REPRODUCTIVE EFFICIENCY OF OSTRICHES (*Struthio camelus*)

by

HELET LAMBRECHTS

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PHILOSOPHIAE DOCTOR

Promotor: **Dr. D. Swart**
Co-promotor: **Prof. J.P.C. Greyling**

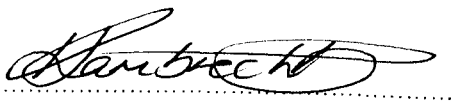
Bloemfontein, May 2004

Dedicated to

'charlie brown'

DECLARATION

I hereby declare that the dissertation, submitted for the degree *Philosophiae Doctor* at the University of the Free State, is my own, unaided work, and has not been previously submitted for any degree or examination at any other University.



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Chapter 1

General introduction

Ostrich farming in South Africa started as early as 1863, and the commercial population amounted to 253 463 ostriches by the end of the 19th century (Van Zyl, 1996). Initially, ostrich feathers were the main commercial product and ranked fourth in value, next to gold, diamonds and wool, as a South African export product (Smit, 1964; Wagner, 1986). The recognition of the commercial potential of ostrich leather and meat led to the establishment of a commercial ostrich abattoir and tannery in 1965 and 1970, respectively, in Oudtshoorn, which was considered as the headquarters of the ostrich industry. Today, South Africa is the largest ostrich producer in the world, producing approximately 60% of the world's 560 000 birds slaughtered annually. Ostrich leather and meat contribute approximately 65% and 27% respectively, to the total GDP of approximately ZAR 5.6 million generated from slaughter birds (Ostrich Section 7 Committee Report, 2003).

The emphasis on producing primarily for the export market and high input costs, together with a fluctuating exchange rate, necessitates that commercial ostrich farmers produce slaughter birds as cost-efficient as possible. The profitability of commercial ostrich production systems is largely determined by the number of eggs produced and chicks hatched per female, feed costs, and the type of production system used. In contrast to commercial poultry production systems, commercial ostrich production systems are characterised by a relatively low reproductive performance, a large variation in the number of eggs produced per female, and high chick mortalities (Van Zyl, 2001). In South Africa, the commercial ostrich industry average of 40 eggs produced per female during an 8-month breeding season (Van Zyl, 2001), a hatchability of 46.2% (Cloete *et al.*, 1998) and a chick survival rate of 95% during the first two days after hatching (Van Zyl, 2001), thus implies that only 18 viable chicks are produced per female during an 8-month breeding season.

Almost 80% of the national ostrich breeding population is maintained as breeding flocks, which can range from 50 to 100 birds in size, at a ratio of 5 to 6 males for every 10 females. Identification of poor or non-producing individuals is complicated by the fact that eggs are laid in communal nests, i.e. more than one ostrich female may lay in a given nest, and an ostrich female may also lay in more than one nest (Lambrechts *et al.*, 2002). The reproductive performance of breeding ostriches may be influenced by the timing of the breeding season, the long interval between pairing and the first oviposition, irregular egg laying sequences, age of breeding birds, nutrition, genetics, breeding behaviour of ostrich males and females, breeding environment, and management procedures.

Feed costs represent almost 83% of the total input costs of a breeding system (Van Zyl, 2001), and the maintenance of poor or non-producing individuals is thus clearly uneconomical. Methods to identify poor or non-producing individuals in commercial ostrich breeding systems, however, are scarce and based on historical performance and observations over time, rather than descriptive

predictions of a breeding ostrich's reproductive potential for future breeding seasons. Such techniques need to be developed to assist commercial ostrich farmers in selecting against low or non-producing ostrich males and females to improve the production performance of the overall farming operation.

The studies were conducted in the arid Klein Karoo region of South Africa, at longitude 22° 15' E and latitude 33° 38' S and at an altitude of 301 m above sea level. The long-term precipitation averages 230 mm, with 54 % of the total rainfall occurring during winter (April to September). The experimental site is characterized by hot summers, with the average maximum temperature exceeding 25°C for the period October to April.

Management aspects (practices) to synchronise and improve overall reproduction efficiency under intensive conditions that were addressed in the study, included the following:

1. Flush feeding and teasing
2. Timing of breeding, and forced breeding rest periods
3. Stocking rate and male:female ratio of breeding ostriches

Behavioural and physiological aspects that were considered as indirect selection criteria for reproduction efficiency of ostriches under commercial conditions were:

4. Shin colour and aggression of male ostriches and its influence on the reproductive performance of companion females
5. Reproductive hormones and their relationship to behavioural and reproductive parameters in breeding ostriches
6. The extent of follicular development before the onset of reproduction, as determined by ultrasound scanning
7. Live weight and body measurements of breeding ostrich females and the relationship with their reproductive performance
8. Semen quality in relation to fertility under commercial breeding conditions

Improving the reproductive performance of ostrich males and females through management strategies and selection for reproductive performance without increasing input costs will directly affect the production cost of day-old chicks. Based on costs calculated during 2000, Van Zyl (2001) concluded that a 10% increase or decrease in the number of day-old chicks hatched per female will amount to a 9.1% decrease or increase in the costs of producing a day-old chick. Similarly, a 10% increase or decrease in feed costs will amount to a 8.3% increase or decrease in the costs of producing a day-old chick (Van Zyl, 2001). The ability to manipulate the reproductive cycles of breeding ostriches, i.e. onset and cessation of breeding, as well as the reinitiation of reproductive activities, may enable the commercial ostrich farmer to manage a breeding flock optimally to ensure the timely production of slaughter birds according to market demand.

With the implementation of the various management, behaviour and physiological aspects addressed in this study, it is hoped that a contribution can be made to increase the overall reproduction efficiency of commercial ostrich production systems.

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Chapter 2

Flush feeding, teasing, and a mid-season breeding rest as management practices to improve the reproductive performance of ostriches (*Struthio camelus* var. *domesticus*) in commercial farming systems

Abstract

Flush feeding, teasing, and a mid-season breeding rest were investigated as management practices to synchronise and improve the overall reproductive performance of ostrich breeding pairs (n=136) during breeding seasons. Teasing significantly improved ($P \leq 0.05$) egg production performance of ostrich females. Maintaining breeding ostriches as single-sex flocks and visually isolating them during the pre-breeding period, and the re-introduction of the ostrich males to neighbouring camps two weeks before being joined, could thus have illicit neuro-endocrine responses in ostrich females that resulted in the initiation of ovulation and an earlier onset of egg production. Joining breeding ostriches after the winter solstice may be necessary to ensure the use of flushing and teasing as management practices to synchronise the reproductive cycles of breeding ostriches for an early onset of reproduction. A mid-season breeding rest improved ($P \leq 0.05$) the egg production performance and consequent chick production of the breeding pairs. A mid-season breeding rest was however, only effective in decreasing the time to onset of production when the breeding pairs were separated before the photoperiod started to decrease, i.e. when birds were still photosensitised enough to react to this management practice. Subjecting breeding ostriches to a breeding rest during the middle of spring in the Southern Hemisphere (i.e. mid-October), when ostriches have already started to become photorefractory, will most probably limit the effectiveness of this management practice to reinitiate reproduction in ostrich males and females. It is thus advisable to separate breeding ostriches for a mid-season breeding rest before daylight length starts to decrease to ensure the effectiveness of this management practice to overcome the seasonal quiescence in reproduction that is characteristic of breeding ostriches.

INTRODUCTION

Timing of breeding in temperate-zone birds is determined by the minimum photoperiod or critical day length, and the development of photorefractoriness (Leitner *et al.*, 2003). Photoperiod is considered as the most reliable and predictive cue that can exert an influence weeks to months before the onset of a breeding season. Photoperiodic stimulation has to attain a certain threshold before gametogenetic development is initiated and achieved, and additional factors bring the gonads to full functional maturity, with subsequent nest-building and egg production. A lack of appropriate environmental stimuli may prevent reproduction from occurring. This differs from tropical and opportunistic breeders where the reproductive system remains in a 'ready to breed' state for a large part of the year, with non-photoperiodic cues determining the onset of reproduction.

Ostriches can be regarded as temperate-zone breeding birds that have developed a rigid form of proximate control of their reproductive cycles to ensure the survival of their offspring during the time of the year when conditions are favourable (Shanawany, 1995). The timing and the duration of ostrich breeding seasons may vary with latitude and altitude (Shanawany, 1994). In birds, melatonin is mainly used as a 'circadian clock', and plays a role in the regulation of diurnal activities (Siopes, 1983; Lumineau *et al.*, 2002). At the molecular level, the avian circadian clock functions in a similar manner to that of the mammalian clocks. In birds, however, photoperiodic responses appear not to involve an interaction between the eyes and the pineal gland, but a direct interaction between photoreceptors and GnRH neurons (Saldanha *et al.*, 2001). It is unsure whether melatonin occurs in ostriches (Skadhauge and Dawson, 1999).

An emphasis towards the cost-effective production of ostrich leather and meat requires that commercial ostrich farmers produce slaughter ostriches as cost-effectively as possible (Huchzermeyer, 1998; Van Zyl, 2001). The characteristic low egg production performance of, and large variation in numbers of eggs produced in ostrich breeding systems necessitate the establishment of management practices to assist the ostrich farmer to manage his/her breeding flock to improve and optimize reproductive performance under commercial conditions (Cloete *et al.*, 1998; Bunter, 2002). Several factors may contribute to the low reproductive performance reported for ostriches under commercial breeding conditions, and may include a long interval between pairing and the first oviposition, irregular egg laying sequences, the age of breeding birds, nutrition, genetics, breeding environment, and management. During a breeding season, ostrich males and females may experience a period of quiescence that may last as long as 3-4 weeks. This quiescent period is characterised by a break in production and a loss of shin colour in the males (Stewart, 1989). Separation of ostrich males and females during this quiescent period is said to improve overall seasonal fertility and reproductive performance. Contradicting results, however, exist on the influence of a mid-season breeding rest period on the production and fertility of ostriches. Soley *et al.* (1991) found a positive relationship between good semen quality and fertility of birds not subjected to a breeding rest.

Flush feeding (flushing) and the use of teaser rams are two management practices used in sheep to overcome the seasonal limitation of lamb production (Martin *et al.*, 1986; Signoret, 1990; Nowers *et al.*, 1994; Smith *et al.*, 1996; Yildiz *et al.*, 2001). Flushing refers to the management practice where feed quantity and quality is increased before the onset of a breeding season, with a stimulatory effect on the reproductive system of especially the female (McDonald, 1980). In some sheep breeds, ewes are preconditioned during spring, with the rams being isolated from the ewes. Introduction of the rams will then illicit a neuro-endocrine response that results in estrus, ovulation and conception (Lishman and De Lange, 1975; Martin *et al.*, 1986). Ovulation rate can also be increased by subjecting ewes to flushing for at least one estrous cycle before being mated - providing that the ewes are in a moderate body condition (Lishman and De Lange, 1975; Haresign, 1981; Gunn *et al.*, 1991).

Management practices that can aid in the synchronization of breeding ostriches in order to identify and select early producing females will possibly contribute to the improvement of the overall performance of ostrich breeding birds under intensive breeding conditions. Flushing and teasing, and a mid-season breeding rest, were consequently investigated as potential management practices to synchronize and improve the overall seasonal production of breeding ostriches under commercial conditions.

MATERIALS AND METHODS

Experimental animals

An ostrich breeding flock consisting of breeding pairs (n=136) maintained at the Little Karoo Agricultural Development Centre outside Oudtshoorn, South Africa, were used for the study. The management of the breeding flock was documented by Van Schalkwyk *et al.* (1996) and Bunter and Graser (2000). The ages of the males and females used in the study varied between 2 and 12 years.

Experimental design

The influence of flushing, teasing, and a mid-season breeding rest, was investigated in a 2X2X2 factorial design trial, with breeding pairs allocated to their respective treatments groups at the beginning of each pre-season rest period. The treatment groups for the flushing and teasing management practices were as follows:

- Control-group: Breeding males and females not subjected to either flushing or teasing
- FT-group: Breeding males and females subjected to both flushing and teasing
- F-group: Breeding males and females subjected to flushing only
- T-group: Breeding males and females subjected to teasing only

In addition to the above-mentioned treatments, approximately half of the number of breeding pairs allocated to each respective treatment group, was also subjected to a mid-season breeding rest.

Management practices investigated

Nutritional flushing:

During each pre-season rest period of 4 months, males and females received a maintenance diet (8.5 MJ ME/kg DM and 9.1% protein) that satisfied the daily maintenance requirements of the breeding birds. Two weeks before a breeding season, the diet of the breeding males and females was changed to an *ad lib* flushing diet (9.5MJ ME/kg DM and 16% protein) to prepare both males and females for the increased physiological stress experienced during the breeding season.

Teasing:

During each pre-season rest period of 4 months, the males and females were pre-conditioned by visually isolating the males from the females, i.e. males and females were not allowed any visual contact. Two weeks before being joined for the breeding season, the males were introduced to camps neighbouring that of the females, and maintained in these camps until being joined.

During the 2000/2001 breeding season, the ostrich males and females allocated to the FT and T-groups were maintained as single-sex flocks during the pre-season and mid-season rest periods. During the 2001/2002 breeding season, the ostrich males and females allocated to the Control- and F-groups, i.e. not subjected to teasing, were maintained as mixed-sex flocks during the pre-season and mid-season rest periods.

A mid-season breeding rest

Breeding pairs that were experiencing a decline in egg production were separated for a mid-season breeding rest approximately in the middle of each breeding season, i.e. when ostriches are said to experience a natural quiescence in production. Each mid-season rest period lasted 6 weeks. The first 4 weeks of the breeding rest simulated a stage where the production cycles of the breeding birds were completely arrested by removing the breeding birds from their breeding territories, and by placing them on a maintenance diet. No visual contact was allowed between the breeding males and females during this period. During the remaining 2 weeks of the breeding rest, the males and females were again subjected to the flushing and/or teasing practices, as set out above.

During the 2000/2001 breeding season, the breeding pairs were removed from their breeding camps at the end of October 2000, and joined again for breeding at the beginning of December 2000. During the 2001/2002 breeding season, the breeding pairs were removed at the beginning of October 2001, and joined again for breeding in mid-November 2001.

Reproductive parameters recorded

Data recorded during the 2000/2001 and 2001/2002 breeding seasons, included the following:

- Number of days from being paired to the first oviposition - recorded for the pre- and mid-season rest periods
- Daily and total egg production
- Egg production performance (EPP) - expressed as percentage, and calculated on a fortnightly basis by using the following equation (Van Schalkwyk *et al.*, 1996):

  EPP = [total number of eggs produced/(0.5 X number of breeding days)] X 100
- Number of clutches – a clutch being defined as a set of eggs laid, where each set of eggs are laid within 4 days of each other; recorded for pre- and mid-season rest periods
- Egg weight on day of production (g)

- Day-old chick weight (g)
- Number and percentage of chicks hatched. The percentage of chicks hatched was calculated by using the following equation:

  Percentage of chicks hatched = [Number of chicks hatched / number of eggs set] X 100

Statistical analysis

Fixed effects in the analysis on the overall performance of females during the breeding seasons included treatment and female age. Least squares procedures were used to account for uneven subclasses (Harvey, 1990). The interaction of treatment with female age was included in the analysis, but found to be not significant.

Random animal effects were computed to account for the covariance introduced by the repeated sampling of the same animal when the fortnightly egg production performance data were analysed. ASREML, computer software that is capable of computing random effects in animal breeding, whilst also predicting least squares estimates of fixed effect means, was used for this purpose (Gilmour *et al.*, 1999). Owing to the fact that typical seasonal patterns were expected for the fortnightly production means, these trends were modeled using cubic splines (Verbyla *et al.*, 1999). The splines consisted of a fixed linear component, as well as random deviations from linearity conforming to a smooth trend. Random deviations from linearity not following a smooth trend were also included in initial runs, but were found to be insignificant ($P \geq 0.05$), as judged by the likelihood ratio test. These trends were interacted with treatment and female age where applicable, and the results were presented graphically.

Various measures of performance were assessed in the 4 treatment groups that were subjected to a mid-season breeding rest period during the breeding seasons. As these analyses were also based on the same animals being sampled repeatedly, the same basic analyses used for the fortnightly egg production data were also applied. The fixed effects in this case included treatment, the production period involved (before or after breeding rest) and female age. The interactions between these main effects were computed where significant ($P \leq 0.05$). Between female and residual variance ratios were obtained from the data sets subjected to the latter two sets of analyses. These estimates were used to obtain variance ratios depicting the within season repeatability estimates for the respective female traits considered (Turner and Young, 1969).

RESULTS

Flushing interacted with teasing during the second year of the study, thus complicating the separation of the respective influences of the two management practices. Therefore, the influence of flushing and teasing on the reproductive performances of the breeding pairs during both breeding seasons are presented in Tables 1 and 2.

Influence of flushing and teasing

The reproductive performances of the breeding pairs were not influenced by either flushing or teasing during the 2000/2001 breeding season. The reproductive cycles of the breeding males and females allocated to the FT-group tended ($P \leq 0.10$) to be more synchronised, with time to first oviposition being shorter than that of the Control-, F- and T-groups (Table 1).

Table 1. The influence of flushing and teasing on the reproduction traits (mean ± SE) of ostrich breeding pairs during the 2000/2001 breeding season.

MANAGEMENT PRACTICE	TREATMENT			
Flushing (F)	Control	Control	Treatment (F)	Treatment (F)
Teasing (T)	Control	Treatment (T)	Control	Treatment (T)
TRAIT				
Number of breeding pairs	31	33	32	35
Days to first oviposition	54.6 ± 11.0	58.6 ± 10.8	66.7 ± 10.9	45.8 ± 10.5
Total EPP (%)	35.6 ± 4.0	36.8 ± 3.9	35.8 ± 3.9	42.8 ± 3.8
Number of clutches	5.3 ± 0.6	5.2 ± 0.6	5.7 ± 0.6	5.4 ± 0.6
Egg weight (g)	1426.1 ± 20.2	1432.1 ± 20.4	1450.1 ± 20.1	1464.8 ± 18.4
Number of chicks hatched	21.8 ± 3.6	24.9 ± 3.6	26.6 ± 3.6	31.1 ± 3.4
Chicks hatched (%)	19.5 ± 3.2	22.4 ± 3.2	23.5 ± 3.2	27.9 ± 3.1
Chick weight (g)	877.7 ± 17.3	883.4 ± 17.5	885.7 ± 17.2	916.7 ± 15.8

When the egg production performance (EPP) of the breeding pairs not subjected to a mid-season breeding rest is considered, neither flushing nor teasing significantly influenced the fortnightly EPP of the breeding pairs (Figure 1). Although not significant, the T-group had a lower EPP throughout the breeding season. The FT- and F-groups tended ($P \leq 0.10$) to have higher EPP's throughout the breeding season, with the Control-group being intermediate to the above-mentioned three groups.

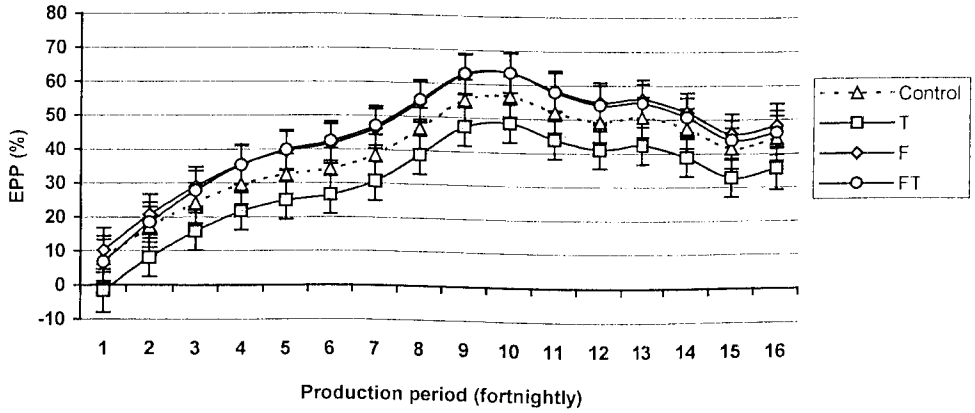

Figure 1. The influence of flushing (F) and teasing (T) on the fortnightly egg production performance (EPP; mean ± SE) of ostrich breeding pairs not subjected to a mid-season breeding rest during the 2000/2001 breeding season.

Similar to observations in the 2000/2001 breeding season, almost all reproductive parameters were unaffected by either flushing and/or teasing during the 2001/2002 breeding season. The only exception was the teasing management practice that improved (P≤0.05) the EPP of the ostrich breeding pairs (Table 2).

Table 2. The influence of flushing and teasing on the reproduction traits (means ± SE) of ostrich breeding pairs during the 2001/2002 breeding season.

MANAGEMENT PRACTICE	TREATMENT			
Flushing (F)	Control	Control	Treatment (F)	Treatment (F)
Teasing (T)	Control	Treatment (T)	Control	Treatment (T)
TRAIT				
Number of breeding pairs	32	34	33	35
Days to first oviposition	56.1 ± 8.9	40.2 ± 9.1	54.9 ± 8.7	61.5 ± 8.6
Total EPP (%)	35.6 ± 3.8^{b}	49.9 ± 3.9^{a}	47.7 ± 3.7^{a}	44.3 ± 3.7^{b}
Number of clutches	4.1 ± 0.6	4.6 ± 0.6	4.3 ± 0.6	3.5 ± 0.6
Egg weight (g)	1450.7 ± 20.9	1474.4 ± 20.6	1451.9 ± 20.76	1456.3 ± 18.7
Chick production (number)	22.2 ± 3.5	31.4 ± 3.5	26.3 ± 3.4	31.8 ± 3.3
Chicks hatched (%)	19.4 ± 3.0	28.8 ± 3.1	23.3 ± 2.9	29 8 ± 2.9
Chick weight (g)	890.7 ± 16.2	903.8 ± 15.9	890.6 ± 15.9	886.5 ± 14.4

$^{a, b}$: Columns with different superscripts differ significantly (P≤0.05)

Flushing and/or teasing did not influence the fortnightly EPP of the breeding pairs not subjected to a mid-season breeding rest during the 2001/2002 breeding season (Figure 2). There was a tendency (P≤0.10) for the breeding pairs subjected to flushing, teasing, or a combination thereof, to have higher EPP's. However, the relative advantage of flushing and teasing decreased as the breeding season progressed.

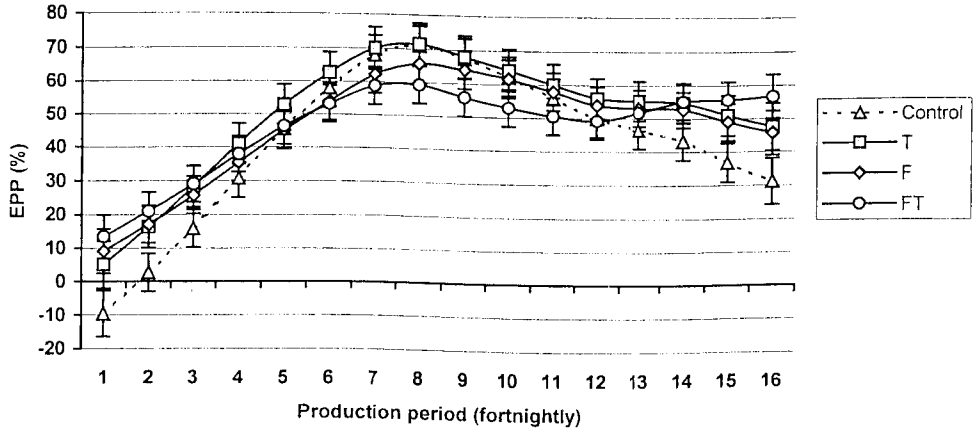

Figure 2. The influence of flushing (F) and teasing (T) on the fortnightly egg production performance (EPP; mean ± SE) of ostrich breeding pairs not subjected to a mid-season breeding rest during the 2001/2002 breeding season.

The mid-season breeding rest also had no detrimental influence on the reproductive performance of the rested breeding pairs during the 2001/2002 breeding season. The rested breeding birds recovered swiftly from the forced break in production, and tended ($P \leq 0.10$) to have improved EPP's for the remainder of the production period, when compared to the non-rested breeding pairs (Figure 5).

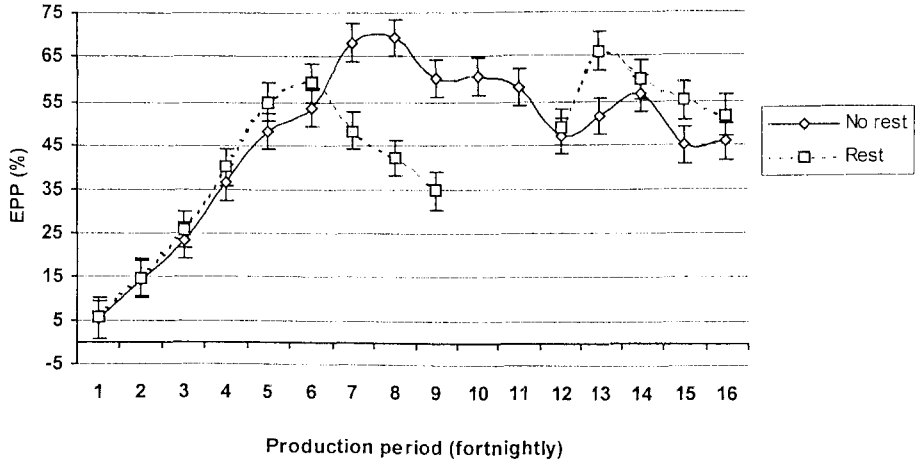

Figure 5. The influence of a mid-season breeding rest on the fortnightly egg production performance (EPP; mean ± SE) of ostrich breeding pairs during the 2001/2002 breeding season.

The beneficial influence of flushing and teasing became evident when the reproductive performances of the treatment and control groups were considered. The EPP of the breeding pairs was quickly restored to levels observed during the pre-rest period, and the FT-, F-, and T-groups producing significantly ($P \leq 0.01$) more eggs than the Control-group during the post-rest period (Figure 6).

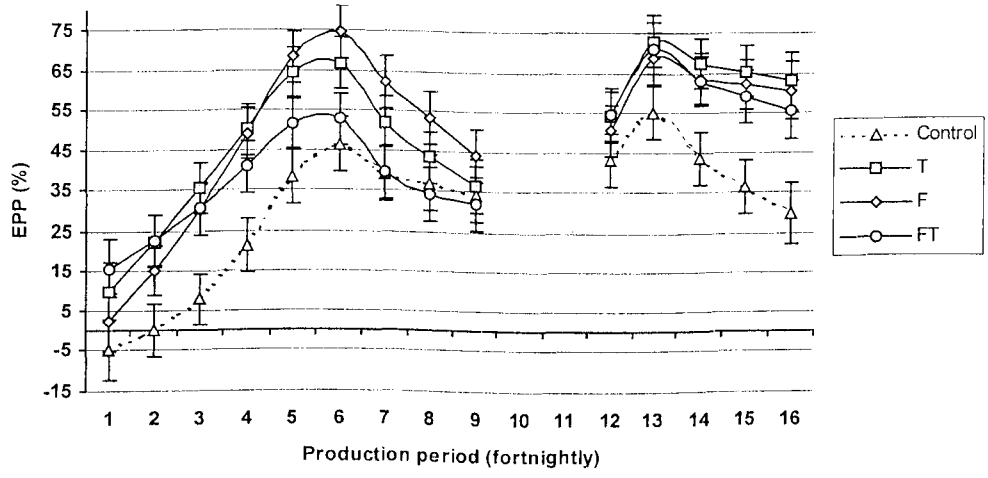

Figure 6. The influence of a mid-season breeding rest on the fortnightly egg production performance (EPP; mean ± SE) of ostrich breeding pairs subjected to flushing (F) and teasing (T) management practices during the 2001/2002 breeding season.

The mid-season breeding rest improved (P≤0.01) the post-rest EPP of the FT-group during the 2000/2001 breeding season (Table 3). The breeding rest also synchronized the reproductive cycles of the FT males and females, resulting in an increase (P≤0.01) in clutch size, i.e. a higher EPP together with a lower number of clutches produced, during the post-rest period (Table 3).

Table 3. The effect of a mid-season breeding rest on the reproduction traits (means ± SE) of ostrich breeding pairs subjected to flushing and teasing during the 2000/2001 breeding season.

TRAIT	Before rest	After rest
EPP (%)		
Control	34.4 ± 7.0	47.7 ± 7.0
Flushing only	32.2 ± 6.6	36.3 ± 6.9
Teasing only	41.3 ± 7.1	42.1 ± 7.1
Flushing and teasing	36.5 ± 6.4[a]	56.2 ± 6.7[b]
Chick production (%)		
Control	19.4 ± 6.1	29.8 ± 6.1
Flushing only	20.7 ± 5.7	21.3 ± 5.9
Teasing only	25.3 ± 6.2	28.2 ± 6.2
Flushing and teasing	24.4 ± 5.5	36.7 ± 5.8
Days to first oviposition	73.3 ± 14.2	69.8 ± 14.5
Number of clutches	3.3 ± 0.3[a]	1.9 ± 0.3[b]

[a, b]: Rows with different superscripts differ significantly (P≤0.01)

A mid-season breeding rest significantly (P≤0.01) improved the post-rest EPP of the F-, T-, and FT-groups during the 2001/2002 breeding season. A combination of flushing and teasing had to effect that fertility and hatchability was improved, resulting in more chicks (P≤0.01) being hatched for the FT-group during the post-rest period. The interval between being joined and the first oviposition during the post-rest period was also significantly (P≤0.01) decreased (Table 4).

Table 4. The effect of a mid-season breeding rest on the reproduction traits (means ± SE) of ostrich breeding pairs subjected to flushing and teasing during the 2001/2002 breeding season.

TRAIT	Before rest	After rest
EPP (%)		
Control	20.9 ± 6.8	32.9 ± 6.8
Flushing only	42.4 ± 6.6[a]	57.2 ± 6.6[b]
Teasing only	44.8 ± 6.4[a]	60.8 ± 6.4[b]
Flushing and teasing	29.5 ± 6.5[a]	62.1 ± 6.5[b]
Chick production (%)		
Control	11.4 ± 6.2	18.3 ± 6.2
Flushing only	18.3 ± 6.0	24.9 ± 6.0
Teasing only	25.3 ± 5.8	32.5 ± 5.8
Flushing and teasing	23.0 ± 5.9[a]	41.3 ± 5.9[b]
Days to first oviposition	54.9 ± 4.5[a]	10.9 ± 4.4[b]
Number of clutches	2.7 ± 0.3	2.2 ± 0.3

[a, b]: Rows with different superscripts differ significantly (P≤0.01)

DISCUSSION

The peak production period observed for ostriches in this study confirms the photoperiod dependent reproduction pattern of ostriches (Jarvis *et al.*, 1985; Hicks, 1992; Mellett, 1993; Horbaυczuk and Sales, 1999). Results in this study are in agreement with Hicks-Aldredge (1993), i.e. that ostrich breeding seasons are characterised by two production peaks. The first production peak occurred in late September to late October (spring in the Southern Hemisphere) and the second less pronounced production peak during late December (summer in the Southern Hemisphere). However, the peak breeding season reported in the study differs from the peak production season reported for Kenyan Red ostriches (*Struthio camelus massaicus*), i.e. from July to October (Bertram, 1979), or from September to December, with a peak in November (Hurxthal, 1979). This supports the contention that the length and timing of ostrich breeding seasons may vary with latitude and altitude (Shanawany, 1994). Results also support the studies of Sauer (1972), Leuthold (1977), Jarvis *et al.* (1985), Cloete *et al.* (1998) and Bunter and Graser (2000), in that peak egg production occurred during August to December. A quiescent period in egg production that lasted approximately 4 weeks during the 2000/2001 and 6 weeks in the 2001/2002 breeding season, respectively, was characterised by a decline in egg production. This period of quiescence is longer than the 3 to 4 week period of quiescence described by Stewart (1989).

Average egg production of ostrich females during this study varied between 44 and 61 eggs for an 8-month breeding season. This is considerably higher than the average egg production reported by Jarvis *et al.* (1985) for ostriches in Zimbabwe (16-17 eggs) under commercial farming conditions but falls well within the range of 0 to 120 reported by Van Schalkwyk *et al.* (1996) and Cloete *et al.* (1998). Egg weights reported in the present study fall within the range of 963-1827.8g reported by Bunter and Graser (2000). Chick production percentages in this study ranged from 19.37% to 36.71%, which falls within the range of 0-78.2% reported by Bunter (2002). Another ratite species in which reproduction is influenced by photoperiod, the geater rhea (*Rhea americana*), produce on average 24-40 eggs, and 18 eggs per female under captive and natural breeding conditions, respectively (Navarro and Martella, 2002), which is lower in both cases than the egg production reported for ostrich females in this study.

Onset of egg production, i.e. days from pairing to first laying, ranged from 40.2 to 66.7 days. This differed from the mean of 35.9 days, and the range of 0 to 230 days reported by Bunter (2002). The number of clutches during a breeding period ranged from 3.5 to 5.7, which is less than the average of 6.3 clutches reported by Bunter and Graser (2000). The average clutch size reported in this study ranged between 7.7 to 15.3 eggs/clutch, which supports the clutch sizes reported by Leuthold (1970), Jarvis *et al.* (1985) and Horbaυczuk and Sales (1999). Jarvis *et al.* (1985) reported clutch sizes of 12-13 eggs /clutch and 16 eggs/clutch for Zimbabwean ostriches in the wild and under commercial farming conditions, respectively. Horbaυczuk and Sales (1999) reported clutches of 12-16 eggs, and Leuthold (1970) a clutch size of 22 eggs under natural breeding conditions for a single female in Tsavo National Park, Kenya. In emus (*Dromaius*

novaehollandiae), artificially inseminated females layed on average 6.7±1.6 eggs per clutch (Malecki and Martin, 2002).

The influence of flushing and teasing

Flushing, teasing, or a combination of flushing and teasing did not have a significant influence on most of the reproductive traits recorded during the study. Teasing was the only management practice that significantly influenced the egg production performance of the breeding pairs. Visually isolating of ostrich males and females during the pre-breeding rest period, and by allowing visual contact two weeks prior to being paired for breeding, elicited a significant physiological response in the ostrich females that resulted in an improved egg production performance. The visual presence of the male, together with a display of reproductive behaviour, stimulated the females to lay sooner after pairing and to produce larger clutches. Total egg and chick production, as well as egg and day-old chick weight, were not significantly influenced by flushing and/or teasing.

Ostrich males and females may differ in terms of their response to proximal stimuli, in this case the presence of the opposite sex and the availability of feed with a higher nutritional value. It has been found in birds that mere light stimulation may induce complete spermatogenesis in males, whereas a female's complete reproductive state may only be achieved under the influence of various complementary factors (Immelman, 1972). In many bird species, it has been found that a female will ovulate only after being together with the male for some time, which indicates that the male stimulates the female by means of for example courtship and other behavioural interactions. The behavioural activities of an ostrich male may thus aid in synchronizing the reproductive cycles of a breeding pair. In Houbara bustards (*Chlamydotis undulata macqueenii; C. u. undulata*) that breed in arid to semi-arid areas in Saudi Arabia, males displayed reproductive behaviour well in advance of the onset of egg production (Saint Jalme *et al.*, 1996). The stimulatory effect of male courtship behaviour and vocalization has been confirmed in several studies for a variety of species (Lehrman, 1965; Brockway, 1965; Hinde, 1967). Tactile stimulation as well as auditory signals have been proven to be of great significance in stimulating female starlings (*Sturnus vulgaris*), with female courtship influencing spermatogenesis in the male starling to a lesser extent than *visa versa* (Burger, 1953).

Although contradictory results were obtained during the two breeding seasons, it stands to reason that the omission of flushing and teasing will have a retarding effect on the development and synchronization of ostrich males and females. In sheep, the omission of flushing and teasing management practices adversely affected the breeding performance of spring mated ewes (Nowers *et al.*, 1994). In chickens, auditory and visual contact with males accelerated the sexual maturation of hens reared adjacent to or in mixed-sex flocks. Hens came into lay earlier and had larger combs than isolated females (Widowski *et al.*, 1998). Egg production was significantly higher in turkey hens under natural mating conditions than in females visually exposed to turkey males or completely isolated from males. Females visually exposed to males also laid more eggs than those completely isolated from males (Jones and Leighton, 1987).

The influence of a mid-season breeding rest

The return of reproductive viability can be regarded as a parameter for determining the successful recycling of turkey breeder hens (Moore and Siopes, 2003). In ducks, forced moulting is often used to stimulate egg production after a senescence in production (Olver, 1995). Arresting egg production in laying chickens by means of a forced moulting had a stimulatory effect on the post-arrest egg production (Hurwitz *et al.*, 1975). A mid-season breeding rest did not appear to negatively influence the EPP of the ostrich breeding pairs, with reproductive activity following the rest quickly restored during both years. The extent to which post-rest production was improved, however, differed between the two breeding seasons. The breeding rest seemed to have a more beneficial effect during the 2001/2002 breeding season, with rested breeding pairs tending to have an improved EPP for the remainder of the post-rest period. This tendency was not observed during the 2000/2001 mid-season breeding rest; the EPP of the rested breeding pairs appeared improved for only a short period and then declined to reach almost the same level as that of the non-rested groups.

This difference in the effect of the breeding rest could be ascribed to the time of the breeding season when the breeding rest was enforced. A decreased sensitivity of the pituitary during the photorefractory state results from a decreased LHRH output from the hypothalamus (Wingfield *et al.*, 1979; Storey and Nicholls, 1983). When the ostrich breeding pairs were separated for a breeding rest in 2000/2001, photoperiod was possibly still long enough to support reproduction when the breeding pairs were separated. The ostrich males and females, however, had already possibly started to enter a state of photorefractoriness due to a gradual decrease in day length. During 2001/2002, the breeding rest was enforced one month earlier. The rested breeding pairs were possibly still under strong photoperiodic control, and thus still photosensitized. Turkey breeder hens showed varying degrees of relative photorefractoriness early in a breeding season, with photorefractoriness increasing in severity as the breeding season progressed (Siopes and Proudman, 2003). Kumar and Kumar (1991, 1993) concluded that the time to onset of gonadal recrudescence and subsequent regression in brahminy myna (*Sturnus pagodarum*) depended on the length of the photophase as well as the time of year when exposed to stimulatory photoperiods. Season had a significant effect on the sensitivity of turkey hens to become photorefractory, with winter delaying the onset of photorefractoriness (Siopes, 2002). Age did not influence the mean time to become photorefractory, with second cycle turkey hens more inclined to become photorefractory than younger first cycle hens (Siopes, 2002).

CONCLUSIONS

Teasing of breeding ostriches significantly improved the egg production performance of the breeding ostrich females. It is possible that the effectiveness of flushing and teasing to synchronise the reproductive cycles of breeding ostriches is limited by pairing breeding ostriches before the winter solstice. Joining breeding ostriches after the winter solstice, i.e. when

photoperiod has increased sufficiently to initiate reproduction, may be necessary to ensure the successful implementation of flushing and teasing to synchronise the reproduction cycle of breeding ostriches. A mid-season breeding rest proved successful in reinitiating and synchronising the reproductive cycles of both the ostrich males and females, and also to recycle the ostrich females that were experiencing a decline in egg production. The extent to which the reproductive cycles of both sexes can be manipulated are most probably influenced by the state of photorefractoriness that the birds have reached during that specific stage of the breeding season.

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Chapter 3

The influence of season and breeding rest periods on the reproductive performance of breeding ostriches (*Struthio camelus* var. *domesticus*) under commercial farming conditions

Abstract

Breeding ostriches were paired for breeding at different times of the year to investigate the influence of onset of breeding on the reproductive performance under commercial farming conditions. Breeding was also interrupted at different times during the breeding season to determine the potential of forced rest periods to reinitiate reproduction and thus improve overall reproductive performance and fertility of breeding ostriches. Peak egg production was recorded during July and January, with lower production recorded during February to June. The time of the year when the ostriches were paired for breeding had a significant influence on the reproductive traits, with pairing off after the winter solstice positively influencing almost all of the reproduction parameters. A pre-season rest period was beneficial in terms of all reproductive traits, with breeding pairs subjected to a continuous breeding season performing worse throughout the entire breeding period. Forced rest periods during breeding significantly improved egg production but not fertility in the treatment groups. This implies that reproduction could be successfully reinitiated in ostrich females, and maintained in ostrich males. Although fertility was not improved by the forced rest periods, the ostrich males had to successfully fertilize the higher number of eggs produced by their companion females and accomplished this without a compromise in fertility. The time of year when the forced rest period was applied determined the extent to which reproductive activities could be reinitiated in ostriches. The fact that the reproductive activities of the breeding pairs that were paired before the winter solstice were synchronised to a higher degree than breeding pairs joined after the solstice, implies that the inhibitory effect of a short photoperiod, i.e. insufficient to stimulate reproduction, may be overcome by subjecting breeding ostriches to enforced breeding rest during a time of the year when the birds are still photosensitive enough to respond to this management practice. This may enable commercial ostrich farmers to manipulate the reproductive cycles of their breeding ostriches in order to ensure the timely production of slaughter birds according to market demand.

INTRODUCTION

Reproductive cycles of birds breeding in temperate regions are regulated by the seasonal change in photoperiod, and these species have become almost completely dependent on a change in photoperiod to initiate gonad development and reproductive activities (Immelman, 1972). Photoperiod is by far the most reliable and predictive factor that can exert its influence weeks to months before the onset of breeding, with timing of breeding determined by the minimum photoperiod or "critical day length" and the development of photorefractoriness (Leitner *et al.*,

2003). Critical day length varies between seasons and are a dynamic characteristic of photoperiodic processes (Siopes, 1994).

An increase in photoperiod is thought to initiate two processes, firstly the secretion of the gonadotrophic hormones and subsequent gonadal maturation and reproduction, and secondly the development of photorefractoriness (Dawson *et al.*, 1986; Foster *et al.*, 1987). Photorefractoriness represents a period where the reproductive system becomes insensitive to photoperiod, with subsequent gonadal regression to a pre-breeding state (Nicholls *et al.*, 1988; Rani *et al.*, 2001; Leitner *et al.*, 2003; Siopes and Proudman, 2003). Photorefractoriness represents almost the most highly developed termination scheme, and is a special adaptation to prevent birds from maintaining production when environmental conditions become unfavourable to the rearing of young, which usually takes place during the months after the summer solstice (Lofts and Murton, 1968). Once in a state of photorefractoriness, exposure to short day lengths is necessary to render birds sensitive to photoperiod again, thus initiating gonadal recrudescence and steroid synthesis and secretion (Wingfield and Farner, 1980; Farner, 1986; Kumar and Kumar, 1991).

Photoperiodic responses in birds appear to involve direct interaction between photoreceptors and GnRH neurons, with the photic cues that regulate the timing of seasonal reproductive cyclicity detected by non-retinal, non-pineal deep brain photoreceptors. These photoreceptors communicate directly with GnRH-neurons, and represents a means by which photoperiodic information reaches the reproductive axis (Saldanha *et al.*, 2001). In mammals, photoperiod also serves as an important proximate cue to initiate the onset of breeding, with the pineal gland and melatonin being an integral part in the regulation of the breeding cycle of e.g. ewes (Bittman, 1984; Kennaway *et al.*, 1982; Nowers *et al.*, 1994; Bentley *et al.*, 2000). In birds, however, melatonin does not fulfill the same role and is mainly used as a circadian 'clock' to regulate diurnal or daily activities (Siopes, 1983; Lumineau *et al.*, 2002). It is unsure whether melatonin occurs in ostriches (Skadhauge and Dawson, 1999).

Ostriches are generally regarded as seasonal breeders, with breeding seasons coinciding with an increase in photoperiod (Hicks, 1992; Mellett, 1993; Horbaυczuk and Sales, 1999). Timing and duration of ostrich breeding seasons can also vary with latitude and altitude (Shanawany, 1994; Bertram, 1979; Sauer and Sauer, 1966a, 1969b). In southern Africa, ostrich breeding seasons are normally from June to January, with some seasons extending to the end of February (Jarvis *et al.*, 1985; Mellett, 1993). Deeming and Ar (1999), however, postulated that because ostriches occur in arid areas on both sides of the equator that their laying season is opportunistic rather than determined by daylength, they are good candidates for domestication and all-year round laying.

The cost-effectiveness of a commercial ostrich enterprise is hampered by the low number of eggs produced and chicks hatched per female, and chick mortalities (Van Zyl, 2001). When compared to commercial poultry production systems, ostrich production systems are characterised by a low reproductive performance and large variation in egg and chick production, and consequently

slaughter bird production. Commercial ostrich producers need to manage their commercial breeding flocks optimally to ensure the cost-effective production of slaughter birds. The present study had a dual purpose, firstly to determine the influence of onset of breeding on the reproduction performance of ostriches over a breeding season, and secondly to determine the potential of rest periods during the breeding season to reinitiate reproduction in ostrich males and females. The ability to manipulate the reproductive cycles of breeding ostriches, i.e. onset and cessation of breeding, as well as the reinitiation of reproductive activities, may enable the commercial ostrich farmer to manage his or her breeding flock to ensure the timely production of slaughter birds according to market demand.

MATERIALS AND METHODS

Experimental animals

An ostrich breeding flock maintained at the Little Karoo Agricultural Development Centre outside Oudtshoorn, South Africa, were used for the study. The management of the breeding flock is documented by Van Schalkwyk *et al.* (1996) and Bunter and Graser (2000). The ages of the males and females used in the study varied between 2 and 12 years.

Experimental procedure

Influence of season

Continuous breeding (CS): At the end of the 2001/2002 breeding season, 24 randomly selected breeding pairs were treated for external and internal parasites, vaccinated for the Newcastle Disease virus, and returned to their breeding camps. These breeding pairs were not subjected to either a pre-season rest period, or flushing and teasing management practices (Materials and Methods, Chapter 2). The breeding pairs were paired on 1 March 2002 and separated on 8 April 2003.

Early season (ES): Fifty-one randomly selected breeding pairs were subjected to a 3-month rest period, as well as flushing and teasing management practices, before being paired for a 10-month breeding season. The breeding pairs were joined on 2 May 2002 and separated on 19 February 2003.

Late breeding season (LS): Fifty-three randomly selected breeding pairs were subjected to a 5-month rest period, as well as flushing and teasing management practices, before being paired for a 10-month breeding season. The breeding pairs were joined on 1 July 2002 and separated on 8 April 2003.

Influence of forced rest periods

Twenty five breeding pairs of the ES group, and 26 breeding pairs of the LS group, respectively, were subjected to a series of forced rest periods throughout their respective breeding seasons. The time table for the separation and re-joining of the breeding pairs is set out in Table 1.

Table 1. Time table of the forced breeding rest periods implemented during the 2002/2003 breeding season.

	Early breeding season	**Late breeding season**
Number of breeding pairs	25	26
First forced rest period		
Start	10 July 2002	16 September 2002
End	21 August 2002	21 October 2002
Second forced rest period		
Start	30 October 2002	20 December 2002
End	11 December 2002	28 January 2003

The management of the breeding pairs during the rest periods consisted of the ostrich males and females being visually isolated during the first 4 weeks, and the males and females being fed a standard maintenance diet. Two weeks before being joined again with their respective breeding companions, the breeding pairs were subjected to standard flushing and teasing management practices (Materials and Methods, Chapter 2).

Data recorded

Data recorded for all breeding pairs included:

- *Time to first lay* - defined as the number of days between the pairing of a breeding pair and the production of the first egg; recorded for each production period.
- *Number of clutches* - defined as groups of eggs laid, where each set of eggs comprises of eggs laid within four days of each other; calculated for each production period
- *Daily egg production*
- *Number of chicks* - defined as number of chicks hatched from eggs set
- *Egg production performance* (EPP) – to standardize for the difference in the number of production days, the total number of eggs produced was expressed as a percentage by using the following equation (Van Schalkwyk *et al.*, 1996):

  EPP = [total number of eggs produced/(0.5 X number of production days)] X 100
- *Fertility* – fertility of eggs produced by the companion female was regarded as the fertility of the breeding pair; expressed as a percentage and calculated by using the following equation:

  Fertility = [(number of eggs set - number of infertile eggs) / number of eggs set] X 100
- *Male territorial aggression* - territorial aggression exhibited by the ostrich males was evaluated on a monthly basis (Materials and Methods, Chapter 5).

