is considerably longer than for other poultry species (Ipek & Sahan, 2004), thus making it difficult to compare ostrich breeding to the smaller domestic poultry species traditionally used for egg production. Egg production of female ostriches starts at 2 - 2.5 years, with a peak in both egg- and chick production at 8 - 9 years, whereafter it declines, more so for chick production than for egg production (Cloete *et al.*, 2006). The proportion of embryonic mortality also accelerates for females older than 10 years (Brand *et al*., 2007; 2009).

Egg storage prior to incubation also affects hatching success, with both fresh eggs and eggs stored for periods > 7 days experiencing impaired hatchability (Benton & Blake, 1996). Morphological changes take place in the blastoderm during the prolonged storage of eggs, which leads to a lower growth rate of the embryo in other domestic poultry species (Fasenko *et al*., 1992; Meijerhof, 1992). For the best hatchability, and to prevent any early blastoderm development, the recommended storage temperature for ostrich eggs is ~17 C° (Van Schalkwyk *et al.*, 1999b; Sahan *et al.*, 2003; Malecki *et al*., 2005). Deeming & Ar (1999) reported that the lower hatchability of ostrich eggs could be ascribed to an increase in early embryonic mortalities for eggs stored between 12 and 14 days, while both Ar & Gefen (1998) and Sahan *et al.* (2004) reported increased embryonic mortalities for eggs stored for extensive periods of > 20 days.

Genetic factors can also influence the performance of individuals (Petitte & Davis, 1999). Currently, estimates of genetic parameters for egg, chick and reproductive traits in ostriches are still limited to only a few studies (Bunter *et al.*, 1999; 2001; Bunter & Cloete, 2004; Cloete *et al.*, 2004; 2005; Lambrechts, 2004). In all these studies reproduction was modelled as a trait of the female. Stewart (1995) suggested that egg quality in ostriches has a significant genetic component. Genetic parameters for ostrich incubation traits have recently been estimated by Brand *et al*. (2008a; 2009). It was also found that embryonic mortality was

influenced by breed combination involving pure and crossbred South African Black (SAB) ostriches and Zimbabwean Blue (ZB) ostriches (Brand *et al.*, 2007), again hinting at possible genetic variation in embryonic mortality.

The aim of this study was to estimate environmental and genetic parameters for embryonic mortality of ostrich eggs. A better understanding of these factors may contribute to an improved hatching success and assist in selective breeding programs aimed at improving hatchability during the incubation phase of ostrich production.

2. Materials and Methods

The experimental population used during the period of the study (1998 to 2010) was a commercial, pair-bred ostrich flock at the Oudtshoorn Research Farm, South Africa. The flock consisted of 188 breeding pairs, aged between 2 and >11 years. The husbandry and management of the flock has been described previously by Brand *et al.* (2008a, b; 2009). The flock mostly included birds of the South African Black genotype, but birds from the Zimbabwean Blue and Kenyan Red Necks (KAR) strains were also introduced recently to study crossbreeding between these genotypes (Engelbrecht *et al*., 2008). The present analysis, however, was confined to South African Black ostriches, for which complete pedigree records of their progenitors were available over a relatively long period. Unless specified otherwise, each breeding bird received a production ration of 2.5 - 3.0 kg dry matter/day throughout the breeding season, which lasted from the beginning of June until the end of January for 1998 to 2007. Outside the breeding season (February - May), male and female birds were kept in separate flocks for resting. From 2008 the breeding season started in mid-May and ended in mid-December.

The methods of egg collection, sanitation and storage of eggs on the research farm are well documented (Van Schalkwyk *et al.*, 1998; 1999b; Brand *et al*., 2007). The fate of each individual egg was recorded as described by Bunter (2002). Eggs were artificially incubated at 36 ^{o}C and 24% RH in Buckeye®, Prohatch®, Natureform® or African International® incubators, with all incubators set to turn eggs automatically through 60 - 90° hourly. Eggs were randomly placed into the different types of incubators. The capacity and operation of the incubators, as well as the hatchery practices have been described by Cloete *et al*. (2001) and Brand *et al.* (2007). Due to high egg production during the mid-season, eggs were occasionally transferred between incubators to make optimal use of incubator space. A new model Buckeye® incubator with a capacity of 1 000 eggs was acquired for the 2010 breeding season. The new incubator also operated at 36 °C and at a RH of 24%. During the breeding season, eggs were stored for no more than 6 days at a temperature of 17 °C and relative humidity (RH) of 75%. The exception to this was during the beginning of the breeding season when egg production was still low and eggs were consequently stored for longer periods (not exceeding 20 days) before setting. 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, expressed as percentages of initial egg weight, up to 21 and 35 days of incubation. On day 35 of incubation, the eggs were transferred from the incubators to a hatcher, which also operated at 36 °C and of 24% RH. Eggs not showing any macroscopic embryonic development after 21 days of incubation were regarded as infertile and

were not considered in any analyses, whereas those with clear evidence of embryonic development that had subsequently ceased were considered as early embryonic mortalities. Subsequent mortality (> 21 days of incubation) was classified as late embryonic death.

Data of overall shell-deaths were available for 48 126 eggs. These records represented 459 individual females that were mated to 463 individual males to form 737 unique breeding pair combinations. The pedigree file included all the animals that were available for selection during the course of the study, as well as the animals that were present since recording started in 1990. Based upon year of production and nutritional treatment, these data were allocated to 49 contemporary groups, while season of egg collection (winter, spring and summer) was also recorded.

The embryonic mortality data so derived were subjected to genetic analysis, using ASREML software (Gilmour *et al.*, 1999). The random effects that were fitted included the direct, additive effect of animal (h^2; fitted as default for all traits), as well as maternal genetic effects and dam permanent environmental effects (pe^2). Fixed effects considered included contemporary groups (n = 49), seasons (winter, autumn and summer), female age (2 to > 11 years), storage time (0 days - 6+ days) and incubator (as specified previously), as well as the interaction of contemporary group with season. Due to the small number of records for females > 11 years, all data from females in this category were combined and grouped as females aged > 11 years. Subsequent analyses also included egg weight and water loss to 35 days of incubation as linear and quadratic covariates.

ASREML was used to fit fixed effects while simultaneously deriving variance components for each trait in single-trait analyses. Likelihood Ratio Tests (LRT) was used to assess the significance of the contribution of each random term to improvements in the model. The LRT is based on testing the increase in Log-likelihood resulting from adding an additional random term to the model of analysis as a Chi^2 statistic. In two models including the same number of random terms, the model with the higher value for the Log-likelihood was preferred. Traits considered were early embryonic mortalities (EEM), late embryonic mortalities (LEM) and overall embryonic mortalities (OEM). Variance components for each trait were estimated in single-trait analyses. ASREML employs an average information algorithm (concurrently providing estimates of standard errors for parameters) to estimate variance components for mixed models by residual maximum likelihood (Gilmour *et al.*, 1995).

Animal solutions reflecting estimated breeding values were available as a byproduct of the genetic analysis. Estimated breeding values for embryonic mortalities from three selection lines (control line, live weight line and chick production line, as described by Cloete *et al*., 2008) were averaged (±S.E.) within hatching years to establish line specific regressions of breeding values for overall embryonic mortalities upon hatching year over a period of 12 years. The selection of the different lines in short entail the annual selection of replacement breeders for the flock from parents with the highest breeding values for chick output for the chick production line, the selection of individuals with the highest breeding values for live weight at about 16 months of age, as well as the selection of individuals with average parental reproductive performance and

average weights at about 16 months for the control line. Genetic trends were tested for differences between the lines using standard errors obtained for the respective regression coefficients.

3. Results

Contemporary group-season effects were significant ($P < 0.05$) in all analyses. Year and seasonal effects commonly occur in livestock breeding. Such effects are, however, often transient and dependent upon unique climatic or management practices (Cloete *et al*., 2002). Although such effects are important to be accounted for in genetic analyses, they often have limited practical application. These results are thus not presented in detail, although the effects were retained in the analyses for the reduction in the mean square for error they afforded.

The effect of female age on embryonic mortalities is presented as proportions in Figure 1. Although female age had no significant effect on EEM, there appeared to be a slight reduction in females of the oldest age group (> 11 years of age). An initial increase in the frequency of late embryonic mortalities occurred with an increase in female age, resulting in a significant lower LEM for females < 5 years (28%) compared to females > 7 years (36%) ($P < 0.05$). It is interesting to note that females > 11 years sustained slightly lower embryonic mortalities than 10 and 11 year old females for all traits (Figure 1). However, this reduction was generally not significant ($P < 0.05$), except for EEM. Overall embryonic mortalities follow the same trend as LEM, with OEM being significantly higher for females aged 10 - 11 years if compared to females < 7 years.

