

**IMPROVEMENT OF FERTILITY  
AND HATCHABILITY OF ARTIFICIALLY  
INCUBATED  
OSTRICH EGGS IN THE  
LITTLE KAROO**

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**BY**

**SALMON JACOBUS VAN SCHALKWYK**

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## **DECLARATION**

I, the undersigned, hereby declare that the work contained in this thesis project is my own original work and has not previously in its entirety or in part been submitted at any university for a degree."

**SJ VAN SCHALKWYK**

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# ABSTRACT

Ostriches are an important commercial species in South Africa and are becoming increasingly so in other parts of the world. Fertility and hatchability of artificially incubated ostrich eggs, however, is generally regarded as low compared to other poultry species and to ostriches in the wild. Investigation into specific farming practices at present indicated scope for an overall improvement in productivity through a sound breeding strategy. This thesis investigated factors that affect egg production, fertility, and hatchability of artificially incubated eggs in the Little Karoo region of South Africa. Specific breeding pair combinations accounted for the major variations in egg weight, hatchability, chick production and offspring weight at slaughter age. An appreciable proportion of variation in reproductive traits was attributable to the repeatable nature of breeding pair performance from year to year, even from first breeding attempts, suggesting that selection of good breeding stock can be made from an early age. Artificially incubated eggs showed improved hatchability when eggs were collected two to three hours after lay rather than the following morning. Storing position of eggs did not significantly effect hatchability when eggs were stored for a maximum of one week. The critical zero temperature for ostrich eggs, below which no embryonic development takes place, was found to be  $\pm 25^{\circ}\text{C}$  and cooling eggs to temperatures below  $20^{\circ}\text{C}$  for complete cessation of embryonic development during storage resulted in better hatchabilities compared to eggs stored at  $25^{\circ}\text{C}$  room temperature. Hatchability decreased when incubator temperatures were raised from  $36$  to  $37.3^{\circ}\text{C}$ . Large temperature fluctuations and gradients, which encompass detrimental temperatures, persist within forced draught wooden incubators of the type most commonly in use in the Little Karoo region. The highest temperatures occurred at the top of these incubators and will consequently have a negative impact on hatchability. The ontogeny of ostrich egg metabolism showed an exponential increase during the first 70 % of incubation followed by a decline to 75 % of the peak value between days 31 and 38 of incubation. From peak levels of embryonic development it was calculated that single stage incubators needs an airflow of  $54.4$  l/egg.hour to maintain oxygen levels just below 21 % and carbon dioxide levels below 0.5 %. Lower embryonic mortalities were observed when eggs were turned twenty-four times/day in an electronic incubator compared to hand turning twice a day. Eggs rotated through increasing angles between  $60$  and  $90^{\circ}$  resulted in a linear improvement in hatchability. In incubators where turning angles were fixed

at 60°, lower hatchabilities were overcome by incubating eggs for 2 - 3 weeks in a horizontal position before placing them vertically. No specific farming practice could be singled out as the main cause of low fertility or hatchability but rather a combination of certain practices applied wrongly.

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# **CHAPTER 1**

## **INTRODUCTION**

## **HISTORICAL BACKGROUND TO THE OSTRICH INDUSTRY IN SOUTH AFRICA**

The ostrich feather trade is several thousand years old, dating back to the period of the early Egyptian, Assyrian and Babylon civilisations of the world. Feathers of wild ostriches were exported from Senegal, Morocco, Algiers, Sudan, Libya and Egypt with the Sahara desert as the main hunting ground for wild ostriches, the birds being killed solely for their plumes.

The African continent appears to be natural home of the ostrich. Towards the middle of the last century ostriches could still be found in large numbers practically all over southern Africa, East Africa and Somalia. Ostriches also inhabited the Arabian Desert, Palestine and Persia (Smit, 1963).

### **1850 - 1913**

Farming with domesticated ostriches, as practised in the Little Karoo and elsewhere in South Africa today, was started between 1857 and 1864 as a completely new branch the agricultural industry (Smit, 1963). Many claim the credit for the domestication of the ostrich but relevant literature is somewhat forgotten, widely scattered and mostly dating back about half a century. In light of available information, Smit (1963) concluded that as far as southern Africa is concerned, several farmers in the Karoo and Eastern Cape succeeded more or less simultaneously in breeding and rearing ostriches during the early 1860's. Arthur Douglass maintained that he was the first to make ostrich farming his sole occupation in 1867 and improvised the first incubator for ostrich eggs, naming it the Eclipse. According to censuses of the Cape Colony there were 80 domesticated ostriches in 1865, 32 247 in 1875 and 253 463 in 1895. This increase took place in spite of a severe drought and a concurrent unknown epidemic during 1889 to 1909, when literally thousands of birds died (Smit, 1963).

Ostriches were initially farmed for their feathers, which were highly prized in the fashion industry. The feathers of the Cape wild birds were, however, by no means the best on the markets of Europe and better feeding and care apparently did little to improve them. Birds were consequently imported into the Cape from Tripoli, Syria, Algeria, Morocco and Tunisia to improve the local strain through crossbreeding. By 1911 the Cape feathers were superior to any other wild feathers and many well known strains such as the Evans, Barber and Riempié

were developed. By 1913 ostrich feathers ranked fourth in value after gold, diamonds and wool on the list of all exports from the Union of South Africa.

#### **1914 - 1944**

With the advent of World War I in 1914 the feather industry collapsed overnight. From an estimated one million ostriches in 1914 the numbers decreased to a mere 23528 in 1930. Killing was done rigidly and only the very best breeding birds were kept. In 1925 the "Suid Afrikaanse Volstruisboere Koöperasie Beperk" was established in order to regulate the market. Having gone from bad to worse for some time this enterprise eventually failed.

#### **1945- 1975**

When the feather market revived during 1945 - 1946 practically all the ostrich farmers, with the exception of the Little Karoo had dropped out of the business. The Little Karoo Agricultural Co-operative was established in 1945 at Oudtshoorn and the feather duster trade experienced rapid expansion with sales being initiated during February, 1947. The Co-operative subsequently gained control over all ostrich products in South Africa on 1 August 1959. This period also saw an increased interest in ostrich meat products and the biltong (dried meat) trade boomed. As a result, an abattoir was built in Oudtshoorn in 1965. In 1970 the Co-operative built a tannery for the export of finished ostrich leather. Of the total value of ostrich products during 1973/74 between R4 and R5 million (SA rands) was from feathers.

#### **1975 - 1995**

During 1975/76 there was a shift away from feathers towards hides and meat. From the R7 million revenue during this period only R3 million was from feathers. The abattoir was upgraded and modernized during 1980/81 to satisfy the meat market and the industry in the Little Karoo expanded rapidly (Table 1). The drop in wool, mohair and meat prices has subsequently also stimulated ostrich farming in the areas outside the Little Karoo since 1985.

**TABLE 1: GROSS TURNOVER OF OSTRICH PRODUCTS IN SOUTH AFRICA BETWEEN 1982 AND 1992. *Nel in litt. (1994)***

YEAR	FEATHERS	OTHER PRODUCTS (R1000)
1982	5194	8779
1983	4889	13173
1984	3904	18603
1985	4154	28919
1986	5217	33544
1987	6696	52892
1988	8287	54128
1989	9119	68434
1990	8222	75825
1991	13913	118035
1992	14011	145700

As new ostrich farming areas developed the Ministry of Agriculture was pressurized to deregulate the ostrich industry and this finally came about on 26 October 1993. Although the majority of ostrich farmers are still found within the Little Karoo ( $\pm 70\%$  of the estimated 35 000 breeding females) ostrich farming has now expanded throughout the whole South Africa and a National Ostrich Production Organization was formed in May 1994 to develop the industry on a national basis.

By 1995 about 170 000 birds were being slaughtered annually at six European Union-approved abattoirs throughout South Africa, of which 75% were slaughtered at the Klein Karoo Co-operative in Oudtshoorn. Ostrich skins are presently tanned at five tanneries while about six processing plants vacuum-pack meat for export, mainly to Europe. Switzerland is currently the biggest importer of ostrich meat while Japan and the United States are the largest importers of tanned ostrich skins. In 1993, when the ostrich industry deregulated, ostrich farmers received about R230 million as payment for slaughter birds. Van Zyl (1996)

suggested that the income from ostrich products for 1995 was around R300 million. Of this total, 75 % is from skins, 20 % from meat and 5 % from feathers.

The income derived from the slaughter of 160 000 - 200 000 birds per annum at present seems relatively little compared when compared to other agricultural enterprises. In the Little Karoo, however, ostrich farming is still the main source of income for most farmers and it has become increasingly important in other areas of South Africa.

## **OSTRICH FARMING IN OTHER COUNTRIES**

European countries have been developing ostrich farming since 1990. Ostrich farming activities in Europe are centred mainly in the United Kingdom, Belgium, Holland and France. In each of these about 200 small ostrich farms are operational. These farms are geared mainly to the production of breeding birds and the total number of ostriches in Europe is currently estimated to be less than 10 000. (Deeming and Angel, 1996). Van Zyl (1996) suggested that between 15 000 and 20 000 birds were slaughtered during 1995 in the rest of the world

According to a survey done by the American Ostrich Association in 1995, the total number of ostriches in the United States was about 250 000 (Ball, 1995). Prices in the United States vary greatly by region. Proven breeders ranged from below \$5,000 to \$15 000 a pair while day old chicks sold for about \$100 to \$150 each in 1996. Ostrich skins coming from slaughter plants in the United States are processed in the United States but also in Mexico where labour is cheap and facilities abundant.

According to Van Zyl (1996), the number of ostriches in Australia was estimated to be more than 50 000. Because the industry in Australia is still very young it is currently based on a breeder market. Although ostriches are also farmed in Canada, Van Zyl (1996) suggested that the Canadian ostrich industry cannot be considered a commercial market as breeding bird numbers are still too low. It is doubtful whether it will become a viable industry because of the high cost of housing facilities for young birds during the long cold winters.

Israel has an estimated 25 000 ostriches which includes 6000 breeding females. In 1995, 9000 birds were slaughtered. Raw hides were exported around the world because no tannery exists in the country (van Zyl, 1996). Commercial ostrich farming was introduced a decade ago in the Guangdong province of the People's Republic of China with a estimated 7 500 bird in 1996. Although ostrich production has developed rapidly, marketing of ostrich products is hampered by inadequate processing facilities.

A growing ostrich industry is also to be found in our neighbouring countries. Zimbabwe has an 230 to 250 ostrich farmers and an estimated 33 000 ostriches. There are two main abattoirs and two tanneries. Exported ostrich products, either finished or crust skins, are mainly exported to markets in Europe, Japan and Singapore. Namibia is believed to have more than 21 000 ostriches which includes about 5 000 breeding females. During 1995 between 5000 and 6000 birds were slaughtered for the exported market. Because of severe droughts in Namibia many farmers see ostrich farming as a viable alternative for the future because of the adaptations of these birds to arid and semi-arid environments. In Botswana only 10 commercial ostrich farms were operational in 1996 with a total of 5000 birds which includes breeders and chicks. Breeding females numbered about 500. Botswana is a breeder sales-orientated industry that exports breeding material to countries around the world. During 1996 no birds were slaughtered in Botswana and the Botswanan government is presently actively promoting ostrich farming and is developing the necessary infrastructure for a slaughter industry.

As in South Africa, income derived from ostrich farming in other countries is relatively little compared with other agricultural enterprises but it is seen as a viable alternative to other agricultural products.

## **OSTRICH RESEARCH IN SOUTH AFRICA**

As early as 1881 investigations were conducted into various diseases and parasitic infections, of which tape worm and "rotten stomach" were the most prominent. Crossbreeding experiments with the aim of improving feather quality of the birds in South Africa was conducted by Deurden at the Grootfontein Agricultural College in the Eastern Cape. Various

studies on these aspects were published (Deurden 1910, 1912a, 1912b) and crossings  $\frac{3}{4}$  Cape and  $\frac{1}{4}$  North Africa seemed to produce the best feather crop. The drop in the feather industry in 1914 brought a halt to all ostrich research until the late 1940's and 1950's, when the need for research was initiated again due to renewed interest in the industry.

Research in the early sixties centred around feather production and dietary needs, especially for optimal feather production (Smit, 1963). The quest for scientific information on ostrich farming led to the purchase by the then Department of Agriculture of the farm Roodeheuveld in Oudtshoorn in 1964. Initially farmers donated 25 breeding birds which formed the basis of the experimental flock in those years. Until the late seventies feather production was still important thus resulting in Swart (1979) quantifying the quality norms and the economical importance of aspects for the ostrich feather.

During the 1980's, when the industry changed from a feather towards a slaughter industry, the need for information on various aspects of ostrich biology initiated several studies. Swart (1981) evaluated the economical value of meat, skin and feathers of the live slaughter bird. This research resulted in the establishment of the optimal slaughter age as being 14 months for quality leather, feather and meat production. The increasing number of farmers who turned towards artificial incubation in the mid-1980's led to the study of the nest microclimate to establish optimal humidity and temperature settings for artificial incubation. A temperature of 36 ° C and a water loss of about 13 % was shown to be optimal (Swart *et al.* 1987; Swart & Rahn, 1988). Studies on the growth and energy metabolism of ostrich chicks by Swart (1988) suggested the ability of ostriches to digest cellulose in the lower intestine and there have been numerous studies on the characterisation and purification of digestive enzymes of ostriches (Oelofsen *et al.*, 1991). During 1990-1995 the industry boomed and the breeding flock on the experimental farm was gradually increased to about 260 breeding birds. The lack of scientific information on optimal dietary needs for ostriches led to the evaluation of various feedstuffs to establish scientifically formulated rations for different age groups (Cilliers, 1994). There have, in addition, been numerous studies on general ostrich anatomy and physiology, behaviour, and diseases, adequately summarised in a recent bibliography (van der Westhuizen & Earle, 1993).

## **BACKGROUND TO THE THESIS**

This thesis details studies on adult breeding ostrich conformation and productivity and on various aspects of egg storage and artificial incubation carried out over a period of four years.

Although during the 1980's and early 1990's more eggs were being produced from larger numbers of breeding stock, ostrich chick numbers were still low, mainly because of high rates of infertility among breeding stock and low hatchability due to high embryonic mortality. Nel and Visser (Personal communication) suggested that hatching performance of farmed ostriches had not improve substantially in spite of scientifically formulated breeder diets and an increase in the number of artificially incubated eggs in the Little Karoo region. They also suggested that because 70 % of all ostriches are still flock-mated, egg production/female is low and variable ( $45 \pm 20$  eggs per breeding female). These numbers are consistent with results from the experimental farm from 1991 - 1995, which suggested that 75 % of all chicks produced in the flock were generated by only 50 % of the breeding birds. Furthermore, with a flock-mating system there is no convenient way for farmers to identify the most productive birds and, because no or poor records are maintained by many farmers, selection for future replacement stock is done subjectively, mostly on body conformation and feather quality. In a trial run on subjective scoring of the experimental flock for body conformation by ten farmers before the birds were allocated to breeding camps at the beginning of the breeding season, farmers significantly scored top producers lower than poor producers. Aspects of adult body conformation and production were consequently investigated to assess whether correlations existed between these variables in ostriches. Correlations between different desirable economic traits as well as possible monthly and yearly trends were also investigated.

As mentioned above, in South Africa, more and more farmers are changing to system of smaller breeding camps with pairs or trios of birds rather than the flock-mating systems. As a result of this, more eggs are collected more regularly and the number of artificially incubated eggs is on the increase. Egg collection in the Little Karoo is usually done every day where small breeding camp of 0.25 ha/breeding pair or trio is used. In instances where flock-mating prevails, egg collection is usually two or three times per week. Egg collections are done mostly in the morning and in rare occasions in the late afternoon, 2 - 3 hours after lay. Eggs

are usually set only once per week, necessitating storage of eggs for periods of 1-7 days. Although there is a reasonable amount of information on optimum egg storage conditions for poultry, little is known about ideal storage conditions for ostriches and other ratites (Lundy, 1969, Deeming, 1996, Wilson, *et al* 1997). Current practices are rather varied but eggs are generally stored at temperatures ranging from 15 - 30°C in a horizontal position and are turned by hand once a day. The effect of collection time, storage position and storage conditions on early embryonic development and on egg hatchability were thus also investigated.

Swart (1988) indicated that hatchability of artificially incubated eggs during the 1980's was low and variable ( $40 \pm 30 \%$ ) compared to eggs incubated under natural conditions. Despite a greater understanding of factors affecting hatchability of artificially incubated eggs, high embryonic mortality persists (Brown *et al.* 1996, Deeming 1995, Philbey *et al.* 1991). Predominant causes of this are excessive oedema and malpositioning, the former of which results from incorrect water loss and the latter probably through incorrect positioning and incorrect turning (Brown *et al.* 1996).

More than 70 % of eggs that are artificially incubated in the Little Karoo are incubated in wooden incubators which, being of older manufacture, are not equipped with automatic turning mechanisms or cooling systems. Eggs are set either horizontally or vertically, depending on the design of the incubator. Turning angles ranges from 60° to 90° and turning intervals vary from 2 - 6 times day. Even in converted electronic incubators, turning angles and frequencies are variable. Little is known about the optimum position and turning regime for ostrich eggs (Brown *et al.* 1996). The effect of different setting position, turning angle, turning position and gaseous environment on the hatchability of ostrich eggs were consequently also investigated.

Extremely variable temperature fluctuations prevail within wooden incubators, resulting in incubation temperatures that may range from 35.8 to 38°C. The effect of incubation temperature on hatchability was thus also investigated. Airflow through ten incubators was measured with a flowmeter and ranged from 20 000 litre/h. 1000 eggs to 130 000 litre/h. 1000 eggs. Such variable ventilation rates may have marked effects on the gaseous environment of

developing embryos. Embryonic oxygen consumption and carbon dioxide excretion were measured and used to produce a model of optimum incubator ventilation rates.

The thesis is divided broadly into two parts. The first describes phenotypic characteristics in relation to production in adult breeding birds (Chapters 1 and 2) and the second describes aspects of storage and incubation conditions, egg turning and incubation temperature on the hatchability of ostrich eggs (Chapters 3 - 7). Some of the chapters have been published, are in press or have been submitted for publication. This has, inevitably and unavoidably, resulted in some repetition between chapters, especially in references cited. The publication status of chapters, along with any co-authors, are indicated at the beginning of each chapter.

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# CHAPTER 2

## PRODUCTION PARAMETERS FOR TRAITS OF ECONOMIC IMPORTANCE IN OSTRICHES

This chapter has been submitted to *Animal Science* with  
S.W.P. Cloete, C.R. Brown and Z. Brand as junior co-authors.

## ABSTRACT

The effects of breeding pair, year and month of production were assessed during 1991 to 1996 for 22 977 ostrich eggs and 10 805 chicks hatched from these eggs. A total of 3 274 breeding pair-month-year combinations were available to study egg production performance (EPP). Hatchability was investigated on 2 115 records, including breeding pair-month-year combinations where more than four eggs were set. Productivity was defined as the product of EPP and hatchability. Progeny weights at 10 months and slaughter age ( $\pm 14$  months) were recorded for 857 and 696 birds, respectively. Mean ( $\pm$ SD) egg weight and chick weight averaged, respectively,  $1.42 \pm 0.084$  and  $0.85 \pm 0.07$  kg. EPP averaged  $45.7 \pm 33.7$  %, hatchability  $48.0 \pm 28.7$  % and productivity  $22.2 \pm 24.3$  %. Progeny weights averaged  $82.6 \pm 12.5$  kg at 10 months and  $109.3 \pm 12.9$  kg at slaughter. Breeding pair accounted for the major portion of the known variation ( $P < 0.001$ ) in all traits. In the case of egg weight, it accounted for 64.3 % of the total phenotypic variation. For chick weight, the percentage amounted to 57.4 %. Breeding pair accounted for 25.0 % (EPP) to 45.8 % (hatchability) of the total phenotypic variation for the reproductive traits. The contribution of breeding pair to the total phenotypic variation in progeny weight amounted to 26.2 % at 10 months and 24.9 % at slaughter. Year, month and their interaction controlled less of the variation (albeit a generally significant proportion), amounting to  $< 1$  % for egg weight and chick weight. For reproductive traits, the percentage of variation accounted for by these individual effects were below 5 %. Seasonal trends for EPP and chick production revealed that both parameters increased ( $P < 0.05$ ) from July to August, and remained at approximately the same level for September. This was followed by a gradual decline towards January. A comprehensive study on ostrich breeding and genetics is of vital interest to the industry, as the large contribution of breeding pairs to the known variation implicates genetic variation that may well be worth exploiting.

