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Review Article

OSTRICH (*Struthio camelus*) EGG EMBRYONIC DEATH DURING ARTIFICIAL INCUBATION

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Abstract

Intensification of ostrich farming revealed that egg hatchability was remarkably lower than the wild. This review considers the factors leading to, as pertaining to the ostrich, egg and incubator. Ostrich genotype, age, season and congenital problems affect clutch and egg sizes and egg quality- fertility to lead a successful hatch. Egg treatment prior incubation can later reduce hatchability, affected by storage conditions and duration. Most detrimental factors lie in the incubator and hatcher management. Egg correct positioning and turning in the appropriate incubator humidity and temperature are likely to yield high hatch. Variability in egg size, shell quality, pore sizes and numbers govern the water loss and exchange of gases. The hatcher management is important when chicks need intervention. Dead-in-shell embryos, early or late were likely to be affected by all of the above factors plus egg microbial contamination or be merely nutritional.

Keywords: Ostrich eggs; artificial incubation; embryonic death; hatchability

Introduction

Hatching success of ostrich eggs in artificial incubators is considerably below that found in wild ostriches (Hurxthal, 1979 and Bertram and Burger, 1981). Stewart (1995) attributed the difficult in ostrich artificial incubation to the great variation in the egg quality between the incubated eggs compared to chicken eggs which were more consistent in size and shell characteristics. Deeming (1993 and 1995a) reported that the hatchability depends firstly on the characteristics of the eggs themselves which were determined prior to laying and then on the management practices and the environmental conditions imposed after the egg was laid.

The many factors known to influence incubation success in eggs of domestic fowl also affect ostrich eggs hatchability. These factors include duration of egg storage, pre-incubation environmental conditions, egg size, shell thickness and porosity, and incubation criteria like temperature, humidity and frequency of egg turning (Rahn and Paganelli, 1979; Board, 1980; Tullett, 1984; Wilson, 1991 and Fasenko *et al.*, 1992). Problems with embryonic mortality during artificial incubation are still one of the main constraints to the development of ostrich industry worldwide, when in South Africa as a well-established industry, on average 50% of all eggs hatch and only 40% of hatchings survive to slaughter age. (Brown *et al.*, 1996 and Deeming and Ar, 1999). Some of the identified causes of hatchability depression (36.5%) included 0.9% infertile eggs, 8.3% early embryonic death, 7.3% egg rots and 5.9% dead-in-shell (Mushi *et al*., 2008).

The ostrich industry suffers from a high rate of embryonic mortality during artificial incubation of eggs. Ostrich eggs often have low hatchability rates because they do not lose sufficient weight during incubation. Egg size, eggshell porosity and thickness and length of pre-incubation egg storage are known to affect egg weight loss during incubation and hatch of ostrich eggs. Temperature, humidity, ventilation and rotation during the incubation period markedly affect the hatchability of fertile eggs and chick quality. Artificial incubation of ostrich eggs as practiced in intensively managed farms requires a relatively high degree of managerial skills. 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 importance and effect of systematic factors like females' age, incubators and season on the hatchability of ostrich eggs, as well as factors associated with incubation, are to a large extent unknown. The above mentioned problems will be discussed under three titles, the bird, egg and incubator.

The ostrich

Genotype

Genetic make-up is a major factor influencing the performance of individuals and genetic improvement may be achieved by selection for certain traits. No information is available on genetic parameters for ostrich incubation traits. According to Brand *et al*. (2007) genotype had no significant effect on the proportion of chicks that pipped, pipped in the correct position or their survival.

Embryonic mortality arising from genetic problems can negatively influence hatchability, but this has not yet been demonstrated in ostriches (Badley, 1997). Brand (2012) reported on embryonic development and hatchability of ostrich eggs from a combination of South African Black (SAB) male ostriches crossed with Zimbabwean Blue (ZB) female ostriches, to have 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%). The unexpectedly high level of embryonic mortalities in SAB male x ZB female combination is a cause of concern, especially since the best hatchability results in absolute terms were achieved in reciprocal cross.

Wilson (1996) suggested that the marked variation in shell porosity implies genetic variation in ostriches. Fertility and hatchability are major parameters of reproductive performance which are most sensitive to environmental and genetic influence (Stromberg, 1975). Heritability estimates for fertility and hatchability in chickens range from 0.06-0.13% (Sapp *et al.,* 2004), that is, non- genetic factors have a higher influence on these traits. Inbreeding is an important factor which affects hatchability. Hermes (1989) reported that high level of inbreeding causes embryonic mortality as a result of genetic mutations and negatively affected hatchability but the specific lethal genes have not yet been recorded in ostriches. Hatchability to a large extent is a derivative of fertility and the presence of major genes and strain/breed differences that affect embryonic livability (Peters *et al.,* 2005). Geneticists always suggest that the performance of individuals at a point is a function of genotype and environment interaction (Peter, 2000).