Statistical analysis

The fixed effects in the analyses on the egg production performance (EPP) of the ostrich females over the entire breeding season included treatment and female age. Least squares procedures were used to account for uneven subclasses (Harvey, 1990). The interaction of treatment with female age was included in the analyses, but was found to be insignificant. The results pertaining to female age were consistent with literature reports (Bunter, 2002; Cloete *et al.*, 1998), and are thus not reported. Selected linear contrasts were estimated between treatments to assist in the interpretation of the results.

The analyses of monthly EPP, as well as male aggression and shin colour were complicated by the fact that the same animals were sampled repeatedly. These observations could thus not be considered as independent, as required by analysis of variance. Random animal effects were computed to account for the covariance introduced by the repeated sampling of the same animal. ASREML, computer software that is capable of the computation of various random effects in animals breeding, while also predicting least squares estimates of selected treatment means, was used for this purpose (Gilmour *et al.*, 1999). The fixed effects included in the analyses were treatment, female age and month of production. Owing to the fact that typical seasonal patterns were expected in monthly production means, these trends were modeled using cubic splines (Verbyla *et al.*, 1999). The splines consisted of a fixed linear component, as well as random deviations from linearity conforming to a smooth trend. Random deviations from linearity not following a smooth trend were also included in initial runs, but were found to be insignificant, as judged by the likelihood ratio test. These trends were interacted with treatment and female age where applicable.

Various measures of performance were assessed in the two treatment groups that were subjected to three forced rest periods during the breeding season. Since these analyses were also based on the same animals being sampled repeatedly, the same basic analyses used for the monthly data were applied. Fixed effects in this case included the timing of the commencement of breeding (early or late), the production period involved (first, second or third) and female age. Interaction between these main effects were computed where significant ($P \leq 0.05$). Between female and residual variance ratios were obtained from the data sets subjected to the latter two sets of analyses. These estimates were used to obtain variance ratios depicting the within season repeatability of the respective female traits considered (Turner and Young, 1969).

RESULTS

The time when the breeding pairs were joined for breeding had a significant influence on almost all of the reproduction traits. The time from being joined to the first laying was significantly ($P \leq 0.01$) shorter for the breeding pairs paired for a late breeding season (LS), compared to the breeding pairs joined for an early breeding season (ES), and with a continuous breeding season (CS) (Table

2). When the ES females were compared to the CS females, the onset of production was not stimulated (P=0.26) by the time when the breeding pairs were paired for breeding (Table 2). The number of clutches was also not influenced by the time when the breeding pairs were paired for breeding. However, the LS females produced more eggs per clutch (P≤0.05) than either the ES or CS females. The ES and CS females, on the other hand, produced clutches of similar sizes (Table 2).

When egg and consequent chick production are considered, the CS breeding pairs performed worse (P≤0.01) than the ES and LS breeding pairs that were subjected to a pre-season rest period (Table 2). The CS breeding pairs also had lower (P≤0.01) egg production performances (EPP's) than the breeding pairs subjected to, and not subjected to forced breeding rest periods, respectively (Table 2). Chick production was similarly affected. Average egg weight and average day-old chick weight was not influenced (P≥0.15) by the onset of breeding or the forced breeding rest periods (Table 2). The fertility of eggs produced by the CS breeding pairs was significantly (P≤0.05) lower than that of breeding pairs subjected to a pre-season rest (Table 2). The fertility of the ES breeding pairs were improved (P≤0.01) to a higher degree, when compared to the CS breeding pairs. The fertility of the LS breeding pairs tended (P≤0.10) to be higher than that of the CS breeding pairs. The fertility of the breeding pairs not subjected to forced breeding rest periods was significantly higher than that of the CS (P≤0.01) and rested (P≤0.05) breeding pairs.

Table 2. Reproduction and egg traits (means ± SE) of ostrich breeding pairs with a continuous breeding season (CS), and breeding pairs paired before (early) or after (late) the winter solstice, and subjected to forced breeding rest periods.

	TREATMENT				
	Continuous	Early breeding season		Late breeding season	
TRAIT	season	Not rested	Rested	Not rested	Rested
No. of breeding pairs	24	26	25	27	26
Days to first egg	85 ± 11	60 ± 12	79 ± 12	24 ± 13	20 ± 13
Number of clutches	5.7 ± 0.8	5.9 ± 0.8	5.2 ± 0.8	5.5 ± 0.9	5.7 ± 0.9
Eggs/clutch	6.6 ± 1.3[a]	7.32 ± 1.4[a]	7.8 ± 1.3[a]	9.8 ± 1.5[b]	9.9 ± 1.5[b]
EPP (%)	16.9 ± 3.6	30.2 ± 4.0	33.5 ± 3 8	34.2 ± 4.4	45.7 ± 4.3
Chick production	6.6 ± 3.0	17.9 ± 3.3	18.8 ± 3.1	20.0 ± 3.6	23.0 ± 3.6
No. of observations	20	25	22	27	26
Egg weight (g)	1392 ± 39	1430 ± 27	1428 ± 32	1372 ± 29	1446 ± 29
Water loss (%)	12.0 ± 0.9	11.7 ± 0.6	12.0 ± 0.8	12.1 ± 0.7	11.4 ± 0.7
Chick weight (g)	852 ± 27	881 ± 19	869 ± 22	843 ± 20	893 ± 20
Fertility (%)	66.3 ± 4.4[a]	86.9 ± 5.0[b, c, d]	78.0 ± 4.6[b, c, e]	84.1 ± 5.3[b, d]	69.2 ± 5.3[b, e]

a, b and **d, e**: Rows with different superscripts differ significantly (P≤0.05)

a, c: Rows with different superscripts differ significantly (P≤0.01)

When the average monthly EPP's of the treatment groups are considered, it is evident that treatment interacted with the linear and non-linear components of the spline for month (Figure 1). The breeding pairs subjected to a continuous breeding season performed on average worse than

the ES and LS breeding pairs. The EPP of CS breeding pairs oscillated between 16.4% and 31.8% for the first 8 months of the breeding season, before declining to below 10%. Joining breeding pairs two months later had to effect that the EPP of ES females not rested, increased from 16.8% to 39.0% in the month after being joined. The EPP of the ES, not rested group, continued to be higher than that of the CS group, and ranged between 42.4% and 48.9% in the following 4 months, declining to 2.4% at the end of their 10-month breeding season. The LS breeding pairs had an average higher EPP of 45.9% in the month of joining, which increased to between 52.6% and 60.5% for the following 3 months of their breeding season. Although their EPP declined in the following month, it continued to be higher than that of the CS and ES females during the 11th month of the study (Figure 1).

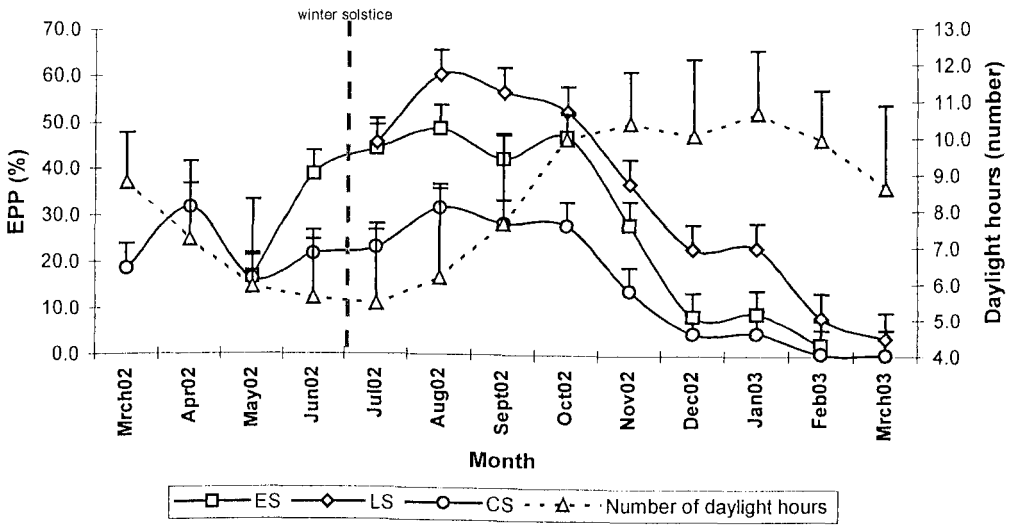

Figure 1. The influence of timing of breeding on the egg production performance (EPP) of ostrich breeding pairs with a continuous breeding season (CS), and breeding pairs joined before (ES) and after (LS) the winter solstice.

The response curves for the ES and LS females subjected to forced rest periods were initially similar to ES and LS females not rested (Figure 2). The forced rest periods had a stimulatory influence on the EPP of the ES and LS females, for their EPP was elevated to levels generally exceeding those of the corresponding groups of females not subjected to resting. For example, the average EPP of rested ES females 1 month after the first rest period was 61.4%, compared to 42.4% of the non-rested ES females. However, the relative advantage of the time when the breeding pairs were joined for breeding, and forced breeding rest periods, disappeared almost at the end of the study period (Figure 2). The EPP of the rested ES females was on average 33.8%, compared to 26.4% of the rested LS females.

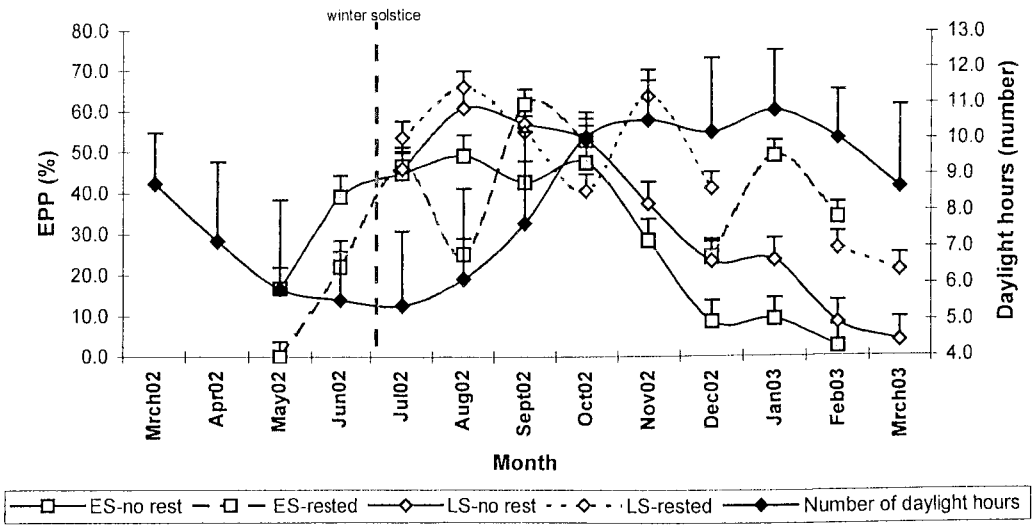

Figure 2. The influence of forced breeding rest periods on the monthly egg production performance (EPP) of ostrich breeding pairs joined before (ES) and after (LS) the winter solstice.

The benefit of a pre-season rest on the reproductive behaviour of ostrich males became evident during the later half of the breeding season, when the CS males exhibited territorial aggression to a lesser extent than the males with a pre-season rest period. The males subjected to forced breeding rest periods tended ($P \leq 0.10$) to exhibit lower levels of territorial aggression than their non-rested contemporaries (Figure 3).

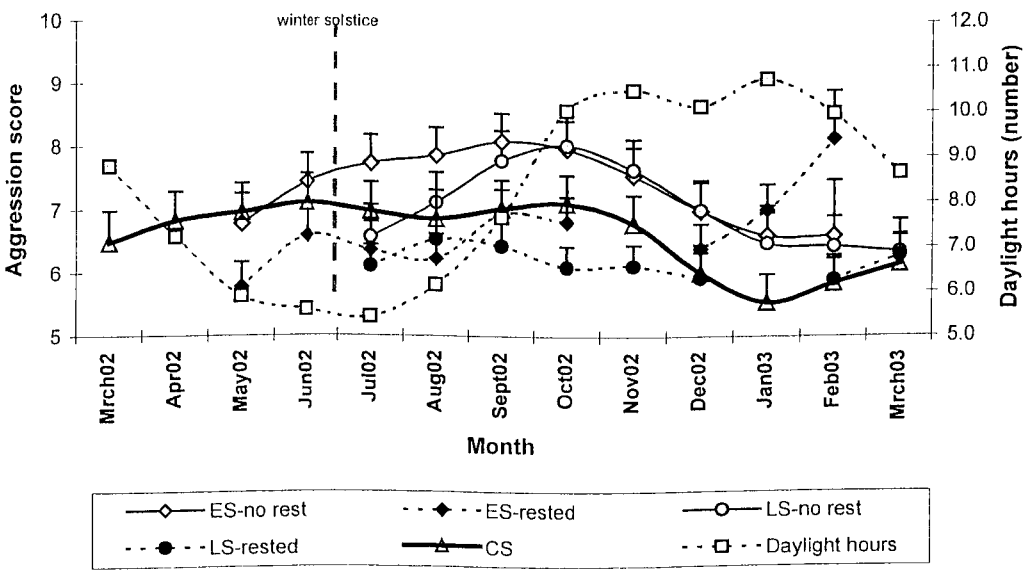

Figure 3. The influence of season and forced breeding rest periods on the territorial aggression of ostrich males with a continuous breeding season (CS), and ostrich males joined before (ES) and after (LS) the winter solstice.

The time when the breeding pairs were joined for breeding generally interacted with the first, second and third productive periods following the forced break in production of the breeding pairs (Table 3). The interval between pairing and the first laying of the breeding pairs paired after the winter solstice were shorter ($P \leq 0.01$) than that of the breeding pairs paired before the winter solstice. The same effect was observed after the second forced rest period. The reproductive behaviour of the ES males and females, however, tended to be more synchronised after the second forced rest, with the number of days from being joined till the first laying being shorter than that reported for the LS males and females. The number of clutches produced, as well as eggs produced per clutch, followed roughly the same trend (Table 3).

The EPP and consequent chick production of the LS females was, respectively 4.7 and 5.1 higher ($P \leq 0.01$) than that of the ES females during the first production period (Table 3). The beneficial influence of joining the breeding pairs after the winter solstice seemed to disappear as the season progressed, for no significant differences were found in terms of the egg and chick production of the ES and LS breeding pairs during the second production period (Table 3). A second forced breeding rest had a significant ($P \leq 0.01$) influence on the reproductive performance of the ES females, for the EPP of the ES females was almost double that of the LS females (Table 3). Chick production of the ES breeding pairs was similarly affected, and was 3.5 times higher than that reported for the LS breeding pairs.

Table 3. The influence of timing of breeding and forced breeding rests on the reproduction traits (means ± SE) of ostrich breeding pairs during the 2002/2003 breeding season.

TRAIT AND TREATMENT	PRODUCTION PERIOD		
	First	Second	Third
Days to laying			
Early breeding season	$73.7 \pm 4.1^{a, 2}$	15.5 ± 4.5^{1}	18.0 ± 4.7^{1}
Late breeding season	$16.4 \pm 4.3^{b, 1, 2}$	12.5 ± 4.3^{1}	25.3 ± 5.0^{2}
Number of clutches			
Early breeding season	$1.2 \pm 0.3^{a, 1}$	2.2 ± 0.3^{2}	$1.9 \pm 0.3^{1, 2}$
Late breeding season	$2.5 \pm 0.3^{b, 2}$	$1.9 \pm 0.4^{1, 2}$	1.4 ± 0.3^{1}
Eggs/clutch			
Early breeding season	$3.2 \pm 1.6^{a, 1}$	9.7 ± 1.6^{2}	$7.7 \pm 1.6^{a, 2}$
Late breeding season	$12.2 \pm 1.6^{b, 2}$	8.8 ± 1.7^{2}	$3.6 \pm 1.7^{b, 1}$
EPP (%)			
Early breeding season	$13.7 \pm 5.0^{a, 1}$	51.6 ± 5.0^{3}	$39.3 \pm 5.0^{a, 2}$
Late breeding season	$64.1 \pm 5.0^{b, 3}$	49.8 ± 5.2^{2}	$20.1 \pm 5.2^{b, 1}$
Fertility (%)			
Early breeding season	85.2 ± 5.0	85.9 ± 4.6	89.4 ± 4.8
Late breeding season	72.3 ± 4.6	76.5 ± 4.6	77.2 ± 5.0
Chick production			
Early breeding season	$5.8 \pm 4.3^{a, 1}$	30.2 ± 4.3^{2}	$26.4 \pm 4.3^{a, 2}$
Late breeding season	$29.3 \pm 4.4^{b, 2}$	24.8 ± 4.4^{2}	$7.6 \pm 4.4^{b, 1}$

$^{a, b}$: values in columns differ significantly ($P \leq 0.01$)

^{1,2,3}: values in rows differ significantly ($P \leq 0.01$)

The repeatabilities of intra-season EPP and chick production were low to moderate, i.e. 0.20 and 0.41. The repeatability of the number of days from joining to the first oviposition, number of clutches and clutch size was weakly repeatable at 0.11, 0.24, and 0.10, respectively. Fertility and male aggression were highly repeatable, 0.41 for fertility and 0.56 for male aggression, respectively.

DISCUSSION

The influence of time of season when joined for breeding

A delicate balance exists between a variety of environmental factors that protect and regulate the gametogenetic activity in birds (Lofts, 1975). Only after the reproductive system has been activated by photoperiod can it be sensitive to additional stimuli such as food availability and rainfall that then exert an influence on the hypothalamo-pituitary-gonad axis (Wingfield, 1983; Wingfield *et al.*, 1992). An increase in photoperiod leads to a so-called neuro-endocrine cascade that is initiated by the secretion of the gonadotrophic releasing hormones from the hypothalamus (Murton, 1975; Hau, 2001). This in turn stimulates the secretion of the pituitary hormones such as FSH and LH, which in turn stimulates gonadal growth and steroid hormone production (Ball, 1993).

The extent to which the production traits of ostriches responded clearly illustrates their photoperiod-dependent breeding strategy. This differs from the general breeding strategy of birds in arid or desert environments, where breeding tends be dissociated from photoperiodic control, and breeding may be more opportunistic (Immelman, 1972; Lofts, 1975; Vleck, 1993) in the present study. Peak production of ostriches in the study occurred between the winter and summer solstice, with lower production observed between April and June. This was supported by the findings of Jarvis *et al.* (1985), but not that of Leuthold (1977). Jarvis *et al.* (1985) reported that for Zimbabwean ostriches, peak production occurred between July and end December-mid January. Leuthold (1977) speculated that ostriches in the Tsavo National Park in Kenya were more opportunistic in their breeding strategy, with rainfall playing a role in triggering reproductive activities. According to Vleck (1993), breeding schedules of birds in arid areas do not operate uniformly over all regions, and may differ in breeding periodicity.

In the present study, onset of reproductive activity was significantly influenced by the time of year when the birds were paired for breeding. Pairing the birds after the winter solstice, i.e. after daylength starts to increase, had a beneficial influence on almost all the reproductive traits considered. The reproductive cycles of the ostrich males and females joined after the winter solstice were synchronized to a greater degree, with time to first laying being significantly shorter. Clutch size was also larger for birds paired after the winter solstice. The number of clutches produced, however, were unaffected by the time when the birds were paired for breeding.

The EPP and consequent chick production were significantly higher for breeding pairs joined after the winter solstice. This supports various studies that reported on the photoperiodic nature of ostrich reproduction (Smith *et al.*, 1995; Bertram, 1979; Sauer and Sauer, 1996a, 1966b). The earlier onset to egg production and the higher EPP of the birds paired after the winter solstice must have resulted from the gonads that were fully matured because the breeding ostriches were in a fully photosensitized state when they were paired for breeding. This is in contrast to the breeding pairs joined before the winter solstice that were still presumably in a photorefractory state, and daylight length was too short to allow birds to react to the stimulating influence of the long daylengths, and thus allow the gonads to undergo recrudescence. The incubation parameters, i.e. egg weight and day-old chick weight, were not influenced by the timing of pairing.

Short days, i.e. less than twelve hours of daylight can terminate the photorefractory state in birds and lead to the restoration of the photosensitive state (Wilson, 1992). This was clearly observed in this study for the birds paired before the winter solstice. The gradual increase in photoperiod had the effect that birds pairs paired off before the winter solstice gradually recovered from being photorefractory, with egg production gradually increasing after being paired for breeding. The decrease in egg production before the summer solstice is a result of the breeding pairs becoming less photosensitive and entering a photorefractory state, even though photoperiod was still sufficiently long enough to support production. In brahminy myna (*Sturnus pagodarum*), it appears that birds do not enter a refractory state unless full gonadal maturation is achieved and that photorefractoriness develops in advance of gonadal regression. However, it was unsure whether photorefractoriness was fixed by daylength before the gonads have developed fully or whether it is fixed by the available daylight at the end of the gonadal growth period, as in starlings (Kumar and Kumar, 1991).

The fertility of the breeding ostriches joined before the winter solstice, was significantly higher than breeding pairs with a continuous breeding season. The fertility of breeding pairs joined after the winter solstice, however, only tended to be higher than breeding pairs with a continuous breeding season.

The influence of a pre-season rest period

The beneficial influence of a pre-season rest period became evident when the reproductive performance of the breeding flock are considered. The CS males and females performed worse in terms of almost all reproductive traits. The CS breeding pairs had significantly lower EPP's than their ES and LS contemporaries. The physiological stress of the previous breeding season presumably depleted the reserves of the CS breeding pairs. The absence of a pre-season rest period, in which the ostrich males and females could restore their body reserves, possibly contributed to the lower EPP's observed. The EPP of the CS females oscillated between approximately 16 and 32% for the first two thirds of the breeding season, and declined and remained below 20% for the remainder of the season. The ES females performed better, with EPP

increasing from 16.4% to 39.0% in the first month after being joined. The LS females, however, outperformed the CS and ES females, with egg production 17.5% higher than that observed for the ES females during the first month after being paired for breeding. Although the EPP of the LS females declined as the season progressed, it was found to be higher than that of the CS and ES females.

The influence of forced breeding rest periods

The production curves of the ES and LS females subjected to forced breeding rest periods initially followed the same pattern as that of their non-rested contemporaries. When the production curves after each respective rest period are considered, however, it was found that the EPP of the rested breeding pairs generally exceeded that of their non-rested contemporaries. The advantage of the forced rest periods seemed to be almost absent at the end of the breeding period.

The breeding males not subjected to forced breeding rest periods had significantly higher fertilities than males without a pre-season rest and males subjected to breeding rests. Fertility of the males subjected to forced breeding rests however, were maintained or even improved, for the breeding males joined before the winter solstice had to fertilize the higher number of eggs produced by their breeding companions during the post-rest periods. This was not the case for the ostrich males paired after the winter solstice, where fertility was unaffected by, and the EPP of their companion females not improved by the forced breeding rests. The maintenance and even potential improvement in the fertility of the breeding birds paired for an early breeding season, is contradictory to findings of Van Schalkwyk (1991). In the latter study, egg production was significantly improved for breeding birds paired before the winter solstice. The fertility of the breeding pairs, however, was compromised in the latter study, because more infertile eggs were reported for the post-rest period.

The effectiveness of forced breeding rest periods and degrees to which the respective reproductive traits were affected, were influenced by the time of the breeding season when the forced breeding rest was implemented. A forced breeding rest period before the traditional quiescent period, i.e. from middle August to September, had a more pronounced effect on the reproductive traits than a forced breeding rest implemented later during the breeding season. This can be explained by the fact that daylight length was still sufficiently long to ensure that the breeding birds remained in a photosensitive state, and that the gonads have not yet started to regress. Forced rest periods had the effect that almost all of the reproduction traits of ES breeding pairs were improved in the second and third production periods. Reproductive traits that were further improved after the first forced rest, included days to nest, days to first egg, EPP, and number of clutches. Forced rest periods did not have a beneficial influence on the reproductive traits of the LS breeding pairs. The onset of a decrease in daylength had the effect that the LS males and females presumably entered a photorefractory state, and were becoming less sensitive to photoperiod that was still long enough to ensure and support reproduction.

The development of photorefractoriness (PR) is not an 'all-or-nothing' response. It can be considered as a complex interaction of age and previous experience, and may possibly be influenced by genotype (Siopes and Wilson, 1981). The incidence and onset of PR were found to be highly variable in first-year turkey hens. Turkey hens also differed in terms of becoming absolutely or relatively photorefractory (Siopes, 2001). The incidence of PR was influenced by age of female turkey breeders, with 100% of recycled hens expressing PR, and 89% of first year breeders expressing PR (Siopes, 2002). First and second year hens did not differ in terms to the time to onset of PR (Siopes, 2002). The onset of PR is influenced by season, with hens stimulated in winter, taking longer to becoming photorefractory, when compared to hens stimulated in spring (Siopes, 2002). Varying degrees of PR may be experienced throughout a breeding season, with incidence and severity increasing as a breeding season progresses (Siopes and Proudman, 2003). Relative PR is experienced early in a breeding season, and increases in incidence as well as severity as a breeding season progresses. At the end of a breeding season when the photoperiod is still long enough to support the reproductive system, birds become refractory, with a subsequent decrease in gonadal steroid secretion and an decreased sensitivity of the reproductive system to long day lengths (Nicholls *et al.*, 1988). It is thus possible the gonads of the LS males and females were less likely to respond to any changes in the steroid hormones that might have resulted from the potential stimulatory influence of the flushing and teasing management practices. The second forced rest, applied when photoperiod was progressively decreasing, had no significant influence on production, for the LS birds had already entered a relative to absolute refractory state, rendering them insensitive to any efforts to reinitiate reproduction. In turkey hens, longer intervals between and greater variation in LH surges were found late in the breeding season when compared to earlier in the season. This increase in intervals and variation in LH surges were also associated with lower egg production (Liu *et al.*, 2002). Baseline LH levels were found to be low at the end of a long laying period, and exposure to long day photoperiod (14h:10h) had the effect that overall and baseline LH concentrations were increased (Bacon and Long, 1996).

Light management is used in poultry species to stimulate reproduction and can be used to identify females of superior laying ability during the first cycle of lay (Lewis and Perry, 1995). The application of such lighting programs however, is impractical in the case of ostriches given their physical size as well as their specific behavioural requirements (Lambrechts *et al.*, 1998). Selecting for responsiveness in broiler males and females resulted in a positive influence on egg production in females, and selection for early sexual maturity has potential for positively affecting reproductive performance and photosensitivity of female broilers (Eitan and Soller, 2000). Selecting for ostrich males and females that comes into production earlier may result in improving the overall performance of a commercial breeding flock.

CONCLUSIONS

The study confirms the photoperiod-dependent breeding strategy of ostriches. Peak production occurred between July (winter in the southern hemisphere) and January (late summer in the southern hemisphere). Given the absence of reproductive performance-related selection in the ostrich industry, it is advisable that ostrich breeding flocks are joined after the winter solstice, when breeding birds have achieved full gonadal maturation. Subjecting breeding birds paired before the winter solstice to forced breeding rest periods, however, provided a means to overcome the influence of photoperiod on the reproductive activities of breeding ostriches. This may enable commercial ostrich farmers to manipulate the reproductive cycles of their breeding birds in order to ensure the timely production of slaughter ostriches. Forced breeding rest periods, however, were only effective in reinitiating reproductive activities during the time of season when ostriches are still photosensitive and have not yet entered a relative to absolute state of photorefractoriness.

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Chapter 4

The influence of stocking rate and male:female ratio on the reproductive performance of breeding ostriches (*Struthio camelus* spp.) under commercial farming conditions[1]

Abstract

The reproductive performance of breeding ostriches, as influenced by stocking rate and male:female (M:F) ratio, were investigated under intensive breeding conditions. The stocking rates for large flocks ranged from 114 to 210 birds per hectare, and the stocking rates for smaller flocks ranged between 9 and 13 birds in 0.13ha (1250 m^2) and 0.30ha (3000 m^2) camps, respectively. The different M:F ratios investigated were 1M:1F (pairs), 1M:2F (trios) and 1M:3F (quads), for breeding systems maintained in 0.06ha (625 m^2) camps. Total and average egg production, fertility, and hatchability were compromised when the stocking rate was increased in almost all the breeding systems. High stocking rates were detrimental to the reproductive performance and reproductive behaviour of the flocks. Increasing the number of females per male had no negative influence on the reproductive traits, with a significantly higher production observed for breeding quads. These findings indicated that ostrich breeding flocks can be maintained at stocking rates higher than those presently used on commercial ostrich farms. Ostrich breeding pairs, trios and quads can also be maintained on smaller areas, with acceptable production levels. Increasing the stocking rate of breeding flocks will potentially have a inhibitory effect on the establishment of territories and use of space by breeding ostriches, thereby impacting negatively on the reproductive behaviour of ostrich females and males in large flocks, respectively.

INTRODUCTION

Ostriches are the main farming interest in the Little Karoo region of South Africa, and used to occur in areas of the Little Karoo that are used mainly for agricultural purposes today. At present, almost 65% of the South African ostrich breeding population is found in the Little Karoo region (Van Zyl, 2001). On commercial ostrich farms where the entire or part of the production cycle, i.e. breeding birds, artificial incubation and raising of chicks and slaughter birds are represented, ostriches are concentrated on relatively small areas (South African Ostrich Business Chamber, 2003). The natural vegetation in the Little Karoo is of low nutritional quality and natural veld areas are most often used only as holding areas for breeding flocks, which are then fed commercial breeder diets. This practice differs from those employed for other livestock farming systems, e.g. sheep and beef cattle, where natural vegetation serve as the main food source.

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The maintenance of breeding ostriches in large flocks is a well-known practice in South Africa and Israel. Breeding flocks can range in size from 50 to 200 birds, respectively (Deeming and Bubier, 1999). Flocks are usually maintained in camps several hectares in size, with no clear guidelines on the optimal stocking rate for ostriches under free-range conditions. Male:female ratios (M:F) in South African breeding flocks are 5-6 males for every ten females, which is similar to the 1M:2F ratio in Israel (Deeming and Bubier, 1999). Guidelines of the South African Ostrich Business Chamber (2003) require a maximum camp size of 0.25 ha for breeding pairs or trios when birds are fed a breeding diet. In the case of larger breeding flocks, the minimum stocking rate is one breeding bird/10ha for an 8-month breeding season. Breeding pairs, trios and quads are used in breeding systems in Australia, with the breeding quads consisting of 2M:2F (More, 1997). In Poland, ostriches are bred as pairs, trios or small flocks and most farmers pay special attention to the space requirements of breeders to create optimum exercise conditions (Horbañczuk, 2002). According to the draft recommendations of the Standing Committee of the European Convention for the Protection of Animals Kept for Farming Purposes, the minimum space requirement for the maintenance of ratite breeding birds is 1200 m^2 per bird, with larger areas recommended to ensure well-being and proper exercise conditions (Horbañczuk, 2002). According to the Code of Conduct for animal welfare in New Zealand (Animal Welfare Advisory Committee, 1998), the stocking rate for ostrich breeding pairs in open conditions should not exceed 20 birds per ha for breeding flocks. The minimum camp size required for breeding pairs and trios is 0.06 ha and 0.20 ha, respectively. No status in terms of percentage vegetation cover was mentioned in this document.

The intensive nature of ostrich farming presents both the commercial farmer and the conservationist with a challenge to ensure that conservation of natural resources can co-exist together with commercial ostrich farming. Scientific research on the production of ostriches in large commercial breeding systems are scarce, and this complicates the development of best practice techniques to enable producers to farm as cost-effectively as possible (Adams and Revell, 2003). Most of the available literature on the egg production performance of ostriches under intensive farming and wild conditions are compiled for breeding pairs, breeding trios, and small flocks (Hurxthal, 1979; Jarvis *et al.*, 1985; Foggin and Van Niekerk, 1995; Hicks-Aldredge, 1996; More, 1997; Deeming and Bubier, 1999; Bunter and Graser, 2000; Lambrechts *et al.*, 2002). This study presents results on the reproductive performance of a large commercial breeding population, and the influence of different stocking rates and male:female ratio on reproductive traits.

MATERIALS AND METHODS

Experimental animals

Production data was obtained from an ostrich breeding flock of 4500 birds during two consecutive breeding seasons. The breeding flock were maintained as a single breeding operation near

Ladismith, in the Western Cape Province of South Africa. The breeding flock consisted of birds maintained continuously on the farm, and breeding birds translocated from another commercial breeding farm in the North-Western Province of South Africa. The North-Western birds were translocated to the Ladismith breeding farm approximately two months before the onset of the 2000/2001 breeding season.

Experimental design

The different stocking rates and M:F ratios studied during the two consecutive breeding seasons are presented in Table 1.

Table 1. Stocking rates and male:female (M:F) ratios implemented in the breeding program of a commercial ostrich breeding operation maintained near Ladismith in the Western Cape, South Africa.

Stocking rate (breeding system)	M:F ratio	Camp size (ha)	Camp size (m^2)	Breeding season studied
Breeding pair	1:1	0.06 and 0.13	625 and 1250	2000/2001 & 2001/2002
Breeding trio	1:2	0.06 and 0.13	625 and 1250	2000/2001 & 2001/2002
Breeding quad	1:3	0.06	625	2000/2001
9-bird flock	1:2	0.13 and 0.30	1250 and 3000	2000/2001 & 2001/2002
13-bird flock	1:2.25	0.35	3500	2000/2001
114 bird flock	1:2	1	10 000	2001/2002
120-bird flock	1:2	1	10 000	2000/2001
130-bird flock	1:2	1	10 000	2001/2002
141-bird flock	1:2	1	10 000	2001/2002
150-bird flock	1:2	1	10 000	2000/2001
210-bird flock	1:2	1	10 000	2000/2001

During the 2000/2001 breeding season, the breeding birds maintained continuously on the Ladismith farm were allocated to only large breeding units, and the imported breeding birds were allocated to both large and small breeding units. The breeding birds were joined in July 2000 for a 7-month breeding season. At the end of August 2000, 40 females and 20 males were removed from each of four 210-bird flocks to establish two 120-bird and four 150-bird flocks. As a result of the shorter breeding season for the 120-bird and 150-bird flocks, only reproductive data for the remaining 5 months of the 2000/2001 breeding season are presented.

Based on the results of the 210-bird flocks during the 2000/2001 breeding season, three breeding systems, i.e. 114-bird, 130-bird, and 141-bird flocks, were established. The breeding quads and 210-bird flocks did not form part of the experimental groups in 2001/2002 breeding season. Due to the allocation of a larger number of birds to large breeding flocks (results not presented here) that were maintained in approximately 40-70ha camps, only 114 and 141 birds were allocated to the 120-bird and 150-bird breeding systems, respectively. The breeding birds were joined in July 2001 and results presented here represent data recorded for the entire 7-month breeding period.

During both breeding seasons, the breeding birds received a complete balanced breeder diet at 2.5kg/bird.day and had free access to fresh, clean water. Eggs were collected daily and identified by means of camp number and date of production. Production was recorded individually for each breeding system. After collection, the eggs were cleaned by dry wiping and disinfected by fumigation (80g potassium permanganate in 130ml 40% formaldehyde solution per m^3 for 20min; Smith *et al.*, 1995). All eggs not suitable for incubation were noted for each breeding system and removed from storage. Reasons for rejection included broken/cracked eggshells, chalky eggshells and loose air cells (i.e. where the air cell was damaged during transport to the incubation facilities).

All eggs were stored in an upright position with the air cell uppermost for a maximum of 10 days at 17-18 °C and 75% relative humidity, and were turned once daily through an angle of 45°. The eggs were artificially incubated in Buckeye® electronic incubators at 36°C and 28% relative humidity for a period of 38 days before being transferred to the hatchers for the remainder of the incubation period. Eggs were candled on day 14 to establish early embryonic deaths and fertility, and on day 38 to establish late embryonic deaths. After hatch, chicks were kept in the hatcher for a period of 24h to allow sufficient time for the navel to close, before being dispatched to contract growers. All eggs and reasons for not hatching during incubation were noted throughout the study.

Definitions for reproduction traits are as follows:

- *Total egg production* = total number of eggs produced per breeding system during the production period
- *Chicks (number)* = number of day-old chicks hatched
- *Average EP/female* = average number of eggs produced per female during the production period
- *Fertility (%)* = [(Total eggs set – number of infertile eggs)/total eggs set] x 100
- *Hatchability (%)* = (Number of chicks hatched/Total number of eggs incubated) x 100

Statistical analysis

The influence of stocking rate and male:female ratio were assessed by one-way analysis of variance procedures. In cases where trends were expected and where degrees of freedom for treatments exceeded one, the degrees of freedom for the number of birds were partitioned in orthogonal polynomials depicting linear tendencies. The LSMLMW computer program (Harvey, 1990) was used for this purpose. When more than two means were compared, least significant differences were computed, provided that the comparisons were protected by a significant value in the ANOVA table (Snedecor and Cochran, 1967)). When only two means were compared, differences between means were tested for significance by using the F-test derived from the ANOVA table (Snedecor and Cochran, 1967).

RESULTS

The 210-bird flocks had on average a higher total egg production during the 2000/2001 breeding season, when compared to the 150-bird and 120-bird flocks (Table 2). Although the F-value for treatment (flock size) in the analysis of variance table did not reach statistical significance (P=0.13), there was a suggestion of a linear increase (P≤0.05) in total egg production per camp with an increase in colony size. This increase in egg production amounted to 11.9±5.4 eggs per female, and accounted for 99.6% of the variation associated with treatments. The same tendency was observed for the number of chicks hatched, with an increase of 3.11±1.86 chicks hatched per breeding bird (P≥0.12). However, fertility and hatchability declined significantly P≤0.05) with an increase in stocking rate. The linear regression coefficient for fertility amounted to -0.06±0.02% per bird (R^2=0.99), and for hatchability to -0.11±0.03% per bird (R^2=0.96). Average egg production was independent of stocking rate.

Table 2. Influence of stocking rate on the reproductive traits (means ± SE) of ostriches maintained in 1 ha camps during the 2000/2001 breeding season.

PARAMETER	SIZE OF BREEDING FLOCK (1M:2F)		
	120 birds	**150 birds**	**210 birds**
Number of breeding systems	2	4	8
Total EP	1748 ± 515	2181 ± 364	2859 ± 257
Number of chicks hatched	515 ± 176	707 ± 124	844 ± 88
Average EP/female	20.0 ±4.1	22.9 ± 2.9	20.9 ± 2.0
Fertility (%)	85.8 ± 1.9[a]	83.2 ± 1.4[a]	79.9 ± 0.9[b]
Hatchability (%)	70.1 ± 2.6[a]	64.6 ± 1.9[b]	59.4 ± 1.3[c]

[a, b, c:] Columns with different superscripts differ significantly (P≤0.05)

During the 2001/2002 breeding season, egg production was compromised with an increase in stocking rate in the 1 ha camps. Total egg production for the 141-bird flock declined significantly (P≤0.01) by 21.3% and 20.2%, when compared to the 130-bird and 114-bird flocks, respectively. Chick production was similarly affected, and declined by 21.2% and 22.0%, when compared to the 130-bird and 114-bird flocks, respectively (Table 3). The average egg production declined significantly (P≤0.01) by 0.44 ± 0.01 eggs per bird as flock size increased (R^2=0.94). Fertility and hatchability did not differ between the respective treatments (P=0.13 and P=0.29, respectively). However, when the degrees of freedom were partitioned in orthogonal polynomials, a linear regression coefficient amounting to –0.15 ± 0.07% per breeding bird were observed for fertility (P≤0.05; R^2=0.99). Hatchability similarly tended to decline (-0.09 ± 0.05%, P=0.10; R^2=0.99) with an increase in stocking rate.

Table 3. Influence of stocking rate on the reproduction traits (means ± SE) of breeding ostriches maintained in 1 ha camps during the 2001/2002 breeding season.

PARAMETER	SIZE OF BREEDING FLOCK		
	114 birds (38M:76F)	**130 birds** (43M:87F)	**141 birds** (47M:94F)
Number of breeding systems	8	4	8
Total EP	2793 ± 121[a]	2834 ± 171[a]	2230 ± 121[b]
Number of chicks hatched	1938 ± 87[a]	1917 ± 123[a]	1510 ± 87[b]
Average EP/female	34.3 ± 1.4[a]	30.5 ± 1.9[a]	22.1 ± 1.4[b]
Fertility (%)	83.6 ± 1.3	81.2 ± 1.9	79.6 ± 1.3
Hatchability (%)	78.4 ± 1.0	77.3 ± 1.5	76.0 ± 1.0

[a, b]: Columns with different superscripts differ significantly (P≤0.05)

Under a constant male to female ratio of 1:2, fertility was improved by 9.5% for the 9-bird flocks maintained in the 0.13 ha camps, when compared to the 9-bird flocks maintained in 0.30ha camps (Table 4). A similar tendency was observed for hatchability, which was 6.0% higher for the 9-bird flocks maintained in the smaller 0.13ha camps. Nine-bird flocks maintained in 0.13ha camps produced on average 23.5 more chicks (P≤0.05) than the 9-bird flocks maintained in the larger 0.30 ha camps.

Table 4. Reproduction traits (means ± SE) for 9-bird breeding flocks (1M:1F) maintained in 0.13 ha and 0.30 ha camps during the 2001/2002 breeding season.

PARAMETER	CAMP SIZE	
	0.13 ha	**0.30 ha**
Number of breeding systems	50	50
Total EP	256 ± 11[a]	233 ± 11[b]
Number of chicks hatched	182.6 ± 8.4[a]	147.8 ± 8.4[b]
Average EP/female	34.1 ± 1.4[a]	29.7 ± 1.4[b]
Fertility (%)	81.6 ± 1.4[a]	74.5 ± 1.4[b]
Hatchability (%)	75.1 ± 1.0[a]	70.6 ± 1.0[b]

[a, b]: Columns with different superscripts differ significantly (P≤0.05)

Average egg production per female was 68% higher (P≤0.01) for breeding trios, when compared to the 9-bird flocks (Table 5). However, fertility and hatchability of eggs produced by 9-birds flocks was significantly improved by 19.6% and 28.9%, respectively (Table 5). When the average egg production per female are compared on a per trio basis, i.e. the 9-bird flock can be considered equivalent to two breeding trios, the 9-bird flocks produced on average 15.8% more eggs on a per trio basis than did the breeding trios.

Table 5. Influence of stocking rate on the reproductive traits (means ± SE) of breeding ostriches maintained in 0.13 ha camps during the 2001/2002 breeding season.

PARAMETER	FLOCK SIZE (M:F)	
	Trios (1M:2F)	**9-bird colony** (3M:6F)
Number of breeding systems	83	50
Total EP	114.7 ± 5.8[a]	255.6 ± 7.4[b]
Number of chicks hatched	67.7 ± 4.6[a]	182.6 ± 5.9[b]
Average EP/female	57.4 ± 1.8[a]	34.1 ± 2.4[b]
Fertility (%)	68.2 ± 1.3[a]	81.6 ± 1.7[b]
Hatchability (%)	58.2 ± 1.3[a]	75.1 ± 1.6[b]

[a, b:] Columns with different superscripts denote significance (P≤0.05)

As can be expected, total egg and chick production increased linearly (P≤0.01) with an increase in the number of females per breeding system (Table 6). Average egg production per female, and fertility of eggs produced, however, did not differ between the different treatments. Hatchability was significantly higher (P≤0.05) for eggs produced by the breeding quads (Table 6).

Table 6. Influence of male:female ratio on the reproduction traits (mean ± SE) of breeding ostriches maintained in 0.06ha breeding camps during the 2000/2001 breeding season.

PARAMETER	BREEDING SYSTEM (M:F)		
	Pairs (1M:1F)	**Trios** (1M:2F)	**Quads** (1M:3F)
Number of breeding systems	50	125	8
Total EP	25.9 ± 2.3[a]	52.1 ± 1.4[b]	92.6 ± 5.7[c]
Number of chicks hatched	21.9 ± 2.0[a]	42.6 ± 1.3[b]	80.5 ± 5.0[c]
Average EP/female	21.6 ± 1.1	21.7 ± 0.7	25.8 ± 2.7
Fertility (%)	74.3 ± 2.1	77.6 ± 1.3	83.8 ± 5.2
Hatchability (%)	70.0 ± 2.2[a]	73.5 ± 1.4[b]	81.1 ± 5.4[c]

[a, b, c:] Columns with different superscripts differ significantly (P≤0.05)

Although maintained in slightly larger camps (0.30 vs. 0.35 ha) and at a higher stocking rate, the 13-bird flocks performed on average better in terms of all reproduction traits, when compared to the 9-bird flocks (Table 7). Total egg production was significantly (P≤0.01) increased for the 13-bird flocks. A similar tendency (P≤0.007) was observed for average egg production per female. Thirteen-bird flocks produced on average 233.7 ± 8.6 eggs in total and 21.7 ± 0.9 eggs/female, compared to 129.7 ± 8.6 eggs in total and 18.0 ± 0.9 eggs/female for the 9-bird flocks. Fertility and hatchability was also improved (P≤0.01) in the 13-bird flocks.

Table 7. Reproduction traits (means ± SE) recorded for 9-bird and 13-bird breeding flocks maintained in 0.30 and 0.35 ha camps, respectively, during the 2000/2001 breeding season.

PARAMETER	FLOCK SIZE (M:F)	
	9-birds (1M:2F)	**13-birds** (1M:2.25F)
Number of breeding systems	25	25
Total EP	129.7 ± 8.6[a]	233.7 ± 8.6[b]
Number of chicks hatched	107.9 ± 7.4[a]	198.3 ± 7.4[b]
Average EP/female	18.0 ± 0.9[a]	21.6 ± 0.9[b]
Fertility (%)	84.9 ± 1.3[a]	90.3 ± 1.3[b]
Hatchability (%)	79.4 ± 1.4[a]	86.1 ± 1.4[b]

[a, b]: Columns with different superscripts differ significantly (P≤0.01)

DISCUSSION

The influence of stocking rate

During the 2000/2001 breeding season, egg production was higher for the 210-bird per hectare stocking rate, with egg production increasing linearly as the stocking rate was increased. There also was a tendency for the number of chicks hatched per bird to be increased as stocking rate was increased. Fertility and hatchability, however, decreased as stocking rate increased. The respective stocking rates did not influence average egg production. During the 2001/2002 breeding season, however, egg production was compromised when stocking rate was increased. Total egg production decreased in the 130-bird and 141-bird flocks, when compared to the 114-bird flocks maintained in the 1 ha camps. Average egg production decreased by 0.44±0.007 eggs per bird as stocking rate was increased. Fertility was lower and hatchability tended to decrease for the higher stocking rates.

The contradictory results obtained during the two breeding seasons can be explained by the translocation of a proportion of the breeding population to a new breeding environment two months before the onset of breeding during 2000/2001 breeding season. These newly imported birds were allocated to a number of the 210-bird flocks. These breeding birds may not have had sufficient time to adapt to their new environment. Translocation to a new and unfamiliar environment results in disorientation and the consequent manifestation of abnormal behavioural patterns (Lambrechts *et al.*, 1998b). Results from this study suggest that the respective flocks were still acclimatizing to the new breeding environment that differed in terms of vegetation, climate and management. With the onset of breeding during the 2001/2002 breeding season, the imported birds had sufficient time to acclimatize and this was reflected in the production figures of the flock at the lower 114 birds per hectare stocking rates.

The tendency for fertility and hatchability to be negatively influenced in the larger flocks during both breeding years, support the reasoning that too high a stocking rate adversely affects the

reproductive performance of ostriches. It is also possible that corticosterone levels were higher in females housed at the higher stocking rates and this may have had a negative influence on ovulation and subsequent oviposition. Corticosterone levels were found to be higher in laying hens housed in higher rates (Mashaly *et al.*, 1984). Abnormal doses of corticosteroids resulted in premature LH release and an irregular laying patterns in laying hens (Gilbert, 1971).