## INTRODUCTION

Ostrich farming was started in South Africa during the late 1800's (Smit, 1963), mainly for the feathers. With the collapse of the feather trade ostrich farming underwent a slump in the early part of this century, although commercial ostrich farming for meat and leather production later saw a resurgence in the industry, which is presently well established. The ostrich slaughter industry gained momentum from 1980, and the number of breeding females rapidly increased to an estimated 35 000 in the Klein Karoo area of South Africa by 1993 (Department of Agriculture, 1993). Before 1986, commercial ostrich production was virtually unknown to the rest of the world. The high prices of ostrich products led to an increased demand for breeding stock, and a global expansion of the industry (Van Zyl, 1996). In times of economic hardship, many potential farmers saw the ostrich industry as a lucrative farming venture into which they could diversify. It was also demonstrated that the potential meat production capacity of ostriches exceeded that of commercially farmed sheep, deer and beef cattle in New Zealand (Brown and Thompson, 1996).

Despite being an established commercial industry, data suitable for the estimation of production parameters of South African ostriches are scant. Ostrich egg production was shown to be exceedingly variable (van Schalkwyk *et al.*, 1996; Deeming 1996). Seasonal influences on egg production under free-ranging (Jarvis *et al.*, 1985) and commercial (Degen *et al.*, 1994; Deeming, 1996) conditions generally found large differences between seasons, with relatively short periods of peak production. Hatching performance of ostrich eggs in artificial incubators was similarly found to be poor relative to that expected from poultry (Deeming *et al.*, 1993; Smith *et al.*, 1995). Large data bases to estimate production parameters for egg and chick weights up to slaughter are virtually nonexistent. In a competitive economy, an industry can ill afford this state of affairs.

Against this background I studied production parameters in a commercial South African ostrich flock. Potential sources of variation included in the analyses were breeding pair, as well as year and month of production.

## **MATERIALS AND METHODS**

### **Experimental material**

Experimental material was drawn from the commercial ostrich breeding flock maintained on the Klein Karoo Agricultural Development Centre near Oudtshoorn. Data were accumulated over a six year period from 1991 to 1996. The maintenance and management of the breeding stock has previously been described by van Schalkwyk *et al.* (1996). The collection, subsequent treatment and incubation of eggs were as described by Van Schalkwyk *et al.* (1998). After hatching chicks were reared under intensive housing conditions. After three weeks of age chicks were allowed outdoors to graze on lucern during the day time. From three months of age chicks were allocated to feedlots of 75 birds/ha. Chicks were grown out on complete diets, containing respectively 12 % crude protein and 7 MJ ME/ kg under feedlot conditions. Chicks were slaughtered at an approximate age of 14 months. Due to constraints with regard to infrastructure and diet costs, only a small proportion of chicks was grown out to slaughter age. The majority were given out on contract to commercial ostrich chick growers.

### **Measurements**

Upon arrival at the hatchery, individual eggs were labelled with paddock number and date of collection, weighed and stored at 17 °C for a period not exceeding seven days. After setting, eggs were treated as described earlier (van Schalkwyk *et al.*, 1996; 1998). Only eggs produced from July to January were considered, as breeding birds were paired off in different weeks of June for the years under consideration. The breeding season was accordingly allowed to progress to February in some years. Monthly production records were, however, complete for the period July - January in all years. Data recorded included egg weight, chick weight at hatching and cause of failure to hatch. Apart from infertility and embryonic deaths, which were major causes of failure to hatch (van Schalkwyk *et al.*, 1996; 1998), alternative classifications included deformity (eggs too small, too large, or deformed), accident (shell cracked or egg accidentally broken) or other causes (eggs used for experimental purposes e.g. opened to study embryonic development).

These data were used to calculate egg production performance (EPP), hatchability and productivity for specific breeding pairs within months and years. The traits were defined as follows (van Schalkwyk *et al.*, 1996):

$$\text{EPP (\%)} = \text{Number of eggs produced} / (\text{Number of days paired off} \times 0.5) \times 100$$

$$\text{Hatchability (\%)} = \text{chicks hatched/eggs incubated} \times 100$$

$$\text{Productivity (\%)} = \text{Number of chicks produced} / (\text{Number of days paired off} \times 0.5) \times 100$$

Batches of chicks grown out at the experimental farm were weighed at irregular intervals. It was thus possible to obtain live weight at 9-11 months and at 13-15 months (corresponding to average slaughter age). Mean age (i.e. respectively 10 months and 14 months) were used to denote approximate age at weighing. In addition to year and month of hatching, sex was also known for these birds.

### Statistical analysis

The following mixed, linear model (Harvey, 1990) was fitted to the data:

$$y_{ijkl} = \mu + P_i + j_j + m_k + j_j m_k + e_{ijkl}$$

where

$y_{ijkl}$  = the  $ijkl$ 'th production measurement on one of the traits analysed,

$\mu$  = the overall mean,

$P_i$  = the random effect of the  $i$ 'th breeding pair,

$j_j$  = the fixed effect of the  $j$ 'th year ( $j = 1991, 1992, \dots, 1996$ ),

$m_k$  = the fixed effect of the  $k$ 'th month ( $k = \text{July, August, } \dots, \text{January}$ )

$j_j m_k$  = the two-factor interaction between month and year,

$e_{ijkl}$  = the random residual variance, as an error term to test the other effects for significance.

For egg weight, chick weight and the reproduction traits, years ranged from 1991 to 1996.

Only eggs produced from July to January were considered in the analysis of these traits. In the case of progeny weight at 10 months or at slaughter, years included 1991 to 1995, and months

of hatching August to November. It was impossible to compute the interaction between month and year for these traits, as progeny hatched in different months were retained for growing out at the experimental farm in consecutive years. In the analyses on progeny weight, the fixed effect of sex (male or female) were also included in the analyses.

## RESULTS

### Means and standard deviations

The coefficients of variation of egg weight and chick weight at hatching were <10 % (Table 1). Reproductive traits, on the other hand, were extremely variable. In the case of chick production, the standard deviation even exceeded the mean value. The coefficients of variation (standard deviation / mean expressed as a percentage) for progeny weight at later ages ranged from approximately 12 to 15 %.

**TABLE 1: NUMBERS, MEANS AND STANDARD DEVIATIONS (SD's) FOR TRAITS UNDER CONSIDERATION**

TRAIT	MEAN $\pm$ SD	NUMBER OF OBSERVATIONS
Reproduction:		
Egg weight (kg)	1.420 $\pm$ 0.084	22977
EPP (%)	45.7 $\pm$ 33.7	3274
Hatchability (%)	48.0 $\pm$ 28.7	2115
Productivity (%)	22.2 $\pm$ 24.3	3274
Chick weight (kg) at:		
Hatching	0.851 $\pm$ 0.065	10805
10 months	82.6 $\pm$ 12.5	857
slaughter	109.3 $\pm$ 12.9	696

### **Analyses of variance**

Year, month and the year X month interaction significantly affected egg and chick weight ( $P < 0.001$ ), but each effect accounted for less than 1 % of the overall variation (Table 2). The contribution of the random effect of breeding pair to the overall variation amounted to 64.3 % for egg weight and 57.4 % for chick weight. Reproductive traits were similarly affected by the fixed effects (Table 3). Year, month and the year X month interaction contributed respectively 3.7, 2.6 and 4.6 % to the overall variation for EPP ( $P < 0.001$ ). Corresponding percentages were 1.9, 1.8 and 3.7 % for chick production and 1.7, 0.8 and 2.7 % for hatchability. The largest contributor to the overall variation was once again the random effect of breeding pair, the percentages of variation accounted for amounting to 25.0 % for EPP, 45.8 % for hatchability and 33.2 % for productivity. Live weight at 10 months was independent of sex and month of hatching (Table 4). Sex similarly did not influence slaughter weight. The contribution of the random effect of breeding pair to the overall variation amounted to 26.2 % for live weight at 10 months and 24.9 % for slaughter weight. From these analyses it was clear that breeding pair accounted for the major part of the known variation for all the traits analysed. The effects of year and month and their interaction, albeit significant, were of minor importance.

### **Effects of year and month**

It is conceded that the interaction of year X month was significant ( $P < 0.001$ ) for the traits where it could be computed. The biological significance of this interaction could, however, not be ascertained. Scrutiny of the data revealed no conclusive and biologically acceptable explanation for the interaction. The effects of year and month on EPP and chick production were thus presented graphically. Year means revealed no conclusive trend in any direction (Figure 1). Both reproduction parameters increased ( $P < 0.05$ ) from July to August, and remained at approximately the same level for September (Figure 2). This was followed by a gradual decline towards the end of the pairing off period.

FIGURE 1: THE EFFECT OF MONTH ON EPP AND PRODUCTIVITY

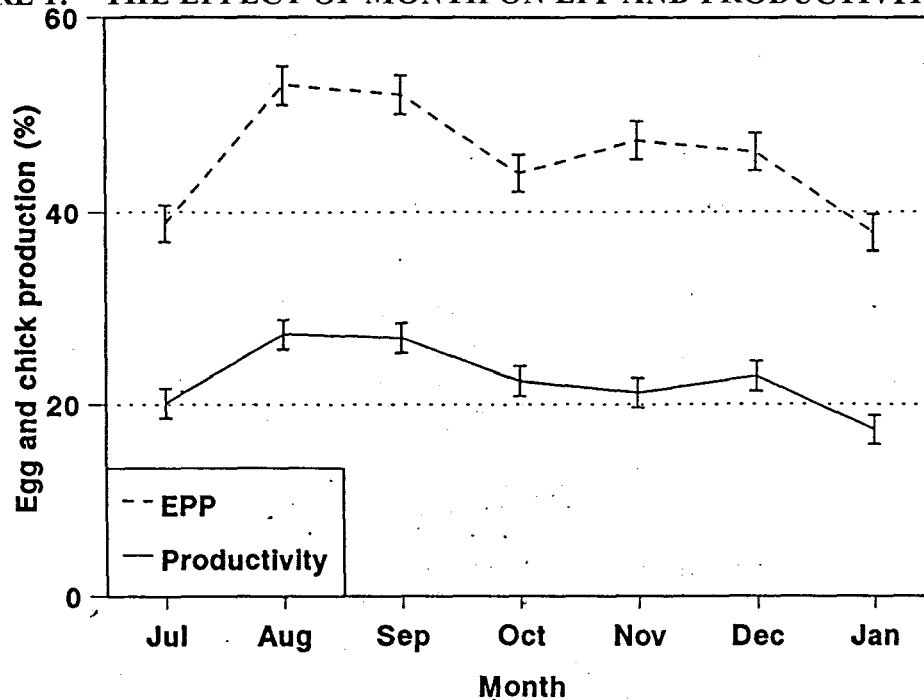
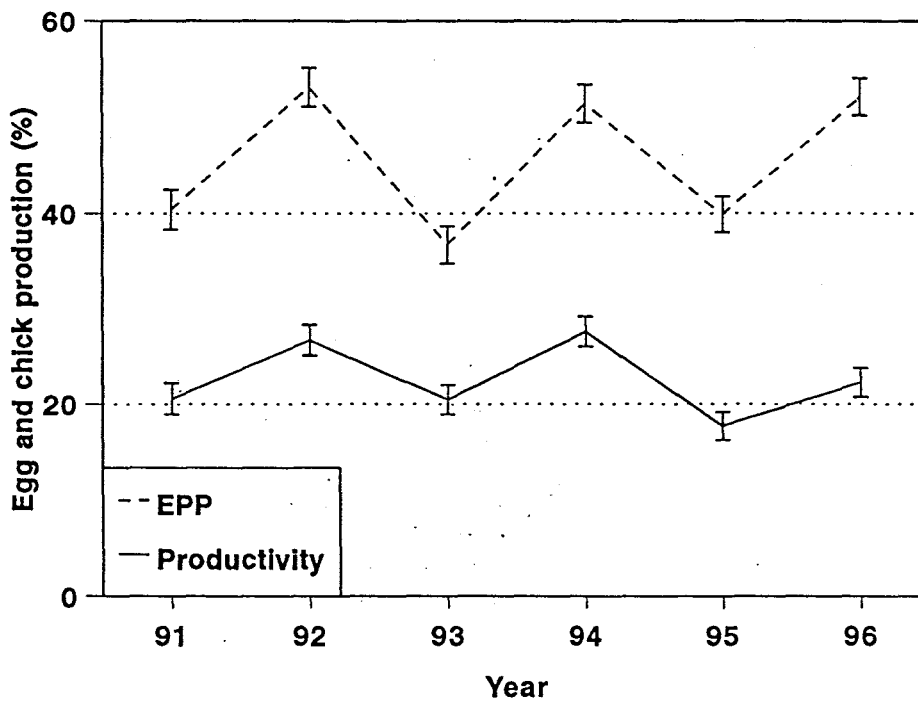


FIGURE 2: THE EFFECT OF YEAR ON EPP AND PRODUCTIVITY



## DISCUSSION

### Means and standard deviations

Deeming (1996) reported a mean weight of 1.46 kg with a coefficient of variation of 11.5 % for ostrich eggs produced in the United Kingdom. Both parameters were slightly higher than

corresponding measurements in the present study (Table 1). The fact that the birds studied by Deeming (1996) originated from diverse backgrounds (Zimbabwe, Israel, Namibia and France), probably contributed to the larger variation in egg weight. Regional differences in the size of mature adults from different regions of southern Africa have previously been reported (Jarvis, 1995), supporting this contention. Egg production performance, hatchability, and chick production were extremely variable in the present study, as reported previously in the literature (Deeming, 1996; van Schalkwyk *et al.*, 1996). Deeming (1996) reported EPP to range from 25.7 to 91.2 % for individual females and corresponding figures for hatchability were 3.8 to 63.2 %. More (1996) reported a range of 0 to 90 eggs per female in Australian ostriches, with 54.3 % of all females of breeding age producing no eggs. No figures for live weight in young ostriches corresponding to those in the present study were found. van Schalkwyk *et al.* (1996), however, obtained coefficients of variation amounting to 11.2 and 13.2 % , respectively, for live weight in mature males and females, which closely resembled the present estimates.

### **Analysis of variance**

The major portion of the known variation in all traits were associated with differences between breeding pairs (Tables 2 to 4). Mature live weight and egg production traits were previously reported to be moderately to highly repeatable in South African ostriches (van Schalkwyk *et al.*, 1996), lending support to the present results. Deeming (1996) similarly reported large differences in egg weights between different females. In his study, mean ( $\pm$ SD) egg weight ranged from  $1.30 \pm 0.10$  kg to  $1.73 \pm 0.10$  kg for 12 females with known records. Since progeny descended from different breeding pairs were grown out together within age groups, it can be assumed that the contribution of possible environmental effects to differences between breeding pairs is minimized. Live weight at 10 months and slaughter age are thus probably heritable in ostriches, although they can presumably be modified by rearing conditions. Apart from the direct implication with regard to meat yield, live weight at slaughter is also closely correlated with hide yield (Swart and Koen, 1983), another important product that contributes to the overall economic yield from ostriches.

**TABLE 2: ANALYSIS OF VARIATION FOR THE EFFECTS OF BREEDING PAIR, YEAR AND MONTH ON EGG WEIGHT AND CHICK WEIGHT**

EFFECT	EGG WEIGHT (KG) <sup>2</sup>		CHICK WEIGHT (KG) <sup>2</sup>	
	df <sup>1</sup>	SUM OF SQUARES	df <sup>1</sup>	SUM OF SQUARES
Breeding pair	154	291.726*	144	60.511*
Year	5	1.442*	5	0.615*
Month	6	3.509*	6	0.381*
Year X Month	30	2.619*	30	0.727*
Remainder	22781	154.366	10619	42.445
Total	22971	453.662	10805	105.404

\* Significant (P<0.001)

<sup>1</sup> Degrees of freedom

**TABLE 3: ANALYSIS OF VARIANCE FOR THE EFFECTS OF BREEDING PAIR, YEAR AND MONTH ON EGG PRODUCTION PERFORMANCE (EPP), PRODUCTIVITY (% OF CHICKS HATCHED) AND HATCHABILITY**

EFFECT	df <sup>1</sup>	EPP (%) <sup>2</sup>	PRODUCTIVITY (%) <sup>2</sup>	df <sup>1</sup>	HATCHABILITY (%) <sup>2</sup>
Breeding pair	158	922 094*	639 426*	153	802 169*
Year	5	136 505*	35 683*	5	29 194*
Month	6	95 557*	33 667*	6	13 897*
Year X Month	30	168 837*	71 623*	30	47 210*
Remainder	3074	2 371 530	1 143 315	1920	860 371
Total	3274	3 694 522	1 923 713	2115	1 752 842

\*Significant (P<0.001)

<sup>1</sup>Degrees of freedom

**TABLE 4: ANALYSIS OF VARIANCE FOR THE EFFECTS OF BREEDING PAIR, YEAR AND MONTH ON CHICK LIVE WEIGHT AT 10 MONTHS OR AT SLAUGHTER**

EFFECT	LIVE WEIGHT (KG) <sup>2</sup> AT:			
	df <sup>1</sup>	10 MONTHS	df <sup>1</sup>	SLAUGHTER
Breeding pair	80	34 095.3*	86	26 570.1*
Sex	1	58.7	1	14.1
Year	2	4 985.4*	3	3 425.9*
Month	4	680.0	4	4 324.8*
Remainder	769	90 406.4	601	72 558.8
Total	856	130 225.8	695	106 893.7

\*Significant (P<0.001)

<sup>1</sup> Degrees of freedom

Years, months and their interaction contributed less to the known phenotypic variation for all traits studied. Live weight of progeny was also independent of sex up to slaughter age (14 months), as was also reported by Mellet (1992). At later ages, male ostriches were reported to be generally heavier than females (Degen *et al.*, 1994; van Schalkwyk *et al.*, 1996).

#### **Month and year trends**

The tendency for ostrich egg production to increase, peak and subsequently decline as the breeding season progresses is well established, both in wild populations (Jarvis *et al.*, 1985) and domesticated birds (Degen *et al.*, 1994; Deeming, 1996; More, 1996). Degen *et al.* (1994) found peak egg production in May and June in the northern hemisphere. Deeming (1996), reported flock egg production to increase from very low levels in mid-March to approximately 90 eggs per week in June-July. This was followed by a gradual decrease back to base levels towards the end of November. In the southern hemisphere, information from nest records suggested a peak in egg laying activity during July-August, followed by a steady decline towards November (Jarvis *et al.*, 1985). Egg production peaked during August-September in the present study, but subsequently declined slowly towards January. It ended

up at fairly high levels at the end of the pairing-off period. This slower decline after peak production, when compared to the literature, probably stems from the shorter, fixed breeding season employed at the Klein Karoo Agricultural Development Centre. Although the significant interaction of month with year could not be explained in biological terms, it may also have contributed to the observed difference with the other studies cited. Most of the studies cited were conducted over only one year. Effects closely linked to a specific year-month (i.e. climate) could have a profound influence on the various production traits, contributing to the observed difference with the existing literature, as well as the year X month interaction.

## **CONCLUSIONS**

This study has shown that the major contribution to variation for variables associated with egg production, hatchability and chick mass at specific ages was associated with breeding pair. This variation is probably largely controlled by gene action. Studies on the genetic improvement of production in ostriches are scarce, although the potential value of selection has been highlighted in previous work at this institution (van Schalkwyk *et al.*, 1996). There appears to be ample phenotypic variation to work with in well-designed breeding programmes for the improvement of egg production (Deeming, 1996), provided that some of this variation is under genetic control. A comprehensive study on ostrich genetics and breeding thus appears to be a prerequisite for further progress in the industry. Seasonal effects were found to be relatively unimportant in the present study. Month-to-month variation was less than in other studies cited, with less distinct egg production peaks. These differences could possibly be related to conditions (i.e. climate) specific to given month-year combinations. Since ostrich production is generally practised under much less intensive conditions than other poultry enterprises, it is imperative that the influence such conditions on production be studied.