Female Age

The age of the female ostrich appear to be one of many factors influencing the number of eggs produced as well as the hatchability of eggs. Female age affected egg and chick weights, peaking in 4-5 years-old females, while other females (> 11 years) produced lighter chicks than the 2 years–olds (Bunter *et al*., 2001). In both broiler breeders and quail, young females had a higher proportion of early embryonic mortalities than mature females (Scott and Mackenzie, 1993; Reis *et al*., 1997; Hocking and Bernard, 2000; Yildirim, 2005), while for ostriches the opposite seems to be true in the sense that embryonic survival decreases over subsequent laying seasons (Badley, 1997). Female age had a significant effect on the proportion of chicks pipped, as well as on early and late embryonic mortalities (Brand, 2012).

Bunter (2002) and Cloete *et al*. (2006) reported that older ostrich females are still capable of good egg production, chick production declined overall due to higher levels of embryonic mortality, while Brand *et al.* (2007) reported that fertile eggs produced by older females are less likely to hatch than eggs produced by younger females. 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 (Brand *et al.,* 2007).

Season or Year of Production

Timing and duration of the ostrich breeding season can vary with latitude and altitude (Bertram, 1979). Several authors have reported significant season or production year effects on the reproductive performance of ostriches, egg and chick production (Van Schalkwyk *et al.,* 1996; Bunter *et al.,* 2001). Most authors reported that hatchability of fertile eggs increased as the breeding season progress from winter to summer (More *et al*., 1994; Bunter *et al.,* 2001) while these results differ from those of Wilson *et al*. (1997) that hatchability for the set number of eggs decreased linearly as the breeding season progressed. According to Brand *et al.* (2007) study, winter and summer showed that the proportion of chicks that pipped was higher ($p<0.05$) than in eggs hatched during the spring and embryonic mortalities declined by 1.8% towards summer. Conversely, Chowdhury *et al*. (2004) reported that the winter season resulted in the best hatching results for duck eggs.

Congenital Problems

Deeming (1996 d) stated that the congenital problems cause high rates of early embryonic deaths. Hormonal imbalances, such as that with the follicle stimulating hormone, have been shown to cause infertility in the ostrich as a result of interference with sperms production (Degen *et al.,* 1994). Ostriches are gregarious, mismatching and incompatibilities between the pairs, groups or colonies have been shown to result in copulation failures (Deeming, 1995b). Subsequently, eggs lay after such mating is infertile. Aiello (1998) reported that inter sexism resulted in reproductive incompatibilities among ostriches as it contributes to infertility. Some cocks had perhaps not developed functional spermatozoa at the time of mating when hens attain sexual maturity before cocks mature (Black, 1995).

Weakness or poor development of the pipping muscle *Musculus complexus* that has been shown to be under the

influence of a maternally derived testosterone could result in failure to pipe (Lipar and Ketterson, 2000).

The Egg

Pre-incubation Conditions

The storage of ostrich eggs prior to incubation is highly recommended due to the seasonal nature of egg laying in ostriches, particularly in the beginning and at the peak of the laying season to overcome incubator capacity problems (Ar, 1996). Ostrich eggs storage at temperatures of 18–23°C for 10 days was found to have no negative effect on the hatchability of ostrich eggs whereas storage for up to 14 days showed little decrease in viability (Gonzalez *et al.*, 1999). Ostrich eggs stored at 25°C show a significant increase in late embryonic mortality (Van Schalkwyk *et al*., 1999). According to Sahan *et al. (*2003a) embryonic mortality was higher in a group of eggs stored at 25°C and acceptable hatching performance was found in ostrich eggs stored immediately after collection at 21°C or less for seven days. However, storage for longer periods was found to cause a significant depression in hatchability (Swart, 1988).

Van Schalkwyk (1998) showed that storage temperature was a key factor in determining embryonic viability. Embryonic mortality was found to be lower at a storage temperature of 17°C than at 25°C. Lower embryonic mortality associates at lower storage temperatures and the temperature of storage also affected the size of blastoderm after 7 days storage with diameter doubling with each degree increase in storage temperature from 25 to 27°C. Storage of eggs at 15-19°C for a period of 1-7 days showed a depression in hatchability of fertile eggs up to 4% compared to eggs stored for either 1 or 6-7 days. This depression was associated with an increase in early mortality (Brinsea, 2003).