The negative influence of the higher stocking rates on normal reproductive behaviour also possibly contributed to the lower fertility observed for the large flocks during both years. Courtship and mating behaviour of ostriches have been well-documented, with reproductive behaviour in captive environments closely resembling that observed in natural environments (Bolwig, 1973; Stewart, 1994; Berendsen, 1995; Hicks-Aldredge, 1996; Deeming, 1997; Bubier *et al.*, 1998). Any change in the immediate environment may disrupt normal reproductive behaviour (Lambrechts *et al.*, 1998b). The establishment of a male's territory is usually initiated by the making of a nest, and borders of males' territories seldom overlap (Sauer and Sauer, 1966b; McKeegan and Deeming, 1997). During breeding seasons, males spend most of their daily activity budget on territorial defense, and this is more pronounced in small breeding groups. The high stocking rates prevented males from establishing clear territories, which may resulted in them spending relatively more time on aggressive interactions with other males and less time on territorial defense and reproductive interaction with females. Although no behavioural study was done to substantiate this, instances were observed where more dominant males interrupted mating sessions by chasing or running a copulating male off a female. Aggressive displays between males and towards onlookers were also observed. The aggressive encounters that were more prevalent in the 210-bird flocks during the study period may be indicative that the defense and maintenance of territories were more difficult in the larger flocks. The increase in fighting bouts between males also possibly disturbed the laying activity of females in these flocks. Wechsler and Schmid (1998) found that aggressive interactions were significantly increased in multiple-male groups of Japanese quail (*Coturnix japonica*). Aggressive encounters between females, as observed by Sauer and Sauer (1966a, 1966b), were seldom observed during the present study.

The high stocking rates of 150 birds, 141 birds and 210 birds per hectare represented stressful conditions that had a negative influence on the normal behaviour of the breeding ostriches. Cases were observed where females and males displayed abnormal homosexual behaviour and this was more prevalent at the highest stocking rate of 210 birds per hectare. Homosexual behaviour was also observed during the non-breeding season, when males and females were separated and maintained in separate-sex flocks at a rate of approximately 150 birds per hectare. Feather pecking was observed for both genders maintained at the higher stocking rates, with females being pecked to a greater extent than males. McKeegan and Deeming (1997), Lambrechts and Cloete (1998) and Lambrechts *et al.* (1998a) indicated that ostriches tend to have rather conservative behavioural patterns and maintaining breeding ostriches in large flocks, may give rise to certain behavioural problems (Stewart, 1994; Hicks-Aldredge, 1996; Deeming,

1997). Abnormal homosexual behaviour in female ostriches in captive environments (Sambraus, 1994) and imprinting on humans (Bubier *et al.*, 1998) have also been reported. Our observations in this study lend support to suggestions of Stewart (1994), Hicks-Aldredge (1996), Deeming (1997) and Sambraus (1995 *cite* Deeming and Bubier, 1999) that abnormal behaviour patterns may develop as a result of too high stocking rates. The abnormal behaviour patterns observed at the higher stocking rates agree with findings in poultry where high stocking rates had a negative influence on the reproduction performance and well-being of laying hens (Hughes and Wood-Gush, 1977; Sherwin and Kelland, 1998; Blokhuis and Wiepkema, 1998; Martrenchar *et al.*, 1999; Savory *et al.*, 1999; Bilcik and Keeling, 2000; Klein *et al.*, 2000). Feather pecking was found to be transmitted socially in groups of laying hen chicks (Zeltner *et al.*, 2000). Odén *et al.* (1999) found that the presence of males had a reducing effect of agonistic behaviour in laying hens, but not on the degree of feather pecking among laying hens housed at high stocking rates.

In contrast to the influence of too high stocking rate of the production of the large flocks, the 9-bird flocks maintained in the smaller 0.13ha camps outperformed the 9-bird flocks in the larger 0.30ha camps in terms of almost all production parameters. Total eggs produced tended to be 9.5% higher ($P \geq 0.15$) for 9-bird flocks maintained in the smaller 0.13ha camps. Average egg production was improved by 14.5% in the 9-bird flocks maintained in the 0.13 ha camps. The fertility and hatchability of 9-bird flocks in the 0.13ha camps were improved by 9.5% and 6.4%, respectively. Chick production was 23.5% higher for the 9-bird flocks maintained in the smaller camps. A possible explanation for this improvement in the respective production traits is that the increased frequency of encounters between males and females in the smaller camps were more frequent than in the larger 0.3ha camps. The smaller camp size of 0.13ha did not appear to influence reproductive behaviour of the birds. It would appear that the establishment of clear territories were not inhibited during the study period. The smaller 0.13ha camps actually might have contributed to females visiting different territories more frequently than in the larger 0.30 ha camps and this possibly resulted in a higher frequency in sexual encounters, which in turn possibly contributed to the higher production performance of the 9-bird flocks in the smaller 0.13 ha camps.

When the production of breeding trios and 9-bird flocks in 0.13ha camps are considered, the 9-bird flocks performed on average better for all reproduction traits, with the exception of average egg production per female. The improvement in almost all the reproduction traits in the 9-bird flocks can be ascribed to the higher number of females and males present in this breeding system, when compared to the trios. Average egg production however, was compromised for the higher stocking rate of 9 birds per 0.13 hectare. The improved fertility and hatchability for the 9-bird flocks maintained in 0.13ha camps can be ascribed to the stimulating effect of more than one male in this breeding system. Females had the opportunity to visit more than one male's territory and this possibly contributed to more frequent sexual encounters and the higher fertility and hatchability recorded for the 9-birds flocks.

When production of the smaller 9-bird and 13-bird flocks are compared, the 13-bird flocks performed significantly better than the 9-bird flocks in terms of all reproduction traits, i.e. total egg production, average egg production per female, number of chicks hatched, fertility and hatchability. When the two breeding systems are compared on an area/bird basis, it became evident that reproductive performance is not only stocking density dependent. The higher density in the 0.35 ha camps (269.2m^2/bird vs. 333.3m^2/bird), possibly had to effect that sexual encounters between the males and females were more frequent, which in turn contributed to the improved fertility and hatchability. It is difficult; however, to reach a conclusion with regard to the improved production of the 13-bird flocks, for the slight increase in camp size might also have had a positive influence on the production performance of the 13-bird flocks.

The influence of male:female ratio

Increasing the number of females per male in the 0.06ha camps had to effect that egg production increased linearly with 29.1± 2.3 eggs per camp, with a corresponding linear increase of 24.2±2.0 chicks per camp. Average egg production per female, however, was not similarly affected. Fertility and hatchability were significantly improved for the breeding quads. An important observation was that neither fertility nor hatchability was compromised when the number of females was increased. A possible explanation for this is that the breeding quads are similar to a breeding harem that is usually established by males during a breeding season. Ostriches are gregarious by nature and the stimulus of an increase in the number of individuals in the respective breeding systems is reflected in the improvement in almost all the reproduction traits of the breeding quads in the study. Deeming (1996) found that breeding pairs and trios were more productive than larger flocks. Results obtained in this study support the findings of Deeming (1996). His study however, did not include a comparison of breeding quads with the larger flocks. Based on the results in the present study it can be assumed that breeding quads will be more productive on a per female basis than larger flocks. However, it has to be conceded that the number of replicates studied for the breeding quads was relatively small when compared to other studies (Craig *et al.*, 1977; Bates *et al.*, 1987; Deeming and Wadland, 2001; Campo and Davilla, 2002). It is therefore possible that the breeding quads could have performed exceptionally well under these circumstances.

The present results are contradictory to results reported by More (1997), who found that the laying performance of breeding pairs tended to be better than that of both trios and a breeding flock. However, although female age significantly influenced egg production, no mention was made on the composition of the respective breeding systems. The improvement in the fertility and hatchability for the breeding quads are contradictory to the decline in fertility reported when the number of females were increased in single-male groups of Japanese quail (*Coturnix japonica*) and commercial pheasants (*Phasianus colchinus*) (Bates *et al.*, 1987; Wechsler and Schmid, 1998; Deeming and Wadland, 2002). Single-male groups containing 8, 12, 16 and 20 hens had fertility percentages of 92%, 84%, 77% and 69%, respectively (Wechsler and Schmid, 1998).

Increasing the number of females from 1M:12F to 1M:18F in pheasants had to effect that egg production was increased, with a subsequent decrease in fertility. Campo and Davilla (2002) investigated the influence of four different mating ratios on stress and fearfulness indicators in chickens. Their results suggested that too high mating ratios increased physiological and psychological stress responses. Deeming and Wadland (2002) investigated the effect of two mating ratios, i.e. 1M:8F and 1M:12F, in commercial pheasant flocks. Flocks with a mating ratio of 1M:8F produced significantly more eggs, and fertility and hatchability were improved significantly for the 1M:8F flocks. The higher M:F ratio of the breeding quads therefore indicates that ostrich males can successfully keep and service a harem consisting of at least three females.

CONCLUSIONS

The results indicate that breeding ostriches could be maintained at stocking rates higher than what is currently used on commercial ostrich farms. This has important implications in terms of the intensification of ostrich farming, especially in areas that are characterised by vegetation that are exposed to the trampling effect of ostriches. However, too high stocking rates may have a negative influence on normal reproductive behaviour, and thereby may not be beneficial for reproduction as well as well-being of ostriches under intensive farming conditions.

Results indicated that a "trade-off" in performance was observed when either the stocking rate was increased, or when the number of females per male was increased. For example, when average egg production per female was improved, fertility and hatchability was compromised. The 114-breeding flocks performed the best in 1 ha (10 000m^2) breeding camps, in terms of all the reproductive traits. Increasing the stocking rate in smaller 0.13 ha (625m^2) breeding camps had the effect that, although average egg production/female was lower, fertility and hatchability were improved, possibly as a result of more frequent sexual interaction of males and females. Breeding pairs, trios and quads could be maintained on smaller areas than what is normally prescribed, with acceptable production levels. Although the number of quads in the study was relatively small, it indicates that harem size under intensive conditions may be as high as three females per male, which supports the polygamous nature of ostrich males.

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Chapter 5

Genetic and environmental (co)variance estimates for ostrich (*Struthio camelus* var. *domesticus*) male aggression, shin colour and egg production performance of companion breeding females

Abstract

Monthly records on male aggression and shin colour scores, as well as for female egg production performance (EPP), were obtained for ostrich breeding pairs (n=136) over the period 1999 to 2002. All traits were affected by animal age, generally increasing up to intermediate ages of 4-7 years, and decreasing subsequently. Direct heritability estimates (±SE) were 0.30±0.04 for male aggression score, 0.03±0.02 for shin colour score and 0.08±0.04 for EPP. The animal permanent environmental variance (PE – association of records obtained across years) was significant for EPP only, at 0.07±0.03. The animal temporary environmental variances (TE – associations between records obtained within a breeding season) amounted to 0.23±0.02, 0.29±0.02, and 0.13±0.02, respectively. When assessed on a per breeding pair level, phenotypic correlations of male aggression score and male shin colour score with female EPP were estimated at 0.29±0.02 and 0.19±0.02 respectively. The partitioning of these correlations indicated that the TE components contributed most to the observed phenotypic relationships, at 0.42±0.07 and 0.51±0.07, respectively. On the PE and genetic levels, the observed correlations were low, mostly negative in sign, and not significant. The derived phenotypic relationships would thus not be valuable as indirect selection criteria for the improvement of egg production in ostriches in the current flock or future generations. The significant genetic variation for male aggression score, however, suggests that it could be possible to change the temperament of ostriches, to enable them to adapt better to routine husbandry operations, without impacting negatively on the egg production performance of companion breeding females.

INTRODUCTION

In Africa, ostriches have been hunted for centuries for their meat and feathers (Holtzhausen and Kotzé, 1990; Drenowatz *et al.*, 1995; Deeming, 1999). The first record of commercial ostrich farming in South Africa dates back as far as the 1860's, with feathers being the main source of income (Smit, 1964). Since then, the emphasis has shifted to leather and meat as the major sources of income, with feathers contributing the least to the total income generated from a slaughter bird (Wagner, 1986; Van Zyl, 2001).

The intensification of commercial ostrich farming and an increase in the emphasis on the welfare of animals under intensive farming conditions during the last few decades, had the effect that commercial ostrich farmers need to integrate the specific behavioural requirements of ostriches into their management programs to ensure an optimum breeding environment (Stewart, 1994; Reiner *et*

al., 1996; Lambrechts *et al.*, 1998; Mohamed *et al.*, 2003). Identification and integration of the behavioural patterns of ostriches in commercial farming systems may reduce potential stress-causing environments that may have an adverse effect on production performance. Overt aggressive behaviour in ostrich males is considered as an abnormal behaviour, and may contribute to lowered reproductive performance in ostrich breeding systems. There are, however, few studies to support these contentions.

Reports on ostrich behaviour have mostly been concerned with natural environments (Sauer, 1972; Bolwig, 1973; Hurxthal, 1975; Bertram, 1992; Sambraus, 1995). Ostriches are gregarious in nature during non-breeding seasons (Sauer and Sauer, 1966a, 1966b; Bertram, 1980, 1992; Burger and Gochfeld, 1988). During breeding seasons, male and female behaviour changes considerably, with both genders exhibiting characteristic behavioural repertoires (Sauer and Sauer, 1966b; Sambraus, 1994; Deeming and Bubier, 1999). Behaviour exhibited under commercial farming conditions closely resembles that observed in the wild (Bolwig, 1973; Berendsen, 1995; Hicks-Aldredge, 1996; Deeming, 1997, 1999; Bubier *et al.*, 1998). Display of territorial aggression, together with the change in colour of the shins, are commonly used by ostrich farmers as an indication of the readiness of a male to enter a breeding season. Both traits were previously shown to be positively related to egg production of female companions on a phenotypic level (Lambrechts *et al.* (2000). Ostrich males appear to develop courtship behaviour later than females, with colouration of the beak, thighs and shins only after the onset of breeding (Bolwig, 1973; Hicks, 1990; Samson, 1996; Deeming and Bubier, 1999). During breeding seasons the shins of ostrich males change in colour from light pink to crimson red.

Territorial behaviour in adult ostrich males, which is most often combined with aggression and vocalisation, is observed to a higher degree at the onset of and during breeding seasons (Sauer, 1972; Bolwig, 1973; McKeegan and Deeming, 1997; Lambrechts, 1998). This increased level of aggression during a breeding season is associated with elevated levels of testosterone (Degen *et al.*, 1994). Male aggression is mainly associated with territorial behaviour, and is characterised by males ramming each other with their chests and by forward kicking (Huchzermeyer, 1997), and by kantling displayed to other males and intruders in or near their territory (Bertram, 1992). The aim of this study was to determine the genetic and environmental (co)variance components for ostrich male aggression, male shin colour, and the correlation of these traits with the reproductive performance of their companion breeding females under commercial conditions.

MATERIALS AND METHODS

Experimental animals

An ostrich breeding flock consisting of 136 breeding pairs, maintained at the Little Karoo Agricultural Development Centre outside Oudtshoorn, South Africa, was used for the study. The

management of the breeding flock is documented by Van Schalkwyk *et al.* (1996) and Bunter and Graser (2000). The ages of the males used in the study varied between 2 and 12 years.

Experimental procedure

Each male was evaluated on a monthly basis throughout the 1999/2000, 2000/2001, 2001/2002 and 2002/2003 breeding seasons, by using a subjective scale to assign aggression and shin colour scores.

Assignment of aggression score:

The study focussed on territorial behaviour exhibited by ostrich males, i.e. aggressive behaviour displayed towards intruders in its breeding territory. Aggression scores were assigned by evaluating males on a monthly basis in terms of the extent of aggressive behaviour displayed throughout the breeding season. Scores were assigned on a scale, according to the following criteria:

- Low aggression score (5-6): assigned to males exhibiting no territorial aggression in a specific production month, and eggs could be collected without additional assistance to divert a male's attention away from the nest. Males appeared subdued and also disinterested in the activities in and around their breeding territories.
- Medium aggression score (7-8): assigned to males exhibiting a more extensive degree of territorial aggression, i.e. additional assistance were required to collect eggs on certain, but not all, occasions during a specific production month. Males frequently mock-charged any intruders, but on confrontation retired to another part of his breeding territory.
- High aggression score (9-10): assigned to males that exhibited extreme territorial aggression towards any intruders into their breeding territories in a specific month. In all cases, additional assistance was required to collect eggs.

Assignment of shin colour score:

A shin colour evaluation chart was compiled by using commercial paint colour charts. The colour chart used to assign shin colour scores is represented by Plate1. A score of 0 represented a white shin colour, with 5 being light pink and 10 a bright crimson red.

Plate 1. Colour chart used to assign monthly shin colour scores to ostrich males. Shin colour score indicated in each respective colour box.

Egg production

Daily egg production was noted for each pair throughout the breeding season. During the 2000/2001, 2001/2002 and 2002/2003 breeding seasons, the breeding pairs were subjected to different management practices, resulting in the breeding pairs differing in the number of production days. Breeding pairs were also subjected to mid-season breeding rests during the respective breeding seasons, resulting in their overall breeding shorter than their non-rested contemporaries. To standardize for the number of production days per breeding pair and also to accommodate months that differed in terms of production days, the egg production performance (EPP) for each breeding pair was calculated by using the following equation (Van Schalkwyk *et al.*, 1996):

EPP = [(total number of eggs produced/(0.5 X number of production days)] X 100

Statistical analysis

The ASREML program (Gilmour *et al.*, 1999) was used for the estimation of the fixed effects, and also subsequently to derive variance components for female egg production, and male aggression and shin colour scores in univariate analyses. Fixed effects that were considered included production year (2000-2002) and treatment (rested or not). Trends with regard to animal age and month of the breeding season were modelled, using cubic splines (Verbyla *et al.*, 1999). The cubic splines consisted of a fixed linear component and a random non-linear component depicting random deviations conforming to a smooth trend. Initially, random deviations from linearity not conforming to a smooth trend were also fitted, but this component was not found to be significant ($P \geq 0.05$) for any of the variables. The first analyses involved fitting various combinations of fixed effects, random spline components and interactions between them to obtain an operational model (termed as Model 1). Effects found to be significant ($P \leq 0.05$) in these preliminary analyses were retained in subsequent analyses. Random terms were then added to the operational model, resulting in the following genetic models for analyses (in matrix notation):

$$y = Xb + Z_{1a} + e \quad (2)$$

$$y = Xb + Z_{2PE} + e \quad (3)$$

$$y = Xb + Z_{3TE} + e \quad (4)$$

$$y = Xb + Z_{1a} + Z_{2PE} + e \quad (5)$$

$$y = Xb + Z_{1a} + Z_{3TE} + e \quad (6)$$

$$y = Xb + Z_{2PE} + Z_{3TE} + e \quad (7)$$

$$y = Xb + Z_{1a} + Z_{2PE} + Z_{3TE} + e \quad (8)$$

In these models, y was a vector of observations for female production or reproduction traits; b, a, permanent environment (PE) and temporary environment (TE) were vectors of fixed effects, direct additive genetic effects, animal permanent environmental effects and animal temporary environmental effects, respectively. PE represented the factors that could contribute to variation observed between breeding seasons, whereas TE represented factors that could contribute to the

variation observed within a breeding season. X, Z_1, Z_2, and Z_3 were the corresponding incidence matrices relating the respective effects to y, and e the vector of residuals.

It was assumed that:

$$V(a) = A\sigma^2_a;\ V(_{PE}) = I\sigma^2_{PE};\ V(_{TE}) = I\sigma^2_{TE};\ V(e) = I\sigma^2_e,$$

with A being the numerator relationship matrix, I being an identity matrix; and σ^2_a, σ^2_{PE}, σ^2_{TE} and σ^2_e being the direct genetic variance, animal permanent environmental variance, animal temporary environmental variance and environmental (residual) variance respectively. All analyses included the full pedigree file, consisting of 601 individuals, the progeny of 135 males and 132 females.

Likelihood ratio tests (LRT) were conducted to determine the most suitable model for each trait in univariate analyses. The LRT was based on testing twice the increase in Log likelihood resulting from adding an additional random term to the model of analysis as a Chi^2 statistic. Alternatively, for two models with the same number of random effects, and assuming identical fixed effects models, the one with the higher likelihood fits the data better.

Subsequently, two-trait animal models were fitted to the data in an attempt to estimate genetic, environmental and phenotypic correlations between the respective traits. Direct additive variance components in the study were relatively small, particularly in the case of shin colour score. It was attempted to use the numerator relationship matrix to estimate genetic correlations between sex-limited traits measured in either males or females, *viz.* between EPP and aggression score, as well as between EPP and shin colour score. Since these traits were not recorded on the same gender, these correlations were not stable, with relatively large standard errors. To add to these results, within and between breeding pair (co)variance components were also obtained to estimate permanent and temporary environmental correlations between these traits on a breeding pair level. Temporary environmental correlations were seen as associations between measures of breeding pair performance within breeding seasons, while permanent environmental correlations were defined as associations across breeding seasons.

RESULTS

The monthly egg production performance (EPP) of the ostrich females was exceedingly variable, as indicated by a standard deviation exceeding the mean (Table 1). A relatively large proportion of females had monthly EPP records of zero in some months, contributing to the observed variation. The corresponding coefficient of variation for male aggression score was 30%. Male shin colour score was less variable, with a coefficient of variation below 10%.

Table 1. Means and standard deviations, and number of observations for reproductive and behavioural traits assessed for ostrich males and females.

TRAIT	Mean (SD)	Number of observations
Monthly EPP (%) (♀)	32.1 (34.2)	4025
Aggression score (♂)	6.62 (2.0)	4406
Shin colour score (♂)	7.75 (0.68)	4406

Male aggression increased with age, with young males (i.e. 2 years of age) exhibiting less aggressive behaviour than their older contemporaries. Aggression reached a peak in males aged 6 to 7 years, and declined with an increase in age. When shin colour scores are considered, males aged 5 to 7 years received the highest scores for shin colour (8.02±0.04). Two-year old males received lower shin colour scores of on average 7.36±0.09 (Figure 1).

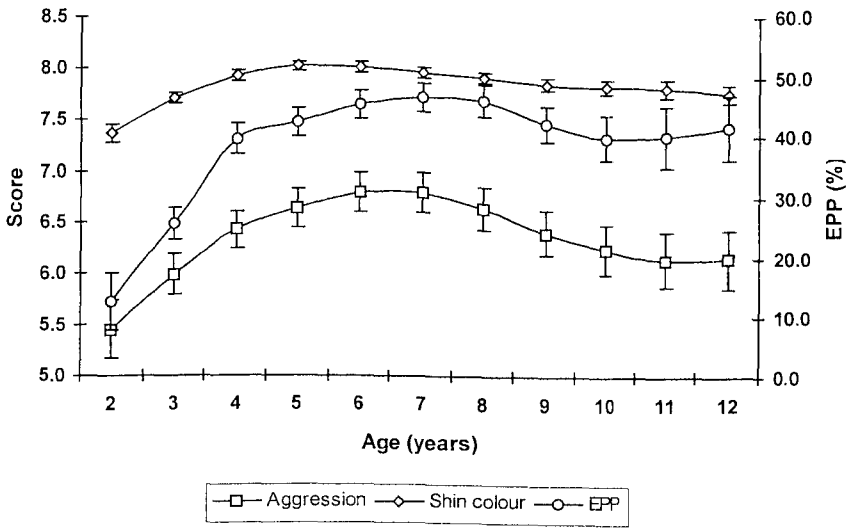

Figure 1. The relationship between male age, aggression and shin colour scores, and female egg production performance (EPP).

The likelihood ratio tests revealed that Model 8 of the statistical analysis, including the direct additive genetic effects, female permanent environmental effects and female temporary environmental effects as random factors, fitted the data best ($P \leq 0.01$) for monthly EPP (Table 2). Model 7, i.e. the model including only the direct additive genetic and male temporary environmental effects, fitted the data best ($P \leq 0.01$) for aggression and shin colour scores of males (Table 2).

Table 2. Log likelihood values for the respective random effect models (model in brackets) considered in the analyses, with the best models indicated in bold.

EFFECT	EPP	Aggression score	Shin colour score
Fixed only (1)	-18857.3	-5454.9	-525.83
+ h^2 (2)	-18525.9	-5464.22	-349.34
+ PE (3)	-18525.3	-5457.9	-339.02
+ TE (4)	-18465.8	-5351.1	-154.18
+ h^2 + PE (5)	-18519.8	-5451.7	-349.34
+ h^2 + TE (6)	-18429.9	**-5302.5**	**-152.10**
+ PE + TE (7)	-18431.8	-5408.0	-153.22
+h^2 + PE + TE (8)	**-18426.8**	-5301.1	-152.10

Heritability (h^2) of the male traits ranged from 0.03 to 0.30 for shin colour and aggression, respectively (Table 3). The monthly EPP of ostrich females were lowly heritable at 0.08. The female permanent environmental variance ratio (PE) was only significant ($P \leq 0.05$) for EPP. The animal temporary environmental variance ratios were significant ($P \leq 0.05$) for all traits, and exceeded 0.10 throughout. These results suggested that the animal temporary environmental variances (i.e. the correspondence between monthly records on the same animal within a season) contributed markedly to all the traits measured on male and female ostriches in the present study.

Table 3. Variance components and ratios (±SE) for the egg production performance (EPP) of ostrich females, and aggression and shin colour scores assigned to ostrich males.

VARIANCE	EPP	Aggression score	Shin colour score
Component			
σ^2_A	80.623	1.2919	0.0155
σ^2_{PE}	74.282		
σ^2_{TE}	138.030	0.9829	0.1347
σ^2_E	752.026	1.9657	0.3217
σ^2_P	1044.961	4.2405	0.4719
Ratios			
h^2	0.08 ± 0.04	0.30 ± 0.04	0.03 ± 0.02
PE	0.07 ± 0.03*		
TE	0.13 ± 0.02*	0.23 ± 0.02*	0.29 ± 0.02*

*$P \leq 0.05$

The significant phenotypic correlation between monthly EPP and aggression score (Table 4), could mostly be attributed to a highly significant ($P \leq 0.01$) TE component. This result suggests that short-term, seasonal differences mostly accounted for the positive phenotypic and environmental correlations. The PE correlation (i.e. the long-term correlation between breeding pair relationship across breeding seasons) was positive, low, and smaller in magnitude than the corresponding standard error. The genetic correlation between male aggression and shin colour was low, at 0.08 (Table 4). The positive and significant phenotypic correlation once again depended mostly on a significant ($P \leq 0.01$) TE correlation.

Table 4. The permanent (PE) and temporary (TE) environment correlations of the egg production performance (EPP) of ostrich females with aggression and shin colour of ostrich males, as estimated between breeding pairs and on an individual basis

TRAITS AND CORRELATIONS	BETWEEN BREEDING PAIR		INDIVIDUAL	
	PE	TE	Environmental	Phenotypic
Number of combinations	299	546	4406	4406
EPP ×				
Aggression score	0.15 ± 0.12	0.42 ± 0.07**	0.31 ± 0 02	0.29 ± 0.02
Shin colour score	-0.12 ± 0.35	0.51 ± 0.07**	0.12 ± 0.02	0.19 ± 0.02
	Genetic	**TE**	**Environmental**	**Phenotypic**
Aggression score ×				
Shin colour score	0.08 ± 0.24	0.32 ± 0.07**	0.15 ± 0.02	0.18 ± 0.02

**P≤0.01

When the numerator relationship matrix was used for the estimation of a genetic correlation, the derived figure was negative, correspondingly low and not different from zero at –0.10 ± 0.31. Results pertaining to the between breeding pair correlation of monthly EPP with shin colour score yielded estimates that were broadly in agreement. The estimated genetic correlation was larger in magnitude and negative, but still not significantly (P≤0.05) different from zero at –0.55 ± 0.42.

The average monthly EPP of the breeding pairs showed a typical seasonal pattern during the study, with the lowest EPP of 22.2±2.7% reported during June (i.e. the first month of a breeding season). The highest EPP of 49.1±2.7% was reported for September, and egg production declined progressively to reach an average of 30.0±2.7% during January (Figure 2).

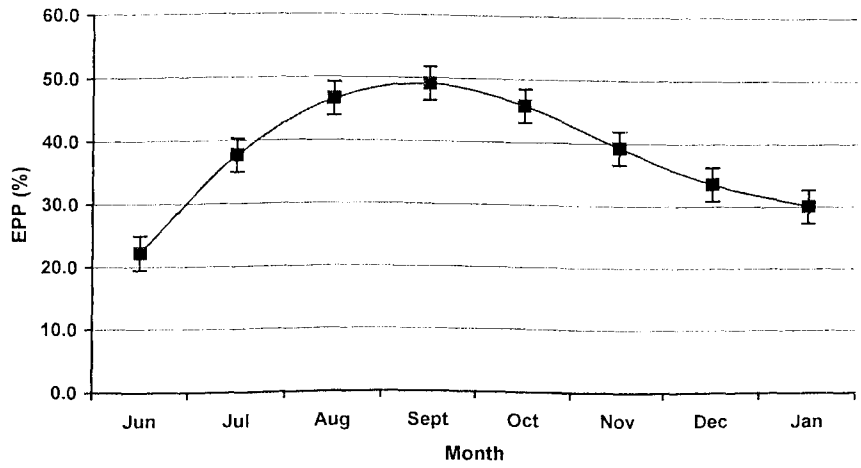

Figure 2. The average monthly (mean ± SE) egg production performance (EPP) of ostrich breeding pairs.

DISCUSSION

Annual egg production records of ostrich females were previously found to be highly variable, with coefficients of variation exceeding 50 % (Cloete *et al.*, 1998; Bunter *et al.*, 2001). In the present study, monthly figures showed even more variation and the derived standard deviation exceeded the mean. No corresponding literature estimates were found for male aggression and shin colour scores, but these traits generally showed less variation.

The increase in territorial aggression with age may be ascribed to previous breeding experience of the older males. Kim and Zuk (2000) stated that previous breeding experience and aggression may determine the position of a male in the dominance hierarchy in a breeding environment of junglefowl. Broiler females showed distinct preferences for certain males, using male behaviour and not male morphology as a basis for their choices (Millman and Duncan, 2000). Rintamaki *et al.* (1998) found that female black grouse (*Tetrao tetrix*) mating with the males with the highest social ranking produced the biggest clutches, with females in good conditions mating with the higher ranking males. The tendency towards a decline in aggression in ostrich males older than 7 years may simply reflect that such males are past their prime. It coincides with a finding that fertility of ostrich eggs depends on male age, with maximum fertility at intermediate ages of 4 – 6 years (Bunter, 2002). The mates of younger and older males produced eggs with a lower level of fertility.

The heritability of monthly egg production figures of females was below 0.10. Estimates based on annual female performance ranged from 0.12 – 0.23 (Bunter *et al.*, 2001; Cloete *et al.*, 2004). The suggestion of a lower estimate for monthly EPP in the present may be related to the inherently greater variability of the trait. The significant genetic variation for EPP, however, opens up avenues for the use of part-records obtained over a fraction of the normal breeding season for the selection of ostrich females for egg production. In a previous study, Lambrechts *et al.* (2004) correspondingly found that EPP recorded over as short as 14 days were highly related to egg production over the entire season on a genetic level. Genetic correlations with annual egg production were unity for part-records derived over periods as short as one month of an 8-month breeding season. Female PE effects in the literature were generally higher than in the present study, at 0.18 – 0.32 (Bunter *et al.*, 2001; Cloete *et al.*, 2004).

No comparable results for ostriches were found in the literature as far as the significant genetic variation for male aggression score was concerned. Other domestic livestock species were subjected to extensive selection for temperament, resulting in modern genotypes that are well adapted to general husbandry routines (Boissy *et al.*, 2002). It is reassuring that the present results suggest that an aspect of temperament in ostriches can also be modified by selection. The implications of such a program should, however, be understood properly before it could be recommended unconditionally.

In a previous investigation, Lambrechts *et al.* (2000) reported that male aggression and shin colour scores were positively related to the EPP of their companion females on a phenotypic level. Respective phenotypic correlations amounted to 0.29 and to 0.21 for aggression and shin colour scores, values that were in close agreement with those obtained in the present study. The previous findings suggested that these traits could play a part in the phenotypic selection of breeding pairs for egg production. In the present study, it was possible to partition the covariance components to show that the correlations reported earlier depended mostly on the temporary environment common to both traits. Short term climatic and other environmental stimuli thus probably played the major role in these relationships, rendering it of no value as far as selection is concerned. Permanent environment (for progress in the current flock) and genetic correlations (for future generations) with EPP, were low and not significant, both for male aggression and shin colour scores. On the other hand, these findings indicate that continued selection for egg production is unlikely to result in excessive levels of aggression in breeder males as a correlated response. The significant phenotypic correlation between male aggression score and male shin colour score, also partitioned mostly towards the TE component. On a genetic level, these traits will unlikely depend on the same sets of genes.

CONCLUSIONS

Male behavioural traits do not seem to have value as indirect selection criteria for egg production in females, either in the current flock or in future generations. It is nevertheless important to recognize the fact that a measure of temperament exhibited genetic variation in ostrich males. This opens up the possibility that the temperament of ostriches may be changed by genetic means, to allow them to adapt better to routine husbandry routines, without impacting negatively on the egg production performance of companion breeding females. This is, however, an entirely new concept in the keeping of ostriches. Extensive research on the genetic components of ostrich behaviour is needed for the further development of this train of thought. Monthly egg production records from the ostrich females in the study also exhibited significant genetic variation. The selection of breeding ostrich females on partial egg production records, i.e. obtained over part of a breeding season, in ostrich breeding programs should be also considered in future research.

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Chapter 6

The relationship between serum testosterone, progesterone, LH and prolactin levels, and behavioural and reproduction parameters measured in breeding ostriches (*Struthio camelus* var. *domesticus*)

Abstract

A total of 669 ostrich (320 female and 349 male) serum samples was assayed to determine the relationship between serum levels of testosterone, LH, progesterone, and prolactin, male aggression and shin colour, and female egg production performance (EPP). The serum levels of almost all the hormones, with serum prolactin being the exception, were exceedingly variable, with the coefficients of variation exceeding 60% in almost all cases. Serum testosterone and LH levels varied on a permanent environmental level, in contrast to progesterone and prolactin that were influenced by the time of sampling and/or the gender of the bird. The animal permanent environment contributed significantly to the variation observed for testosterone, LH, aggression, live weight, and EPP. The only significant ($P \leq 0.01$) correlation reported in this study was that of serum LH with the EPP of ostrich females (0.40). Serum LH levels increased with an increase in male age, in contrast to serum LH levels of females, which declined with an increase in age. Serum testosterone levels tended to decline with an increase in male age, and the highest levels were reported for 6 year old ostrich males (0.7 ± 0.1 ng.mL^{-1}). The highest female testosterone levels were reported for 3- (0.6 ± 0.1 ng.mL^{-1}) and 10-year old (0.7 ± 0.2 ng.mL^{-1}) females, respectively. Ostrich males and females did not differ in terms of serum prolactin levels. Serum progesterone concentration tended to increase with an increase in female age, with highest levels measured in 11-year old females (0.7 ± 0.1 ng.mL^{-1}). Gender and time of sampling interacted for serum LH and testosterone concentrations, implying that the extent of gonad differentiation and the degree of photorefractoriness the breeding ostriches were experiencing influenced the serum testosterone and LH levels measured at the beginning and end of breeding. The maintenance of territorial aggression in males at the end of a breeding season, when lower levels of serum testosterone were measured, imply that factors other than LH may contribute to the maintenance of reproductive behaviour at the end of a breeding season. The study also reports on the sexual dimorphism observed in the breeding ostriches. The larger size of ostrich males reflects the increased parental investment in the care of their young. Heritabilities reported for live weight and EPP amounted to 0.30 and 0.31, respectively. The potential of, and the extent to which the reproductive cycles can be manipulated by means of exogenous gonadotropins and steroid hormones to improve the reproductive performance of breeding ostriches under commercial farming conditions warrants studies to determine baseline values of the gonadotropins and steroid hormones for breeding ostriches.

INTRODUCTION

Birds may use a variety of breeding strategies to enable them to breed under diverse conditions and environments. Synchronisation of the endocrine and behavioural factors involved in reproductive activities is necessary to ensure successful reproduction. Several studies in birds have been performed to illustrate this, but literature on endocrine factors that may influence behaviour and reproduction in ostriches, are however, scarce. Previous studies on ostriches focussed mainly on determining haematological parameters (Van Heerden *et al.*, 1985; Dawson *et al.*, 1996; Mushi *et al.*, 1998; Spinu *et al.*, 1999; Romdhane *et al.*, 2000), and biochemical properties of the hypothalamic and pituitary hormones (Papkoff *et al.*, 1982; Bonna-Gallo *et al.*, 1983; Licht *et al.*, 1983; Powell *et al.*, 1987; Koïde *et al.*, 1996; Maney *et al.*, 1997; Skadhauge and Dawson, 1999). In another ratite species, Williams *et al.* (1998) investigated the potential of surgical and hormonal gonadectomy to overcome the seasonal effect on productivity of emus. Degen *et al.* (1994) presented the first information on the level of LH, testosterone and estradiol in breeding ostriches. Prolactin levels were measured in a few cases in ostriches, with the prolactin levels being higher in an incubating male (Skadhauge and Dawson, 1999). Marked seasonal changes in circulating levels of prolactin occur in most birds species studied, but have not been established for ostriches.

Two forms of gonadotropin-releasing hormone (GnRH) are found in birds, i.e. GnRH-I that elicits gonadotropin release, and GnRH-II that plays a role in reproductive behaviour, apparently independent from GnRH-I (Maney *et al.*, 1997). An increase in photoperiod stimulates the release of hypothalamic GnRH, with a subsequent secretion in pituitary LH and FSH (Deviche *et al.* 2000). These three hormones induce gonadal development and the subsequent secretion of gonadal hormones that play a role in behavioural changes during a breeding period, and also the development of the secondary sexual characteristics (Paster, 1991; Deviche *et al.*, 2000). FSH is responsible for the maturation of follicles in the female and the Sertoli cells in the testes of the male, while LH stimulates the interstitial cells of the ovary and the Leydig cells of the testes to produce the steroid hormones.

Estrogens, androgens and progesterone are the most important steroid hormones involved in avian reproduction, and have a variety of effects that may include sexual differentiation, the development of secondary sexual characteristics, behaviour, metabolism, gamete production, moult and feedback inhibition of LH secretion (Howarth, 1995). In the avian female, progesterone is produced by the granulosa layers of the largest preovulatory follicles in reaction to LH, and has a positive feedback on the pituitary that results in an LH surge that results in subsequent ovulation. The estrogens are synthesised by the theca layer, with maximum levels during ovarian development and egg laying. The estrogens stimulate the liver to produce vitellogenin that is transported via the blood to the ovary where it is deposited as yolk in the developing follicles, and is also involved in oviduct development and induction of female reproductive behaviour (Farner and Wingfield, 1980; Etches, 1995). Prolactin is a

pituitary peptide hormone, with secretion stimulated by a decrease in photoperiod. High circulating levels of prolactin coincide with the development of photorefractoriness and subsequent gonadal regression (Deviche *et al.*, 2000). Vaso-active intestinal polypeptide (VIP) primarily regulates the synthesis and release of prolactin in birds, with increased VIP levels associated with decreased GnRH expression (El Halawani *et al.*, 1995; Deviche *et al.*, 2000).

Testosterone is the most important androgen produced by the testes, and secretion shows a typical seasonal pattern (Donham, 1979; Dittami, 1981; Fraissinet *et al.*, 1987; Degen *et al.*, 1994). Testosterone, in the presence of FSH, plays a role in the regulation and maintenance of spermatogenesis, with increased levels associated with the onset and the later stages of spermatogenesis. The Sertoli cells are also under the control of FSH, and may play a role in the conversion of androgens to estrogens via a process of aromatization (Howarth, 1995). The testes become functional earlier than the ovary in birds (Farner and Wingfield, 1980). Increased male testosterone levels are associated with aggressive, territorial displays at the onset of courtship behaviour, and is responsible for libido and mating behaviour (Farner and Wingfield, 1980; Howarth, 1995).

Behavioural patterns exhibited during the reproduction period can provide a good indication of an individual's reproductive ability (Ottinger and Mench, 1989). The present study investigated the relationship between male aggression and shin colour, female egg production performances recorded for, and serum levels of testosterone, LH, progesterone, and prolactin sampled in ostriches at the beginning and end of breeding seasons.

MATERIALS AND METHODS

Experimental animals

An ostrich breeding flock consisting of breeding pairs (n=136) maintained at the Little Karoo Agricultural Development Centre outside Oudtshoorn, South Africa, were used for the study. The management of the breeding flock is documented by Van Schalkwyk *et al.* (1996) and Bunter and Graser (2000). The ages of the breeding birds varied between 2 and 12 years.

Experimental procedures

Collection and processing of blood samples

Blood sampling was performed at the beginning and end of each breeding season. Blood samples were collected by venipuncture of the wing vein (Van Heerden *et al.*, 1985) and processed as soon as

possible after collection. The blood samples were not collected at a set time and collections were performed throughout the day. In cases where samples were not processed on the day of collection, samples were stored at 4°C for a maximum of one day. Blood samples were centrifuged at 3000 rpm for 10 minutes and serum was transferred to sterile vials, and stored at –20°C for later analysis.

Freeze drying of samples

One millilitre of each sample was aliquoted to sterile glass serum vials, labelled and transferred to a deep freeze at –80°C, where the samples were kept until subjected to freeze-drying. After completion of the freeze drying process, the samples were stored at 4°C until analysis.

Hormone assays

All radio-immuno assays were performed at the Faculty of Agriculture, University of Western Australia, Perth, Australia. After being subjected to gamma irradiation, serum samples were reconstituted by adding 1mL of sterile, distilled water (Millipore) to each sample. Before the reconstituted serum samples were subjected to radio-immuno assay (RIA) procedures, samples were clarified by polyethyleneglycol (PEG) precipitation, according to a method described by Van Cleeff *et al.* (1998). Emu serum samples were clarified for RIA with PEG, with minimal hormonal loss (Van Cleeff *et al.*, 1998). Serum samples destined for testosterone and progesterone assays were not subjected to PEG precipitation. The respective media preparation guides and assay procedures are set out in Appendix A. All assays were performed in duplicate.

LH assay

Serum concentrations of LH were measured by the RIA method described by Sharp *et al.* (1987), using chicken-LH (PRC-AE1-1), prepared and used for antisera production, as described by Talbot *et al.* (1988). The antiserum was raised in a rabbit and designated anti-ch-LH 3/3 (anti 3/3). The PRC-AE1-1 was purified by gel filtration to remove contaminating proteins of high and low molecular weight, and the resulting PRC-AE1-1-s-1 was labelled with ^{125}I using chloramine-T. On day one, 50µl of the first antibody was added to all the tubes and incubated at 4 °C for 24h. On day two, 50µl of tracer (^{125}I-PRC-AEI-s-1) was added to each tube and incubated for 48h. On day four of the assay, 50µl of normal rabbit serum (NRS) and 50µl of the second antibody were added to all tubes, except the total counts. On day five, 1mL of basic LH buffer with 6% PEG was added to all tubes, except the total counts, and centrifuged at 3000rpm for 30 minutes. The supernatant was then aspirated with a Pasteur pipette attached to water pump. Tubes were then counted for 60 seconds.

Prolactin assay

Serum prolactin concentrations were assayed as described by Talbot and Sharp (1994). Recombinant-derived chicken prolactin was prepared as described by Hanks *et al.* (1989 *cite* Talbot and Sharp, 1994) and was used for tracer and antibody production. On day one, 50µL of the first antibody was added to all tubes except the total count (TC) and non-specific binding (NSB) tubes, and incubated to 4°C for 24h. On day two, 50µL of tracer (radioactive prolactin) was added to all tubes and incubated at 4°C for 24h. On day three, 50µL of NRS and 50µL of the second antibody was added to all tubes, vortexed and incubated at 4°C for 24h. On day four, 1mL of PRL buffer containing 6%PEG was added to all samples and centrifuged at 4°C and 3000rpm for 25 minutes. The supernatant was then aspirated off and all tubes were counted.

Testosterone assay

Serum testosterone concentrations were measured in unextracted samples, as described by Malecki *et al.* (1998). An antiserum raised against testosterone-3-carboxymethyloxime-human serum albumin, 4-androsten-17β-ol-3-one as standard and 1, 2, 6, 7-^{3}H-testosterone as tracer was used in the assay. On day one, 100µL of the first antibody was added to all tubes except the NSB tubes, to which 100µL GPB was added. On day two, 100µL of the tracer was added to all tubes and samples were vortexed and incubated at 4°C. On day three, 100µL NRS and 100µl of the second antibody was added to all tubes. On day four, 1mL of PBS with 2%PEG was added to all tubes except the total counts. All samples, with the exception of the total counts, were centrifuged at 4°C and 3000rpm for 25 minutes. After centrifugation, the supernatant was aspirated and 0.5mL 0.05M HCl added to tubes. The samples were then vortexed and dispensed into the counting vials that contained 2mL of scintillant each. Each sample was gently shaken and left in the dark for at least 1h before being counted.

Progesterone assay

Serum progesterone concentrations were measured in unextracted samples by using a method developed by the Animal Science RIA laboratory of the University of Western Australia. Samples were extracted by adding 2mL of hexane to 100µL of serum and vortexed for 3 minutes. The aqueous phase was frozen by using an acetone-dry ice bath. The solvent phase was then transferred to assay tubes (10X75 mm) and was then dried under nitrogen gas. After extraction on day one, 200µL of the first antibody were added to samples, vortexed and incubated at 4°C overnight. On day two, 100µL tracer was added and samples were again vortexed and incubated overnight at 4°C. On day three, 100µL of the second antibody (15µL donkey serum 5 batch 1 + 90µL PBS) was added, with samples again vortexed and incubated overnight at 4 °C. On day four, 1mL of 2% PEG was added and samples were spinned for 25 minutes at 4°C and 3000rpm. The supernatant was aspirated and 0.5mL

0.05M HCl was added to all tubes, except the total count tubes. Samples were then vortexed and dispensed into counting vials with 2mL of scintillant. Samples were shaken and left in dark for a minimum of one hour before being counted.

Data recorded

Each male was evaluated on a monthly basis in terms of territorial aggression and shin colour, as described in *Materials and Methods*, Chapter 5. Daily egg production was recorded for each breeding pair throughout the breeding season. Post-collection treatment and subsequent artificial incubation of eggs was performed as described by Van Schalkwyk (1998). To account for any differences in the number of production days, the number of eggs produced per breeding pair was calculated as egg production performance (EPP) by using the following equation, and expressed as a percentage (Van Schalkwyk *et al.*, 1996):

EPP = [(total number of eggs produced/(0.5 X number of production days)] X 100

Statistical analysis

The ASREML program (Gilmour *et al.*, 1999) was used for the estimation of the fixed effects, and also subsequently to derive the variance components for live weight, hormone concentrations, EPP and hormone concentrations in univariate analyses. The fixed effects that were considered in all the analyses included sampling time (at the beginning and end of breeding), and gender where applicable (progesterone concentrations and EPP were measured in females only, and aggression and shin colour scores in males only). Age was not included as a fixed effect and could be described as longitudinal data. Correlations were estimated only for traits where one or more of the random effects exceeded 5 % of the overall phenotypic variance, since spurious results may be obtained with very small variance components.

On the assumption that a specific trend would be discernible, a smoothing spline was fitted to the data where applicable (Verbyla *et al.*, 1999). The spline consisted of three components, i.e. a fixed linear component, random deviations from linearity following a smooth trend, and random deviations from linearity not conforming to a smooth trend. The latter effects were found to be not significant, and were excluded from the final analyses. Random terms were then added to the operational model, resulting in the following models for analyses (in matrix notation):

$$y = Xb + Z_1a + e \quad (1)$$

$$y = Xb + Z_2c + e \quad (2)$$

$$y = Xb + Z_1a + Z_2c + e \quad (3)$$

In these models, y was a vector of observations for production traits or hormone concentrations; b, a and c were vectors of fixed effects, direct genetic effects and permanent environmental effects; X, Z_1

and Z_2 were the corresponding incidence matrices relating the respective effects to y, and e the vector of residuals.