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# CHAPTER 3

## REPEATABILITY AND PHENOTYPIC CORRELATION FOR BODY WEIGHT AND REPRODUCTION IN COMMERCIAL OSTRICH BREEDING PAIRS

This chapter as been published in *British Poultry Science* 37: 953 - 962 with  
S.W.P. Cloete and JA de Kock as junior co-authors.

## ABSTRACT

Reproduction is an important aspect of ostrich farming, where income is mainly derived from hides and meat. No estimates of repeatability or phenotypic correlations for reproduction and body weight are currently available for commercial ostriches. Means, standard deviations, repeatability coefficients and phenotypic correlations for and among reproductive traits and body weight were computed for the average yearly production of 42 to 67 mixed age ostrich breeding pairs maintained on the Klein Karoo Agricultural Development Centre from 1990 to 1994. The among-breeding-pair variance component was used in the repeatability estimations, as the pairing off of the same male : female combinations repeatedly resulted in the confounding of these effects. Phenotypic correlations of male body weight with egg production performance (- 0.20) and female body weight with hatchability percentage (-0.16) were negative. Correlations of egg production performance with infertility (-0.20) and hatchability (0.23) percentages were favourable. The repeatability of annual adult body weight was  $0.68 \pm 0.05$  in male ostriches and  $0.61 \pm 0.05$  in females. Ostrich reproductive traits were extremely variable. An appreciable portion of this variation could be attributed to the repeatable nature of breeding pair performance from year to year. All the reproductive traits analysed were moderately repeatable, ranging from  $0.38 \pm 0.07$  (hatchability percentage) to  $0.51 \pm 0.06$  (percentage of embryonic deaths). Egg production performance during the first breeding season of 17 breeding pairs for which data were available predicted subsequent performance satisfactorily, suggesting that selection decisions can be made at quite an early age.

## **INTRODUCTION**

Reproduction is considered to be the cornerstone in virtually all farmed livestock species. The ostrich industry, where income is mainly derived from the selling of hides and meat, is no exception. The success rate with the artificial hatching of ostrich eggs is, however, low (Swart, 1978; Burger and Bertram, 1981) and variable (Deeming *et al.*, 1993) when compared to poultry (Hodgetts, 1991). Hatchability was found to be heritable in turkeys (Cook *et al.*, 1962; Buss, 1989). So far, no concerted effort has been made to determine if the same situation applies to ostriches.

Egg production is extremely variable in captive ostriches, ranging from 0 to 167 consecutive eggs produced during the breeding season (Hicks-Allredge, 1993). The possibility of improving this trait in domestic ostriches by means of selection was mentioned nearly 90 years ago (Deurden, 1908), but so far no further progress in this regard has been reported. When the success of improving egg production in chickens (Gowe and Fairfull, 1985) and turkeys (Nestor *et al.*, 1969) by means of selection is considered, it is clear that it should receive serious attention in ostriches.

Against this background, we studied the year-to-year variation in reproductive traits of farmed South African ostriches paired off in consecutive breeding seasons. The repeatability of the performance of established pairs was derived from mixed model analyses, to consider the possibility of improving current flock reproduction by means of selection.

## **MATERIAL AND METHODS**

### **Experimental animals**

The ostrich breeding flock at the Klein Karoo Agricultural Development Centre near Oudtshoorn was used. Male and female ostriches were paired off for 188 d (July to January) to form 42 breeding pairs in 1990. Breeding paddock facilities were expanded to accommodate 67 breeding pairs in subsequent years. Animals were paired off for longer periods (224 to 274 d) in 1991-94. The mating season commenced in June, and extended to

March in 1993/94, the longest breeding season. Two lines were represented; commercial breeding stock at the Centre and animals bought in from flocks previously selected for feather production and quality. The former were mostly over 10 years of age when first paired off during 1990. The latter group was 3 years of age when paired off for the first time during 1991. A number of young animals entered the breeding flock at 2 or 3 years of age in later years because of death, injury, sickness or ill-thrift of existing birds. Breeding pairs mostly consisted of the same 2 individuals, although combinations were sometimes changed owing to the reasons given above.

### **Management**

Male and female ostriches ran in separate flocks during the off-season (February to June). After mating ceased in February, all animals received hammer-milled lucerne (12 mm screen) *ad libitum*, together with a mineral-vitamin premix. They were also clipped and plucked (white plumage only) at this stage, before being spray-treated for external parasites and drenched for internal parasites. They were kept on lucerne pasture for the rest of the off-season. Two weeks prior to the breeding season, birds were flushed by providing 500 g/bird.d of a strategic supplement. They were also treated against external and internal parasites at this stage, and vaccinated against Newcastle disease and avian influenza. At the beginning of the breeding season, female ostriches were introduced to individual breeding paddocks of 0.25 ha each. Males followed two days later. Established breeding pairs occupied the same breeding paddock from year to year. During breeding a complete diet (140 g crude protein, 9 MJ metabolisable energy, 25 g calcium and 9 g phosphorus per kg) was provided *ad libitum*.

### **Recordings**

Identity was confirmed and body weight recorded for all animals when paired off in June. Eggs were collected daily and identified. After being washed and disinfected with a Virkon S® solution, eggs were stored for a period not exceeding 7 days at a constant temperature of 17°C and 75 % relative humidity (RH). Eggs were artificially incubated at 36.0°C and 28 % in Buckeye® electronic incubators. The capacity of incubators was 1000 eggs, and the eggs were turned hourly, 24 times a day. Eggs were candled at 21 days, using a 150 watt candling lamp. Eggs showing no sign of embryonic development were opened at the area of the air sac and inspected for embryonic development. In the absence of embryonic development, eggs

were recorded as infertile. Eggs showing evidence of embryonic development at this stage were regarded as having sustained early embryonic deaths. Deaths occurring after 21 days were regarded as late embryonic deaths, and pooled with early deaths. The hatching of live chicks was recorded within 15 h of hatching.

These data were firstly used to calculate the egg production performance (EPP) of individual breeding pairs, within years, defined as:

$$\text{EPP (\%)} = \text{Number of eggs produced} / (\text{Number of days paired off} \times 0.5) \times 100$$

The number of days paired off was halved to account for the fact that female ostriches are expected to lay only on alternate days (Deurden, 1908; Hicks-Allredge, 1993). Incubation results were used to calculate percentages of infertile eggs, embryonic deaths and hatchability, all expressed as percentages of the total number of eggs incubated. Only data of breeding pairs where more than 5 eggs were incubated in a specific breeding season were considered in the case of the latter three traits. The productivity of individual pairs was defined as follows:

$$\text{Productivity (\%)} = \text{Number of chicks produced} / (\text{Number of days paired} \times 0.5) \times 100$$

These indications of reproductive performance were obtained for individual breeding pairs within breeding seasons.

### **Statistical analysis**

Means and standard deviations were calculated for annual adult male and female body weights, as well as EPP, infertility, embryonic deaths, hatchability and productivity. Normal or near normal distributions were found for male and female weight, number of eggs produced, EPP and productivity. Percentages infertility and embryonic death were positively skewed. Hatchability percentage was negatively skewed. Kurtosis was normal, except in the case of infertility percentage, which was leptokurtic. The distributions of these traits were normal or near normal after the application of the power transformation (Van Ark, 1981). The untransformed and transformed data were subsequently analysed, using the following mixed model (Harvey, 1990):

$$y_{ijk} = \mu + a_i + p_{ij} + b_k + e_{ijk}$$

where:

$y_{ijk}$  = an individual body weight or reproductivity record,

$\mu$  = overall mean for the trait considered,

$a_i$  the fixed effect of the  $i$ th line,

$i$  being either commercial or feather-producing birds,

$p_{ij}$  = the random effect of the  $j$ th breeding pair nested within the  $i$ th line,

$b_k$  = the fixed effect of the  $k$ th production year ( $k = 1990, 1991, \dots, 1994$ ), for which degrees of freedom were partitioned in non-orthogonal polynomials depicting linear, quadratic, cubic and quartic trends,

$e_{ijk}$  = the residual variance, used as random error term to test the other effects for significance.

The effects of breeding line and age of both members of the breeding pair were confounded. It was therefore impossible to assess these effects separately and only the line effect was retained in the final analysis. Because the same individuals were mostly paired off repeatedly, the effects of males and females were also confounded. It was thus impossible to distinguish between possible male-specific or female-specific influences on reproductivity. Specific male : female combinations available for at least two breeding seasons were thus treated as separate entities. A total of 85 individual breeding pairs with records in at least two breeding seasons were available. Pairs with 2 to 5 records numbered 29, 21, 20 and 15 respectively. Where an existing male or female was combined with a new mate, the combination was treated as a new breeding pair. Preliminary analyses, based on either males or females being available for at least two breeding seasons yielded results that were essentially similar to those reported in this paper. Given the extent of confounding of male and female effects, this should not be surprising. The effect of breeding paddock was also confounded with that of breeding pair, and it could thus not be assessed separately.

Repeatability ( $t$ ) was calculated as follows (Turner and Young, 1969):

$$t = \sigma_p^2 / \sigma_p^2 + \sigma_e^2$$

where:  $\sigma_p$  = the among breeding pair variance component, and  $\sigma_e^2$  = the residual variance component (error variance).

Standard errors for repeatability coefficients were obtained as described by Harvey (1990). Phenotypic correlations among weight and reproductivity traits were obtained from the same analyses used to estimate repeatability. Subsequent EPP (average of performance over at least two subsequent breeding seasons) was regressed on EPP in the first breeding season for specific pairs. Data from 17 pairs entering the breeding flock as young replacements at 2 or 3 years and being available for 2 to 3 subsequent breeding seasons, were used for this purpose.

## **RESULTS**

### **Data transformation**

Although normality of the data was improved by the application of the power transformation to infertility, embryonic death and hatchability percentages, results and conclusions were essentially similar to those obtained using untransformed data. Only results of untransformed data will thus be reported, for ease of comprehension.

### **Phenotypic variables**

Male ostriches were slightly heavier than females (Table 1), with coefficients of variation just higher than 10 % in both cases. Marked variation occurred in all the measures of reproductivity, resulting in large standard deviations for all traits. Mean yearly egg production for all pairs averaged 55.5 eggs/year (SD = 26.2). This trait was closely related to EPP ( $r_p = 0.99$ ). As the length of the breeding season differed from year to year, further results will be given for EPP only.

Line effects were computed, but will not be presented. Line and age effects were confounded, as mentioned previously, and discussion is thus not warranted. Effect of breeding season was significant ( $P < 0.01$ ), for male and female weight. The partitioning of the degrees of freedom for breeding season in non-orthogonal polynomials suggested linear increases from 1990 to 1994. These increases amounted to 3.3 (SE = 0.4) kg per year ( $R^2 = 0.87$ ) for males and  $3.6 \pm 0.5$  kg per year for females ( $R^2 = 0.98$ ). Trends for EPP and productivity were more compiled, revealing quadratic response curves ( $P \leq 0.01$ ). Means for both traits increased ( $P \leq 0.05$ ) from 1990 (EPP =  $37.9 \pm 3.4$  %; Productivity =  $19.8 \pm 2.7$  %) to 1992 ( $50.1 \pm 2.6$  % and  $34.2 \pm 2.3$  % respectively). This was followed by a decline ( $P \leq 0.05$ ) to respectively  $53.0 \pm 2.9$  % and  $30.6 \pm 2.5$  % in 1994. Trends for embryonic death and hatchability percentages were quadratic

( $R^2 > 0.90$ ). The curve for embryonic deaths revealed minima in the 1991 and 1992 breeding seasons, with higher levels prior to 1991 and after 1992. An opposite trend was found for hatchability percentage.

**TABLE 1: MEANS, STANDARD DEVIATIONS AND RANGES FOR ANNUAL BODY WEIGHT AND REPRODUCTIVITY FIGURES OF OSTRICH BREEDING PAIRS FOR THE PERIOD 1990 -1994**

TRAIT	MEAN	STANDARD DEVIATION	RANGE
Body weight (kg)			
Males	116	13	84-159
Females	107	14	72-142
Egg production (%)			
EPP <sup>1</sup>	46.1	20.8	0-93.2
Infertility	17.1	21.9	0-100
Embryonic deaths	21.1	13.4	0-55.6
Hatchability	61.8	21.4	0-100
Productivity <sup>2</sup>	29.1	17.6	0-83.7

<sup>1</sup> EPP (%) = Number of eggs produced / (Number of days paired off X 0.5) X 100

<sup>2</sup> Productivity (%) = Number of live chicks produced / (Number of days paired X 0.5) X 100

The phenotypic correlations of male weight with EPP and productivity were negative (Table 2), while hatchability was negatively related to female live weight. Higher EPP figures were generally associated with lower infertility and better hatchability. Phenotypic correlations among the other measures of reproductivity were as expected.

**TABLE 2: PHENOTYPIC CORRELATIONS MATRIX FOR BODY WEIGHT AND REPRODUCTIVITY TRAITS**

Trait	Female weight (kg)	EPP (%)	Infertility (%)	Embryonic deaths (%)	Hatchability (%)	Productivity (%)
Body weight (kg)						
Male	0.07	-0.20**	-0.04	0.03	0.02	-0.16*
Female		0.04	0.13	0.05	-0.16*	-0.07
Egg production (%)						
EPP			-0.20**	-0.04	0.23**	0.81**
Infertility				-0.36**	-0.80**	-0.58**
Embryonic deaths					-0.26**	-0.21**
Hatchability						0.73**

\*\* P<0.01; P<0.05

### Repeatability

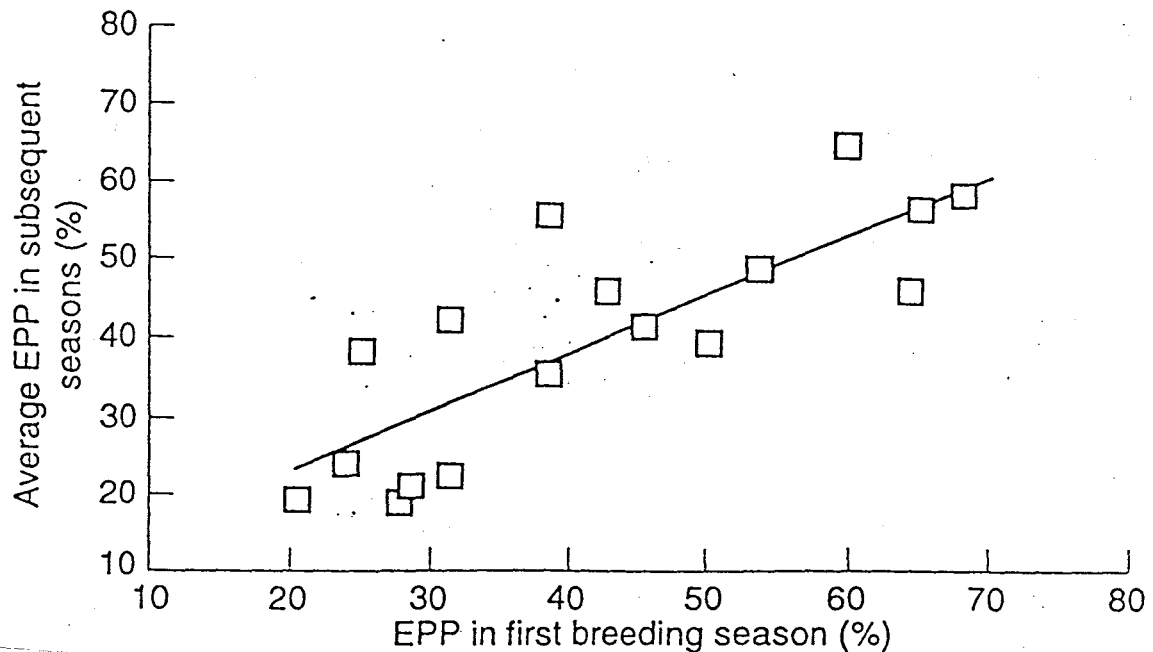
The variation among breeding pairs was significant ( $P < 0.0001$ ) in all analyses. The repeatability coefficient for male weight approached 0.70, while it was slightly above 0.60 for females (Table 3). All measures of reproductivity analysed were moderately repeatable, coefficients ranging from 0.38 (hatchability) to 0.51 (embryonic deaths and productivity). Average EPP in subsequent seasons were related ( $P < 0.01$ ) to EPP in the first breeding season for the 17 breeding pairs for which data were available (Figure). Early performance thus predicted subsequent EPP satisfactorily.

**TABLE 3: VARIANCE COMPONENTS AND REPEATABILITY COEFFICIENTS (*t*) FOR BODY WEIGHT AND REPRODUCTIVITY FIGURES OF OSTRICH BREEDING PAIRS**

Trait	Variance components <sup>1</sup>		<i>t</i> ± SE
	Among breeding pairs	Residual	
Live weight (kg)			
Males	110.96	52.30	0.68 ± 0.05
Females	100.50	65.64	0.61 ± 0.05
Egg production (%)			
EPP	174.32	200.78	0.47 ± 0.06
Infertility	211.16	254.90	0.45 ± 0.07
Embryonic deaths	90.75	86.61	0.51 ± 0.06
Hatchability	164.21	270.26	0.38 ± 0.07
Productivity	149.60	144.26	0.51 ± 0.06

<sup>1</sup>Based on 83 df for pair:line, 187 df error and a *k*-value of 3.23 for weight, EPP and productivity. Corresponding figures for the other traits are 82, 173 and 3.08.

**FIGURE 1: AVERAGE SUBSEQUENT EGG PRODUCTION PERFORMANCE (EPP) OVER AT LEAST TWO BREEDING SEASONS REGRESSED ON EPP IN THE FIRST BREEDING SEASON IN 17 OSTRICH BREEDING PAIRS  $Y = 7.81 + 0.763X(R^2 = 0.66)$**



## DISCUSSION

### Phenotypic variables

Extreme variability in EPP is apparent from the present results. Captive ostriches in Texas produced from 0 to 167 eggs during the breeding season when eggs were removed daily (Hicks-Allredge, 1993). The average production of 42.1 eggs per female per year was marginally lower than the production observed in the present experiment. Average egg production of 4 mating groups of ostriches recorded by Deurden (1908) was higher than the mean performance of birds in the present study. Each mating group consisted of two females and one male, with production figures ranging from 75 to 100 eggs per mating group produced over a 122-d mating period. Conversion to EPP for comparison with the present results gives a range of 62 % to 82 %, expressed on a per female basis. The fact that the ostriches recorded by Deurden (1908) were a small sample and only evaluated over a 4-month period may have contributed to the difference.

Percentages of infertility, embryonic deaths and hatchability of eggs set in the present study corresponded to those reported by Deeming *et al.* (1993) for the first of two imported batches of ostrich eggs. The percentage of infertile eggs in the second batch was higher (33.1 %), resulting in a corresponding decline in hatchability of eggs set. An Australian study similarly reported that fertility and hatchability were variable and in many cases relatively low in commercial ostrich hatcheries (Philbey *et al.*, 1991).

In poultry, selection for increased body weight commonly results in reduced egg numbers (Mukherjee and Friars, 1970; Nestor, 1984; Nestor and Bacon, 1986; Nestor and Noble, 1995) and impaired hatchability (Ogasawara *et al.*, 1963; McCartney, *et al.*, 1968; Verghese and Nordskog, 1968). In the study of Nestor (1984), the decline was particularly severe in the 4th and 5th generation of selected birds. Funk (1950) reported that larger turkey hens produced eggs with a lower hatchability than smaller hens. At this stage it is uncertain if the negative phenotypic correlations between body weight and male and female ostriches and reproduction traits (Table 2) could be related to the results mentioned previously. Further research is required to verify these correlations and to establish whether the origin of the negative covariation between weight and reproduction are genetic or environmental.

