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&
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Novel Research - Functional Farming

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The contributed abstracts were submitted electronically, and were subjected to a peer review process. The Organizing Committee cannot be held responsible for any views expressed in the abstracts, or in the presentation thereof.

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FOREWORD AND WELCOME

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I am delighted to have been invited to write the foreword for this book of abstracts. Further, I am particularly pleased that the 6th International Ratite Symposium is for the first time being held under the auspices of working Group 3 (Ratites) of the Asia Pacific Federation of World's Poultry Science Association branches and in conjunction with the 49th South African Society of Animal Science Congress.

This collection of abstracts is the product of a great deal of effort by numerous people on the Organizing Committee of the Symposium and by all presenters at the event, both invited speakers and those who's submitted papers and posters were accepted. My thanks go out to them all for the many hours of preparation to make this symposium such a success.

The presentations abstracted here report the latest in scientific research outcomes. These can be employed directly in industry to make a positive impact on productive performance in both the larger commercial and smallholder sectors.

With the theme of "Novel Research - Functional Farming", the symposium covers many issues of importance to the global ratite industries including Genetics, Reproduction and Breeding, Husbandry, Nutrition, Veterinary Health, Welfare and Product Quality. This compilation is a valuable record of proceedings of the symposium and is a further step along the pathway to sophistication in a sustainable ratite industry. It is entirely consistent with the objectives of the World's Poultry Science Association.

FEED PREFERENCE OF GROWER OSTRICHES CONSUMING DIETS DIFFERING IN *Lupinus angustifolius* INCLUSION LEVELS

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Background: Feed costs make the largest proportion (*ca*. 75 %) of the input costs of slaughter birds in an intensive ostrich production unit. Studies to evaluate local raw products with the potential to improve the efficiency of production in this species are therefore needed.

Aim: To determine the feed preference of ostriches when diets containing different levels of lupins (sweet and bitter cultivars) are fed to establish to what degree lupins can be included in ostrich rations without affecting intake.

Methodologies: Sixty South African Black ostriches of 43 weeks of age, were randomly divided into ten groups of six birds per group. Three trials with five experimental diets per trial were conducted to investigate the diet preference of grower ostriches in a free-choice system. Feed and water was supplied *ad libitum*. The position of the diets in the successive paddocks housing the groups of six birds was changed by rotating five feed troughs in a clockwise direction. However, within each paddock the position of troughs containing the diets stayed the same throughout the three trials. In the first two trials, respectively sweet and bitter lupins replaced soybean oilcake meal to provide 0%, 7.5%, 15%, 22.5% and 30% inclusion levels of lupins. During the third trial soybean oilcake meal was replaced to provide 0% lupins, 15% sweet, 15% bitter, 30% sweet and 30% bitter inclusion levels of lupins in the diets. All the diets were formulated to be iso-nutritious in terms of metabolisable energy (12.8 MJ/kg feed), protein (15.1%), fat (2.8%), fibre (6.9%), calcium (1.2%), total phosphorus (0.7%), lysine (0.8%), methionine and cysteine (0.5%), threonine (0.6%) and tryptophan (0.2%). The initial average body weight of the birds was 73.6 ± 0.5 kg. The daily intake per group for each diet was monitored over a period of five days each. Ethical clearance for the project was obtained from the Western Cape Department of Agriculture (clearance number R14/108).

Results: No interaction ($P > 0.05$) was found between day and diet using a multifactor ANOVA ($P = 0.45, 0.88, 0.99$ for the three trials, respectively). A one-way ANOVA analysis was conducted to evaluate feed intake by diet and the results showed that dry matter intake (DMI) did not differ between the five treatments for each of the three trials ($P = 0.27, 0.11, 0.25$, respectively). During the second trial a tendency ($P = 0.11$) was observed where birds showed a preference for the 7.5% bitter lupin inclusion level, while discriminating to some extent against the 15% and 30% bitter lupin inclusion levels. Regression analysis of DMI on lupine inclusion rate revealed no significant trends.

Discussion: This study showed that soybean oilcake meal in the diets of grower ostriches can be replaced with different inclusion levels of lupins (both sweet and bitter) without any significant detrimental effect on feed intake.

Conclusions and recommendations: Results from this study may assist in establishing a potential market for lupins as a locally produced protein source. The use of cheaper protein sources such as lupins will improve profit margins of ostrich farmers.

IMPROVING EARLY WEIGHT, IMMUNO-COMPETENCE AND SURVIVAL OF OSTRICH CHICKS THROUGH POSITIVE HUMAN-BIRD INTERACTIONS

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Background: Highly variable early weight gain and poor chick survival are important constraints in ostrich farming. This can be attributed in part to poorly defined husbandry practices, disease management and inappropriate stockman ship. Extensive research on developing good husbandry practices can assist to address these constraints, thereby improving ostrich production and welfare.

Aim: To study the effect of different husbandry practices on weight gain, survival and immuno-competence of ostrich chicks.

Methodology: A study conducted during 2013 and 2015, using a total of 216 and 200 ostrich hatchlings respectively, investigated the influence of contrasting husbandry practices on ostrich chick traits from day-old to 3 months of age. Three husbandry practices were investigated including standard husbandry (S; exposure to humans limited to providing feed and fresh water); human imprinting (S+I1; lengthy exposure to humans including touch [hand feeding], audio [human voices], and visual [human presence] cues); and human imprinting (S+I2; lengthy exposure to humans, with only visual and audio cues). Imprinting took place over a 30 - day period. A Hemaglutination Inhibition (HI) test was carried out on 5-months-old chicks (N = 196) in 2013 to determine the humoral response 21 days after vaccination according to the standard Newcastle Disease protocol. Live weight, survival, and immuno-competence at 6 and 12 weeks after hatching were recorded at weekly and monthly intervals, to determine the influence of husbandry practices. Ethical clearance was granted by the Western Cape Department of Agriculture (Ref No.: R9/24) and SAS Enterprise Guide 5.1 was used to analyse data.

Results: At 6 weeks old, S+I1 chicks had higher live weights compared to the S+I2 and S treatments (7.47 ± 0.18 kg vs 7.06 ± 0.15 kg and 6.21 ± 0.13 kg, respectively; $P < 0.001$), but no difference was observed between the three groups when they reached 12 weeks of age (S+I1: 22.67 ± 0.63 kg; S+I2: 22.80 ± 0.67 kg; and S: 22.10 ± 0.66 kg respectively; $P > 0.05$). Survival to 6 weeks was improved in the S+I1 and S+I2 group, when compared to the S group (87.5% and 86.9% vs. 83.7%; $P = 0.04$), while no difference was observed between the three groups at 12 weeks of age ($P > 0.05$). Furthermore, the HI test revealed that, more S chicks tested positive to Newcastle disease vaccination compared to the imprinting treatments (S: 69.1%; S+I1: 49.9%; and S+I2: 63.6%; $P < 0.05$), suggesting that S+I2 and particularly S+I1 chicks had an improved immuno-competence.