It was assumed that:

$$V(a) = A\sigma^2_a;\ V(c_{PE}) = I\sigma^2_C;\ V(e) = I\sigma^2_e,$$

with A being the numerator relationship matrix, I being an identity matrix; and σ^2_a, σ^2_C, and σ^2_e being the direct genetic variance, permanent environmental variance, and environmental (residual) variance respectively. All analyses included the full pedigree file, consisting of 601 individuals, the progeny of 135 males and 132 females. Random terms were added to analytical models sequentially, as noted above, with Likelihood Ratio Tests (LRT) performed to assess the significance of their contribution to improvements in the model for analysis. The LRT is based on testing twice the increase in Log-likelihood resulting from adding *n* random terms to the model of analysis as a χ_n^2 statistic. Alternatively, for two models with the same number of parameters, and assuming identical fixed effects models, the one with the higher likelihood fits the data better. Subsequently, 2-trait animal models were fitted. These analyses allowed the calculation of all relevant correlations between traits, together with their appropriate standard errors.

RESULTS

The coefficient of variation for live weight of the breeding birds was 12.4% (Table 1). Serum hormone levels were exceedingly variable, with the respective coefficients of variation exceeding 60% in almost all cases. The exception was prolactin, with a coefficient of variation of below 30%. The coefficient of variation for shin colour score was approximately 10%, while that of aggression score was slightly below 30%. The coefficient of variation of EPP across the two breeding seasons approached 50%.

Table 1. Means (SD) for live weight, egg production performance (EPP), serum hormone concentrations, and male aggression and shin colour measured in breeding ostriches.

TRAIT	Number of observations	Mean (SD)	CV (%)	Range
Live weight (kg)	692	121 (15)	12.4	76 – 160
EPP (%)	220	41.6 (19.4)	46.6	0 – 89.9
Testosterone ($ng.mL^{-1}$)	628	0.52 (0.57)	109.6	0 – 6.4
Prolactin ($ng.mL^{-1}$)	690	0.84 (0.23)	27.4	0.01 – 1.75
LH ($ng.mL^{-1}$)	683	3.25 (2.97)	91.4	0.16 – 17.8
Progesterone ($ng.mL^{-1}$)	320	0.58 (0.40)	68.9	0.14 – 2.68
Shin colour score	349	7.42 (0.77)	0.10	5 – 10
Aggression score	349	6.20 (1.82)	29.4	5 – 10

The ostrich males were slightly heavier (P≤0.05) than females overall, i.e. 125 ± 2 kg vs. 119 ± 2 kg, respectively (Figure 1). Live weight and EPP of ostriches generally increased with animal age, to reach a maximum at 5-6 years of age for females and 6 years for males. As the trends at older ages (9-11 years) were based on fewer observations, they were more variable as indicated by the larger standard errors (Figure 1), and did not differ from live weight at 6 years of age (P≤0.05).

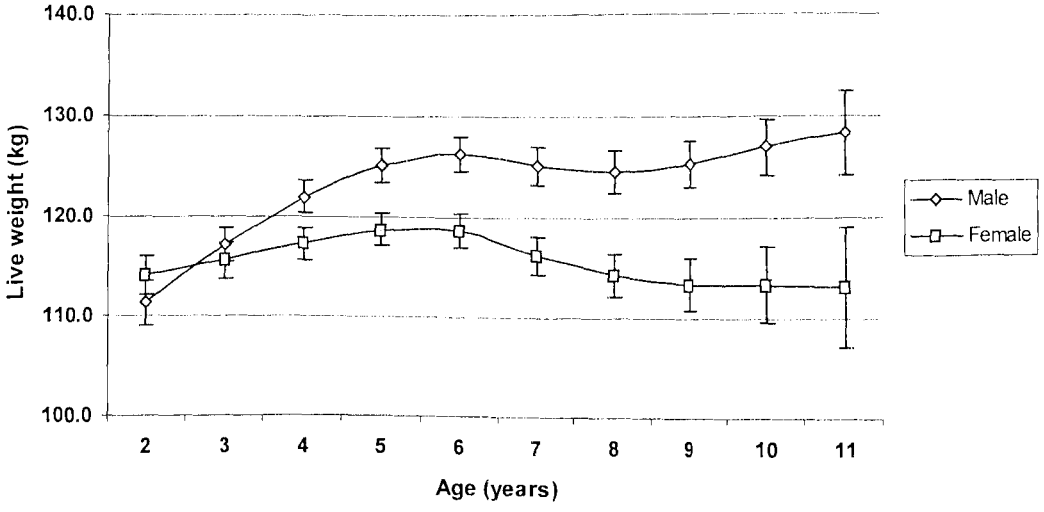

Figure 1. The influence of age on the live weight of male and female ostriches.

The observed likelihood ratios indicated that the models fitting both direct genetic and animal permanent environmental variances fitted the data best for the production traits of animal live weight and EPP (Table 2). The animal permanent environment was the only significant (P≤0.05) random effect in the cases of serum testosterone and LH concentrations. Serum prolactin and progesterone concentrations were independent of the random sources of variation considered, and a fixed effect model fitted the data best. The best model for shin colour score included the direct additive effect as a significant (P≤0.05) additional random effect. The male permanent environment contributed significantly to aggression score.

Table 2. The log likelihood ratios for the respective random effect models fitted to the data set, with the best model indicated in bold.

TRAIT	EFFECT(-S) IN MODEL			
	Fixed only	+ h^2 (1)	+ c^2 (2)	+ h^2 + c^2 (3)
Live weight	-2151.3	-2001.7	-2002.5	**-1994.3***
Egg production performance	-806.3	-789.6	-790.0	**-787.5***
Shin colour score	-64.97	**-62.58***	-62.83	-62.58
Aggression score	-393.2	-363.3	**-363.0***	-361.4
Serum LH	-791.7	-755.1	**-771.8***	-770.8
Serum testosterone	46.84	47.46	**49.01***	49.01
Serum progesterone	**127.5***	128.1	127.5	128.1
Serum prolactin	**642.6***	642.8	643.6	643.6

*P≤0.05

The heritability (h^2) for live weight amounted to 0.30, whereas the animal permanent environment (c^2) contributed 42 % towards the overall phenotypic variance (Table 3). The h^2 for EPP was 0.31, with a corresponding c^2 estimate of 0.29. The animal permanent environment contributed 9%, 29% and 43% respectively, to the variation observed for serum testosterone, LH, and male aggression score, respectively. Although the log likelihood ratios in Table 2 indicated a significant h^2 estimate for shin colour score, this estimate was below 0.05 (Table 3) and smaller than the corresponding standard error.

Table 3. The variance components and ratios for live weight, serum testosterone and LH, male aggression and shin colour, and egg production performance (EPP) measured in breeding ostriches.

COMPONENTS AND RATIOS	TRAIT					
	Live weight	Testosterone	LH	Shin colour	Aggression	EPP
Component						
σ^2_a	57.37			0.0164		129.3
σ^2_{PE}	80.82	0.0265	1.027		1.405	118.8
σ^2_e	54.26	0.2814	2.509	0.4918	1.897	167.7
σ^2_P	192.44	0.3079	3.536	0.5082	3.302	415.9
Ratio						
h^2	0.30 ± 0.11*			0.03 ± 0.04		0.31± 0.16
c^2	0.42 ± 0.10**	0.09 ± 0.04*	0.29 ± 0.05**		0.43 ± 0.06**	0.29 ± 0.16

*P≤0.05; **P≤0.01

The genetic correlation between live weight and EPP were positive, and relatively high (Table 4). Live weight was negatively correlated with EPP on a permanent environmental level and in the medium range, compared to most of the other correlations, which were below 0.30. Environmental correlations were in most cases below 0.15, with the only exception the correlation between serum LH

concentration and EPP (0.40; Table 4). The phenotypic correlations between live weight and EPP (0.17) were significant (P≤0.05) and the phenotypic correlation between LH concentration and EPP (0.16) approached significance.

Table 4. The genetic, permanent environmental, environmental, and phenotypic correlations between behavioural and reproduction traits measured in breeding ostriches, where at least one random effect exceeded 5 % of the overall phenotypic variance.

TRAITS INVOLVED	CORRELATION			
	Genetic	Permanent environmental	Environmental	Phenotypic
Live weight x				
Serum LH		-0.01 ± 0.12	0.05 ± 0.05	0.02 ± 0.04
Serum testosterone		-0.17 ± 0.20	0.09 ± 0.05	0.01 ± 0.04
Aggression score		-0.07 ± 0.15	0.11 ± 0.07	0.02 ± 0.06
EPP	0.67 ± 0.22**	-0.34 ± 0.42	0.03 ± 0.10	0.17 ± 0.08*
Serum testosterone x				
Serum LH		0.20 ± 0.23	0.05 ± 0.05	0.07 ± 0.04
Aggression score		-0.04 ± 0.25	-0.02 ± 0.07	-0.02 ± 0.05
EPP		0.30 ± 0.44	-0.14 ± 0.11	-0.04 ± 0.07
Serum LH x				
Aggression score		-0.07 ± 0.14	-0.04 ± 0.06	-0.05 ± 0.05
EPP		-0.22 ± 0.33	0.40 ± 0.11**	0.16 ± 0.09

*P≤0.05; **P≤0.01

The influence of age

Serum LH levels increased almost linearly with age in males, and ranged between 4.9±0.2 ng.mL^{-1} (2-year old) to 6.8±0.4 ng.mL^{-1} (11-year old). Female serum LH levels, however, did not follow the same pattern (Figure 2). The highest serum LH concentrations were reported for young 2-year old females, and the lowest serum LH concentrations reported for older 11-year old females. The serum LH concentrations reported for females ranged between 0.9±0.2 ng.mL^{-1} and 0.8±0.5 ng.mL^{-1}, for 2-year and 11-year old females, respectively.

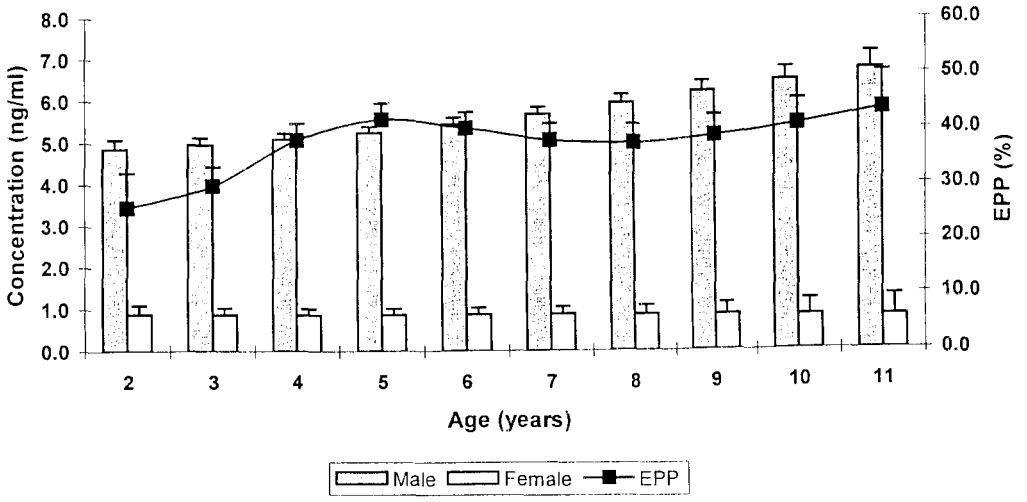

Figure 2. The influence of age on the serum LH levels (mean ± SE) measured for and egg production performance (EPP; mean ± SE) of breeding ostriches.

The serum testosterone concentrations measured in the ostrich males was higher in birds 6 and 8 years, respectively, and varied between 0.7±0.1 ng.mL^{-1} and 0.8±0.1 ng.mL^{-1} (Figure 3). Serum testosterone levels tended (P≤0.10) to decline with an increase in age, and measured 0.3±0.3 ng.mL^{-1} in 11-year old males. There was a tendency (P≤0.10) for the serum testosterone levels of females aged 9 years and younger to be lower, when compared to that measured in the males. Exceptions were in the 3-year old and 9 years and older age groups, where the ostrich females had higher levels of serum testosterone than the males (Figure 3).

Serum prolactin levels ranged between 0.8±0.1 ng.mL^{-1} and 0.9±0.1 ng.mL^{-1}. The serum prolactin levels measured in males ranged between 0.7±0.1 ng.mL^{-1} and 0.9±0.1 ng.mL^{-1} (Figure 3).

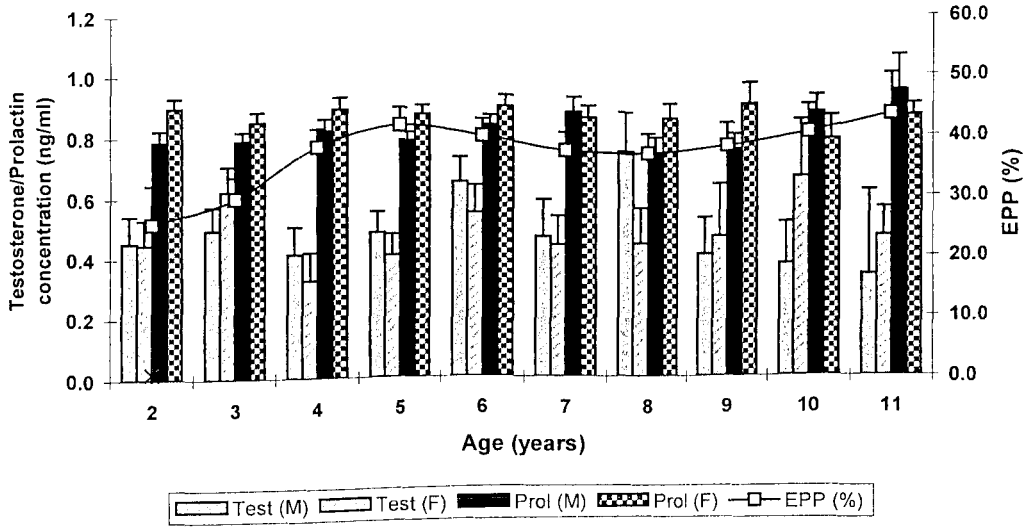

Figure 3. The relationship between age, serum levels of testosterone and prolactin (mean ± SE), and the egg production performance (EPP; mean ± SE) of breeding ostrich males and females.

Serum progesterone levels tended (P≤0.10) to increase with age, and ranged between 0.5±0.04 ng mL^{-1} in 2-year old females, to 0.7±0.1 ng.mL^{-1} in 11-year old females (Figure 4).

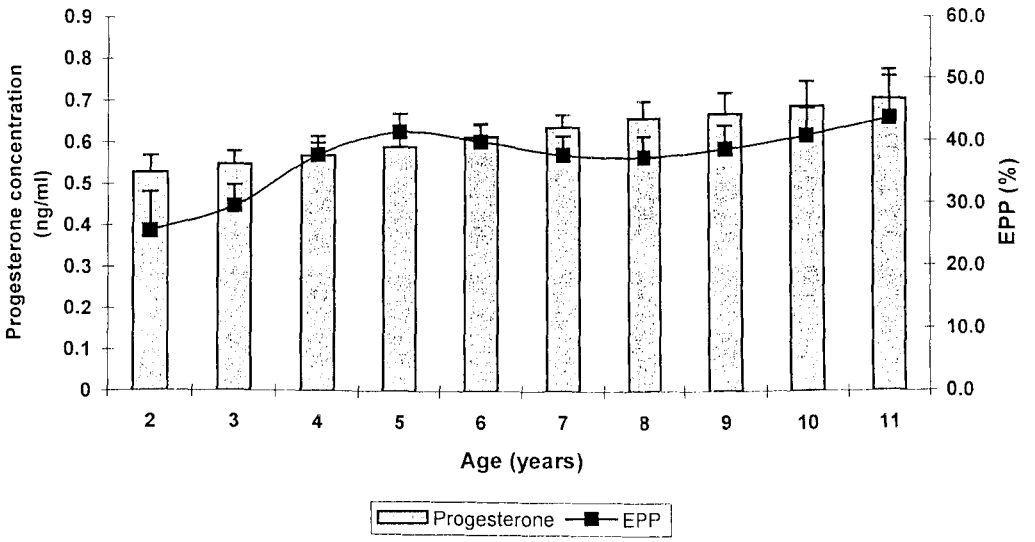

Figure 4. The relationship between age, serum progesterone concentration (mean ± SE) measured in and egg production performance (EPP; mean ± SE) of ostrich females.

The influence of gender and time of sampling

Gender and the time of sampling interacted (P≤0.05) for serum testosterone and LH. Serum testosterone concentrations measured in the breeding males and females were similar at the beginning of breeding in 2000 (Table 5). Female serum testosterone concentrations tended to be higher at the end of breeding in January 2001, whereas it was approximately 30 % higher (P≤0.05) in the males at the beginning of the 2001 breeding season. Male serum LH concentrations tended to increase during the 2000 breeding season, being higher at the end of the season. The opposite trend was observed for the breeding females (Table 5). Male serum LH concentrations measured at the beginning of the 2001 breeding season stayed at almost the same level, while female serum LH concentrations tended (P≥0.05) to be higher at the beginning of the 2001 breeding season (Table 5). The serum progesterone concentrations measured in the ostrich females at the end of the 2000 breeding season were higher (P≤0.05) than that measured at the beginning of the 2001 breeding season (Table 5). The serum prolactin concentrations were not affected by the stage of sampling, but the ostrich females had slightly higher concentrations than the males overall (0.9 ± 0.01 vs. 0.8 ± 0.01 ng.mL^{-1} respectively). The total EPP during the 2001 breeding season was 26 % higher (P≤0.01) than in the 2000 breeding season. Male shin colour scores assessed at the end of the 2000 breeding season were higher (P≤0.01) than scores obtained at the beginning of breeding (Table 5). No significant tendency was found for male aggression scores.

Table 5. The influence of time of sampling on the live weight, egg production performance (EPP), male aggression and shin colour, and the serum hormone levels measured for breeding ostriches. The interaction of gender and time of sampling are presented where significant ($P \leq 0.05$).

TRAIT AND GENDER FOR SIGNIFICANT INTERACTIONS	SAMPLING PERIOD		
	Beginning 2000	End 2000	Beginning 2001
Live weight (kg)	122.0 ± 2.0	123.0 ± 1.0	121.0 ± 1.0
Total EPP (%)	35.5 ± 3.0		44.6 ± 3.0
Shin colour score	7.11 ± 0.08	7.80 ± 0.06	7.21 ± 0.07
Aggression score	5.92 ± 0.19	6.23 ± 0.16	6.20 ± 0.16
Serum testosterone ($ng.mL^{-1}$)			
Male	0.53 ± 0.06	0.51 ± 0.05	0.46 ± 0.05
Female	0.50 ± 0.07	0.57 ± 0.05	0.31 ± 0.05
Serum LH ($ng.mL^{-1}$)			
Male	4.84 ± 0.21	5.49 ± 0.17	5.44 ± 0.18
Female	0.91 ± 0.24	0.66 ± 0.18	0.79 ± 0.17
Serum progesterone ($ng.mL^{-1}$)	0.59 ± 0.05	0.67 ± 0.04	0.50 ± 0.03
Serum prolactin ($ng.mL^{-1}$)	0.82 ± 0.02	0.84 ± 0.01	0.85 ± 0.01

DISCUSSION

Serum levels of testosterone, LH, and progesterone were extremely variable, with the coefficient of variation exceeding 60% in all cases. Serum prolactin was the only exception, showing the least variation. The animal permanent environment contributed significantly ($P \leq 0.05$) to the observed variation in the serum testosterone and LH concentrations in both males and females. Variation in the serum levels of prolactin in both the breeding males and females, and serum progesterone in the breeding females could be attributed to the time of sampling and/or the gender of the animal. The observed variance in male aggression and shin colour (both traits measured only in the ostrich males) could be attributed to the direct additive variation and the permanent environment, respectively. When the correlations of the behavioural and reproduction traits with the various hormones measured are considered, no significant correlations were evident for the various traits considered. The only exception was the correlation between serum LH concentration and the EPP of the breeding females, which was found to be significant and positive on a genetic, environmental, and phenotypic level.

An interesting observation was the sexual dimorphism in terms of live weight, with males being the larger gender. This presents the first evidence of sexual dimorphism in ostriches, as sexual dimorphism is generally not found for live weight in ostriches (Cloete *et al.*, 1998a, b; Bunter *et al.*, 1999; Cloete *et al.*, 1999a, b; Bunter and Graser, 2000; Cloete *et al.*, 2002). The latter studies focussed on slaughter ostriches of up to 14 months of age. Variation in sexual dimorphism in birds is generally attributed to differences in social mating systems, i.e. polygamy leads to the competitive sex

being larger and more ornate (Owens and Hartley, 1998). This is, however, not the case in all bird species, for it can also be associated with gender differences in parental care (Owens and Hartley, 1998). Males and females can also differ in less overt ways such as levels of activity, regulation of body weight and aggression level, with some gender-specific actions associated with systematic dissimilarities in certain parts of the brain (Crews, 1994). The observed dimorphism in size in the breeding males and females in the present study is supported by the fact that the ostrich male invests more time and energy in the raising of chicks than the female does. Characteristic of all ratite species is an increased parental investment by the male, with ostriches being the only group where the female will assist in incubating the eggs, as well as participating in raising of the chicks.

The heritability reported for live weight was in the medium range, while the permanent environment contributed 42% to the overall phenotypic variance. Live weight was positively and correlated ($P \leq 0.05$) with EPP on a genetic level, although a negative correlation was reported with EPP on a permanent environmental level. This negative correlation support previous studies by Van Schalkwyk *et al.* (1996), where a negative correlation was reported for live weight and EPP.

The influence of age

Serum LH concentration measured in the breeding males tended to increase almost linearly with age, with highest levels reported for 11-year old males. This tendency was not observed for the breeding females, for highest concentrations were reported for the 2-year old females and lowest concentrations for the 11-year old females. Serum LH levels measured in the males were considerably higher than those measured in the females, which indicates the extent of involvement of LH in the various reproductive processes. In males, LH is involved in steroid synthesis, as well spermatogenesis, and in females LH is involved in steroid synthesis and ovulation. Serum testosterone levels measured in the breeding ostriches varied between 0 and 6.4 $ng.mL^{-1}$, with levels being higher in males in most cases. Male serum testosterone levels tended to increase with in age, with highest levels reported for males aged 5-6 years. Serum prolactin levels did not differ between the males and females, with the females having slightly higher concentrations than the males. Serum progesterone levels measured in the breeding females increased with age, and ranged between 0.1 $ng.mL^{-1}$ and 2.7 $ng.mL^{-1}$. Age did not influence the level of circulating progesterone and estradiol in domestic chicken hens, with the older hens producing larger but fewer eggs than younger females (Joyner *et al.*, 1987).

The influence of gender and time of sampling

The ostrich males tended to have higher serum testosterone concentrations at the beginning of a breeding season, with the females having higher concentrations at the end of a breeding season. The establishment of territories, attracting breeding partners, and high serum LH levels are possible factors

contributing to the higher serum testosterone levels measured. Several studies have highlighted the relationship between circulating levels of testosterone, reproduction, and territorial behaviour of males during breeding periods in birds [song sparrows (Wingfield, 1984), Northern pintail ducks (Penfold *et al.*, 2000); Korean ring-necked pheasants (Kim and Yang, 2001)]. In male Houbara bustards, the highest testosterone and LH levels were associated with territorial display before onset of lay and after cessation of laying (Saint Jalme *et al.*, 1996). In the current study, serum testosterone levels were slightly lower at the end of breeding, possibly as a result of decreased LH activity due to the ostrich males becoming photorefractory. In male emus, plasma LH and testosterone concentrations were highest during the breeding season, with a gradual decrease in LH and a rapid decrease in testosterone concentrations at the end of a breeding season (Malecki *et al.*, 1998). Males of another ratite species, the North Island brown kiwi (*Apteryx australis mantelli*), had low levels of testosterone during the non-breeding season (<0.18ng.mL^{-1}), which rose to a maximum of 1.9±0.8 ng.mL^{-1} during the peak breeding season. In contrast, testosterone levels in the kiwi females remained lower than 0.10 ng.mL^{-1} (Potter and Cockrem, 1992). Values reported for the ostrich males were intermediate to that reported for the kiwi males, and values reported for ostrich females in this study were higher than that reported for kiwi females. The relative higher testosterone values reported for the ostrich females, when compared to the kiwi females, can possibly be ascribed to the fact that the ostrich female participate in nest-defence as well as incubation behaviour, whereas the female kiwi does not.

Studies of Gomez *et al.* (1998) suggested that complex mechanisms regulate steroid synthesis in the avian ovary. In the avian female, androgens of ovarian origin is also thought to be involved in oviduct maturation (Gilbert, 1971). The extent of follicular development of a female's ovary at the time of sampling could possibly have contributed to the differences observed for beginning and end of season measurements. Follicular development is less extensive at the onset of breeding, and becomes more extensive as a breeding season progresses. Testosterone is synthesised by the theca cells of the avian ovary, and a less extensive follicular development would possibly have the effect that levels were lower than when measured at the end of a breeding season when follicular development is more extensive. At the end of the breeding period, it is possible that the ovary was still sensitive enough to the given daylength and continued to synthesise testosterone to support the oviduct. Testosterone stimulated progesterone synthesis in cultured granulosa cells of Japanese quail (Sasanami and Mori, 1999). Higher female testosterone levels observed at the end of the breeding season might also be the result of a decrease in aromatase activity and estradiol production. Female zebra finches had higher aromatase activity, but lower testosterone levels than males (Vockel *et al.*, 1990). It is thought that the steroid modulation of aromatase activity observed in the latter study may be related to the activation of sexual, aggressive, and nest-building behaviours (Vockel *et al.*, 1990). Tanabe *et al.* (1983) found that plasma levels of testosterone and 17β estradiol were higher in female than in male embryos. Unfortunately, aromatase activity and estradiol levels were not measured in this study.

The lower serum- LH levels measured in the ostrich females at the end of a breeding season can be ascribed to the ostrich females becoming photorefractory, with a subsequent decrease in serum LH concentration. LH levels show a characteristic seasonal cycle in domestic Hungarian geese, with a definite decrease at the onset of photorefractoriness (Hargitai *et al.*, 1993). In rose-ringed parakeets (*Psittacula krameri*), plasma LH decreased at the end of a breeding season (Sailaja *et al.*, 1988). A sharp decline in plasma LH levels was also observed in wild Svalbard ptarmigans (*Lagopus mutus hyperboreus*), a non-photoperiodic breeder (Stokkan *et al.*, 1986). The higher LH levels measured in males at the end of the breeding season can be ascribed to the fact that ostrich males do not react as strongly as ostrich females to a decrease in photoperiod, and that LH synthesis was maintained in order to support the synthesis of testosterone and reproductive behaviour. Males tend to defend their breeding territory throughout the breeding season, which is usually accompanied by territorial aggression. Testosterone levels in brown dipper (*Cinclus pallasii*) males were associated with intensive territory-defence behaviour (Kofuji *et al.*, 1993), and free-living white ibises (*Eudocimus albus*) had higher testosterone levels before a breeding season, and lower levels throughout the remaining stages of the reproductive season, i.e. copulation, incubation and chick rearing.

Degen *et al.* (1994) found that peak LH levels in ostrich males and females coincided with the initiation of an egg production cycle. However, the amplitude for the reported annual cycle was small, when compared to that of other avian species. Malecki *et al.* (1998) reported a similar subdued cycle in LH for emus. Degen *et al.* (1994) measured both testosterone and estradiol levels in ostriches and found peak levels to coincide with egg laying. The values however, were lower than values reported for other seasonally breeding birds (Degen *et al.*, 1994).

Lower serum levels of progesterone were measured before the onset of breeding than at the end of breeding. This can possibly be ascribed to the degree of development on the ovary which is not as extensive as during the later stages of the breeding period. Progesterone is synthesised by the larger yellow or pre-ovulatory follicles in the follicular hierarchy, and a lower number of the pre-ovulatory follicles may contribute to the lower levels of progesterone measured in the study. The presence of a larger number of large follicles towards the end of breeding, partly as a result of failure to be ovulated as a result of lower LH levels may contribute to the higher progesterone levels measured at the end of breeding. In two species of Houbara bustard, seasonal variation in LH and progesterone in females was synchronised with egg production (Saint Jalme *et al.*, 1996). In female white ibises, progesterone levels increased during display and remained high throughout the breeding season, whereas male progesterone levels did not show the same seasonal pattern, and were more variable (Heath *et al.*, 2003).

Prolactin is a pituitary peptide hormone that is involved in the development of broodiness and regression of gonads, with marked seasonal changes in circulating levels of prolactin occurring in most bird species studied. Prolactin levels were measured in a few cases in ostriches, with prolactin level

being higher in an incubating male (Skadhauge and Dawson, 1999). In this study, eggs were collected on a daily basis that suppressed the development of broody behaviour in both males and females. It can thus be accepted that the range reported for breeding ostriches in this study (0.80 - 0.87 $ng.mL^{-1}$) represents low values for breeding, non-incubating ostriches, and that levels measured in incubating ostriches could probably be higher. Malecki *et al.* (1998) found prolactin levels to increase in non-breeding emu males, with the highest levels coinciding with a rapid decrease in testosterone level, which marked the end of the breeding season. Serum prolactin concentration peaked several weeks after the peak of the breeding season (Malecki *et al.*, 1998). Prolactin levels measured in male emus varied according to the number of eggs present in the nest, i.e. between 1.2±0.5 $ng.mL^{-1}$ (6 eggs in the nest) and 6.6±1.8 $ng.mL^{-1}$ (8 and more eggs in nest), respectively (Van Cleeff *et al.*, 1999). In mallards, prolactin levels were higher during incubation, and LH levels remained high for longer than that observed in females (Goldsmith and Williams, 1980).

Laying and non-laying canvasback duck (*Aythya valisineria*) females were compared in terms of levels of serum LH, prolactin, estradiol, and progesterone during the pre-laying, laying and incubation periods (Bluhm *et al.*, 1983). Lower levels of LH, prolactin, estradiol, and progesterone were recorded for non-laying females during the respective three periods. Bluhm *et al.* (1983) suggested that the lack of reproduction could be ascribed to the failure of the hypothalamus to release GnRH hormones and not the inhibitory effect of prolactin, for injection with LHRH had the effect that serum LH levels were significantly elevated. In domestic hens, plasma LH levels were found to be significantly higher in hens with a high rate of egg production, when compared to hens with a poor rate of egg production (Wilson, 1978). Serum LH levels varied between 1.7±0.3 $ng.mL^{-1}$ and 2.6±0.7 $ng.mL^{-1}$ for arrested and laying turkey hens, respectively, and progesterone levels between 1.8±0.2 $ng.mL^{-1}$ and 4.7±1.3 $ng.mL^{-1}$ for normal and arrested laying turkey hens, respectively (Liu *et al.*, 2001).

Domestication may have an influence on the hormonal cycles of certain avian species, which may lead to certain reproductive problems (Paster, 1991). Seasonal changes in the production of the steroid hormones seem to control the seasonal changes in sexual behaviour of birds, with influences on e.g. courtship, copulation and territorial displays (Gahr, 2001). The sex steroids may influence the development and expression of sexual behaviour by direct actions on the central nervous system (Schultz and Schlinger, 1999). Selecting ostriches for production performance should not have a negative impact on the hormonal profiles of females. Turkey hens selected for high growth had multiple ovulations, and low plasma concentrations of oestrogen, with a correlated response in the steroidogenic capacity of the ovarian tissue (Buchanan *et al.*, 2002).

CONCLUSIONS

The only significant correlation reported was between serum LH concentration and the egg production performance of the ostrich females, on an environmental level. The fact that the blood samples were collected throughout the day in this study and not at a specific time may have affected the serum LH concentrations measured in this study. It is known that LH secretion follow a diurnal pattern in female birds, and it is possible that peak levels were measured in some females and low levels on other may have contributed to the observed variation.

Gender and time of sampling interacted for serum LH and testosterone concentrations, implying that the extent of gonad differentiation and the degree of photorefractoriness the breeding ostriches were experiencing influenced the serum testosterone and LH levels measured at the beginning and end of breeding. The environment thus played a major role in the regulation of reproduction and reproductive performance of the breeding birds. The maintenance of territorial aggression in males at the end of a breeding season, when lower levels of serum testosterone were measured, imply that factors other than LH may contribute to the maintenance of reproductive behaviour at the end of a breeding season. The potential of, and the extent to which the reproductive cycles can be manipulated by means of exogenous gonadotropins and steroid hormones to improve the reproductive performance of breeding ostriches under commercial farming conditions warrants studies to determine baseline values of the gonadotropins and steroid hormones for breeding ostriches.

Future studies need to focus on establishing the circulating levels of LH, testosterone, estradiol, progesterone, as well as prolactin, in ostriches during non-breeding and breeding seasons, and also on a diurnal basis. This may provide better insight into the endocrine control of reproduction in both males and females, and how this is linked to reproductive behaviour exhibited by both genders. This will also enable the potential application of reproductive technologies, as in the case of breeding emu male and females, to improve overall reproduction efficiency of ostriches in commercial breeding systems (Williams *et al.*, 1998).

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Chapter 7

Preliminary results on the use of diagnostic ultrasonography as a management tool to quantify egg production potential in breeding ostrich (*Struthio camelus australis*) females[1]

Abstract

An ostrich breeding flock, joined as individual breeding pairs (n=136 pairs), was used to investigate the possibility of diagnostic ultrasonography as a method to predict the reproductive performance of ostrich females during a breeding season. Follicular activity was easily detected and quantified using diagnostic ultrasonography. One to eight follicles were recorded in 25% of the females when scanned at the beginning of the nine-month breeding season. At the end of the breeding season, one to three follicles were observed in 28.7% of the females. Females in which follicular activity was observed before paired came into production earlier than their contemporaries in which no follicles were observed, with the mean (± SE) number of days to the production of the first egg being 22.3 ± 12.5 and 87.4 ± 7.2 days, respectively. Females in which follicular activity was observed with scanning at the beginning of the breeding season, produced on average 181% more eggs during the first month of the breeding season ($P<0.01$) when compared to females in which no follicular activity was observed (6.67 ± 0.70 vs. 2.37 ± 0.41 eggs). Egg production over the first two months of breeding and over the entire breeding season were similarly affected ($P < 0.01$), with the mean number of eggs produced over the first two months of the breeding season being 14.7 ± 1.5 for females with observed follicular activity and 7.4 ± 0.9 eggs for females with no observed follicular activity. Females in which follicular activity was observed at the end of the breeding season produced on average 108% more eggs ($P<0.01$) during the last month of the breeding season than females in which no follicular activity was observed (2.77 ± 0.43 vs. 1.33 ± 0.27 eggs). There was a tendency ($P=0.06$) for egg production over the last two months to be similarly affected (6.10 ± 0.85 vs. 4.19 ± 0.54 eggs). No relationship with egg production over the entire breeding season was found for the end-of-the-breeding-season observations. Diagnostic ultrasonography can thus be used as a management tool to identify reproductively healthy ostrich females and also females with a higher egg production potential over a period of two months after or prior to assessment. Future studies should focus on the development of the technique to predict reproductive performance over entire breeding seasons for selection purposes.

INTRODUCTION

Egg production in commercially farmed ostriches were shown to be extremely variable (Van Schalkwyk *et al.*, 1996; Cloete *et al.*, 1998). Egg production over an 8-month breeding season was shown to vary from zero to more that 100 eggs per female. It is estimated that 70 – 80 % of ostrich eggs produced in South Africa originate from breeding flocks in free-ranging systems. Male and

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female ostriches are commonly kept in flocks with a size of 50-100 birds at a sex ratio of 6 males to 10 females (Swart, 1986). Eggs are laid in communal nests and several females may visit and lay in a single nest. Commercially viable methods for the identification of eggs according to the female that produced them are presently not in place. It is therefore difficult to determine either egg production on a per female basis or the parentage of the eggs produced. It is, however, most likely that the same large variation in egg production observed under pair-breeding conditions will prevail under flock mating conditions.

Breeder birds are fed a complete breeder diet during the breeding season, with the average daily feed consumption of ostrich females estimated at 2.5 – 3.0 kg feed (*ca.* 3% of live mass) per bird per day. It is evident that the maintenance of breeding birds is a major source of expenditure for commercial breeders and the maintenance of females with a poor egg production would be uneconomical. The repeatability of egg production in ostriches were shown to be fairly high ($t \approx 0.45$) (Van Schalkwyk *et al.*, 1996; Cloete *et al.*, 1998), meaning that female ostriches would tend to achieve similar rankings for egg production both within and across production seasons. It is likely that feed costs may be reduced substantially without a marked reduction in egg production if poor producers in breeding flocks can be identified and culled. No practical system for this purpose is in place at present. The success of diagnostic ultrasonography in the determination of reproduction success in for example domesticated farm species (Wani, 1981; Fowler and Wilkins, 1982, 1984; Owens and Armstrong, 1985; Fukui *et al.*, 1986; Grace *et al.*, 1989; Kilgour, 1992; Knopf *et al.*, 1989; Lambrechts and Pfister, 1999), llamas (Bourke *et al.*, 1992) and camels (Skidmore *et al.*1996) triggered the idea of using diagnostic ultrasonography as a tool to assess the reproductive fitness of the ostrich under commercial farming conditions.

Against this background we investigated the feasibility of diagnostic ultrasonography for the quantification of egg production potential of adult breeding ostrich females. This paper presents preliminary findings with regard to the method as well as the relationship of the ultrasonograms with female egg production.

MATERIALS AND METHODS

Animals, management and recordings

The ostrich breeding flock (n=136 breeding pairs) at the Little Karoo Agricultural Development Centre, Oudtshoorn, was used in the study. The flock, its origin and management are well documented by Van Schalkwyk (1998) and Van Schalkwyk *et al.* (1996). The entire breeding flock was subjected to a 3-month rest period, before paired in 0.25 ha camps. The breeding birds received a balanced breeder diet at 2.5kg/bird.day and had free access to clean drinking water. The breeding season commenced on 24 May 1999 (day 1) and ended on 29 February 2000 (day 281). The ages of the females in this study ranged between 2 and 13 years.

All females were subjected to diagnostic ultrasonography at the beginning and end of the 1999/2000 breeding season. A Pie Medical 100LC Vet scanner fitted with a 3.5/5.0 MHz dual frequency curved array transducer (Philips South Africa (Pty) Ltd.) was used for this purpose. Females were scanned at a frequency of 3.5 MHz and ultrasound coupling gel was used during each scanning procedure to ensure sufficient lubrication between the surface of the transducer and the body of a female. The transducer was placed on the ventral, non-feathered area, caudal to the right thigh of each female. During each scanning procedure, as many as possible follicles in the scanning field (i.e. a single plane image) were captured and stored on disc for later analysis. Each ultrasonogram was printed out at 100% of the size of the on-screen version and was used to count the number of follicles and to measure follicle diameter. The presence and number of discernable follicles were noted for each individual ultrasonogram, and the average diameter (in millimeters) of the observed follicles was measured in each case.

Eggs produced by each female were collected daily during the afternoons and were recorded on an individual basis for each female.

Statistical analysis

Monthly egg production was averaged for all breeding females. Average egg production of females with different numbers of follicles discernable at the beginning of the breeding season were calculated for the first month of production, the first two months of production, and the entire nine month breeding period. One-way analysis of variance procedures were used for this purpose (Snedecor and Cochran, 1967). The average subsequent egg production of females where ≥ 1 follicle was observed was also compared to that of females where no follicles could be discerned. The same basic procedure was followed for relating ultrasonograms at the end of the breeding season to egg production during the last month of breeding, the last two months of breeding and during the entire breeding season.

RESULTS

Follicles (or ova) of different sizes and stages of maturity were detected in the ostrich females during scanning. The different views obtained during the imaging are depicted in Figures 1 and 2 that represent ultrasonograms of ovaries with an extensive degree of follicular development. A developing follicle appears as a round anechoic image with a slightly more hyperechoic central area in the middle of the follicle. This hyperechoic area appears to be the developing follicle's attachment to the ovary. A normal ostrich ovary will contain a number of follicles of different sizes, ranging from 12.6mm to 120 mm in diameter. The extent of follicular development of an ovary determined the number of follicles that could be captured in each field.

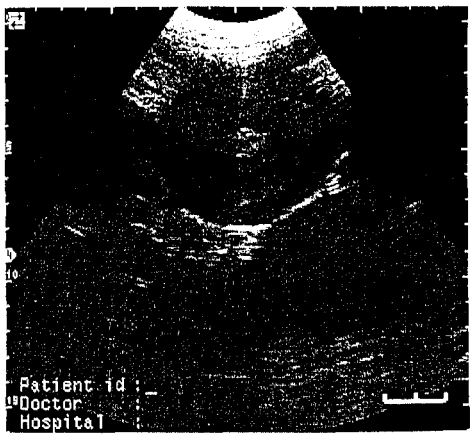

Figure 1. An ultrasonogram of the ovary of a breeding ostrich female, with five follicles distinguishable in the ultrasonogram. The largest follicle has a diameter of 63mm.

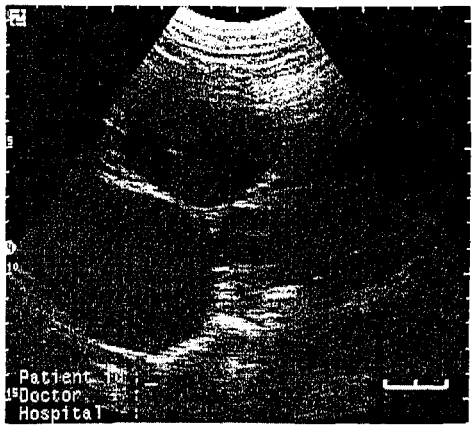

Figure 2. An ultrasonogram of the ovary of a breeding ostrich female, with five follicles distinguishable in the ultrasonogram. The largest follicle has a diameter of 58mm and the smallest follicle a diameter of 18mm.

One or more follicles could be discerned in 34 females (25%) at the beginning of the breeding season and in 39 females (28.7%) at the end of breeding season. At the beginning of the breeding season, females of three years and younger generally showed considerably less follicular development than females aged 4 years and older, with no follicles observed in 2-year old females (Figure 3). The number of follicles observed was unrelated to female age when assessed at the end of the breeding season.

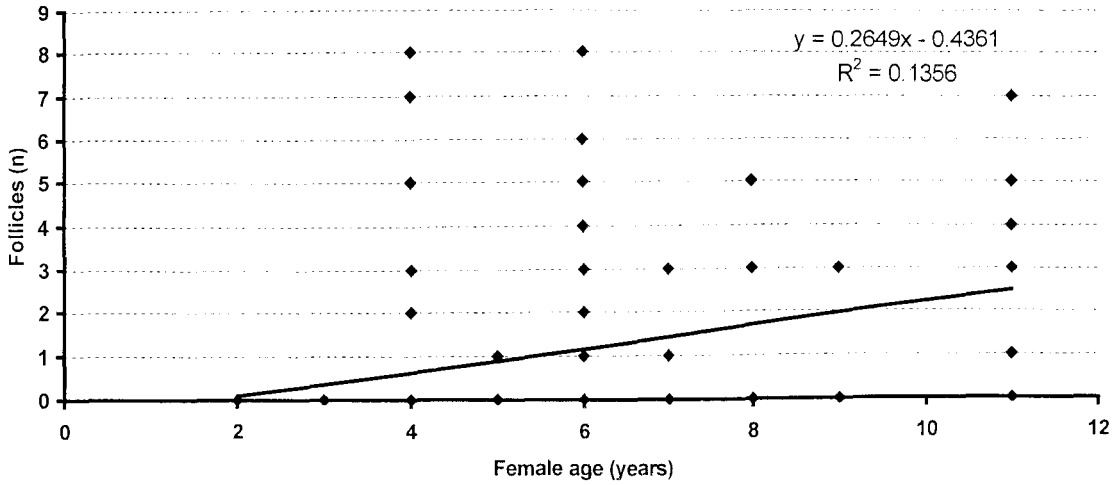

Figure 3. Relationship between female age and number of follicles noted with diagnostic imaging at the beginning of the 1999/2000 breeding season.

Egg production over the first month and the first two months generally was greater ($P<0.05$) in females where two or more follicles were observed during scanning at the beginning of the breeding season. When females where at least one follicle was observed (Group A, n=34) were pooled, this group deviated from those where no follicles were observed (Group B, n=102) (Table 1). Group A came into production earlier than Group B, with the mean (± SE) period in days from the commencement of the breeding season to the production of the first egg being 22.3 ± 12.5 and 87.4 ± 7.2 days, respectively. Group A produced on average 181 % more eggs during the first month of breeding (mean ± SE) than Group B ($P \leq 0.01$; 6.7 ± 0.7 vs. 2.4 ± 0.4 eggs). Egg production over the first two months of breeding and over the entire breeding season were similarly affected ($P<0.01$). The mean number of eggs produced over the first two months of the breeding season were 14.7 ± 1.5 for Group A, compared to 7.4 ± 0.9 eggs produced by Group B. Corresponding mean egg production was, respectively, 48.5 ± 4.4 and 35.3 ± 2.5 eggs over the breeding period of 281 days.

Table 1. Mean (± SE) egg production during the first month, first 2 months, and for the entire breeding season, classified according to the number of follicles observed at the beginning of the breeding season.

NUMBER OF FOLLICLES OBSERVED	NUMBER OF OBSERVATIONS	EGG PRODUCTION		
		First month	First 2 months	Entire season
0	102	2.4 ± 0.4	7.4 ± 0.9	35.1 ± 2.5
1	4	2.8 ± 2.0	7.5 ± 4.3	41.3 ± 12.7
2	4	7.5 ± 2.0	16.5 ± 4.3	52.0 ± 12.7
3	12	6.3 ± 1.2	14.3 ± 2.5	43.4 ± 7.4
4	3	10.0 ± 2.4	23.3 ± 5.0	66.0 ± 14.7
5	5	9.0 ± 1.8	16.6 ± 3.9	42.8 ± 11.4
6 +	6	6.0 ± 1.7	13.2 ± 3.5	53.3 ± 10.4

The number of eggs produced during the last month of the breeding season, during the last two months of the breeding season and during the entire breeding season in relation to the number of follicles observed at the end of the breeding season are presented in Table 2. Fewer follicles, but with a larger diameter, were observed at the end of the breeding season. Females where follicular activity was observed at the end of the breeding season produced on average 108% ($P<0.01$) more eggs during the last month of the breeding season than females where no follicular activity was observed (2.8 ± 0.4 vs. 1.3 ± 0.3 eggs). There was a tendency ($P=0.06$) for egg production over the last two months to be similarly affected (6.1 ± 0.9 vs. 4.2 ± 0.5 eggs). Egg production over the entire season was unrelated ($P \geq 0.05$) to the number of follicles observed at the end of the breeding season.

Table 2. Mean (± SE) egg production during the last month, the last two months, and during the entire breeding season, when classified according to the number of follicles observed at the end of the breeding season

NUMBER OF FOLLICLES OBSERVED	NUMBER OF OSTRICHES	EGG PRODUCTION		
		Last month	Last 2 months	Entire season
0	97	1.3 ± 0.3	4.2 ± 0.5	37.4 ± 2.6
1	18	3.3 ± 0.6	7.8 ± 1.3	40.7 ± 6.1
2	8	2.4 ± 1.0	4.8 ± 1.9	44.8 ± 9.2
3 +	13	2.2 ± 0.7	4.5 ± 1.5	38.0 ± 7.2

The number of follicles observed was negatively correlated ($P<0.01$) with mean follicle diameter, with the correlation between number of follicles observed and mean follicle diameter amounting to –0.69 at the beginning of the breeding season and –0.72 at the end of the breeding season. The phenotypic correlation between the number of follicles observed at the beginning of the breeding season and that observed at the end of the breeding season was relatively low ($r=0.20$; $P<0.05$).

