

# **Research Project Summaries – Ostriches 2012/13**

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## Foreword

The first and foremost challenge of the South African Government is to alleviate poverty and ensure sufficient and safe food for our people. South Africa and thus the agricultural sector, is confronted with the additional challenges of the worldwide decrease in food production and subsequent effect on imports to South Africa (availability and price); the worldwide recession (job losses); the growth in malnutrition and HIV/Aids infected people in our country (loss of breadwinners); changes in climate (more disasters and sporadic weather patterns). The message is clear: increase agricultural production to supply our needs, and safeguard against the impending effects of climate change. In order for agricultural producers (commercial and small holder) to increase their production, lower input technology (lower input cost) and higher output (production) technology is required. The role of research and development in these two factors is of critical importance. New and adaptive technology generated from cutting-edge research efforts will ensure that our producers are sustainable and competitive with limited natural resources and are able to adjust to the changing environment. The aim is to secure the foundation to increase agricultural production by 10% over the next ten years.

Agriculture is one of the most important and largest knowledge based sectors in South Africa. Research and development with innovation focused on the farmers' needs is important to underpin agrarian economic growth and to ultimately address food security and rural development. The Directorate: Animal Sciences of the Department of Agriculture Western Cape is geared to meet these challenges. The dedicated group of animal science experts and supporting staff has established this research team as one of the most important and productive groups in South Africa. Three specialist scientists from the Directorate: Animal Sciences are appointed as professors extraordinaire at the University of Stellenbosch, Department of Animal Sciences. Their appointments endorse their outstanding professional and scientific status and their major contribution to human capital development in terms of post-graduate students and mentorship. The specialist scientists are recognized for their contribution to the international scientific community with both local and international research collaborations. Research is supported by extensive infrastructure and research support services.

The Oudtshoorn Research Farm celebrates its 50<sup>th</sup> anniversary this year. The Department has worked closely with the ostrich industry over the years and is committed to continue to support the respective agricultural industries and commodities.

**Dr Ilse Trautmann**

**Chief Director: Programme: Research and Technology Development Services  
Western Cape Department of Agriculture**

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# 1. Die studie van die bestuurs-aspekte om die oorlewing en produksie van volstruiskuikens te verhoog

**Projekleier: Prof TS Brand**

Volstruisboerdery is 'n belangrike landbou bedryf wat hoofsaaklik in die Wes-Kaap beoefen word. Die sukses van volstruisproduksie is in 'n groot mate afhanklik van die produksie van gesonde kuikens wat so ekonomies as moontlik groei tot slaggewig. Die suksesvolle grootmaak van volstruiskuikens tot 'n ouderdom waarop die voëls as onafhanklik en gehard beskou kan word, is 'n groot struikelblok in die ontwikkeling van volstruisboerdery. Die projek poog om volstruisproduksie te verhoog deur praktiese bestuursoplossings te vind en te ontwikkel, wat oorlewing van volstruiskuikens onder kunsmatige toestande sal verbeter. Dit sal ook waardevolle kennis verskaf van die gedrag van volstruiskuikens onder kunsmatige toestande en sal dus die behoeftes van kuikens uitwys. Die moontlike impak wat resultate van hierdie projek op die volstruisbedryf kan hê, kan enorm wees. Die geskatte produksie van slagvoëls in Suid-Afrika in 2004/2005 was 291 000. Indien vrektes met 5% verminder kan word, beteken dit 'n toename van 14 550 slagvoëls per jaar, met 'n monetêre waarde van ongeveer R29 miljoen.

## **Publikasies vir meer inligting op die projek:**

Kruger, A.C.M., 2007. The effect of different management practices on the feed intake and growth rate of ostrich chicks. M.Tech. Thesis, Nelson Mandela Metropolitan University.

Brand, T.S., 2008. Studies oor voedingsfaktore wat die oorlewing van volstruiskuikens kan verhoog. *Landbou Weekblad*, Oktober 2008.

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Janse van Vuuren, M., 2008. Faktore wat die oorlewing van volstruiskuikens verhoog. M.Tech. Thesis, Nelson Mandela Metropolitan University.

## 2. Development of a mathematical optimization growth model for ostriches

**Project Leader: Prof TS Brand**

Optimising growth models were built for example for poultry, turkeys and pigs and are nowadays used all over the world with great success. These models have the ability to consider various biological and animal related factors, feed factors, the environment as well as interactions between these factors. An recommendation in economic terms are then provided together with a least-cost diet that will provide nutrient requirements of the bird at that specific stage and will result in a bird that will produce end products with specific qualities.

The models will thus help the producer to produce products that will fulfil requirements of the market at that specific stage. This will specifically help the Ostrich Industry which provide a series of high quality products in terms of skin, with different characteristics according to market requirements, high priced meat and feathers (current income ratio of 50:40:10), that will change from time to time according to market demand. The nutritive values of feedstuffs used in ostrich diets are currently based on values obtained with other species (for example poultry, turkeys or pigs) although it is was demonstrated several times that these values are not applicable to ostriches.