Discussion: Extensive exposure of ostrich chicks to humans impacted positively on chick weight gain, survival and immuno-competence during the first 3 months after hatching, a critical time in the intensive rearing of ostrich chicks. The exact mechanisms involved in improving the viability and survivability of ostrich chicks still need to be clarified but it is assumed that chicks accustomed to human presence may experience lower levels of stress during routine farming operations.

Conclusion/recommendations: Further studies are needed to evaluate the effect of different husbandry practices on long- and short-term stress responses, docility, reproductive performance and meat quality of ostriches.

PRELIMINARY RESULTS ON THE EFFECTS OF CLIPPING THE TOENAILS OF OSTRICH CHICKS

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Background: Research indicated that ostrich skin grading was markedly improved when day-old chicks were declawed. However, declawing chicks raised welfare concerns due to the permanent removal of the toenails, as it involved removing part of the toe to remove the growth point. The alternative practice of only clipping the toenails has consequently been adopted by the local ostrich industry to improve welfare, while minimizing injuries and improving skin quality. The toenails regrow quickly with this method, but are shorter and less likely to inflict lacerations after regrowth. However, there are still welfare concerns because of the pain that the clipping of the toenails is thought to cause due to the presence of spongy bone tissue from the last toe digit in the part of the nail that is clipped.

Aim: To report preliminary results on the effects of clipping the toenails of ostrich chicks on welfare and production and to determine whether this husbandry practice is beneficial or detrimental.

Methodologies: In Trial 1 the clipping of the toenails of ostrich chicks at day-old and again at one month of age was investigated and compared to a control treatment where toenails were left intact. The experimental treatments involved the clipping of toenails of some chicks at day-old, while others were clipped at day-old and again at one month of age. Toenails were clipped with an electrical debeaker with a heated blade according to a standardised methodology. Each of the three treatments was replicated in five groups of chicks. Wellness was assessed according to a wellness score based on behaviour and wound healing. Growth and mortalities of chicks were also monitored. The skins of experimental birds were evaluated for evidence of toenail-related injuries after slaughter at 10 months of age. The ratio of heterophils to lymphocytes (H/L) was used to assess stress as a result of toenail clipping with and without the use of a non-steroidal anti-inflammatory drug (NSAID) and a topical anaesthetic in Trial 2. Blood smears were taken before and 48 hours after treatment to determine whether treatment influenced the H/L ratio of individual chicks. The data of both trials were analysed according to a completely randomised design. Ethical clearance for the project was obtained from the Western Cape Department of Agriculture (clearance number R14/106).

Results and discussion: Growth and mortality were not influenced by treatment. The number of toenail-related lesions on skins as well as skin grading was significantly reduced in both clipped treatments in Trial 1. However, clipping the toenails again at one month of age did not have any additional benefits. Toenails regrew quickly to reach about the same length as unclipped toenails within 14 weeks. In Trial 2, no treatment differences were evident in H/L ratios after toenail clipping, either with or without the use of a NSAID and a topical anaesthetic.

Conclusion and recommendation: Clipping the toenails of day-old ostrich chicks does not seem to impact negatively on the long-term welfare and production of ostriches, while it improved skin quality by decreasing toenail-related skin lesions. Research on the refinement of the technique and alternative strategies should continue.

EVALUATION OF A DNA-VACCINE AGAINST MYCOPLASMA INFECTIONS IN OSTRICHES

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Background: Three *Mycoplasma* species are associated with respiratory infections in South African ostriches and are in short referred to as Ms01, Ms02 and Ms03. Mycoplasmas are problematic in feedlot ostriches where they cause reduced production, downgrading of carcasses and, in extreme cases, chick mortalities. Infections can be treated with antibiotics, but may require long-term treatment with subsequent unwanted accumulation of antibiotic residues in meat. The development of whole-organism vaccines, as alternative, is impractical and not economically feasible. Knowledge of the Mycoplasma genome of Ms01 allowed a different approach where a Ms01-antigen could be identified as vaccine target for use in a DNA-vaccine against Ms01 infections in ostriches.

Aim: This study investigated the ability of DNA vaccines developed against Ms01 to elicit an antibody response in ostriches during a vaccination trial.

Methodologies: The OppA protein was identified as a possible antigen and its gene cloned into two DNA-vaccine vectors, pCIneo and VR1020. Both vectors include an origin of replication, selection marker and eukaryotic promoter, intron and polyadenylation signal. In addition, VR1020 has a signal peptide allowing export of the expressed protein for better immune activation. The trial consisted of 140, 3 - 4 month old ostriches that were raised in Fraserburg and moved to Oudtshoorn once they reached 40 kg. Group one (n = 60) was vaccinated with pCI-neo_*oppA*, group two (n = 60) with VR1020_*oppA* and group three (n = 20) acted as control and did not receive any vaccine. Each of the vaccinated groups was subdivided into three groups (20 per group) with each receiving either a 100, 300 or 600 µg/ml dose of plasmid intramuscularly at week 0 and 7. Blood was drawn at week 0, 4, 7 and 10 and anti-OppA antibodies measured using an ELISA developed for this purpose. Tracheal swabs were also taken from all ostriches during the course of the trial to determine background mycoplasma infections using PCR. Ethical clearance was obtained from the University of Stellenbosch Animal Ethics Committee (Ref: SU-ACUM13-00019) and the Director of Animal Health (DAFF) (Ref: 12/11/1/1/3) respectively.

Results: The control group showed no increase in antibody titres over the 10-week 10 weeks. Compared to this, pCI-neo_*oppA* was not able to elicit a statistically significant antibody response irrespective of the dose administered. In contrast, VR1020_*oppA* elicited an anti-OppA antibody response. Based on the calculated LSD (0.1759), only the groups that received a 300 or 600 µg/ml plasmid dose had titers significantly different from the control group at week 7 and 10. Background mycoplasma infections increased at week 10 and only after movement of ostriches to Oudtshoorn. Ms02 and Ms03 infections were dominant with overall fewer infections in the VR1020_*oppA* groups using a dose of 600 µg/ml plasmid, and the most in the control group. Background infections still need to be evaluated, but are not expected to have an influence on the observed antibody titers before week 10. No increase in Ms01 infections was observed, but this included the control group and therefore it cannot be concluded that the lack of Ms01 infections was only due to the VR1020_*oppA* vaccine.

Discussion: Despite having the same antigen, only the VR1020_*oppA* construct was elicited a dose-dependent antibody response. This highlights the role of the plasmid backbone in the antibody response. A lack of cross-reactivity between the anti-OppA antibody of Ms01, Ms02 and Ms03 could explain the dominance of Ms02 and Ms03 infections.

Conclusion and recommendation: The use of DNA-vaccine technology in ostriches is a viable approach. The vaccination schedule and ability of the chosen antigen to confer protection against mycoplasma infections need to be further evaluated.

HUMORAL IMMUNE RESPONSES TO DIETARY L-CARNITINE SUPPLEMENTATION IN OSTRICH CHICKS

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Background: Proper nutrition has direct and indirect benefits for the immune system.

Aim: The objective of this study was to determine whether supplemental dietary L-carnitine has an effect on immune responses in ostrich chicks.