DISCUSSION

Follicle activity in ostrich females was clearly discernable with diagnostic ultrasonography. During imaging, it is impossible to capture all possible follicles in one image field and in each case, as many follicles as possible were captured and stored on disc for later analysis. Because only a limited number of follicles (i.e. less than what actually could be observed in each female) could be captured in each ultrasonogram, each female was given a score (see Chapter 8) in terms of the extent of ovary development. These data, however, are not presented in this paper but will be discussed in a subsequent paper. In this study, females were scanned on the right side only. Although the ovary is located in the left half of the female's body, better ultrasonograms have been obtained in most cases from the right hand side. In a follow-up phase of this study, females will be scanned on both sides to determine the correlation between ultrasonograms obtained from the left and right sides of a female.

The number of follicles observed was related to the egg production of individual females over the short term, i.e. within one or two months of assessment at the beginning of the breeding season. Selection of females based on the visibility of one or more follicles at the onset of the breeding season would consequently have resulted in marked improvements in egg production over the first two months following assessment. A similar conclusion was reached with regard to scanning at the end of the breeding season. Although lower levels of egg production were attained during the last month of the breeding season, it was still possible to identify females with higher levels of egg production over the last month prior to scanning. The ability to accurately identify high producing individuals over a longer term (> two months), however, was compromised to an extent. This can be ascribed to the fact that egg production of ostrich females start to decline at the specific time of year, i.e. end of January, when the end of season scanning was performed.

This study is in agreement with previous reports that egg production in ostriches is highly variable (Deeming, 1996; Van Schalkwyk *et al.*, 1996; Cloete *et al.*, 1998). The wide variation in egg production within female age groups is clearly illustrated in Figure 3. The fact that no follicles were observed in the 2-year old females that were in their first breeding season, forced the regression of number of follicles scanned on female age in a positive direction. The absence of follicular activity in two-year old females at the beginning of the breeding season and their consistently lower egg production can probably be ascribed to the age at which females become reproductively mature. Culling of ostrich females solely on egg production and / or ultrasonograms is therefore likely to discriminate against young females under circumstances where flock breeding is practiced, especially when their age is unknown. Previous results have shown that egg production of ostrich females at a young age predicted subsequent egg production fairly well (Van Schalkwyk *et al.*, 1996) and that egg production generally increased with age to reach a maximum at approximately nine years of age (Cloete *et al.*, 1998). It is advisable to take this information into account under conditions where ostrich females of unknown age are scanned for egg production.

The correlation between ultrasonograms obtained at the beginning and end of the breeding season was relatively low (r=0.20). Factors contributing to this observation is that older, higher producing birds entered a natural rest period at the end of the breeding season, and the 2-year old females only came into production towards the end of the breeding season.

Two aspects remain as cause for concern with regard to the practical application of the ultrasonography technique in ostriches. First, in the present study only a small percentage of females that were scanned actually showed follicular activity at assessment. It is clearly not viable to retain such a minority of the total number of females available when breeding under commercial conditions. The only possible exception may be a breeding operation with an almost unlimited access to resources in terms of female breeding birds. Given the high cost of growing and finishing breeder birds, such conditions are unlikely. Second, the ability to predict egg production over the entire breeding season was limited, especially when the females were scanned at the end of the breeding season. Based on the results in the present study, diagnostic ultrasonography can be

used to identify reproductively healthy females but cannot be used as a selection tool to cull females at the end of a breeding season.

In order for the practice of diagnostic ultrasonography to become a viable proposition in the management of commercial ostrich breeding flocks, these issues need to be addressed. It is also necessary to ensure that a greater proportion of the breeding females is in a stage of ovulation when scanning is performed. The objective should thus be to find ways of strategically stimulating ostrich females to higher levels of sexual activity at the beginning of a breeding season. This could be done by for example, delaying the onset of the breeding season, when natural sexual activity ought to be higher. Alternatively, other practices like teasing and flushing that were found to enhance sexual activity in other livestock species (Pearce and Oldham, 1984; Scaramuzzi and Campbell, 1990; Signoret, 1990) and ostriches (Chapter 3) can also be considered. These practices are also widely advocated in the ostrich industry (Swart, 1986; Lambrechts *et al.*, 1998).

CONCLUSIONS

Diagnostic ultrasonography can be used to identify reproductively healthy females and can be used as a diagnostic tool to identify potentially high producing females at the beginning of a breeding season. Certain issues, however, needs to be addressed before it can be applied on a large scale in commercial ostrich enterprises. In the present study, only a small proportion of females displayed follicular activity when scanned, making these results less suitable for selection purposes under most conditions where only small numbers of breeding females are available. It was also complicated by the fact that younger females were less likely to show follicle development.

Farming practices to maximize and synchronize ovulation and egg laying cycles at the commencement of the breeding season should also be investigated. The investigation of teasing and flushing effects on ostrich egg production could not only provide clarity with regard to the importance of these practices to efficient production systems, but could also prove to be indispensable for the practical application of diagnostic ultrasonography in the broader industry. It is also necessary to address the need to improve long term predictions in terms of egg production for ostrich females, based on a single scanning measurement. These aspects should be addressed in the further development and adaptation of diagnostic ultrasonography as a management tool to improve reproduction efficiency in commercial ostrich breeding flocks.

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Chapter 8

Genetic variability of ultrasound scanning parameters and egg production traits in breeding ostrich (*Struthio camelus* var. *australis*) females[1]

Abstract

The use of ultrasound scanning as a management tool to identify reproductively healthy ostrich females and to determine the genetic variability of ultrasound parameters was investigated during four consecutive breeding seasons. A total of 1334 records was analysed, with follicle diameter only measured in 900 cases where follicular activity was observed. Total egg production records were analysed in 491 cases where scanning was performed before the onset and at the end of the breeding seasons. Heritabilities were low, but significant ($P \leq 0.05$) in the case of follicular classification score (0.07) and number of follicles observed (0.06), and increased in magnitude as a breeding season progressed. Overall egg production and ultrasound recordings at the beginning of a breeding season were highly correlated (0.41±0.06), suggesting that females may be selected on partial production records for use in ostrich breeding programmes. Ultrasound parameters were closely related to short-term egg production on a genetic level, indicating the potential of the technique to identify females that start producing earlier in a breeding season. Duration of lay after commencement of a breeding season was also strongly associated with reproductive success. Results also indicate that age of ostrich females needs to be considered when performing ultrasound scanning when breeding selection is based on phenotypic data alone to prevent discrimination against young breeding female ostriches.

INTRODUCTION

Commercial breeding systems for ostriches can range from extensive free-range flock mating to intensive pair or trio breeding systems. Ostrich farming in South Africa is mostly based on open range flock mating systems, as well as breeding pairs and trios, with almost 70% of ostrich eggs produced in flock breeding systems. In flock breeding systems, more than one female contributes to a single nest and females may also lay in more than one nest (Jarvis *et al.*, 1985; Lambrechts *et al.*, 2002a). This behaviour of ostrich females complicates the identification of females with low or no egg production.

Commercial ostrich breeding systems are characterised by low reproductive performance, with egg production varying between 0 and 100 eggs produced per female in an 8-month breeding season (Van Schalkwyk *et al.*, 1996; Van Schalkwyk, 1998; Deeming and Ar, 1999; Cloete *et al.*, 2002a).

[1] Submitted for publication in Livestock Production Science, with co-authors **D Swart, SWP Cloete, JPC Greyling** and **AP Pfister**

Low average production performance of ostrich females and the extreme variation in egg production is major constraints in the reproduction efficiency of ostrich breeding systems. Egg production in ostriches has been found to be highly repeatable ($t \approx 0.45$), implying that females with a certain egg production ranking would tend to achieve similar rankings in subsequent breeding seasons (Cloete *et al.*, 1998). Feeding costs of breeding ostriches contribute up to almost 80% of input costs, and the identification and culling of poor or low producers has the potential to reduce feeding costs substantially in a commercial ostrich breeding system (Brand, 2002).

The ostrich female's reproductive tract consists of the single left ovary and accompanying left oviduct, and lies ventral to the cranial part of the left kidney and dorsal to the abdominal air sacs (Duerden, 1912; Bezuidenhout, 1986; Fowler, 1991; Hicks, 1993; Soley and Groenewald, 1999). As in poultry, the ostrich female's reproductive tract is completely internalised, making visual evaluations of the reproductive status of the female difficult (Jull, 1952; Gilbert, 1971; Gilbert, 1979; Solomon, 1983). The reproductive status of the female or the extent of follicular development on the ovary can be determined if a female is killed, and thus only lifelong egg production can be inferred. This, together with the cost of sacrificing breeding females, necessitated the development of a commercially viable technique for the identification of poor or non-producing ostrich females and the determination of the reproductive potential of ostrich females for selection purposes.

The success of ultrasound scanning as a tool to assess reproductive fitness in humans and animals is well documented (Allan and Meredith, 1981 (dogs); Fowler and Wilkins, 1982 (sheep); Sengoku *et al.*, 1985 (humans); Bourke *et al.*, 1992 (llamas); Boyd, 1995 (cattle); Skidmore *et al.*, 1996 (camels); Casares *et al.*, 1997 (tortoises); Krautwald-Junghans *et al.*, 1998; Shaffer *et al.*, 1998 (rhinoceros); Hildebrandt *et al.*, 2000). In domestic chickens, ultrasound has been used successfully to identify individual males and females with acceptable testicular development and well-formed, normal follicular hierarchies (Kirby *et al.*, 2001; Melnychuk *et al.*, 2002). In ostriches, Lambrechts and Pfister (1999) and Lambrechts *et al.* (2002a) investigated the potential of ultrasound scanning as a management tool to predict the reproductive performance of ostrich females. Ultrasound scanning could be used successfully to identify reproductively healthy females, as well as females with a higher egg production during the first two months after or prior to scanning. Bronneberg and Taverne's (2003) findings supported the potential of the technique as a management tool to identify actively producing ostrich females.

The application of genetic selection as a tool to improve reproduction and production performance has been well illustrated in livestock industries, with the poultry industry mostly used as a model to demonstrate the possible benefits of selection in the ostrich industry. The ostrich industry, however, has a short history in terms of selection. Literature reports on genetic parameters for ostriches are scarce, and are in general agreement with genetic correlations and selection responses reported for poultry (Petitte and Davis, 1999; Van Schalkwyk *et al.*, 1995; Van Schalkwyk *et al.*, 1996; Horbañczuk and Sales, 1999; Cloete *et al.*, 1998; 2002a; 2002b; Bunter, 2002). The use of poultry genetic parameters is, however, not ideal, given the basic physiological

differences between poultry and ostriches and the different management conditions under which these species are maintained. The long generation interval in ostriches and the age at which sexual maturity is attained suggest that genetic progress will be slower than that reported for commercial poultry species. Large numbers of breeding birds with unknown pedigree and lack of production records infer the hazard of inbreeding depressions (Hicks-Aldredge, 1993; Badley, 1997; Deeming and Ar, 1999). The determination of genetic effects in ostriches is complicated by the fact that paternal and maternal effects are confounded. The fact that the same male is often mated repeatedly with the same female under the same management conditions also hampers the partitioning of the direct and maternal genetic effects (Cloete *et al.*, 1998; Bunter, 2002).

When the successes that have been achieved in poultry are considered, it is evident that there is considerable scope for improvement of egg production in commercial ostrich breeding programs (Nestor *et al.*, 1969; Gowe and Fairfull, 1985; Poggenpoel *et al.*, 1996). Cloete *et al.* (2002a) recently reviewed the potential role of genetic improvement in the ostrich industry. The review reported estimates for several reproduction-related parameters, but no studies have yet been conducted on the genetic variability of ultrasound scanning parameters. The aim of this study was therefore to establish genetic and environmental estimates for ultrasound scanning parameters for ostrich females that may assist the commercial ostrich farmer in making sound selection decisions.

MATERIAL AND METHODS

Experimental animals and management

An ostrich breeding flock (n=136 breeding pairs) maintained at the Little Karoo Agricultural Development Centre outside Oudtshoorn, South Africa, were used in the study. The management of the breeding flock is well documented (Van Schalkwyk *et al.*, 1996; Bunter and Graser, 2000). The ages of females used in the study ranged between 2 and 13 years. Data were recorded during four consecutive (1999/2000, 2000/2001, 2001/2002 and 2002/2003) breeding seasons.

The breeding birds were maintained in single-sex flocks during the 4-month rest period preceding breeding (approximately February to May). Two weeks prior to being paired as breeding pairs in 0.25ha camps, males and females were placed in adjoining camps (i.e. teasing) and were fed a flushing diet (Lambrechts *et al.*, 2002b). Females were introduced to their breeding camps before the males.

During the 1999/2000 and 2000/2001 breeding seasons, 90 of the 136 breeding pairs were subjected to a feeding trial that investigated the influence of different energy and protein levels on the production of the males and females. During the 1999/2000 breeding season, the three energy levels were 8.5 MJ ME/kg DM, 9.5MJ ME/kg DM and 10.5MJ ME/kg DM. The three protein levels were s13.5%, 15.0% and 16.5%. During the 2000/2001 breeding season, the energy levels were

7.5MJ ME/kg DM, 8.5MJ ME/kg DM and 9.5MJ ME/kg DM. The three protein levels were 10.5%, 12.0% and 13.5%.

During the 2000/2001 and 2001/2002 breeding seasons, 63 of the 136 breeding pairs were subjected to a mid-season rest period of 6 weeks. During this rest period, the males and females were visually separated and placed onto a maintenance diet for a period of four weeks. Two weeks prior to being joined again, the males and females were subjected to flushing and teasing, as described above.

During the 2002/2003 breeding season the breeding flock were divided into five treatment groups. Group 1 (n=25 breeding pairs) was not subjected to a rest period. Breeding pairs in this group were paired immediately after being treated for external and internal parasites, and vaccinated for Newcastle Disease. They were separated on 8 April 2003. Groups 2 (n=25 breeding pairs) and 3 (n=25 breeding pairs), after being subjected to flushing and teasing, were joined on 1 May 2002 and separated on 19 February 2003. Group 3 was subjected to a series of simulated production and rest periods. The duration of each production and rest period was approximately 10 weeks and six weeks, respectively. During each rest period, males and females were separated visually and placed on a maintenance diet. During the last 2 weeks before being joined again, males and females were subjected to flushing and teasing, as described above. Groups 4 (n=25 breeding pairs) and 5 (n=25 breeding pairs), after being subjected to flushing and teasing, were joined on 1 July 2002 and separated on 8 April 2003. Group 5 was subjected to a series of production and rests cycles, with treatment during each rest period the same as for Group 3.

Diagnostic imaging

A Philips Pie Medical Scanner 100LC (Pie Medical Equipment BV, Maastricht, The Netherlands), fitted with a 3.5/5.0 MHz dual frequency curved array transducer, was used to obtain the diagnostic images. Females were scanned at a frequency of 3.5 MHz and a hypoallergenic water-based ultrasonic coupling gel (*Ultra/Phonic Conductivity Gel*, Pharmaceutical Innovations Inc., Newark, New Jersey, 07114, USA) was used to ensure sufficient contact between the transducer surface and the body of the female. The transducer was placed on the ventral non-feathered area, caudal to the right thigh of a female (Lambrechts and Pfister, 1999). During each evaluation, as many as possible follicles were captured in each scanning field, i.e single plane image (Figure 1), and stored on computer for later analysis. Each sonogram obtained was used to calculate the number of follicles and to measure follicle diameter (Lambrechts *et al.*, 2002a).

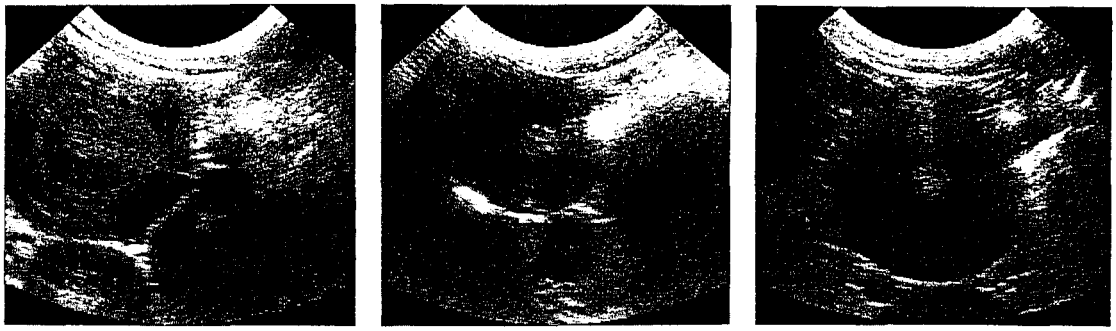

Plate 1. Sonograms obtained during ultrasound scanning of ostrich females.

As it was impossible to capture all follicles in a given sonogram, each female was assigned a follicular classification score (FCS), depending on the number of follicles observed during each examination. The follicle classification scores assigned to each female are set out in Table 1.

Table 1. Follicle classification scores assigned during ultrasonic examination of breeding ostrich females

Number of follicles observed during examination	Follicle classification score
No follicles observed	0
1 to 3 follicles observed	1
4 to 6 follicles observed	2
7+ follicles observed	3

Ultrasonograms were obtained at the beginning and end of each respective breeding season (Lambrechts *et al.*, 2002a). In addition to the pre- and post-season scanning, approximately half of the breeding flock's females were subjected to scanning before and after a forced mid-season rest during the 2000/2001, 2001/2002 and 2002/2003 breeding seasons. During the 2002/2003 breeding season, the females used in the study were subjected to ultrasound scanning on both the left and right sides to determine the correlation between the FCS and the number of follicles observed in each sonogram.

Data recorded

Eggs laid by the breeding females were collected daily during the afternoons and recorded for each female throughout each breeding season. Egg collection, handling, disinfecting and incubation procedures were performed as documented by Van Schalkwyk (1998). The number of eggs produced were expressed as egg production percentage (EPP, Van Schalkwyk *et al.*, 1996) which were calculated using the following equation:

EPP = [(number of eggs produced)/(0.5Xnumber of production days)] X 100

Statistical analysis

The ASREML program (Gilmour *et al.*, 1999) was used for the estimation of the fixed effects, and to derive variance components for the respective follicle and egg production parameters in univariate analyses. Although fixed effects of treatments within production years (as defined previously) were included in the model of analysis, these effects are not presented or discussed in detail because they formed part of separate studies, to be reported elsewhere. Initially, all available scanning and egg production records were incorporated (numbering 1334 observations in total). Recordings made at the onset and end of each mating season were analysed separately, with these data sets consisting of 491 records each. Total egg production during the breeding season was only considered in analyses involving scanning at the beginning or end of the mating season.

The analysis of discrete data such as follicle classification score and the number of follicles observed with parametric methods used in the present study are not optimal, as was outlined by Purvis and Hillard (1997). The close approximation of outcomes from linear models to that derived from non-linear methods resulted in recommendations that the former methods could be employed until alternative software packages become available (Jorgensen, 1994; Brien *et al.*, 2002). Fixed effects that were considered in all the analyses included year (1999-2002) and age of the female. When scanning results at the beginning of the season were incorporated, teasing and flushing treatments were also considered during the 2000/2001 and 2001/2002 breeding seasons. Random terms were then added to the operational model, resulting in the following models for analyses (in matrix notation):

$$y = Xb + Z_1a + e \quad (1)$$

$$y = Xb + Z_2c_{female} + e \quad (2)$$

$$y = Xb + Z_1a + Z_2c_{female} + e \quad (3)$$

In these models, y was a vector of observations for female follicle or egg production parameters; b, a and c_{female} were vectors of fixed effects, direct genetic effects and female permanent environmental effects; X, Z_1 and Z_2 were the corresponding incidence matrices relating the respective effects to y, and e the vector of residuals.

It was assumed that:

$$V(a) = A\sigma^2_a;\ V(c_{PE}) = I\sigma^2_{female};\ V(e) = I\sigma^2_e,$$

where A represents the numerator relationship matrix, I an identity matrix, σ^2_a, σ^2_{female}, and σ^2_e the direct genetic variance, female permanent environmental variance, and environmental (residual) variance, respectively. All analyses included the full pedigree file, consisting of 578 individuals, the progeny of 129 males and 127 females. Log likelihood tests were conducted to determine the most suitable model for each parameter in univariate analyses, and subsequently, two-trait animal models were fitted (Snyman *et al.*, 1996). These analyses allowed the calculation of all relevant

correlations between parameters, together with their appropriate standard errors. The Analysis Toolpack of the Microsoft Excel™ package was used to calculate the correlation between the number of follicles observed on both flanks and the assigned FCS.

RESULTS

A total of 1334 records were analyzed, with follicle diameter (FD) measured in only 900 cases where at least one follicle was observed. Total egg production records were analyzed in only 491 evaluations where scanning was performed at the beginning and end of a breeding season. The fixed effect of year was significant ($P \leq 0.05$) in some instances. It is, however, not tabulated for this report as differences between year means resulted from various interacting management and husbandry practices, with the prevailing climate also possibly exerting an influence.

The number of follicles (FN) observed ranged between 0 and 9, with an average of 1.92±1.77 (Table 2). Follicle diameter measured ranged between 2.0 and 120mm, with an average of 45.3±19.2mm. All egg production (EP) parameters were highly variable, as suggested by the high coefficients of variation and extreme ranges. Standard deviations exceeding the mean and coefficients of variation exceeding 50%, were commonly found.

Table 2. Number of observations, means and standard deviations (SD), and ranges for the respective ultrasound parameters and egg production traits for breeding ostrich females.

Parameter	Number	Mean (SD)	CV (%)	Range
Follicle classification	1334	1.07 (0.91)	85.1	0 – 3
Number of follicles	1334	1.92 (1.77)	92.2	0 – 9
Follicle diameter (mm)	900	45.3 (19.2)	42.4	2.0 – 120.0
EP % over 14 days (EPP_{14d})	1334	23.9 (32.8)	137.2	0 – 133.3
EP % over 1 month (EPP_{1m})	1334	31.6 (32.8)	103.8	0 – 115.2
EP % over 2 months (EPP_{2m})	1334	33.4 (29.6)	88.6	0 – 109.7
EP% for entire breeding season (EPP_{total})	491	43.1 (24.0)	55.7	0 – 109

Sonograms obtained on the right flanks of ostrich females provided a better indication of the follicular development in the ovary, with correlation of the follicle classification score (FCS) with the sonograms obtained on the left and right flanks being 0.43 and 0.90, respectively (Table 3).

Table 3. The correlation of follicle classification score (FCS) with the number of follicles observed on the left (L) and right (R) flanks of ostrich females, respectively; and of the number of follicles observed on the left flanks (FN-L) with number of follicles observed on the right flanks during the 2002/2003 breeding season.

	FCS	L	R	L+R	FN-L
Number of follicles observed on:					
Left flank only (L)	0.01				
Right flank only (R)	0.01	-0.19			
Left and right flanks (L+R)	0.37	-0.37	-0.42		
Number of follicles observed on:					
Left flank (FN-L)	0.43	0.25	-0.44	0.59	
Right flank	0.90	-0.25	0.10	0.39	0.25

The production and reproduction traits of females in the older age groups were generally higher than the performance levels of 2-year old females. Two-year old females performed worse ($P \leq 0.05$) than their older contemporaries in terms of FCS and egg production performance (EPP) for the first 14 days after or prior to scanning, one month and 2 months after or prior to scanning and also for the entire breeding season (Table 4). Mean FN and FD did not differ significantly between the three age groups (Table 4, $P \geq 0.05$).

Table 4. Least square means (±SE) depicting the influence of age on the ultrasound parameters and egg production traits of breeding ostrich females.

Parameter	Age group		
	2 Years	3–6 Years	7+ Years
Follicle classification score	0.81 ± 0.07^{a}	1.02 ± 0.04^{b}	1.10 ± 0.06^{b}
Number of follicles	1.57 ± 0.14	1.82 ± 0.08	1.92 ± 0.11
Follicle diameter (mm)	44.1 ± 1.8	45.8 ± 0.9	47.2 ± 1.2
EPP_{14d}	11.1 ± 2.7^{a}	23.3 ± 1.6^{b}	24.0 ± 2.1^{b}
EPP_{1m}	14.4 ± 2.7^{a}	30.5 ± 1.7^{b}	34.2 ± 2.2^{b}
EPP_{2m}	14.0 ± 2.7^{a}	32.8 ± 1.9^{b}	35.3 ± 2.2^{b}
EPP_{total}	21.7 ± 3.2^{a}	44.8 ± 2.4^{b}	45.4 ± 2.8^{b}

$^{a, b}$: Different superscripts denote significance ($P \leq 0.05$)

Models fitting the data best in the overall analysis mostly included the direct additive genetic variation as the only significant random effect (Table 5). The only exception was FD, where none of the additional random effects fitted to the data improved the log likelihood. The same was found when the production traits were assessed at the beginning (Table 6) and end of the breeding season (Table 7). EPP_{14d} was the exception when end of season results were analysed. The model fitting additional random effects did not improve the log likelihood.

Table 5. Log-likelihood values for models fitting different random effects for ultrasound parameters and egg production traits obtained for entire breeding seasons.

Parameter	Fixed effects	h^2 + PE	h^2	PE
Follicle classification score	-435.6	-420.9	**-421.2***	-424.4
Number of follicles	-1340.5	-1329.4	**-1329.4***	-1333.6
Follicle diameter (mm)	**-2990.8***	-2989.2	-2989.4	-2989.4
EPP_{14d}	-5279.5	-5270.2	**-5269.9***	-5274.2
EPP_{1m}	-5266.7	-5249.3	**-5249.0***	-5255.1
EPP_{2m}	-5125.1	-5086.9	**-5087.3***	-5091.9

h^2 = heritability; **PE** = permanent environment, * = P≤0.05

Table 6. Log-likelihood values for models fitting different random effects for ultrasound parameters and egg production traits obtained at the beginning of the breeding seasons.

Parameter	Fixed effects	h^2 + PE	h^2	PE
Follicle classification	-151.673	-145.153	**-145.156***	-148.432
Number of follicles	-501.873	-499.193	**-498.756***	-501.84
Follicle diameter (mm)	**-1028.51***	-1027.85	-1028.48	-1027.85
EPP_{14d}	-1733.20	-1729.28	**-1729.48***	-1730.77
EPP_{1m}	-1814.16	-1806.00	**-1806.00***	-1809.96
EPP_{2m}	-1827.75	-1805.54	**-1805.60***	-1811.34
EPP_{total}	-1757.48	-1721.76	**-1722.83***	-1726.87

h^2 = heritability; **PE** = permanent environment, * = P≤0.05

Table 7. Log-likelihood values for models fitting different random effects for ultrasound parameters and egg production traits obtained at the end of the breeding seasons.

Parameter	Fixed effects	h^2 + PE	h^2	PE
Follicle classification	-166.852	-162.089	**-161.990***	-165.36
Number of follicles	-467.732	-462.403	**-462.404***	-465.463
Follicle diameter	**-979.182***	-977.679	-978.262	-977.689
EPP_{14d}	**-1904.28***	-1903.24	-1903.26	-1903.52
EPP_{1m}	-1830.82	-1827.34	**-1827.35***	-1828.42
EPP_{2m}	-1763.06	-1760.20	**-1760.19***	-1761.25
EPP_{total}	-1764.88	-1728.32	**-1729.66***	-1732.58

h^2 = heritability; **PE** = permanent environment, * = P≤0.05

Heritability estimates (in bold and on the diagonal) in the overall analysis were generally low, in most cases < 10% (Table 8). In the case of EPP, estimates tended to increase as data were recorded over a longer period. The genetic correlations (*above the diagonal*) of FCS and number of follicles with EPP were generally positive, but failed to reach statistical significance (i.e. a level of double the corresponding standard error). Genetic correlations among EPP parameters were very high and outside the parameter space in some instances. Environmental correlations (*below the diagonal*) between the various measures of egg production were generally high. The environmental correlation between FCS and number of follicles observed approached 80%. The

latter parameters were generally positively related to the various measures of EPP ($P \leq 0.05$) on an environmental level, although the estimates failed to reach 0.20.

Table 8. Genetic and environmental (co)variance estimates and ratios for follicle classification score (FCS), number of follicles (FN), follicle diameter (FD), and egg production performance (EPP) over all available records.

Variances and parameters / traits	FOLLICLE PARAMETERS			EGG PRODUCTION TRAITS		
	FCS	FN	FD	14 days	1 month	2 months
Variances						
Phenotype	0.6926	2.700	268.4	1017.5	997.4	815.6
Genotype	0.0517	0.158	7.9	53.7	77.4	121.4
Environment	0.6409	2.542	278.5	963.8	919.0	694.2
Parameter						
FCS	**0.07±0.02**	0.96±0.04	0.00±0.41	0.23±0.23	0.27±0.21	0.19±0.18
FN	0.78±0.01	**0.06±0.02**	-0.64±0.36	0.26±0.24	0.30±0.22	0.27±0.19
FD	0.16±0.03	-0.30±0.03	**0.03±0.02**	0.25±0.45	0.14±0.39	0.25±0.31
EPP_{14d}	0.09±0.03	0.11±0.03	0.08±0.04	**0.05±0.02**	0.96±0.05	1.00±0.04
EPP_{1M}	0.12±0.03	0.12±0.03	0.09±0.04	0.70±0.02	**0.08±0.02**	1.05±0.01
EPP_{2M}	0.14±0.03	0.15±0.03	0.05±0.04	0.69±0.02	0.87±0.01	**0.15±0.03**

Both measures of follicular activity were highly correlated with EPP close to the assessment, and declined and became insignificant ($P \geq 0.05$) as data were recorded over a longer period. When related to overall egg production, genetic correlations were negative in sign, but small in magnitude (Table 9). Environmental correlations resembled those reported in Table 8, but were generally higher when the measures of follicular activity were related to egg production. Heritability of overall egg production was fairly high at 41%.

Table 9. Genetic and environmental (co)variance estimates and ratios for follicle classification score (FCS), number of follicles (FN), follicle diameter (FD), and egg production performance (EPP) when recorded at the beginning of the breeding season.

Variances and parameters/traits	FOLLICLE PARAMETERS			EGG PRODUCTION TRAITS			
	FCS	FN	FD	14 days	1 month	2 months	Overall
Variances							
Phenotype	0.6402	2.722	190.2	446.7	623.5	665.0	521.2
Genotype	0.0809	0.193	2.34	39.2	85.9	182.0	215.3
Environment	0.5596	2.529	187.8	507.5	537.6	483.0	305.9
Parameter							
FCS	**0.13±0.05**	1.11±0.13	n.e.	1.04±0.26	0.56±0.26	0.25±0.22	-0.14±0.21
FN	0.69±0.03	**0.07±0.04**	n.e.	0.99±0.34	0.48±0.33	0.19±0.28	-0.13±0.25
FD	n.e.	n.e.	**0.01±0.05**	n.e.	n.e.	n.e.	n.e.
EPP_{14d}	0.17±0.05	0.14±0.05	n.e.	**0.09±0.05**	1.00±0.05	0.97±0.09	0.74±0.17
EPP_{1M}	0.23±0.05	0.19±0.05	n.e.	0.81±0.01	**0.15±0.05**	0.98±0.03	0.68±0.14
EPP_{2M}	0.21±0.05	0.20±0.05	n.e.	0.60±0.03	0.84±0.02	**0.27±0.06**	0.78±0.08
EPP_{total}	0.12±0.06	0.15±0.05	n.e.	0.25±0.05	0.37±0.05	0.50±0.04	**0.41±0.06**

n.e. = not estimable

Results pertaining to h^2 estimates and environmental correlations at the end of the breeding season corresponded with those presented in Table 8 and Table 9 in most instances (Table 10). Genetic correlations between follicle parameters and egg production however, were different from those obtained for the entire season and at the beginning of the breeding season. All end of season correlations were negative and substantial in magnitude for EPP_{14d}.

Table 10. Genetic and environmental (co)variance estimates and ratios for follicular classification score (FCS), number of follicles (FN), follicle diameter (FD), and egg production performance (EPP) recorded at the end of the breeding season.

Variances and parameters/traits	FOLLICLE PARAMETERS			EGG PRODUCTION TRAITS			
	FCS	FN	FD	14 days	1 month	2 months	Overall
Variances							
Phenotype	0.6890	2.383	252.8	895.9	665.9	502.4	521.2
Genotype	0.0634	0.235	29.9	46.8	72.2	48.5	215.3
Environment	0.6256	2.148	222.9	849.1	593.0	445.9	305.9
Parameter							
FCS	**0.09±0.04**	0.97±0.05	-0.85±0.35	-0.80±0.56	-0.20±0.36	-0.30±0.37	-0.16±0.24
FN	0.86±0.01	**0.10±0.04**	-0.76±0.27	-0.64±0.54	-0.06±0.34	-0.15±0.36	0.05±0.23
FD	-0.22±0.07	-0.39±0.06	**0.12±0.07**	0.74±0.55	-0.26±0.46	0.09±0.08	-0.22±0.30
EPP_{14d}	0.11±0.05	0.13±0.05	-0.04±0.07	**0.05±0.04**	1.04±0.20	0.97±0.10	0.78±0.18
EPP_{1M}	0.13±0.05	0.14±0.05	0.09±0.07	0.46±0.04	**0.11±0.05**	1.01±0.04	0.97±0.09
EPP_{2M}	0.15±0.05	0.15±0.05	0.03±0.07	0.75±0.02	0.86±0.01	**0.10±0.05**	0.94±0.09
EPP_{total}	0.16±0.06	0.18±0.05	-0.01±0.07	0.38±0.05	0.45±0.04	0.47±0.04	**0.41±0.06**

DISCUSSION

Ultrasound scanning proved to be an effective and stress-free method to assess the extent of follicular development on the ostrich ovary. The scanning procedure is non-invasive and can be performed easily and time-efficiently, subjecting the bird to minimal stress if the proper handling techniques are employed. Observations on ovarian appearance and different sized follicles during the study are in agreement with observations on the female reproductive tract in poultry and ratites (Duerden, 1912; Waddington *et al.*, 1985; Bezuidenhout, 1986; Hocking *et al.*, 1987; Hicks, 1992; Soley and Groenewald, 1999; Williams, 1998).

A prominent feature of the data is the variability of all ultrasound parameters and egg production traits considered, as reflected by the high coefficients of variation and extreme ranges in Table 2. Despite the large variation, no evidence of skewness in the distributions of any of the parameters or traits was detected by standard descriptive statistics methods. Egg production percentages calculated for the various production periods after or prior to scanning showed extreme variability and agree with the marked variation in EPP recorded in previous studies (Van Schalkwyk, 1998; Bunter and Graser, 2000; Lambrechts *et al.*, 2002a, b; Cloete *et al.*, 2002a). The range of follicles observed was larger than that observed by Lambrechts *et al.* (2002a) and Bronneberg and Taverne (2003). Follicles with a minimum size of 30mm were considered in the latter study, with no cases

where no follicles were observed. In this study, a larger range of different sized follicles was observed than reported by Bronneberg and Taverne (2003). The number and diameter of follicles observed in this study also differs from that observed in domestic chicken and turkey hens (Gilbert *et al.*, 1983; Hocking *et al.*, 1987; Melnychuk *et al.*, 2002). Although the ostrich ovary is located on the left side of the body, sonograms obtained on the right flank of the ostrich females provided a better indication of the extent of follicular development on the ostrich ovary.

Females younger than 3 years in this study performed worse in terms of all ultrasound parameters and egg production traits than their older contemporaries. This supports findings that ostrich females younger than 3 years produced significantly less eggs than older females and contemporaries and suggested that immaturity was the most important factor affecting the laying performance of the females (More, 1997; Cloete *et al.*, 1998; Bunter, 2002). It also agrees with findings of Lambrechts *et al.* (2002a) that ostrich females of three years and younger showed considerably less follicular development than females four years of age and older. In the latter study, two-year old ostrich females showed no follicular activity at the beginning of the breeding season. Ovary size is in part determined by the age of an ostrich female, with the ovary of a young immature female being thin, flattened and almost rectangular in appearance. The ovary of a mature female consists of a stroma with a series of embedded, developing follicles, which is absent in reproductively immature females (Cho *et al.*, 1984).

In the absence of reliable data on ostriches, it is appropriate to consider information regarding the laying period in other avian species. The laying period of domestic hens can be divided into three phases, i.e. the onset of lay, the main period of lay and the end of laying. During the onset of lay, the ovary might be very sensitive to gonadotropic levels and action. The ovary and the oviduct appear to be asynchronized, as about 40% of ovulations did not result in oviposition during this phase in domestic hens (Gilbert and Wood-Gush, 1971). The hormones responsible for oviductal growth and function, i.e. androgens, progestagens and oestrogens, are dependent on the hormones responsible for follicular maturation and ovulation. Lack of synchronization between or co-ordination of the ovary and oviduct may be a result of bringing birds into lay precociously. During the main production period, the egg production pattern becomes regular, with each hen having a characteristic production pattern, and at the end of the laying period production ceases as a result of the cessation of ovulation and the production of ova. The infindibulum also fails to engulf almost 40% of the ova that are shed (Gilbert and Wood-Gush, 1971). The reason for this is unclear since the oviduct was shown to remain active longer than the ovary (Gilbert and Wood-Gush, 1971). The duration of the processes involved from ovulation to oviposition in the ostrich is unknown, but it can be assumed that it takes 48 h to travel through the oviduct (Irons, 1995).

It is possible that the reproductive system of the young ostrich females in this study were not yet sufficiently developed to ensure the production of the reproductive hormones at levels high enough to support follicular recruitment, development, and the subsequent ovulation processes (Card and Nesheim, 1972; Hafez, 1987). This is reflected in the significantly lower egg production observed in

this study. The presence of follicles in young (in this study 2-year old) females does not necessarily indicate that a female will produce eggs. In cases where the ovaries of 2-year old females might have been sufficiently developed to support the synthesis and secretion of the reproductive hormones, the ovary and oviduct might not have been synchronised at the beginning of and throughout the breeding season to ensure successful egg production. Very small follicles that occur in young female chickens are indicative of the absence of gonadotropin support by the follicles. In laying hens, it appears that the maturation and the initiation of egg-laying is dependant on an age-related increase in ovarian sensitivity to gonadotropin stimulation (Etches, 1995). The circulating levels of FSH and LH may be sufficient to ensure follicular recruitment and development, but not ovulation and subsequent egg production in the young females. The first oviposition is not necessarily the first indication of sexual maturity since certain sexual organs may be functional before others (Gilbert, 1971). No correlation of significance has been found between the number of visible ova and the egg-laying ability of the hen (Card and Nesheim, 1972). Laying hens (*Gallus domesticus*) of all ages were able to produce mature follicles. Birds with poor laying performance had reduced ovulation rates due to the loss of large follicles by atresia, which was rarely observed in birds with good laying performances (Waddington *et al.*, 1985).

The fact that not all females come into lay at the beginning of a breeding season implies that genetic factors are involved. The h^2 estimates for the ultrasound parameters and EP traits reported in this study were generally lower than that reported for poultry species, but significant for almost all ultrasound parameters and EP traits considered. The exception was follicle diameter. Heritability estimates of EP reported for domestic chickens were approximately 0.10 and for common ducks (*Anas platyrhynchos*) between 0.15 and 0.47 (Gowe and Fairfull, 1995; Brun and Larzul, 2003). Heritability reported for the total number of eggs produced by three Catalan poultry breeds, were estimated to be 0.20, 0.31 and 0.33 respectively (Francesh *et al.*, 1997). Heritability estimates for egg number for purebred and crossbred chicken lines were 0.54 to 0.74 and 0.04 to 0.51, respectively (Wei and Van der Werf, 1995). A long-term goose breeding program for egg production and crammed liver weight (Shalev *et al.*, 1991) found that heritability estimates were found to be high for most of the parameters, with phenotypic and genetic correlation coefficients being rather low.

There was a suggestion for h^2 estimates to be higher when assessed at the beginning of a breeding season. No literature could be found to support or refute these trends. Overall EP had a higher h^2 of 0.41. Previous studies on the same flock yielded h^2 estimates of approximately 0.15, with permanent environmental variance ratios of 0.25-0.30 (Bunter, 2002; Cloete *et al.*, 2002b). The best model in the present study however, did not partition the genetic and permanent environmental variances as the previous studies did. The size of the present data set was not large enough to provide the necessary degrees of freedom to allow for the partitioning of genetic and permanent environmental effects. Alternatively, a lack of pedigree depth could also have contributed to the lack of partitioning genetic effects from the overall female variances in the earlier studies. Bunter (2002) stressed this aspect since relatively few of the hens that were assessed

were descendants from females with production records. Total between-female variance ratios (i.e. the sum of the direct additive and female permanent environmental effects), however, remained relatively stable at 0.40-0.45 across all studies.

All genetic correlations between FCS and number of follicles were effectively unity, illustrating that it is effectively the same parameter on a genetic level. The same reasoning is applied to part-season records for egg production. The very high genetic correlation of parameters for follicular activity with egg production during the first 14 days or month into a breeding season indicate that ultrasound can be used to predict egg production accurately over the short term. The relationship however, grew weaker as the interval between scanning and the recording of egg production increased. Lambrechts *et al.* (2002a) reported similar results on a phenotypic level.

The discrepancy between parameters for follicular activity and egg production at the beginning and end of the breeding season is possibly the most striking feature of the data set. Although the general trends observed in the present study agree with each other, the derived genetic correlation differed in sign. It would appear that follicular activity at the end of a breeding season was associated with a low egg production just prior to scanning at the end of the breeding season on a genetic level. It is possible that the decrease in day length had the effect that synthesis of the gonadotropin decreased to such a level that follicle recruitment and growth could be supported, but that the circulating levels were not high enough to support ovulation and oviposition at the end of the season. Therefore the presence of follicles on the ovary would not necessarily result in successful oviposition at the end of the season. Further research is required for the proper understanding of this phenomenon.

Price *et al.* (1988) also stated that pairs of birds that start to breed earlier in the season have higher reproductive success than those who start breeding later. When the high genetic correlations between part-season records for egg production and overall egg production is considered, our results would support this contention. The results from this study did not, however, hold true for follicular activity reflected in the sonograms, with activity being poorly related to overall egg production. Environmental correlations between follicular activity and egg production were positive in all instances, suggesting that an environment favourable for follicular activity would also benefit egg production. The derived correlations however, were too low for accurate predictions of egg production. The potential of ultrasound scanning to predict the egg production for an entire breeding season is therefore limited.

CONCLUSIONS

The study presents novel information on genetic estimates of ultrasound parameters and their relationship with egg production traits in breeding ostrich females. Ultrasound scanning can be used to identify females that come into lay early in a breeding season, and the duration of lay after

the commencement of the breeding season was strongly associated with reproductive success. The correlation reported for early season scanning and overall egg production suggest that females can be selected on partial production records for use in breeding programs.

Progress can be made in the current flock through the selection of ostrich females with well-developed follicular hierarchies at the beginning of a breeding season. Purvis and Hillard (1997) reported acceptable rates of genetic progress under similar conditions in sheep. Partial records for a given season may, however, not be a reliable indicator of the ability of a female if the commencement of lay were delayed for the specific female or if the age of the female was unknown. The age of females needs to be considered when selection decisions are based on phenotypic data. Failure to do so may discriminate against young females.

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Chapter 9

(Co)variance estimates for live weight, body measurements and reproductive traits of pair-mated ostrich females[1]

Abstract

Estimates of genetic parameters for live weight, body measurements and reproductive traits were obtained using data from a pair-mated ostrich flock located at Oudtshoorn in South Africa. Live weight, chest circumference and tail circumference were recorded at the commencement and end of each breeding season. Reproductive traits included total egg and chick production, along with the percentage of infertile eggs. The data set consisted of approximately 1000 ostrich female-year records, from 283 hens, obtained for the period 1990-2001. Estimates of heritabilities (h^2) were 0.20 to 0.34 for live weight, 0.12 for chest circumference and 0.30 to 0.38 for tail circumference. In the case of the reproductive traits, h^2 was 0.23 for egg production, 0.20 for chick production and 0.10 for hatchability. Estimates of female permanent environmental effects (c^2) were 0.32 to 0.36 for live weight and 0.23 to 0.32 for chest circumference; 0.18 for egg production, 0.18 for chick production and 0.21 for hatchability. Service sire exerted significant effects only on hatchability (0.22) and consequently chick production (0.09). Correlations between live weight recorded at the beginning and end of the breeding season were unity for additive genetic and permanent environmental effects, but the residual correlation was substantially lower. Genetic correlations of live weight or the body measurements with reproductive traits were generally low to moderate and variable and failed to reach a significant difference from zero. Egg and chick production were highly correlated genetically and phenotypically, with the genetic correlation exceeding the theoretical limit. Hatchability was positively related to chick production among others at the male (service sire) level. Selection gains in the current flock and future generations are likely in ostriches. No significant adverse relationships were found between live weight, body measurements and reproductive traits. This will enable the development of a dual-purpose ostrich strain, where emphasis may be placed on both growth traits and reproduction.

INTRODUCTION

Marked advances in the genetic evaluation and sustained improvement of production traits in livestock have been achieved over the past few decades, particularly in the more intensive industries. The ostrich industry is an exception in this regard, and limited knowledge of genetic parameters of growth and reproduction is characteristic of the industry (Pettite and Davis, 1999). Genetic parameter estimates for live weight and reproductive traits are limited to a few studies, generally on the same resource population (Bunter *et al.*, 1999; 2001a; Cloete *et al.*, 2002a). This is in stark contrast to

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experience with small domestic poultry species, where extensive knowledge is readily available for as growth and development (Marks, 1995) and egg production (Gowe and Fairfull, 1995) of commercial stocks.

There is general consensus that growth and development is unfavourably correlated genetically with several reproductive traits in domestic poultry species (Marks, 1995; Gowe and Fairfull, 1995; Bunter, 2002). At this stage, there is almost a total lack of knowledge in this regard as far as ostriches are concerned. Phenotypic correlations between live weight or body measurements and reproductive traits have been reported (Van Schalkwyk and Cloete, 1996; Van Schalkwyk *et al.*, 1996; Lambrechts *et al.*, 1998; 1999). These studies were inconclusive as far as the sign and magnitude of correlations of growth traits with reproduction were concerned, possibly because they were estimated from relatively small databases. The nature of this correlation is, however, very important for developing an appropriate breeding policy for the broader industry (Bunter, 2002). The formation of a single, dual-purpose line, where emphasis is placed on both growth and reproductive performance would become feasible if this correlation is more favourable. Alternatively, the formation of specialist lines, one for growth and one for reproduction, could be contemplated, with crossbreeding of these lines at the commercial level (Cloete *et al.*, 2002a).

The present study provides estimates of genetic and environmental parameters for mature female live weight and body measurements, as well as reproductive traits for ostrich females, including estimates of genetic correlations between these traits.

MATERIALS AND METHODS

Experimental animals and location

Data were obtained from the commercial ostrich breeding flock at the Klein Karoo Agricultural Development Centre, recorded during the breeding seasons from 1990 to 2001. The experimental site is situated in the arid Klein Karoo region of South Africa, at longitude 22° 15' E and latitude 33° 38' S and at an altitude of 301 m above sea level. The long-term precipitation averages 230 mm, with 54 % of the total rainfall occurring during winter (April to September). The site is characterized by hot summers, with the average maximum temperature exceeding 25°C for the period October to April.

The origin and management of the ostrich population is well-documented (Van Schalkwyk *et al.*, 1996; Bunter, 2002). The data used for this paper were obtained from between 33 and 136 pair-breeding paddocks over the period 1990 to 2001. In total, 1177 female-year records of 283 females were represented in the reproductive data. The animals were paired off (one male to one female) for a period of approximately 8 months in most years included in the study, but the breeding season was

extended to a 9th month in 1999. The actual mean (SD) number of production days of individual females in the study was 235 (36) days per annum. These data included part-records in cases where females were replaced due to death or injury during the course of the breeding season. The females in the study were between 2 and 22 years of age. The age structure of the breeding flock differed considerably during the experimental period. Initially, the breeding stock was relatively old, with few age groups represented. From 1992 onwards, a breeding flock with a balanced age structure and balanced age groups was strived for. During the most recent breeding season (2001), female age groups ranging from 2 to 11 years were represented in the data.