The phenotypic correlations of EPP with infertility and hatchability were favourable. Literature reviewed by Buss (1989) and research conducted by Nestor and Noble (1995) correspondingly suggested that the genetic correlations of egg production with fertility and hatchability were positive in turkeys. If further research verifies the present results, it is reassuring to know there are no antagonistic relationships.

### **Repeatability**

The body weight of ostriches could be expected to predict weight in subsequent years fairly accurately. Given that weight is heritable in turkeys (Buss, 1989) and responds readily to selection (Cook *et al.*, 1962; Mukherjee and Friars, 1970; Nestor, 1984) it is likely that the same situation will apply to ostriches. Selection of turkeys for 12-week body weight was effective in increasing body weight at subsequent ages, suggesting that it was repeatable (Mukherjee and Friars, 1970).

Selection of ostrich breeding pairs on their reproductive performance in one season is likely to lead to substantial current flock gains in subsequent years. Repeatability of egg production agreed with coefficients reported for hens by Beaumont *et al.* (1992). Performance in the first breeding season predicted subsequent reproduction fairly well and use of repeated records to improve predictive ability appears to be unnecessary. The contribution of permanent environmental effects (including the possible effect of breeding paddock) to the observed repeatability coefficients could not be determined, but it is reasonable to assume that at least part of the among-breeding-pair variance can be attributed to genetic effects. The average heritability of egg production in turkeys was reported to be 0.22 in earlier work (Arthur and Abplanalp, 1975) while estimates in excess of 0.30 were reported more recently (Buss, 1989). Egg production also responded to selection in chickens (Gowe and Fairfull, 1985) and turkeys (Nestor, 1980), resulting in lines differing markedly in this respect (Lillpers, 1991; Nestor and Noble, 1995). The evidence from poultry species suggest that it is likely that selection of ostriches on egg production traits will also result in the improvement of the performance of future generations. This contention, however, needs to be verified in future research.

Phenotypic correlations reported in this study agree reasonably well with selection responses and genetic correlations in poultry. It is necessary to test the assumption that ostriches would respond in the same way as poultry to selection, for it would have a marked influence on future selection strategies. Furthermore, ostrich breeding pairs differed markedly with regard to reproductive traits. A significant proportion of this variation could be attributed to the repeatable nature of breeding pair performance from year to year. Selection will thus result in improved reproduction in the current flock and probably also in future generations. At this stage it is uncertain whether reproductive traits are also repeatable within a breeding season, or if selection decisions can be based on records accumulated over part of a breeding season. In chickens, Ibe (1995) found that weekly egg production from ages of 21 to 30 weeks and 31 to 40 weeks was poorly correlated (0.06 to 0.16). When records accumulated from 21 to 30 weeks were compared with those obtained from 31 to 40 weeks, repeatability coefficients were considerably higher ( $> 0.31$ ). The possibility of selecting ostrich breeding pairs on records obtained over a part of the breeding season should be considered. Earlier selection has obvious advantages as far as the cost required to maintain breeding stock is concerned.

A high priority should be placed on obtaining data suitable for deriving genetic and environmental parameters for the production of eggs, hides and feathers in ostrich breeding populations, which will help in formulating a sound, scientifically based breeding policy for farmed ostriches.

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# CHAPTER 4

## **THE EFFECTS OF TIME OF EGG COLLECTION AND PRE-INCUBATION STORAGE CONDITIONS ON BLASTODERM DEVELOPMENT AND EMBRYONIC MORTALITY IN OSTRICH EGGS**

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SWP Cloete, CR Brown and Z Brand as junior co authors

## ABSTRACT

I investigated the effects of collection time, storage position and temperature as well as storing conditions on blastoderm development and embryonic mortalities in ostrich eggs. Eggs collected  $\pm 2$  to 3 hours after lay at 16:00 - 18:00 tended to sustain lower levels of embryonic deaths than eggs left overnight in nests to be collected between 09:00 and 11:00 in the morning  $\pm 16$  - 18 hours after lay (16.6 vs 22.9%; SE mean = 2.9; P = 0.15). Embryonic deaths were not affected by storing eggs for 1 week in either a vertical position (with the air cell at the top or the air cell at the bottom) or in a horizontal position. Blastoderm development was studied in eggs stored for 7 days at 20°C (5 eggs) and 25, 26 and 27°C (10 eggs each). Storage at 25°C resulted in a significant increase in blastoderm size compared to eggs stored at 20°C (Means  $\pm$  SE = 12.1  $\pm$  0.6 mm vs 6.0  $\pm$  0.9 mm respectively). Batches of ostrich eggs were subjected to five treatments; 1. Stored for  $\leq 7$  days at 17°C immediately after collection (control); 2. Stored for  $\leq 7$  days at 25°C immediately after collection; 3. Incubated at 36°C for 12 h prior to storage at 25°C; 4. Incubated at 36°C for 12 h prior to storage at 17°C; 5. Incubated at 36°C for 48 h prior to storage at 25°C. The latter three treatments simulated eggs that were subjected to high summer temperatures for varying periods prior to collection. Embryonic mortality was lowest in batches of eggs stored at 17°C immediately after collection and when incubated at 36°C for 12 h before storage at 17°C (26.7 % and 31.8 %, respectively). Embryonic mortality of eggs stored at 25°C immediately after collection averaged 45 %, but increased (P < 0.05) to exceed 50 % in batches of eggs incubated at 36°C for 12 or 48 h before being stored at 25°C. It was concluded that ostrich eggs should be stored at a temperature causing the cessation of all embryonic development after collection, even after being exposed to high environmental temperatures for 12 h.

## INTRODUCTION

Commercially farmed ostriches in South Africa are mostly flock-mated (van Schalkwyk *et al.*, 1996). Because of the size of the breeding paddocks (20 - 100 ha), eggs are generally collected two or three times per week. Eggs are generally collected in the morning, when it is still cool, particularly during the summer months of November to February, when daytime temperatures can reach above 40°C. Eggs are collected daily only on farms where small groups of breeding birds are maintained in much smaller paddocks. Baxter-Jones (1991) suggested that the frequent collection of nest-laid chicken eggs is essential for the production of eggs with a low bacterial count. Deeming (1996) showed that microbial contamination was a major cause of failure of ostrich eggs to hatch in the United Kingdom. Although the importance of prompt collection of ostrich eggs after lay was stressed in the latter study, no comparison on hatchability was made between eggs collected during the early evening (18:00 to 20:00) and those collected at feeding time (08:00 to 10:00).

Eggs collected from flock-mating systems as well as those coming from small pens are generally set once a week. Eggs may thus have to be stored for up to six days before setting. Storage conditions should be sufficiently cool to prevent embryonic development during this period. For most birds, the critical temperature for the initiation of embryonic development appears to be about 25 - 27°C (Drent 1975). For commercially incubated species, Funk and Bellier (1944) reported that blastodermal growth of chicken eggs was inhibited until storage temperatures approached 26.7°C. Later findings of Lundy (1969) confirmed that the temperature threshold for development of chicken embryos was between 25 and 27°C, although Mayes and Takeballi (1984) recommend substantially lower storage temperatures of 16 - 17°C for chicken eggs stored for 3 - 7 days. There is, however, no information available in the literature on the minimum temperature required to initiate blastoderm development in ostrich eggs. Furthermore, although it has been demonstrated that storage periods exceeding 7 days results in reduced hatchability of ostrich eggs (Wilson *et al.*, 1997), no information for optimum storage temperatures is currently available. Storage temperatures are consequently variable between farms, ranging from 17°C to 30°C, depending on storage facilities. Because nests in large paddocks are sometimes overlooked and daytime temperatures and solar

radiation can be high, some eggs may be exposed to temperatures sufficiently elevated to initiate embryonic development for varying times prior to collection, resulting in the collection of eggs at unknown stages of development. Apart from storing time and temperature, the position of eggs during storage may effect hatchabilities. Proudfoot and Hulan (1983) and Butler (1991) suggested that hatchability was improved when chicken eggs were stored with their pointed end up. No such information on storing positions for ostrich eggs could be found in the literature.

Against this background, I investigated the time of collection and blastoderm development in ostrich eggs stored at 20 to 27°C and the effects of elevated temperatures prior to storage as well as the position of eggs during storage on embryonic mortality of ostriches.

## **MATERIALS AND METHODS**

Eggs used in the study were obtained from the commercial ostrich breeding flock at the Klein Karoo Agricultural Development Centre near Oudtshoorn. The management of the breeding flock and the treatment of eggs were described by van Schalkwyk *et al* (1996). Four separate trials were conducted on batches of eggs incubated in electronic incubators:

### **Trial 1: Time of egg collection**

Batches of ostrich eggs were collected according to two treatments, namely late afternoon between 16:00 and 18:00 h ( $\pm 2 - 3$  hours after being laid) and in the morning from 09:00 to 11:00 ( $\pm 16 - 18$  hours after being laid). Thirty breeding pairs were allocated at random to each treatment. Eggs were stored for a maximum of 6 days at 17°C and 75 % RH before being set. Each treatment was replicated eight times, with 13 - 63 eggs constituting a replication, depending on egg numbers available. Eggs were incubated at 36°C and a relative humidity of 28 % in an 1000 egg capacity electronic Buckeye<sup>®</sup> incubator (Buckeye Poultry Equipment, PO Box 1749, Krugersdorp, 1749, South Africa) after storage for a week. Eggs were incubated in the horizontal position for two weeks, before being placed in the vertical position with the aircell at the top (van Schalkwyk *et al.*, in press). Eggs were rocked hourly through 60° throughout. The eggs were candled at 21 days, using a 150 watt candling lamp.

Eggs not fitting the developmental stage of ostriches at that time (van Schalkwyk *et al.*, 1994), were opened and inspected for embryonic development. Eggs not showing any development were regarded as infertile, and those with embryonic development that had ceased as embryonic deaths. Subsequent shell deaths were also classified as embryonic deaths. Infertility, embryonic deaths and the hatching of live chicks were recorded individually, and totalled for batches of eggs. Infertility was expressed as percentage of eggs set, and embryonic deaths as percentage of fertile eggs within batches.

### **Trial 2: Storage position**

Batches of ostrich eggs were stored at a constant temperature of 17°C and a relative humidity of 75 % in a store room for a maximum of 6 days. The eggs were placed into Buckeye® trolley trays which were turned once a day through an angle of 90°. Batches of 30 - 68 eggs were subsequently allocated to one of the following 3 treatments at random:

- \* Vertical with air cell at the top
- \* Vertical with air cell at the bottom
- \* Horizontal

Eggs collected on day 7 were set as fresh eggs 3 - 5 hours after collection as a fourth treatment, together with eggs from the three treatments mentioned previously.

Before storage, the position of the air cell was determined by candling the egg with a 3 volt torch with a head circumference of 2.5 cm. By placing the light source tightly against the shell surface on either side the air cell of approximately 3 cm could clearly be discerned. The position of the air cell was subsequently marked by a pencil on each egg.

After storage for a week these batches of eggs were set in Buckeye® electronic incubators. Incubation procedures as well as the recording of infertility and embryonic deaths were as described for Trial 1. Each treatment were replicated four times.

### **Trial 3: Critical Zero temperature**

Eggs were stored at constant temperatures of 20 (n = 5; control), 25, 26 and 27°C (n = 10 each) in La Nationale® incubators. After storage for seven days, the eggs were opened and

the diameter of the blastoderm of each egg was measured to the nearest 0.1 mm with a pair of callipers. Increases in blastoderm size were regarded as the sole indication of embryonic development. Morphological changes in the embryo were not studied.

#### **Trial 4: Storage temperature conditions**

Depending on numbers of eggs available, batches of 8 - 17 eggs were allocated to one of the following 5 treatments at random:

- \* Stored at 17°C immediately after collection (control)
- \* Stored at 25°C immediately after collection
- \* Incubated at 36°C for 12 hours before being stored at 25°C
- \* Incubated at 36°C for 12 hours before being stored at 17°C
- \* Incubated at 36°C for 48 hours before being stored at 25°C

The latter three treatments simulated eggs that were subjected to high summer temperatures in paddocks for varying periods prior to collection. Each treatment was replicated six times. Subsequent incubation procedures and the recording of infertility and embryonic deaths were as described in Trials 1 and 2.

#### **Statistical evaluation**

The effects of collection time, storage position and storage temperature was assessed in completely randomized statistical designs. Since a number of replications fell outside the 0.3 - 0.7 range, proportions were transformed to angles in preliminary analysis, using the arc sine transformation (Snedecor and Cochran, 1967). Conclusions derived from the analysis on transformed data were, however, similar to those stemming from untransformed data. The analysis that were presented were based on untransformed percentages, which was preferred for ease of comprehension. All treatment means were compared by using the Least Significant Difference (LSD) method. In line with recommendations by Snedecor and Cochran (1967) the LSD test was only used when it was protected by a significant F-value in the analysis of variance table.

## RESULTS

### **Trial 1: Time of egg collection**

Infertility ( $\pm$  SD) averaged  $10.9 \pm 7.6$  %. Embryonic mortality of eggs collected between 16:30 and 18:30 in the afternoon tended to be slightly lower ( $16.6$  vs  $22.9$  %; SE mean =  $2.9$ ;  $P = 0.15$ ) than eggs collected in the morning between 09:00 and 11:00.

### **Trial 2: Storage position**

No statistical differences were found between treatments when embryonic deaths were split into early ( $\leq 21$  days) and late embryonic deaths ( $> 21$  days). All eggs that failed to hatch (excluding infertile eggs) were subsequently pooled.

The mean ( $\pm$  SD) infertility during this trial was similar between treatments and averaged  $25.3 \pm 8.7$  %. Embryonic deaths were not significantly affected by the storing position ( $P \geq 0.05$ ). The percentage embryonic deaths for eggs stored with the air cell at the top, eggs stored with the air cell at the bottom, eggs stored in the horizontal position and eggs that were set immediately after collection were  $21.9$ ,  $21.1$ ,  $22.9$  and  $25.3$  % respectively. The corresponding SE mean was  $2.7$  %.

### **Trial 3: Critical zero temperature**

No development of the ostrich embryo was expected to take place in eggs kept at  $20^{\circ}\text{C}$  and this treatment was regarded as the control. Blastoderm size ( $\pm$  SE) of eggs stored at  $20^{\circ}\text{C}$  for a week averaged  $6.0 \pm 0.9$  mm. Eggs incubated at  $25^{\circ}\text{C}$  for a week had blastoderms that averaged  $12.1 \pm 0.6$  mm, significantly larger ( $P < 0.01$ ) than those of the control eggs. Increasing the storage temperature to  $26$  and  $27^{\circ}\text{C}$  resulted in further significant increases in blastoderm size to  $20.2 \pm 0.6$  and  $42.1 \pm 0.6$  mm, respectively ( $P$ 's  $< 0.01$ ).

### **Trial 4: Storage temperature conditions**

Infertility ( $\pm$  SD) of eggs averaged  $17.6 \pm 2.5$  %. Embryonic mortality was lowest ( $26.7$  %) in the control group of eggs stored at  $17^{\circ}\text{C}$  immediately after collection (Table 1). Embryonic mortality of eggs incubated for 12 h prior to storage at  $17^{\circ}\text{C}$  was slightly, but not significantly

higher than controls, as was that of eggs stored at 25°C without prior incubation ( $P < 0.05$ ). In contrast, embryonic mortality was significantly elevated to 53 and 58 % respectively, in eggs exposed to high temperatures for 12 and 48 h before storage at 25°C (Table 1).

**TABLE 1: EFFECT OF DIFFERENT STORING CONDITIONS ON THE HATCHABILITY PERFORMANCE OF OSTRICH EGGS. MEANS ARE BASED ON 6 REPLICATIONS EACH**

TREATMENT	INCUBATION PERIOD (hours)	STORAGE TEMPERATURE (°C)	EMBRYONIC DEATHS
Control (17 ° storage)	-	17	26.7 <sup>a</sup>
25° storage	-	25	44.8 <sup>a,b,c</sup>
12h Incubation; 25 ° storage	12	25	53.8 <sup>b,c</sup>
12h Incubation; 17 ° storage	12	17	31.8 <sup>a</sup>
2d Incubation; 25 ° storage	48	25	59.0 <sup>c</sup>
SE mean			6.0

<sup>a,b,c</sup> Means followed by different superscripts were significantly ( $P \leq 0.05$ ) different.

## DISCUSSION

We found no conclusive difference in embryonic mortality between eggs collected in the afternoon or the morning, but embryonic mortalities did tend to be lower in eggs collected during the afternoons. In the poultry literature, Fassenko *et al.* (1994) suggested that nest holding time significantly affected post oviposition pre-incubation embryonic growth in chicken eggs. Eggs collected 6 hours after lay had significantly larger blastoderm diameters ( $p \leq 0.001$ ) than those collected 1 hour after lay. Abnormalities were observed more frequently when embryo development was arrested prior to or during early gastrulation or one day or more after gastrulation was completed (Hutt and Pilkey, 1930; Hays and Nicolaidis, 1934). Arora and Kosin (1966) postulated that the extent of gastrulation at oviposition may

influence the capability of the chick blastoderm to survive storage. On the other hand, prolonged exposure to nest conditions in the poultry house increased the possibility of microbial infection. North (1984) recommended that chicken eggs should be collected at least four times daily for optimum embryonic viability and hatchability.

As far as ostriches are concerned, the microbial spoilage of ostrich was regarded as the biggest single problem facing commercial production of chicks in the United Kingdom (Deeming, 1996). The absence of any organic cuticle on the external shell surface of the ostrich egg makes it especially prone to microbial infection if not collected soon after laying (Deeming, 1996). The ostrich flock in my study usually starts laying from 12:00 until 17:00 (Lambrechts, Personal communication). This pattern of lay accorded with patterns reported by Deeming (1996) in the United Kingdom.

Deeming (1996) observed that microbial contamination significantly increased as the breeding season progressed. The fact that ostriches in small breeding camps use the same nesting site throughout the year may contribute to such increased microbial contamination. The birds used in the present study not only used the same nesting site throughout the breeding season but more than 80 % of the pairs used the same nest every year (van Schalkwyk, 1996, personal observation).

Although nest holding time might be important for the ostrich embryo to reach a certain degree of development, which may increase its ability to withstand storage conditions, egg contamination under intensive farming conditions poses a more serious threat, possibly affected by the tendency towards lower levels of embryonic deaths in eggs collected during the afternoon. Since ostrich nests are situated in open paddocks, it is impossible to implement nest hygiene measures as recommended for the poultry industry. Our recommendation is consequently in agreement with that of Deeming (1996) who suggested that ostrich eggs should be collected soon after lay, in order to reduce the period of exposure to soil and other debris which may increase microbial spoilage and therefore impair hatchability.

Our results show no significant difference in hatchability when ostrich eggs were stored "small end up" compared with other storage positions. In commercial ostrich farming, eggs are not usually stored for longer than one week and hatchability seems not to be effected by the position of the eggs prior to setting. Storing eggs for longer periods may result in differences in hatchabilities for the various storing positions. Wilson *et al.* (1997) and Deeming (1996) reported a reduction in the hatchability of ostrich eggs when stored for longer than seven days. The position of chicken eggs during storage have been reviewed extensively. Storing eggs small end up without turning resulted in increased hatch, which was not further improved by turning (Proudfoot and Hulan, 1983; Mayes and Takeballi, 1984; Butler, 1991). It was suggested that storing eggs small end up may result in the centring of the yolk in the albumen, giving the embryo greater protection from dehydration and adhesions (Mayes and Takeballi; 1984).

The size of the blastoderm more than tripled when eggs were stored between 25 and 27°C, suggesting that the critical zero temperature for ostrich eggs is broadly similar with that of chicken embryos as reported by Lundy (1969). It was also in agreement with findings of Miller and Wilson (1975) who concluded that the temperature required to initiate blastoderm development of Bobwhite quail embryos was between 24.4°C and 25.6°C.

