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Problems Associated with Artificial Incubation and Hatching of Ostrich (*Struthio camelus*) Eggs in Botswana

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Abstract: A study to investigate problems associated with hatchability of artificially incubated ostrich (*Struthio camelus*) eggs was conducted on a farm in Lobatse district, Botswana. Out of 1935 eggs set in the entire breeding season, 1306 chicks hatched, thus depicting a 67.5% hatchability rate. Some of the identified causes of hatchability depression (32.5%) included infertile eggs (0.9%), early embryonic death (8.3%), egg rots (7.3%) and "dead in shell" (5.9%). Hatchability depression was maximal during the month of November 2001 at a rate of 42.7 and lowest (20.4%) in August, 2001. These findings suggest that breeding should be maximised during the month of August when hatchability depression would be relatively minimal.

Key words: Hatchability, *Struthio camelus*, hatchability depression, infertility, egg rots dead-in-shell, early embryonic death

INTRODUCTION

Whereas commercial ostrich (*Struthio camelus*) production in Europe, United States of America.and Australia has been described as emerging (Deeming *et al*., 1993) in Botswana, it is not well developed. There is a dearth of information pertaining to reproductive performance of the ostriches. Artificial incubation of ostrich eggs as practised in intensively managed farms, requires a relatively high degree of managerial skills. The sustainability of such a production system depends on a high level of hatchability, which is the percentage of eggs that hatch into viable chicks out of the total number that is laid. Various authors have identified some of the major factors affecting hatchability (Doneley, 1994; Deeming, 1995; More, 1996; Samson, 1997; Gonzalez *et al*., 1999).

Various environmental conditions namely duration of storage period of eggs prior to incubation, temperature, humidity, season of lay, gaseous environment and orientation of eggs have been shown to influence ostrich hatchability (Gonzalez *et al*., 1999).

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). The author also found out that oedematous chicks were more prone to malpositioning. The author reckoned that the latter would culminate inembryonic death due to hypoxia.

Seasonal infertility whereby oviposition may occur early in the breeding season before the cock has produced functional spermatozoa may result in the hen laying infertile eggs (Black, 1995). Hormonal imbalances resulting in low levels of testosterone in the cocks during the breeding season has been described (Black, 1995). Hormonal imbalances such as the Follicular Stimulating Hormone (FSH) have been shown to cause infertility in the ostrich as a result of interference with spermatozoa production (Degen *et al*., 1994). Although, ostriches are gregarious, mismatching and incompatibilities between the pairs, groups or colonies have been shown to result in copulation failure (Deeming, 1995). Subsequently, eggs laid after such mating are infertile.

Intersexism resulting in reproductive incompatibility ha also been reported among ostriches elsewhere as contributing to infertility in ostriches (Aiello, 1998). In some of these cases, black pigmented hens may be rejected by males at mating since they may have rudimentary male sexual organs and are recognised as fellow males.

Microbial spoilage of eggs culminating in infertility has been attributed to dipping or washing of ostrich eggs with liquid disinfectants making eggs vulnerable to microbial attack (Huchzermeyer, 1996; Richards *et al*., 2002). It is imperative that egg hatchability depression is minimised to ensure sustainability of the

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ostrichindustry in the country. Therefore, the purpose of this study was to investigate problems associated with hatchability of artificially incubated ostrich eggs and possible preventive measures. This is the first report on problems associated with hatchability of ostrich eggs in Botswana.

MATERIALS AND METHODS

Information on the problems encountered during artificial incubation of ostrich eggs and subsequent hatching was obtained from an ostrich farm in Lobatse district of south eastern Botswana. The farm was located in Lobatse with a grid reference of 25° 13'E and 25° 55'S at an altitude of 1192 m with an annual rainfall of 500 mm (data obtained from Botswana Meteorological Services). The birds in the breeding camps were supplemented with a commercial ostrich feed for breeders (Table 1).

Every two weeks, visits were made to the farm in order to collect data from the farm manager. The data included total number of chicks hatched as a percent of all eggs set; hatchability depression was calculated using the following expression:

$$\text{Hatchability depression} = \frac{\text{Total no problems encountered}}{\text{Total number of eggs set}} \times 100$$

Breeding birds were kept in breeding camps in a cock: hen ratio of 1:2 prior to the onset of the breeding season, that lasts from August to April.

Attempts were made on the farm to cross breed three subtypes mainly, the less domesticated Kalahari Blue neck, indigent to Botswana, the more docile, Black neck otherwise known as the Cape ostrich and the Red neck, thought to have originated in Masailand, Kenya (Jarvis *et al*., 1985).