Storage Duration

The importance of storage duration of ostrich eggs cannot be underestimated (Deeming and Ar, 1999). 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. Ar and Gefen (1998) suggested that ostrich eggs may benefit from a storage period of only 3-4 days. Chicks from eggs stored for intermediate periods, i.e. 3,4 and 6 days prior to being set, were more likely to pipe than chicks from those eggs set directly after collection without storage (Brand, 2012).The author also reported that embryonic mortality was increased in eggs that were set directly (32.0%) or subjected to longer than 6 days of storage (43.5%).

Pre-incubation storage leads to morphological changes in the blastoderm and to lower growth rate of the embryo in small domestic poultry (Meijerhof, 1992 and 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 and Yildirim, 2005). This Coincides with the results of Badley (1997), Ar and Gefen (1998), Deeming and Ar (1999), Gonzalez *et al. (*1999), Nahm (2001), Sahan *et al.* (2004) and Hassan *et al.* (2005) that lower hatchability in ostrich eggs that could be attributed to an increase in early mortalities was for eggs stored between 10-12-14 days .

Deeming (1996 b) showed that for 12-14 days of storage, there was only 50% hatch of fertile eggs and this was associated with high early mortality (1-7 days) rather than late mortality. Long-term storage appears to be most successful at or near 12°C (Funk and Forward, 1960). Mayes and Takeballi (1984) concluded that the shorter the storage period, the higher the storage temperature required for maximum hatchability. Woodard (1982) reported that hatchability of eggs decrease quickly after 7 days of storage period for pheasant. Fertility, hatchability and hatchability of fertile eggs decrease with increasing storage time (Woodard and Morzenti, 1975; Woodard, 1982; Fasenko *et al.*, 2001 and Elibol *et al.*, 2002). Brake and Walsh (1993) and Brake *et al.* (1994) reported that albumen quality have an important role in embryo development. They demonstrated that setting fresh ostrich eggs particularly from young hens resulted in increased early embryonic mortality. Williams (1992) and Aaron (1994) cleared that storage of eggs for too long or those with poor quality; the albumen (in older or late season hens) loses water too quickly and causes early embryonic failure.

The incubator

Embryonic Mortality

There were substantial advances in incubator design and incubation techniques since the ostrich industry began in South Africa during the 1800s .The physiological requirement of the developing ostrich embryo can be met during artificial incubation by providing an appropriate temperature (Van Schalkwyk *et al.*, 1999), humidity(Swart *et al.*,1987), the correct gaseous environment and the proper turning of eggs (Van Schalkwyk *et al.*, 2002). The range in egg weight and shell water vapour conductance may be restricted by discarding eggs with extreme values to limit variations (Ar, 1996). Exposure to heat stress can impair hatchability (Ande and Wilson, 1981) and high temperature can lead to an increase in early-and late embryo mortalities as well (French, 1997; Badley, 1997; Hassan *et al*., 2005; Brand *et al*., 2011).

Jensen *et al.* (1992) reported that the standard mortality curve for a set of eggs maintains that one third of the total mortality occurs during the first trimester of incubation (the period of organ system development) and the remainder will occur during the last trimester of incubation (as the chick prepares to hatch). Mortality example in an 80% hatch, about 7% of the total mortality will occur during the first trimester and 13% of the total mortality will occur during the last trimester. A deviation in which 10% are lost during

the middle trimester might indicate a nutritional problem. Late deaths were related to the high temperature (Van sckalkwyk *et al.*, 1999), incorrect setting and inadequate turning of eggs (Deeming, 1996a; 1997; Brown *et al.*, 1996).

Calvert (1982) reported that in chicken eggs there are three critical periods in the embryonic development which causes the major mortality to occur throughout the incubation period. An early period from the third to the fifth day post lay covers 25% of mortality that is related to the poor hygiene and storage conditions prior to incubation. Thereafter, mortality is imposed by increasing or decreasing incubation temperature or insufficient turning of eggs. In a mid-period (day 12-14) during the second week of incubation, most cause will be faulty nutrition in the breeding flock which consequently leads to deficient essential nutrients in the egg, such as the deficiency of riboflavin which causes leg clubbed down and generalized edema of the embryo. Late period (from 18-20 days) contributed about 50% of the total mortality. It is a sequel of earlier weakening of the embryo during the early period or caused by incorrect setting of temperature, humidity or ventilation in the incubator. Mortality can result also from careless handling when transporting chicks to the hatchery compartment which which causes death by chilling. Insko and Martin (1933) showed that embryonic deaths in avian species appears to follow a pattern typified by the fowl embryo, with one mortality peak during the first few days of development and a second larger peak during the last few days of incubation, while Deeming (1995a and 1996b) described the same pattern for the ostrich, allowing for pathological variations depending on the causes of mortality, e.g. storage or microbial contamination. Assisted to-hatch chicks with poor growth rates, report high mortality with least survivals (Deeming and Ayres, 1994).