Methodologies: The response of the immune system to supplementary dietary L-carnitine (LC) as an immunomodulatory was investigated in day-old ostrich chicks from Days 1 to 81. The Newcastle disease (ND) antibody titer determination in ostrich sera was used as a humoral immune response indicator after vaccination. Thirty-two day-old Black Neck ostrich chicks were randomly divided into four treatments with four replicates, each containing two chicks. All birds received the same basal diet supplemented with 0 (T0, control), 125 (T125), 250 (T250) or 600 (T600) mg/kg LC. Chicks were vaccinated against inactive ND Virus (NDV) with an inactive vaccine at Day 30 as primary immunisation, and at Day 51 as booster immunisation. Blood samples were taken at Days 30, 51, 70 and 81. ND antibody responses were monitored over three phases: day 51 before the booster was administered; Day 70, which included the residual effect of the initial immunization and the effect of the booster and Day 81, which had the same combined effects as for Day 70. To determine serum antibody production in response to vaccination, a commercially available chicken anti-NDV enzyme-linked immunosorbent assay (ELISA) was modified for the detection of anti-NDV antibodies in ostrich serum.

Results: The different levels of treatments and the time periods were influenced ($P<0.01$) by ND antibody responses. Moreover, interactions between treatment levels and time periods affected ND antibody responses ($P<0.05$). The birds that were fed a diet containing 125 and 250 mg/kg of LC had the highest level ($P<0.05$) of ND antibody responses compared to the other groups over the total period. There were no differences ($P>0.05$) in ND antibody response between T0 (control) and T600 as well as T125 and T250. A response curve fitted to the treatment means appears hyperbolic with an optimum between 125 and 250 mg/kg.

Discussion: The means show that Days 70 and 81 had higher ($P<0.05$) ND antibody responses compared to Day 50. Likewise, the highest ND antibody responses were recorded on Day 70.

Conclusions and recommendations: The results suggest that LC supplementation at levels of 125 and 250 mg/kg have positive effects on antibody production and immune response in Black Neck ostrich chicks.

A REVIEW ON FACTORS INFLUENCING OSTRICH SKIN QUALITY

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Background: Leather is the main source of income for ostrich producers. Due to it competing in the luxury leather market, quality is of utmost importance and influences producer income to a large extent. It is therefore important to know and understand the factors that influence ostrich leather quality to be able to produce leather of premium quality.

Aim: To review and summarize the current knowledge on factors influencing leather quality.

Review: The size of the skin, the occurrence of visible defects and the appearance of the feather nodules collectively determine the quality of ostrich leather. Skin size is influenced by fixed effects such as age and weight, while heritability estimates ranging from 0.14 ± 0.04 to 0.51 ± 0.09 have been estimated for crust skin size. Crust skin size is also highly correlated with animal live weight on the genetic level. Skin grading is determined by the presence of visible skin damage and the appearance of the nodules. It has been established that skin damage is mostly influenced by environmental factors, while nodule shape, size and distribution display genetic variation. Nodule size and shape were also influenced by age and gender, with males having bigger and better shaped nodules. Heritability estimates for nodule size and shape were 0.34 ± 0.08 and 0.37 ± 0.08, respectively. There is also a positive genetic correlation between live weight and nodule size and nodule shape, respectively. Specific intrinsic factors, such as the presence of hair follicles, can compromise skin quality. Scores for the presence of hair follicles were highly heritable (0.50 ± 0.09), with males having more hair follicles than females, and the presence of hair follicles increasing with age. The most common cause of skin damage, namely scratches, was related to toenail-related injuries. Clipping the toenails soon after hatch can significantly reduce these lesions resulting in substantially improved grading results. Injuries during hatching, handling and transport should also be minimized, since these injuries result in permanent lesions on the skins of slaughter ostriches. Another increasingly problematic defect on ostrich skins, namely pitting, were related to external parasites and were alleviated by the regular use of an effective ectoparasitic control program, specifically aimed at targeting visiting insects. Treatment with deltamethrin compounds was more effective than flumethrin treatments, presumably because of its more potent effect on insects. Research is underway to refine chemical treatments as part of an integrated insect control programme to possibly combat pitting damage.

Conclusions and recommendations: It is possible to substantially improve ostrich leather quality through improved management practices and a proper genetic selection programme. Owing to generally favourable genetic correlations among key traits of economic importance, it was shown that selection based solely on live weight will still be effective in improving skin size and nodule quality even at the low level of recording practiced in the South African ostrich industry at present. Research on genetic components of skin traits, and well as combating the presence and extent of pitting and other skin damage should continue.

THE EFFECT OF VARYING SWEET LUPIN INCLUSION LEVELS ON THE PRODUCTION AND SLAUGHTER TRAITS OF SLAUGHTER OSTRICHES (*Struthio camelus* var. *domesticus*)

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Background: Nutrition contributes 70 – 80% of the total input costs of an intensive ostrich production unit. An increase in the price of traditional protein sources, such as soybean oilcake meal, compels producers to find cheaper alternatives to ensure the cost efficient production of slaughter ostriches.

Aim: To determine to what levels soybean oilcake meal can be replaced by locally produced sweet lupin seed in the diets of slaughter ostriches (*Struthio camelus* var. *domesticus*). Also to evaluate the effect of increased levels of lupins in ostrich diets on the end weight, feed intake (DMI), average daily gain (ADG) and feed conversion ratio (FCR).

Methodologies: For this trial 141 chicks of 83 days of age of were randomly divided into five dietary treatments with three replications each. Two iso-nutrient diets were formulated for each production stage (starter, grower and finisher) to contain either soybean oilcake meal (control diet) or sweet lupins. Soybean oilcake meal as protein source was gradually replaced (0%, 25%, 50%, 75% and 100%) with sweet lupins. Feed and water were supplied *ad libitum*. Biweekly feed intake, as well as the weights of the ostriches was recorded up to slaughter at approximately 10.5 months of age. Ethical clearance for the project was obtained from the Western Cape Department of Agriculture (clearance number R14/108).

Results: The initial average body weight of the chicks was 13.8 ± 0.6 kg. No difference (P = 0.39) was found in the mean weights of the birds between the respective dietary treatments at the end of the trail. The end weights of the birds were 87.5 ± 3.0 kg, 92.3 ± 3.0 kg, 94.6 ± 3.0 kg, 95.2 ± 3.4 kg and 88.9 ± 3.1 kg, respectively, for the diets with 0%, 25%, 50%, 75% and 100% lupins. For the different diets DMI, ADG and FCR did not differ (P = 0.51, 0.21, 0.96, respectively) between the five treatments. A mean DMI and ADG of respectively 2021 ± 62 and 352 ± 8 g/bird/day were observed with a mean FCR of 5.7 ± 0.1 kg feed/kg weight gain over all treatments. Over the entire experimental period regression analysis of the data indicated that ADG was quadratically related to increased lupin inclusion level (R^2 = 42% and P = 10%), with a tendency for a higher growth rate with the intermediate diets combining soybean oilcake and lupins.

Discussion: Results from the study showed that soybean oilcake meal in the diets of slaughter ostriches can be replaced with up to 30% lupins without any significant detrimental effect on production.