Savings of more than 10% in the dietary costs of the Industry, due to the use of an optimising model and least-cost program with appropriate feed values, will lead to huge savings for the Industry. No such model and feeding systems exists for the Ostrich Industry at present. End products in the ostrich industry (skin, meat and feathers) also differ from other Industries and the same models are not applicable. Since no such technology exists in the Industry, producers and the Feed Manufacturing Industry had no alternative than to apply it to retain their competitive edge in the Industry. The purpose of this project is to develop a feed optimising model for ostriches. In the process work is also being done to get physiological as well as economical information on the species.

Up to now ranges of information are collected like:

- Growth curves for ostriches
- Carcass composition of ostriches
- Effect of different nutritional levels on different production parameters
- Effect of different nutritional levels on end products (meat, skin and feathers) of ostriches
- Effect of slaughter age on production
- Effect of slaughter age on profitability
- Production studies with breeding birds

- The effect of dietary amino acid levels on the production of breeding birds
- The effect of breeder genotype on growth and production
- Growth of reproductive organs of breeding birds during the breeding season
- Studies on the digestive physiology of the bird
- The effect of different dietary energy and protein levels on the digestive physiology of the bird
- The effect of feed composition on the digestive physiology of the bird
- Studies on feed processing
- The effect of feather harvesting on production and end products
- The effect of supplementary feeding on the production of grazing ostriches
- Behavioural studies with ostrich chicks
- Feather and skin development studies
- Allometric description of body component growth
- The effect of bulk density on feed intake

Up to now a model was developed to predict the ideal slaughter age of slaughter ostriches. This model is currently in the process of revision to incorporate different growth curves. A model to predict the nutritional need for slaughter birds is being developed and is currently under revision to correct feed intake values. A new model is currently in the development phase to determine the nutritional needs of breeding birds.

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### **3. The evaluation of raw materials in monogastric nutrition**

#### **Project Leader: Prof TS Brand**

The project involves the evaluation of alternative feed sources in monogastric nutrition (pigs, poultry and ostriches).

A near infrared spectroscopy laboratory as well as a high pressure liquid chromatography (HPLC) laboratory are also managed under this project.

Several studies were completed under this project for example:

- Evaluation of full-fat canola in pig nutrition.
- Evaluation of full-fat canola in poultry nutrition
- Evaluation of lupins in pig nutrition.
- Evaluation of lupins in poultry nutrition.
- Supplementary feeding of ostriches grazing lucerne pasture.
- Cottonseed oilcake meal as alternative protein source compared to soybean oilcake meal for ostriches.
- Raw full-fat soybean meal as alternative protein source for ostriches.
- Diets with high levels of fibre in diets for growing and finishing ostriches.
- Several studies with ostrich chicks were performed to describe the digestive physiology of the young bird when fed diets with different nutrient levels

A total of 1152 samples were analysed by NIRS during the year and 100 samples were analysed by HPLC during the year (mainly amino acids)

Several NIRS calibration curves for the analysis of different raw materials were developed and updated under this project.

Two manuscripts were submitted on the nutrition of ostrich chicks, two other submitted manuscripts include work on the development of NIRS curves, one manuscript focused on the effect of cottonseed oilcake inclusion on the live performance and chemical composition of ostrich meat.

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#### **4. Development of a mathematical growth model for slaughter ostriches: Verification of growth data and the testing of the effect of feather harvesting as well as the provision of shade**

**Project Leader: Prof TS Brand, W Kritzinger and Mr PD Carstens**

Feeding costs are the largest expense in an ostrich production system. A simulation model was developed by Gous and Brand to predict the nutrient requirements of growing ostriches during different production stages, in an attempt to optimise feeding costs. In the evaluation phase of the model this study was done to determine the effect of three different dietary protein concentrations (with a specific associated amino acid content) on certain production traits in growing ostriches. Measured parameters included feed intake, feed conversion ratio, and growth rate. Basic abattoir weight, *post mortem* measurements of the commercial cuts of meat and measurements on the feathers were also done. The crude protein and amino acid requirements of ostrich chicks for the different production phases (pre-starter, starter, grower and finisher) were predicted by the simulation model. Three basic diets were formulated to be 20% below and 20% above predicted levels for lysine, sulphur-containing amino acids, threonine, tryptophan and arginine (named diets with a low, medium or high protein concentration). The three diets were fed to ostriches during each of the four production phases from hatching up to slaughtering. Feed and water was available *ad libitum*. Significant differences were found for the final live mass of birds at slaughter (300 days old), cold carcass mass, thigh weight as well as for most of the weighed muscles. Concerning the feed related parameters, only feed intake was influenced by dietary treatment ( $P < 0.05$ ). No significant differences were found for any of the measured parameters on the feathers. Results indicated that birds on the diet with the medium protein performed optimal. No further increase in production levels were observed with the diet with the highest level of protein (and associated amino acids). This study showed that feeding diets with a higher protein and amino acid content than that predicted by the model developed by Gous and Brand (2011) was unable to further increase performance levels for growing ostriches.