Ar *et al*. (1996) found that it was difficult to distinguish between early dead embryos and infertile eggs but showed a definite mortality peak is in the last week of incubation. Brown *et al*. (1996) reported that only seven embryos out of a total of 111 studied showed gross deformities, with four embryos having leg deformities, two having defects of the bill and one being ophthalmic.

During the eighties Swart (1988) had observed more eggs were produced from large number of breeding stock, but numbers of live chicks produced were still low. He suggested that the low and variable (40+30%) hatchability was due to the high embryonic moralities and infertility. Jensen *et al.* (1992) summarized that the two main reasons for poor incubator hatching results are poor hatchability potential of the egg (genetic, nutritional and physical components) and incubator environment factors (temperature, humidity and the carbon dioxide/oxygen balance). According to the study conducted by Brand (2012), transfer of eggs between setters (i.e. disturbance of eggs) during incubation reduced the number of ostrich chicks pipping in the correct position.

In a study conducted by Obied (2006) in red-necked ostrich eggs incubation for three consecutive seasons showed that the mortality of ostrich chick's embryo is the major problem in the incubation of ostrich egg especially in the first trimester of incubation. Early embryonic mortality was higher in the first season of incubation than the two successive seasons. Late embryonic deaths were different within groups in the season and within seasons. The overall means of total percent embryonic deaths were high in the first season and then decreased towards the third season. Results from the work done by both Deeming (1996 a; b) and Ar (1996) were corresponding with this pattern.

Agab (2005) conducted study in two ratite farms, (ostrich and Emu) in Al-Qassim area, Central Region of Saudi Arabia to record, analyze and discuss the day-to-day practical aspects and constraints affecting the commercial production of ostriches *(Struthio camelus*) and emus (*Dromaius novaehollandiae*). He found that the incidence of early embryonic mortality in ostrich eggs for four consecutive seasons (1999-2002) were 47.3, 36.7, 43.6 and 32.8 and for late embryonic mortality were 52.7, 63.3, 56.4 and 62.2. The majority of embryonic mortalities in ostrich eggs (59.9%) were in late developmental stages during the last two weeks of incubation, whereas 40.1% were mortalities happened during early embryonic development in the first four weeks of incubation as indicated by the dead embryo size. An average of 60% late embryonic mortality was recorded in ostrich and emu eggs compared to 40% early embryonic mortality. The high percentage of late embryonic mortalities in ostrich and emu eggs could be due to insufficient water loss during incubation. This produces a too small air cell that brings about some difficulties in the hatching process such as suffocation (Ley *et al.*, 1986; Horbanezuk and Sales, 1998).Some other workers, on the other hand, incriminated the vertical positioning method of the longitudinal axis of the eggs, which has been used in these two farms, as a cause of embryo death (Smith *et al.*, 1995).

Pre-incubation of eggs, particularly by the male breeders, due to late egg collection with subsequent cooling and storage of the pre-incubated egg, was considered a main cause for early embryonic mortality (Shane and Tully, 1996). Other possible causes of early embryonic mortality include poor quality eggs, microbially contaminated eggs, poor storage conditions and incorrect incubation temperature (Deeming, 1997; Hassan *et al.*, 2004).

Malpositioning

Many factors can interfere with the success of incubation or the quality of hatched chicks, such as egg positioning during artificial incubation. Ghodsi *et al*. (2010) conducted a study to investigate the effect of setting eggs with small end up,

on hatchability and embryo mortality in the Japanese quail. They found that mid and late embryonic mortality was higher in small end up eggs. They concluded that setting eggs small end up has significant effects on hatchability and cause higher embryonic mortality at late incubation period in the Japanese quail. Deeming (1991) and Tiwary and Maeda (2005) reported that chicken eggs stored with the small end up (opposite position) had significantly higher hatchability as compared with large end up eggs (normal position). They concluded that egg position changes the exposed surface area, changes loss of water from the egg and finally affects hatchability indirectly. The rejected candled eggs were divided into infertile eggs (Showing no development) and early embryonic deaths (where some development took place, but ceased before 21 days) and all shell–deaths after candling were classified as late embryonic deaths (Van Schalkwyk *et al.*, 1999).

