

TABLE OF CONTENTS

EFFECT OF PROTEIN INTAKE ON KIDNEY FUNCTION IN ADULT FEMALE OSTRICHES (*STRUTHIO CAMELUS*)

Darin C. Bennett and Yutaka Karasawa vii

FILOPLUMES AND PINHOLES IN OSTRICH HIDES

D. Black and P. C. Glatz viii

VACCINE STRATEGIES AGAINST OSTRICH MYCOPLASMAS

A. Botes, B. Pretorius, A. J. Olivier, W. P. Burger and D. U. Bellstedt ix

PRELIMINARY RESULTS ON THE EFFECT OF GENOTYPE ON EMBRYONIC POSITION IN DEAD-IN-SHELL OSTRICH EGGS

Z. Brand, S. W. P. Cloete, I. A. Malecki and C. R. Brown x

GEOMETRIC LIMB SIMILARITY BETWEEN TWO FLIGHTLESS BIRDS: AN EXTINCT TERROR BIRD (PHORUSRHACINAE GEN.) V. THE OSTRICH (*STRUTHIO CAMELUS*)

R. G. Cooper and A. Tennett xi

THE EFFECT OF DIETARY PROBIOTIC SUPPLEMENTATION ON PRODUCTION PERFORMANCE OF OSTRICH CHICKS

F. Foroudi, A. Afsar and M. E. Kolli xii–xiii

INFLUENCE OF DIFFERENT PROCESSING METHODS ON NUTRITIONAL ASPECTS OF OSTRICH (*STRUTHIO CAMELUS*) MEAT

R. G. Garcia, E. A. Silva, A. C. Orrico, A. A. Mendes, A. B. Neto and I. C. L. A. Paz
xiv

A METAPOPOPULATION MODELLING APPROACH FOR WILD GREATER RHEAS IN CENTRAL ARGENTINA

P. F. Giordano, J. L. Navarro and M. B. Martella xv

PHYSIOLOGICAL STRESS RESPONSE IN CAPTIVE GREATER RHEAS (*RHEA AMERICANA*)

A. Lèche, J. M. Busso, C. Hansen, J. L. Navarro, R. H. Marín and M. B. Martella
xvi

NEST POPULATION DYNAMICS IN COLONY BREEDING OSTRICHES

J. M. Ledger and I. A. Malecki xvii

EMU OIL: A POTENTIAL TREATMENT FOR CHEMOTHERAPY INDUCED MUCOSITIS?

R. J. Lindsay, M. S. Geier, R. Yazbeck, K. A. Lymn, R. N. Butler and G. S. Howarth
xviii–xix

ARTIFICIAL INSEMINATION OF FEMALE OSTRICHES USING VOLUNTARY CROUCH

I. A. Malecki and P. K. Rybnik xx

CROUCHING BEHAVIOUR AND OVIPOSITION RATE IN FEMALE
OSTRICHES REARED WITHOUT MALES

I. A. Malecki, P. K. Rybnik, E. Lukaszewicz and J. O. Horbanczuk xxi

BONE AND EGG QUALITY OF BREEDER OSTRICHES FED A MAINTENANCE
DIET (LOW CALCIUM) AND A LAYER DIET (HIGH CALCIUM)

*I. C. L. Almeida Paz, A. A. Mendes, A. Balog, C. M. Komiyama, I. C. L. Almeida and E.
L. Milbradt* xxii

THE FORMULATION OF A NEAR-NATURAL DIET FOR CAPTIVE KIWI

C. J. Minson, M. A. Potter, D. V. Thomas and R. G. Lentle xxiii

PRESENT STATUS OF EMU FARMING IN INDIA

D. Narahari, N. Ramamurthy, R. Kumararaj and P. T. Gnanaraj xxiv

SEMEN CHARACTERISTICS OF 2- AND 3-YEAR-OLD MALE OSTRICHES

P. K. Rybnik, I. A. Malecki, J. O. Horbanczuk and E. Lukaszewicz xxv

CHARACTERISTICS OF OSTRICH EJACULATE IN THE SECOND HALF OF
THE BREEDING SEASON

P. K. Rybnik, I. A. Malecki, J. O. Horbanczuk and E. Lukaszewicz xxvi

HAEMATOLOGY AND SERUM PROFILE IN GROWING OSTRICHES

S. T. Selvan, T. Sivakumar, P. Vasan, Deepan Kishore and R. Prabakaran xxvii

PRODUCTION PERFORMANCE OF OSTRICHES IN INDIA

S. T. Selvan, T. Sivakumar, P. Veeramani and R. Prabakaran xxviii

REPRODUCTIVE AND HATCHING PERFORMANCE OF OSTRICHES IN INDIA

S. T. Selvan, T. Sivakumar, S. Nagarajan and R. Prabakaran xxix

EFFECT OF PROTEIN INTAKE ON KIDNEY FUNCTION IN ADULT FEMALE OSTRICHES (*STRUTHIO CAMELUS*)

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Ostriches are the largest living birds and are endemic to hot arid and semi-arid climates. Their kidneys are relatively large and can produce concentrated urine, but no detailed renal studies have been conducted. In mammals, a high protein diet increases both glomerular filtration rate (GFR) and urine flow rate (UFR). Three adult female ostriches (mean body mass 108 kg) were fed four pelleted diets containing 1.2 to 2.4 % N (7.5-15 % CP). Each trial consisted of 10-day adaptation, followed by a 6-day period during which all excreta was collected. Creatinine concentration of the plasma ($[Cr]_{pl}$), urine ($[Cr]_u$) and excreta were measured. GFR was calculated as the total daily excreted creatinine divided by $[Cr]_{pl}$. UFR was calculated as the total daily excreta creatinine output divided by the $[Cr]_u$.