The same data were used to evaluate the growth response of ostrich chicks fed the three diets containing the three different levels of protein and amino acids. The study deals with the analysis of ostrich body weight (BW) by modelling growth with linear and nonlinear functions for all the data across treatments. In total 3378 BW recordings of 90 animals were collected weekly from hatch (day 1) to 281 days of age. Seven nonlinear growth models and two polynomial functions were applied to the data. The growth models were compared by using Akaike's information criterion (AIC). The Bridges model had the smallest

AIC value, indicating the best fit for the ostrich data when all the measurements collected were fitted to the model without taking treatments into account. Data of different treatments were also modelled and it was found that for the L treatment, the Bridges and Janoschek models had the lowest AIC values; for the M treatment, the Janoschek had the lowest value and for the H treatment, the 3<sup>rd</sup> degree polynomial had the lowest AIC value. The results from this study aid in describing the growth of ostriches subjected to optimum feeding conditions. This information can also be used in research when modelling the nutrient requirements of growing birds.

The effect of different dietary energy concentrations on ostrich production parameters were examined in two different separate trials in the second study. The first trial included measurements from the pre-starter phase through the starter phase till the grower phase. The second trial was on the finisher phase. In both trials, the influence of dietary energy on feed intake, feed conversion ratio and growth parameters were assessed. Basic abattoir mass, measurements of the feathers and skin as well as *post mortem* meat quality measurements on commercial cuts of the meat were investigated. In both trials, three diets with different levels of dietary energy (Low, Medium, and High for each phase) were fed respectively for each phase: 11.6, 14.5, and 17.4 MJ/kg feed pre-starter, 10.8, 13.5, 16.2 MJ/kg feed starter, 9.2, 11.5, 13.8 MJ/kg feed grower and 7.6, 9.5, 11.4 MJ/kg feed finisher. Feed and water were available *ad libitum* in both trials. Overall dietary levels provided in the pre-starter, starter and grower phases indicated better growth, FCR, skin size and grade, thigh mass, live mass and cold carcass mass for the birds fed the medium energy diet.

Dietary energy levels provided during the finisher phase indicated that the energy level above the medium level (9.5 MJ/kg feed) resulted in improved growth rate and tanned skin size. Gender of the birds influenced cold carcass mass, growth rate, and certain feather parameters significantly. The effect of dietary energy level on feed intake is important to compare the feed intake of birds in the simulation model.

Further work on the different dietary crude protein levels will be done, since the current conclusions are still incomplete.

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## 5. Development of an mathematical optimization model for breeding ostriches

**Project Leaders: Prof TS Brand, T Olivier and Mrs GA Tesselaar**

In the first phase of the study, two different protein sources, cottonseed oilcake versus soya oilcake, were compared in breeding ostrich diets. Ninety six breeding pairs were divided into two groups to determine the effects of the two different protein sources during the breeding season. Gossypol levels in the feed were tested in the different breeder diets. All data relating to breeding ostrich production was monitored. The percentage gossypol in the cottonseed oilcake was determined to be 10-20 ppm. The inclusion of cottonseed oilcake meal in diets had no significant effect on the total number of eggs produced ( $47.86 \pm 3.58$  vs.  $50.31 \pm 3.66$ ) or infertile eggs ( $31.46 \pm 3.90$  vs.  $39.30 \pm 4.00$ ), while a tendency ( $P=0.06$ ) was observed that the dead-in-shell chicks increased ( $20.17 \pm 2.44$  vs.  $26.79 \pm 2.50$ ). The inclusion of cottonseed oilcake meal in diets of breeding birds however led to a 47.6% reduction in chick production ( $17.2$  vs.  $36.1$  chicks/hen/breeding season). It was concluded from this study that the inclusion of cottonseed oilcake meal in the diets of breeding ostriches will have a detrimental effect on chick production.

Data to be incorporated in the model includes the seasonal breeding pattern of breeding ostriches. Collected data were thus analysed to determine the seasonal production pattern as well as the seasonal variation in egg quality characteristics over the breeding season. During the breeding season, from mid-May to mid-December, sixteen eggs were randomly selected per month from 96 SA Black ostrich breeding pairs. The total weights as well as the different components of the ostrich egg were determined. When looking at the total egg weight of an ostrich egg, the eggs produced in May ( $1271.8 \text{ g} \pm 37.8$ ) differ significantly from eggs produced in September ( $1496.2 \text{ g} \pm 33.8$ ,  $P \leq 0.0$ ) as well as December ( $1472.1 \pm 32.7$ ,  $P \leq 0.0$ ). The total egg weight for the month of June ( $1343.3 \pm 32.7$ ) also differs significantly from September ( $1496.2 \text{ g} \pm 33.8$ ,  $P=0.04$ ). The yolk: albumin ratio of eggs produced in May ( $2.13 \pm 0.17$ ) differs significantly from the yolk: albumin ratio of the eggs of November ( $2.69 \pm 0.13$ ) and December ( $2.29 \pm 0.10$ ). A positive relationship between the two characteristics was observed. No linear tendency in either egg weight ( $R^2 = 0.40$ ) or yolk: albumin ratio ( $R^2 = 0.20$ ) was observed over the breeding season.