The incidence of malpositioning in ostrich eggs was attributed to the improper turning and errors in positioning the eggs in the incubator because of difficulties in identifying the blunt end of the egg (Deeming, 1991). Deeming (1995a and 1997a) reported malpositioning as a common problem in dead-in-shell eggs, of incidence 36.9%, with most malpositions being of rotational problems, where the embryo was incorrectly positioned relative to the air space. Deeming (1995a), Ley *et al.* (1986) and Button *et al.* (1994) reported that head-in- the small end is the commonest malposition, but Brown *et al.* (1996), Deeming (1997) and Ipek and Sahan (2004) found a high incidence of malpositioning (51 of 93 embryos examined) with rotational problems being commonest. The authors examined ostrich embryos that died in the last 10-14 day of artificial incubation to assess the causes of mortality. The predominant symptoms of dead-in-shell embryos were malposition 55% and severe edema 41%. Of these, 22 embryos (24%) showed both symptoms. Malpositioning of the ostrich embryo may results from incorrect setting of eggs or inadequate turning, and dead-in-shell embryos of eggs whose weight exceeded 2kg were found to be in malposition and were edematous, with the beak facing away from the air cell. Malpositioning of embryos with respect to the air cell generally results failure to hatch (Button *et al*., 1994; Brown *et al.*, 1996; Deeming, 1997).

Deeming (1995a) reported that malpositions in the egg may have been misinterpreted in the past because of the unusual hatching position and the commonest malposition described here was head-in-the small end. Horbanozuk *et al.* (1999) recorded eggs from a batch inculcated at the highest (50%) initial relative humidity had the greatest number of malpositioned chicks most of them with unabsorbed yolk sac. Deeming (1996d) reported that towards the end of development, malpositioning is a major reason for late term mortality with head-in the-small end being the commonest malposition in ostrich eggs, while excessively high incubation temperature, too low or too high weight losses or hypoxia can all cause embryonic death during the late stage of incubation.

The malposition generally resulted from incorrect setting of the eggs or inadequate turning, and edema was significantly correlated with the amount of water lost from the eggs which was dependent on the egg size, while less than 10% of chicks examined were affected by myopathy, gross lesions of internal organs, hemorrhage, bacterial infection and congenital deformities. Brown *et al.* (1996) and Van Schalkwyk *et al.* (1996) suggested that these losses are mainly caused by inadequate incubation equipment, which results in high relative humidity, overheating and often inadequate hygiene management. About 25% of all fertile eggs that failed to hatch contain chicks in one or more of several malpositions (Byerly and Olsen, 1936).

Microbial Contamination

Bacterial contamination is an important factor for ostrich egg infertility, but it is not the only factor of early embryonic mortality (Deeming, 1995b). In South Africa, bacterial contamination was observed in 13.4% of the eggs examined and the organisms isolated were typical of soil and faecal environments (Brown *et al.*, 1996). Fungal contamination was found to be higher in eggs in which the embryo had survived to the end of development. Microbial spoilage of ostrich eggs has been shown to result in embryonic mortality (Deeming, 1996b), the author showed that bacterial contamination was the major microbial one, 18 - 20% in a variety of eggs sources, being the major cause of ostrich eggs' hatch failure in the United Kingdom. The properties of the shell remark important factors in determining the risk of microbial contamination. Shells with high conductance values have a higher risk of contamination and had higher average percentage mass loss values compared to uncontaminated eggs (Deeming, 1996b).

Sanitation programmes for ostrich eggs are based on procedures employed for poultry eggs and vary from simple brushing off soil to thorough egg washing (Deeming, 1997). However, the washing disinfection procedures were noticed to depress hatchability by 6– 10% due to increased early mortality (Van Schalkwyk *et al.*, 1998). Severely contaminated eggs can be washed but this procedure will reduce hatchability due to the removal of the outer protective cuticle. Immersion in a disinfectant solution may facilitate penetration of bacteria through shell pores. However, eggs can be dipped for 30 – 45 seconds in a freshly prepared solution of an approved phenolic or quaternary ammonium disinfectant warmed to 100° F. It is advised that eggs should not be immersed in an antibiotic solution as a routine, as this practice may lead to development of drug resistant strains of pathogens (Shane, 1996).

Several researchers have emphasized the importance of yolk sack infection as a cause of mortality which could increase the number of so-called dead- in shell eggs (Dzoma and Dorrestein, 2001; Walker *et al*., 2002). Dzoma and Dorrestein (2001) reported that 42% of the dead-in-shell ostrich embryos contained bacteria, and that the most common isolate was *E. colli*. Faecal contamination of eggs was considered to be the most important source of yolk sac infection (Rajesh *et al.,* 2001). Yolk sac infections can also result from translocation of bacteria from the chick's intestine or from the bloodstream (Saif *et al.,* 2003), also by topical contaminates which penetrate the egg shell (Huchzermeyer, 1998). According to Jahantigh (2010), Cabassi *et al.* (2004) and Deeming (1995a), detection of bacterial flora of the dead-in-shell ostrich chicks revealed *E. colli*, being most, plus Proteus spp, Kliebsiella spp, Staphylococcus spp and Bacillus spp were the isolated ones.