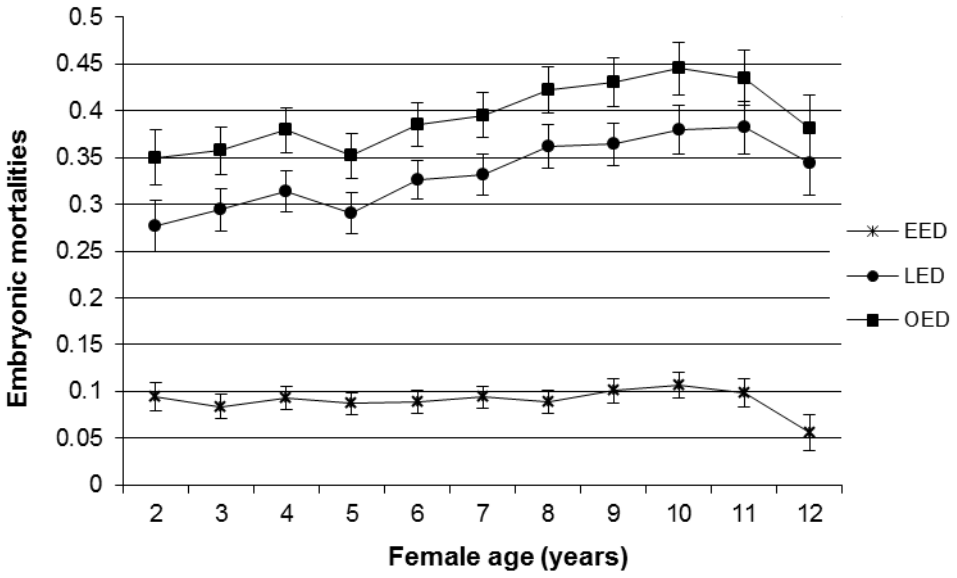

Figure 1 Least squares means depicting the effect of female age on early embryonic mortality (EEM), late embryonic mortality (LEM) and overall embryonic mortality (OEM) presented as frequency of fertile eggs set. Vertical lines about means represent standard errors.

Different incubators resulted in significant differences ($P < 0.05$) for all categories of embryonic mortalities (Table 1). It appeared as if embryonic mortalities during the late developmental phase were affected more

by the different incubators than during the EEM stage. The exception was the significantly ($P < 0.05$) higher EEM of 12% for eggs that were moved between incubators at some stage during the 42-day incubation process. OEM of the Natureform® and the new Buckeye® incubators were the highest at 43% and 42% respectively, while old Buckeye® incubator had the best overall performance with a hatchability of fertile eggs that amounted to 64%.

Table 1 Least squares means (± S.E.) depicting the effect of different incubators on early embryonic mortality (EEM), late embryonic mortality (LEM) and overall embryonic mortality (OEM) presented as a frequency of fertile eggs set.

Incubator	Trait		
	EEM	**LEM**	**OEM**
Buckeye®	0.07 ± 0.01	0.27 ± 0.02	0.36 ± 0.02
Prohatch®	0.08 ± 0.01	0.35 ± 0.02	0.37 ± 0.02
African Incubator®	0.10 ± 0.02	0.30 ± 0.03	0.37 ± 0.03
Natureform®	0.09 ± 0.02	0.37 ± 0.04	0.43 ± 0.04
Combinations	0.12 ± 0.01	0.35 ± 0.02	0.43 ± 0.02
New Buckeye®	0.08 ± 0.02	0.33 ± 0.03	0.42 ± 0.03

Storage time had no effect on EEM (proportions ranging between 0.08 and 0.09), the exception being the significant increase in EEM for eggs stored for more than 6 days (0.12). LEM for fresh eggs set and eggs stored for more than 6 days were significantly higher at 0.37 and 0.42 respectively, than for eggs stored for 1 to 6 days (0.30 - 0.32) ($P < 0.05$; Figure 2). Eggs that were set directly (0 days storage) accordingly sustained higher levels of embryonic deaths than those stored for intermediate periods. OEM followed the same trend as LEM.

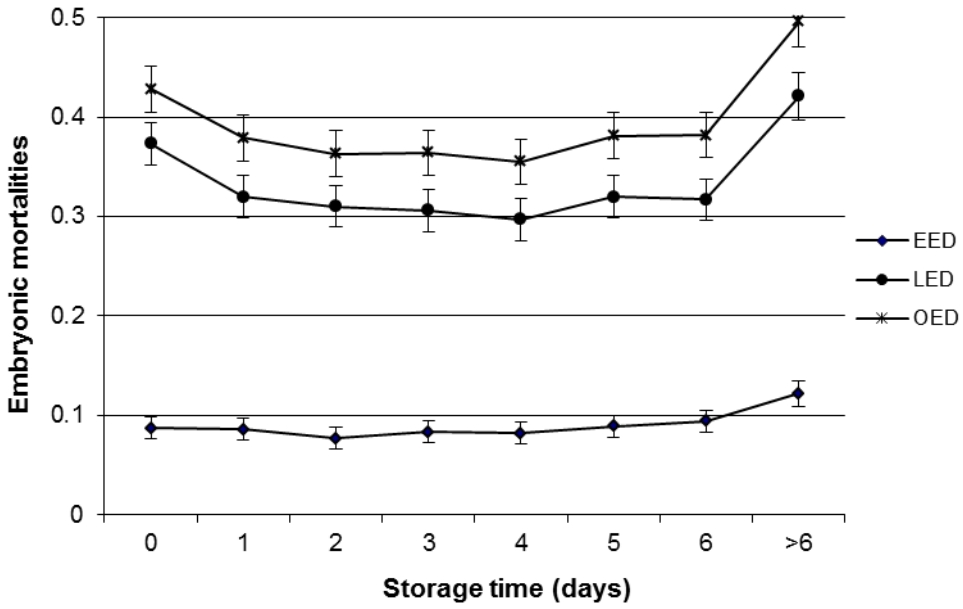

Figure 2 Least squares means depicting the effect of storage time on early embryonic mortality (EEM), late embryonic mortality (LEM) and overall embryonic mortality (OEM) presented as a frequency of fertile eggs set. Vertical lines about means represent standard errors.

Log-likelihood values under alternative random effects models are presented in Table 2. The addition of the direct additive effect as a single random source of variation resulted in significant improvements in the LRT throughout. When further random effects were added, the LRT indicated that models for all the traits, EEM, LEM and OEM, should include only the direct additive and dam permanent environment effects as random sources of variation.

Table 2 Log-likelihood values for the respective traits under different random effects models, with the best model for each trait represented in bold italic figures

Trait	Fixed effects only	+ h^2 (Model 1)	+ h^2 + c^2 (Model 2)	+ h^2 + m^2 (Model 3)	+ h^2 + m^2 + c^2 (Model 4)
EEM	731.86	1144.76	***1156.23***	1150.54	1156.23
LEM	4591.57	5486.89	***5507.1***	5503.08	5507.16
OEM	3371.45	4499.4	***4527.83***	4523.51	4527.92

h^2 = direct heritability, c^2 = dam permanent environment, m^2= maternal heritability, EEM = early embryonic mortality, LEM = late embryonic mortality, OEM = overall embryonic mortality

Variance components and ratios were estimated for all the traits in single-trait analyses (Table 3). Heritability (h^2) estimates were medium to high (low constitutes a value < 10%, medium ranges between 10% and 20% and high > 20%; Turner & Young, 1969) for all three embryonic mortality traits and ranged between 0.16 for EEM and 0.22 for OEM. All heritability estimates were significant (at least double the corresponding S.E.). Dam permanent environmental effects (c^2) were low but associated with a small standard error and thus

significant for all traits, ranging between 0.021 ± 0.005 and 0.046 ± 0.003. The correlation between direct effects and maternal effects (r_{am}) were not considered as maternal variation generally partitioned towards c^2 and not towards m^2. Derived variance components and ratios for all traits were generally similar to those estimated from analyses in which eggs without WL35 data were removed when the latter was included as a covariate. The inclusion of egg weight and WL21 or WL35 as linear and quadratic covariates (as appropriate) did not have a marked impact on h^2 estimates, which amounted to 0.178 ± 0.023 for EEM, 0.165 ± 0.022 for LEM and 0.211 ± 0.025 for OEM. Corresponding c^2 estimates amounted, respectively, to 0.021 ± 0.006, 0.034 ± 0.007 and 0.040 ± 0.008.

Table 3 Estimated variance components and ratios (±SE) of early embryonic mortality (EEM), late embryonic mortality (LEM) and overall embryonic mortality (OEM) as derived from single-trait analyses.