Acceptable hatching performance was found in ostrich eggs stored at a temperature below 20°C for  $\leq 7$  days immediately after collection. This corresponds with the observation of Wilson (1991) who suggested that fertile chicken eggs can be stored for several days without a major loss in hatchability, providing that optimal storage conditions prevail. However, Johnson and MacIlraith (1967) observed that cooling of turkey eggs to 13°C depressed hatchability in comparison with those held at 22 - 26.5°C during the first day.

Jones (1986) and McDaniel (1990) suggested that storage temperatures above the physiological zero allow the embryo to continue growing and that this treatment resulted in better hatchability than that of embryos from eggs cooled immediately after collection. Ar (1996) suggested that, as with chicken eggs, ostrich eggs may hatch better when stored at room temperature for 3 - 5 days prior to setting. In contrast, Scott *et al.* (1993) reported

higher levels of early and late embryonic deaths when chicken eggs were incubated at 30°C for 24 h prior to storage at 20°C for 7 days. Our results are consistent with those of Scott *et al* (1993). Kosin (1956) as well as Becker and Bearse (1958) found that warming of chicken eggs for 5 hours at 37.8°C prior to storage has a beneficial effect on hatchability. Lancaster and Jones (1986) found that if prewarming periods prior to storage increased beyond 5 hours, hatchability of chicken eggs stored for long periods is impaired. Hatching performance of ostrich eggs was impaired when stored at temperatures approaching the critical zero temperature (25°C) and exposed to elevated temperatures prior to storage. Kaufman (1948) and Lundy (1969) reported that cooling of eggs exposed to optimal incubation temperature to temperatures above the physiological zero should be avoided, as embryonic development still proceeds. They suggested that cooling to a temperature that does not produce a complete cessation of development may be more harmful than cooling to a temperature that brings about complete stoppage. This finding was in accordance with earlier work by Romanoff *et al* (1938). Taylor *et al.* (1933) and Romanoff (1960) suggested that the sensitivity of the embryo to cooling from just below the physiological zero to above freezing point increases with age. In my study the effect of cooling on the viability of embryos beyond the 48 hour developmental stage was not investigated. Older ostrich embryos may suffer even more from cooling to temperatures below the physiological zero as reported by Landauer (1967) who suggested that chicken embryos has a much higher resilience to cooling during the first two weeks than during the last two weeks of incubation.

In conclusion, the present study suggests that commercial ostrich farms should collect eggs from the nest as soon as possible, with the aim of lowering bacterial counts. The position during storage did not seem to affect hatchability if eggs are stored for less than one week. Hatcheries should store eggs after collection at temperatures below 20°C to ensure cessation of all embryonic development, even if eggs were possibly exposed to high environmental temperatures prior to collection.

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## **CHAPTER 5**

# **THE EFFECT OF TEMPERATURE ON THE HATCHING PERFORMANCE OF OSTRICH EGGS, AND ITS IMPLICATIONS FOR ARTIFICIAL INCUBATION IN FORCED DRAUGHT WOODEN INCUBATORS**

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SWP Cloete, CR Brown and Z Brand as junior co-authors

## ABSTRACT

Batches of 60 - 65 ostrich eggs were incubated in electronic incubators at 36.0, 36.5 and 37.3°C. The proportion of live chicks hatched relative to eggs set at 37.3°C (22/65 = 0.338) was impaired ( $P < 0.05$ ) relative to those incubated at 36.0 and 36.5°C (respectively 38/60 = 0.633 and 36/60 = 0.600). Shell deaths were higher ( $P < 0.05$ ) in fertile eggs incubated at 37.3°C than in those incubated at 36.0°C (28/50 = 0.560 vs 14/52 = 0.269 respectively). Temperatures were recorded in three horizontal (front, middle and back) and three vertical (top, middle and bottom) positions in 10 forced draught wooden incubators. In general, a linear temperature gradient was found from the front to the back of these incubators. Temperatures recorded in the top tray of the incubators were higher ( $P \leq 0.05$ ) than those recorded in the bottom and middle trays and temperatures recorded in the front and back of the bottom tray were higher ( $P \leq 0.05$ ) than those measured in corresponding positions of the middle tray. Mean temperatures of  $\geq 37^\circ\text{C}$  were measured at the back of the top and bottom trays, as well as in the middle of the top tray. The hatching performance of ostrich eggs is likely to be impaired in these positions. It was assumed that younger embryos are less susceptible to excessive heat than older embryos. It was thus suggested that freshly laid eggs should routinely be placed in those positions where temperatures exceeded  $37^\circ\text{C}$  in forced-draught wooden incubators.

## INTRODUCTION

The physiological requirements of the developing avian embryo, which can be controlled in artificial incubators are the correct temperature, optimal humidity, correct gaseous environment, proper turning angle and egg orientation. Of these, temperature is the most critical factor. The effect of temperature on hatchability has been the subject of several reviews (Barott, 1937; Orlov, 1962; Kosin, 1964; Landauer, 1967; Lundy, 1969; Swart, 1988; Ar, 1996; Deeming, 1997). These reports indicated that temperature variation within incubators should be minimised. Barott (1937), for example, concluded that optimum temperature conditions for the best quality chicks and hatchability were 37.8°C for chicken eggs and that temperature variation should not exceed 0.3°C.

Egg temperatures under natural incubation conditions, however, may vary considerably between species, suggesting variability in temperature requirements (Romanoff, 1934; Huggins, 1941; Martin and Insko, 1935; Wilson *et al.*, 1979; Kühn *et al.*, 1982). The incubation temperature for ostrich eggs under natural conditions was reported to be 36°C (Swart and Rahn, 1988).

Early research on temperature effects on incubation involved exposing developing embryos to temperatures above that considered optimal (Alsop, 1919; Orlov, 1962; Landauer, 1967; Lundy, 1969). Lundy (1969) concluded that the effect of high temperature on hatchability increased with increased temperature. Landauer (1967) concluded that the upper temperature for normal development is closer to the optimum temperature than the lower limit for normal growth. This suggested a greater sensitivity of the embryo to temperatures increased above optimum levels than to temperatures below optimum levels.

No research has been done so far with regard to the exposure of ostrich embryos to high temperatures. The effect of increased temperatures on the hatchability of ostrich eggs was therefore investigated. It is estimated that more than 70% of all ostrich eggs in the Little Karoo area are incubated in forced-draught wooden incubators (Visser, Personal communication). The temperature fluctuations in such forced-draught wooden incubators have

also not been studied adequately. This study was thus undertaken to determine whether heat distribution was evenly spread within such incubators.

## **MATERIAL AND METHODS**

The study on the effect of increased temperatures on hatchability was conducted in the incubation facilities of the Oudtshoorn experimental farm. Facilities and procedures for the collection and storing of eggs were described in the literature (Van Schalkwyk *et al.*, 1996; 1998). Three 75 egg capacity La National® electronic incubators were used with settings at 36°C, 36.5°C and 37.3°C. The relative humidity was kept at a constant 28% throughout. The number of eggs set per incubator ranged from 60 to 65.

Ten 200-egg forced-draught incubators of the same design and manufacturer were monitored on five farms. Air circulation and heat distribution in these incubators (height 2 m and width 1 m) are maintained by a paddle fan with air intakes on either side of the incubator, 50 cm from the bottom. The correspondingly outlets are 50 cm from the top. Heat generation is by two elements situated on the floor of the incubator. The thermostat probe is situated halfway between the middle and the top of the incubator. Temperature setting is usually done according to a hand-operated thermometer hanging in the middle against the door window.

In this study, temperatures were recorded with an electronic Hennia thermometer in the top, middle and bottom trays of each incubator. Within each tray these recordings were done just behind the observation window, in the middle and at the back of the tray. The temperature measurements were taken individually after a period of 20 minutes was allowed for the equilibration of temperatures within incubators. The thermometer was placed in the incubator in each case, and the temperature was recorded through a closed glass window in the incubator door.

### **Statistical analysis**

Numbers of infertile eggs and chicks hatched were expressed as proportions of eggs set. Dead-in-shell eggs and eggs with malpositioned embryos (assessed according to the

descriptions of Deeming 1995) were correspondingly expressed as proportions of fertile eggs. Chi<sup>2</sup> procedures were used to compare these proportions, utilizing the Bonferroni correction to account for the fact that three comparisons were made.

The analysis of the temperatures recorded in each of the 10 wooden incubators was complicated by the fact that measurements were made in nine different locations within each incubator. The covariation arising from repeated measurements in the same incubator was accounted for by fitting the following mixed linear model to the data, (Harvey, 1990):

$$y_{ijkl} = \mu + B_i + h_j + v_k + h_j v_k + e_{ijkl}$$

where  $y_{ijkl}$  = an individual temperature recording,

$\mu$  = the overall mean,

$B_i$  = the random effect of the i'th incubator,

$h_j$  = the fixed effect of the j'th horizontal position (j = front, middle or back),

$v_k$  = the fixed effect of the k'th vertical position (k = top, middle or bottom),

$h_j v_k$  = the two-way interaction between horizontal and vertical position,

$e_{ijkl}$  = the random error term, used to test the other effects for significance.

The intraclass correlation derived from the between incubator variance component could be used to estimate the repeatability of temperature recorded within incubators.

## RESULTS

**TABLE 1: THE HATCHING PERFORMANCE OF OSTRICH EGGS  
SUBJECTED TO INCUBATION AT DIFFERENT TEMPERATURES**

Parameter	Temperature			Chi <sup>2</sup> *
	36°C	36.5°C	37.3°C	
Number of eggs set	60	60	65	
Number of fertile eggs	52	53	50	
Proportions relative to eggs set				
Fertile eggs	0.867	0.883	0.769	3.53
Chicks hatched	0.633 <sup>a</sup>	0.600 <sup>a</sup>	0.338 <sup>b</sup>	13.21
Proportions relative to fertile eggs				
Shell deaths	0.269 <sup>a</sup>	0.321 <sup>a,b</sup>	0.560 <sup>b</sup>	10.37
Malpositions	0.231	0.283	0.440	5.57

\* Critical Chi<sup>2</sup> (P = 0.05) with 2 degrees of freedom = 5.99

<sup>ab</sup> Proportions with different superscripts differ (P < 0.05) in rows

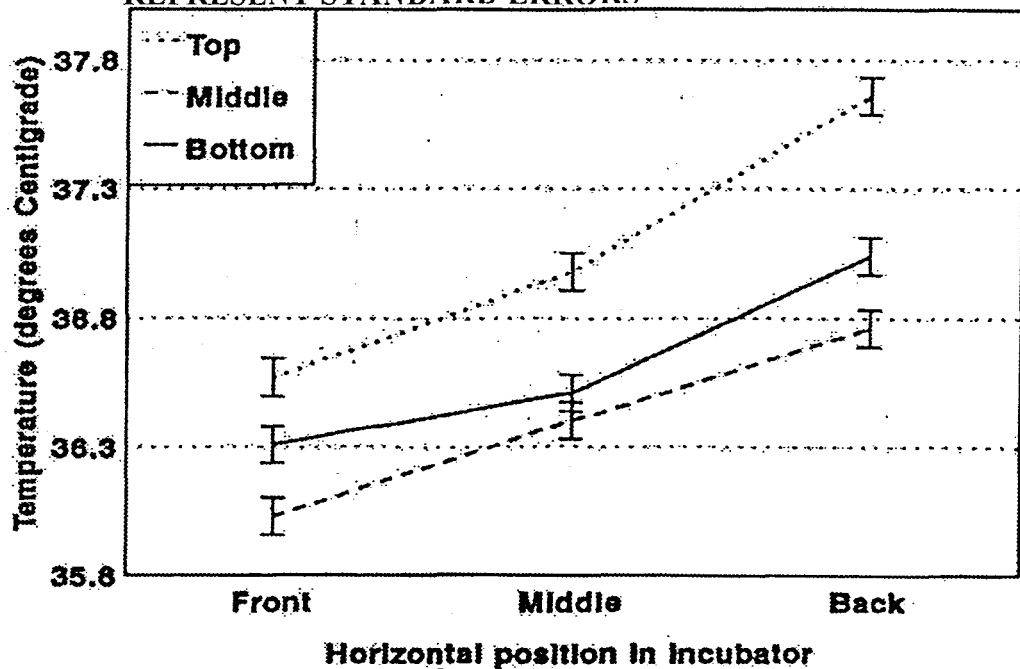
The proportion of fertile eggs was not affected by different incubation temperatures (Table 1). The proportion of live chicks hatched expressed relative to eggs set was lower (P ≤ 0.05) in eggs incubated at 37.3°C than in those incubated at lower temperature. Expressed relative to fertile eggs, shell deaths were higher (P ≤ 0.05) in eggs incubated at 37.3°C than in those incubated at 36°C. There was a suggestion (P ≤ 0.10) for the incidence of malpositioned embryos to increase with increased incubation temperature, but significance could not be demonstrated.

The model fitted to the temperatures recorded in different positions in the wooden incubators accounted for 85.2% of the overall variation. A small, albeit significant (P = 0.042), portion of this variation was associated with the covariation of the same incubator being measured repeatedly. The intraclass correlation (repeatability) derived from the variance components

were small ( $0.11 \pm 0.10$ ). The measurements were thus assumed to be sufficiently uncorrelated to validate analysis of variance procedures. Despite being significant, the interaction between horizontal and vertical position only accounted for 2.4% of the temperature variation, compared to 48.2% for horizontal position and 30.8% for vertical position.

There was a linear gradient from the front to the back of the incubators, successive means for the front, middle and back differing ( $P \leq 0.05$ ) in the top (36.6, 37.0 and 37.7°C respectively;  $SE = 0.07$ ) and bottom (36.3, 36.5 and 37.0°C respectively;  $SE = 0.07$ ) (Figure 1). In the middle, temperatures recorded in the front (36.0°C) and middle (36.4°C) differed ( $P \leq 0.05$ ) from those recorded in the back (36.8°C). Temperatures recorded in the top of the incubators were higher ( $P \leq 0.05$ ) than those recorded in the middle and bottom. In the back of the incubators temperatures recorded at the bottom (37.0°C) were higher ( $P \leq 0.05$ ) than those recorded in the middle (36.8°C).

**FIGURE 1: THE INTERACTION BETWEEN VERTICAL POSITION (TOP TRAY OR BOTTOM TRAY) AND HORIZONTAL POSITION (FRONT, MIDDLE OR BACK) FOR TEMPERATURES RECORDED IN 10 FORCED DRAUGHT WOODEN INCUBATORS. VERTICAL BARS ON THE LINE REPRESENT STANDARD ERRORS**



## DISCUSSION

Elevated incubator temperatures in the present study resulted in an increase in embryonic mortality and abnormalities in ostrich embryos. These results are consistent with previous studies on a variety of avian species. As incubation temperature is raised above the optimum level for maximum hatchability the mortality and the number of crippled and deformed chicks increased (Romanoff, 1936; Barott, 1937; Romanoff, 1938). Leighton *et al.* (1964) found that heart and kidney enlargement and heart failure was the result of 11- and 12-day old embryos being exposed to 42.5°C for 1 week. An embryonic mortality of 50% or more resulted from exposure of 16-day broiler embryos to 43.3°C for 9 hours. Henderson and Brody (1927) observed that growing chicken embryos were very sensitive to small changes in incubator temperature between 35 and 37°C up to the 13th day of development. Romanoff (1936) experienced the same results with chicken embryos up to 16 days of incubation. It was concluded that chicken embryos were more affected within the first week of incubation than later. Romanoff (1938) suggested, however, that mortality could be appreciably reduced by a slightly higher temperature of 0.25°C above optimum during the early part and a slightly lower temperature of 0.2°C below optimum during the latter part of incubation. These findings were in agreement with those reported by Romijn and Lokhorst, 1955; Tullett, 1990; Wilson, 1991) and are presumably related to temperatures experienced by embryos during natural incubation. Under natural incubation conditions the young embryo floats just under the brood patch enjoys a warmer temperature than the average (Ar, 1996). Overheating of embryos is a key problem in commercial incubation of any species of bird because metabolic heat production by the embryo during the second half of development can raise the egg temperature as much as 2°C above that of the incubator temperature (Deeming, 1997). Ar (1996) suggested that overheating of the embryo in constant temperature incubators can be circumvented in modern single stage incubators by the changing of the incubator temperature as incubation progress to offset changes in embryonic heat production.

Four of the ostrich chicks hatched at 37.3°C in the present study had eye abnormalities. Nilsen (1968), suggested that eye abnormalities were caused by circulation disturbances when chicken eggs were exposed to temperatures of between 40 and 42.2°C during the first six days of incubation. Ande and Wilson (1981) observed that chicken poults hatched after severe

heat stress were not only weaker than contemporaries incubated normally but that there was also an increased incidence of clubbed wry down and the exhibition of an unsteady gait. Barott (1937) found that chicks incubated at 38.9 were smaller, less lively with more abnormalities than chicks incubated at 37.3 to 37.8°C. French (1994) observed that turkey eggs incubated at 38.5°C hatched significantly less well than eggs incubated at 37.5°C. Overheated embryos had a mortality peak between 15 and 20 days and an increased mortality after 24 days of incubation. Overheated eggs were characterised by a high incidence of embryos with heads in the small end, excess albumen, ruptured yolk sacs, oedematous heads, eye cataracts and swallow down plumules.

The increased temperatures in the top of the incubators can probably be related to natural convection, as the heat-generating elements are situated in the bottom of the incubators. The proximity to these elements probably resulted in the suggestion of higher temperatures recorded in the bottom of the incubators compared to those recorded in the middle (Figure 1). It is important to note that temperatures of  $\geq 37^{\circ}\text{C}$  were recorded in three positions. Hatchability of artificially incubated ostrich eggs may be impaired at these temperatures. Ar (1996) suggested that the growth of the early embryo is much more sensitive to changes in temperature than the late embryo. Oxygen uptake increases earlier in eggs incubated at a higher temperatures. Later in incubation the rate of increase in  $\text{O}_2$  uptake become similar to that of eggs incubated at a lower temperatures. These findings were supported by Meir and Ar (1990) and Deeming and Ferguson (1991).

## **CONCLUSION**

For ostrich farmers setting eggs in small wooden incubators it is suggested that older eggs should be set in those parts of the incubator that do not exceed a temperature of 36.5°C. The setting of older eggs at extreme temperatures of 37°C and higher will potentially result in late embryonic deaths and an increased incidence of abnormalities if the temperature sensitivity displayed by ostrich embryos corresponds to those reported for chickens and turkeys. Younger eggs can tolerate higher temperatures better, and should be set in trays at the top and bottom where higher temperatures were recorded. Further research is required to elucidate the effect of high temperatures on newly set ostrich eggs.

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# **CHAPTER 6**

## **EMBRYONIC METABOLISM AND THE GASEOUS ENVIRONMENT OF THE OSTRICH EMBRYO IN RELATION TO INCUBATOR DESIGN**

## ABSTRACT

Oxygen consumption of ostrich embryos increased exponentially during the first 70% of incubation but reached a plateau at approximately day 31, and maintained this plateau until day 38 of incubation. During peak metabolism about 180 ml/h of oxygen was consumed and 120 ml/h carbon dioxide was excreted, respectively. This stage was followed by a decline in metabolic rate to approximately 75 % of the peak value. The ontogeny of ostrich eggs incubated in this study at 36°C was compared with studies where incubation temperatures of 35, 35.5 and 36.3°C were used. Although the time of hatch differed between these studies (41, 44.6 and 47 days compared to the 42 day incubation period in the present study), the general trends in oxygen consumption and carbon dioxide excretion were broadly similar, although there were slight differences in the plateau phases. From the data on oxygen consumption and carbon dioxide excretion, it was calculated that single stage incubators needs a peak airflow of 54.4 l/egg. hour to prevent reduction in oxygen levels below 21 % and a CO<sub>2</sub> concentration exceeding 0.3 %. These results enable incubator operators to adjust ventilation rates to accommodate embryonic age and metabolism and avoid excessive costly heat loss because of over ventilation.