Conclusions and recommendations: The hind-gut fermentation ability of ostriches most likely enables them to utilise lupins, with their higher fibre content compared to soybean oilcake meal, efficiently. The results from this study will contribute to the limited knowledge on the formulation of ostrich diets, as well as the optimal inclusion level of lupins in ostrich diets. These findings may also assist in creating a potential market for locally produced protein sources such as lupins and broaden our knowledge with regard to the potential of this raw material as feed ingredient for animals.

ASSISTED REPRODUCTION TECHNOLOGY FOR RATITES – PROGRESS AND CHALLENGES FOR FARMING

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Background: Ostriches, emus or rheas are attractive for commercial production of meat, leather, oil and feathers and farming of these species has been most successful in countries that have species' native environments. However, production of ratites in general is not very efficient, as little progress has been made in genetic improvement for traits important to economics of production and reproduction. Constrained by biology of the species ratite production is seasonal and involves monogamous or polygamous mating systems with a relatively low male to female ratio and long generation interval that are not suited to rapid genetic progress. The research of the last 10-15 years involving naturally mated ostriches has demonstrated that traits important to reproduction and economics of production are at least moderately heritable, which could also be true for the emu and rhea. With the assisted reproduction technology genetic improvement could be accelerated, offering better economics of production as demonstrated for other livestock species (poultry, pigs or cattle) for which breeding programs have been established.

Aim: To review recent advances in assisted reproduction techniques (ART) with specific reference to ostriches and emus.

Methodologies: The success of ART in other species has always depended on a good scientific foundation in behaviour and reproductive physiology. Until recently development of in these techniques in ratites did not seem feasible. However, recent advances in techniques to collect semen from trained ratite males for insemination of trained females resulted in marked progress towards a workable protocol for ART in these species. Papers reporting this progress are reviewed.

Results: Today, the practices of semen collection and artificial insemination have been well established for emus and ostriches and semen preservation technology has advanced considerably to an extent that short-term liquid storage of semen, long-term semen freezing and artificial insemination with fresh or stored semen has already been implemented fairly successfully. Research in this area has uncovered marked variation in male and female reproductive behaviour and in seasonality of gamete production, thereby rendering the development of reliable protocols for semen preservation and artificial insemination challenging. These developments, on one hand, indicate marked opportunities for continued progress. On the other hand, it may be a challenging and cumbersome task to screen many individuals in search of those having the most desirable characteristics to suit assisted reproduction technology. On the positive side, traits important for ART, like semen output, libido and female egg production in the absence of a male, were all repeatable, opening up possibilities for current flock gains. It should also be considered that animals selected for ART should, above and beyond desirable behavioural repertoires, also have desired genotypes for other economically important traits, like reproduction, survival, growth and product yield, as well as product quality.

Conclusions and recommendations: While progress is being made it is yet too early to speculate on semen extension and potential genetic improvement rates achievable with ART. Genetic and genomic technologies could greatly assist this process and help driving selection that ultimately could lay foundations for structured breeding programs and wide spread adoption of ART in ratite farming.

EFFECT OF SHORT-TERM STORAGE OF OSTRICH (*Struthio camelus*) SEMEN ON FERTILITY AFTER ARTIFICIAL INSEMINATION

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Background: Poor sperm supply due to male-female incompatibility, between-male variation in sperm output and seasonality are some of the primary reasons for low fertility in ostriches. Additionally, the current natural mating system (6:10 male to female ratio dominating in colonies) does not allow the identification of lowly reproducing individuals nor chick pedigrees. Consequently, inadequate genetic improvement is still limiting the expansion of the commercial ostrich industry. Artificial insemination (AI) would relieve the impact of these constraints but protocols for storage of liquid and frozen-thawed semen must first be developed.

Aim: To develop a protocol for short-term storage of ostrich semen for AI.

Methodologies: Ejaculates of eight South African Black males, collected using the dummy female method, was diluted 1:6 regardless of live sperm concentration at 21°C with a synthetic diluent analogous to ostrich seminal plasma. Semen was cooled at 1°C/minute to 5°C and stored for 24 hours. The Sperm Class Analyzer® was used to evaluate sperm motility. Semen was pooled for AI to supply 16 females split into a control (fresh diluted) (N = 8) and a treatment (chilled) (N = 8) group. Insemination dosages consisted out of 800 x 10^6 total sperm cells/mL (control group) and 1000 x 10^6 total sperm cells/mL (treatment group). Insemination was carried out for four consecutive days with a follow-up insemination every 6th day for 3 consecutive 6 - day cycles. Fertility was determined by examining the status of the germinal disc and the number of trapped sperm in the outer perivitelline layer (OPVL sperm/mm^2).

Results: OPVL sperm was the only fixed effect that contributed ($P < 0.001$) to the variation in fertility status. Semen treatment did not affect ($P > 0.05$) the number of OPVL sperm obtained or fertility status of eggs given the fact that sperm concentration was adjusted for chilled semen. Fertilised eggs had a mean (± SE) number of 11.01 ± 2.27 OPVL sperm/mm^2 compared to 0.92 ± 0.39 OPVL sperm/mm^2 for eggs not fertilised. Females produced fertilised eggs for up to 11 days after the last insemination. Variation between females was evident for OPVL sperm number and fertilisation status, with some females consistently producing eggs with OPVL numbers between 13.08 ± 4.56 and 7.00 ± 3.45 sperm/mm^2 of which 75 to 100% were fertilized while egg fertility was compromised in other females.

Discussion: The relationship between fertilisation status and trapped OPVL sperm is consistent with other avian studies. Low OPVL numbers are generally associated with low sperm numbers reaching the semen storage tubules of the female tract after an insemination. *In vivo* evaluation was complicated because of variation among females for OPVL numbers and fertilisation status.

Conclusion and Recommendations: Insemination of females with either fresh or stored semen resulted in comparable fertility figures, showing that ostrich semen can be stored in a synthetic diluent for at least 24 hours. Extension of semen storage time and between-female variation in sperm acceptance and fertile period duration need to be addressed in future studies.

CRYOPRESERVATION OF OSTRICH SPERM BY FAST FREEZING AND FIRST RESULTS FROM ARTIFICIAL INSEMINATION WITH FROZEN-THAWED SEMEN

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Background: Assisted reproduction technology (ART) can be a valuable tool for the ostrich industry as avian influenza represents a risk to valuable genetic resources. It may also help to speed up genetic improvement and increase fertilization rates in high egg production months (July/August) when males produce semen of low quality. Deriving an optimal freezing rate is thus fundamental to a successful semen preservation protocol for ART in ostriches.

Aim: To develop a protocol for long-term storage of ostrich semen for the purpose of artificial insemination.