The tendency of ostrich producers to continuously mate the same male-female combinations in the same breeding paddock completely confounded data obtained from earlier analyses (e.g. Van Schalkwyk *et al.*, 1996; Cloete *et al.*, 1998; Lambrechts *et al.*, 1999). In later years breeding pairs were intentionally reallocated to different paddocks, while natural attrition of both males and females resulted in different male-female combinations becoming available (Bunter, 2002). These changes facilitated the partitioning of variances for animal (or female), breeding paddock and service sire effects in later years. The breeding stock were mostly commercial birds obtained from a variety of sources, including birds derived from South African feather strains. The classification of birds as commercial or feather strains was somewhat arbitrary, and no significant differences between these groups or indications of heterosis in crossbred individuals has been observed previously for reproduction, egg traits and live weight to slaughter (Bunter, 2002). Against this background, strain effects were not considered in the present study.

Breeding pairs were housed in paddocks of 250 m^2 when paired off for breeding. Although some vegetation in the paddocks was available for browsing, all birds received a breeding diet at a level of 2.5 kg per bird per day throughout the entire breeding season. During the course of the data recording period, a number of experiments were carried out on the experimental flock. During 1998 and 1999, the flock were subjected to a feeding trial involving a series of dietary energy and protein concentrations (Brand *et al.*, 2000; 2003). Studies on the flushing and teasing, as well as a mid-season rest, of breeding birds were conducted during 2000 and 2001 (Lambrechts *et al.*, 2002; Chapter 2). These treatments were considered as fixed effects in this study, solely to account for the variation in performance of breeding females it controlled.

Data recorded

Live weight and body measurements were recorded on individual females at the beginning and end of each breeding season for the entire experimental period. Live weight at the commencement of mating was not available for females introduced in mid-season to replace birds incapacitated due to death or injury. Birds leaving the breeding flock during the mating period were likewise not weighed at the cessation of breeding. Body measurements followed previous definitions provided by Van Schalkwyk

and Cloete (1996), and included the thoracic circumference measured cranial to the hind limb (chest circumference) and the largest circumference of the most caudal aspect of the abdomen in the vicinity of the caudal callus (tail circumference). These data were recorded selectively on predominantly younger birds during the initial years of the experiment, but were recorded routinely for all potential breeders from the 1996-breeding season onwards. Eggs produced during the breeding season were collected daily, and incubated artificially, as described by Van Schalkwyk (1998) and Bunter (2002). Daily egg production was known for individual breeding paddocks and was used to derive production records of individual females within a specific season. During the artificial incubation process, the outcome of individual eggs (hatched, infertile, dead in shell) was known (Van Schalkwyk, 1998; Van Schalkwyk *et al.*, 2000; Bunter, 2002). This information was used to derive the hatchability of eggs set, as well as the number of chicks produced per breeding female for a breeding season. Hatchability was only considered in females where more than five eggs were set during a specific breeding season.

Statistical analyses

The ASREML program (Gilmour *et al.*, 1999) was used for the estimation of the fixed effects, and subsequently to derive (co)variance components for each trait in univariate analyses. ASREML estimates variance components for mixed models by restricted maximum likelihood, employing an average information algorithm that concurrently provides estimates of standard errors for parameter estimates (Gilmour *et al.*, 1995). A series of two-trait analyses were then performed to estimate correlations between traits. From the outcomes of these analyses, it was decided to perform three-trait analyses, involving live weight at the commencement and cessation of breeding, as well as egg or chick production.

Systematic effects

Fixed effects that were considered for all traits included the production year (1990-2001), age of the female at the start of each breeding season, dietary treatments (1998 and 1999), as well as flushing, teasing and resting (reproductive management) treatments (2000 and 2001). Two-factor interactions between main effects were considered initially, where applicable. Interactions were, however, found to be not significant, and were excluded from the final, operational models. Length of the breeding season (in days) was fitted as a linear covariate for egg and chick production traits.

The means for females belonging to the respective age groups could be described as longitudinal data. On the assumption that a specific trend would be discernable, a smoothing cubic spline was initially fitted to the data (Verbyla *et al.*, 1999) using ASREML (Gilmour *et al.*, 1999). The spline consisted of three components, namely: a fixed linear component, random deviations from linearity following a smooth trend, and random deviations from linearity not conforming to a smooth trend. In some cases this approach did not result in marked improvements in the interpretation of the results. The final

operational models thus included female age as a fixed effect, although outcomes of the analyses involving splines will be presented where applicable. In view of the change in the age structure of the breeding flock, it was decided to obtain age trends for the period from 1996 to 2001, when all age groups from 2 to 10 years were represented annually. The data file used for this purpose consisted of 708 female-year records for reproduction and between 665 and 692 records for live weight and body measurements. The approach to use data on a limited number of age groups is motivated further by recommendations that there is little to be gained from the maintenance of ostrich female age groups older than 10 to 11 years of age (Cloete *et al.*, 1998; Bunter 2002).

Random effects

Parameter estimates for each trait were obtained under an animal model, attributing each record to an individual female. Repeated records were accommodated by fitting a random permanent environmental effect for each trait. In initial analyses, random paddock effects were also fitted to the data. This term was, however, not significant and dropped from the final operational models. The following models were considered for analyses of female live weight and body measurements (in matrix notation):

$$y = Xb + Z_1a + e \quad (1)$$

$$y = Xb + Z_2c_{female} + e \quad (2)$$

$$y = Xb + Z_1a + Z_2c_{female} + e \quad (3)$$

The additional random effect of service sire was assessed for all the reproductive traits, resulting in the following model:

$$y = Xb + Z_1a + Z_2c_{female} + Z_3c_{service\ sire} + e \quad (4)$$

where y is a vector of observations; b, a, c_{female} and $c_{service\ sire}$ are vectors of fixed effects, additive genetic effects and permanent environmental effects of hens or their mates; X, Z_1, Z_2, and Z_3 are the corresponding incidence matrices relating the records to effects; and e is the vector of residuals

It was assumed that:

$$V(a) = A\sigma^2_a;\ V(c_{female}) = I\sigma^2_{female};\ V(c_{service\ sire}) = I\sigma^2_{service\ sire}\ V(e) = I\sigma^2_e,$$

where A is a matrix describing relationships between animals (i.e., the numerator relationship matrix), I is an identity matrix; and σ^2_a, σ^2_{female}, $\sigma^2_{service\ sire}$ and σ^2_e are the variation attributable to additive genetic effects, permanent environmental effects of the female and her mate and environmental (residual) effects respectively.

Random terms were added to analytical models sequentially. Likelihood Ratio Tests (LRT) were performed to assess the significance of the contribution of each random term to improvements in the model for analysis. The LRT is based on testing twice the increase in Log-likelihood resulting from adding a random term to the model of analysis as a Chi^2 statistic. Alternatively, for two models with the same number of random terms, and assuming identical fixed effects modeling, the model with the higher value for the Log-likelihood fits the data better. Variance components were expressed relative to the overall phenotypic variance for each trait to derive estimates of the appropriate variance ratios. All analyses included the full pedigree file, consisting of 601 individuals, the progeny of 135 males and 132 females.

RESULTS

Data description

Female live weight had coefficients of variation ranging from 12.2 % at the beginning of the breeding season and 15.3 % at the end of breeding (Table 1). Corresponding coefficients of variation for body measurements were below 10 %. Reproductive traits were exceedingly variable, with coefficients of variation ranging from 51.6 to 82.5 %.

Table 1. Descriptive statistics for the traits assessed in ostrich breeding females for the productive years 1990 to 2001

TRAIT	Number of observations	MEAN (SD)	CV (%)
At the beginning of mating:			
Live weight (kg)	1131	115 (14)	12.2
Chest circumference (cm)	846	122 (6)	4.9
Tail circumference (cm)	849	102 (10)	9.8
At the end of mating:			
Live weight (kg)	1112	111 (17)	15.3
Chest circumference (cm)	836	128 (12)	9.4
Tail circumference (cm)	815	100 (8)	0.1
Reproduction traits:			
Eggs produced (n)	1177	46.3 (26.5)	57.2
Chicks produced (n)	1177	22.9 (18.9)	82.5
Hatchability (%)	1089	51.0 (26.3)	51.6

Systematic effects

The effects of diet, flushing, teasing and a mid-season rest are not considered, since it formed part of other studies (Brand *et al.*, 2000; 2003; Chapter 2). Effects due to production year are mediated through a number of interacting climatic and husbandry factors. Since year effects tend not to be repeatable, these effects are not reported. The length of breeding season affected egg and chick production. The production of eggs increased ($P \leq 0.01$) by 0.166 ± 0.016 per day in the period paired off. Chick production correspondingly increased ($P \leq 0.01$) by 0.088 ± 0.013 per additional day paired off.

Live weight at the commencement of breeding was relatively stable from 2 years of age to 7 years of age, and no significant differences between age groups were evident (Figure 1). In subsequent years, live weight tended to decline with a further increase in age. When live weight at the cessation of breeding was considered, there was a suggestion of an increase up to an age of five years, followed by a general decline in later years. Differences between age groups were, however, relatively small and generally not significant ($P \geq 0.05$). Linear body measurements showed less variation with female age than live weight, and no conclusive age trends were noted. These results are thus not presented.

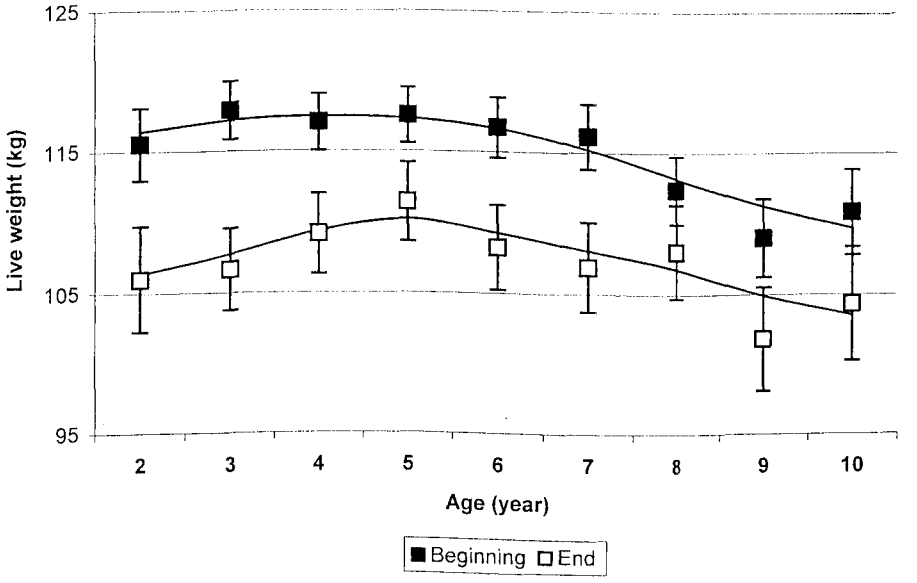

Figure 1. Mean (±SE) live weight at the commencement and cessation of mating in female age groups from 2 to 10 years. Trend lines were derived from initial analyses where age trends were modeled by cubic splines.

When age effect on egg and chick production were considered, the main contribution in preliminary analyses came from the random non-linear component of the spline not conforming to a smooth trend. On this basis, it was decided to include age as a fixed effect in the appropriate operational models. Egg

and chick production increased (P≤0.05) from 2-year-old females to 3-year-old females (Figure 2). At later ages, egg production tended to remain stable with no significant differences between age groups. In the case of chick production, on the other hand, there was a suggestion towards a slight decline at later ages.

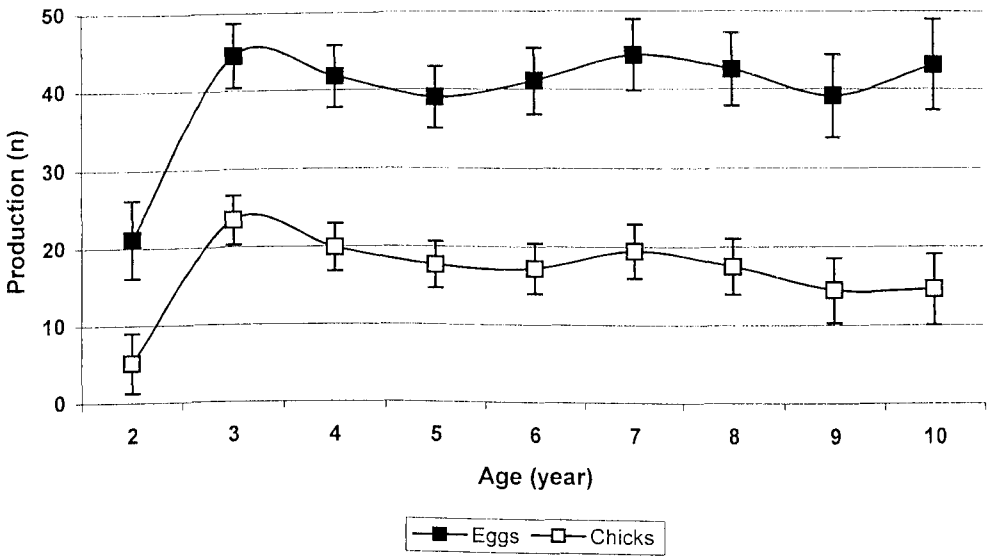

Figure 2. Means (±SE) for egg and chick production in female age groups from 2 to 10 years.

Hatchability increased (P≤0.05) from 2-year-old females to 3-year-old females (Figure 3). A gradual decline was observed at subsequent ages.

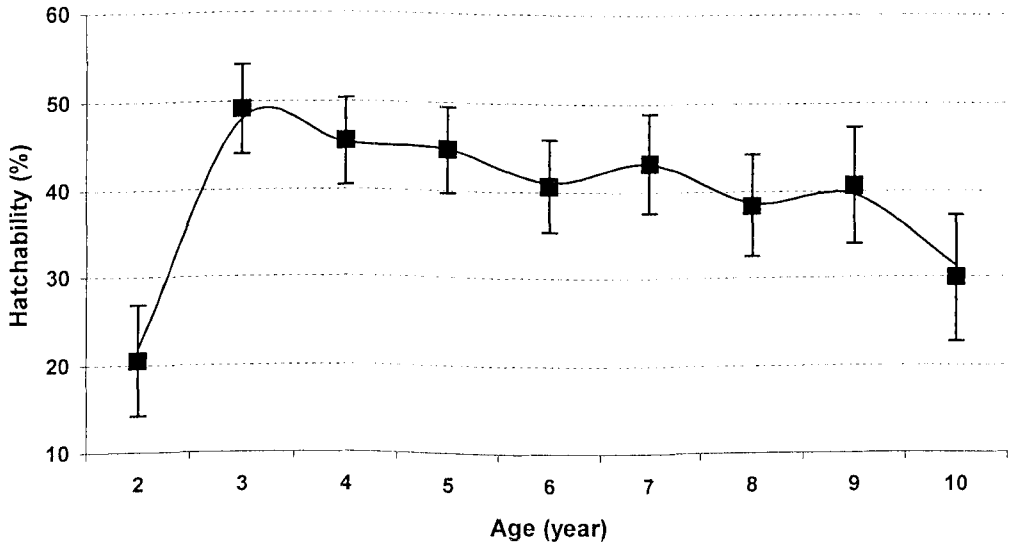

Figure 3. Mean hatchability (±SE) in female age groups from 2 to 10 years. The trend line was derived from an initial analysis, where the age trend was modelled by fitting a cubic spline.

Random effects

The likelihood ratio tests indicated that models including additive genetic and permanent environmental effects due to the female, fitted the data best for live weight and chest circumference at the beginning and end of breeding (Table 2). Only additive genetic effects were required for analyses of tail circumference. Models for chick production and hatchability included additive genetic, permanent environmental (female) effects along with the additional random effect for service sire. Not surprisingly, service sire effects were not significant ($P \geq 0.05$) for egg production.

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

Trait	Fixed only	+ h^2 (Model 2)	+ c^2 (Model 1)	+ h^2 + c^2 (Model 3)	+ h^2 + c^2 + ss^2 (Model 4)
At the beginning of mating:					
Live weight (kg)	-3457.6	-3100.3	-3104.5	**-3094.2**	–
Chest circumference (cm)	-1914.6	-1827.3	-1821.8	**-1819.4**	–
Tail circumference (cm)	-2138.9	**-2088.7**	-2097.6	-2088.4	–
At the end of mating:					
Live weight (kg)	-3489.9	-3297.4	-3297.0	**-3290.2**	–
Chest circumference (cm)	-1968.4	-1912.3	-1910.6	**-1907.7**	–
Tail circumference (cm)	-2010.4	**-1940.5**	-1952.7	-1940.3	–
Reproduction traits:					
Eggs produced (n)	-4187.1	-4078.6	-4082.5	**-4075.9**	-4074.8
Chicks produced (n)	-3915.4	-3788.7	-3789.3	-3783.5	**-3779.3**
Hatchability (%)	-6349.1	-6260.8	-6256.6	-6255.2	**-6236.9**

h^2 – Direct heritability c^2 – Female permanent environment ss^2 – service sire

Ratios of variance components

Estimates of heritability (h^2) for female live weight were 0.34 at the beginning of mating and 0.20 at the end of mating (Table 3). Estimates derived for chest circumference were lower at 0.12, while the h^2 for tail circumference ranged from 0.30 to 0.38 at the beginning and end of breeding. Estimates of h^2 were slightly above 0.20 for egg and chick production, and 0.10 for hatchability. All estimates were significant ($P \leq 0.05$), with the exception of h^2 values for chest circumference and hatchability.

Table 3. Variance components and ratios (± SE) for the respective traits assessed in ostrich breeding females over the period from 1990 to 2001.

TRAIT	VARIANCE COMPONENTS				VARIANCE RATIOS		
	σ^2_A	σ^2_C	σ^2_{SS}	σ^2_E	h^2	c^2	ss^2
Beginning of mating:							
Live weight (kg)	62.2	66.3	–	55.5	0.34±0.11*	0.36±0.10	–
Chest circumference (cm)	4.25	11.7	–	20.1	0.12±0.07	0.32±0.07	–
Tail circumference (cm)	19.1	–	–	44.6	0.30±0.04*	–	–
End of mating:							
Live weight (kg)	43.5	69.9	–	102	0.20±0.09*	0.32±0.08	–
Chest circumference (cm)	5.39	10.1	–	28.6	0.12±0.07	0.23±0.07	–
Tail circumference (cm)	22.0	–	–	35.9	0.38±0.05*	–	–
Reproduction traits:							
Eggs produced (n)	121.	97.9	–	314	0.23±0.09*	0.18±0.08	–
Chicks produced (n)	64.1	57.1	28.28	176	0.20±0.08*	0.18±0.08	0.09±0.04
Hatchability (%)	74.0	153	162.39	336	0.10±0.07	0.21±0.08	0.22±0.05

__: Effect not considered or not significant

σ^2_A: Direct additive variance component

σ^2_C: Female permanent environmental variance component

σ^2_{SS}: Service sire variance component

σ^2_E: Environmental variance

h^2: Direct heritability; * = P≤0.05

c^2: Female permanent environment

ss^2 Service sire

Permanent environmental variation (c^2) accounted for between 0.23 and 0.36 of the phenotypic variation for chest circumference and live weight . Estimates of c^2 effects for the reproduction traits were relatively stable, at approximately 0.20 for egg and chick production. Service sire (ss), as expected, only exerted a significant influence (P≤0.05) on hatchability and subsequently chick production, accounting for between 0.22 and 0.09 of the observed phenotypic variation in these traits.

The estimates of h^2 (upper value) and c^2 (lower value) are represented by bold figures on the diagonal in Table 4. The genetic (upper value) and permanent environmental (lower value) correlations are given *above* the diagonal. The environmental (upper value) and phenotypic (lower value) correlations are given *below* the diagonal. The estimates of h^2 and c^2 for live weight at the beginning of mating in the three-trait analyses with final live weight and egg or chick production closely resembled estimates derived from the two-trait analyses (Table 4). Variances for live weight at the end of breeding were partitioned somewhat more towards h^2 and less towards c^2 in the three-trait analyses, although changes were not significant (P≥0.05) according to the appropriate standard errors. Estimates of h^2 and c^2 for egg and chick production were within 0.02 of those derived from the two-trait analyses. The ss^2 estimate for chick production was slightly higher (0.11 ± 0.04) in the three-trait analysis, compared to the corresponding estimate obtained from the two-trait analysis.

Table 4. (Co)variance ratios (± SE) depicting estimates of h^2 and c^2 as well as genetic, permanent environmental, environmental and phenotypic correlations for live weight at the beginning (B) or end (E) of the mating season, egg production and chick production, as assessed by three-trait analyses in breeding ostrich females.

TRAIT	LIVE WEIGHT (B)	LIVE WEIGHT (E)	EGG PRODUCTION
Live weight (B)	**0.33 ± 0.11**	0.97 ± 0.04	0.10 ± 0.27
	0.36 ± 0.10	0.90 ± 0.05	-0.07 ± 0.23
Live weight (E)	0.17 ± 0.03	**0.25 ± 0.09**	-0.14 ± 0.27
	0.62 ± 0.03	**0.26 ± 0.08**	-0.04 ± 0.24
Egg production	0.18 ± 0.03	-0.06 ± 0.04	**0.22 ± 0.08**
	0.09 ± 0.05	-0.08 ± 0.05	**0.19 ± 0.07**
TRAIT	**LIVE WEIGHT (B)**	**LIVE WEIGHT (E)**	**CHICK PRODUCTION**
Live weight (B)	**0.33 ± 0.11**	0.97 ± 0.04	0.07 ± 0.28
	0.36 ± 0.10	0.90 ± 0.05	-0.08 ± 0.24
Live weight (E)	0.17 ± 0.03	**0.25 ± 0.09**	-0.16 ± 0.28
	0.62 ± 0.03	**0.26 ± 0.08**	-0.12 ± 0.25
Chick production	0.08 ± 0.04	-0.11 ± 0.04	**0.22 ± 0.09**
	0.03 ± 0.05	-0.12 ± 0.04	**0.19 ± 0.09**

Correlations

Correlations between additive genetic and permanent environmental effects for live weight and the linear body measurements were high to very high (Table 5). When the same trait was assessed at the beginning and end of the breeding season, these correlations did not differ from unity. The genetic correlation between chest circumference at the beginning and end of the breeding season went outside the boundary of the parameter space (i.e. exceeded one). A similar result was obtained for the permanent environmental correlation between live weight and chest circumference at the end of the breeding season. Environmental correlations among the live weight and body measurements were generally lower (below 0.30). Overall, moderate to strong estimates of phenotypic correlations between live weights and body measurements were evident, with the strongest correlations generally between the same trait recorded at different points in time.

The genetic and phenotypic correlations between female live weight or body measurements at the commencement of breeding with reproductive traits were generally favourable but low when compared to the corresponding standard errors (Table 5). The only genetic correlations between body measurements with reproductive traits that actually exceeded their standard errors were those for tail circumference measured at the beginning of breeding with egg and chick production. Corresponding permanent environmental and residual correlations were low and variable in sign. The environmental correlations between live weight and egg production, and the phenotypic correlations between tail

circumference with egg and chick production, were positive and significantly ($P \leq 0.05$) different from zero.

The genetic and permanent environmental correlations of live weight or body measurements recorded at the end of the breeding season with reproductive traits were low and generally negative (Table 5). Corresponding environmental and phenotypic correlations were consistently negative. Environmental correlations of live weight with chick production and hatchability were significant ($P \leq 0.05$), but relatively low (-0.10 to -0.12). Phenotypic correlations between live weight and egg production or hatchability also reached significance ($P < 0.05$), at -0.08 and -0.12 respectively.

Genetic correlations among reproductive traits were very high, and outside the boundary of parameter space in the case of egg and chick production (Table 5). The high genetic correlation between egg production and hatchability is notable, suggesting that females with a genetically high egg production are also likely to produce eggs with a high hatchability. However, correlations between these two traits for other random effects were only moderate, contributing to a moderate phenotypic correlation between egg production and hatchability overall. Egg and chick production were highly correlated at all levels of random effects, as were chick production and hatchability. The ss correlation between hatchability and chick production was high, as might be expected, at 0.77 ± 0.09.

When compared to the two-trait analyses, the three-trait analyses yielded identical correlations between initial and final live weight of breeding hens at all levels. Genetic correlations between live weight at the commencement of breeding and reproduction were similar in sign, but the magnitude was approximately half that of corresponding correlations derived from two-trait analyses in absolute terms. Correlations of live weight at the cessation of breeding with reproduction traits were similar in sign and within 0.02 in magnitude from those derived from two-trait analyses. Female permanent environmental correlations obtained from the three-trait analyses were similar in sign, but consistently smaller in magnitude than those obtained from the two-trait analyses. All environmental and phenotypic correlations between live weight and reproduction closely resembled those derived from two-trait analyses.

Table 5. Estimates of genetic, permanent environmental, environmental and phenotypic correlations (± SE) between traits, as assessed by two-trait analyses in breeding ostrich females. Live weight and body traits were recorded at the beginning (B) or end (E) of mating.

TRAITS	CORRELATION			
	Genetic	Female PE	Environment	Phenotypic
Live weight (B) ×				
Chest circumference (B)	0.89 ± 0.09	0.96 ± 0.05	0.30 ± 0.04	0.63 ± 0.03
Tail circumference (B)	0.93 ± 0.06	–	0.13 ± 0.04	0.42 ± 0.04
Live weight (E)	0.97 ± 0.04	0.90 ± 0.05	0.17 ± 0.03	0.62 ± 0.03
Chest circumference (E)	0.80 ± 0.15	0.87 ± 0.11	0.18 ± 0.04	0.49 ± 0.03
Tail circumference (E)	0.92 ± 0.05	–	0.05 ± 0.04	0.43 ± 0.04
Egg production	0.21 ± 0.28	-0.19 ± 0.22	0.17 ± 0.03	0.08 ± 0.04
Chick production	0.15 ± 0.28	-0.16 ± 0.24	0.07 ± 0.04	0.03 ± 0.05
Hatchability	-0.15 ± 0.34	0.13 ± 0.28	-0.01 ± 0.04	-0.01 ± 0.05
Chest circumference (B) ×				
Tail circumference (B)	0.93 ± 0.06	–	0.12 ± 0.04	0.32 ± 0.04
Live weight (E)	0.83 ± 0.14	0.95 ± 0.08	0.11 ± 0.04	0.48 ± 0.03
Chest circumference (E)	1.08 ± 0.15	0.92 ± 0.09	0.14 ± 0.04	0.45 ± 0.03
Tail circumference (E)	0.88 ± 0.10	–	0.03 ± 0.04	0.28 ± 0.04
Egg production	0.09 ± 0.37	0.01 ± 0.20	0.01 ± 0.04	0.02 ± 0.05
Chick production	0.16 ± 0.37	-0.01 ± 0.22	-0.06 ± 0.04	-0.01 ± 0.05
Hatchability	-0.15 ± 0.49	0.01 ± 0.22	-0.05 ± 0.05	-0.04 ± 0.05
Tail circumference (B) ×				
Live weight (E)	0.94 ± 0.08	–	0.03 ± 0.04	0.31 ± 0.04
Chest circumference (E)	0.87 ± 0.10	–	-0.01 ± 0.04	0.23 ± 0.04
Tail circumference (E)	0.93 ± 0.05	–	0.04 ± 0.04	0.35 ± 0.04
Egg production	0.25 ± 0.17	–	0.05 ± 0.04	0.09 ± 0.04
Chick production	0.26 ± 0.17	–	0.03 ± 0.04	0.08 ± 0.04
Hatchability	0.21 ± 0.24	–	-0.00 ± 0.04	0.04 ± 0.05
Live weight (E) ×				
Chest circumference (E)	0.80 ± 0.16	1.05 ± 0.10	0.14 ± 0.04	0.48 ± 0.03
Tail circumference (E)	0.94 ± 0.06	–	0.11 ± 0.04	0.42 ± 0.04
Egg production	-0.12 ± 0.31	-0.11 ± 0.21	-0.04 ± 0.04	-0.08 ± 0.04
Chick production	-0.14 ± 0.30	-0.18 ± 0.23	-0.10 ± 0.04	-0.12 ± 0.04
Hatchability	-0.26 ± 0.37	-0.08 ± 0.22	-0.12 ± 0.04	-0.12 ± 0.04
Chest circumference (E) ×				
Tail circumference (E)	0.85 ± 0.11	–	0.11 ± 0.04	0.31 ± 0.04
Egg production	0.03 ± 0.37	0.03 ± 0.23	-0.02 ± 0.04	0.00 ± 0.04
Chick production	0.11 ± 0.37	-0.07 ± 0.25	-0.04 ± 0.04	-0.02 ± 0.04
Hatchability	-0.11 ± 0.46	-0.14 ± 0.25	-0.02 ± 0.05	-0.05 ± 0.04
Tail circumference (E) ×				
Egg production	0.10 ± 0.17	–	-0.06 ± 0.04	-0.01 ± 0.05
Chick production	-0.01 ± 0.16	–	-0.04 ± 0.04	-0.03 ± 0.05
Hatchability	-0.10 ± 0.23	–	-0.06 ± 0.05	-0.05 ± 0.05
Egg production ×				
Chick production	1.16 ± 0.11	0.71 ± 0.11	0.75 ± 0.02	0.78 ± 0.02
Hatchability	0.80 ± 0.26	0.24 ± 0.25	0.22 ± 0.04	0.29 ± 0.04
Chick production ×				
Hatchability	0.76 ± 0.17	0.82 ± 0.11	0.77 ± 0.09	0.74 ± 0.02

– Female permanent environment not significant in both traits

DISCUSSION

Data description

Previous studies on ostriches have reported coefficients of variation of 11 to 12 % for live weight at slaughter (Cloete *et al.*, 1998; Bunter *et al.*, 1999) and between 12 and 13 % for mature live weight (Van Schalkwyk *et al.*, 1996; Lambrechts *et al.*, 1999). These values are relatively low compared to coefficients of variation for weight in other species (~20%), perhaps due to selective recording of breeding animals only. Chest and tail circumferences of two-year old females had coefficients of variation of slightly below 10 % (Lambrechts *et al.*, 1998). Low coefficients of variation for these traits could also suggest a developmental or structural optimum in these body dimensions at a given live weight.

Ostrich reproductive traits have previously been shown to be highly variable. Coefficients of variation ranged between 45 and 52 % for annual egg production and between 61 and 81 % for annual chick production (Van Schalkwyk *et al.*, 1996; Cloete *et al.*, 1998; Lambrechts *et al.*, 1999; Bunter *et al.*, 2001). Deeming (1996) reported a coefficient of variation of 58 % for weekly egg production, and of 66% for hatchability for breeding ostriches in the United Kingdom. Despite the observed large coefficients of variation, the present data still largely conformed to normality, with low levels of skewness and kurtosis. Similar conclusions were made by Van Schalkwyk *et al.* (1996) and Bunter (2002), on smaller subsets of the present data.

Systematic effects

No comparable results pertaining to the effects of age on ostrich female live weight at the commencement or cessation of breeding were found in the literature. The present results suggest that ostrich females are fairly mature in terms of live weight when they are mated for the first time at 2 years of age, as live weight generally did not increase much with breeding age. In poultry, Leghorn pullets are expected to increase from 1250 to 1350 g at the commencement of laying to 1650 g at peak production to ensure sufficient reserves for the production process (Summers, 1995). Expressed as a percentage of the initial live weight, it indicates increases of between 22 and 32 %. Ostrich females obviously do not achieve anything remotely in this order, but it has to be conceded that the production process in ostriches are much less intensive than in commercial poultry. In other species where females are maintained over consecutive breeding seasons, live weight generally increases markedly with age until a relatively stable mature live weight is attained. In females maintained for a longer period, live weight would subsequently decline. Further studies on the effect of age on the live weight of ostrich females are indicated, including guidelines pertaining to the growing out of replacement birds as well as further studies on the nutrition of breeding birds. Brand *et al.* (2003) found that live weight

was reduced in breeder birds maintained on energy concentrations of 7 5 and 8.5 MJ/kg ME, while egg production was only impaired on the former diet.

Previous results pertaining to egg and chick production of ostrich females showed a significant increase from 2 to 3 years of age (Cloete *et al.*, 1998; Bunter 2002). Two-year-old females took longer to commence laying than older hens, resulting in a shorter duration of lay in the study of Bunter (2002). More (1996) correspondingly related low productivity levels in ostriches kept in Queensland (Australia) to a large proportion of young non-laying females in the breeding operations. Peak egg and chick production levels were achieved at 8-9 years of age (Cloete *et al.*, 1998, Bunter, 2002), when reproduction was recorded at higher ages than in the present study. These levels of peak production were followed by a general decline in reproductive performance at older ages, which was more pronounced for chick than it was for egg production. Bunter (2002) reported divergence for egg production and chick production at older ages. A corresponding tendency was evident in the present study. The age trend obtained for hatchability in the present study refutes a previous allegation that this trait is largely independent of female age (Cloete *et al.*, 1998). It is, however, in close agreement with a more recent trend reported by Bunter (2002), involving a larger data base, and may be related to changes in egg weight and shell quality with female age, which ultimately influence hatchability of eggs under a constant incubation environment.

From recent results, there is little motivation for ostrich females to be kept in the breeding flock for more than 10-11 years (Bunter, 2002). Although the oldest age groups in the latter study were still capable of a good egg production, chick production declined with female age above 10 to 11 years of age. Average egg and chick weights also showed a decline for females aged 10 years and older. Bunter (2002) suggested that this could lead to higher chick mortality levels in commercial breeding systems. In this regard, it has been demonstrated that lighter day-old chicks were more likely to succumb before one month of age than heavier contemporaries (Cloete *et al.*, 2001). Apart from considerations pertaining to flock performance, too many age groups will affect genetic progress deleteriously, with an increase in the generation interval (Cloete *et al.*, 1998).

Estimates of genetic parameters

Estimates of h^2 for ostrich live weight at a slaughter age of approximately 14 months ranged between 0.17 and 0.45 (Bunter *et al.*, 1999; Bunter, 2002). The only previous h^2 estimates for mature live weight in ostriches were derived from the current data set, involving fewer records (Cloete *et al.*, 2002b). Estimates for h^2 and c^2 thus agreed with those reported in the present study for both initial and final live weight. Previous estimates for the repeatability of mature live weight ranged from 0.61 to 0.72 (Van Schalkwyk *et al.*, 1996; Lambrechts *et al.*, 1999), corresponding well with estimates derived from the present data set (0.69 for initial live weight and 0.52 for final live weight). Combining initial and final live weight in a three-trait analysis with reproduction traits resulted in slightly more animal variance

being partitioned to h^2 in the case of final live weight. Information pertaining to the initial live weight recordings probably resulted in the slightly higher h^2 estimate in this instance. No other estimates were available in the literature for comparison with the h^2 or c^2 of linear body measurements.

Previous estimates of h^2 and repeatabilities for parameters of egg and chick production were derived from part of the data set used for the present investigation (e.g. Bunter *et al.*, 2001a, Bunter, 2002, Cloete *et al.*, 2002b). Thus, estimates reported here are basically similar to those previously reported, confirming moderate heritabilities (h^2 ~ 0.13-0.22) for egg and chick production traits. Parameter estimates involving the three-trait analyses including egg or chick production were generally in agreement with single trait estimates. The current study also confirms a significant service sire effect (ss^2 = 0.22) on hatchability, but suggests a low heritability (0.10) and only moderate repeatability (0.31) for this trait. Previously estimated variance ratios for hatchability were 0.01 for h^2, 0.35 for female permanent environment (c^2), and 0.13 for variation due to service sire (ss^2) (Bunter, 2002).

When results from previous studies are considered, a generally higher proportion of the phenotypic variation seemed to be partitioned to h^2 in the present study, at the expense of lower c^2 estimates. Changes in genetic parameters were, however, not significantly different to previous estimates due to fairly large standard errors. An increase in h^2 as more data become available was not entirely unexpected, since the pedigreed portion of the population and the number of parents with reproduction records in the data increases all the time (Bunter, 2002). When h^2, c^2 and ss^2 variance ratios (where applicable) were summed to provide repeatability estimates, these estimates were 0.41 for egg production, 0.45 for chick production and 0.53 for hatchability. All estimates closely resembled previous estimates ranging from of 0.37 to 0.47, 0.51 to 0.55 and 0.38 to 0.56 respectively (Van Schalkwyk *et al.*, 1996; Cloete *et al.*, 1998; Lambrechts *et al.*, 1999). Heritability estimates for ostrich reproductive traits were furthermore in accordance with estimates for poultry reviewed from the literature (Gowe and Fairfull, 1995). When the marked genetic improvement in the reproduction of poultry are considered, it is clear that similar successes could be expected from well-constructed breeding programs involving ostriches.

The confirmed influence of service sire on the reproductive performance of companion females agrees with results reported by Bunter *et al.* (2001a), Bunter (2002) and Cloete *et al.* (2002b). It can thus now be accepted that the influence of service sire upon egg production of females is limited. Service sires do, however, affect chick production by exerting an influence on the fertility of eggs (Bunter, 2002) and thereby influencing the hatchability records for individual females.

Correlations

High to very high genetic correlations were previously reported for live weight of slaughter ostriches between six and approximately 14 months of age (Bunter *et al.*, 1999; Bunter, 2002). No genetic

correlations between ostrich live weight and linear body measurements were found in the literature. It is, however, generally accepted that ostrich live weight can be fairly accurately predicted from functions derived from linear body measurements (Bezuidenhout and Van Schalkwyk, 1996; Deeming *et al.*, 1996). The high genetic and permanent environmental correlations between the same measurements made at the commencement and cessation of breeding are readily explicable, as they are the same traits but measured at different times during the reproductive cycle.

It is generally accepted that reproduction and growth traits are unfavourably correlated in small domestic poultry species (Marks, 1995; Gowe and Fairfull, 1995). Phenotypic correlations in earlier studies by Van Schalkwyk and Cloete (1996) and Van Schalkwyk *et al.* (1996) seemed to support the argument that the same applies in ostriches. It has, however, been argued that these relationships in mixed age flocks could have resulted from unproductive birds becoming fatter with an increase in age. Phenotypic correlations derived from data on two-year old females (where confounding age-productivity relationships are not present) also did not conform to this generalization (Lambrechts *et al.*, 1998). The present study confirms that there are no marked unfavourable genetic correlations between female live weight at the beginning of breeding and the measures of reproduction assessed here. Phenotypic correlations between initial female live weight and egg production in this study (0.08) were similar to previous estimates of 0.04 (Van Schalkwyk *et al.*, 1996; Lambrechts *et al.*, 1999). Corresponding correlations for chick production in the literature were generally opposite in sign (–0.04 and –0.07) but of similar magnitude to that of the present study (0.03). The phenotypic correlation between initial live weight and hatchability of –0.16 estimated by Van Schalkwyk *et al.* (1996) was much larger in magnitude than the present correlation, which is based on a much larger and more informative data set.

Estimates of genetic correlations between live weight recorded at the end of the breeding season and reproductive traits were negative in sign, but not significantly different from zero. This change in sign compared to live weight at the commencement of breeding is difficult to explain, given the near unity genetic correlation between live weight recorded at the respective stages. It has to be considered that sampling issues could have caused this result. The lack of an influence due to the inclusion of both initial and final live weight together with egg or chick production on the genetic correlations of final live weight with reproduction in three-trait analyses does not support this line of reasoning. From these analyses it appears that the negative genetic covariance between final live weight and reproduction is real. Much more data are, however, required for the estimation of genetic correlations with sufficiently low standard errors to confirm or refute the contentions listed above.

On the female PE level, live weight was generally negatively related to reproduction. These correlations were somewhat smaller when assessed in three-trait analyses. It could be argued that the negative sign of these covariance components could be related to the demands of egg production on the metabolism of the breeding female being carried over to subsequent seasons. In other species,

correction for previous reproduction were found to impact on the magnitude of the female permanent environmental correlation between live weight and reproduction (Cloete *et al.*, 2004). In the study of Lambrechts *et al.* (1999), it was also shown that live weight change within a breeding season was negatively related to egg (–0.20) and chick production (–0.17) on a phenotypic level. Overall, the derived genetic and permanent environmental correlations resulted in a low but significant negative association of reproductive traits and hatchability with final live weight on the phenotypic level.

Genetic correlations between chest circumference and reproductive traits tended to be similar to those between live weight and reproductive traits, but of lower magnitude. Phenotypic correlations of chest circumference with reproductive traits were essentially zero and consistent with those available in the literature (Van Schalkwyk and Cloete, 1996; Lambrechts *et al.*, 1998). On a genetic basis, there was evidence of a stronger positive relationship between tail circumference and reproductive traits, although significance could not be demonstrated. Initially, significant negative phenotypic correlations of this trait measured at the beginning of the breeding season with reproductive traits were thought to hold promise with regard to the prediction of the reproductive capacity of ostrich females (Van Schalkwyk and Cloete, 1996). These correlations were –0.20 for egg production, –0.12 for chick production and –0.22 for hatchability. Phenotypic correlations in the present study were, however, much lower in magnitude and different in sign in the majority of cases. The differences in the sign and magnitude of the phenotypic correlations between studies are most likely to be associated with differences in the age structure of the breeding flock. During the time Van Schalkwyk and Cloete (1996) conducted their study, most breeding females were older than 10 years, with ages exceeding 20 years in some instances. Breeding females in the present flock were mostly younger than 12 years of age. When only two-year old females were considered, there was little evidence of a strong phenotypic relationship between tail circumference and reproductive performance (Lambrechts *et al.*, 1998). This can be ascribed to the two-year old females becoming sexually mature later in a breeding season, resulting in the number of eggs produced being generally low because they come into lay later than their older contemporaries.

The high correlations between reproductive traits were not unexpected. Previous studies suggested high correlations between additive genetic (Bunter, 2002; Cloete *et al.*, 2002b), and female permanent environmental (Bunter *et al.*, 2001b; Bunter, 2002; Cloete *et al.*, 2002b) effects, culminating generally in moderate to high phenotypic correlations (Van Schalkwyk *et al.*, 1996; Lambrechts *et al.*, 1999; Bunter, 2002). Lower environmental and phenotypic correlations between egg production and hatchability were due to the influence of service sire effects on the level of egg fertility. Service sire solutions for infertility were shown to be unfavourably related to chick production, suggesting that service sire infertility or mate incompatability deleteriously affected the chick production of companion females (Bunter *et al.*, 2001b, Cloete *et al.*, 2002b). The favourable correlation between service sire effects for hatchability and chick production in the present study supports this line of reasoning.

CONCLUSIONS

Significant genetic parameters for egg and chick production of commercial ostriches were evident in this study. Genetic progress in reproductive traits thus seems feasible in this livestock species. When it is considered that the production of leather and meat constitutes the outputs of any commercial ostrich operation (Cloete *et al.*, 1998; 2002a), it is evident that reproductive performance is of paramount importance in a commercial breeding program. Comparatively high repeatability estimates also suggest that worthwhile progress is also achievable in the current flock (Bunter *et al.*, 2001a). Moreover, reproduction does not seem to be unfavourably related to live weight prior to breeding on a genetic or phenotypic level. This result seems to open the avenues for the development of a dual-purpose ostrich strain, where emphasis may be placed on selection for both growth and reproductive traits. Other traits, like hide attributes, still need to be considered for the development of a breeding strategy that addresses all significant traits in the breeding goal.

It is important to state that the material used for the present study, as well as the bulk of research cited, was essentially derived from the same resource population. The results may not necessarily be robust for extension to other populations and regions (Bunter, 2002; Cloete *et al.*, 2002a). There is an urgent need for similar studies on other resource populations to confirm or refute the results obtained from the present study.

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Chapter 10

Semen quality of ostrich males (*Struthio camelus* var. *domesticus*) in relation to fertility in a commercial breeding system

Abstract

Phallus traits and semen quality were determined in 421 ostrich males, before the onset and at the end of breeding, and for four consecutive breeding seasons. Phalluses could be extracted in 386 males. Aspermic ejaculates, and urine and faeces contamination were recorded in 72 and 64 cases, respectively. Aspermic ejaculates were more prevalent before the onset of breeding. Phallus length and circumference were normally distributed, and moderately repeatable (0.30 and 0.28, respectively) and correlated on a permanent environment, environmental, and phenotypic level. Phallus size tended to increase with an increase in age, and was positively correlated to sperm concentration. Sperm concentration and percentage abnormal spermatozoa were exceedingly variable. Sperm concentration increased with an increase in age, but tended to decline in males aged 6 years and older. The percentage of abnormal sperm per ejaculate was not influenced by age, while sperm concentration was moderately repeatable (0.18), and the percentage abnormalities lowly repeatable (0.14). Ejaculate colour and viscosity increased linearly with an increase in age, and ejaculate colour tended to be lowly repeatable (0.05). Viscosity of ejaculates obtained from ostrich males subjected to flushing management practices, were higher than that obtained from males not subjected to flushing management practices. Sperm concentration was positively and significantly correlated with fertility at the end of breeding. Fertility of 75% and higher was reported for males between 5 and 8 years of age, and tended to decline in males older than 8 years. The most common sperm abnormalities included damage to the acrosome, swollen heads, and broken and loose tails. Aggregations of sperm were observed in samples obtained from different males, and possibly represent a form or mechanism of sperm competition in ostriches. No reliable male trait could be identified that can be used as a reliable indicator of male fertility in commercial breeding flocks. Traits that can aid in the development of a semen quality index that can be used in commercial breeding program to select potentially fertile ostrich males, need to be identified. The role of endocrine and behavioural mechanisms, such as sperm competition and cryptic female choice, that may contribute to an individual's reproductive success, also warrants further investigation.

INTRODUCTION

Puberty in the avian male can be defined as the age when spermatozoa first appear in the ejaculate. Puberty and sexual maturity, however, is not synonymous. Sexual maturity is defined as the time when testis growth reaches its maximum, and the number and quality of spermatozoa is at a peak (Howarth, 1995). Ostrich males are considered sexually mature at approximately 3-4 years of age, with factors such as breed and time of the season when hatched, influencing the onset of sexual maturity (Hicks-Aldredge, 1994; Smith *et al.*, 1995; Horbañczuk and Sales, 1999). Spermatogenic activity, however, has been reported for prepubertal ostriches, with

spermatogenesis undergoing seasonal changes as observed in mature breeding ostrich males (Madekurozwa *et al.*, 2002).

The ostrich male has an intromittant phallus which lies folded up into the proctodeum of the cloaca. The ostrich phallus is characterised by a dorsal sulcus or groove that runs on the dorsal surface, and the absence of a urethra. The dorsal groove directs the semen into the cloaca of the female during copulation (Fowler, 1991). In the mature ostrich male, phallus length varies from 20cm when flaccid, to 40 cm when erect. Kreibich and Sommer (1995) reported phallus lengths of between 25 to 39 cm for immature ostrich males. When erect, the ostrich phallus projects from the cloaca in a ventro-cranial curve, and deviates to the left due to the presence of asymmetrical fibrolymphatic bodies (McCracken, 2000; Fowler, 1991).

Several factors may influence a male's fertility, and these may include genetic, behavioural and environmental aspects. Soley and Roberts (1994) determined that ostrich spermatozoa are of the sauropsid type that are characteristic of non-passerine birds, and in general resembles chicken, turkey, guinea fowl, tinamou and budgerigar spermatozoa. A variety of sperm defects are known that adversely affect fertility in domestic animals, with defects well documented by Oettlé and Soley (1988), Hafez (1987), Marquez and Ogasarawa (1975), Saeki and Brown (1962), and Saeki (1960). Various studies have reported parameters used for ostrich semen evaluation (Soley *et al.*, 1996; Hemberger, 1996; Irons *et al.*, 1996; Irons, 1995; Bertschinger *et al.*, 1992; Hicks, 1990; Von Rautenfeld, 1977). Studies performed by Soley (1992; 1996) and Von Rautenfeld (1977) also reported on the histology of the ostrich male reproductive tract and ostrich sperm morphology.

Very little, however, is known about the relationship between phallus traits and various ejaculate parameters, and the relationship with fertility of eggs produced by companion females. This study investigated the relationship between male phallus traits and semen quality, and the fertility of eggs produced by companion females under commercial breeding conditions, as well as the influence of age, season, and management on the phallus and sperm traits measured in ostrich males.