## INTRODUCTION

The ontogeny of O<sub>2</sub> consumption of the domestic chicken, *Gallus gallus*, has been well described (see, for example, Wagensteen and Rahn, 1970.) and similar data are available for many other avian species (Kendeigh, 1940; Lewis *et al.*, 1965; Hoyt *et al.*, 1979; Rahn, 1981; Burton and Tullet, 1983; Meir *et al.*, 1984). Several studies have investigated embryonic metabolism in ostriches, *Struthio camelus*, emus, *Dromaius novaehollandiae*, and Rheas, *Rhea americana* (Hoyt, *et al.*; 1978, Vleck, *et al.*; 1980, Meir and Ar; 1990, Reiner and Dzapo, 1995). These studies show different patterns of oxygen consumption with age. These different patterns occur in altricial and precocial birds and it was suggested that these patterns may also vary with egg size and incubation period. Egg of the common Rhea, *Rhea americana* and the Emu, *Dromaius novaehollandiae*, are approximately the same size, but the two species differ significantly in incubation period and egg shell conductance (Hoyt, *et al.*; 1978, Vleck, *et al.*; 1980). One of the factors that can alter the pattern of embryonic metabolism is incubation temperature. Decuypre *et al.* (1979), for example, demonstrated that the metabolic rate of chicken embryos increases when exposed to increasing temperatures between 35.9 and 38.8°C and the same phenomenon has been observed in the studies with ostrich eggs (Hoyt *et al.*, 1978; Meir and Ar, 1990; Reiner and Dzapo, 1995, Ar 1996) where temperatures of 35°C, 35.5°C and 36.3°C were used, respectively. The results from these papers have previously been compared in the review of Ar (1996.). These temperatures differ from the 36°C incubation temperature used in the present study and which is typical for artificially incubated ostrich eggs. Incubation period also varied considerably between these studies (41, 44.6 and 47 days) as opposed to the typical 40 - 44 days with an average of 42 days under natural conditions (Sauer and Sauer, 1966; Leuthold, 1970; Swart and Rahn, 1988). This variation is presumably related to the different temperatures at which the eggs were incubated.

Proper air circulation in an incubator is important to provide an even temperature distribution throughout the incubator environment and proper incubator ventilation prevents accumulation of water vapour, limits undesirable increases in incubator temperature as a result of metabolic heat production by the embryos, maintains the oxygen (O<sub>2</sub>) concentration high in spite of O<sub>2</sub> uptake by the developing embryos, and carbon dioxide (CO<sub>2</sub>) concentrations low by removing CO<sub>2</sub> produced in the egg.

Optimum gaseous conditions for hatching chicken eggs have been discussed in early studies (Romanoff, 1930; Barott, 1937; Wilgus and Sadler, 1954). Little, however, is known about optimum gaseous conditions for ostrich eggs, in part because information on ostrich embryonic metabolism has only recently become available.

The aim of this study was thus to establish whether the same values for O<sub>2</sub> uptake and CO<sub>2</sub> excretion would have been obtained when average commercial incubation temperature of 36 °C was used instead of temperatures used in previous studies. Because little attention has been paid to proper incubator ventilation in the past, guidelines for a more cost effective incubator operation, based on embryonic metabolism of ostrich embryos, are presented.

## **MATERIALS AND METHODS**

### **Collection and handling of the eggs**

Eggs were collected from the experimental ostrich flock at the Klein Karoo Agricultural Development Centre near Oudtshoorn. Details regarding the maintenance and management of breeding stock as well as the collection and incubation of eggs are provided in papers by van Schalkwyk *et al.* (1996; 1998). Eggs used for this investigation were collected early in the morning, fumigated with formalin, and stored at 17°C and 75 % relative humidity. Eggs were set weekly in a Buckeye® electronic incubator, which was set to turn eggs hourly through an angle of 60°. For the measurements of embryonic metabolism, a wooden incubator, of the type described in Chapter 5 and used by 70% of ostrich farmers in the Little Karoo region, was set up at Rhodes University in Grahamstown. Eggs from the Development Centre were subsequently transferred at various stages of development < 27 days in insulated cool boxes to the incubator in Grahamstown, where they were incubated at 36°C and a RH of 28 %. Turning was done manually, twice daily, 08:00 and 16:00 respectively. The weight range of eggs used in the study were from 1252 to 1588 gram. Eggs for this study was collected from a flock mating system (15 males : 25 females) thus resulted in unknown initial weights at setting into the incubator on the Oudtshoorn Experimental farm.

Oxygen consumption ( $V_{O_2}$ ) and carbon dioxide excretion ( $V_{CO_2}$ ) of embryos between five and 10 d old were measured in a closed respirometry system at 36°C and  $V_{O_2}$  and  $V_{CO_2}$  of older

embryos were measured using an open-flow respirometry system.

### **Closed respirometry**

Each egg was weighed and its volume measured by water displacement. For volume measurement, eggs were sealed in a plastic bag, with the air sucked out before immersion in water at approximately incubation temperature. Eggs were then placed inside a gas-tight plastic chamber of predetermined volume. The lids of the chambers contained an inlet with a stopcock valve, and an outlet with a 3-way valve.

After being placed in the chambers, egg temperatures were allowed to restabilise to incubator temperature for at least 15 minutes before being sealed. A 60 ml gas-tight syringe, with its plunger drawn back to the 50 ml mark, was attached to the 3-way outlet valve of the chamber. After the initial 15 minutes the chambers were sealed and the inlet stopcock closed. Eggs were left in the respirometer for a period that depended on their age and ranged from 30 to 180 min. At the end of the time the syringe was pumped several times to ensure thorough mixing of the air with that in the respirometer before a 50 ml gas sample was drawn up. The 3-way valve was then sealed and the syringe and valve disconnected from the outlet. Barometric pressure was measured after each run.

The gas sample was analysed for O<sub>2</sub> and CO<sub>2</sub> as described by Lighton (1991). Baseline (atmospheric) air was pulled through plastic tubing connected to the outside of the building past an injection port (3-way valve) and a gas analysis system by an Applied Electrochemistry R1 flow controller. The airstream passed through a silica gel tube to remove water vapour before entering an Applied Electrochemistry Model CD-3A CO<sub>2</sub> analyser. The air was subsequently scrubbed of both water and CO<sub>2</sub> by a carbosorb/silica gel tube before entering an Applied Electrochemistry Model S-3A/1 O<sub>2</sub> analyzer. The gas analysers were set assuming concentrations of O<sub>2</sub> of 20.95% and CO<sub>2</sub> concentrations of 0.03% in the baseline airstream. Flow rate through the system was measured using a bubble flowmeter and converted to STPD as described by Levy (1964).

O<sub>2</sub> and CO<sub>2</sub> concentrations of the samples were recorded directly onto a microcomputer using Datacan V data acquisition software (Sable Systems Inc, Salt Lake City) The syringe containing the gas sample was inserted into the injection port and the port valve opened.

After recording a 30 point baseline the syringe valve was opened and the gas sample injected as a bolus into the air flow. Percentage O<sub>2</sub> and CO<sub>2</sub> were recorded at 1 second intervals until they returned to baseline levels. After running a second 30-point baseline, recording was terminated. The volume of O<sub>2</sub> depletion and CO<sub>2</sub> enrichment were calculated by integrating areas under the curves using equation 6 and 7 as described by Lighton (1991). These values were substituted into equations 1 and 2 below to calculate  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$ .

$$\dot{V}_{O_2} \text{ (ml/h)} = (VC-VE)/VS \times (VI/t) \times 60 \quad (1)$$

where VC = respirometer chamber volume, VE = egg volume, VS = sample volume, VI = fractional volume of O<sub>2</sub> depleted and t = time the egg was in the chamber (min).

$$\dot{V}_{CO_2} \text{ (ml/h)} = (VC-VE)/VS \times VI/t \times 60 \quad (2)$$

where VI is the fractional volume of CO<sub>2</sub> produced and other variables are the same as above.

### **Open-flow respirometry**

Eggs were weighed to 0.01 g and placed in an air tight perspex chamber (33 x 17 x 20 cm) in a constant temperature cabinet. Temperature in the chamber (T<sub>a</sub>) was set to 36°C and was monitored by a thermocouple inserted through a rubber bung into the chamber. Dry, CO<sub>2</sub>-free air was pumped through a mass flow meter (Aalborg GFM 1700) at a flow rate of 750-850 ml/ min before entering the chamber. Air leaving the chamber passed through a tube of silica gel, and Applied Electrochemistry CD-3A CO<sub>2</sub> analyser and another tube of carbosorb and silica gel before entering an Applied Electrochemistry S-3A/1 O<sub>2</sub> analyser. Eggs were left for approximately 30 minutes prior to measurement of  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$ . Flow rate, percentage CO<sub>2</sub>, and O<sub>2</sub>, and chamber temperature were then recorded every 20 seconds throughout the run on a computer using Datacan V data acquisition software. Each experimental run lasted 2 - 3 h.  $\dot{V}_{O_2}$  was calculated for each run from the lowest stable period of metabolism without activity using equation 4a of Withers (1977).

The age of the embryo concerned was recorded for each measurement. Some eggs were sampled repeatedly at different stages of incubation. For the purpose of this investigation, repeated measurements on the same egg were assumed to be uncorrelated. Measurements included oxygen consumption and carbon dioxide excretion for each egg measured and the

ratio of CO<sub>2</sub> produced to O<sub>2</sub> consumed (Respiratory Quotient) over the 42 day incubation period.

### **Statistical analysis**

Standard nonlinear regression techniques were used to describe the rate of change in oxygen consumption and carbon dioxide excretion over the first 31 days of incubation (see below). Maximum levels of carbon dioxide excretion were used to estimate air flow rates through incubators that would be sufficient not to impair embryonic growth by interpolation. For this purpose several non linear regressions were fitted to obtain a regression of CO<sub>2</sub> concentration on airflow rate. A hyperbolae was eventually found to give the best fit. The nonlinear regression of carbon dioxide concentration on airflow rate in an incubator was computed to determine the airflow rate required to keep carbon dioxide concentrations at acceptable levels.

## **RESULTS**

### **Oxygen consumption and carbon dioxide excretion**

Oxygen consumption and carbon dioxide excretion increased exponentially over the first 31 days of incubation (Figure 1). Pooled data on 102 measurements of 45 eggs resulted in the following regression ( $\pm$  SE) for oxygen consumption:

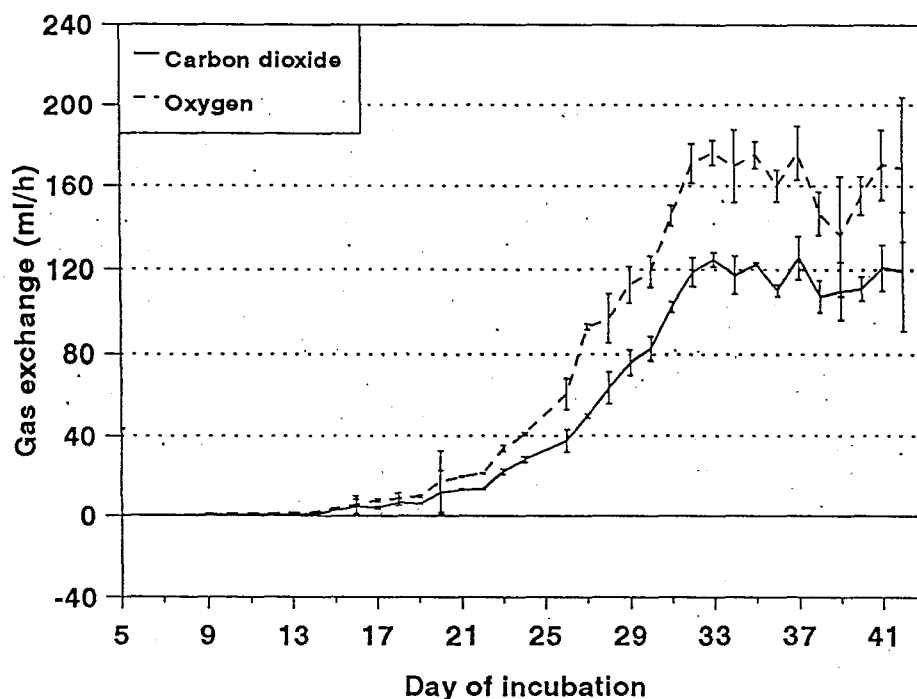
$$V_{O_2} = 0.434 \pm 0.096e^{0.189 \pm 0.007t} \quad (R^2 = 0.96)$$

where  $V_{O_2}$  = oxygen consumption (ml/h), and  $t$  = day of incubation. The corresponding equation for carbon dioxide excretion was:

$$V_{CO_2} = 0.225 \pm 0.052e^{0.198 \pm 0.008t} \quad (R^2 = 0.97)$$

where  $V_{CO_2}$  = carbon dioxide excretion (ml/h) and  $t$  = day of incubation.

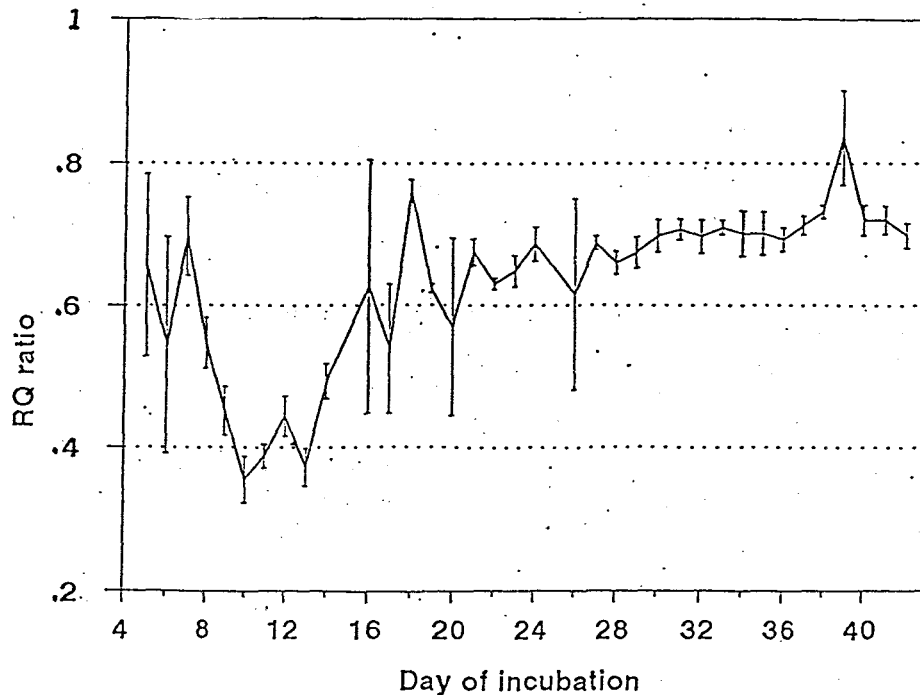
**FIGURE 1: OXYGEN CONSUMPTION AND CARBON DIOXIDE EXCRETION OF THE OSTRICH EMBRYO OVER 42 DAYS INCUBATED AT 36°C**



This exponential increase was followed by a plateau phase, during which oxygen consumption and carbon dioxide excretion stabilised at approximately 180 and 120 ml/h respectively (Figure 1). This was followed by a suggestion of a reduction in oxygen consumption during the period from 38 to 40 days of incubation to 140 ml/h oxygen consumption. The last two days of incubation was characterized by a return of oxygen consumption to plateau level. Both oxygen consumption and carbon dioxide excretion were extremely variable on the 42nd day of incubation, presumably reflecting different levels of activity of embryos just prior to hatching.

The respiratory quotient (RQ) average 0.68 until day 7 of incubation at which point it declined to reach its lowest level (0.55) at 10 days of incubation. From day 10 it gradually increased until day 21, after which it averaged 0.72.

**FIGURE 2: THE RESPIRATORY QUOTIENT (RQ) OF OSTRICH EGGS INCUBATED AT 36°C**



## DISCUSSION

In eggs of chickens and ducks, the rate of oxygen consumption increases nearly exponentially during the first 80 % of incubation and remains relatively constant during the remaining plateau phase (Rahn, *et al.*; 1974). The plateau phase is followed by a rise in oxygen consumed before pipping of the shell hours later. Visschedijk, (1968) suggested that the rise at the end of the plateau phase occurs when the embryo penetrated the air shell. This pattern is typical of precocial species of birds (Vleck, *et al.* 1979).

Ar, (1996) compiled 3 data sets from the literature (Hoyt, *et al.*, 1978; Meir and Ar, 1990; Reiner & Dzapo, 1995), obtained using three different temperatures and three methods of measurement. He conclude that the rate of oxygen consumption for ostrich eggs also increased exponentially during the first part of incubation. In the present study, the steepest area of the sigmoidal curve lay between days 26 and 31. Studies by Reiner and Dzapo (1995) also found that increased embryonic metabolic rate started at day 25 and lasted until day 36 with sharpest rise of 30 % per day prior to day 26. Hoyt *et al.* (1978), who incubated eggs at 35°C, found that increased metabolic rate only started at day 30 and reached a peak at 41 days. This delay is probably due to the lower than normal incubation temperature. The steep exponential phase in the study of Hoyt *et al.* (1978) and Reiner and Dzapo (1995) was

followed by a decline of about 25 % during the following 4 - 6 days. In our study the steep exponential phase was also followed by a more definite plateau phase for the next six days until day 38 when oxygen consumption and carbon dioxide excretion decreased by about 22% and 10%, respectively to 140 and 110 ml/h. The reason for the differences in the plateau phase may be related to the fact that the same egg was sampled in succession. A plateau phase followed by a decline was also observed in studies with the rhea and emu. Vleck *et al.* (1979) suggested that the decline in growth rate and O<sub>2</sub> consumption late in incubation may indicate that development is essentially complete several days prior to pipping which enables synchrony of hatching. Finally, the rate of oxygen consumption increased just before pipping, in the present study on day 42 and in that of Hoyt *et al.* (1978) and Reiner and Dzapó (1995) on days 44 and 40, respectively. This increase in metabolic rate has been suggested to coincide with internal pipping and the beginning of the transition from chorioallantoic to pulmonary respiration (Ar, 1996). Ar (1996) suggested that the total amount of O<sub>2</sub> which is consumed throughout incubation amounts to 5.2 l (STPD)/kg fresh egg mass, a value lower than the 6.5 l/kg reported by Hoyt & Rahn (1980). Both these values are among the lowest reported among birds and show that ostrich embryonic growth can be considered very efficient. Because initial egg weights in the present study were unknown gas exchange could not be expressed as ml/initial egg weight as was demonstrated by Ar (1996).

Slight differences in incubation temperatures in the different studies on ostrich embryonic metabolism appear to have relatively little effect on the overall pattern of metabolism but increased incubation temperatures lead to a shift of the curve to the left whereas decreased temperatures lead to a flattening of the steep increases in metabolism observed in the present study in the middle period of incubation and a delay in hatching. Ar, 1996 translates the rate of O<sub>2</sub> uptake into heat production and suggested that nett heat production must be taken into account in the overall balance of heat in the incubator. Metabolic heat production of embryos is low early in incubation and must be compensated for by heat from the incubator. During the second halve of incubation, the temperature gradient is inverted due to the substantial increase of heat production in the older embryos. The high heat production of hatchlings in the hatcher due to an eight fold increased in O<sub>2</sub> uptake rate compared to an unpipped egg during the last day of incubation may even require active cooling or at least high ventilation rates to prevent hyperhemia.

The average RQ calculated from data in the present study was 0.72, slightly lower than the RQ of 0.79 measured by (Meir and Ar, 1990) but the same as that recently reported by Prinzinger *et al.* (1997). An RQ of 0.72 is indicative of predominantly lipid catabolism and is typical of avian embryos (Meir and Ar 1990). The reduction in RQ to low levels between days seven and 10 of incubation is unusual and, to the best of my knowledge, has not previously been reported for avian embryos. Such patterns have been reported for animals entering torpor or hibernation and for poikilotherms during changes in body temperature and are indicative of a period of CO<sub>2</sub> storage or change in acid-base status (Stinner and Wardle, 1988). The pattern in ostrich embryos is, however, not readily explicable and may warrant further investigation. The possibility that it may be related to the change in technique used to measure metabolism over this period also cannot be overlooked because such a pattern was not observed in young embryos of the rhea (Prinzinger *et al.* (1997).