In theory dietary energy level suppress feed intake in livestock. This tendency is however not observed with breeding ostriches. This may be due to a certain shortage of certain nutrients in the diet, since animals normally consumed feed in relation to the most limiting nutrient. To try to identify the limiting nutrient eight diets were provided as follows:

- Standard diet without vitamin and mineral premix pack;
- Standard diet with normal vitamin and mineral premix pack;

- Standard diet with normal vitamin and 2 x level of elements (M2);
- Standard diet with normal trace elements and 2 x vitamins;
- Standard diet with premix pack and limestone added;
- Standard diet with premix pack and monocalciumphosphate added;
- Standard diet with premix pack and soybean oilcake added;
- Standard diet with premix pack and linseed added.

Significant differences ( $P<0.05$ ) were found in the quantity of feed ingested by birds between the diet with surplus minerals (M2) ( $2.3\pm 0.3$  kg/bird/day), the diet with no vitamins and minerals added ( $2.7\pm 0.3$  kg/bird/day) and the diet with added fatty acids in the form of linseed ( $2.9\pm 0.5$  kg/bird/day). This may indicate the possibility that ostriches ingest feed to satisfy mineral requirements. No statistically significant relationship was found between the thickness and strength of the shell. Significant ( $P\leq 0.05$ ) differences in shell strength were found between treatments, but no specific pattern could be identified. Results further revealed no effect of dietary treatment on egg production, dead-in-shell eggs, infertile eggs or chick production.

Further studies will focus on the effect of dietary energy levels and dietary protein levels on the production of breeding ostriches.

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## **6. The incidence and extent of pitting on ostrich skins in the South African ostrich leather industry**

**Project Leaders: Prof SWP Cloete and Dr A Engelbrecht**

Visible leather damage is an important financial factor for the ostrich industry, with many skins being downgraded. Surface pitting on ostrich skins is becoming an increasing problem for the ostrich industry that results in large financial losses due to the impact on leather quality. The aim of the project is to determine the cause or origin of pitting damage on ostrich skins and to find a solution that will decrease pit marks on ostrich skins.

Approximately 50 ostriches infested with ostrich feather lice were used to test various spray products. Four treatments were given, and treatments were duplicated. The treatments were a control group (sprayed with clean water), a group sprayed with an Amitraz spray (Taktic® Cattle Spray), a group sprayed with a Deltamethrin spray (Deca-tix 3®) and a group sprayed with a Spinosad spray (Extinosad®). The birds were evaluated for lice infestation before and after treatment. They were slaughtered 4 months after treatment. The skins were evaluated for pitting damage after being processed to the chrome-crust phase. The data have been analysed and will be reported in two papers. Four pour-on products (Bodygard, Cypertraz, Deadline and Taktic) were also evaluated with regard to their effect on the skins of the ostriches. The skins were evaluated after slaughter and in the chrome-crust phase for signs of skin damage. A follow-up trial will be done to investigate this further. The other trials could not be done yet due to lack of a suitable location and funding.

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## **7. Conservation of the ostrich genetic resource of the Oudtshoorn Research Farm**

**Project Leaders: Prof SWP Cloete and Dr A Engelbrecht**

The project aims to protect the ostrich genetic resource of the Oudtshoorn Research farm by moving a representative sample of the genetic resource to a different location that is geographically removed from the high risk area. This will spread the risk and thereby prevent loss of the entire resource population in the event of an avian influenza outbreak at one location.

Nortier Research Farm has been identified as a suitable location for safe-keeping a sample of the ostrich genetic resource from the Oudtshoorn research flock. Nortier Research Farm is in the process of erecting the necessary infrastructure for this purpose and will be registered for keeping ostriches. The first birds will be sent to Nortier once these preparations are finalized.

## **8. The management, long-term conservation and selection of the Ostrich Resource Flock at Oudtshoorn Research Farm**

**Project Leaders: Prof SWP Cloete and Dr A Engelbrecht**

The ostrich flock at the Oudtshoorn research farm was developed as a research resource from a donation of commercial breeder birds made by local producers in 1964. As such, the Department has a responsibility to maintain this unique and valuable resource, and to provide information and breeding material to the ostrich industry. This project aims to ensure the management, long-term conservation and selection of the ostrich flock at the Oudtshoorn Research Farm to aid ostrich research and to benefit the ostrich industry as a whole.