Effect	Trait		
	EEM	LEM	OEM
Components			
σ^2_a	0.011	0.036	0.049
σ^2_c	0.001	0.007	0.010
σ^2_e	0.057	0.156	0.166
Ratios			
h^2	0.159 ± 0.021	0.183 ± 0.023	0.219 ± 0.026
c^2	0.021 ± 0.005	0.037 ± 0.007	0.046 ± 0.008

σ^2_a = direct additive variance, σ^2_c = dam permanent environmental variance, σ^2_e = environmental (residual) variance, h^2 = direct heritability, c^2 = dam permanent environment

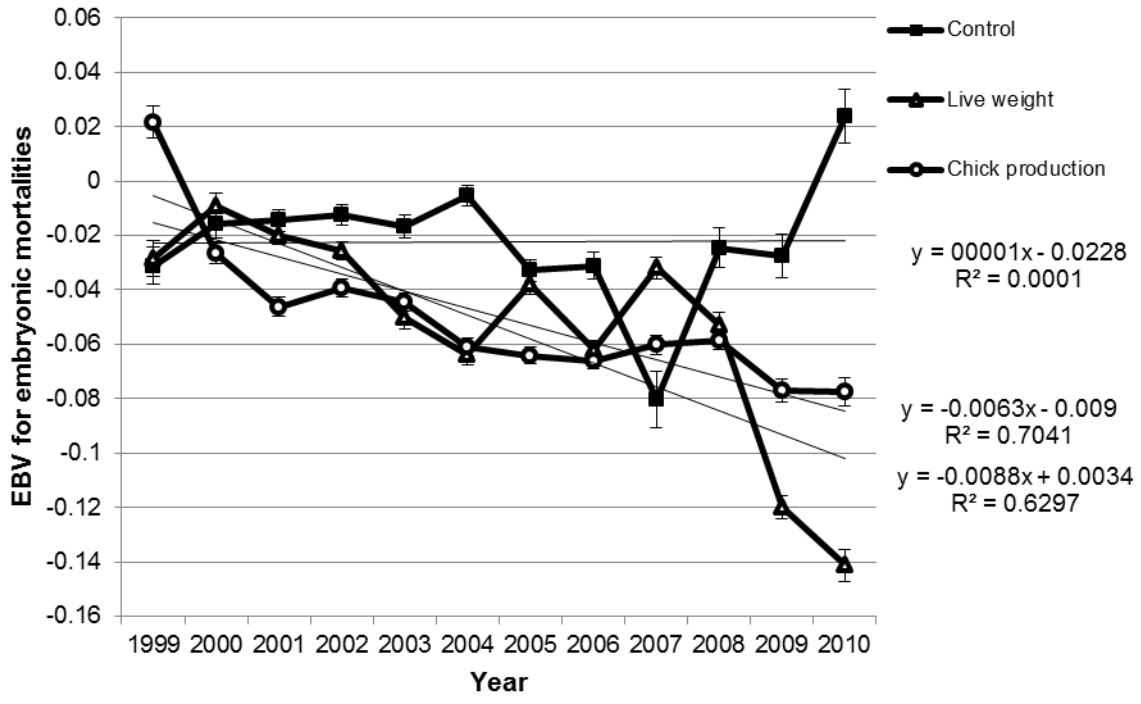

Figure 3 Estimated breeding values (EBV) for embryonic mortality for the control line, live weight line and the chick production line from 1999 to 2010. Means are accompanied by standard errors within years.

The regressions of estimated breeding values for embryonic mortality within selection lines (live weight and chick production, as well as the unselected control line) upon hatching years are shown in Figure 3. It is evident that no conclusive genetic change could be detected in the Control line. The regression (±S.E.) of breeding values for overall embryonic mortalities on year of hatch amounted to 0.00001 ± 0.00210 per annum (R^2 = 0.0001). In contrast, reductions (P < 0.01) in embryonic mortalities were evident for both the live weight line (-0.0088 ± 0.0021 fewer mortalities per fertile egg per annum; R^2 = 0.63) and the chick production line (-0.0063 ± 0.0013 fewer mortalities per fertile egg per annum; R^2 = 0.70). The derived regression coefficients for both selection lines differed from that of the control line (P < 0.05).

4. Discussion

In both broiler breeders and in quail, eggs of young females had a higher proportion of early embryonic mortalities than those produced by mature females (Reis *et al.*, 1997; Hocking & Bernard, 2000; Yildirim, 2005). In contrast, eggs from older chickens had poorer albumen quality, worse hatching rates and produced a lower proportion of high-quality day-old chicks compared to eggs of younger breeders (Decuypere & Bruggeman, 2007). The hatching rate results were consistent with those of the present study, and also in line with previous findings reported by Badley (1997) and Brand *et al.* (2007). In this study, water loss to 35 days of incubation only tended to increase with female age (P = 0.06; Chapter 13), while eggshell permeability showed a clear increase as female age increased (P < 0.01; Chapter 15). The latter change may possibly contribute to the increased level of embryonic mortalities as female age increases, but further research is required to confirm or refute this contention.

The success of artificial incubation also depends on the quality of the incubator and the provision of an optimal environment for incubation by applying the correct settings. Physiological requirements of the developing ostrich embryo include the correct hatching temperature, the correct humidity, optimal gaseous environment and proper turning of eggs (Swart *et al.*, 1987; Van Schalkwyk *et al.*, 1999a; 2000; 2002). Despite the fact that all the incubators were set to provide the same conditions with regard to temperature and humidity, there was differential embryonic mortality between them. This difference in hatchability also has economic implications in the long term. The Natureform® incubator had the highest proportion of mortality in the latter stages of incubation and also the highest overall embryonic mortality (Table 1). The increased handling of eggs during the incubation process by moving them between different incubators appears to also contribute to increased embryonic mortality, as was previously reported by Brand *et al.* (2007). The present results also suggest that eggs are more sensitive to incubator performance during the second half of incubation, which corresponds with findings by Brand *et al.* (2007). It futher could be speculated that the incubator settings for temperature (36°C) and humidity (24%RH) may not reflect the micro-environment across all the incubating eggs set in different parts of the incubator, and thus could have caused different hatching success for different incubators. The poorer hatching performance of eggs moved between incubators is consistent with previous results (Chapter 3), and may be related to increased handling of the eggs.

In agreement with previous studies (Deeming, 1996; Wilson *et al.*, 1997; Ar & Geffen, 1998; Hassan *et al.*, 2005), embryonic mortality increased significantly in those eggs stored for more than 7 days. Sahan *et al*. (2004) correspondingly found that late embryonic mortalities increased from 14.3% in eggs stored for one day to 18% in eggs stored for 10 days. Results previously reported by Brand *et al.* (2007) also showed higher embryonic mortalities, early as well as late, for freshly laid eggs compared to eggs with intermediate storage. Benton & Blake (1996) reported both a significant greater albumen height and lower pH for fresh broiler eggs than for stored eggs, exposing the developing embryo to an inappropriate trans-vitelline membrane pH gradient, as well as to thick albumen that may slow vital gas diffusion, causing an increased incidence of embryonic mortalities. The results suggest that the ideal storage time for ostrich eggs is between 2 and 4 days, as previously indicated by Van Schalkwyk *et al*. (1999b) and Brand *et al.* (2007).

There is little published information on heritability (h^2) estimates for embryonic mortality in domestic avian species in general, and ostriches in particular. In a comprehensive review, Kinney (1969) reported low heritability estimates (0.10) for both early and late embryonic mortality for chickens. The h^2 estimates of overall shell-deaths in our current study (Table 2) were significant and higher than the value 0.06 ± 0.06 reported by Brand *et al*. (2008a), when embryonic mortality was assessed as a trait of the female. The fact that higher h^2 estimates were derived when embryonic mortality was assessed as a trait of the egg needs to be studied further, as well as how these findings will find application in practical ostrich production. The h^2 of embryonic mortalities at different stages (EEM and LEM) were similar, indicating that the contribution of the genetic makeup of the egg to the overall phenotype did not differ substantially according to stage of incubation. Given the significant h^2 estimates, selection against embryonic mortality may assist to increase hatchability by selecting the appropriate replacements, based on breeding values derived from all known relationships among animals. This contention is supported by the derived genetic trends for the two selection lines in the present study. Cloete *et al*. (2008) reported that estimated breeding values for hen liveweight increased at 0.635 ± 0.059 kg per annum in the line selected for live weight ($P < 0.01$), and at 0.680 ± 0.024 chicks per annum in the chick production line ($P < 0.05$). As embryonic mortalities would impact negatively on chick production, the genetic reduction in embryonic mortalities in the latter line is understandable. However, the reason for the genetic improvement in chick survival in the live weight line is not clear. A lack of comparable results in the literature accordingly did not assist in providing deeper insight in this matter.

5. Conclusions

Results of the present study indicate that embryonic mortality in ostrich eggs was influenced by a number of factors, including female age, storage time and different types of incubators. More importantly, there appears to be an inherited genetic component that can potentially be exploited. As only chicks from eggs that hatched are eligible for selection, the process of selecting for reduced embryonic mortalities needs to be refined. When the large, predominantly full sib family structure of ostriches are considered, it seems reasonable that such a system could be based upon breeding values, where information on parental information could be exploited fully. As a matter of fact, the obtained selection responses in chick production of ostriches in the study of Cloete *et al*. (2008) were based on maternal performance.

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Chapter 17

Genetic (co)variances between egg weight, water loss and shell-deaths in ostrich eggs, using threshold-linear models

Abstract

The hatchability of artificially incubated ostrich eggs is relatively low and highly variable, with embryonic mortalities being a major contributor to hatching failure. Data for obtained from a pair-mated ostrich flock from 1998 to 2010. Data of 47 598 eggs were available and these records represented 459 individual females that were mated to 463 individual males to form 737 unique breeding pair combinations. Heritability (h^2) was significant for water loss at a level of 0.32 to 0.34 (both after 21 and 35 days of incubation). Estimates of h^2 for embryonic survival on the underlying scale were significant, and ranged from 0.21 to 0.22 for early embryonic survival (up to 21 days of incubation) to 0.30 to 0.32 for late embryonic survival. The genetic correlation of water loss to 35 days of incubation with late embryonic survival was negative at -0.22. Based on these results, it can be expected that genetic change in the hatchability of ostrich eggs would be achieved with an appropriate selection program.