Methodologies: Ejaculates of four South African Black males with good quality ejaculates were collected using the dummy female method and diluted 1:1 at ~24°C with a synthetic diluent analogous to ostrich seminal plasma. Semen was cooled at 1°C/minute to 5°C, then diluted 1:1 in a pre-cooled dimethylacetamide (DMA) solution as cryoprotectant to a final concentration of 16%. Following equilibration for 15 minutes at 5°C, 250 µl straws were loaded, sealed and frozen. Different freezing rates of 1°C/minute and 10°C/minute to temperatures of -19, -22, -28, -30, -60 and -80°C, before liquid nitrogen immersion, were investigated. Straws were thawed 24 hours later in a pre-cooled water bath set at 5°C for 12 seconds. After evaluation of sperm motility with Sperm Class Analyzer® software semen from the protocol yielding the highest quality sperm was pooled according to sperm numbers to include equal numbers of each male and used for artificial insemination. Inseminations were carried out for three consecutive days with a follow-up insemination every 6th day for 3 cycles. The frozen-thawed semen doses contained a higher dose of 1000 x 10^6 total sperm compared to the fresh semen dose 800 x 10^6 sperm, and 5 females were inseminated in each treatment. Fertility was determined by counting trapped sperm in the outer perivitelline layer (OPVL sperm/mm^2) and by determining the fertility status of the germinal disc.

Results: The freezing rate of 10°C/minute to an end temperature of -30°C prior to liquid nitrogen immersion was best to preserve sperm. After thawing, sperm motility was reduced by approximately 29% compared to fresh samples. Following artificial insemination with fresh or cryopreserved semen the number of OPVL sperm and fertilisation status of eggs did not differ between treatments ($P > 0.05$). Females produced fertilised eggs for up to nine days after the last insemination. The OPVL sperm number was the only fixed effect contributing ($P < 0.001$) to the variation in fertility status. Fertilised eggs from fresh and cryopreserved semen had a mean ± standard error (geometric mean) of 2.28 ± 0.141 (91.7) OPVL sperm/mm^2, compared to 2.01 ± 0.004 (2.9) OPVL sperm/mm^2 for unfertilised eggs.

Discussion: The freezing rate of 10°C/min to -30°C, appeared to cause less damage to sperm than slower rates or freezing to lower end temperatures. This treatment probably resulted in less chilling damage than other treatments, suggesting that the transition temperature was nearly optimal.

Conclusion and Recommendations: Ostrich semen can be preserved successfully for indefinite storage at -196°C in liquid nitrogen. Further studies are necessary to investigate ways to further improve the present protocol.

INTERACTIONS BETWEEN SPERM AND SEMINAL PLASMA INFLUENCE THE SEMEN QUALITY OF MALE OSTRICHES

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Background: Fertilisation success is likely to be influenced by interactions between sperm and seminal plasma, particularly when competition over mates and fertilization occurs. However, limited research has been undertaken to understand whether these interactions could explain the poor fertility rates commonly observed in ostrich flocks.

Aim: To assess if in vitro measures of sperm motility and viability are influenced by the interactions between sperm and seminal plasma of ostrich males.

Methodologies: Sperm motility of 13 ostriches was investigated after reconstituting semen with: seminal plasma of the same male (SPS) and seminal plasma of another male (SPD). Semen was collected from 27 pair combinations of males using the dummy female method. Each male was replicated across 3 to 4 different males and each pair replicated once. Semen was centrifuged for 1 minute at 10 000 rpm and seminal plasma removed. Sperm was then re-suspended in SPS or SPD to a concentration of 10 - 15 million sperm/mL. After incubation for 15 min at room temperature, sperm motility was video recorded and the average path velocity (VAP) of each sample was estimated using a Sperm Class Analyzer. Sperm viability was measured using the nigrosine-eosin staining protocol to calculate the proportion of live normal (LN), live abnormal (LA) and dead sperm (D). Generalized Linear Mixed Models were used to evaluate the effect of seminal plasma on sperm motility and viability. Ethical clearance was granted by the Western Cape Department of Agriculture (R09/24).

Results: No difference was observed in sperm velocity between SPS and SPD ($P > 0.05$). Re-suspension of sperm had a negative effect on the proportion of LN sperm ($P < 0.05$) relative to neat samples, but no difference was observed between SPS and SPD for the proportions of LN, LA or D sperm ($P > 0.05$). In SPS, ejaculate volume and concentration significantly affected VAP median: sperm from larger ejaculates swam faster ($P < 0.05$), whereas highly concentrated ejaculates showed a lower velocity ($P = 0.021$). In SPD, sperm velocity was positively correlated to the velocity of the sample from which the seminal plasma originated ($P < 0.001$). The ejaculate volume from which the seminal plasma originated also had a positive effect on VAP median ($P < 0.05$), where the velocity of sperm mixed with seminal plasma from larger ejaculates was increased. Preliminary analysis also showed that there was no difference in effect of SPD and SPS on sperm viability ($P > 0.05$). However, sperm velocity was positively related to the proportion of LN sperm in the same ejaculate, and the ejaculate from which the SPD was taken ($P < 0.001$). Finally, significant inter-male variation was observed in the response of their sperm to SPS and SPD ($P < 0.05$).

Discussion: These results show that sperm mixed with seminal plasma from an ejaculate with a higher velocity had a higher velocity than when they were mixed in SPS. This outcome suggests that fertilization ability of ostriches may be influenced not only by the size and quality of ejaculates, but also by the composition of seminal plasma added.

Conclusion and recommendation: Further investigations are needed to evaluate whether males can adjust the sperm and seminal fluid in their ejaculates and the influence thereof on fertilization success.

THE EFFECT OF FLOCK STRUCTURE ON THE REPRODUCTIVE OUTPUT OF OSTRICHES *Struthio camelus domesticus*

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Background: The ratio of males to females in commercial ostrich group-mating systems is historically very narrow at approximately 6:10. We failed to find any scientific study that justifies the use of this ratio in terms of production efficiency. The inherently high feeding costs in the industry could be reduced markedly if fewer males per female could be maintained. The ostrich industry has also been plagued by inefficient egg production, high embryo mortality, low chick survival and industry-wide poor responses to selective breeding. This is despite the fact that all traits of economic importance exhibit significant levels of genetic variation, which should facilitate responses to artificial selection. An important barrier to solving these problems has been a lack of information on how the reproductive output of individuals changes across different flock structures, partly because it has been difficult to established parentage in flocks.

Aim: To examine how reproductive output of individuals changes across different sizes of social groups with different levels of relatedness between males, and between females.

Methodologies: The resource flock of SA Black ostriches (SAB) at Oudtshoorn Research Farm was used. Group composition was manipulated to create eight main treatments (6 to 9 replicates of each treatment over 3 breeding seasons): (1) pairs; (2) one male, three females; (3) one male, four females; (4) two males, two females; (5) two males, three females; (6) two males, four females; (7) three males, three females; and (8) three males, four females. The mating performance of individuals was monitored through behavioural observations (c. 20 x 3 hour observations per group). The fertilisation success of individuals was measured by taking blood samples from chicks and adults, as well as from the embryos of unhatched dead-in-shell eggs. Microsatellite analyses were used to assign paternity and maternity in groups containing multiple males and females. Although infertile eggs were recorded, they could not be assigned to specific females as it is very difficult to gain sufficient DNA for maternity analysis. The number of day-old chicks hatched from specific mating combinations was defined as chick production.