MATERIALS AND METHODS

Experimental animals

Ostrich males maintained in a breeding flock (n=136 breeding pairs) at the Little Karoo Agricultural Development Centre outside Oudtshoorn, South Africa, were used in the study. Management of the breeding flock have been documented by Van Schalkwyk *et al.* (1996), and Bunter and Graser (2000). The ages of the males used in the study ranged between 2 and 10 years. During the second and third year of the study, the breeding pairs were subjected to flushing and teasing management practices (Materials and Methods, Chapter 2).

Measurement of phallus length and circumference

Phallus length and circumference were measured with a tape measure. The phallus was everted from the proctodeum and extended distally, and phallus length was obtained by measuring the distance (in cm) from the opening of the dorsal groove (A, Figure 1) to the distal tip of the phallus (B, Figure 1). Phallus circumference was obtained by measuring the circumference (in cm), as indicated by C and D (Figure 1).

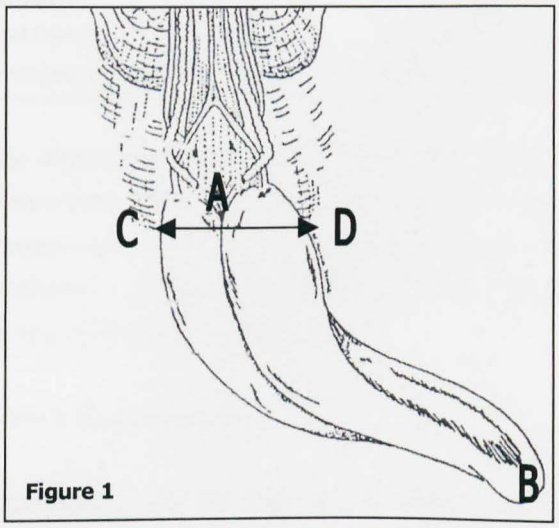

Figure 1

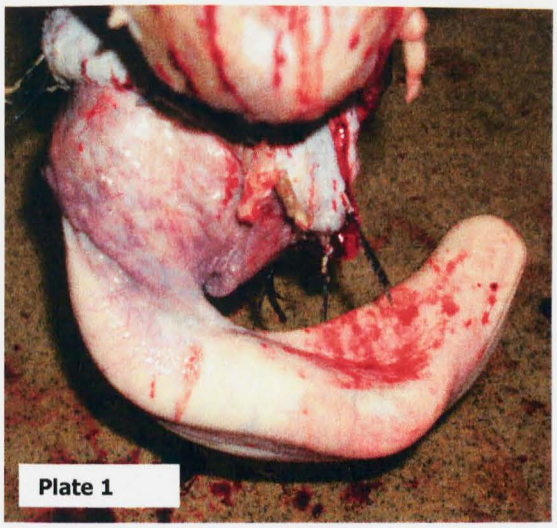
Plate 1

Figure 1 and Plate 1. The drawing on the left represents an ostrich phallus, with A, B, C and D indicating the measurement points for phallus length and circumference (adapted from Jensen *et al.*, 1992). Plate 1 represents a post-mortem photograph of an ostrich phallus, indicating the deviation of the phallus to the left-hand side when everted (photograph taken from the dorsal side).

Semen collection and evaluation

Due to the lack of sexual imprinting in the ostrich males used in this study, the forced massage method, as described by Soley (1992), with a minor modification, was used to collect the semen samples. Each male was hooded and injected with 5 IU of oxytocin 5 minutes prior to stimulation and collection (Bertschinger *et al.*, 1992). In several cases it was found that the reaction period of 5 minutes was too short to allow collection to be satisfactory performed. Instead of administering a second dose of oxytocin, the period between injection and collection was extended to 7-10 minutes and this proved to be sufficient to allow successful collection in most cases. Semen samples were obtained from ostrich males before the onset and at the end of the 1999/2000, 2000/2001, 2001/2002 and 2002/2003 breeding seasons.

Macroscopic evaluation

Semen samples were macroscopically evaluated on a scale of 0-4 for colour, and on a scale of 1-5 for viscosity (Table 1). Ejaculate colour ranged from creamy white (4) to almost transparent (0), and ejaculate viscosity ranged from thick creamy (5) to watery (1). Viscosity and colour of

ejaculates were only evaluated during the 2000/2001, 2001/2002 and 2002/2003 breeding seasons.

Table 1. A scale for the macroscopic assessment of colour and viscosity score of ostrich semen samples.

TRAIT	SCORE	TRAIT	SCORE
Colour		Viscosity	
Creamy white colour	4	Thick creamy	5
Creamy colour	3	Creamy	4
Light grey	2	Thin creamy	3
Light brown	1	Milky	2
Almost transparent	0	Watery	1

After macroscopic evaluation, nigrosin-eosin (1-% eosin and 5% nigrosin in 3% sodium citrate) smears were prepared by adding 1 drop of the staining reagent to 1 drop of semen. The sperm-nigrosin-eosin droplet was gently mixed and after approximately a minute a drop of the mixture was transferred to a clean microscopic slide and a smear was made. Each smear was allowed to air-dry and stored for later analysis.

Microscopic evaluation

Spermatozoa with the head gently curved, crescent-shaped or convoluted, and that formed a complete unit with the mid-piece and tail, were regarded as normal, viable spermatozoa (Hemberger *et al.*, 2001; Irons *et al.*, 1996; Soley and Roberts, 1994; Soley, 1992). Spermatozoa exhibiting any of the following defects were regarded as abnormal:

- → Swollen heads, i.e. bulb-headed spermatozoa
- → Broken head-mid-piece junctions, i.e. crooked neck spermatozoa
- → Damage to the acrosome, i.e. translucent head where nucleus could be observed under segments of remaining acrosome
- → Damage to the tails, i.e. bent or broken tails
- → Dead spermatozoa, i.e. spermatozoa completely stained by the staining reagent

Male fertility was not directly determined, and therefore the fertility of eggs produced by a male's companion female was regarded as the fertility of that specific breeding male. Only breeding pairs where females produced 5 or more settable eggs, were considered in the calculation of fertility. Fertility of eggs produced by companion females was expressed as a percentage, and calculated according to the following equation:

Fertility (%) = [(number of eggs set-number of infertile eggs)/number of eggs set] X 100

The analySIS™ program (Version 3.00; Soft Imaging System GmbH, Germany) was used to determine the number of sperm per ejaculate, and to evaluate sperm morphology. Nine fields were counted and averaged to obtain a representative count for each smear. Counts were performed at X200 magnification, and examination of abnormalities were performed at X400 magnification.

Statistical analysis

Data obtained on an all-or-none scale (i.e. whether it was possible to extract or not extract a phallus, contamination of semen with urine or faeces or no contamination, and aspermic or no aspermic samples) were expressed as proportions and analysed by standard Chi^2 procedures (Siegel, 1956). Proportions at the commencement of breeding were compared to those at the cessation of breeding, using 2 X 2 analysis of variance. Analyses of the phallus traits and semen quality were complicated by the fact that the same individuals were sampled repeatedly. The covariance arising from the repeated sampling of the same animal was accounted for by the inclusion of random animal effects in the mixed models used during the analyses. In view of the fact that only 192 to 232 observations, depending on the trait considered, were available for analysis, it was not attempted to partition the between animal effects into direct genetic and permanent environmental components.

ASREML (Gilmour *et al.*, 1999) was used for the analyses. The software is suitable for the analysis of mixed models, and allows for the estimation of variance components of selected random effects, as well as the prediction of least squares means for specific fixed effects. Fixed effects included in the present study included breeding season, management practice (flush feeding and/or teasing), and stage of the breeding season when ejaculates were obtained (before onset of or end of breeding). Trends with regard to animal age were longitudinal by definition and modelled by using cubic splines (Verbyla *et al.*, 1999). The cubic splines consisted of a fixed linear component and a random non-linear component depicting random deviations conforming to a smooth trend. Initially, random deviations from linearity not conforming to a smooth trend were also fitted, but this component was found to be not significant ($P \geq 0.05$) for any of the variables. The first analyses involved fitting various combinations of fixed effects, random spline components and interactions between them to obtain an operational model. When this model was established, random animal effects were added. Between and within animal variance components were used for the estimation of repeatability for the respective phallus traits and sperm characteristics. Two-trait models were subsequently fitted to obtain all relevant between animal, environmental and phenotypic correlations between the phallus and sperm traits.

RESULTS

It was possible to extract the phalluses of 368 (87.4%) of the 421 males that were sampled during the study period, i.e. between 1999 and 2002. No difference was found between males sampled successfully at the beginning (86%) or end (89%) of the breeding season (0.86 vs. 0.89 respectively). Of the 368 males that were sampled, 64 semen samples (17.4%) were discarded due to contamination with urine and/or faeces, with no difference between the proportions at the beginning or end of the breeding season. Of the remaining 304 individuals that were sampled, 72 (23.7%) were found to be aspermic. These proportions differed ($P \leq 0.01$) according to the time of the breeding season (0.26 vs. 0.13 at the beginning and end of breeding, respectively).

In the calculation of the descriptive statistics of the anatomical and sperm traits, only data recorded for males where both the phallus could be retrieved and an ejaculate with sperm could be obtained, were used. Phallus length and circumference were normally distributed, with coefficients of variation of 14.1 and 11.3 %, respectively (Table 2). Sperm concentration and the percentage of abnormal sperm showed considerable variation. The transformation of sperm concentration to the natural logarithm scale assisted to stabilise the variance for this trait. The percentage of abnormal sperm was also transformed, for similar reasons, using the arc sine transformation prior to analysis (Snedecor and Cochran, 1967). Visually assessed sperm traits (viscosity and colour) were normally distributed and fairly variable, with coefficients of variation just exceeding 30%. The fertility of eggs produced by companion females varied considerably, ranging between 0 and 100%.

Table 2. Phallus traits and semen quality parameters (mean and SD) measured in ostrich males during four consecutive breeding seasons.

TRAIT	N	Mean (SD)	Range	CV (%)
Anatomical traits				
Phallus length (cm)	228	27.0 (3.8)	13 - 35	14.1
Phallus diameter (cm)	228	18.6 (2.1)	12 - 24	11.3
Sperm traits				
Sperm concentration per mL ($X10^4$)	232	830 (978)	26.7 - 6588.3	1.18
Abnormalities (%)	232	23.5 (22.7)	1.0 - 84.4	96.6
Viscosity (score)	151	4.01 (1.26)	1 – 5	31.4
Colour (score)	151	2.84 (1.05)	0 – 4	36.9
Fertility (%)	171	74.2 (26.8)	0 - 100	36.1

Influence of management practice and period of collection

Almost all phallus and sperm traits were unaffected by the flush feeding and teasing management practices. The only exception was the viscosity of ejaculates that were significantly ($P \leq 0.05$) increased by flush feeding (Table 3). Phallus traits varied significantly ($P \leq 0.05$) between years, with phallus measurements obtained at the end of breeding being bigger ($P \leq 0.01$) than measurements obtained before the onset of breeding (Table 3).

Sperm concentration tended to be the highest during the first and last years of the study, and lower prior to the onset of breeding (Table 3). The percentage of abnormalities was also considerably lower ($P \leq 0.01$) during 1999 than in any of the other years. The percentage of abnormal sperm also tended to be lower prior to the onset of breeding than at the end of a breeding period (Table 3). The viscosity of ejaculates did not differ between breeding seasons, with viscosity of ejaculates obtained prior to the onset of breeding being lower ($P \leq 0.01$) than viscosity of ejaculates obtained at the end of breeding. The colour scores for ejaculates obtained prior to the onset of breeding, however, was higher ($P \leq 0.01$) than that of ejaculates obtained at the end of breeding. The colour scores assigned to ejaculates were highest ($P \leq 0.01$) during the 2000/2001 breeding season, and lower during the 2001/2002 and 2002/2003 breeding seasons.

Table 3. Means (± SE) of phallus length (PL), phallus circumference (PC), and sperm traits measured in ostriches, as influenced by management practice, breeding season, and time of collection.

PARAMETER	PHALLUS TRAITS		SPERM TRAITS			
	PL (cm)	PC (cm)	Sperm concentration	Abnormalities	Viscosity	Colour
Overall mean	26.9±0.4	18.9±0.2	6.3±0.2 (528.5X10^4)	24.8±2.0 (17.6)	4.1±0.1	2.6±0.1
Management practice						
Flushing	ns	ns	ns	ns	*	ns
No	26.4±0.6	18.9±0.3	6.3±0.2 (550.0X10^4)	24.1±2.7 (16.7)	3.9±0.2	2.7±0.2
Yes	27.4±0.4	19.0±0.2	6.2±0.2 (512.9X10^4)	25.4±1.9 (18.4)	4.3±0.1	2.5±0.1
Teasing	ns	ns	ns	ns	ns	ns
No	26.8±0.6	18.6±0.3	6.3±0.2 (523.2X10^4)	25.5±2.7 (18.5)	3.9±0.2	2.6±0.1
Yes	27.1±0.4	19.3±0.3	6.3±0.2 (539.2X10^4)	24.0±2.0 (16.5)	4.3±0.2	2.6±0.1
Breeding season	*	**	ns	**	ns	**
1999/2000	28.2±0.7	17.6±0.4	6.4±0.3 (584.1X10^4)	10.2±3.0 (3.1)	----	----
2000/2001	26.3±0.4	19.0±0.2	6.2±0.2 (473.4X10^4)	28.2±2.1 (22.3)	3.8±0.1	3.4±0.1
2001/2002	25.4±0.5	18.3±0.3	6.2±0.2 (492.7X10^4)	32.7±2.2 (29.2)	3.9±0.1	2.3±0.1
2002/2003	27.8±0.5	20.8±0.6	6.4±0.4 (584.1X10^4)	28.0±4.6 (22.0)	4.5±0.4	2.0±0.3
When collected	**	**	ns	ns	**	**
Before onset of breeding	25.6±0.5	18.0±0.3	6.2±0.2 (473.4X10^4)	25.9±2.2 (19.1)	3.6±0.2	2.8±0.1
At the end of breeding	28.3±0.6	19.9±0.3	6.4±0.2 (595.9X10^4)	23.6±2.4 (16.0)	4.6±0.2	2.4±0.1

Note: The sperm concentration and percentage abnormalities values represent the transformed ln- and arcsine values, respectively (non-transformed values in brackets); ns = Not significant (P≥0.05); * = Significant (P≤0.05); ** = Highly significant (P≤0.01)

Influence of age on phallus and sperm traits

The average phallus length and circumference measured in two-year old males was 26.7±0.7 cm, compared to 27.1±0.5 cm in 10 year old males (Figure 2). This difference in phallus circumference, however, was found to be not significant.

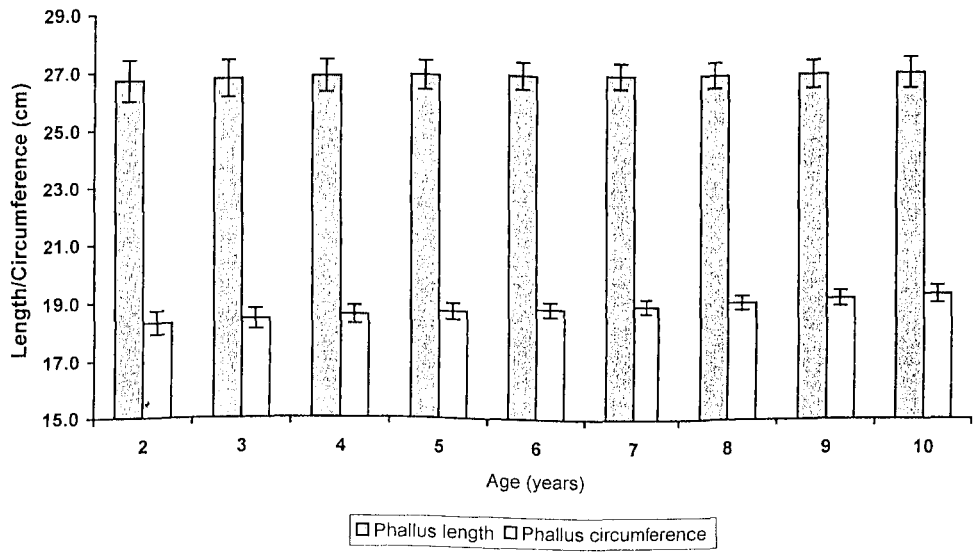

Figure 2. The influence of age on phallus length and circumference (±SE) measured in breeding ostrich males.

Sperm concentrations generally increased (P≤0.05) with an increase in male age to reach a maximum in five-year old ostrich males (Figure 3), and declined in males aged six years and older.

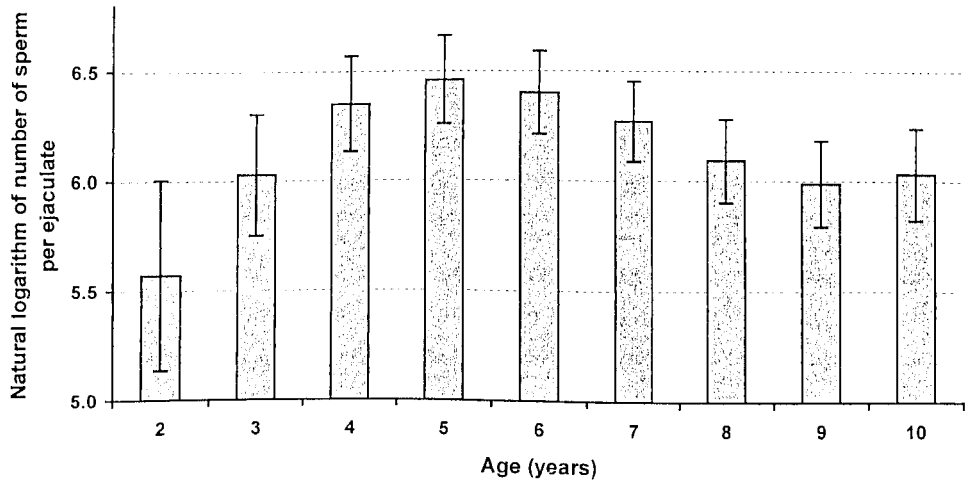

Figure 3. The effect of age on the sperm concentration (±SE) of ejaculates obtained from breeding ostrich males.

Age groups did not differ in terms of the percentage of abnormal sperm (Figure 4).

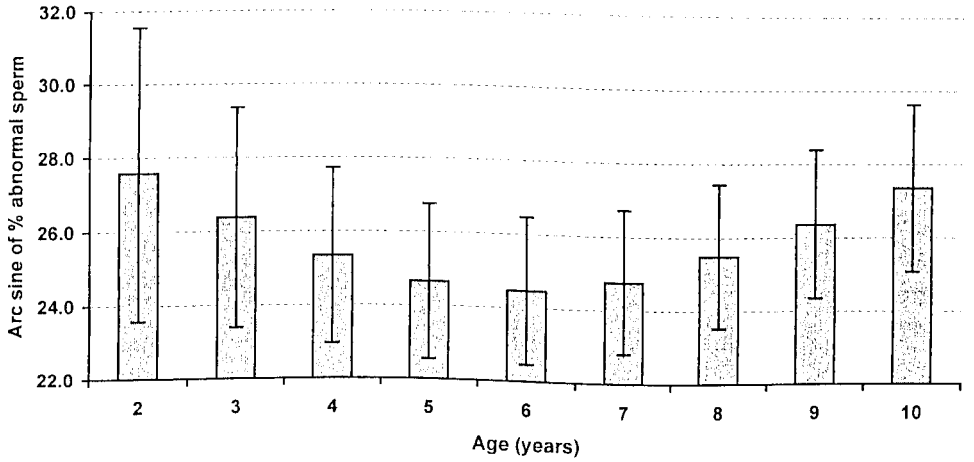

Figure 4. The influence of male age on the percentage of abnormalities in ejaculates obtained from breeding ostrich males.

Subjective scores of ejaculate viscosity and colour increased (P≤0.05) linearly with an increase in age (Table 4).

Table 4. Relationship between age, and colour and viscosity scores (±SE) recorded for ostrich semen samples.

Age (years)	Colour score [Scale=1-4]	Viscosity score [Scale=1-5]
2	1.9±0.2	3.9±0.2
3	1.9±0.2	3.9±0.2
4	2.1±0.2	4.1±0.2
5	2.2±0.2	4.1±0.1
6	2.3±0.1	4.2±0.2
7	2.3±0.1	4.2±0.2
8	2.4±0.1	4.3±0.2
9	2.5±0.2	4.4±0.2
10	2.5±0.2	4.4±0.2

Fertility of eggs produced by companion females generally increased when the females were paired with males aged 3 years and older (Figure 5). Fertilities of 75% and higher were reported for males between 5 and 8 years of age. Fertility tended to declined below 75% in ostrich males older than 8 years.

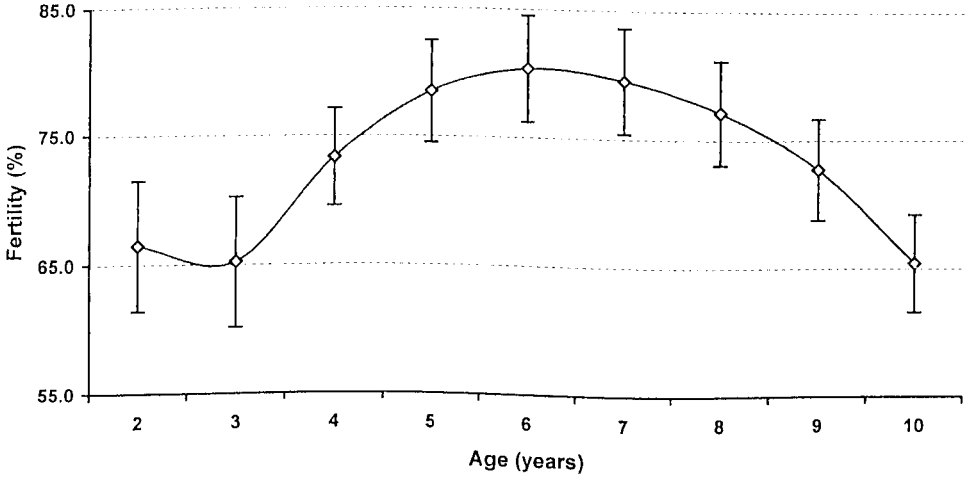

Figure 5. The effect of male age on the fertility of eggs produced by companion females.

Phallus length and circumference measurements were moderately repeatable (Table 5), and with the exception of number of spermatozoa per ejaculate, none of the estimates for sperm traits exceeded a level of more than double the corresponding standard error. The residual or environmental component of the phenotypic variance was consistently higher for all traits. The between animal variance for viscosity score went to the boundary of parameter space (zero), and is thus not presented.

Table 5. Variance components and repeatability (t) estimates for phallus traits and semen parameters of breeding ostrich males.

VARIANCE COMPONENTS	PHALLUS		SPERM		
	Length	Circumference	Concentration	Abnormalities	Colour
Between males	3.339	0.969	0.268	29.72	0.037
Environment	7.992	2.513	1.214	186.48	0.732
Phenotype	10.331	3.482	1.492	216.20	0.769
t ± SE	0.30±0.08	0.28±0.08	0.18±0.08	0.14±0.08	0.05±0.11

Phallus length and phallus circumference was significantly (P≤0.05) correlated on a permanent environment (0.67), environmental (0.50), and phenotypic (0.55) level (Table 6). Phallus length and circumference were also positively correlated (P≤0.05) with sperm concentration. Correlations of phallus traits with abnormalities and subjective colour score were generally low. Sperm concentration could not be related to colour score, and was closely related to the percentage of sperm abnormalities per ejaculate.

Table 6. The correlation of phallus length and circumference with semen parameters of breeding ostrich males.

TRAIT	CORRELATION		
	Between males	Environment	Phenotypic
Phallus length X			
Phallus circumference	0.67±0.15	0.50±0.07	0.55±0.05
Sperm concentration	0.20±0.28	0.12±0.09	0.14±0.07
Colour	-0.43±0.71	0.06±0.11	-0.01±0.09
Abnormalities	0.10±0.33	-0.14±0.09	-0.09±0.07
Phallus circumference X			
Sperm concentration	-0.12±0.30	0.17±0.09	0.10±0.07
Colour	0.00±0.66	-0.01±0.11	-0.01±0.08
Abnormalities	-0.01±0.33	-0.09±0.09	-0.07±0.07
Sperm concentration X			
Colour	0.42±1.16	-0.15±0.11	-0.10±0.08
Abnormalities	-1.01±0.14	-0.69±0.05	-0.74±0.03
Colour X			
Abnormalities	-0.68±1.49	0.06±0.10	0.01±0.08

Repeatability estimates for fertility of the ejaculates obtained before the onset and at the end of breeding were highly repeatable (0.40±0.12 and 0.41±0.12, respectively). The repeatability estimates for fertility with colour score was 0.39±0.12 and 0.40±0.12, for the period before and at the end of breeding, respectively.

The correlations reported for phallus and sperm traits with fertility were insignificant in most cases. Phallus length was negatively correlated (P≤0.05) with fertility when assessed prior to the onset of breeding, on a phenotypic level (Table 7). The only exception was the correlation of sperm

concentration with fertility, on an environmental level and measured at the end of breeding (Table 7). Fertility tended (P≤0.10) to be negatively correlated with the percentage of sperm abnormalities on a permanent, environmental, and phenotypic level. In most cases, higher correlations were reported at the end of a breeding season (Table 7). Fertility tended (P≤0.10) to be positively correlated with sperm concentration only on an environmental and phenotypic level, with higher correlations at the end of breeding. Fertility tended (P≤0.10) to be negatively correlated with sperm concentration on a permanent environmental level, and negatively correlated with colour of ejaculates on a permanent environmental and phenotypic level before onset of breeding, and on a permanent environmental level at the end of breeding (Table 7). Similarly, ejaculates with a higher colour score tended (P≤0.10) to be more fertile when assessed prior to the onset of breeding, and also on both an environmental and phenotypic level when assessed at the end of breeding (Table 7).

Table 7. The correlations of phallus traits and semen parameters with fertility of eggs produced by companion females, determined for ejaculates obtained before the onset of and at the end of breeding for ostrich males.

PARAMETER AND TIME OF COLLECTION	CORRELATION WITH FERTILITY		
	Permanent environment	**Environment**	**Phenotypic**
Prior to breeding			
Phallus length	-0.28±0.30	-0.15±0.14	-0.19±0.09*
Phallus circumference	-0.04±0.27	-0.27±0.14	-0.18±0.09
Sperm concentration/mL	-0.05±0.35	0.15±0.15	0.08±0.10
% Abnormalities	-0.57±0.34	-0.29±0.18	0.05±0.12
Colour	-0.28±0.32	0.13±0.24	-0.06±0.13
End of breeding			
Phallus length	-0.35±0.41	0.20±0.13	0.04±0.09
Phallus circumference	0.04±0.26	0.07±0.14	0.05±0.09
Sperm concentration/mL	-0.18±0.39	0.36±0.14*	0.18±0.09
% Abnormalities	-0.25±0.46	-0.12±0.16	-0.14±0.10
Colour	-0.36±1.21	0.26±0.18	0.14±0.11

*: P≤0.05

Microscopic sperm abnormalities

Plates 2 and 3 represent micrographs depicting normal morphology of ostrich spermatozoa, at X200 and X400 magnification, respectively.

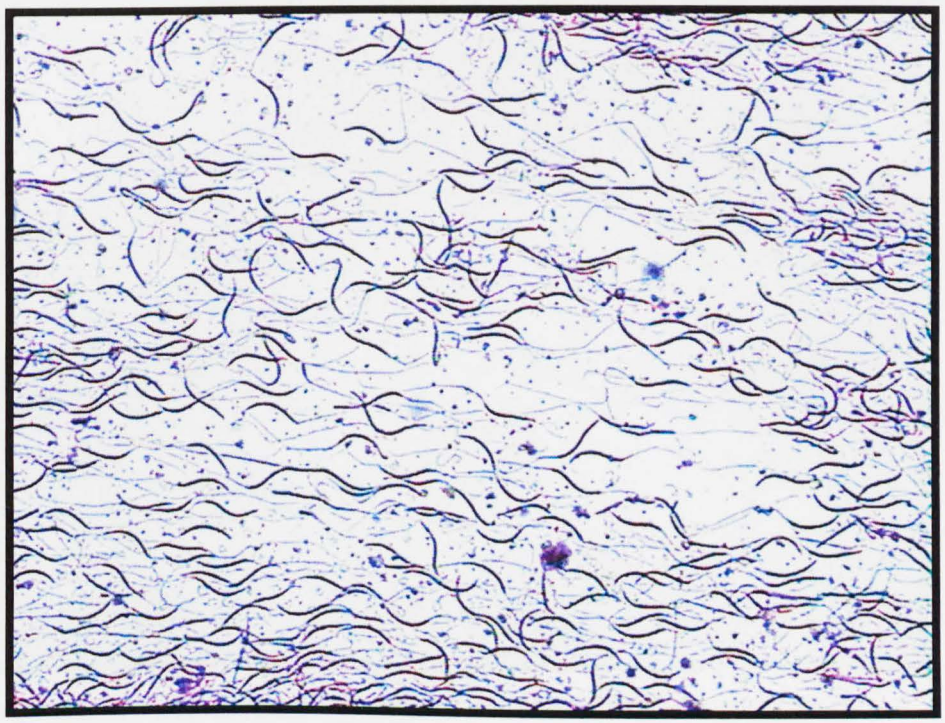

Plate 2. A micrograph depicting morphologically normal ostrich sperm (X200 magnification).

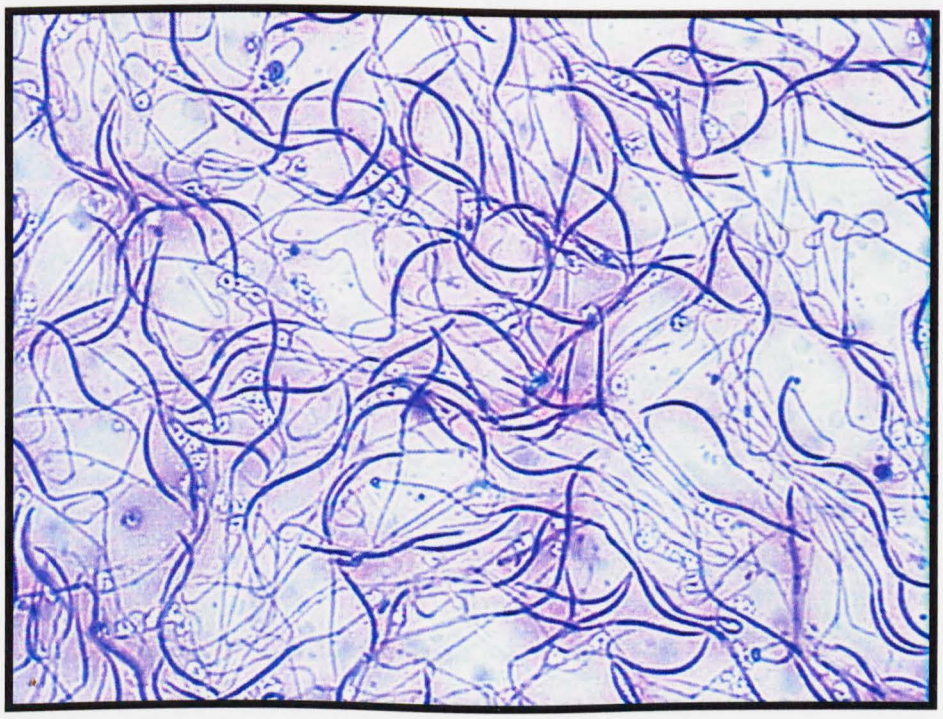

Plate 3. A micrograph depicting morphologically normal ostrich sperm (X400 magnification).

Sperm abnormalities observed during evaluation were sperm with acrosomal damage (Plate 4; X400 magnification), swollen heads (Plate 5; X400 magnification), and damage to tails (Plate 6; X400 magnification).

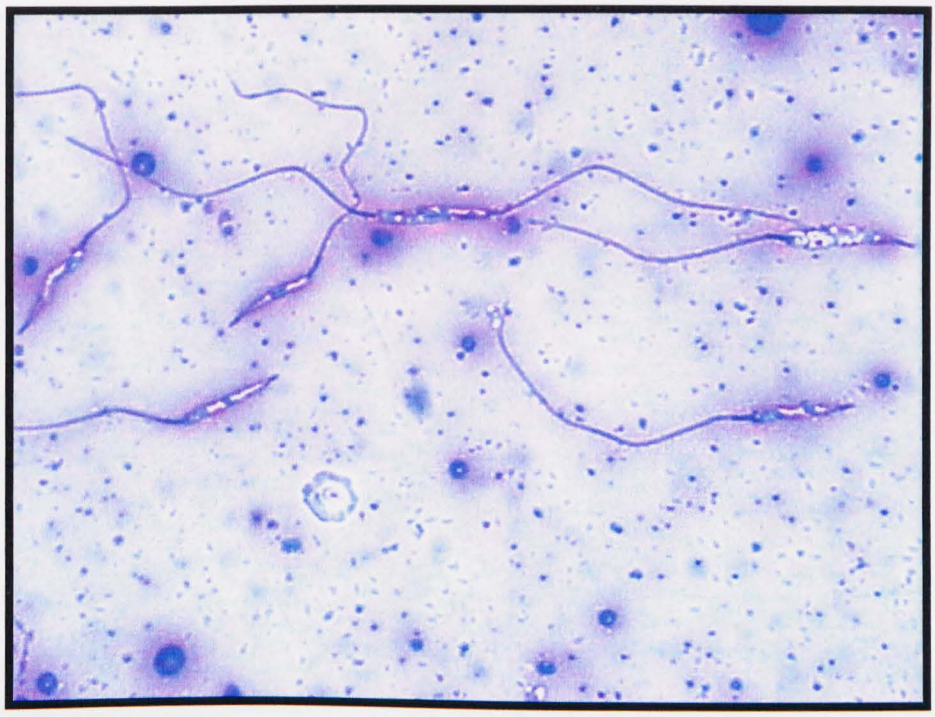

Plate 4. A micrograph depicting acrosomal damage to ostrich sperm (X400 magnification).

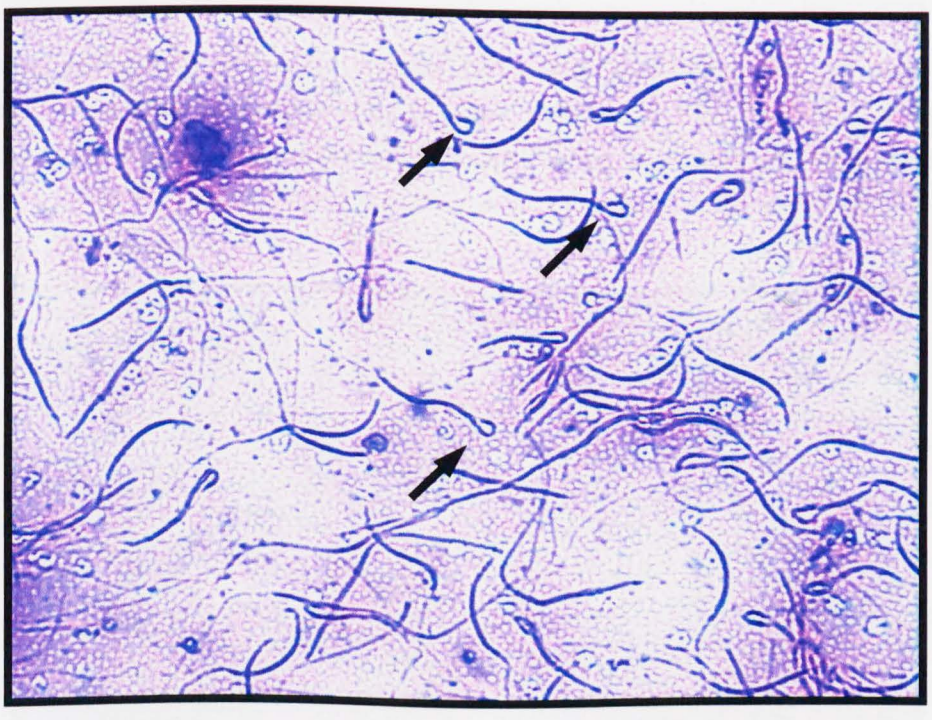

Plate 5. A micrograph depicting ostrich sperm with swollen heads (arrows) (X400 magnification).

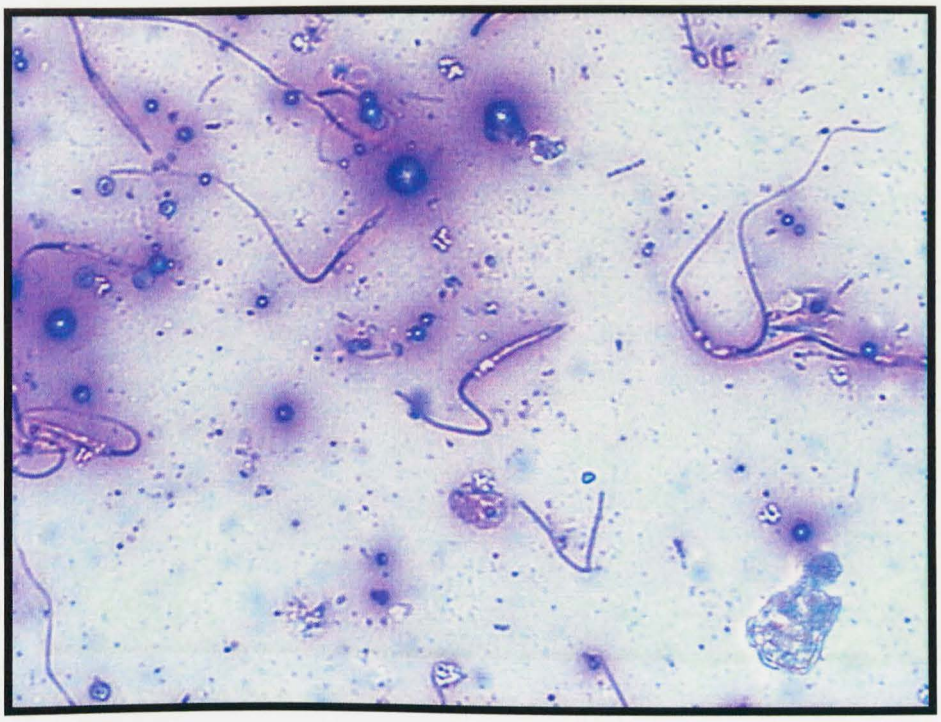

Plate 6. A micrograph depicting ostrich sperm with abnormal morphology, i.e. broken and loose tails (X 400 magnification).

An interesting observation during the microscopic semen evaluation, was the agglutination of spermatozoa. Hundreds of spermatozoa clumped together to form "clusters" of sperm, and spermatozoa in the vicinity of such clusters appeared to be orientated with their heads towards such clusters. Plates 7 and 8 depict micrographs of agglutinated sperm (X200 and X400 magnification, respectively).

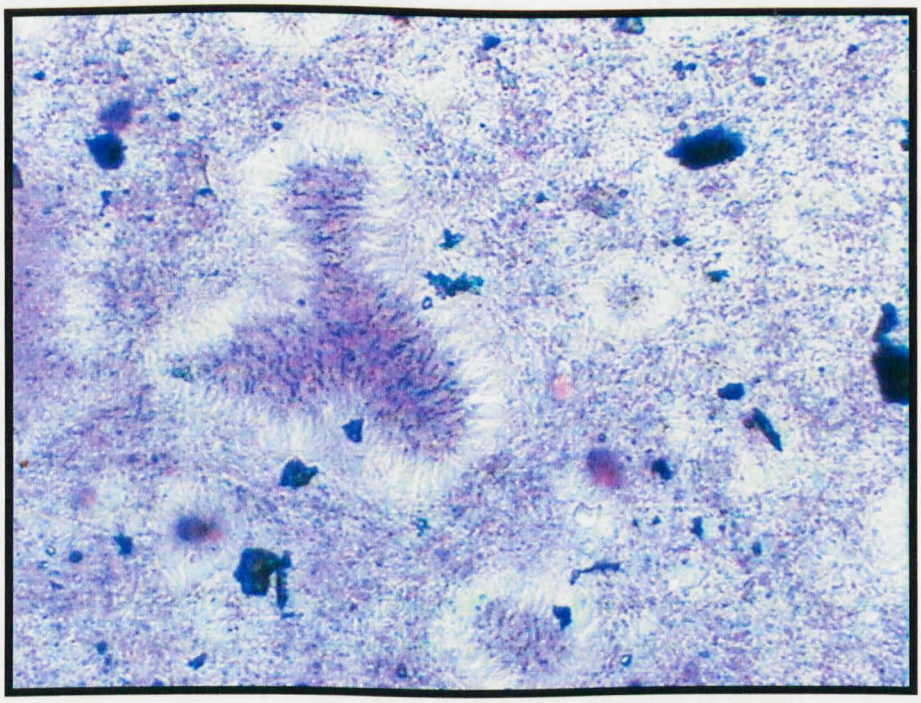

Plate 7. A micrograph depicting the agglutination of ostrich sperm (X200 magnification).

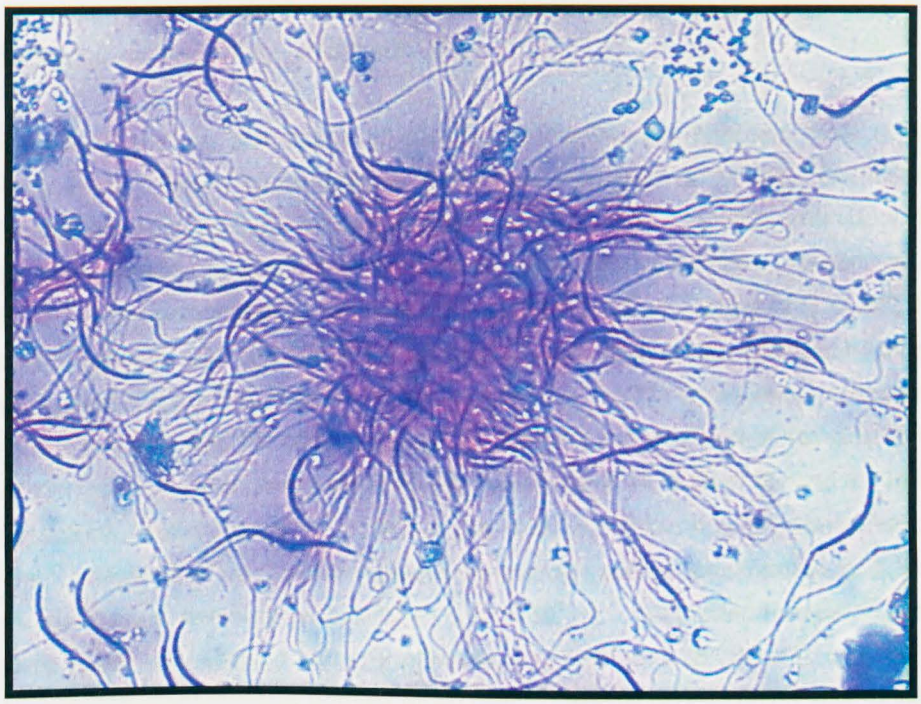

Plate 8. A micrograph depicting the agglutination of ostrich sperm (X400 magnification).

DISCUSSION

Phallus traits

Phallus length and circumference measured ranged between 13-35cm and 12-24cm, respectively. The minimum phallus length reported in this study is shorter than that reported by Kreibich and Sommer (1995) and Fowler (1991). Kreibich and Sommer (1995) reported a phallus length of 25cm for males of 16-18 months, and of 29-39 cm for males older than 2 years of age, and Fowler (1991) reported phallus lengths of up to 40cm when erect.

Phallus size differed significantly between the respective years, being the largest during the first year of the study. A higher proportion of young males that were included in the breeding population during the second year of the study possibly attributed to this significant difference in phallus size. Larger phalluses measured at the end of breeding can be possibly be ascribed to the fact that several males were still sexually passive or inactive before being paired for breeding. Ostrich males tend to come into production later than females, and circulating levels of gonadotrophins and steroid hormones may have been too low to result in phallus development before the onset of breeding (Deeming and Bubier, 1999).

Phallus length was significantly correlated with phallus circumference on all levels of assessment. Phallus size was also positively correlated with sperm concentration. In waterfowl that also possess an intromittant copulatory organ, both testes size and phallus length were related to a higher frequency of extra-pair copulations in males (Coker *et al.*, 2002).

Sperm concentration and percentage of abnormalities

Sperm concentration and the percentage of sperm abnormalities per ejaculate were extremely variable in this study. Sperm concentrations ranged between 26.7 X 10^4.mL^{-1} and 6588.3 X 10^4.mL^{-1}. Rozenboim *et al.* (2003), Hemberger *et al.* (2001), Irons *et al.* (1996), and Hicks (1990) also reported large variation in sperm concentration of ostrich semen samples. Hemberger *et al.* (2001) reported sperm concentrations of between 8.9 and 78.1 X 10^6.μL^{-1}, Irons *et al.* (1996) sperm concentrations of between 1.67 and 636 X 10^6 sperm.mL^{-1}, and Hicks (1990) sperm concentrations of between 2.5 and 3.2 X 10^9.mL^{-1}. In the study of Irons *et al.* (1996), some samples had too few sperm to count. This was also the case in this study. Sperm concentrations of samples collected by means of the funnel, vacuum, and tube methods, were 0.66±0.14, 2.35±0.26, and 2.13±0.27 X 10^9.mL^{-1}, respectively (Rozenboim *et al.*, 2003). YaJie *et al.* (2001) found sperm concentrations of 1.57 X 10^9.mL^{-1}. Malecki *et al.* (1996a) reported an average sperm concentration of 3.34±0.14 X10^9.mL^{-1} for male emus, and a range of 0.87 to 4.67 X 10^9.mL^{-1}. In frizzled Hungarian ganders, sperm concentrations also showed extreme variation, i.e. 0.26 to 2.25X10^6.μl^{-1}, with 84% live spermatozoa and 37% abnormalities (Almasi *et al.*, 2002). Sperm concentration was moderately repeatable, whereas the repeatability of percentage abnormalities did not reach significance. Sperm concentration was negatively correlated with percentage abnormalities, on a permanent environment, environment, and phenotypic levels.

Generally, higher sperm concentrations were reported for males aged five to eight years, with a tendency for sperm concentration to decline in males aged 9 years and older. Sperm concentrations tended to be higher at the end of breeding, which is contradictory to that observed by Bertschinger *et al.* (1992). Bertschinger *et al.* (1992) reported high sperm concentrations at the beginning of breeding, with increased sperm concentrations associated with the peak breeding season and elevated testosterone levels. Rozenboim *et al.* (2003) on the other hand, found semen characteristics to show no seasonal pattern, with peak sperm concentrations reported during June-July in Israel. In Northern pintail ducks, semen production were correlated with testosterone concentrations, with highest levels associated with the peak mating period (Penfold *et al.*, 2000). Bah *et al.* (2001) also reported a positive correlation between sperm concentration, total sperm count, and semen volume in the domestic fowl.

The percentage of abnormalities was highly variable and ranged between 1.0 and 84.4%. Hemberger *et al.* (2001) reported 5-26% abnormal and 4-28% dead spermatozoa, Rozenboim *et al.* (2003) 5.8±1.8%, 4.7±1.2%, and 7.1±1.7% abnormalities for the funnel, vacuum, and tube collection methods, respectively, and YaJie *et al.* (2001) 13.3% percentage abnormalities. Age did not significantly influence the percentage of sperm abnormalities per ejaculate. The percentage abnormalities were significantly lower during the first year of the study, however, possibly resulting from a higher proportion of sexually mature males in the breeding population. The percentage abnormalities were also lower before the onset of breeding than at the end of a breeding season.