In a study to investigate the effect of micronutrients and of disinfection on hatchability of ostrich eggs, Musara and Dziva (1999) found 124 eggs condemned for failure to hatch after 46 days of incubation and when examined for gross pathological lesions they suspected streptomyces to have caused early embryonic mortality. Albumen protects the embryo from infection, therefore storage conditions and duration need to be adjusted to consider the quality of albumen (Badley, 1997).

Incubator Temperature

Temperature, humidity, ventilation and rotation during incubation period markedly affect the hatchability of fertile eggs and chick quality. The temperature experienced by a developing embryo depends on three factors; incubator temperature, ability of heat to pass between the incubator and the embryo and metabolic heat production of the embryo itself (French, 1997). The effect of temperature on the hatchability of fertile eggs was investigated by many researchers (Lundy, 1969 and Meir and Ar, 1990). Egg temperature varies greatly among the species in natural incubation (Kosin, 1964; Wilson *et al*., 1979). Deeming *et al*. (1993) reported that ostrich eggs could be incubated at temperatures between 35 and 37ºC. Landauer (1967) reported that the increase in temperature during incubation was very critical for chick embryos. Wilson (1991) reported that growth was retarded or ceased and the incidence of poor second quality chicks increased as the temperature was raised. The effect of different incubation temperatures on the incubation performance of ostrich eggs and hatchability characteristics are investigated by Ipek *et al*. (2003). Data obtained by the research indicated that the temperature applied during the growth period in artificial incubation of ostrich eggs significantly affected the hatchability of fertile eggs, embryo mortalities and incubation period. The authors reported that embryonic mortality tended to increase at 37.2ºC and the deformed chicks were observed only at37.2 ºC. Also the authors reported that the late embryonic mortality rate in the eggs incubated at 37.2^0C temperature was found to be high. Decuypere and Michels (1992) noted that older embryos were more affected by low temperatures. In South Africa and Australia, improved hatchability was found with a humidity of 34% – 37% at a recommended incubator temperature of 36ºC (Horbanezuk and Sales, 1998). Van Schalkwyk *et al.* (1999) found that dead-in-shell embryos were significantly higher in fertile eggs incubated at 37.30ºC than those incubated at 36.00ºC, and he suggested malpositions to increase with increasing incubation temperatures but significance could not be demonstrated.

Exchange of Water Vapor and Gases

The rate of water loss from eggs incubated artificially was found to be lower than the loss observed in eggs in the nest which was considered as a significant problem contributing to lower hatchability in artificial incubation. Moreover, low mass loss usually results in oedemic hatchlings or dead-in-shell embryos (Ley *et al*., 1986).Therefore matching the humidity of the incubator to the shell conductance is a way to optimize weight loss of individual eggs. On the other hand, insufficient water loss was found to produce a small air cell which may bring about difficulties in the hatching process leading to late embryonic mortality due to suffocation (Ley *et al*., 1986). Generally, it is observed that between 8% and 18% water loss, hatchability was relatively high whereas for eggs of both lower and higher water loss, mortality was usually close to 100% (Deeming and Ar, 1999). 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. However, Horbanezuk and Sales (1998) mentioned that the acceptable range of humidity during the incubation of ostrich eggs is 25 – 40%, although 40% humidity gives a loss of only 11% of the initial egg weight. Therefore, Sahan *et al.* (2003b) recommended that incubator humidity should be low (25%) to allow enough mass loss from the eggs during incubation while hatcher humidity was advised to be around 80%. Mortality of late stage embryos was found to be related to the percentage of water loss and mass specific water vapour conductance of the shell with extreme ranges causing the highest mortality(Deeming ,1996a). Eggs with low water vapor loss reflect on the chick being edematous or semi edematous. The edema degree determines the fate of the chick, if intense can lead to death unless assisted by making a whole in the top of the wide area in the egg to facilitate water vapor loss and relief hypoxia (Deeming, 1995b; Ar, 1991; Brinsea, 2003). Egg water loss also varies directly with the air cell volume.

Studies by Swart *et al*. (1987), 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%. Blood *et al.* (1998) indicated that there is a sharp increase in embryonic mortality below 10% and above 18% water

loss at 35 days of incubation. Davis et *al.*(1988) reported that excessive water loss results in dehydration of embryo, while Musara *et al.*(1999) reported that insufficient water loss from the egg can result in water retention by chicks, potentially causing embryonic mortality through respiratory insufficiency and a high proportion of chicks that are malpositioned at the point of hatch or have unabsorbed yolk sacs (Horbanczuk *et al.,* 1999). On average 15% of the initial egg weight is lost during incubation and an additional 6% is lost between pipping and hatching (Ar and Rahn, 1980).