Results: Mating behaviour (number of copulations, displays by males and displays by females) was significantly affected by flock structure. Specifically, mating displays and copulation rates per individual increased with the number males in the group, but not with the number of females. We also found that males had more successful copulations when they were in groups with a brother as opposed to unrelated competitors, particularly when there were more than three males in a group. We found no differences in the average total number of live and dead-in-shell chicks produced per female as the number of males and number of females increased in groups. However, we found that, as the number of males and the number of females increased, infertility rates decreased leading to higher chick production. This outcome suggests that having more males in a flock, where females can choose their mates, acts as insurance against infertility and low chick production.

Discussion: The results from this study suggest that substantial improvements to reproductive output can be made by simply manipulating the number of males and females in groups. Previous work has examined the reproductive output of pairs, trios and quads, but this is the first study, to our knowledge, to systematically quantify the reproduction of individuals in groups across a range of flock structures.

Conclusion and recommendations: Groups containing 1 male and 3 to 4 females had the highest chick production per breeding bird maintained, making this the most efficient flock structure of the groups we tested. Furthermore, by using genetic parentage, 'free-loading' individuals can be identified and removed, reducing production costs and further improving future efficiency. Further studies on even wider male: female ratios seem validated by these results.

UPDATED GENETIC AND CROSSBREEDING PARAMETERS FOR OSTRICH REPRODUCTION TRAITS

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Background: Structured breeding of ostriches is constrained by the lack of systems to record parentage and production performance in colonies. Previous analyses suggested genetic variation in key reproduction traits, while responses to selection for egg and chick production have also been demonstrated. Updates to these existing genetic parameters have not been done for about a decade, thus necessitating the present paper.

Aim: To estimate genetic and crossbreeding parameters for ostrich reproduction traits.

Methodologies: The pair bred ostrich resource flock at Oudtshoorn, consisting of SA Black (SAB), Zimbabwean Blue (ZB) and Kenyan Red (KR) breeders and their crosses were used. Approximately 3 500 repeated hen-year records of ostrich females for the interval between the first and last eggs of the season, the interval between the commencement of mating and the production of the first egg, the number of clutches (NCL), total egg production (TEP), total chick production (TCP), mean egg weight (MEW) and mean chick weight (MCW) were recorded. The data were analysed to estimate genetic parameters for the traits mentioned. A subset of the data was used to get an indication of heterosis for the SAB and ZB and their reciprocal cross. Ethical clearance for the project was obtained from the Western Cape Department of Agriculture (clearance number R12/48).

Results: All reproduction traits were heritable with estimates ranging from 0.05 for NCL to 0.16 for TEP. Heritability estimates for qualitative offspring traits (MEW and MCW) were higher at > 0.50. It should be noted than the quantitative reproduction traits exhibited high coefficients of variation (CV's) exceeding 50%, while MEW and MCW had CV's in the 10 – 15% range. Animal permanent environmental and service sire effects also affected most of the reproduction traits, estimates ranging from 0.10 - 0.25 and 0.04 - 0.12, respectively. No substantial unfavourable genetic correlations were found among traits. However, the direction of the genetic correlations of MEW and MCW with TEP and TCP were consistently unfavourable. With respect to heterosis we found that the reproductive performance of crossbred females largely resembled that of SAB females instead of ZB females. However, when the performance of crossbred females was compared to the mid-parent value of the pure breeds there was no significant estimate of heterosis for any of the traits.

Discussion: The present study confirms earlier findings that female reproduction traits in ostriches are variable and heritable. It should thus be feasible to improve these traits by directional selection, using conventional means. The most striking discrepancy between the present analyses and previous research in the same resource flock is that there were previously no indications of potential unfavourable genetic correlations of egg and chick production with egg and chick weight, as previous estimates were variable in sign with absolute values mostly below 0.10. Potential unfavourable genetic correlations between these traits may not be entirely unexpected, as these correlations are typically unfavourable in domestic poultry.

Conclusions and recommendations: The results suggest that industry-wide improvement of ostrich reproduction traits would be feasible if an affordable service for the assignment of progeny to specific parents would become available. Further research should strive to establish a system to make this feasible. Further studies on larger databases are likely to provide unbiased estimates for heterosis in ostrich reproduction traits.

(CO)VARIANCE RATIOS FOR EGGSHELL TRAITS IN OSTRICHES MODELED AS A TRAIT OF THE FEMALE

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Background: At present, there is little known about either genetic parameters or responses to selection for specific incubation traits in ostriches. Egg and chick weights are reported to have a significant genetic component, while eggshell traits directly affect both egg and chick weight through their effect on water loss and gas exchange.

Aim: To estimate covariance ratios for eggshell traits in ostriches, modeled as a trait of the ostrich female.

Methodologies: Eggshells for this study were collected during the 2005 - 2008 breeding seasons from the commercial, pair-bred ostrich breeding flock at the Oudtshoorn Research Farm of the Western Cape Department of Agriculture in South Africa. Data of 14 146 eggs, including infertile eggs, hatched eggs and dead-in-shell eggs were condensed to 700 hen-year records by deriving means for specific male-female combinations. Traits averaged for hen-male-year combinations were pore count (PC), average pore diameter (APD), total pore area of all the pore clusters in a given area (TPA), shell thickness (ST) and permeability (pore area/shell thickness - PERM), as well as egg weight (EWT), water loss up to 21 days of incubation (WL21) and water loss up to 35 days of incubation (WL35). (Co)variances were derived from ASREML analyses and ethical clearance was obtained from the Western Cape Department of Agriculture (clearance number R12/48).

Results: Heritability estimates for eggshell traits of females were high and ranged from 0.63 for pore count to 0.72 for shell thickness. The heritability estimates for EWT, WL21 and WL35 days of incubation was also high at 0.69, 0.66 and 0.40, respectively. On a genetic level, the correlations of PC with TPA and PERM, 0.58 ± 0.06 and 0.60 ± 0.06 respectively, indicated that an increased PC will lead to a larger pore area with an increased permeability. The genetic correlation (r_g) of APD was positive with all traits. TPA was highly correlated with PERM (0.97 ± 0.01) suggesting a very close relationship between these traits. The negative genetic correlations of shell thickness with both PERM and water loss indicates that an increased permeability occurred in eggs with thinner shells, which resulted in higher water loss. This was also confirmed by the high r_g of PERM with WL21 and WL35 (0.56 and 0.57 respectively). Water loss was not genetically related to egg weight but negatively related to chick weight. The phenotypic correlations for the egg traits were mostly similar in sign to r_g, but in most cases they were somewhat smaller in magnitude.

Discussion: Parameters indicate that it should be possible to select for the measured eggshell traits in ostrich eggs, or for permeability and water loss. However, as traits with likely intermediate optima, direct selection for permeability and other eggshell traits would not be straightforward and require further study.

Conclusions and recommendations: The possible application of these results to improve hatchability of ostrich eggs needs consideration. In all probability efforts will need to be directed at reducing the variation in traits like permeability and water loss to an optimal mean value. Such a strategy may possibly enhance embryonic survival.