Nests of birds incubated under natural conditions are periodically ventilated by the adults who raise themselves off the eggs and expose them to convective air movements. Under conditions of artificial incubation, however, ventilation conditions generally remain relatively constant and gas concentrations in the incubator can consequently change as embryonic metabolism increases, especially in the wooden incubators that predominate in the Little Karoo area. Ar (1992) suggested that the partial pressure of O<sub>2</sub> around the ostrich egg is important in maintaining high hatchabilities. Oxygen (and carbon dioxide and water vapour) cross the eggshell by diffusion (Rahn *et al.* 1974). This diffusion of gases is dependent on gas concentration gradients between the inside and outside of the eggshell. As development proceeds the rising demand for oxygen and increasing production of carbon dioxide produces changes in concentrations of the respiratory gases within the egg. Taylor *et al.* (1971) suggested that egg shells with a low conductance resulted in accumulation of CO<sub>2</sub> and a corresponding decrease on O<sub>2</sub> that causes hypercapnia and hypoxia. On the other hand, excessive loss of CO<sub>2</sub> from the egg might lead to a loss of blood buffering capacity, which can change the acid base balance of the embryo. Hypocapnia, however, appears not to significantly affect the oxygen consumption, growth, or hatchability of embryos. Landauer (1967) suggested that good hatchabilities in chicken eggs can be obtained between 18 and 50 % oxygen but not greater than 0.5 % CO<sub>2</sub> and the maximum hatchability has been reported when oxygen concentration was maintained at 21 %.

Wilgus and Sadler (1954) observed the greatest hatchability in chicken eggs at 0.5 % CO<sub>2</sub> levels and they suggested that CO<sub>2</sub> may have a stimulating effect on embryonic development within certain limits. In contrast, Gildersleeve and Boeschen (1983) reported better hatchabilities of Turkey eggs when incubator CO<sub>2</sub> concentrations were not more than 0.3 %. Whereas slightly elevated levels of CO<sub>2</sub> may have a slight stimulating effect on embryos, levels higher than 0.5% appear to be detrimental. Romanoff (1930) and Barott (1937) found that CO<sub>2</sub> concentrations above 1% resulted in slow growth, a high incidence of abnormalities, and early embryonic deaths.

Development of the chick embryo is influenced by oxygen availability from the beginning of incubation. Smith *et al* (1969) reported that embryo growth is retarded by incubation at high altitude, where oxygen partial pressures are lower than at sea level. Bartels *et al.* (1973) showed that embryonic dry weight of chickens is reduced by day 3 of incubation when the oxygen concentration in the incubator is lowered, whereas the dry weight of the area vasculosa of the yolk sac is not affected. Evidence for changes in the timing of the appearance of definitive red cells in the circulation resulting from hypoxia is given in the work of Baumann *et al.* (1983), who showed that switching from embryonic to adult hemoglobin is accelerated by hypoxic incubation.

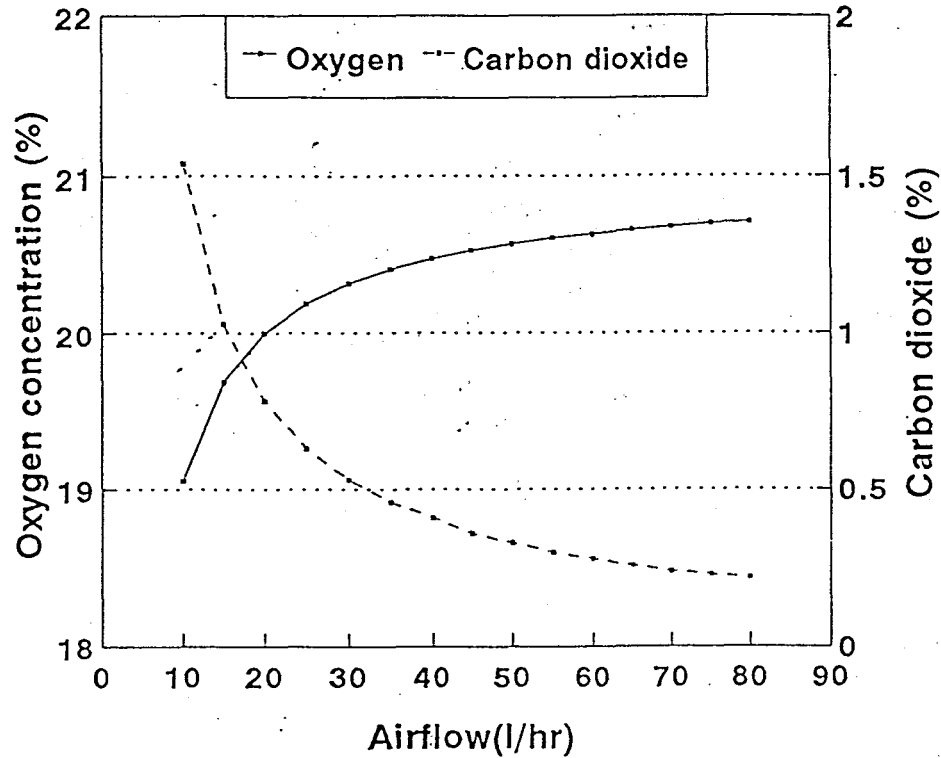
Synergetic interactions between carbon dioxide and oxygen concentrations were found to be present in incubator air. When levels of carbon dioxide were high (> 0.05) and the concentrations of oxygen in the incubator air were maintained at normal atmospheric levels of approximately 21%, hatchability of fowl eggs was significantly improved (Wilgus and Sadler, 1954). Conversely, hatchabilities were reduced when carbon dioxide levels were high (> 0.05) but oxygen levels (< 19 %) were low. A reduction in hatchability was also observed when both oxygen and carbon dioxide concentrations were high. (Taylor *et al.* 1956, Taylor *et al.* 1971). More important is the decrease of incubator O<sub>2</sub> which may cause hypoxia.

In ostriches, the peak rates of O<sub>2</sub> consumption and CO<sub>2</sub> excretion during the plateau phase are unlikely to exceed approximately 200 ml/egg.h and 150 ml/egg.h, respectively. Assuming these maxima, and an oxygen concentration of approximately 21 % in atmospheric air, incubator air quality could be calculated accordingly for a single egg (Figure 3). In order to maintain carbon dioxide concentrations below 0.3%, a function fitting the CO<sub>2</sub> curve was

developed. The nonlinear function that fitted the data best was the following hyperbolae:

$\text{CO}_2$  concentration in incubator air (%) =  $1/(0.046 + 0.0607 \cdot \text{Flow})$  ( $R_2 = 0.9993$ ),  
where flow is the airflow rate through the incubator in litres/h.

**FIGURE 3: OXYGEN AND CARBON DIOXIDE CONCENTRATION IN THE INCUBATOR FOR DETERMINING OPTIMAL VENTILATION RATES**



By substitution, it can be calculated from this equation that an ostrich embryo at peak metabolic rate requires an airflow of 54.15 litres/hour. Ar (1996) suggested that the reduction in oxygen partial pressure in the incubator environment should not change by more than 3 Torr (0.4 %) at sea level. He calculated that the ventilation needed at various stages of incubation to maintain optimum O<sub>2</sub> uptake for ostrich eggs amounts to about 47 litres/hour per egg of 1.4 kg. This result is consistent with our estimates. Extrapolation of this result to the incubator situation indicates that at least 54 200 litres of air should pass through a 1000 egg single-stage incubator per hour at times of peak embryonic metabolism. This airflow requirement may be halved if a multi-stage incubator is used.

Most electronic incubators presently used for ostrich egg incubation are chicken incubators with converted trolleys. A 1000 egg ostrich incubator (Buckeye)<sup>®</sup> occupies the same space as 19 000 chicken eggs. The ventilation rate required for maximum removal of CO<sub>2</sub> for

chicken eggs is approximately 3400 litre/1000 sixty gram eggs (Owen, 1991). Therefore the incubator ventilation rate needed for optimal hatchability of 1000 ostrich eggs is lower (54 200 vs 64 600 l/h) than the same incubator filled with chicken eggs. No adjustment is normally made to reduce ventilation rate which potentially results in the loss of costly heat energy because of overventilation (Ar, 1996).

## CONCLUSION

Although the tolerance of ostrich embryo to various concentrations of carbon dioxide during incubation is not known, our results present single stage incubator operators with a means to regulate ventilation during times of low embryonic metabolism in such a way that CO<sub>2</sub> can be purge on a daily basis by simply incrementing either fan speed or some control device e.g. baffle or recirculation flaps. Such adjustments may allow savings through reduced heat loss from the incubator.

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# CHAPTER 7

## HATCHING SUCCESS OF OSTRICH EGGS IN RELATION TO SETTING, TURNING AND ANGLE OF ROTATION

This chapter has been submitted for  
publication to *British Poultry Science* with  
S.W.P. Cloete, C.R. Brown and Z. Brand as junior co-authors.

## ABSTRACT

1. Three trials were designed to study the effects of axis of setting, turning frequency and axis and angle of rotation on the hatching success of ostrich eggs. The joint effects of axis of setting and angle of rotation were investigated in a fourth trial.
2. Trial 1: The hatchability of fertile ostrich eggs artificially incubated in electronic incubators (turned through 60° hourly) was improved substantially ( $P < 0.05$ ) in eggs set in horizontal positions for two or three weeks and vertically for the rest of the time.
3. Trial 2: The hatchability of fertile eggs set in the horizontal position without any turning was very low (27%). It was improved ( $P < 0.05$ ) to approximately 60% by manual turning through 180° over the short axis and through 60° over the long axis at 8 and 16 hour intervals. A further significant improvement ( $P < 0.05$ ) to approximately 80% was obtained in eggs automatically turned through 60° over the long axis in the incubator. Additional turning through 180° over the short axis twice daily resulted in no further improvement.
4. Trial 3: The hatchability of fertile eggs set vertically in electronic incubators and rotated hourly through angles ranging from 60° to 90°, increased linearly over the range studied ( $P < 0.05$ ). The response amounted to 1.83% for an increase of 1° ( $R^2 = 0.96$ ).
5. Trial 4: The detrimental effect of rotation through the smaller angle of 60° could be compensated for by setting ostrich eggs in the horizontal position for two weeks before putting them in the vertical position.

Keywords: Incubation, hatching success, setting position, turning frequency, angle of rotation, ostrich eggs.

## INTRODUCTION

It is well known that almost all avian eggs, with a few exceptions like the megapodes, need to be turned throughout incubation for correct embryonic development to take place (Drent, 1975; Deeming, 1991). Eycleshymer (1907) appears to be one of the first authors to report the detrimental effects of not turning fowl eggs (*Gallus gallus*), citing hatchabilities of only 15% in unturned eggs. Although a substantial body of information is now available for many aspects of the setting and turning of eggs (Kaupp and Dearstyne, 1926; Landauer, 1967; Proudfoot *et al.*, 1981; Deeming, 1989a), the majority of studies to date have been concerned with poultry (Lundy, 1969; Buhr, 1989). Comprehensive and well-proven guidelines can therefore be compiled for poultry species (Deeming, 1989 a, b, c; Wilson, 1991 a), but few comparable results have so far been reported for other species. Ostriches are an important commercial species in South Africa and are also becoming so in other parts of the world. The hatchability of artificially incubated ostrich eggs is, however, low and variable compared to other avian species, ranging from less than 30% to approximately 60% (Deeming *et al.*, 1993; Deeming, 1995; Brown *et al.*, 1996; Deeming, 1996; More, 1996; van Schalkwyk *et al.*, 1996). This relatively poor hatchability of artificially incubated ostrich eggs may, in part, be related to improper treatment with regard to setting and turning. Although other aspects of ostrich incubation and poor hatchability have been studied previously (Burger and Bertram 1981; Jarvis *et al.* 1985; Swart and Rahn 1988; Deeming *et al.* 1993; Deeming 1995; Brown *et al.* 1996), egg turning has so far been largely neglected.

Against this background, we conducted a series of 4 trials to investigate aspects of the setting, as well as the frequency, axis and angle of rotation of ostrich eggs.

## MATERIALS AND METHODS

Eggs for the study were obtained from the commercial ostrich breeding flock at the Klein Karoo Agricultural Development Centre near Oudtshoorn. The management of the breeding flock and eggs before incubation was as described by van Schalkwyk *et al.* (1996). Four separate trials were conducted on batches of eggs incubated in electronic incubators.

### **Trial 1: Axis of egg setting**

Batches of 24 ostrich eggs were set in electronic Buckeye® (Buckeye Poultry Equipment, PO Box 1749, Krugersdorp, 1749, South Africa) incubators in order to correspond to 7 treatments:

- In the vertical position for 6 weeks
- In the horizontal position for one week and the vertical position for five weeks
- In the horizontal position for two weeks and the vertical position for four weeks
- In the horizontal position for three weeks and the vertical position for three weeks
- In the horizontal position for four weeks and the vertical position for two weeks
- In the horizontal position for five weeks and the vertical position for one week
- In the horizontal position for six weeks

Each treatment was replicated 2–5 times. Eggs were incubated at a temperature of 36°C and a relative humidity of 28%. All eggs were rotated hourly through an angle of 60°.

Numbers of infertile eggs, embryonic deaths, and chicks hatched were obtained for each replication.

### **Trial 2: Frequency and axis of rotation**

Batches of ostrich eggs were set in the horizontal position in Buckeye® electronic incubators as described previously, according to 5 treatments. For the first two treatments, the trays were disconnected and the eggs were turned manually, twice daily, at 8 and 16 hour intervals. The first treatment involved turning of eggs through 180° over the short axis. In the second treatment eggs were turned through 60° over the long axis. The third and fourth treatments were carried out in trays subjected to conventional turning through 60° over the long axis in the incubator every hour. In the third treatment, eggs were also manually rotated through 180° over the short axis twice daily, as described previously. The fifth treatment (control) was not subjected to any turning. Each treatment was replicated three times. In this trial, the eggs were candled at 21 days using a 150 watt candling lamp. All those eggs that did not fit the normal developmental stage of ostrich embryos at that time were removed from the incubator. At 21 days the aircell should occupy about 16% of the total length of the egg, with an embryo weight of approximately 18 g and a length of 85 mm, with the feather buds just appearing (van Schalkwyk *et al.*, 1994). Eggs removed were divided into infertile eggs (showing no

development) and early embryonic deaths (where some development took place, but ceased before 21 days). Eggs where an element of doubt was involved, were regarded as infertile. All shell-deaths after candling were classified as late embryonic deaths. The subsequent treatment of eggs and records were as described for Trial 1.

### **Trial 3: The angle of rotation**

Batches of ostrich eggs were set in the vertical position in electronic Buckeye® or Prohatch® (Prohatch Ostrich Incubation Systems, Somchem, Reeb Avenue, PO Box 187, Firgrove, Somerset West, 7129, South Africa) incubators as described previously. Trays were modified to enable the hourly rotation of the eggs over the short axis, through angles of 60°–90°, with 10° intervals (60°, 70°, 80° or 90°). Batch sizes were considerably larger in this trial, ranging from 30–90 eggs. The reason for the difference in batch sizes was in the design of the incubators. The modification to the incubators resulted in the trays of the centre two of the four trolleys being fixed together to rotate through the same angle, resulting in a larger batch size for that angle. The outside trolleys could each be set to rotate through different angles, involving smaller batch sizes. Since larger sized batches could merely add to the accuracy with which percentages could be estimated, this was not seen as a limitation. In this trial, egg weight was recorded for some batches at setting and at 35 days of incubation. These weights were used to estimate evaporative water loss. The time of hatching and chick weight at hatching were recorded for chicks hatched in some batches. Other records were as described for Trial 2.

### **Trial 4: Axis of setting and planes of rotation**

Batches of eggs were set in electronic Prohatch® incubators. The four treatments involved the setting of eggs either vertically for the entire incubation period, or horizontally for two weeks and vertically for the remainder of the incubation period as well as angles of rotation of either 60° or 90°. These treatments were structured according to a 2 x 2 factorial experimental design with six replications for each treatment. Subsequent handling of eggs and records were as described for Trials 2 and 3.

### **Statistical evaluation**

Numbers of infertile eggs were expressed as percentages of eggs set prior to analyses. Infertility percentage was not expected to depend on any of the treatments, but were presented

for the sake of literature comparisons. Numbers of eggs with early or late embryonic deaths and live chicks were correspondingly expressed as percentages of fertile eggs prior to analyses. Since a number of replications fell outside the 0.3–0.7 range, proportions were also transformed to angles, using the arc sine transformation (Snedecor and Cochran, 1967). Conclusions derived from the transformed means were, however, similar to those made on the untransformed data. For ease of comprehension, only untransformed means are given.

Trials 1–3 were analysed according to a one way analysis of variance in a completely randomized design (Snedecor and Cochran, 1967). During the course of Trials 1 and 3, it happened by coincidence that batches of eggs were not treated to conform to a balanced design. This was mainly caused by competition for the available facilities by other high priority research programmes. Since these trials involved completely randomized designs, with no interactions that could be complicated by unequal subclasses, it was not expected to influence conclusions in any way. In Trial 3, where the treatments corresponded to a gradient, the degrees of freedom for treatments were partitioned into orthogonal polynomials depicting linear, quadratic and cubic trends. It was attempted to elucidate mechanisms contributing to the results obtained in Trial 3, by comparing egg weight at setting and after 35 days of incubation, as well as evaporative water loss, hatching date and chick weight for eggs rotated through 60° or 90°. One way analysis of variance procedures were used for this purpose including linear covariables where appropriate. Trial 4 was analysed according to a 2 x 2 factorial design, including the effects of axis of setting (vertically throughout or horizontally for two weeks and vertically for four weeks) and angle of rotation (through 60° or 90°).

## **RESULTS**

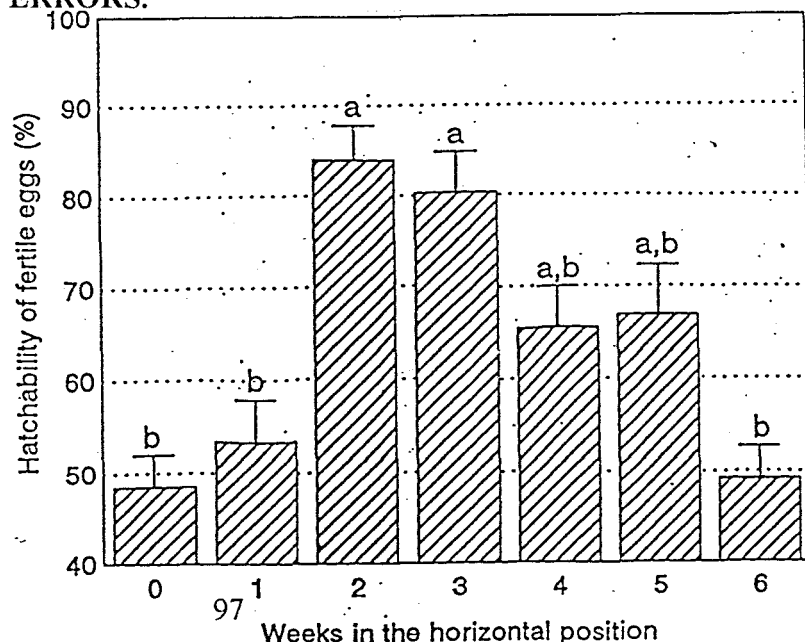
### **Trial 1**

Infertility of eggs used in this trial were similar across treatments and ranged from  $9.3 \pm 2.4\%$  to  $12.5 \pm 2.9\%$ . The setting of eggs in the vertical or horizontal position for different periods had a marked effect on the hatchability of fertile eggs. Hatchability was up to 36% higher ( $P \leq 0.05$ ) in batches of eggs incubated for two or three weeks in the horizontal position and the rest of the period in the vertical position than it was in batches of eggs incubated either horizontally or vertically throughout, as well as in batches incubated for one week in the horizontal position and five weeks in the vertical position (Figure 1).