In artificially incubated eggs, there are then two main factors that affect water loss from the eggs; relative humidity in the incubator and the porosity of the eggshell itself to water vapour. Relative humidity near 75% is recommended to prevent excess evaporative water loss from the eggs during storage (Stewart, 1995).

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. Bowsher (1992) stated that the hatching difficulty in the assisted groups may be caused by the low porosity; resulting in weak watery embryos trapped in. These embryos with marginal eggshell porosity were unable to break through the thicker shell to pip or to hatch. In such situation, chicks can be saved by intervention of the hatchery manger, but the dead in the shell group exhibited no difference in thickness and a decreased number of pores. Upon examination of the embryos, most died during some stage of yolk sac retention, or had completely retracted the yolk sac but died before piping and some were typical of embryos dying during the last stages of incubation due to insufficient respiration exchange. It was concluded that the variation found in regional porosity of ostriches eggs was great. The major contributing factor in the late dead embryo or dead-in shell was the low percentage of water loss from the ostrich egg during the incubation. Blood *et al.* (1998) showed that embryonic deaths due to the less evaporative water loss had occurred to 35 days of incubation. The weight of the eggs at the start of incubation influencing gaseous exchange and water loss has been extensively investigated (Hassan *et al.,* 2005). Large sized ostrich eggs were found to lose less water, had reduced oxygen uptake, abnormal calcium metabolism and were more frequently associated with oedematous chicks (Deeming ,1993).

Movement of water vapour through the avian egg shell is dependent on the functional pore area (Ar and Rahm, 1982) and the rate of loss is proportional to egg mass (Meir et *al.*, 1984; Meir and Ar, 1987). According to Gonzalez et *al.* (1999) ostrich eggs that possess low number of large pores and increased shell thickness hatch poorly and the large pore size numbers were positively correlated to egg weight loss during incubation while an inverse relationship was noted between thick egg shell and hatchability. Also the author reported that failer to pip may have been due to the shell quality and low carbon dioxide tension, and the latter is supposed to stimulate pipping .

Different levels of CO_2 play an important role in the development of embryos. Gildersleeve and Boeschen(1983) reported a higher hatchability , lower embryo mortalities and lower levels of malpositioning for turkey eggs when CO_2 levels were no more than 0.3% . A study conducted by Carlea *et al.* (2012) showed that a gradual increase in CO_2 levels during the first 10 days of incubation of chicken eggs enhanced embryo growth and improved hatchability, but after 10 days of incubation this practice had no effect on hatchability. Everaert *et al.* (2007) found that chicken embryo can tolerate high (4%) concentrations of CO_2 between day 10 and day 18 of incubation without any effect on pre and postnatal growth, embryonic mortality and hatchability.

Egg Turning

Egg turning was found to have a very significant role during development and promoting normal growth of the embryo and is essential during incubation up to transfer to the hatcher. It was found that turning through 30–45 degrees at hourly intervals satisfies normal embryonic development. However, Van Schalkwyk (1998) showed that as the angle of hourly rotation increased, the level of both early and late embryonic mortality decreased. The orientation of the egg during incubation also appears to have an influence, since eggs incubated with their long axis set horizontally for 2 – 3 weeks and then repositioned with their long axis set vertical for the rest of the incubation period yielded higher hatchability results and reduced embryonic mortality (Smith *et al.*,1995 and Van Schalkwyk *et al.*, 2000).

Some researchers, however, reported the low significance of egg turning frequency on hatchability, when Wilson and Eldred (1997) showed that hatchability of eggs turned eight times a day was not significantly different from eggs turned 24 times a day. Khan *et al.* (2012) conducted an experiment to determine the effect of pre-heating and turning during storage period on hatchability and post hatch performance of broilers. They reported that the hatchability of the eggs was highest when given turning and preheating during storage. Lack of egg turning results in poor chick hatch and delays hatch for a few days (Van Schalkwyk *et al.*, 2000; Yoshizaki and Saito, 2003). Elibol and Brake (2004) found increased mortality in malpositioned embryos with complete absence of turning during the first week of incubation.

Conclusion

The above mentioned factors concerning the bird, egg and incubator should all be considered when planning commercial ostrich husbandry and artificial incubation operations to avoid the high rate of embryonic mortalities aforesaid. Though genetic problems can negatively

influence hatchability, no solid information is available as yet on gene-inked traits for ostrich incubation, but non-genetic factors have a higher influence on these traits as their heritability values are low. Young females (4-5 year-old) yield significant proportion of pipping chicks, less early and late embryonic mortalities, more eggs as well as the hatchability. These values decrease over subsequent laying seasons. Controversial findings were cited on the effect of the breeding season progress from winter to summer or during the spring towards summer, on the hatchability of fertile eggs. Declined hatching can arise from congenital affections, leading to copulation failures resulting infertile eggs. Egg storage pre-incubation is necessitated by the seasonal nature of egg laying. Eggs are best stored below 20ºC after a pre-storage heating to incubation temperature for about 4hrs or at temperatures 18–23°C for 10 days but at 25°C eggs will show a significant increase in late embryonic mortality. Longer periods' storage depresses hatchability significantly but lower storage temperatures yields lower embryonic mortality.