The eggs were laid in scrapes or scratches (nest) to simulate their natural habitat in the wild. Using sterile disposable gloves, these eggs were collected in sponge padded boxes twice a day, on the same day when they were laid, to prevent brooding by the hens. Sterile dummy eggs were placed in the nests all the time to encourage the hens to lay eggs.

Eggs were rebuffed with dry steel wool to remove any dried up mud on the shell. The boxes were well ventilated by holes on the sidewalls of the box to reduce a build up of carbon dioxide around the eggs. Extreme caution was exercised in handling eggs, to prevent hairline cracks, which could expose eggs to bacterial contamination due to loss of the protective cuticle. They were transported on their sides. The eggs were weighed and labelled to indicate source, date of collection and mass. Thereafter, eggs were fumigated with a mixture of 40% formaldehyde in 200 g potassium permanganate.

Table 1: Nutrient values of commercial ostrich feed for breeders as indicated by the manufacturer

Ingredients	(%)
Plant protein (cotton seed cake)	15.4
Crude fibre	12
Crude fat (ether extract)	4.0
Calcium	1.7
Phosphorous	1.0
Magnesium	0.43
Manganese	360 mg kg^{-1}
Zinc	175 mg kg^{-1}
Selenium	0.42
Vitamin E	49 mg kg^{-1}

Storage was at 12-18°C in a cold room. Prior to transfer to the incubator, the eggs were pre warmed for 8-12 h at 25°C. The eggs were set on day 10; thereafter, they were incubated at a temperature of 36.2°C and 42% relative humidity. Eggs were weighed weekly to find out the rate of weight loss, which was a reflection of water loss, by the embryo.

Egg incubation and hatching on this farm were carried out in high security premises with restricted access.

Candling to check for embryonic development was done on day 14 after collection.

While in the setting room, the eggs were turned about 6 times daily, on each occasion vertically through 45° each side of vertical. Eggs were subsequently transferred to the hatcher on their side about 6 h before hatching on the 42nd day. After this day on wards, candling was done every day, to ascertain embryo viability. In some cases, cracking the egg open helped chicks that failed to pip. Non-hatching eggs, infertile eggs and dead-in-shell embryos were submitted to the laboratory for further investigation such as bacteriological and fungal culture using previously described micro-biological methods (Quinn *et al*., 1998).

Briefly, swabs from the above eggs were cultured both aerobically and anaerobically on blood and MacConkey agar respectively, at 37°C for 24 h. Some swabs were plated on Sabouraid dextrose agar for fungal culture and incubated aerobically for one week at 37°C. The colonies were characterised based on morphology and appearance as previously described (Buchanan and Gibbons, 1974). Analytical Profile Indices (API) used as an adjunct to bacterial identification was carried out as described by the manufacturer of the kits (Bio Meriaux, Lyon, France). The coagulase, oxidase and catalase tests were conducted on selected colonies using conventional methods (Quinn *et al*., 1998). The data was analysed for means and differences between means using Student' t- test.

RESULTS

Out of a total of 1935 eggs set in the entire breeding season, 1306 chicks hatched, thus depicting a 67.5% hatchability rate. Some of the causes of hatchability depressions are depicted in Fig. 1. Major causes of hatching depression rated at 32.5% included infertile eggs, 10.9% (n = 211); early embryonic deaths, 8.3% (n = 162), egg rots, 7.3% (n = 144) and dead-in-shells, 5.9% (n = 142).

When hatchability was considered on a monthly basis (Fig. 2), November had the highest hatchability depression (42.7%) followed by October (38.5%). The lowest hatchability depression of 20.4% was in August. The average monthly depression of hatchability was 32.9%.

It was observed that by the Kalahari blue necks were dominant and less docile than the Black necks and exhibited aberrant mating behaviour. They spent most of their mating time in the morning in threat and territorialism related activities. Kalahari blue necks laid associated with infertile eggs.

Noteworthy was the inhibited mating behaviour of the Kalahari Blue cocks in contrast with the carefree Black necks (Cape ostriches). The latter sub species were less prone to disruption during copulation. Only a few breeding camps cross breeding the Blue, Black and Red necks experienced problems of cock-hen incompatibility.

Kalahari Blue neck hens were found to lay very large eggs (over 2.0 kg) in contrast to the Black neck hens, which laid medium-sized eggs with weights ranging from 1.3-1.6 kg. Eggs from one breeding camp with sub species cross breeding, were large with a characteristic double ridge. Such eggs were associated with poor hatchability. Furthermore, chicks hatching from such eggs were weak and had poor survivability. Some of the "dead-in-shells" looked oedematous.

Microbial flora cultured from non-living hatching eggs included Bacillus cereus, Aeromonas hydrophila, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, Aspergillus and Penicillium species (Table 2).