1. Introduction

Embryonic mortalities amounts to between 21% and 29% loss of fertile ostrich eggs that fail to hatch (Deeming *et al*., 1996; Van Schalkwyk *et al*., 1996; Brand *et al*., 2008a), which makes it a major contributor to hatching failure and lossof productivity. Shell deaths are usually defined to include both early- and late embryonic mortality (Badley, 1998). It is important to understand all the contributing factors leading to shell deaths, because even small improvements in the hatchability of the ostrich eggs can result in important economic gains.

Evaporative water loss (WL) occurs naturally during the incubation process and achieving the correct WL during artificial incubation is a challenge. WL is a multifactorial trait, being influenced by both incubator conditions, the physical properties of the eggshell, as well as internal factors as the embryo develops (Ar, 1991). Studies by Swart *et al.* (1987), Deeming (1995), Ar (1996) and Blood *et al.* (1998) showed that the optimal water loss in artificially incubated, as well as in ostrich eggs incubated in natural nests, amounts to about 13% to 15% of initial egg weight. Blood *et al*. (1998) reported that, although there is some variation in the percentage WL at which ostrich eggs will still successfully hatch, a sharp increase in embryonic mortality occurs below 11% WL and above and 15% WL at 35 days of incubation. Ostrich embryos may also be particularly susceptible to incorrect water loss during specific periods of the hatching process. Snyder & Birchard (1982) reported that the critical period for water loss is during the first half of incubation in chicken eggs, whereas embryos in the second half of incubation appear to be able to tolerate water losses up to 25% of their total body water without embryonic survival being severely compromised.

Petitte & Davis (1999) suggested that genetics influence the performance of individual ostriches, while Wilson (1997) argued that hatchability of ostrich eggs could be substantially improved by selecting females that produce eggs with good shell qualities and with an adequate, uniform shell porosity. Currently, estimates of genetic parameters for ostrich egg, chick and reproductive traits are still limited to a few studies (Bunter *et al.*, 1999; 2001; Bunter & Cloete, 2004; Cloete *et al.*, 2004; 2005; 2008; Lambrechts, 2004,). Brand *et al*. (2008a; 2009) have more recently estimated genetic parameters for ostrich incubation traits.

The aim of this study was to estimate genetic (co)variances for embryonic survival (as a binary trait), egg weight and WL of ostrich eggs, using a threshold-linear animal model. A better understanding of these interrelated factors may contribute to an improved hatching success and assist in selective breeding programs.

2. Material and Methods

The experimental population used during the period of the study (1998 - 2010) was a commercial, pair-bred ostrich flock at the Oudtshoorn Research Farm, South Africa. The flock consisted of 188 breeding pairs of ostriches at the end of the study, and the age of the breeding birds in the flock ranged between 2 and 11+-years-old. The husbandry and management of the flock has been described previously by Brand *et al.* (2008a; b; 2009). The flock mostly included birds of the South African Black genotype, but birds from the

Zimbabwean Blue and Kenyan Redneck strains were also introduced recently to study crossbreeding between these genotypes (Engelbrecht *et al*., 2008). The present analysis was, however confined to the South African Black genotype, where sire and dam identities were known for all progeny hatched since 1990, ensuring that the majority of birds had >2 generations of pedigree depth. The methods of egg collection, sanitation and storage on the research farm are well documented (Van Schalkwyk *et al.*, 1998; 1999; Brand *et al*., 2007). The fate of each individual egg was recorded as described by Bunter (2002). Eggs were artificially incubated at 36 ºC and 24% RH in automatic incubators, with all incubators set to turn eggs automatically through 60 - 90° hourly. During the breeding season eggs were stored for no more than 6 days at a temperature of 17 °C and relative humidity (RH) of 75 %. The exception to this was during the beginning of the breeding season when egg production was still low and eggs were consequently stored for longer periods, not exceeding 20 days, before setting. 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, expressed as percentages of the initial egg weights, up to 21 and 35 days of incubation. On day 35 of incubation, the eggs were moved from the setters to a hatcher, which also operated at 36 °C and a RH of 24%. Eggs not showing any signs of continuing development at candling at 21 and 35 days of incubation were opened and assessed for embryonic development. Eggs not showing any macroscopic signs of embryonic development after 21 days of incubation were regarded as infertile and were not considered in any further analyses. Eggs remaining after the infertile eggs were discarded numbered 47 598. Of these, those eggs with clear evidence of embryonic development that had subsequently ceased were considered as shell-deaths during the first half of incubation. Subsequent shell-deaths were classified as late embryonic mortalities (occurring after 21 days of incubation). These data were used to construct three binomial traits, namely:

- Early embryonic survival (EES): Eggs with embryos that survived to 21 days of incubation were considered as one category, while those eggs with early embryonic mortalities constituted the other.
- Late embryonic survival (LES): Eggs with embryos that survived to 35 days of incubation constituted one category, whereas eggs with embryos that sustained embryonic mortalities after 21 days of incubation were regarded as the other category. Eggs sustaining early embryonic mortalities were excluded from the analysis of this trait.
- Overall embryonic survival (OES): Eggs with embryos that survived to hatching constituted one category, while eggs with embryos that sustained embryonic mortalities at any stage of incubation were regarded as the other category.

Data of egg weight (n = 47 598), WL to 21 days of incubation (WL21; n = 47 598) and WL to 35 days of incubation (WL35; n = 46 706) was accordingly available. These records represented the progeny of 459 individual females that were mated to 463 individual males to form 737 unique breeding pair combinations. The pedigree file included all the animals that were available for selection during the course of the study, as well as the base animals that were present when recording started in 1990. Based upon year of production and nutritional treatment, these data were allocated to 49 contemporary groups, while season of egg collection (winter, spring and summer) was also recorded. A series of 3-trait threshold-linear animal models were constructed. The first of these, included egg weight (EWT), water loss at 21 days of incubation (WL21) and EES. For the analysis that was presented, embryonic survival was defined as a binary trait with two

categories (1 for embryos that died prior to 21 days of incubation and 2 for those that died after 21 days). Fixed effects that were considered included contemporary groups (n=49), female age (2 to 11+ years) and storage time (0 days to 6+ days). Because of the small number of records for females > 11 years, all the data from females > 11 years were combined and grouped as the female age of 11+ years. The equation for the 3-trait model was thus the following:

$$y_{ijklm} = f_{ij} + a_{ik} + m_{ik} + c_{il} + e_{ijkl} \quad (1)$$

In this model, y was a vector of observations for egg weight, WL21 and underlying values for EES; i was indicative of the respective traits (i = 3), f_{ij} was the fixed effect j for the i'th trait, a_{ik} was the direct genetic effect of the k'th animal for the i'th trait, m_{ik} was the maternal genetic effect of the k'th animal for the i'th trait, c_{il} was the maternal permanent environmental effect of the l'th dam for the i'th trait, and e was the vector of randomly distributed residual effects. The correlation between direct and maternal effects was not considered for any of the three traits. Fixed effect solutions are not considered here, as it was already covered in an earlier paper (Brand *et al*., 2011).

The software used was THRGIBBSF90 (Misztal *et al*., 2002; 2008). This software is suitable for the estimation of variance components and genetic parameters in threshold-linear animal mixed models for any combination of categorical and continuous traits (Lee *et al*., 2002). The programme POSTGIBBSF90 was used for Post Gibbs analysis (Misztal *et al*., 2002). The software allows for the prediction of solutions for fixed and random effects, as well as histograms depicting the highest posterior density regions for the respective variance components.

A single chain of 250 000 cycles was run, with the first 50 000 cycles used as the burn-in period. When the sampled values were plotted against the iterations, a stationary stage could be confirmed at this stage by graphical inspection. Every 10^{th} sample was stored after 50 000 iterations, giving a total of 20 000 samples for the computation of posterior means, standard deviations as well as 95% HPD confidence intervals. Point estimates were calculated as the posterior mean from Post Gibbs analysis of the specific variance components, using the results from the final 20 000 samples as set out above. Direct genetic, maternal genetic, dam permanent environmental and environmental (residual) correlations were derived from these analyses.

Subsequent analyses included the combinations of egg weight, WL35 and LES, as well as egg weight, WL21 and OES. Finally, a 2 trait analysis was conducted, with only EES and LES as traits. As none of the eggs that sustained early embryonic mortalities had valid records for LES, it was impossible to derive a residual correlation. This analysis was conducted simply to find out if EES and LES were controlled by largely the same set of genes, which could easily be calculated through the relationships in the numerator relationship matrix.

3. Results

Descriptive statistics for the traits under consideration are provided in Table 1. The number of records for each trait ranged from 44 173 for late embryonic mortalites to 47 957 for overall shell deaths, egg weight and WL to 21 days of incubation. All traits were variable, with coefficients of variation (CV) exceeding 20%. The only exception was egg weight with a lower CV of 8.7%. The distribution of egg weight and WL data did not deviate from normality. Embryonic survival traits (as defined previously) were considered as threshold traits in threshold-linear analyses, as described previously.