No auction of breeder birds was held during the reporting period due to the avian influenza outbreak of 2011 that resulted in most farms not being registered yet, thereby prohibiting the movement of ostriches between farms. The breeding flock was subsequently decreased in number due to budget constraints to consist of only 150 breeding pairs. The breeder females were randomly allocated to camps. A pre-selected male for each female were put in the camps one week later.

The number of pairs for each selection line was as follows:

- 19 pairs of SAB (South African Black) control line
- 20 pairs of SAB weight line
- 21 pairs of SAB reproduction line
- 1 pair of SAB weight x SAB reproduction
- 10 pairs of ZB (Zimbabwean Blue) pure line
- 10 pairs of KR (Kenyan Red) pure line
- 10 pairs of ZB x SAB
- 10 pairs of SAB x ZB
- 4 pairs of SAB x KR
- 10 pairs of KR x SAB
- 10 pairs of SAB x cross ZBxSAB
- 10 pairs of SAB x cross SABxZB
- 10 pairs of SAB x cross KRxSAB
- 5 pairs of SAB x cross SABxKR

The number of eggs and chicks produced during the 2012 breeding season from the pair-bred flock birds were 5060 and 1864, respectively. The number of chicks/slaughter birds on the farm on 31 March 2013 was 477. Excess day-old chicks were sold to farmers.

Appropriate biosecurity measures were put in place during 2012 for protection of the research flock. Separate breeder and export farms were subsequently registered during February 2013.

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## 9. The development of a viable artificial insemination protocol for ostriches

**Project Leaders: Prof SWP Cloete and Dr M Bonato**

### 1) *Progress on the development of a suitable semen diluent for ostriches*

The first feasible ostrich semen extender has been established as that based on the inorganic and organic composition of ostrich seminal plasma. The following chemical components were used: D-Glucose, Magnesium acetate (4H<sub>2</sub>O), Tripotassium citrate (H<sub>2</sub>O), Sodium glutamate (H<sub>2</sub>O), Sodium phosphate dibasic, TES and Calcium chloride and adapted to a pH of 7 with a osmolarity of 295 mOsm/kg. This extender has been found effective, but can be further optimized in the near future with specific focus on organic components, including specific sugars and protein components.

### 2) *Optimal storage conditions for diluted and cryopreserved semen*

Ostrich semen can be stored in the above mentioned extender (adapted to pH7) up to 48 hours with minimal deterioration in sperm quality measured in terms of the % live spermatozoa with no significant difference between 5 and 24 hours post storage. Different dilution rates were investigated (1:1, 1:2, 1:4 and 1:8) and higher dilution rates of 1:4 and 1:8 showed a higher percentage motile, percentage progressive motile sperm over longer storage times of 48 hours. Furthermore, a higher pH of 8 was found to increase the mean sperm velocity as compared to a pH of 7. Preliminary work has been started on the appropriate dilution rate, cooling rate, type of cryoprotectant (CP) and concentration (% of CP) to be used for cryopreservation. It appeared that cooling rates of 5°C and 1°C/min and a 1:4 dilution rate are beneficial for preserving ostrich semen.

### 3) *Optimal insemination dosage and frequency to be used on females*

We investigated the effect of two dilution rates (1:2 and 1:4) with EK diluent (poultry extender) on the duration of the fertile period of 14 female ostriches (aged 2-6 years) following artificial insemination with  $3.00 \times 10^9$  spermatozoa on five consecutive days, using voluntary crouch behaviour. We found that there was no difference in the duration of the fertile period between the two treatments (dilution 1:2, 9 days; dilution 1:4, 12 days,  $F_{1,73} = 0.03$ ,  $P = 0.850$ ). However, the overall number of sperm detected in the perivitelline membrane of the eggs was higher for the 1:4 dilution as compared to 1:2 dilution rate ( $F_{1,68} = 19.68$ ,  $P < 0.001$ ). The rate of sperm loss was also lower in the former than in the latter (1:2,  $y = -0.05x + 1.41$ ,  $P = 0.001$ ; 1:4,  $y = -0.02x + 1.66$ ,  $P = 0.001$ ;  $F_{1,68} = 7.39$ ,  $P = 0.008$ ).

### 4) *Establishment of seasonal changes in semen characteristics and hormone profiles*

As season can potentially influence the quality and quantity of semen collected for artificial insemination and therefore fertility, we are currently examining