BREED AND CROSSBREEDING EFFECTS ON OSTRICH SURVIVAL, SLAUGHTER AND PRODUCTION TRAITS

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Background: Three strains of ostriches are used in the commercial industry, namely the South African Black (SAB), Zimbabwean Blue (ZB) and Kenyan Red (KR). SAB ostriches are most widely used, but are sometimes crossbred with either ZB or KR ostriches in an effort to increase slaughter weight. However, the various strains have not been properly evaluated for production and slaughter traits.

Aim: To compare the three commercially available ostrich breeds and their crosses for survival, production and slaughter traits.

Methodologies: Data from chicks from the Oudtshoorn Research Farm reared and slaughtered from 2008 to 2015 were used to compare mortality, growth and slaughter traits for the various breeds and their crossbreds. Binary chick survival data up to 30 days, 90 days and 365 days of age, respectively, were analysed. The chicks were weighed at day-old and again at approximately 84, 148, 226, 300 and 365 days of age. Age at weighing was included as a linear covariate when analysing the different weight traits due to differences in the exact age at weighing across years. Ostriches were slaughtered at between 275 and 492 days of age and the skins were evaluated for various skin traits during the chrome-crusted stage. Slaughter traits analysed included slaughter weight (with slaughter age as covariate), crust skin size, carcass weight, skin thickness, as well as nodule size and nodule shape, with both slaughter age and slaughter weight as linear covariates. Data for the three pure breeds were analysed as an unbalanced design using GenStat regression, with genotype and slaughter group as fixed effects. Slaughter weight was added as covariate for all slaughter traits. The linear contrast of mean crossbred performance with the mean of the two parental breeds, also known as the mid-parent value, was computed to provide an indication of the significance of heterotic effects for SAB and ZB ostriches and their reciprocal cross, as well as SAB and KR ostriches and their reciprocal cross, respectively. Ethical clearance for the project was obtained from the Western Cape Department of Agriculture (clearance number R12/48).

Results and discussion: The respective genotypes did not follow the same growth pattern. The contrast depicting direct heterosis was not significant for all weight traits in the SAB x ZB combinations, while it was significant for all weight traits in the SAB x KR combinations. Average (±SE) mortalities to 30 days were 0.214 ± 0.007 for SAB, 0.236 ± 0.021 for ZB and 0.260 ± 0.028 for KR ostriches, while mortalities to 365 days were 0.497 ± 0.008, 0.580 ± 0.025 and 0.616 ± 0.031, respectively. Significant heterosis was evident for the SAB x KR combinations for mortality at 30, 90 and 365 days of age. Most of the slaughter traits were either similar or better in the ZB and KR ostrich breeds compared to SAB ostriches. The only slaughter traits showing heterosis were slaughter weight and carcass weight, for both the combinations of the SAB with ZB and KR strains.

Conclusion and recommendation: Making use of the ZB and KR breeds does not seem to be detrimental with regard to slaughter bird production, but survival to slaughter of these pure breeds seem to be lower compared to SAB ostriches. Further studies on the optimal usage of these genetic resources are required.

REVIEW: ADVANCES IN RESEARCH ON THE HATCHABILITY OF ARTIFICIALLY INCUBATED OSTRICH EGGS

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Background: Artificial incubation has become an essential part of the commercial ostrich enterprise. Despite substantial advances in incubator design and incubation techniques, problems with embryonic mortality during artificial incubation still constrain the development of the ostrich industry world-wide. Shell-deaths contribute to a large extent to low hatching rates obtained in the ostrich industry.

Aim: To review recent advances in embryonic development of ostrich chicks as well as managerial and genetic impacts on the hatchability of ostrich eggs.

Methodologies: Papers reporting embryonic development of ostriches from the commencement of incubation to 7 days as well as from 7 days to hatching were reviewed. Initially, assessment was based on assessment on a stereo microscope using image analysis from 0 to 7 days of incubation. Macroscopic evaluation and physical measurements were used subsequently. The levels of early and late embryonic mortalities were quantified and subsequently related to genetic and environmental effects in further papers that were reviewed.

Results: Ostrich embryos exhibited marked growth from 0 to 7 days of incubation. This growth continued subsequently but became differential for embryo and limb length when compared to eye size. A calibration curve was developed to estimate the age of embryonic death based on body measurements. Near-term embryos that were grossly mispresented were very unlikely to hatch. Eggs from older females, exceeding 8 to 10 years of age, were more likely to sustain embryonic mortalities than those of younger females. Eggs not stored prior to setting and those subjected to prolonged storage exceeding 8 days were also more likely to suffer embryonic mortalities. Genetic analyses indicated that embryonic mortalities as a trait of the egg were heritable, while all traits potentially contributing to embryonic mortalities were under various levels of genetic control. Selection for chick production and live weight in replacements resulted in lower levels of embryonic mortality. Genetic correlations of other measureable traits related to incubation with embryonic mortalities were generally weak, and it seems as if indirect selection for correlated traits is unlikely to promote embryo survival.

Conclusions and recommendations: There are numerous opportunities for curbing embryonic mortalities in ostrich chicks. Husbandry practices and knowledge of the stage of incubation when the death occurred should be integrated with genetic solutions to achieve this goal. At the practical level, females older than 8 - 10 years should be culled from the breeding flock. Eggs should be stored for at least 2 days but preferably not more than 7 days. It is foreseen that marked improvement may be achieved in this major source of hatching and reproductive failure in future.

FEED INTAKE OF MALE AND FEMALE BREEDING OSTRICHES

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Background: The South African ostrich (*Struthio camelus var. domesticus*) industry has developed for more than a century. The success and cost-effective production of ostriches starts with the breeder birds. However, the nutritional requirements of these birds are not well defined. During the breeding season male and female ostriches are maintained together. An understanding of the intake requirements of both males and females is needed to optimise the reproductive potential of this species.

Aim: To determine the feed intake of male and female ostriches during the breeding season.

Methodologies: A pelleted breeder diet was provided to male (3 to 9 years of age) and female (2 to 9 years of age) breeding ostriches over a 25-week period. The breeding season extended from 14 May to 23 December 2014. The birds were kept individually in adjacent pens to stimulate breeding behaviour, where they received a breeder diet containing 9.2 MJ metabolisable energy/kg feed, 14.0% protein, 0.6% lysine, 2.5% calcium and 0.6% phosphorus *ad libitum*. The weekly feed intake of 12 male and 21 female breeding birds, maintained in separate paddocks for usage during the development of an ostrich artificial insemination protocol, was recorded over this period. Birds were weighed at the beginning and end of the study period. Ethical clearance for the project was obtained from the Western Cape Department of Agriculture (clearance number R11/40).