Colour and viscosity

Ejaculate colour and viscosity were variable, and comparable to that described by Hemberger *et al.* (2001) and Irons *et al.* (1996). Ejaculates collected before the onset of breeding were awarded higher colour scores than ejaculates obtained at the end of breeding. A lymph-like fluid of cloacal origin, also known as transparent fluid, contributes to semen volume during copulation in poultry, and is derived from lymph folds adjacent to and caudal to the ejaculatory papillae (Howarth, 1995; Jensen *et al.*, 1992). It is possible that this transparent fluid contributed to a limited extent to ejaculate volume due to the absence of copulation in the period before the onset of breeding, compared to ejaculates obtained at the end of a breeding season when this contribution is expected to be more extensive due to a higher mating frequency. Assigned semen colour scores also differed between the respective years, being the highest during the second year of the study. Male age significantly influenced the colour scores of ejaculates, with ejaculates from young males receiving the lowest colour scores. The darker ejaculates produced by the younger males can possibly be attributed to the absence of sexual activity or a lower mating frequency. Ejaculate colour were lowly repeatable.

Ejaculates obtained from ostrich males receiving a flushing diet were more viscous than ejaculates from males not fed a flushing diet. The viscosity of ejaculates produced at the end of breeding was higher than that of ejaculates produced before the onset of breeding. Age had a significant influence on the viscosity of ejaculates, improving with an increase in age. According to Irons *et al.* (1996), viscosity (consistency) of ejaculates obtained from ostrich males is related to the presence of sperm granules, and not to sperm concentration.

Correlation of phallus and sperm traits with fertility

The correlations of phallus measurements and sperm traits with fertility were insignificant in almost all cases. The only exception was sperm concentration, that was positively ($P \leq 0.05$) correlated to fertility when estimated at the end of a breeding season. This is contradictory to the absence of a seasonal effect on the fertilisation rate of ostrich spermatozoa reported by Malecki and Martin (2003). In the latter study, the low fertilisation rates appeared to be a result of a lack of synchrony between timing of laying and sperm production, and a lack of mating. Fertility tended to be negatively correlated with percentage abnormalities, with higher correlations at the end of breeding. The fertility of ejaculates with a high colour score tended to be higher when assessed before the onset and at the end of breeding. Repeatability estimates of the correlation of fertility with assigned colour scores were also moderately repeatable. Repeatability of sperm traits estimated for beginning of season ejaculates were moderately to highly repeatable, in contrast to that of ejaculates obtained at the end of a breeding season.

Fertility is, however, not always associated with high sperm numbers in an ejaculate. In sandhill cranes (*Grus canadensis pratensis*), higher fertility was associated with male sandhill cranes that

produced ejaculates of an inferior quality (Chen *et al.*, 2000). Chen *et al.* (2000) suggested that males that bred successfully with their mates produced ejaculates with lower sperm counts. Bahr and Bakst (1987) found no correlation between male libido and fertility in domestic chickens, and a tendency for frequent copulators to produce aspermic ejaculates.

Fertility increased with an increase in age, and declined in males 6 years and older. Fertilities of 75% and higher were recorded for males aged 5 to 8 years. Bunter and Graser (2000), however, found ostrich males aged 4-6 years produced more fertile ejaculates, and did not consider the fertility of eggs produced by companion breeding females. When the age of the companion female was also taken into account in their study, ostrich males aged 3 to 9 years were found to have higher fertility levels than younger and older males (Bunter and Graser, 2000). Soley *et al.* (1991) reported fertilities ranging between 3 and 55% for males ranked according to the fertility of their breeding companions. Bramwell *et al.* (1996) reported a decrease in the fertilising ability of spermatozoa with an increase in age in chickens.

Fertilities observed in this study was lower than that reported for eggs produced after artificial insemination (AI) in ostriches and emus (Hemberger, 1996; Malecki *et al.*, 1996b). The average fertility of eggs produced 3-5 days after AI in ostriches was 83.2%, with a hatchability of 78.6% (Hemberger, 1996). Fertility of emu eggs produced after AI was 89.9%, with a hatchability of 84.6% (Malecki *et al.*, 1996b). Malecki and Martin (2003) reported fertilities in ostrich eggs being 89.4±3.4% and 94.4±3.1% during two consecutive breeding seasons, with ranges of 78.6 to 98.2%, and 64 to 100%, respectively.

Sperm morphology

The sperm in the semen samples obtained in this study exhibited typical characteristics previously reported for ostrich spermatozoa (Baccetti *et al.*, 1991; Soley, 1989, 1993; Soley and Roberts, 1994; Hemberger, 1996). Abnormalities observed in this study included bulb-headed spermatozoa, crooked-neck spermatozoa (i.e. with a broken mid-piece junction), spermatozoa with damage to the head (i.e. damage to the acrosome), and broken tails. These abnormalities are consistent with abnormalities observed by Irons *et al.* (1996), Hemberger (1996), and Hemberger *et al.* (2001). Another abnormality observed in samples in this study is that of translucent sperm, which was also reported by Soley (1992). Cytoplasmic droplets (Soley *et al.*, 1996) were not observed in ejaculates obtained from the breeding ostriches in this study. Lukaszewiczl *et al.* (2000) observed similar abnormalities in semen samples obtained from ganders during an entire breeding season. The most frequent type of abnormalities observed in frizzled Hungarian geese included different types of acrósomal aberrations and spermatozoa with enlarged nuclei (Almasi *et al.*, 2002). Tail, head, and acrosome and mid-piece defects, were the most common defects observed in turkey semen samples (Alkan *et al.*, 2002).

Aggregation of spermatozoa

An interesting observation during the microscopic evaluation of the ejaculates in this study was the presence of sperm 'clusters' or aggregations of spermatozoa in some ejaculates. Aggregation of spermatozoa has also been reported in quail, where spermatozoa tend to form clusters in the *vas deferens* (Fujihara *et al.*, 1989). Seminal plasma in the male fowl is characterised by a high concentration of glutamate, with glutamic acid being the principle amino acid in the oviduct and *vas deference* (Barna and Boldizsar, 1996; Barna *et al.*, 1996; Howarth, 1995). Glutamate has an inhibitory effect on the respiratory rate and metabolism of spermatozoa, thus maintaining them in a relative state of quiescence within the male and female reproductive tracts (Howarth, 1995). In the male quail, a white frothy or foamy secretion is produced by the dorsal proctodeal gland and is ejected during ejaculation and transferred to the female cloaca during mating. Quail spermatozoa, when exposed to this foamy secretion, are freed, and it thought that this may play an important role in the motility and sperm transport in the oviduct (Fujihara *et al.*, 1989).

Some species, i.e. Desmognathine salamanders and the common wood mouse (*Apodemus sylvaticus*), also show a form of sperm aggregation in their reproductive tracts. Female salamanders have cloacal spermathecae that store sperm before oviposition (Sever and Hamlett, 1998). During storage in the spermathecae, sperm are arranged in parallel arrays or may have no patterned arrangement. Sever and Hamlett (1998) suggested that the aggregations of sperm may present a form of sperm competition in salamanders. Thus, sperm from another male may be excluded from the spermathecae or initial ejaculates are then pushed further into the spermatheca's secretory matrix, thereby minimising or eliminating spermatozoa from the any previous matings' chance of fertilising an egg. Altruistic behaviour of spermatozoa have been observed in the common wood mouse, and were thought to be a mechanism to allow spermatozoa of a given male to gain an advantage when inter-male sperm competition is intense (Moore *et al.*, 2002).

Adelie penguins are considered to be the first evidence of an avian species that allocates ejaculates, like certain insect and fish species (Hunter *et al.*, 2000). Male Adelie penguins withhold ejaculates from their social partners, and are more likely to transfer sperm during extra-pair copulations than during pair copulations (Hunter *et al.*, 2000). Female feral fowl were found to be biased in terms of sperm retention, and when inseminated by subordinate males, differentially ejected the ejaculates of the subordinate males and retained that of the most dominant males (Pizzari and Birkhead, 2000).

Whether this ability of ostrich spermatozoa to aggregate presents a form of sperm competition or mechanism to preserve sperm integrity and viability in ostriches is unsure and warrants further investigation. The presence of a proctodeal gland and possible contribution of its secretion to the preservation of sperm integrity have not been determined in ostriches, and also warrants further research.

CONCLUSIONS

Semen collection in birds can be performed by means of voluntary ejaculation, forced massage, or electro-ejaculation (Rozenboim *et al.*, 2003; YanBo *et al.*, 2001; Irons *et al.*, 1996; Malecki *et al.*, 1996a; Hicks, 1990; Tan, 1980). The successful application of the forced massage semen collection method in this study was, however, hampered by several factors. An inability to retrieve the phallus in several cases, the level of stress that accompanies the procedure, urine and faeces contamination, and also the production of aspermic ejaculates, limits the practical implementation of this collection method. It is thus possible that birds that produce highly fractious samples may be discriminated against, for ejaculates that are obtained may not be a physiological representative sample of the animal (Irons *et al.*, 1996; Hicks-Aldredge, 1994). Rozenboim *et al.* (2003) and YanBo *et al.* (2001) investigated various stress- and restraint-free methods for semen collection in ostriches. These methods, however, involved either the training of males to ejaculate in an artificial vagina, or the interruption of the mating process. Given the current nature of ostrich farming systems in South Africa, and the lack of sexual imprinting in ostrich males, the application of the latter methods are of limited value.

Sperm concentration was positively and significantly correlated with fertility. The study, however, failed to identify any other male trait that could be used as a reliable indicator of female fertility under commercial breeding conditions. Sperm concentration and male phallus traits were found to be repeatable in ostrich males. Phallus traits together with sperm concentration, ejaculate colour and viscosity increased with an increase in male age. Fertility of ejaculates similarly increased with an increase in male age. The phallus traits and semen quality parameters may also show genetic variation, as is the case in other domesticated species. Flush feeding increased the viscosity of ejaculates.

It is important to understand the extent to which sperm characteristics contribute to the insemination success of an ostrich male in order to improve fertility and overall reproduction efficiency in commercial farming systems. Traits therefore need to be identified that can aid in the development of a semen quality index, as used in the commercial poultry industries (Parker *et al.*, 2000). Such an index may potentially be used to reliably indicate the fertility of individual males for the potential use in breeding programs.

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Chapter 11

Management practices and performance indicators of reproduction in ostriches – conclusions and recommendations

South Africa produces approximately 60% of the world's total number of slaughter ostriches. The emphasis on producing primarily for export markets and high input costs, together with a volatile exchange rate, necessitates that commercial farmers produce slaughter ostriches as cost-effectively as possible. The reproductive efficiency of breeding ostriches, i.e. the number of fertile eggs and chicks produced under commercial breeding conditions, plays an integral role in determining the success of a commercial ostrich farming enterprise. Commercial ostrich production systems, however, are characterised by low reproductive performances and a large variation in egg production, as well as high chick mortalities. Identification of poor or non-producing breeding individuals in commercial breeding systems is hampered by the fact that breeding ostriches are maintained in large free-ranging flocks and the communal nesting system of laying ostrich females.

The emphasis on the cost-effective production of slaughter ostriches formed the basis of this study. The aim was to determine possible management practices to improve the overall reproduction efficiency of breeding ostriches under commercial farming conditions. Behavioural, physiological and anatomical parameters were also investigated to determine their potential as performance indicators to allow the identification and selection of breeding ostriches with a better reproductive performance under commercial farming conditions. The findings are summarized in the sections below, with recommendations on the practical implementation of these findings under commercial farming conditions to improve the overall reproduction efficiency of breeding ostrich males and females.

Management of breeding ostriches to improve seasonal reproductive performance

The response of the breeding ostriches in the study clearly demonstrated a photoperiod-dependent breeding strategy. Peak production was recorded during June to January. Breeding ostrich males and females are traditionally joined for an 8-month breeding period at the end of May, which is approximately one month before the winter solstice in the southern hemisphere. Joining breeding ostriches before the winter solstice, i.e. when still in a photorefractory state, had the effect that the reproductive cycles of breeding ostrich males and females were not yet synchronised, resulting in a longer period to first laying and a lower seasonal reproductive performance. Joining ostriches after the winter solstice synchronised the reproductive activities of the breeding males and females shortened the time to the onset of reproduction after being paired and improved the overall reproductive performance of the breeding birds.

In cases where a breeding program necessitates the joining of breeding birds before the winter solstice, certain management practices can be applied to initiate and synchronize the reproductive cycles of breeding ostrich males and females. A pre-season rest, together with flush feeding and teasing, can be used to initiate and synchronize the reproduction in breeding ostrich males and females. Breeding ostriches without a pre-breeding rest period performed worse throughout a breeding season in all the reproductive traits recorded. Maintaining ostrich males and females in single-sex flocks during the non-breeding season and reintroducing males to neighbouring camps two weeks before being paired for a breeding season, significantly improved egg production of the breeding ostriches. The potential of flushing and teasing to synchronize the reproductive cycles of breeding ostriches, however, appears to be limited by the time of the year when breeding ostriches are paired for breeding. Pairing breeding ostriches when photoperiod is sufficiently long to stimulate reproduction may be necessary to ensure the successful implementation of these management practices.

Breeding ostriches may experience a quiescent period in reproduction during breeding seasons. Subjecting breeding ostriches that experience a decline in production to a mid-season rest was successful in reinitiating reproduction and improving egg and chick production of breeding pairs (subjected to flushing and teasing) during the post-rest period. Egg and chick production was significantly improved by 52.5% and 44.0%, respectively, during the post-rest period. The potential of a mid-season breeding rest to reinitiate the reproductive cycles of breeding ostriches, however, is determined by the state of photosensitivity of the birds. Implementing the management practice too late during a breeding period when the breeding birds have already started to become less sensitive, limits the reaction to any management practice to reinitiate reproduction in both the males and the females.

Intra-season rest periods also proved a means to overcome the photoperiodic control of the reproductive cycles of breeding ostriches paired before the winter solstice. This may allow a commercial ostrich farmer to ensure the timely production of slaughter ostriches according to market demand. Intra-season rest periods were effective in synchronizing the reproductive cycles of breeding males and females and improving overall reproductive performance during the post-rest periods. The effectiveness of this management practice, however, is limited by the time of the breeding period when the breeding birds are subjected to a breeding rest. Enforced breeding rest periods are only effective in reinitiating reproductive activities during the time of the year when ostriches are still photosensitive and have not yet entered a state of photorefractoriness

Ultrasonography as a management tool to identify reproductively healthy, and early producing ostrich females:

In domestic fowl, high producing birds have a very long prime sequence early in lay, and the length of the prime laying sequence might be a good indicator of reproductive efficiency of a female. The extreme variation in the egg production potential reported for breeding ostrich females, i.e. between

0 and 121 in an 8-month breeding period, motivated the study to determine the potential of ultrasound scanning (diagnostic imaging) as a management tool to identify actively and early producing ostrich females.

Ultrasound scanning can be used to identify females that come into lay early in a breeding season, and progress can be made in commercial breeding flocks through the selection of ostrich females with well-developed follicular hierarchies at the beginning of a breeding season. The correlation reported for early season scanning and overall egg production suggests that females can be selected on partial production records for use in breeding programs. The duration of lay after the commencement of the breeding season was strongly associated with reproductive success. Ultrasound can thus be used to identify ostrich females with a late onset of lay and lower production success under commercial breeding conditions. The respective ultrasound parameters were closely related to short-term egg production on a genetic level, indicating the ability of the technique to identify healthy reproductive ostrich females. The heritability reported for follicle classification score was low but significant. Females below 2 years of age proved to be too young to be included usefully in breeding programs.

Partial egg production records for a given season, however, may not be a reliable indicator of the ability of a female if the commencement of lay is delayed for a specific female or if the age of the female is unknown. The age of ostrich females needs to be taken into account when selection decisions are based on phenotypic data. Failure to do so may discriminate against young ostrich females. Management practices to initiate and synchronise reproduction in ostrich females (e.g. flush feeding and teasing) will be indispensable for the practical application of diagnostic ultrasonography in the broader industry.

Management of large commercial ostrich breeding flocks, in terms of stocking rate and male:female ratio

Ostriches are the main farming interest in the Little Karoo, and approximately 65% of the South African ostrich breeding population are found in this region. Ostrich breeding flocks are concentrated on relatively small areas, and through trampling have the largest effect on the natural vegetation. The influence of stocking rate and male:female ratio was therefore investigated on a commercial ostrich farm outside Ladismith in the Western Cape Province, South Africa, to determine the influence of these two factors on the overall reproductive performance under intensive breeding conditions.

The findings indicated that ostriches can be maintained at higher stocking rates than is currently advised for ostriches under intensive flock breeding conditions, i.e. 1 breeding bird per 10ha for an 8-month breeding period. This has important implications in terms of the intensification of ostrich farming, especially in areas that are characterised by vegetation that are exposed to the trampling effect of ostriches. A stocking rate of 114 birds (1M:2F) per 10 000 m^2 performed the best in terms

of all reproductive traits. Increasing the stocking rate in smaller 625m^2 (0.13ha) camps resulted in an overall improvement in reproduction, possibly because of more frequent sexual interactions between breeding ostrich males and females.

A trade-off was observed in terms of the reproduction traits, i.e. when average egg production per female was improved, fertility and hatchability was compromised. Breeding pairs, trios and quads could be maintained on smaller areas (312.5 to 625 m^2/bird; 208.3 to 416.7 m^2/bird; and 156.3 m^2/bird, respectively) than normally prescribed with acceptable production levels. Although the number of quads in the study was relatively small, it indicates that harem size under intensive conditions may be as high as three females per male, which supports the polygamous nature of ostrich males. Maintaining commercial ostrich breeding flocks at too high stocking rates will, however, impact negatively on the normal reproductive behavioural patterns of breeding birds, and may not be beneficial for the overall reproductive performance and well-being of ostriches under intensive farming conditions.

Ostrich male aggression and shin colour in relation to the reproductive performance of companion females

Breeding ostrich males were evaluated on a monthly basis in terms of territorial aggression and shin colour, and this was then related to the egg production performance of their companion females. Territorial aggression was moderately heritable, and male shin colour lowly heritable in breeding ostrich males. Territorial aggression was positively correlated with egg production performance of companion females. Male aggression and shin colour was also highly correlated with egg production performance of companion females. However, a negative genetic correlation of male aggression with female egg production performance warrants further studies to quantify the extent to which excessive aggression contributes to overall aggression exhibited by ostrich males. The possible relationship of corticosterone with male aggression may aid in the understanding of ostriches' ability to cope with stress related to an intensive breeding environment, and warrants further investigation.

Serum hormone levels, in relation to male aggression and shin colour, and female egg production performance

The study presents information on the relationship between serum levels of testosterone, LH, progesterone, and prolactin, and male aggression and shin colour, as well as the egg production performance of ostrich females measured at the beginning and end of breeding seasons. In addition, the study also presents novel information on sexual dimorphism in adult breeding ostriches.

The serum levels of the respective hormones were extremely variable, with prolactin being the only exception. Serum testosterone and LH varied on a permanent environment level, and gender and

time of the year influenced serum prolactin and progesterone levels. Animal permanent environment contributed to the variation observed for live weight, LH, testosterone, aggression, and egg production performance. The only significant correlation was reported for LH and egg production of females on an environmental level. Low serum levels of testosterone and high levels of aggression observed at the end of breeding implies that factors other than LH contribute to the maintenance of territorial aggression at the end of a breeding season.

The fact that blood samples were collected throughout the day in the study and not at a specific time, i.e. during the mornings or in the afternoons; may have affected the LH concentrations measured in this study. It is known that LH secretion follows a diurnal pattern in female birds, and it is possible that peak levels were measured in some females and low levels in others, which may have contributed to the observed variation. Future studies should aim at determining base line concentrations of LH, testosterone, estradiol, progesterone, as well as prolactin, in ostriches during non-breeding and breeding seasons and also on a diurnal basis. This may provide a better insight on the endocrine control of reproduction in both males and females, and how this is linked to reproductive behaviour exhibited by both genders.

Female live weight and body measurements, in relation to reproductive performance

Literature on genetic parameters of growth and reproduction in ostriches are scarce. This is in stark contrast to experience with small domestic poultry species, where extensive knowledge is readily available for growth and development and egg production of commercial stocks. The study investigated the potential of the live weight and body measurements of breeding ostrich females, as selection criteria to improve reproduction performance in commercial breeding systems.

The heritabilities reported for live weight, chest circumference, and tail circumference varied between 0.12 and 0.38. The heritabilities reported for egg production, chick production, and hatchability ranged between 0.10 and 0.23. The estimates for female permanent environment effects were 0.18 for egg and chick production, and 0.21 for hatchability. Service sire exerted significant effects on hatchability and chick production. Egg and chick production was highly correlated on a genetic and phenotypic level, while hatchability was positively related to chick production on a service sire level. No adverse relationship was found between live weight, body measurements and reproductive traits.

Relatively high repeatability estimates previously reported for ostriches suggest that worthwhile progress is achievable in commercial ostrich breeding flocks. The reproductive performance of breeding ostrich females does not seem to be negatively related to live weight before breeding on a genetic or phenotypic level. These findings imply the potential development of a dual-purpose ostrich strain, where emphasis may be placed on selection for both growth and reproductive traits. Significant genetic parameters for egg and chick production of commercial ostriches were evident in this study, and genetic progress in reproductive traits thus seems feasible for ostriches.

Reproductive traits of ostrich males in commercial breeding systems:

Ostrich males are sexually mature at approximately 3 to 4 years of age, with factors such as breed (e.g. SA Black vs. Kenyan Red or Zimbabwean Blue) and time of the season when hatched, influencing the onset of sexual maturity. Spermatogenic activity, however, has been reported for prepubertal ostriches, with spermatogenesis undergoing seasonal changes as observed in mature breeding ostrich males. The study investigated the relationship between phallus and sperm traits, and fertility under commercial breeding conditions, as well as the influence of male age, season, and management on these traits.

The study failed to identify a male trait that can be used as a reliable indicator of fertility in ostriches under commercial flock-mating conditions. Male phallus traits and sperm concentration was found to be repeatable in ostrich males. These traits may also exhibit genetic variation, as found in other domesticated species. Sperm concentration was moderately repeatable and sperm abnormalities lowly repeatable. Phallus measurements were positively correlated with sperm concentration. Phallus measurements and sperm concentration increased with an increase in male age. Fertilities of 75% and higher were reported for males between 5 and 8 years of age. Although the study failed to identify a reliable indicator of male fertility, correlations reported for phallus measurements and sperm concentration were in the right direction. Future studies including a larger number of males may aid in establishing reliable indicators to quantify and qualify the reproductive fitness of ostrich males under intensive breeding conditions.

Little is known regarding the mechanisms determining the reproductive success of ostrich males after copulation and insemination, and how these mechanisms interact with one another and with selective mechanisms occurring before insemination, i.e. mate choice and mate acquisition. It is important to understand the extent to which sperm characteristics contribute to insemination success in order to improve reproduction efficiency in commercial farming systems. Traits need to be identified that can aid in the development of a sperm quality index including e.g. sperm concentration, viscosity and fertilising ability as criteria, that may potentially be used to reliably indicate the fertility of individual males.

To conclude:

The implementing of flush feeding and teasing management practices will aid in the synchronization of the reproductive cycles of breeding ostriches, thus enabling the commercial ostrich farmer to optimize the reproductive potential of breeding ostrich males and females under intensive breeding conditions. The use of these management practices, together with ultrasound scanning, will aid in the accurate identification of and consequent selection of breeding ostrich females with an early onset of production. Results from the study indicated that reproductive success is in part determined by an early onset of reproduction, implying that the identification and selection of breeding ostriches with an early onset of production will subsequently lead to an overall

improvement and optimization of the reproductive efficiency and thus reproductive performance of breeding ostriches under intensive breeding conditions. Improving the reproductive performance of breeding ostrich males and females will potentially lead to an increase in the number of day-old chicks produced per female, thereby decreasing the overall costs of producing a day-old chick, as well as contribute to decreasing the feed costs of a commercial ostrich breeding operation.

The observed high degree of genetic variation in the commercial ostrich breeding population implies that considerable genetic improvement is feasible through the identification of breeding ostriches with an improved reproductive performance, given the high repeatability estimates previously reported for ostriches under flock-mating conditions. It is, however, important to keep in mind the long-term goal of a breeding program, i.e. selection must be performed but without compromising production under commercial conditions.

Unravelling the neuroendocrine mechanisms behind the flexibility in reproductive timing in ostriches may aid in the understanding of why ostrich males and females differ in terms of basic reproduction-related mechanisms, e.g. why some come into production earlier than others. This will aid in determining the influence of domestication on the ability of ostriches to adapt to and cope with stressful breeding environments, and also potentially assist the commercial ostrich farmer in the selection of individuals with a better reproduction efficiency under commercial conditions.

SUMMARY

South Africa is the world's largest ostrich producer, producing approximately 60% of the world's total number of ostriches slaughtered annually. Almost 90% of all ostrich products (i.e. leather, meat and feathers) generated are exported. High input costs, together with fluctuations in the exchange rate, places commercial ostrich producers under constant pressure to produce slaughter birds as cost-effectively as possible. The characteristic low reproductive performance of commercial ostrich breeding systems, as reflected in the large variation in the numbers of eggs produced, and high chick mortalities, contribute to the emphasis on the cost-effective production of slaughter ostriches. The identification of poor or non-producing breeding ostriches are hampered by a communal nesting system and the free-range conditions of commercial ostrich breeding systems. Different management practices, and behavioural and physiological aspects were studied to determine their potential to identify and select breeding ostrich males and females with a higher reproductive performance, and thereby improving overall reproductive performance of breeding ostriches under intensive farming conditions. Maintaining ostrich males and females separately as single-sex flocks during the non-breeding resting period improved the overall reproductive performance of the breeding ostriches. The reproductive cycles of breeding ostriches can successfully be manipulated to ensure the timely production of chicks and thus slaughter birds. The successful implementation of intra-season breeding rest periods to synchronize and thus improve overall reproductive performance is however, determined by the time of the breeding season when the breeding rest is enforced. Breeding ostriches can be maintained at stocking rates higher than those currently applied. However, too high stocking densities are detrimental to the well-being of the breeding ostriches under intensive conditions. A trade-off was observed in terms of egg production and hatchability, with hatchability decreasing as average egg production/female increased. The egg production performance of ostrich females was significantly correlated with serum LH levels. Territorial aggression exhibited by breeding ostrich males did not exhibit significant genetic variation to serve as an indirect selection criterion for egg production in companion breeding females. The territorial aggression observed in the breeding ostrich males, however, showed genetic variation, thus indicating that the temperament of ostriches may be changed through genetic selection, potentially improving their ability to adapt better to intensive husbandry practices. Ultrasound scanning of follicle development can be used to identify ostrich females with an early onset of production and selection of females on partial reproduction records will improve overall reproduction efficiency of commercial breeding systems. Significant heritabilities were reported for follicle score during scanning in ostrich females. No negative correlation was reported for female live weight, body measurements, and egg production. Although no reliable indicator was identified to quantify the reproductive fitness of ostrich males under intensive conditions, correlations reported for phallus and semen quality traits with male fertility were in the right direction.

To conclude:

The implementing of flush feeding and teasing management practices will aid in the synchronization of the reproductive cycles of breeding ostriches, thus enabling the commercial ostrich farmer to optimize the reproductive potential of breeding ostrich males and females under intensive breeding conditions. The use of these management practices, together with ultrasound scanning, will aid in the accurate identification and consequent selection of breeding ostrich females with an early onset of production. Results from the study indicated that reproductive success is in part determined by an early onset of reproduction, implying that the identification and selection of breeding ostriches with an early onset of production will subsequently lead to an overall improvement and optimization of the reproductive efficiency and thus reproductive performance of breeding ostriches under intensive breeding conditions. Improving the reproductive performance of breeding ostrich males and females will potentially lead to an increase in the number of day-old chicks produced per female, thereby decreasing the overall costs of producing a day-old chick, as well as contribute to decreasing the feed costs of a commercial ostrich breeding operation.

The observed high degree of genetic variation in the commercial ostrich breeding population implies that considerable genetic improvement is feasible through the identification of breeding ostriches with an improved reproductive performance, given the high repeatability estimates previously reported for ostriches under flock-mating conditions. It is, however, important to keep in mind the long-term goal of a breeding program, i.e. selection must be performed but without compromising production under commercial conditions.

Unravelling the neuroendocrine mechanisms behind the flexibility in reproductive timing in ostriches may aid in the understanding of why ostrich males and females differ in terms of basic reproduction-related mechanisms, e.g. why some come into production earlier than others. This will aid in determining the influence of domestication on the ability of ostriches to adapt to and cope with stressful breeding environments, and also potentially assist the commercial ostrich farmer in the selection of individuals with a better reproduction efficiency under commercial conditions.

Keywords: breeding ostriches, reproduction efficiency, flush feeding, teasing, stocking density, male territorial aggression, serum hormone levels, ultrasound scanning, semen quality

OPSOMMING

Suid-Afrika is die wêreld se grootste produsent van volstruise en produseer ongeveer 60% van die wêreld se totale getal volstruise wat jaarliks geslag word. Die Suid-Afrikaanse volstruisbedryf is primêr 'n uitvoerbedryf - ongeveer 90% van alle volstruisprodukte (leer, vleis en vere) word uitgevoer. Hoë insetkostes, tesame met 'n fluktuerende wisselkoers, plaas kommersiële volstruisprodusente onder gedurige druk om slagvolstruise so koste-effektief as moontlik te produseer. Kommersiële volstruisproduksiestelsels word gekenmerk deur 'n lae reproduksie-doeltreffendheid, soos weerspieël in 'n groot variasie in eierproduksie, asook hoë kuikenmortaliteite. Die identifisering van individuele volstruismannetjies en –wyfies met lae reproduksiedoeltreffendhede word bemoelik deur die feit dat volstruiswyfies in meer as een nes kan lê, asook deur die ekstensiewe tot semi-intensiewe omstandighede en troppparingstelsels waaronder kommersiële volstruisbroeikuddes aangehou word. Die potensiaal van sekere bestuurspraktyke en gedrags- en fisiologiese aspekte om aktief-reproduserende volstruise te identifiseer en sodoende die reproduksiedoeltreffendheid van 'n broeikudde te optimaliseer, is in die studie ondersoek. Die fisiese en visuele skeiding van mannetjies en wyfies tydens die russeisoen het 'n positiewe invloed op die sinkronisasie van die reproduksiesiklusse van die volstruise gehad, met 'n betekenisvolle verbetering in seisoenale reproduksieprestasie. Die reproduksiesiklusse van broeivolstruise kon suksesvol deur middel van gedwonge binne-seisoen rusperiodes gesinkroniseer en verbeter word. Die sukses van dié bestuurspraktyk om reproduksie te sinkroniseer en te verbeter, word egter bepaal deur die tyd van die jaar wanneer die bestuurspraktyk geïmplementeer word. Volstruisbroeikuddes kan teen hoër digthede as wat tans deur die toepaslike riglyne voorgeskryf word, aangehou word. Met 'n toename in die gemiddelde eierproduksie per wyfie, het die uitbroeibaarheid van eiers afgeneem. Volstruisbroeitroppe wat by te hoë digthede aangehou word, kan ook 'n negatiewe invloed op die normale reproduksiegedrag van volstruise hê. Eierproduksie van volstruiswyfies was betekenisvol gekorreleerd met serum LH-vlakke. Territoriale aggressie by volstruismannetjies het daarop gedui dat temperament by volstruise oorerflik is, wat impliseer dat volstruise geneties selekteer kan word om beter by intensiewe volstruisboerderypraktyke aan te pas. Territoriale aggressie het egter geen waarde as 'n indirekte seleksiemaatstaf waarvolgens volstruiswyfies vir verhoogde eierproduksie geselekteer kan word nie. Ultraklankskandering kan suksesvol gebruik word om aktief-reproduserende volstruiswyfies te identifiseer, asook om wyfies met 'n hoër reproduksiepotensiaal tydens 'n broeiseisoen uit te soek. Geen negatiewe korrelasie is gevind tussen die liggaamsmassa, liggaamsmates en eierproduksiepotensiaal van volstruiswyfies nie. Alhowel geen betekenisvolle maatstaf van semenkwaliteit by volstruismannetjies geïdentifiseer is nie, was die korrelasies positief en in die regte rigting.

Om saam te vat:

Die implementering van prikkelvoeding- en koggelpraktyke sal bydra tot die sinkronisasie van die reproduksiesiklusse van broeivolstruise en so die kommersiële volstruisboer in staat sal stel om die

reproduksiepotensiaal van sy broeivolstruise onder intensiewe toestande te verbeter. Die toepassing van hierdie bestuurspraktyke, tesame met die gebruik van ultraklankskandering, sal die identifisering en gevolglike seleksie van broeiwyfies wat vroeg in lê kom, moontlik maak. Bevindinge van die studie het aangedui dat reproduksiesukses deels deur 'n vroeë aanloop van eierproduksie bepaal word, wat beteken dat die identifisering en seleksie van wyfies wat vroeg in lê kom, sal lei tot die algehele verbetering en optimering van die reproduksiedoetreffendheid en -prestasie van broeivolstruise onder intensiewe toestande. 'n Verbetering in die reproduksieprestasie van volstruise sal potensieël die aantal dagoud kuikens per wyfie geproduseer verhoog, wat weer die koste om 'n dagoud kuiken te produseer, sal verlaag. Deur nie-produserende indiwidue in kommersiële broeitroppe te identifiseer, sal ook wesenlik tot aansienlike besparings in voerkoste bydra.

Die hoë mate van genetiese variasie in die kommersiële volstruisbevolking impliseer dat daar aansienlike genetiese vordering gemaak kan word deur die identifisering van volstruise met 'n verhoogde/verbeterde reproduksieprestasie, gesien teen die hoë herhaalbaarhede wat reeds vir volstruise in tropparingstelsels gerapporteer is. Dit is egter belangrik die langtermyn doelwit van so 'n seleksieprogram altyd nagestreef word sonder om produksie in kommersiële sisteme in te boet nie.

Die ontrafeling van die neuro-endokriene meganismes wat 'n rol in die reproduksiesiklusse van volstruise speel, sal bydra tot verklarings waarom volstruismannetjies en -wyfies verskil in terme van basiese reproduksiemeganismes (bv. hoekom sommige vroeër in lê kom as ander, ens.). Dit sal ook bydra om die invloed van domestikering op die aanpassingsvermoë van volstruise om potensiële stresvolle toestande te hanteer en dus beter onder intensiewe kommersiële boerderyomstandighede te vaar, te verstaan. Die seleksie van volstruise met 'n verbeterde aanpassingsvermoë vir kommersiële boerderypraktyke sal potensieël tot 'n algehele verbetering in reproduksiedoeltreffendheid bydra.

Sleutelterme: broeivolstruise, reproduksiedoeltreffendheid, prikkelvoeding, koggelpraktyk, tropgrootte, territoriale aggressie, serum-hormoonvlakke, ultraklankskandering, semenkwaliteit

Appendix

Prolactin assay

Radio-iodination materials and procedure

Chicken prolactin iodination buffers

Tris/Tween 20

→ 1.8g sodiumchloride

→ 2.42g Tris or 3.14g Tris/HCl

→ 0.1g sodiumazide

→ 200µl Tween 20

→ Make up to 200ml with glass-distilled water

0.3M sodium phosphate buffer

→ 3.68g Na_2HPO_4 + 0.64g $NaH_2PO_4.2H_2O$

→ Make up to 100mL with glass-distilled water

→ Adjust pH to 7.5

Chloramine-T

→ 5mg chloramine + 10mL 0.3M Na-phosphate buffer

→ Do not use metal spatula

→ Do not mix more than 10 min before use

2% Bovine serum albumin (BSA) buffer

→ 0.2g BSA + 10mL 0.3M phosphate buffer

Columns

PD-10 – equilibrated with Tris/Tween

G-100 – equilibrated with Tris/Tween

Iodination procedure:

- Add 25µl of glycerol to each of 15 tubes (for the collection from the PD-10 column).
- Add 10mL of the phosphate buffer to the chloramine-T.
- Aliquot 14µl from prolactin stock into eppendorf tubes
- Add 16µl of the phosphate buffer to the recombinant chicken prolactin.
- Add 5µl of the [^{125}I]-NaI to the prolactin.
- Add 10µl of the chloramine-T solution to the prolactin tube.
- Vortex and incubate at room temperature for 60 seconds.
- Stop the reaction by the addition of 200µl of the 0.3M phosphate buffer.
- Apply the mixture immediately to the PD-10 column and allow to drain in.
- Wash out the reaction tube with 250µl of the phosphate buffer and apply to the column.
- Apply fresh Tris/Tween 20 buffer to the column and collect 11 drop fractions into each of the 15 tubes with added glycerol.

- Monitor the tubes for radioactivity – the first peak should be around tubes 6-8 or earlier, and the second peak around tubes 12 or 13.
- Mix the solution in the peak tubes with the glycerol and pool the fractions.
- Allow the G100 column to drain down to the top gel surface.
- Apply the pooled radio-active fraction, and gently top up the fluid with Tris/Tween 20 to the top of the column, then attach either a pump or gravity feed to the column.
- Collect 1 mL fractions and monitor for radio-activity – depending on the column the prolactin should come off at around fraction 40.
- Dilute the peak fractions collected in 1:1 with the 2% BSA in phosphate buffer and store in aliquotes at –70°C until use.

Radio-immuno assay media and procedure

Basic prolactin buffer (PRL buffer)

- 0.1M Na-phosphate buffer, pH 7.5 900mL
- 0.9% NaCl 9.0g NaCl
- 10mM Na_2EDTA 3.72g Na_2EDTA
- 0.1% sodium azide 1.0g NaN_3
- 0.1% BSA 1.0g BSA

→ Adjust the pH to 7.0 with 1M NaOH and make up to 1litre

First antibody

→ Number 31/1, 1:6000, 50µl per tube, for 1200 tubes

→ = 59mL prolactin buffer + 1mL stock of first antibody (1:100)

Tracer

→ 50µl per tube, for 1200 tubes

→ = 60mL prolactin buffer and stock trace to > 12000 counts per 50µl

Normal rabbit serum (NRS)

→ 50µl per tube, for 1200 tubes

→ = 100µl rabbit serum + 59.9mL prolactin buffer

Second antibody

→ 50µl per tube, for 1000 tubes

→ = 30mL donkey serum (doneky 5 batch 2 @ 1:2) + 30mL prolactin buffer

Notes:

- Run samples, B-zero, quality control pools, and standards at 100µl with no added buffer.
- Non-specific binding tubes (NSB) get 150µl buffer
- Add all reagents at 50µl
- For pour-off buffer, use 6% polyethyleneglycol (PEG) at 1mL
- Stored stock for standard curve is 5µm per vial, and working stock of 12500ng.mL^{-1} per 5mL vial
- Top standard is made up by bringing 10µl working stock up to 1mL in PRL buffer, thus = 125ng.mL^{-1}

The prolactin assay was set up in triplicate in 12 X 75mm polypropylene tubes (tubes for standard curve from tube 6 to 15):

→ 1 tube (total count) (empty)
→ 1 tube (NSB, blank) (200µl PRL buffer)
→ 3 tubes for zeros (100µl PRL buffer)
→ 1 tube containing 125ng/ml standard (500µl standard + 500µl PRL buffer)
→ 1 tube containing 62.5ng/ml standard (500µl standard + 500µl PRL buffer)
→ 1 tube containing 31.25ng.ml^{-1} standard (500µl standard + 500µl PRL buffer)
→ 1 tube containing 15.625ng.ml^{-1} standard (500µl standard + 500µl PRL buffer)
→ 1 tube containing 7.8125ng.ml^{-1} standard (500µl standard + 500µl PRL buffer)
→ 1 tube containing 3.9ng.ml^{-1} standard (500µl standard + 500µl PRL buffer)
→ 1 tube containing 1.95ng.ml^{-1} standard (500µl standard + 500µl PRL buffer)
→ 1 tube containing 0.977ng.ml^{-1} standard (500µl standard + 500µl PRL buffer)
→ 1 tube containing 0.4883ng.ml^{-1} standard (500µl standard + 500µl PRL buffer)
→ 1 tube containing 0.24ng.ml^{-1} standard (500µl standard + 500µl PRL buffer)
→ From here on, unknown samples were aliquoted in duplicate and at 100µl per tube for assay

Testosterone assay

Gelatine phosphate buffer (GPB)

→ = 1.0g gelatin + 1 litre PBS

First antibody

→ 100µl at 1:80000dilution in GPB

Tracer

→ Counts = 15000dpm

Normal rabbit serum (NRS)

→ 100µl rabbit serum (1:500) in GPB with 0.1M EDTA

Second antibody

→ Donkey 6 batch 5 – 1:30

On day 1, the assay was set up in 10mm glass tubes and in triplicate (tubes for standard curve from tubes 5-13) as follows:

→ 1 tube (total count) (empty)
→ 1 tube (NSB, blank) (300µl GPB)
→ 3 tubes for zeros (200µl GPB)
→ 1 tube containing 16ng/ml standard (25µl standard + 175µl GPB)
→ 1 tube containing 8ng/ml standard (25µl standard + 175µl GPB)
→ 1 tube containing 4ng.ml^{-1} standard (25µl standard + 175µl GPB)
→ 1 tube containing 2ng.ml^{-1} standard (25µl standard + 175µl GPB)
→ 1 tube containing 1ng.ml^{-1} standard (25µl standard + 175µl GPB)

→ 1 tube containing 0.5ng.ml^{-1} standard (25µl standard + 175µl GPB)

→ 1 tube containing 0.25ng.ml^{-1} standard (25µl standard + 175µl GPB)

→ 1 tube containing 0.0625ng.ml^{-1} standard (25µl standard + 175µl GPB)

Quality controls were prepared in duplicate by using serum collected from 3 ostrich males and 4 ostrich females at a commercial ostrich farm. A set of tubes were made up for each respective ostrich male and female, and were prepared as follows:

→ 1 tube containing 25µl serum (25µl made up to 200µl with GPB)

→ 1 tube containing 20µl serum (20µl made up to 200µl with GPB)

→ 1 tube containing 10µl serum (10µl made up to 200µl with GPB)

→ 1 tube containing 5µl serum (5µl made up to 200µl with GPB)

→ 1 tube containing 2.5µl serum (2.5µl made up to 200µl with GPB)

→ From here on, 1 tube per unknown sample (25µl serum made up to 200µl with GPB)

LH assay

Iodination of RC-AEI-s-1 in chloramine-T

Need:

- 1.5 mL Eppendorf sample cup
- PH-10 (Pharmiacia column) or 2g Sephadex G25 medium and 1 X 10 glass column with top

Prepare on day of use:

0.5M sodium phosphate buffer

→ 6.13% Na_2HPO_4 + 1.06% $NaH_2PO_4.2H_2O$

→ Adjust to pH 7.5

0.1M phosphate gel

→ 1.226% Na_2HPO_4 + 0.212% $NaH_2PO_4.2H_2O$ + 0.25% gelatine

→ Adjust pH to 7.5

0.1M phosphate-BSA

→ 1.226% Na_2HPO_4 + 0.212% $NaH_2PO_4.2H_2O$ + 1.1% bovine serum albumin (BSA)

→ Adjust pH to 7.5

Chloramine-T

→ 5mg chloramine-T in 10mL 0.1M phosphate

→ Adjust pH to 7.5

Sodium metabisulfite

→ 10mg Na-metabisulfite in 100mL 0.1M phosphate

→ Adjust pH to 7.5

Potassium iodide

→ 1g KI in 10mL 0.1M phosphate

→ Adjust pH to 7.5

Procedure for iodination of PRC-AEI-s-1 in chloramine-T

- Remove sealed tip of PD-10 column and allow sotrage solution to run to waste.
- Wash column with 1mL KI then 15mL 0.25% gelatine-phosphate buffer.

OR

- Pour G25 into the glass column (after pre-soaking in 0.25% gelatine-phosphate buffer overnight) and allow to pack down.
- Add the 0.1M phosphate buffer (pH 7.5) to the chloramine-T, sodium-metabisulfite, and KI
- Place an agitator in the sample cup (small piece of paper clip) and start the magnetic stirrer underneath. Add 10µl of the PRC-AEI-s-1 (1.25µg made up in water).
- Add 5µl 0.5mCi $Na^{125}I$
- Add 20µl chloramine-T and vortex
- Incubate for 60 seconds
- Stop reaction with 100µl Na-metabisulfite
- Add 200µl KI
- Drain the column down to the gel surface and place the content of reaction vessel on the column.
- Rinse the reaction vessel with 250µl KI and add to the column. The column effluent from these two steps can be allowed to run to waste.
- The [^{125}I]PRC-AEI-s-1 is eluted from the column by using 0.25% gelatine-phosphate buffer
- 0.5mL fractions are collected into glass tubes containing 0.5mL of 1% BSA-phosphate buffer
- The [^{125}I]PRC-AEI-s-1 is eluted first from the column, in fractions 5-7 from a PD-10 column
- The free iodine is eluted last in fractions 12-18 of a PH-10 column

Radio-immuno assay media and procedure

Basic LH buffer

→ 0.04M phosphate buffered saline (0.15M NaCl)

→ Prepare 1 litre - need 350µl per tube

• 0.5M phosphate buffer	80mL
• 0.15M NaCl	8.75g NaCl
• 0.01M EDTA	2.92g EDTA
• 0.1% sodium azide	1.0g NaN_3
• 0.1% BSA	1.0g BSA

→ Bring to 900mL in double-distilled H_2O

→ Adjust the pH to 7.0 with 1M NaOH

→ Make up to 1L

0.5M phosphate buffer (1 litre)

→ For assay diluent and iodination

→ = 61.3g Na_2HPO_4 + 10.6g $Na_2HPO_4.2H_2O$

→ Bring up to 900mL with double distilled H_2O

→ Adjust the pH to 7.0 with 1M NaOH

→ Make up to 1 litre

First antibody

→ 50µl per tube, 1:17K

→ = 98.823mL basic LH buffer + 1.177mL stock of first antibody @ 1:200

Tracer

→ 50mL per tube

→ = 100µl basic LH buffer + stock trace (>10000counts per 50µl)

Normal rabbit serum (NRS)

→ 50µl per tube

→ = 99.75mL basic LH buffer + 250µl rabbit serum (1:400)

Second antibody

→ 50µ per tube

→ 83.33mL basic LH buffer + 16.67mL donkey serum (donkey 6 batch 7, 1:6)

On day 1, the LH assay was set up in triplicate (tubes for standard curve from tube 5-13) as follows:

→ 1 tube for total count	(leave empty)
→ 1 tube (blank)	(50µl diluent)
→ 2 tubes for zero standard	(200µl diluent + 50µl anti 3/3)
→ 1 tube containing 5ng PRC-AEI-s-1.ml^{-1}	(200µl standard + 50µl anti 3/3)
→ 1 tube containing 2.5ng PRC-AEI-s-1.ml^{-1}	(200µl standard + 50µl anti 3/3)
→ 1 tube containing 1.25ng PRC-AEI-s-1.ml^{-1}	(200µl standard + 50µl anti 3/3)
→ 1 tube containing 0.625ng PRC-AEI-s-1.ml^{-1}	(200µl standard + 50µl anti 3/3)
→ 1 tube containing 0.312ng PRC-AEI-s-1.ml^{-1}	(200µl standard + 50µl anti 3/3)
→ 1 tube containing 0.156ng PRC-AEI-s-1.ml^{-1}	(200µl standard + 50µl anti 3/3)
→ 1 tube containing 0.078ng PRC-AEI-s-1.ml^{-1}	(200µl standard + 50µl anti 3/3)
→ 1 tube containing 0.039ng PRC-AEI-s-1.ml^{-1}	(200µl standard + 50µl anti 3/3)
→ 1 tube containing 0.019ng PRC-AEI-s-1.ml^{-1}	(200µl standard + 50µl anti 3/3)
→ 1 tube containing 20µl of cockerel pool	(20µl made up to 200µl with RIA diluent + 50µl anti 3/3)
→ 1 tube containing 40µl of hen pool	(40µl made up to 200µl with RIA diluent + 50µl anti 3/3)
→ From here on , 1 tube per unknown sample	(200µl of sample + 200µl of RIA diluent)

Notes:

- Use basic buffer to dilute samples
- Run samples at 25-50µl – make up to 200µl with basic buffer
- For pour-off buffer (1mL) use 6% PEG