semen output and male libido of seven ostriches (aged 2 to 4 years) over a period of 24 months (June 2009-2011). The volume of semen (mean  $\pm$  SEM) varied between  $1.03 \pm 0.12$  mL and  $1.85 \pm 0.07$  mL, the concentration between  $3.21 \pm 0.12 \times 10^9$  / mL and  $4.16 \pm 0.74 \times 10^9$  / mL and the number of spermatozoa between  $3.42 \pm 0.28 \times 10^9$  and  $7.66 \pm 0.47 \times 10^9$ . The largest values for ejaculate volume were found during autumn and winter (April-September) while higher numbers of spermatozoa per ejaculate were found during the spring and autumn. As season can potentially influence the quality and quantity of semen collected for artificial insemination and therefore fertility, we are currently examining semen output and male libido of seven ostriches (aged 2 to 4 years) over a period of 24 months (June 2009-2011). The volume of semen (mean  $\pm$  SEM) varied between  $1.03 \pm 0.12$  mL and  $1.85 \pm 0.07$  mL, the concentration between  $3.21 \pm 0.12 \times 10^9$  / mL and  $4.16 \pm 0.74 \times 10^9$  / mL and the number of spermatozoa between  $3.42 \pm 0.28 \times 10^9$  and  $7.66 \pm 0.47 \times 10^9$ . The largest values for ejaculate volume were found during autumn and winter (April-September) while higher numbers of spermatozoa per ejaculate were found during the spring and summer months (October – April), with a peak in spring (October – November). Both motility and libido score peaked in spring and showed lower values in the middle of summer (January-February). Furthermore, we observed high individual variation between males for all variables tested, except for motility. These results indicate that collections conducted in spring will yield higher numbers of spermatozoa, when libido of males is at a maximum. Hence, seasonal variation should thus be considered during the development of artificial insemination programs in this species to optimize fertility. Additionally blood samples have been collected on both males and females for a year (March 2010 – March 2011) to establish hormone profiles (corticosterone, testosterone, luteinizing hormone). Once the hormone profile of each bird will be established, this will be related to semen characteristics of males, and egg production of females. It will also be attempted to established seasonal patterns in serum hormone concentrations.

##### 5) *Imprinting of chicks*

Preliminary investigations on the effect of four different husbandry practices (standard husbandry, two extended human care treatments and foster parent care) performed at an early age on chick survival and growth, reported that chick survival to four weeks was significantly higher for human imprinted chicks when compared to chicks reared using standard husbandry practices validating the positive effect of regular gentle handling on chick welfare. Furthermore, such extensive human care soon after hatching was found to improve the docility of birds at a later stage of development whereby human imprinted birds were found to be more willing to associate with humans and to show more docile behaviour patterns as compared to chicks reared by conventional methods or by foster parents, irrespective of breed and sex. In addition, chicks reared by foster parents were consistently observed to keep their distance from observers, and were extremely difficult to handle during movements within the farm. Hence, habituation to regular and gentle handling

seems to be a powerful method to reduce fear of human beings demonstrated by ostriches, and could also play a major role in not only improving the welfare of commercial birds, but also in promoting human occupational health and safety.

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## 10. Evaluation of ostrich genotypes and crosses for the development of slaughter production systems

**Project Leader: Prof SWP Cloete**

A number of different ostrich lines are available for commercial production. Crossing of these lines are done in an unscientific and often a haphazard fashion. Yet major problems in the ostrich industry, like poor growth and survival, can possibly be resolved by structured crossbreeding based on scientific principles. All planned crossbreeding systems revolve around advantages expected due to heterosis and/or sexual dimorphism. The crossing of SA Blacks, Zimbabwean Blues and Kenyan Rednecks in a structured crossbreeding program is thus under investigation.

In a study on 2-year-old reproduction of ostrich females, it was found that South African Black (SAB) females had an increased interval from the production of their first egg to the production of their last egg compared to their Zimbabwean Blue (ZB) contemporaries. SAB females also started laying earlier in the production season and also recorded a higher total egg production compared to ZB birds. Reproduction was fairly independent of whether females were mated to a male of the same breed or not, although there was a trend for females mated to a male of the same breed to have a higher total egg production than contemporaries bred to a male of a different breed (respectively  $26.4 \pm 2.0$  vs.  $22.0 \pm 2.0$ ).

SAB breeders had wider and longer feathers than both the ZB and Kenyan Redneck (KR) strains. Reciprocal crosses among the breeds were generally intermediate, and mostly near to the midparent value.

Results on both aspects studied were still not very stable, as there are still too few records, particularly involving the KR as a pure breed, and as crosses. The investigation is therefore set to continue.

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## **11. Studies on genetic and environmental factors affecting growth and skin traits in ostriches**

**Project Leaders: Prof SWP Cloete and Dr A Engelbrecht**

The success of genetic improvement as a tool for improved productivity has been well documented in other livestock industries. For the ostrich, however, little is currently known about the genetic basis of observed variation in recorded leather and slaughter traits, and effective breeding programs are yet to be developed. The study aimed to estimate genetic parameters for slaughter, meat and skin traits for ostriches for use in a selection index for slaughter ostriches.

Substantial variation, high and favourable genetic correlations as well as moderate to high heritability estimates were found among, and for distinguished body weight traits of growing ostriches. Heritability estimates of 0.14, 0.22, 0.33, 0.43 and 0.43 for 1-month, 4-month, 7-month, 10-month and 13-month-old ostrich weights were estimated in a five-trait animal model analysis.