Modern ostrich incubators were designed for better embryo development concerning temperature**,** humidity**,** correct gaseous environment and proper eggs turning. All eggs should conform in weights and shell conductance to limit variations. Comparative mortality studies of avian and ratite species revealed almost similar trends in early, mid and late embryonic mortalities apart from genetic and pathological causes. Early mortalities (25-40% of the total) were tied to the poor hygiene and pre-incubation storage conditions. In the mid period mortalities (about 10% of the total), most causes will be due to faulty nutrition in the breeding flock. Late deaths (50-60% of the total) were related to the high temperature**,** incorrect setting and inadequate turning of eggs**.** Mortality rates were found to decrease with advancing seasons of lay**.** The vertical positioning method of the longitudinal axis of the eggs was incriminated as a cause of embryo death**.** Late egg collection with pre-incubation of eggs and subsequent cooling and storage, was considered a main cause for early embryonic mortality beside poor egg quality, microbial contamination, poor storage conditions and incorrect incubation temperature**.** Egg positioning during artificial incubation can affect incubation success with malpositioning (55%) relative to the air space and severe edema (41%) which are common findings in the dead-in-shell embryos, all because of difficulties in identifying the blunt end of the egg and/or inadequate turning. Egg small-end up yielded higher embryonic mortality in the Japanese quail but higher hatchability in the chicken, subject to exposed surface area and consequent water loss. An estimate of 25% of all fertile eggs that failed to hatch contain chicks in one or more of several malpositions.

Microbial spoilage of ostrich eggs has been shown to result in embryonic mortality. Bacterial contamination, typical of soil and faecal environments was estimated at 13.4% of the eggs, causing ostrich egg infertility and early mortality. Shells with high conductance values have a higher risk of contamination compared to uncontaminated eggs**.** Egg sanitation by simple brushing, washing or immersion in a disinfectant depresses hatchability by 6– 10% due to increased early mortality**.** Faecal contamination of eggs was considered to be the most important source of yolk sac infection and about 42% of the dead-in-shell ostrich embryos contained mostly *E. colli* isolates**.** Care for albumen quality, through storage conditions and duration, protects the embryo from fungal early embryonic mortalities.

Incubation temperature was found best between 35-37ºC, when humidity was 34% - 37% with optimal ventilation and rotation during the incubation period. Optimal temperature adjustment can be affected by metabolic heat production of the embryo when egg size differences were high. A slightly higher temperature (37.2^0C) brought about increased deformed chicks and late embryonic mortality. Further increase (37.30ºC) increased dead-in-shell embryos with malpositions. Lower hatchability in artificial incubation compared to nest was attributed to lower rate of water loss (less than 13%). Insufficient water loss (below 10%) produces a small air cell leading to late embryonic mortality by suffocation at 35 days of incubation or oedematous hatchlings that need assistance by making a whole in the top of the wide area in the egg to facilitate water vapor loss and relief hypoxia**.** Egg water loss also varies directly with the air cell volume. Optimal water loss in artificially incubated ostrich eggs was found between 13 and 15% when hatchability was relatively high. Matching the humidity of the incubator to the shell conductance (25- 40%) is a way to optimize weight loss of individual eggs, while hatcher humidity was advised to be around 80%. Excessive water loss (above 18%) results in dehydration of embryos. The second factor of water loss from the eggs is the porosity of the eggshell itself to water vapour. Ostrich eggs hatchability could be substantially improved by selection for good shell qualities and adequate uniform shell porosity**.** Water vapour movement through the egg shell is dependent on the functional pore area and the rate of loss is proportional to egg mass. Poor hatch of ostrich eggs was tied to low number of large pores and increased shell thickness whilst large pore size numbers were positively correlated to egg weight loss during incubation. An inverse relationship exists between thick egg shell and hatchability. Failure to pip may have been due to the shell quality and low carbon dioxide tension. Gradual increase in CO_2 levels during the first 10 days of incubation of chicken eggs enhanced embryo growth and improves hatchability.

Egg turning during incubation is significant in promoting normal growth of the embryo regardless of frequency, whereas lack of yields poor hatch. Turning was suggested through 30–45 degrees at hourly intervals and as the angle increased, the level of both early and late embryonic mortality decreased. Egg orientation (initial horizontal incubation for 2-3 weeks) yielded higher hatchability and reduced embryonic mortality.

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