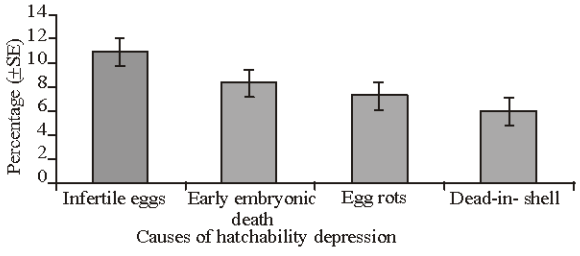

Fig. 1: Causesof hatchability depression among ostriches

Table 2: Micro-organisms isolated from unhatched ostrich eggs

Microorganism isolates	Number of isolates	(%)
Escherichia coli	10	27.7
Bacillus cereus	7	19.4
Aeromonas hydrophila	6	16.6
Staphylococcus aerueus	6	16.6
Streptococcus faecalis	5	13.8
Aspergillus flavus	1	2.7
Penicillium sp.	1	2.7
Total	36	100.0

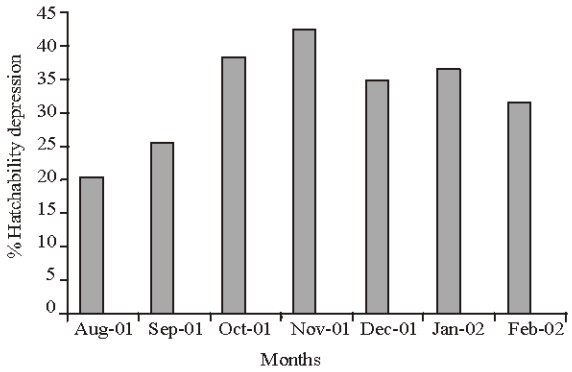

Fig. 2: Monthly hatchability depression

E. coli was the commonest bacterium isolated and accounted for 27.7% of all microorganisms isolated (Table 2).

DISCUSSION

Results in this study showed a hatchability depression of 32.5% caused by various factors as depicted in Fig. 1. The commonest cause of hatching depression was egg infertility. This rate of hatchability was comparable to that obtained from ostrich eggs in Zimbabwe (Foggin and Honywill, 1992), United States of America (Aiello, 1998) and Australia (More, 1996). In South Africa, a high rate of embryo mortality during artificial incubation of ostrich eggs was reported in the last 10-14 days of incubation (Brown *et al*., 1996). Although the hatchability obtained in the present study was higher than that reported elsewhere, it is imperative that a higher figure is obtained and the causes of hatchability elucidated in order to ensure sustainability of the industry.

Breeding took place in the recommended period, August to April which is conducive for maximum reproductive potential (Jarvis *et al*., 1985). Hatchability was maximal during the month of August 2001 in spring and lowest in November 2001, during the summer rains. Perhaps handling of wet eggs may have made eggs vulnerable to microbial penetration. According to eggs laid early in the season have better albumen quality and

thicker shells that allow less oxygen. It was, therefore, paradoxical that hatchability was maximal in August.

Some authors have indicated that long storage period of the eggs before incubationmay lower hatchability (Gonzalez *et al*., 1999; Nahm, 2001; Hassan *et al*., 2005). The period over which eggs were stored in this study was of no consequence since the eggswere consistently set on day 10.

Another possible contributory factor to low hatchability in this study may have been the age of the ostriches at mating. Too old or too young a hen has been shown to compromise egg fertility (Black, 1995). Since culling of breeding stock due to old age was not frequently practised on this farm, it is possible that this may have contributed to infertility. It is also possible that some cocks had perhaps not developed functional spermatozoa at the time of mating since hens attain sexual maturity before cocks mature (Black, 1995). This could not be confirmed for studies on semen evaluation were out of the scope of the present study.

Behavioural problems have been shown to interfere with egg fertility (Doneley, 1994). This may have been minimal since it has been shown that sperm storage occurs in the ostrich hen and fertile eggs can be laid as long as eight days post copulation (Bezuidenhout *et al*., 1995). However, since some of the dominant male Kalahari Blue cocks spent most of their mating time allocation in aggressive and threat-related activities, there was little time for copulation. This could partlyexplainwhy some hens in some of the breeding camps housing dominant cocks were laying infertile eggs.

The lack of human sexual imprinting response in the less domesticated Kalahari Blue neck cocks has been shown to result in lower testosterone levels which would compromise spermatogenesis (Deeming, 1995). It was observed that Kalahari Blue necks in the breeding camps showed inhibited mating instincts in the presence of farm workers. Furthermore, this subspecies were more vulnerable to distraction than either the Red or the Black necks during copulation. Although, staff were discouraged from visiting the breeding camps at mating time, this behavioural problem may have somewhat compromised egg fertility.

Mismatching, incompatibility and intersexism alluded to in the literature as factors likely to reduce egg fertility, were not encountered among ostriches on this farm.