All carcass component weight traits, with the exception of the weight of the liver, showed significant genetic variation. No significant maternal permanent environmental variance was evident for these traits. Heritability estimates ranged from 0.21 (for subcutaneous fat weight) to 0.45 (for neck weight) in multi-trait analyses. The only potentially unfavourable correlation was a high genetic correlation between live weight and subcutaneous fat weight, as fat is considered as a waste product in the present system. The heritability estimates for individual muscle weights ranged from 0.14 to 0.43, while the genetic correlation between these weights and pre-slaughter live weight were all positive, ranging from 0.59 to 0.82.

When meat quality traits were analysed it was evident that lightness ( $L^*$ ) and ultimate pH (pHu) showed significant genetic variation, with heritability estimates of 0.37 and 0.42, respectively.  $L^*$  and pHu were negatively correlated ( $-0.65 \pm 0.19$ ). Since pH is an indicator of various meat quality parameters, it could be considered as an appropriate selection criterion for enhanced meat quality.

With the exception of skin grading and crown length, all quantitative and qualitative skin traits showed significant genetic variation. Nodule traits were accordingly moderate to highly heritable. A negative, but favourable, correlation between weight and hair follicle score was ascertained, as hair follicles is a defect that should be selected against.

This study demonstrated that sufficient genetic variation exists for most slaughter traits to allow sustained genetic progress for these traits, should it be desired as

part of the overall selection objective. Combining some of the current economically important slaughter traits in a provisional selection index, it was clear that weight and crust skin size contributed most to monetary gain (approximately 54 and 38%, respectively). It was also demonstrated with this simple index that monetary gains in slaughter bird production should be easy to achieve at all levels of production performance and data recording.

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## 12. Increasing production in ostriches by understanding compatibility between mating partners

**Project Leaders: Prof SWP Cloete and Dr C Cornwallis**

The aim of this project is to reduce the problem of poor production efficiency in ostriches by increasing the genetic compatibility between breeding males and females.

1) Progress on testing how the breeding success of individuals changes with group composition.

Group composition was experimentally manipulated and range from male female pairs to groups with 3 males and 4 females (total  $n_{\text{groups}}=18$ ). The reproductive behaviour of these groups was recorded for 235 hours during which time 2590 interactions and 812 mating attempts were observed. The groups produced 1353 eggs of which 606 hatched (hatching success = 45%). A multi-locus (7 polymorphic loci) multiplex microsatellite typing scheme was developed to accurately determine the parentage of chicks and DNA was extracted from 506 samples ready for parentage analysis. The genotyping of these samples will be completed by January 2014.

This experiment will be repeated during the 2013 and 2014 breeding seasons after which time the data will be analysed.

2) Identifying genes underlying mate compatibility and variation in production traits

Blood samples were collected from all breeding birds (595 individuals) accurately labelled and stored in 95% ethanol. DNA was extracted from 100 adults. Restricted site Associated DNA (RAD) libraries were prepared for 12 individuals (4 blacks, 4 blues & 4 reds) and sent to Beijing Genomics Institute (BGI) in China for illumina sequencing for single nucleotide polymorphism (SNP) discovery. Of these 12 individuals 6 libraries were successfully sequenced first time, 4 individuals will be re-sequenced and the DNA from 2 individuals was too low quality and will be replaced with two different individuals. SNP analysis is now underway and first indications are that around 30,000 SNPs are presented in the data so far.

### 13. Characterization of adrenal steroidogenesis in the ostrich

**Project Leaders: Prof SWP Cloete and Dr D Hough**

In the continual effort to improve the robustness of ostriches, we suggest the utilization of the hypothalamic-pituitary-adrenal axis function (involved in metabolism and stress response) as breeding tool to ultimately improve the robustness of this species. We set out to identify the pathway for steroid biosynthesis within the adrenal gland and analyze the potential of this breeding tool. It was possible to identify the predominant circulating steroid hormones in ostriches, as described in the results below. Furthermore, adrenal glands were collected during routinely scheduled culling and used to isolate sub-cellular fractions (microsomes and mitochondria). When a spectral assay was used to determine the concentration of the cytochrome P450-type steroidogenic enzymes, the concentration of these enzymes were extremely low. We concluded that the sub-cellular fractionation was unsuccessful, so we repeated the adrenal gland collection and fractionation. We gained the same results and decided to test whether there is any steroidogenic enzyme activity. There was activity, which meant that the fractionation was successful, but that the majority of the steroidogenic enzymes in the adrenal gland of the ostrich are likely to be of the dehydrogenase-type enzymes, rather than the cytochrome P450-type enzymes. This supports the current hypothesized steroidogenic pathway for corticosterone synthesis. Microsomal and mitochondrial fractions have been stored and ready to use in further assays to confirm the pathway for steroid biosynthesis in the ostrich adrenal.