Table 1 Descriptive statistics of eggs traits recorded for breeding ostriches for the 1998 - 2010 production years.

Traits	Number	Mean ± S.D.	CV (%)	Range
EWT (g)	47 598	1431 ± 124	8.67	959 - 2 129
WL 21 (%)	47 598	7.97 ± 1.97	24.7	1 - 37.7
WL 35 (%)	46 706	13.1 ± 3.1	23.3	4 - 48
LES*	44 173	0.74 ± 0.44	59.5	0 - 1
OES*	47 598	0.69 ± 0.46	66.7	0 - 1
EES*	47 598	0.93 ± 0.26	28.0	0 - 1

S.D. = standard deviation; CV = coefficient of variation; EWT = egg weight, WL21 = water loss at 21 days of incubation; WL35 = water loss at 35 days of incubation; LES = late embryonic survival; OES = overall embryonic survival; EES = early embryonic survival; * these traits analysed were coded 1 and 2, as the software treats 0 as missing values.

Posterior distributions for the genetic components for early embryonic survival, late embryonic survival and overall embryonic survival are provided in Figure 1. The genetic variance was significant in all instances, as reflected by 95% confidence limits for the highest posterior density (HPD) excluding zero (also see Tables 2 to 5).

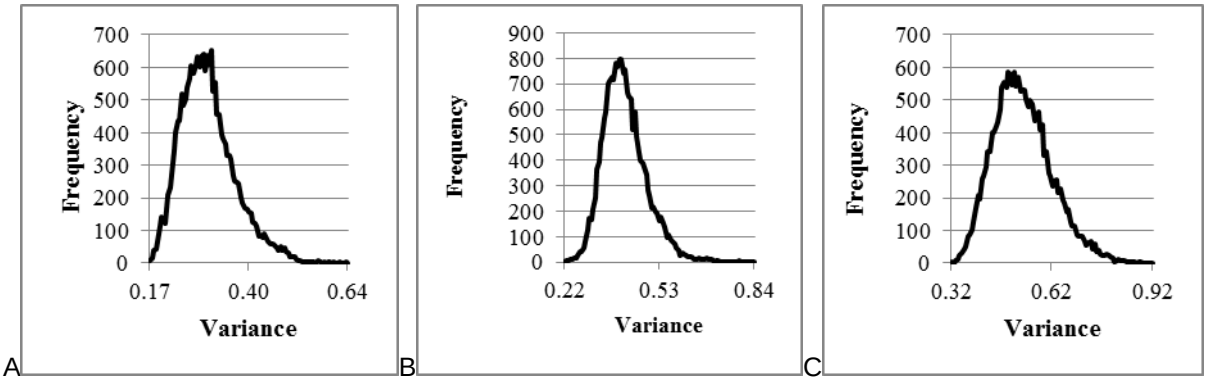

Figure 1 Posterior distibution for the genetic variance components of early embryonic survival (A), late embryonic survival (B) and overall embryonic survival (C) for ostrich eggs (1998 - 2010)

The estimate of heritability (h^2) for EWT was high and significant in the analyses involving egg weight, ranging between 0.26 ± 0.02 and 0.29 ± 0.02 ($P < 0.05$; Tables 2 - 4). Maternal components (m^2) for EWT were greater than double the corresponding SE at respectively 0.44 ± 0.08, 0.55 ± 0.08 and 0.37 ± 0.08 for

analyses 1, 2 and 3. Dam permanent environment (pe^2) for EWT varied considerable between the 3 analyses, to contribute between 0.07 and 0.22 to the observed phenotypic variation. Estimates of h^2, m^2 and pe^2 were moderate to high and significant for WL21 ($P < 0.05$); Table 2 and 4), amounting to 0.34 ± 0.05, 0.21 ± 0.06 and 0.15 ± 0.04 respectively. The same basic trend was found for the analysis including WL35 (Table 3), which recorded values of 0.32 ± 0.04 for h^2, 0.28 ± 0.06 for m^2, and 0.15 ± 0.04 for pe^2. The estimate of h^2 for embryonic survival was moderate to high for the 3 analysis and ranged between 0.21 ± 0.05 for EES and 0.32 ± 0.05 for LES ($P < 0.05$; Tables 2 - 4). The estimate of m^2 and pe^2 for EES were smaller in magnitude and did not exceed 0.07.

The genetic correlations (r_g) and maternal correlations (r_m) of EWT with WL21 and WL35 were small and not significant for any of the three analyses ($P > 0.10$; Tables 2 - 4). Although the r_{pe} for EWT with the water loss were higher, it was not significant. Environmental correlations (r_e) of WL21 and WL35 with EWT were moderate and significant ($P < 0.05$; Table 2 - 4). Very low to zero genetic, maternal, dam permanent environment and environmental correlations were found between EWT and the three embryo survival traits. No significant correlations were accordingly found between water loss and embryo survival.

Table 2 Mean (co)variance components, posterior S.D. (PSD), 95% highest posterior density (HPD) confidence intervals, and variance ratios for egg weight (EWT), water loss to 21 days of incubation (WL21) and early chick survival (EES).

Trait and Item[1]	(Co)variance		95% HPD confidence interval			Ratio ± SE
	Component	PSD	Lower	Upper	Item	
EWT (g)						
σ^2_a	5589	311.9	4978	6200	h^2	0.28 ± 0.02
σ^2_m	8891	1650	5656	12130	m^2	0.44 ± 0.08
σ^2_{pe}	3153	973	1246	5060	pe^2	0.16 ± 0.05
σ^2_e	2383	157.2	2075	2691		
WL21						
σ^2_a	1.626	0.174	1.285	1.967	h^2	0.34 ± 0.05
σ^2_m	0.992	0.296	0.412	1.572	m^2	0.21 ± 0.06
σ^2_{pe}	0.743	0.188	0.375	1.111	pe^2	0.15 ± 0.04
σ^2_e	1.364	0.088	1.191	1.537		
EES						
σ^2_a	0.302	0.065	0.174	0.429	h^2	0.21 ± 0.05
σ^2_m	0.070	0.021	0.030	0.110	m^2	0.05 ± 0.02
σ^2_{pe}	0.037	0.015	0.009	0.066	pe^2	0.03 ± 0.01
σ^2_e	1.001	0.009	0.983	1.019		
Covariance components and ratios between EWT and WL21						
σ_a	-1.510	5.206	-11.71	8.694	r_a	-0.02 ± 0.06
σ_m	-26.23	15.21	-56.04	3584	r_m	-0.28 ± 0.16
σ_{pe}	15.98	9.257	-2.165	34.12	r_{pe}	0.33 ± 0.19
σ_e	-8.713	2.635	-13.88	-3.549	r_e	-0.15 ± 0.05
Covariance components and ratios between EWT and EED						
σ_a	0.375	3.329	-6.15	6.899	r_a	0.01 ± 0.08
σ_m	-11.76	4.842	-21.25	-2.273	r_m	-0.47 ± 0.19
σ_{pe}	2.519	3.033	-3.426	8.463	r_{pe}	0.23 ± 0.28
σ_e	1.390	1.820	-2.178	4.958	r_e	0.03 ± 0.04
Covariance components and ratios between WL21 and EED						
σ_a	0.027	0.078	-0.126	0.180	r_a	0.04 ± 0.011
σ_m	0.030	0.045	-0.059	0.119	r_m	0.11 ± 0.017
σ_{pe}	-0.039	0.036	-0.109	0.031	r_{pe}	-0.24 ± 0.21
σ_e	-0.142	0.042	-0.224	-0.060	r_e	-0.12 ± 0.04

[1] σ^2_a = direct additive genetic variance; σ^2_m = maternal genetic variance; σ^2_{pe} = dam permanent environmental variance; σ^2_e = environmental variance; σ^a = additive covariance; σ^m = maternal covariance; σ^{pe} = dam permanent environmental covariance; σ^e = environmental covariance; h^2 = direct heritability; m^2 = maternal heritability; pe^2 = dam permanent environment; r_g = genetic correlation; r_m = maternal correlation; r_{pe} = dam permanent environmental correlation; r_e = environmental correlation

Table 3 Mean (co)variance components, posterior S.D. (PSD), 95% highest posterior density (HPD) confidence intervals, and variance ratios for egg weight (EWT), water loss after 35 days of incubation (WL35) and late embryo survival (LES).