## Trial 2

Early embryonic deaths averaging 50% were recorded for batches of unturned eggs. This figure was significantly reduced to about 23% by the manual turning of eggs through 180° over the short axis, twice daily ( $P \leq 0.05$ ). A further significant reduction in early embryonic deaths to only 6-8% was obtained in batches of eggs subjected to hourly turning in automatic incubations ( $P \leq 0.05$ ). Differences in late embryonic deaths were generally less between treatments, but treatments involving manual turning of eggs through 60° over the long axis twice daily, and the automatic, hourly turning of eggs without additional turning by hand resulted in significantly fewer late embryonic deaths ( $P \leq 0.05$ ). With regard to overall hatchability, it was clear that eggs automatically rotated hourly through 60° over the long axis in the incubator resulted in a higher proportion of chicks than eggs rotated twice daily by hand ( $P \leq 0.05$ ) (Table 1). The rotation of eggs twice daily through 180° in addition to automatic rotation in the incubator failed to improve hatching performance further. There were, in fact, instances where no significant differences were observed between this treatment and one or both treatment(s) involving manual rotation twice a day ( $P \leq 0.05$ ). Hatching performance was not significantly influenced by axis of rotation in the treatment rotated twice daily by hand.

**FIGURE 1: THE HATCHABILITY OF BATCHES OF FERTILE OSTRICH EGGS SET IN THE VERTICAL POSITION THROUGHOUT INCUBATION OR SET IN THE HORIZONTAL POSITION FOR A GIVEN NUMBER OF WEEKS BEFORE CHANGED TO THE VERTICAL POSITION. VERTICAL BARS UPON THE HATCHED COLUMNS REPRESENT STANDARD ERRORS.**



**TABLE 1: THE EFFECT OF TURNING FREQUENCY AND AXIS OF ROTATION ON THE INCUBATION RESULTS OF OSTRICH EGGS SET IN THE HORIZONTAL POSITION. MEANS ARE BASED ON THREE REPLICATIONS EACH, AND EXPRESSED RELATIVE TO FERTILE EGGS WITH THE EXCEPTION OF INFERTILITY. REPLICATIONS CONSISTED OF 24 EGGS EACH.**

Turning frequency, angle and axis of rotation	Incubation parameter			
	Infertility	Early embryonic deaths	Late embryonic deaths	Hatchability
Manually, two times a day, 8 and 16 h intervals:				
Through 180° over short axis	20.0 <sup>a,b</sup>	23.2 <sup>b,c</sup>	14.5 <sup>a,b,c</sup>	62.3 <sup>b</sup>
Through 60° over long axis	29.9 <sup>b</sup>	38.9 <sup>a,b</sup>	5.6 <sup>c</sup>	55.5 <sup>b</sup>
Hourly through 60° over long axis in the incubator:				
Manually, two times a day, through 180° over the short axis in addition	16.2 <sup>a</sup>	6.8 <sup>c</sup>	19.4 <sup>a,b</sup>	73.8 <sup>b,c</sup>
No additional manual rotation	12.8 <sup>a</sup>	8.2 <sup>c</sup>	9.8 <sup>b,c</sup>	82.0 <sup>c</sup>
Control set horizontally, no rotation	57.2 <sup>c</sup>	50.0 <sup>a</sup>	23.5 <sup>a</sup>	26.5 <sup>a</sup>
<b>SE mean</b>	<b>3.7</b>	<b>6.3</b>	<b>3.7</b>	<b>6.0</b>

<sup>a,b,c</sup> Means followed by different superscripts were significantly ( $P \leq 0.05$ ) different in columns

### Trial 3

The mean ( $\pm$  SD) infertility of eggs in this trial was similar ( $P > 0.50$ ) between treatments and averaged about 20% (Table 2). Early embryonic deaths were not significantly affected by angle of rotation but late embryonic deaths in eggs rotated through 90° were lower than in treatments rotated through  $\leq 70^\circ$  ( $P \leq 0.05$ ; Table 2). When partitioned into orthogonal

polynomials, 91.9% of the variation introduced to late embryonic deaths by angle of rotation was associated with a linear decline of 1.62% (SE = 0.41;  $P \leq 0.001$ ). The hatchability of the treatments rotated through 80° and 90° was correspondingly higher ( $P \leq 0.05$ ) than in those rotated through  $\leq 70^\circ$ . A linear incline of  $1.83 \pm 0.32\%$  was strongly associated with an increased angle of rotation ( $R^2 = 0.964$ ,  $P \leq 0.001$ ).

**TABLE 2: THE EFFECT OF ANGLE OF AUTOMATIC HOURLY ROTATION ON THE HATCHING PERFORMANCE OF OSTRICH EGG SET IN THE VERTICAL POSITION. MEANS ( $\pm$  SE'S) ARE GIVEN, AND ALL PARAMETERS EXCEPT INFERTILITY ARE EXPRESSED RELATIVE TO FERTILE EGGS. REPLICATIONS CONSISTED OF 30 TO 90 EGGS.**

Hatching performance	Angle of rotation			
	Through 60°	Through 70°	Through 80°	Through 90°
Number of replications	6	6	6	12
Infertility (%)	22.7 $\pm$ 3.1	22.1 $\pm$ 3.1	20.2 $\pm$ 3.1	20.3 $\pm$ 2.2
Embryonic deaths (%):				
Early	8.7 $\pm$ 2.1	4.9 $\pm$ 2.1	4.0 $\pm$ 2.1	4.9 $\pm$ 1.5
Late	42.3 <sup>a</sup> $\pm$ 5.4	42.8 <sup>a</sup> $\pm$ 5.4	29.0 <sup>a,b</sup> $\pm$ 5.4	20.6 <sup>b</sup> $\pm$ 3.8
Hatchability (%)	49.0 <sup>b</sup> $\pm$ 4.1	52.4 <sup>b</sup> $\pm$ 4.1	67.1 <sup>a</sup> $\pm$ 4.1	74.5 <sup>a</sup> $\pm$ 2.9

<sup>a,b</sup> Means followed by different superscripts were significantly ( $P \leq 0.05$ ) different in rows

Measurements made on eggs subjected to the extreme angles of rotation (through 60° and 90° respectively) were compared to investigate a possible mode of action for the improvement of hatching performance at 90°. A total number of 497 eggs rotated through 60° did not differ ( $P > 0.30$ ) from 489 eggs rotated through 90° for egg weight at setting ( $1.465 \pm 0.006$  vs  $1.458 \pm 0.006$  kg respectively), egg weight at 35 days ( $1.245 \pm 0.006$  vs  $1.240 \pm 0.006$  kg), percentage of evaporative water loss to 35 days ( $15.05 \pm 0.13\%$  vs  $14.96 \pm 0.13\%$ ) or chick weight at hatching ( $0.843 \pm 0.004$  vs  $0.849 \pm 0.004$  kg). In absolute terms it was observed

that eggs rotated through 60° were slightly heavier at setting and 21 days than those rotated through 90°. The reverse was true for chick weight. After accounting for the effect of egg weight at setting by analysis of covariance, chick weight at hatching was higher ( $P < 0.01$ ) in eggs rotated through 90° than in those rotated through 60° ( $0.852 \pm 0.003$  kg vs  $0.841 \pm 0.002$  kg). Although it was not quantified or considered as important at the time, unabsorbed albumen was commonly observed in the bottom of the eggshell in hatched eggs rotated through 60° whereas unused albumen was rarely found in eggs rotated through 90°. This probably accounts for the difference in chick weights at hatching. The average hatching date after setting was 41.6 (SE = 0.2) days for 385 eggs rotated through 90°, compared to  $42.6 \pm 0.2$  days for 574 eggs rotated through 60° ( $P \leq 0.01$ ).

#### **Trial 4**

Early embryonic deaths were independent of angle of rotation and axis of setting, although there was a suggestion ( $P = 0.12$ ) of a lower proportion of early embryonic deaths in eggs rotated through 90° (1.98% vs 5.03%; SE = 1.31). Late embryonic deaths were significantly higher in the treatment rotated through 60° and set in the vertical position throughout incubation than in the other treatments ( $P \leq 0.05$ ). Angle of rotation interacted with axis of setting for the hatchability of fertile eggs ( $P = 0.045$ ). The detrimental effect of rotation through the smaller angle of 60° was compensated for by the setting of eggs in the horizontal position for two weeks, before putting them in the vertical position (Table 3). Eggs incubated according to the latter schedule and rotated through 60° had a 26% higher hatchability than those incubated in the vertical position throughout ( $P \leq 0.05$ ).

**TABLE 3: THE EFFECTS OF ANGLE OF ROTATION AND THE SETTING OF OSTRICH EGGS IN THE HORIZONTAL POSITION FOR TWO WEEKS BEFORE PUTTING IT IN THE VERTICAL POSITION ON HATCHING PERFORMANCE. ALL PARAMETERS EXCEPT INFERTILITY ARE EXPRESSED RELATIVE TO FERTILE EGGS. REPLICATIONS CONSISTED OF 30 TO 165 EGGS.**

Hatching performance	Rotated through 60°		Rotated through 90°		SE mean
	Vertical	Horizontal-vertical	Vertical	Horizontal-vertical	
Number of replications	6	6	6	6	
Infertility	31.6	19.7	19.2	21.9	3.9
Embryonic deaths (%):					
Early	6.4	3.7	1.5	2.5	1.9
Late	33.8 <sup>a</sup>	20.2 <sup>b</sup>	16.7 <sup>b</sup>	13.5 <sup>b</sup>	3.4
Hatchability (%)	59.8 <sup>b</sup>	76.2 <sup>a</sup>	81.8 <sup>a</sup>	84.1 <sup>a</sup>	3.3

<sup>a,b</sup> Means followed by different superscripts were significantly ( $P < 0.05$ ) different in rows

## DISCUSSION

### Axis of setting

Funk and Forward (1960) obtained better hatchabilities when chicken eggs were incubated blunt end up as opposed to a horizontal setting (75.1% vs 71.3%). In contrast, Kaltofen (1959) obtained significantly better hatchabilities when eggs were turned in a horizontal position than with the blunt end up (77.5% vs 74.5%). He also emphasised that eggs should not be set in a strictly horizontal position but that the angle between the long axis and the horizontal should not be less than 20°. Chicken eggs are presently set vertically with their air sac up and turned around the short axis, whereas best results with the eggs of waterfowl are obtained when set horizontally and turned around the long axis (Wilson, 1991 a, b). No

conclusive advantage of either a horizontal or a vertical setting for the total incubation period could be demonstrated for ostrich eggs when rotated hourly through 60° (Figure 1). Perhaps the most surprising result is the advantage demonstrated for the setting of eggs horizontally for two or three weeks before turning them vertically. No corresponding results were found in the literature for other avian species. Under commercial conditions the vertical setting of ostrich eggs will be preferred, because of obvious advantages with regard to incubator space. The acceptable hatching performance of ostrich eggs set vertically and rotated through 90° (Trials 3 and 4) is thus encouraging.

### **Frequency of turning and axis of rotation**

As with the majority of avian species (Drent, 1975; Deeming, 1991) ostrich eggs have to be turned to obtain an acceptable hatchability. The high level of embryonic deaths in ostrich eggs not subjected to turning in the present study is consistent with results obtained by Deeming (1989 a, b, c) on chick eggs. The higher levels of infertility recorded in unturned batches of eggs probably also constitutes early embryonic deaths not detectable on a macroscopic level. Although manual turning twice a day improved hatching performance, hourly turning in an automatic incubator produced best results. This is consistent with conclusions by Wilson (1991 a, b) that hourly turning in automatic incubators is to be preferred. It is also noteworthy that no further improvement was obtained by the manual turning of eggs incubated in electronic incubators twice daily through 180° over the short axis. No conclusive advantage could be demonstrated for the manual turning of ostrich eggs either over the short axis through 180° or over the long axis through 60°. This is in contrast with significantly improved hatchabilities found by Funk and Forward (1952) when chicken eggs were subjected to one, two or three different planes of rotation. The principal results were a reduction in malpositions and an improvement in chick quality.

### **Angle of rotation**

Funk and Forward (1953) turned eggs through four different angles during incubation, viz. 40°, 60°, 80° and 90°. Hatchabilities increased progressively with angle of turn: 61.3, 69.6, 74.8 and 75.5% respectively. Funk and Forward (1960) extended these observations to cover angles of 120° and 150°. The highest hatchabilities in both experiments were obtained at a turning angle of 90°. Wilson (1991 a, b) concluded that best results in poultry and waterfowl were obtained with turning angles between 40° and 90°. Hatching success of ostrich eggs

improved linearly with increased angles of rotation from 60° to 90°. Our data do not allow extrapolation outside these ranges, but it may be speculated that this improvement will continue for larger angles. Further work is, however, required to test this possibility.

The mechanism involved in the better hatching performance of ostrich eggs rotated through larger angles remains unclear (Wilson, 1991 a). Findings in other avian species suggested that failure to turn eggs resulted in retarded development of the area vasculosa, the extra-embryonic membranes, retarded embryonic growth, reduced oxygen uptake and albumen absorption (New, 1957; Tazawa, 1980; Tullet and Deeming, 1987; Deeming, 1989a,b,c). The benefit of turning through larger angles (Funk and Forward, 1953; 1960), is probably associated with the facilitation of these processes. Based on observations in this study, we hypothesize that this is the case in the ostrich. The earlier hatching date of eggs rotated through 90°C, as well as the suggestion of an increased chick weight at hatching (possibly associated with better albumen utilization) contributed to this line of reasoning. Further work is required to elucidate these aspects to a greater extent.

Another aspect of this work involves the finding that the detrimental effect of a smaller angle of rotation could be compensated for by the setting of ostrich eggs in the horizontal position for two weeks prior to placing them vertically. No support for these findings, nor corresponding results were found in the literature.

## **CONCLUSIONS**

This study goes a long way towards providing guidelines for optimal incubation conditions for ostrich eggs. It is clear that turning of eggs should be as frequently as possible, and that angles of rotation approaching 90° should be adopted. Apart from the direct advantage in hatchability resulting from rotation through an angle of 90°, the earlier hatching date also allowed for better hygienic control in the incubator facility. The detrimental effect of a smaller angle of rotation was largely compensated for by the incubation of eggs horizontally for 2–3 weeks, and then vertically. This finding provides a cheaper alternative than altering incubator trolleys in hatcheries owning electronic incubators in which trays turn less than 90°.

It furthermore has to be conceded that hatchability figures seldom surpassed 70% of eggs set. There thus appear to be ample scope for further improvement in the ostrich industry. Avenues that should be pursued include selection, for which previous work at this institute suggested considerable scope (van Schalkwyk *et al.*, 1996), even if only in the current flock. Since ostrich farming is more extensive in nature than most other avian farming enterprises, a better understanding of environmental impacts on hatching success also appears to be a prerequisite.

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# **CHAPTER 8**

## **SUMMARY AND CONCLUSIONS**

## SUMMARY

This study found extreme variability in egg production performance ( $45.7 \pm 33.7\%$ ), hatchability ( $48.0 \pm 28.7\%$ ) and chick production ( $22.2 \pm 24.3\%$ ) of farmed ostriches. This was, however, consistent with other studies around the world. The major variation in all these traits was associated with differences between breeding pairs.

Although a significant interaction between month and year was observed, the trend for egg production was to start in June, to reach a peak during August - September, followed by a subsequent decline towards the end of the pairing-off period in January.

Reproduction traits analysed were found to be moderately repeatable ranging from  $0.38 \pm 0.07$  for hatchability percentage to  $0.51 \pm 0.06$  for embryonic deaths. The repeatability for annual adult body weight of male and female ostriches was  $0.68 \pm 0.05$  and  $0.61 \pm 0.05$ , respectively. Phenotypic correlations of male body weight with egg production performance ( $-0.20$ ) and female body weight with hatchability percentage ( $-0.16$ ) were negative. Correlations of egg production performance with infertility ( $-0.20$ ) and hatchability ( $0.23$ ) percentages were favourable. Egg production performance during the first breeding season predicted subsequent performance in later breeding seasons. This potentially allows selection of good breeding stock early on without resorting to subjective assessments of body conformation.

Investigations into the effect of collection time (morning versus afternoon), storage position and temperature, as well as pre-incubation storage conditions on blastoderm development and embryonic mortality in ostrich eggs suggested that eggs collected  $\pm 2$  to 3 hours after lay sustain lower levels of embryonic deaths ( $16.6$  vs  $22.9\%$ ) than those eggs left overnight to be collected in the morning. Embryonic deaths were not affected by storing eggs for 1 week in either a vertical (with the aircell at the top or the air cell at the bottom) or in a horizontal position. The critical zero temperature for ostrich eggs was found to be broadly similar to other avian species ( $\pm 25^\circ\text{C}$ ) with storage temperatures below  $20^\circ\text{C}$  causing complete cessation of all embryonic development even after exposed to high environmental temperatures of  $36^\circ\text{C}$  for 12 h. Storage of eggs at room temperature of  $25^\circ\text{C}$  resulted in an embryonic mortality of about  $45\%$  and this increased to  $>50\%$  in eggs exposed to high environmental temperatures before storage. It is recommended that ostrich eggs should be

collected frequently to avoid exposure to high environmental temperatures and that they be stored below 20°C.

Investigations into the tolerance of ostrich embryos to increased temperature regimes as well as possible temperature variations in forced draught wooden incubators indicated that hatchability was nearly halved (63.3 vs 33.8 %) when incubator temperatures were increased from 36 to 37.3°C. Temperatures recorded in the top tray of the wooden incubator was found in some instances to be nearly 1°C higher ( $P \leq 0.05$ ) than the middle or bottom part of the incubator due probably to natural convection. Younger embryos are likely to be less susceptible to temperature variations and it is recommended that, when incubation is in wooden incubators, that these be placed in positions where temperatures are more variable.

The ontogeny of metabolism of ostrich embryos incubated in this study showed broadly similar trends in oxygen consumption and carbon dioxide excretion to that of other studies. Oxygen consumption increased exponentially during the first 70 % of incubation, reaching a maximum between days 31 and 38 of incubation. During peak metabolism about 180 ml/h oxygen was consumed and 120 ml/h carbon dioxide was excreted, respectively. This stage was followed by a decline in metabolic rate to approximately 75 % of the peak value. It was thus calculated that single stage incubator needs an airflow of 54.4 l/egg.hour to prevent reduction in oxygen levels below 21 % and a CO<sub>2</sub> concentration exceeding 0.3 %.

Studies on the effect of axis of setting, turning frequency and axis and angle of rotation on the hatching success of ostrich eggs concluded that hatchability of fertile eggs increased linearly in the order of 1.83 % for an increased of 1° when turning angles increased from 60° to 90°. Hatchability was also improved from 60 % to 80 % when turning frequency increased from hand-turning twice a day to twenty-four times a day in automatic electronic incubators. It was also demonstrated that the effect of rotation through smaller angles of 60° could be compensated for by setting ostrich eggs in the horizontal position for 2 - 3 weeks before putting them in a vertical position.

## CONCLUSIONS

Results from both Chapters 2 and 3 indicated that ostrich production traits have a genetic basis. Selection for increased productivity in future is therefore possible. Negative correlations between male body mass and female productivity, although low, is alarming. With the present subjective selection criteria in which heavier birds are preferred for replacement stock, farmers might unknowingly decrease productivity in the long run. Farmers are therefore urged to change towards small camp systems, which allow assessment of reproductive performance rather than flock-mating.

Results from Chapters 4 - 7 indicated that low fertility and hatchability can be attributed to a combination of farming practices rather than one specific action. This might be a reason why overall productivity has remained low over the years in spite of improved incubator equipment.

Farmers are therefore advised to collect eggs late afternoons rather than in the morning and to store eggs at temperatures below 20 °C for not more than one week. Because of high temperature fluctuations within wooden incubators care must be taken that younger eggs are routinely placed in those position where temperatures are likely to exceeded 37°C. Eggs should be incubated horizontally for 2 - 3 weeks and subsequently incubated vertically with the air cell uppermost. Eggs should be turned as often as practical up to 24 time/day through 60 - 90°. Air flow through incubators should be at least 54 l/egg.hour between days 31 and 38 of incubation.