#### **Results:**

Corticosterone was by far the dominant steroid that was detected in ostrich plasma (20 – 70 ng/mL) and was significantly different among breeds (Figure 1, Addendum A; Two-way ANOVA: Breed  $P = 0.0423$ , Gender  $P = 0.8351$ , Interaction  $P = 0.7325$ ). The South African Blacks (group = "1") produced the lowest corticosterone (Males:  $11.26 \pm 3.29$ , Females:  $8.93 \pm 1.82$ ), followed by the Zimbabwean Blues (group = "2", Males:  $15.85 \pm 2.83$ , Females:  $16.48 \pm 4.59$ ) and the Kenyan Reds (group = "13", Males:  $17.85 \pm 5.12$ , Females:  $21.52 \pm 4.79$ ) that produced the most corticosterone. Since the blood collection for all three breeds was done in the same manner, the differences in corticosterone levels are not the result of stressed vs. unstressed groups of birds. Instead, it seems that these results depict the different baseline concentrations of corticosterone for the three ostrich breeds. Because of the circadian and ultradian variation of corticosterone, it might be worthwhile to examine whether these differences in circulating corticosterone is also present in feathers, which reflects the 'build-up of corticosterone' over a long period of time that is more representative of the true baseline concentrations.

Differences in temperament have been observed between these three breeds, where the Kenyan Red seems have a more agitated and aggressive behaviour compared to the other two breeds (personal communication, Maud Bonato). This agitated behaviour may have a direct correlation with the amount of circulating corticosterone, however, the breed differences in temperament still need to be formally documented. The current results provide evidence that it would be worthwhile endeavouring a study to correlate corticosterone production with temperament among these three breeds, and investigate its implication for production performance, animal health and welfare.

No cortisol or 11-deoxycortisol was detected, which indicates that cytochrome P450 17-alpha-hydroxylase/17,20-lyase (CYP17) is not expressed in the ostrich adrenal gland and corticosterone is therefore the only glucocorticoid produced in response to hypothalamic-pituitary-adrenal axis (HPA-axis) stimulation. Three additional steroids were present in ostrich blood, but at lower concentrations, namely testosterone, progesterone and androstenedione. Two-way ANOVA of testosterone data confirmed that the production of testosterone differs between males and females, but is not different across breeds (Breed  $P = 0.1833$ , Gender  $P = 0.0116$ , Interaction  $P = 0.2915$ ). It should be noted that testosterone, progesterone and androstenedione were present at concentrations just above their limits of quantification.

There were certain steroids that had significant signal-to-noise ratios in some samples, but were absent, or had too low signal-to-noise ratios, in other samples were 11-dehydrocorticosterone, deoxycorticosterone, dehydrotestosterone, 11-ketoandrostenedione and 11-hydroxyandrostenedione. As a result, there were large standard errors of the mean. These steroids are present at too low concentrations in the blood to be accurately measured with the UPLC-MS/MS method. If their quantification is required in future, the 500  $\mu$ L sample volume for serum needs to be increased.

The weak ionization of pregnenolone,  $\beta$ -estradiol and estrone made their detection in ostrich serum difficult. These steroids had low signal-to-noise ratios, but their low response-values resulted in standard curves that overestimate low concentrations with weak signal-to-noise ratios. Data for these steroids could subsequently not be used. Derivatisation for  $\beta$ -estradiol and estrone should be considered if their quantification is desired in future studies.

### **Conclusion:**

Corticosterone is the predominant glucocorticoid produced by ostriches. There are differences among the three ostrich breeds in their corticosterone production. It is worth comparing how these breeds cope with different stressors, how their corticosterone responses correlate with behaviour, production performance and robustness. If the quantification of reproductive steroids is required, then derivatisation, larger sample sizes or RIA should be considered.

## **14. Effect of storage periods for longer than 6 days on the hatchability of artificially incubated ostrich eggs**

**Project Leader: Dr Z Brand**

Artificial incubation has become an essential part of commercial ostrich farming in South Africa. However, our understanding of artificial incubation in ostriches is still poor compared to domesticated poultry. Problems with artificial methods of incubation and chick rearing are currently still among the most important constraints to the development of the ostrich industry. High levels of reproductive failure, particularly during the artificial incubation phase and the subsequent chick rearing phase, are impacting on the economic viability of the commercial ostrich production industry. Low chick production is a significant problem for the ostrich industry due to infertility (~ 20%) and hatching failure (~ 20%) (Deeming & Ar, 1999; Brand *et al.*, 2012).

With the exception of a few studies, the factors influencing fertility, effecting hatchability and incubation have not been investigated in ostriches. The high levels of hatching failure of ostrich eggs needs to be addressed for the ostrich farming to become more profitable. Identification of non-genetic factors affecting hatching success may be useful for improving flock productivity through management and hatchery practices. Storage time is a non-genetic factor that is expected to have an effect on hatchability.

Thus the objective of the present study is to investigate the effect of storage time on embryonic mortality in ostrich eggs. The aim is to increase overall hatchability by storing fresh eggs for up to 14 days prior to incubation. This will not only increase chick production, but will also benefit the ostrich industry as a whole.



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