Genetic relatedness of the ostriches was ruled out since ostriches were tested previously to prevent in-breeding. The possibility of in-breeding as one of the possible causes of egg infertility was thus eliminated by laboratory testing.

Failure of large sized eggs laid by the Kalahari Blue neck hens may have been aggravated by the presence of low density of large pores and thick shells (Gonzalez *et al*., 1999; Christensen *et al*., 1996; Foggin and Honywill, 1992). Chicks hatching from such eggs were weak and had to be assisted out of the shell. One plausible explanation was that large eggs produced large (oedematous) chicks that fail to lose the critical amount of water in order to hatch by day 42 of incubation (Brown *et al*., 1996). Mortality of late stage embryos was found to be related to percentage of water loss and mass specific water vapour conductance of the shell with extreme ranges causing mortality (Deeming, 1996).

It is, therefore, possible that perhaps, the incubation temperature of 36.2°C and a Relative Humidity (RH) of 42% used on this farm was not adequate for the heavy eggs laid by the Kalahari blue (Hassan *et al*., 2005). This reduces pore area and reduced egg shell conductance resulting in a " dead-in shell".

Microbial spoilage of ostrich eggs has been shown to result in embryonic mortality (Deeming, 1996). The microflora recovered from eggs upon culture were similar to that cultured from non-hatching ostrich eggs in Zimbabwe (Foggin and Honywill, 1992). Although, workers on this farm always exercised stringent hygienic measures while handling eggs, microorganisms may have penetrated wet eggs through the pores (Deeming, 1996; Richards *et al*., 2002).

Fumigation of eggs was carried out on this farm as recommended by (Huchzermeyer, 1996). Probably, bacteria and fungi cultured from egg rots could have resulted from inadequate disinfection of contaminated incubators. Although egg incubators on this farm were disinfected regularly before use to prevent contamination, the efficacy of this disinfection was not assessed regularly. Elsewhere, microbial contamination of eggs was a significant problem and varied in eggs from different farms indicating that more attention is needed in both breeder and nest management (Deeming, 1996). In the present study, microbial spoilage accounted for 7.3% of hatchability depression. Reports from other workers cited microbial infections as a minor cause of infertility accounting for less than 1% of non-hatching eggs (Brown *et al*., 1996).

Malpositioning of the ostrich embryo may result from incorrect setting of eggs or inadequate turning (Brown *et al*., 1996). Dead-in-shell embryos of eggs whose weight exceeded 2 kg were found to be in malposition II and were oedematous, with the beak facing away from the air cell. The Kalahari Blue necks hens with their crosses with the counterpart cocks laid most of these eggs. The normal hatching position of ostrich is such that the beak

moves to the air cell during internal piping. In this study, the dead embryos were in the small end of the egg as described by Deeming (1995).

It has also been established that movement of water vapour through the avian eggshell is dependent on the functional pore area (Ar and Rahm, 1982) and the rate of loss is proportional to egg mass (Meir and Ar, 1987).

These heavy eggs seem to have had thick eggshell that would partly explain the failure to lose the expected 12-15% weight within the 42 day incubation period. Ostrich eggs that possess low number of large pores and increased shell thickness hatch poorly (Gonzalez *et al*., 1999). Similarly, gaseous exchange follows the same pattern.

Failure to pipe may also have been due to the shell quality and low carbon dioxide tension. The latter is supposed to stimulate piping. 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 (Gonzalez *et al.,* 1999).

A diagnosis of myopathy based on finding suggestive gross muscle lesions had been cited as a possible cause of infertility in the ostrich (Brown *et al*., 1996). Perhaps weakness or poor development of the piping muscle *Musculus complexus* that has been shown to be under the influence of maternally derived testosterone (Lipar *et al.,* 2000) could result in failure to pipe. The possible role of myopathy in the non-hatching phenomena could not be underestimated considering that cases of Vitamin E/selenium responsive myopathy have been previously described and successfully treated (Mushi *et al*., 1998). Supplementation with "seleron", a preparation containing selenium and vitamin E may be beneficial in increasing the mass of the piping muscle, *M. complexus*.

CONCLUSION

In order to minimise hatching depression, the following recommendations were made:

- Monitoring of water loss from large and small eggs during incubation to minimise the tendency to produce oedematous chicks from former.
- Study specific conductance of thick shelled eggs with a ridge laid by the Kalahari blue neck hens.
- Artificially, increase eggshell conductance to improve hatchability as has been done for turkey eggs (Meir and Ar, 1987) with a high degree of success.
- Regular monitoring of hygiene in the incubators and assessing effectiveness of disinfection.
- Investigatingthe role played by some of the environmental conditions; humidity, water loss from eggs during incubation warrant.

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