Trait and	(Co)variance		95% HPD confidence interval			
Item[1]	Component	PSD	Lower	Upper	Item	Ratio ± SE
EWT (g)						
σ^2_a	5341	319.8	4714	5668	h^2	0.26 ± 0.02
σ^2_m	11210	1577	8117	14300	m^2	0.55 ± 0.08
σ^2_{pe}	1449	743.2	-7.640	2906	pe^2	0.07 ± 0.04
σ^2_e	2356	161.1	2040	2672		
WL35						
σ^2_a	3.908	0.426	3.074	4.743	h^2	0.32 ± 0.04
σ^2_m	3.331	0.779	1.804	4.858	m^2	0.28 ± 0.06
σ^2_{pe}	1.775	0.487	0.821	2.728	pe^2	0.15 ± 0.04
σ^2_e	3.071	0.216	2.647	3.494		
LES						
σ^2_a	0.416	0.072	0.275	0.556	h^2	0.27 ± 0.05
σ^2_m	0.092	0.028	0.038	0.147	m^2	0.06 ± 0.02
σ^2_{pe}	0.042	0.016	0.010	0.073	pe^2	0.03 ± 0.01
σ^2_e	1.001	0.009	0.983	1.020		
Covariance components and ratios between EWT and WL35						
σ_a	-10.34	7.989	-26.00	5.320	r_a	-0.07 ± 0.04
σ_m	-67.44	25.72	-117.9	-17.02	r_m	-0.35 ± 0.13
σ_{pe}	29.09	14.47	0.738	57.40	r_{pe}	0.57 ± 0.29
σ_e	-11.44	4.043	-19.36	3.517	r_e	-0.13 ± 0.05
Covariance components and ratios between EWT and LES						
σ_a	2.800	3.366	-3.797	9.397	r_a	0.06 ± 0.07
σ_m	-1.440	4.733	-10.72	7.835	r_m	-0.05 ± 0.15
σ_{pe}	-3.215	2.438	-7.994	1.564	r_{pe}	-0.42 ± 0.31
σ_e	0.023	1.759	-3.424	3.469	r_e	0.0 0.04
Covariance components and ratios between WL35 and LES						
σ_a	-0.285	0.114	-0.508	-0.061	r_a	-0.22 ± 0.09
σ_m	0.016	0.110	-0.199	0.230	r_m	0.03 ± 0.20
σ_{pe}	0.030	0.064	-0.095	0.155	r_{pe}	0.11 ± 0.24
σ_e	0.028	0.060	-0.089	0.145	r_e	0.02 ± 0.03

[1] σ^2_a = direct additive genetic variance; σ^2_m = maternal genetic variance; σ^2_{pe} = dam permanent environmental variance; σ^2_e = environmental variance; σ^a = additive covariance; σ^m = maternal covariance; σ^{pe} = dam permanent environmental covariance; σ^e = environmental covariance; h^2 = direct heritability; m^2 = maternal heritability; pe^2 = dam permanent environment; r_g = genetic correlation; r_m = maternal correlation; r_{pe} = dam permanent environmental correlation; r_e = environmental correlation

Table 4 Mean (co)variance components, posterior S.D. (PSD), 95% highest posterior density (HPD) confidence intervals, and variance ratios for egg weight (EWT), water loss 21 days of incubation (WL21) and overall chick survival (OES).

Trait and	(Co)variance		95% HPD confidence interval			
Item[1]	Component	PSD	Lower	Upper	Item	Ratio ± SE
EWT (g)						
σ^2_a	5372	309.2	4766	5978	h^2	0.29 ± 0.02
σ^2_m	7000	1453	4151	9849	m^2	0.37 ± 0.08
σ^2_{pe}	4121	1038	2086	6156	pe^2	0.22 ± 0.06
σ^2_e	2340	155.3	2036	2645		
WL21						
σ^2_a	1.481	0.171	1.146	1.817	h^2	0.32 ± 0.04
σ^2_m	1.049	0.300	0.460	1.637	m^2	0.23 ± 0.07
σ^2_{pe}	0.612	0.195	0.229	0.994	pe^2	0.13 ± 0.04
σ^2_e	1.438	0.087	1.268	1.609		
OES						
σ^2_a	0.532	0.087	0.361	0.703	h^2	0.31 ± 0.05
σ^2_m	0.115	0.035	0.046	0.183	m^2	0.07 ± 0.02
σ^2_{pe}	0.052	0.023	0.006	0.098	pe^2	0.03 ± 0.01
σ^2_e	1.002	0.009	0.983	1.020		
Covariance components and ratios between EWT and WL21						
σ_a	-4.062	5.185	-14.23	6.102	r_a	-0.05 ± 0.06
σ_m	-27.100	12.40	-51.41	-2.793	r_m	-0.32 ± 0.15
σ_{pe}	10.14	8.354	-6.238	26.51	r_{pe}	0.20 ± 0.16
σ_e	-7.689	2.626	-12.83	-2.542	r_e	-0.13 ± 0.05
Covariance components and ratios between EWT and OES						
σ_a	1.592	3.627	-5.518	8.701	r_a	0.03 ± 0.07
σ_m	-2.121	5.109	-12.14	7.893	r_m	-0.08 ± 0.18
σ_{pe}	-2.959	3.154	-9.140	3.222	r_{pe}	-0.20 ± 0.22
σ_e	0.934	1.885	-2.759	4.628	r_e	0.02 ± 0.04
Covariance components and ratios between WL21 and OES						
σ_a	-0.106	0.080	-0.262	0.051	r_a	-0.12 ± 0.09
σ_m	0.012	0.069	-0.124	0.149	r_m	0.04 ± 0.20
σ_{pe}	0.022	0.044	-0.064	0.108	r_{pe}	0.12 ± 0.25
σ_e	-0.095	0.041	-0.176	-0.015	r_e	-0.08 ± 0.03

[1] σ^2_a = direct additive genetic variance; σ^2_m = maternal genetic variance; σ^2_{pe} = dam permanent environmental variance; σ^2_e = environmental variance; σ^a = additive covariance; σ^m = maternal covariance; σ^{pe} = dam permanent environmental covariance; σ^e = environmental covariance; h^2 = direct heritability; m^2 = maternal heritability; pe^2 = dam permanent environment; r_g = genetic correlation; r_m = maternal correlation; r_{pe} = dam permanent environmental correlation; r_e = environmental correlation

The direct additive effects for analysis 4, where EES was analysed with LES, was high and significant at 0.22 ± 0.04 for EES and 0.30 ± 0.06 for LES (Table 5). Estimates of m^2 and pe^2, although significant at double the corresponding SE, were low at 0.06 and 0.03 respectively. The correlation estimates between EES and LES were high, and amounted to 0.78 ± 0.17 for r_a, 0.72 ± 0.07 for r_m and 0.75 ± 0.33 for r_{pe} respectively, suggesting that both traits are governed by largely the same set of genes.

Table 5 Mean (co)variance components, posterior S.D. (PSD), 95% highest posterior density (HPD) confidence intervals, and (co)variance ratios for early embryonic survival (EES) and late embryoinic survival (LES).

Trait and	(Co)variance		95% HPD confidence interval			
Item[1]	Component	PSD	Lower	Upper	Item	Ratio ± SE
EES						
σ^2_a	0.306	0.059	0.185	0.418	h^2	0.22 ± 0.04
σ^2_m	0.051	0.023	0.006	0.096	m^2	0.04 ± 0.02
σ^2_{pe}	0.042	0.017	0.009	0.076	pe^2	0.03 ± 0.01
σ^2_e	1.001	0.009	0.983	1.019		
LES						
σ^2_a	0.499	0.092	0.318	0.681	h^2	0.30 ± 0.06
σ^2_m	0.094	0.032	0.031	0.157	m^2	0.06 ± 0.02
σ^2_{pe}	0.053	0.020	0.013	0.092	pe^2	0.03 ± 0.01
σ^2_e	1.001	0.009	0.983	1.020		
Covariance components and ratios between EES and LES						
σ_a	0.302	0.066	0.173	0.431	r_a	0.78 ± 0.17
σ_m	0.051	0.023	0.005	0.095	r_m	0.72 ± 0.08
σ_{pe}	0.035	0.016	0.005	0.066	r_{pe}	0.75 ± 0.33

[1] σ^2_a = direct additive genetic variance; σ^2_m = maternal genetic variance; σ^2_{pe} = dam permanent environmental variance; σ^2_e = environmental variance; σ^a = additive covariance; σ^m = maternal covariance; σ^{pe} = dam permanent environmental covariance; σ^e = environmental covariance; h^2 = direct heritability; m^2 = maternal heritability; pe^2 = dam permanent environment; r_g = genetic correlation; r_m = maternal correlation; r_{pe} = dam permanent environmental correlation

4. Discussion

The mean EWT, WL21 and WL35 found in the present study correspond with previous results from the same breeding population (Bunter & Cloete, 2004; Brand *et al.*, 2008a; b). Swart & Rahn (1988) and Blood *et al*. (1998) reported that WL of approximately 13% up to 35 days of incubation is characteristic of ostriches, which also corresponds with our findings.

The h^2 estimate for EWT of 0.26 to 0.29 is within the range of values reported the literature for EWT, including values of 0.19 (Bunter & Cloete, 2004) and 0.46 (Brand *et al*., 2009). The maternal genetic effect for EWT ranged from 0.44 to 0.55 in the present study. There appeared to be a bit of a trade-off between m^2 and pe^2 in the present study. This is not surprising if the high sampling correlation between these traits is considered (Bunter & Cloete, 2004). The m^2 estimates are, however still substantially higher than the value

of 0.19 reported by Brand *et al*. (2009), but closer to the value of 0.31 estimated by Bunter & Cloete (2004). At 0.07 to 0.16, the dam pe^2 estimates for EWT were also lower than the values of respectively 0.25 and 0.24 to 0.27 reported by Brand *et al*. (2009) and Bunter & Cloete (2004). The repartitioning of pe^2 to h^2 for EWT is the present study is perhaps not surprising, if it is considered the present study included data over a longer period, with a considerably deeper pedigree than the previous studies reported by Brand *et al*. (2009) and Bunter & Cloete (2004).