Results: The average initial live weights of the male and female breeder birds were 132 ± 6 kg and 124 ± 4 kg, respectively ($P = 0.22$). No interaction ($P > 0.05$) was found between stage of the breeding cycle and gender for the following traits: live weight ($P = 0.71$), feed intake ($P = 0.51$) and feed intake as a percentage of live weight ($P = 0.36$). No differences were observed for the live weight of male and female ostriches at the commencement or cessation of breeding ($P = 0.69$). However, differences were observed for live weight between males (134 ± 4 kg) and females (124 ± 3 kg) ($P = 0.04$). Differences in feed intake were observed for gender ($P = 0.04$), and stage of the breeding cycle ($P = 0.03$). The overall feed intake was 3 109 ± 179 and 2 627 ± 135 g/day for male and female breeder birds, respectively. At the commencement of breeding, feed intake was 3 123 ± 159 g/bird/day and at the end it was 2 614 ± 159 g/bird/day. No differences for feed intake as percentage of live weight at 2.4 ± 0.2% for males and 2.1 ± 0.1% for females were observed for gender ($P = 0.28$), while differences ($P = 0.03$) were observed between the start (2.5 ± 0.1%) and end stages (2.0 ± 0.1%) of the breeding cycle. No interaction was observed for feed intake between gender and production week ($P = 0.13$). However, gender and week both had an effect on feed intake ($P < 0.0001$).

Discussion: The study showed that male breeding ostriches had a higher live weight and feed intake than females. However, the feed intake of males and females as percentage of live weight did not differ. Feed intake of the birds was higher at the beginning of their breeding cycle than at the end.

Conclusions and recommendations: In the present economic climate it is important to determine the feed intake of male and female breeder birds to minimise feed wastage and to compensate for the higher feed intake of male ostriches in commercial production systems.

PREDICTING SKIN SURFACE AREA OF OSTRICHES BASED ON AGE AND LIVE WEIGHT USING GROWTH MODELS

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Background: A model is currently being developed to determine the nutritional requirements of ostriches more accurately. To do this, it is important to know the level of heat loss the animal experiences at different stages in its life. By being able to predict the skin surface based on age and/or weight, the amount of heat loss can be predicted. Heat is lost through radiation and convection, the skin surface area affects the rate of heat loss. This can be used to better formulate diets for specific groups of animals based on weight or age.

Aim: To derive the skin surface area of ostriches from their age and/or weight.

Methodologies: Data was collected from 342 South African Black ostriches (from 1 day to 500 days of age and 0.79kg to 147kg live weight) from three experiments respectively conducted at the Kromme Rhee and Oudtshoorn Research farms. The ostriches were slaughtered at different weight and age intervals and the skin surface area of the wet skins was determined at each slaughter interval. Raw data was analyzed with a one-way ANOVA to evaluate the fit of various growth models. The models chosen after analysis were the Gompertz and power model. The study was granted ethical clearance by the Western Cape Department of Agriculture (number: R11/41; S13/93).

Results: The relation between age and skin surface area was best described by the Gompertz model with the following equation: $Y = 123.1*\exp(-\exp(-0.00862*(X-181.4)))$ ($R^2 = 0.94$, $P < 0.01$), where Y is the skin surface area and X is the age of the bird. To describe the relationship between live body weight and skin surface area, the linearized power curve was used, yielding the following equation: $Y = 0.701X + 1.6874$ ($R^2 = 0.98$, $P < 0.01$), where Y is Ln(skin surface area) and X is Ln(live weight) of the bird.

Discussion: The strong correlation between the actual skin surface area and the Gompertz model prediction ($r = 0.97$) indicated that the model accurately predicted skin surface area from age. A high R^2 - value shows that 93.9% of the variation in skin surface area could be accounted for by the age of the birds. By using the linearized power model for the relationship between live weight and skin surface area we can simply state that there is a positive linear correlation between Ln(live weight) and Ln(skin surface area) ($r = 0.99$), without using complicated equations. With this model 97.6% of the variation in skin surface could be accounted for by the live weight of the birds.

Conclusion/recommendations: By using these two models, the skin surface area of the ostrich could be predicted quite accurately at a certain age or body weight. Weight will be the better independent variable considering the higher R^2 - value of the linearized power model where body weight is used to predict skin surface area and the fact that measuring weight is easier than keeping track of age. These models can be used to predict skin surface area and ultimately heat loss capacity in ostriches for future studies.

COMPARISON OF SPERM MOTILITY PARAMETERS BETWEEN INDIVIDUAL AND POOLED EJACULATES IN THE OSTRICH

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Background: Sperm motility is a vital component of sperm quality as it measures the kinematic activity underlying the competing ability of sperm from each male in fertilising an egg. Pooled ejaculates are commonly used in experiments aimed at developing an Artificial Insemination (AI) protocol. However, sperm competition to fertilise eggs from pooled ejaculates might affect sperm motility, yet few studies have been undertaken to estimate its effect on overall motility of pooled samples.

Aim: To evaluate the effect of pooling ostrich ejaculates on sperm motility.

Methodologies: Twenty-two ejaculates were collected from 2 to 4 South African Black male ostriches (2 - 9 years of age) for a period of 3 weeks using the dummy female method. Ejaculates were collected 3 times per week and diluted 1:6 with an ostrich specific semen diluent and assessed for sperm motility using Sperm Class Analyzer software (SCA v.3, Microptic S.L., Spain). One or 2 billion sperm were then drawn and pooled with the same number of sperm from other males. Eight pooled ejaculate samples (1 constituted of 4 males, 3 constituted of 2 males, 4 constituted of 3 males) were then assessed for sperm motility and compared to sperm motility of individual ejaculates. A general linear mixed model was performed using SAS, version 9.3. This study was granted ethical clearance (Reference: R9/24) by the Western Cape Department of Agriculture.

Results: The percentage progressively motile (PMOT) sperm of pooled ejaculates and ejaculates from individual males (mean ± SE) amounted to 55.3 ± 4.4% and 58.9 ± 2.7% respectively ($P > 0.05$). The total sperm motility percentage (MOT) of the pooled ejaculates and individual males amounted to 72.3 ± 4.2% and 77.6 ± 2.5% respectively ($P > 0.05$). Similarly, pooled ostrich ejaculates did not differ from individual ejaculates for average path velocity (VAP: 67.9 ± 3.3 µm/s and 72.8 ± 2.0 µm/s, respectively), curvilinear velocity (VCL: 80.4 ± 3.2 µm/s and 86.6 ± 1.9 µm/s, respectively), straight line velocity (VSL: 55.3 ± 2.8 µm/s and 57.5 ± 1.7 µm/s, respectively), amplitude of lateral displacement (ALH: 2.6 ± 0.1 µm and 2.7 ± 0.1 µm, respectively), linearity (LIN: 69.1 ± 2.6% and 66.5 ± 1.6%, respectively), threshold straightness (STR: 82.2 ± 2.5% and 79.1 ± 1.5%, respectively) and wobble (WOB: 84.2 ± 1.6% and 83.9 ± 0.9%, respectively). Furthermore, no difference ($P > 0.05$) was observed in the overall sperm motility of pooled ejaculates containing either 1 or 2 billion sperm.

Discussion: The results of this study reveal that pooling of diluted ejaculates from different male ostriches does not affect overall sperm motility. Pooled ejaculates can therefore be used in developing a protocol for AI in ostriches without compromising the motility of sperm. However, single male ejaculate should still be preferred once a viable AI protocol is in place for breeding to facilitate the recording of pedigree information, which is a constraint to the industry at present.

Conclusion/recommendations: Further studies are needed to evaluate the effect of pooling ejaculates on the morphology, viability and *in vivo* fertilising ability of sperm.

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