The high h^2 estimates of 0.32 to 0.34 reported for WL21 and WL35 in Tables 2 - 4 compares well with those of, respectively, 0.26 to 0.34 reported by Brand *et al*. (2009). Unfortunately, there is little other published information on h^2 estimates for WL in ostriches. Estimates of m^2 amounted to 0.21 to 0.28 for WL in the present study. The previous study of Brand *et al*. (2009) did not partition variance to m^2, but reported higher values of 0.25 for the pe^2 of WL21 and 0.30 for pe^2 of WL35, compared to the present values of 0.13 and 0.15. It is also feasible that the added pedigree depth in the present study resulted in the better partitioning of the maternal effects, thus adding to this discrepancy.

There is a lack of parameter estimates for embryonic mortalities or embryonic survival on the underlying scale in ostrich embryos, when treated as a trait of the egg. When binomial embryonic mortality data were treated as linear in Chapter 16, h^2 estimates of 0.16 (early embryonic mortalities) to 0.22 (overall embryonic mortalities) were derived. These estimates were slightly lower, but in the same range as the present values of 0.21 to 0.22 for EES and 0.30 to 0.32 for LES. In the absence of other values for ostriches, it is sensible to look at corresponding values in the poultry literature. In a comprehensive review, Kinney (1969) reported low heritability estimates of 0.10 for both early and late embryonic mortalities in chickens.

Studies by Bunter *et al*. (2001), and Cloete *et al*. (2004; 2005), Bunter & Cloete (2004) and Brand *et al.* (2008a; b) showed that traits like EWT and CWT were highly correlated on a genetic level, which indicates that these traits are governed largely by a similarly set of genes. This was also true for the traits WL21 and WL35, for which no genetic correlations were derived in the present study. Genetic correlations between EWT and WL in the study of Brand *et al*. (2009) were low and negative (-0.12 - -0.21). These estimates were somewhat higher than estimates of -0.02 - -0.07 in the present study. The partitioning of a part of the genetic covariance to the maternal genetic component probably contributed to this result. The previous study of Brand *et* al. (2009) did not include maternal genetic variance for WL21 and WL 35, as set out above. A negative correlation between EWT and WL confirmed the theory that heavier eggs are expected to have lower rates of WL due to the surface area : volume ratio. There are no comparable studies pertaining to the maternal relationship of EWT with WL in either ratites or small domestic poultry species.

The present study also attempted to correlate embryonic survival with the WL traits. The negative sign of the r_g of WL35 with LES (Table 3) and of WL21 with OES (Table 3) for analysis 2 and 3 in the present study was consistent with previous findings by Brand *et al*. (2008a), where all traits were modeled as traits of the female. In the case of the r_g between WL35 and LES the value reached a magnitude of twice the corresponding standard error. This correlation seems to be consistent with previous contentions that late-

term embryo are more susceptible to an inadequate WL than to an excessive WL. Findings by Deeming (1995) in that the pattern of mortality in eggs which survived beyond day 36 of incubation were closely linked with the degree of variation in amount of WL from the egg, correspond with these results. Water loss of less than 10% and more than 20% of the eggs initial weight, resulted in a reduction in hatchability in near-term embryos (Brand *et al*., 2011).

Although the r_{pe} of EWT and WL in the three analyses were higher than the 0.11 for WL21 and 0.02 for WL35 as reported in Brand *et al*. (2009), it was lower than the 0.40 and 0.37 respecively for WL21 and WL35 as reported by Brand *et al*. (2008a) when both traits were treated as traits of the female. However, corresponding to findings reported by Brand *et al*. (2009), the r_{pe} between EWT and WL was not significant. Contradictory to results of Brand *et al*. (2008a; 2009), the residual correlation between EWT and WL was high and significant.

Finally, it was important to note that the r_g between EES and LES were quite high, and near to unity. The results seem to suggest that the genes that control EES are largely the same as those controlling LES. This generalisation also appled to r_m although the absolute value of this correlation was lower. No other comparable results were found in the literature.

5. Conclusion

The moderate to high h^2 estimates for WL indicates that it is possible to modify this trait in ostrich eggs by genetic selection. It could be contended that selection to standardise EWT in order to reduce the variation in WL during incubation, may assist in improving embryonic survival. The derived h^2 estimates for embryonic survival on the underlying scale were moderate. These traits also showed substantial phenotypic variation, suggesting that selection for improving embryonic survival could be contemplated. The way whereby this may be accomplished, however, requires further investigation. The lack of robust r_g estimates between WL and EES complicates the implementation of a pragmatic selection program based on indirect selection for a change in WL to improve EES in ostrich breeding flocks. It could be argued that this result is possibly not surprising if the curvilinear relationship of egg weight loss during incubation with embryonic survival is considered.

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CHAPTER 18

General conclusions and recommendations

1. General conclusions

The aim of this study was to identify factors contributing to the failure of ostrich eggs to hatch, to investigate and describe the embryonic development and to establish genetic parameters for different production and egg traits. These studies are seen as vitally important to the alleviation of embryonic mortalities during artificial incubation, using an integrated approach.

1.1. Identification of non-genetic factors affecting reproduction and incubation traits.

Chapters 3 - 5 concentrated upon the effect of different systemic factors and their influence on embryonic mortalities and the subsequent hatching of chicks. Although the causes of embryonic mortalities are probably multi-factorial, several factors can be controlled. These include genotype, female age, year and season of production, storage time prior to setting eggs in the incubator and the incubator used.

Results showed that genotype significantly affected embryonic mortalities with eggs produced by South African Black (SAB) males crossed to Zimbabwean Blue (ZB) females having high levels of embryonic mortalities, whereas the level of embryonic mortality was reduced in the reciprocal cross. Genotype had no significant effect on the proportion of near-term chicks that pipped, the proportion of chicks that pipped in the correct position or the survival of chicks pipping in the correct position. These results suggest that crossing of different strains of breeds can be done without compromising hatchability of late-term eggs (≥ 35 days of incubation).

Although older ostrich females are still capable of good egg production, chick production declined overall due to higher levels of embryonic mortality. Both embryonic mortalities during the 1st half and 2nd half of incubation were proportionally increased in older females, the effect being more pronounced for mortalities during the 2nd half of incubation. The proportion of chicks pipping from eggs laid by 3-year-old females were significant higher than those from 8-year-old females.

Embryonic survival in general (except for deaths post-pipping) was adversely affected by prolonged storage, with embryonic mortalities in the 1st half of the incubation period increasing nearly 2-fold in eggs stored for >7 days. The overall proportions of embryonic mortality were higher at 32.0% in freshly laid eggs that were set directly without any storage as well as in those eggs stored for > 7 days (43.5%). It also appears that fresh eggs have a higher level of embryonic mortalites in the 2nd half of the incubation period compared to eggs stored between 1 and 6 days. In this study, embryonic mortalities during the 2nd part of incubation increased by 4.1% after 6 days of storage, and corresponded to the proportion of mortalities in eggs that were set directly. Storage time prior to setting had a significant influence on the pipping of near-term chicks, with pipping proportion being the highest for eggs stored for between 2 and 5 days. The best performance in terms of chicks pipping in the correct position, as well as survival of chicks pipping in the correct position, was found for eggs stored for 4 days. Apart from a reduced pipping percentage, chicks from eggs that were not stored before setting also had a poorer ($P < 0.05$) survival of hatch when pipping in the correct position.

Despite all the incubators used in this study being set to provide the same conditions with regard to temperature, 36 °C and RH, 24%, there was differential embryonic mortality between incubators. The only category that was not affected by incubator was those deaths that occurred post-pipping, after the eggs had been transferred to the hatcher. The frequency of chicks that pipped in the correct position differed between incubators, owing to a lower pipping frequency of chicks in those eggs that were transferred between incubators compared to chicks from eggs incubated in a single incubator throughout. It also seems that arguments in favour of an impaired pipping ability due to more frequent handling of transferred eggs do not seem to be valid, for such transfers are usually performed during routine husbandry procedures like candling. Eggs returned to the same incubator are thus also subjected to the same set of procedures as those that were returned to other incubators.

There were significant differences in pipping position and survival of hatched chicks for different levels of water loss (WL) to 35 days of incubation. Pipping frequency of incubated eggs, as well as survival of chicks, both from chicks pipping in the correct or incorrect positions were lowest for those eggs where water loss was either below 9% or above 19% over the first 35 days of incubation, i.e. these traits had an intermediate optimum. As water loss increased, the pipping hole was more likely to be situated closer to the equator of the egg.

A definite pattern was evident for the position of each of the different limbs in near-term chicks succumbing prior to hatching. Although a high proportion of dead-in-shell (DIS) chicks in the present study were presented in the correct position, a substantial proportion of DIS chicks that pipped internally, were positioned with their heads towards the equator of the egg. Only a very small proportion (0.9%) was presented with their heads towards the bottom of the egg, away from the air cell. Only 1.2% of chicks pipped internally at the bottom of the egg. Common malpositions were with legs facing upwards, but the head facing the opposite pole and chicks on their sides with their legs at the middle of the egg, with the head either turned towards the air cell or towards the bottom of the egg. Results from our study showed that approximately 50% of the dead-in-shell chicks were correctly positioned, thus making suffocation because of a serious malposition unlikely.

1.2. Developmental aspects

The stages of development of the ostrich embryo up to 7 days of incubation (Chapter 7) in the present study were closely related to the stages in described for chickens by Hamburger & Hamilton (1951). Although the hatchability of ostrich eggs was affected by year, such effects are usually unpredictable. It would suffice to state that year effects are not repeatable, and such effects have therefore little practical application, except for the identification of possible long-term trends.

For the stages of development of the ostrich embryo between 7 and 42 days of incubation (Chapter 8), the present study was closely related to trends reported by Gefen & Ar (2001). This was true, even though the latter authors used a slightly higher incubation temperature compared to the present study (36.5 ºC vs. 36.2 ºC). These results thus appear to be quite robust for ostrich eggs in general.

Egg candling remains a useful tool to assess egg development, and to identify infertile eggs, as well as eggs sustaining early embryonic mortalities (Chapter 9). However, while changes in the air cell volume conformed to expectations, differences between hatched eggs and those with dead-in-shell chicks were not marked. Fairly large levels of variation within classes involving hatched, infertile and dead-in-shell chicks also makes it difficult to draw up robust guidelines that would be of value at the level of the individual hatchery, where similar records are highly unlikely to be noted. It can thus be concluded that, although this study was able to detect subtle differences between hatched and DIS chicks, it is unlikely to find application in the broader industry.

1.3. Genetic and environmental effects on ostrich eggs

Apart from embryonic survival, several other traits were assessed for genetic variation. These included egg weight, water loss during incubation and ostrich shell quality traits.

These ostrich incubation traits were affected by a number of environmental factors, as well as genotype as shown in Chapter 11. These factors therefore need to be considered during routine genetic evaluation. Failure to consider these factors will potentially lead to biased genetic parameters and result in inaccurate breeding values. The average day-old chick weight of the pair-bred flock at Oudtshoorn Research Farm was 854 g, thus amounting to approximately 60% of the fresh egg weight. Zimbabwean Blue (ZB) females laid significantly heavier eggs (5%), which resulted in their chicks being 7% heavier than those of South African Black (SAB) females. Eggs produced by the combination of SAB males x ZB females were also substantially heavier than those produced by SAB females subjected to pure breeding. Incubation time to external pipping (PT) was not different between the different breed combinations, while water loss during incubation was generally independent of sire and/or dam genotype.

Year effects may depend on ambient climate during a specific period, as well as other management factors. Egg (EWT) and chick weight (CWT), as well as weight loss (WL) and external pipping (PT) were affected by an interaction between year and season (winter, spring or summer) of production. Day-old weight of chicks hatched during spring and winter seemed to be higher than those of chicks hatching during the summer. Ostrich chicks hatching from eggs laid during the summer had an earlier PT compared to those hatching from eggs laid in winter. Female age significantly influenced all the traits (EWT, CWT WL PT) under consideration. Ostrich EWT and CWT increased about 7% between 2-year-old and 3-year-old females and peaked at five years of age. A linear increase occurred in pipping time for eggs that were set immediately after collection to those eggs stored for longer than 7 days. Thus prolonged egg storage appears to affect embryonic development that can either result in embryonic mortality or a delayed pipping time.

As presented in Chapters 12 and 13, estimates of h^2 indicate that it is feasible to alter evaporative water loss of ostrich eggs by genetic selection. Egg weight and day-old chick weight, as well as pipping time, were also heritable. It is conceded that the standardisation of egg weight to reduce the variation in evaporative water loss during artificial incubation may assist in alleviating the present high level of embryonic mortalities. However, larger chicks are generally favoured by commercial chick raisers, as they would be more likely to

survive. First-grade, day-old chicks are expected to weigh 850 g, which could be used to derive a minimum egg weight of 1 400 g to allow the hatching of a first-grade chick. The 95% lower confidence limit for eggs used in the present study was 1 156 g, indicating that a substantial number of the eggs used were below this recommended weight. Direct and maternal genetic correlations among EWT, WL and PT traits were either high to very high in the desired direction, or small in magnitude.

The medium to high repeatability estimates for eggshell traits in the present study were partitioned into direct and maternal genetic components (Chapters 14 & 15). Permeability and water loss, as for WL, have an intermediate optimum, which would complicate implementation into future selection programs. Pore count (PC), permeability and shell thickness were negatively affected in eggs of older females. These results suggest culling of older females from the breeding flock may be beneficial to eggshell quality, as was also suggested in previous studies where embryonic mortalities were assessed. It seemed that different PC of ostrich eggs dependent on genotype, but those differences were generally small in magnitude, albeit significant. It is unsure if and how eggshell traits would compromise the standard practice of incubating eggs from different genotypes in the same incubator.

Initial studies on embryonic mortalities, as a trait of the female showed discouragingly low heritability (h^2) estimates (Chapter 13). However, when treated as a trait of the individual egg or chick, embryonic mortalities showed adequate genetic variation to ensure sustainable genetic progress, irrespective of whether it was assessed on the observed scale or the underlying liability scale (Chapter 16 & 17). To support this contention, evidence was provided that selection for chick production resulted in a genetic improvement of embryonic mortalities.

2. Recommendations

- Comprehensive research is required as far as the observed bloodline effects are concerned to establish impact on reproduction traits and hatching successes. Studies thus far focused on the SAB, ZB and the different crosses, but the effects of introducing the Kenyan Red Necks (KAR) into breeding populations still need to be investigated. One of the more direct and immediate recommendations is that females older than 8-10 years should be culled from the breeding flock. Older females may still have a good egg production, but a definite decline in chick production occurs with aging.

- The storage of eggs is part of the regular hatchery practices, but the influence of storage time and conditions on hatchability should be considered. Egg should be stored for at least 2 - 3 day but preferably not more than 7 days. Best hatching results came from eggs stored for between 4 - 5 days, but this is not always practical to implement in a hatchery program. Not setting eggs directly after collection would result in some eggs being stored for > 7 days when eggs are set weekly. It is important to know if there is a sudden increase in embryonic mortality in eggs stored for longer than

7 days. If not, it could well be practical to store those eggs collected 1 day and less before the intended setting date for 7 or 8 days before setting. This needs to be investigated further.

- Incubators should also be set to optimally control water loss within the ranges required for optimal hatching success. Ostrich hatching facilities mostly employs multi-stage incubators. Under such conditions it also seems to be a good management practice to minimize the transfer of eggs between incubators during the incubation process.

- Deviations from the normal hatching position vary greatly in ostrich chicks. Although a substantial proportion of near-term dead-in-shell ostrich chicks were positioned correctly, they still failed to hatch. The reasons causing this situation need to be studied further. Further work is needed to refine the different dead-in-shell positions before trying to classifying them.

- Information stemming from observations on embryo development throughout the incubation process can be used for the identification of incubation problems that could result in a low hatchability of fertile eggs. Results from the present study can be put to practical use when determining whether eggs are infertile or fertile with embryonic development that ceased early.

- Although the heritibility estimates indicate that it is possible to alter evaporative water loss of ostrich eggs, a feasible selection strategy needs to be devised, as it may be challenging to effect genetic change in a trait with an intermediate optimum. Egg and day-old chick weights, as well as pipping time were heritable and should respond to selection. Pipping time may play an important role in post-hatching survival of chicks. This possibility should be studied further.

- Embryonic survival was heritable when estimated as a trait of the individual egg or chick. The inclusion of a measure of embryonic survival in selection indeces for ostriches thus needs to be studied. It should also be considered whether the best strategy would be to select directly for embryonic survival, instead of focusing on traits like water loss or eggshell quality, which also show adequate genetic variation.

- Alternatively, it would also be beneficial to standardize egg size, evaporative water loss and eggshell quality at intermediate optima. It is expected that such an approach would also benefit embryonic survival. As such selection would mostly involve the females that produce the eggs and analyses should be expanded to study these traits as traits of the female.

- Further studies are needed on assessing embryonic survival as a trait of the female. It was noted that, whereas heritibility was discouraging low at 0.06 in Chapter 13, that the animal permanent environmental effect was higher at 0.33. It is feasible that part of the variation between animals may be repartitioned to additive animal effects when more data with a deeper pedigree are available. This would make future selection based on maternal performance feasible.

From the foregoing, it is evident that there are numerous opportunities for reducing embryonic mortalities in ostriches. Husbandry practices and knowledge of the stage of incubation when the death occurred should be integrated with genetic solutions to achieve this goal. It is foreseen that marked improvement may be achieved in this major source of hatching and reproductive failure.