Increasing dietary CP increased the GFR, but had no affect on UFR (Fig. 1). In contrast Goldstein et al. (2001) found that increased protein intake in sparrows increased UFR, but not GFR. GFR and UFR of ostriches in this study averaged 47.2 ± 5.5 and 2.46 ± 0.26 mL/min, respectively. This GFR value is lower than that predicted allometrically for a 100 kg bird, but similar to that predicted for avian species from arid habitats (Bennett and Hughes 2003).

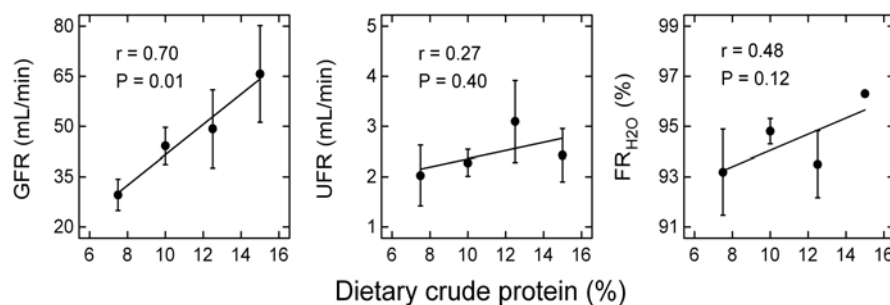


Fig. 1. The effect of dietary crude protein on glomerular filtration rate (GFR), urine flow rate (UFR) and fractional reabsorption of water (FR_{H2O}) of adult female ostriches.

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FILOPLUMES AND PINHOLES IN OSTRICH HIDES

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Filoplumes are hair-like feathers (slender rachis arising from the edge of its superior umbilicus) that are considered to be normal in most orders of birds. One common anatomy text states “filoplumes are absent in ostriches, emus and cassowaries and are said to be absent in pelicans and aningas” (Lucas and Stettenheim, 1972). However feathers that fit a “filoplume” classification do occur in ostriches and mainly comprise 6-8 filoplumes in an arc at the caudal aspect of each main follicle in the crown of the hide. Like filoplumes, some feathers observed on ostriches in Australia could be classified as bristle hairs. These have not only been found around the eyes and ears of ostriches, but also on the feathered areas of the body. Where a significant incidence of these filoplumes and bristle hairs occurs in ostriches it becomes a serious economic issue as they cause “pinhole” defects in tanned ostrich hides. True “pinhole” defects in ostriches have been confused with other similar, less uniform defects in ostrich hides (Cooper, 2001).

The incidence of filoplumes and reported defects due to pinholes varies between flocks and countries. Estimates of the incidence of filoplumes in ostriches in South Africa are approximately 3-5% of processed birds (B. Rayner, pers comm). The incidence of filoplumes in ostriches in Australia in the 1990's was much higher and was as high as 50% in predominantly Australian bird flocks or less than 5% in flocks derived from predominantly non-Australian bloodlines. Selection practices identifying and culling breeding birds with filoplume characteristics have reduced the incidence of this defect in ostriches in Australia and, consequently, the contribution of pinhole defects to downgrading of Australian hides. The heritability of filoplumes has not been reported for ostriches but a significant reduction in pinhole affected hides after heavy culling of filoplume-affected breeder birds in the Australian population provides preliminary anecdotal support for a genetic relationship to exist. It is important to be careful in assessing filoplumes in ostriches less than 12 months of age, as some filoplumes are naturally lost as the bird matures (Black 1999). Further research is required to understand the possible genetic heritability of filoplumes in ostriches. New ostrich industries worldwide should be vigilant in detecting the emergence of filoplumes and their pinhole hide defects to avoid major economic consequences.

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VACCINE STRATEGIES AGAINST OSTRICH MYCOPLASMAS

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In South Africa, three ostrich specific mycoplasmas (*Ms01*, *Ms02* and *Ms03*) have been identified, which are primarily responsible for a complex disease syndrome in ostriches. Symptoms include respiratory infections such as sinusitis and rhinitis, which together with external factors contribute to a reduced growth rate and increased susceptibility to other bacterial and viral infections. The production of conventional vaccines as one of the strategies to control mycoplasma infections in ostriches has not been successful because ostrich mycoplasmas were found to be difficult to cultivate. We are therefore currently investigating alternative approaches. The first is to test the efficacy of poultry mycoplasma vaccines in giving protection against ostrich specific mycoplasmas. The second is the development of ostrich specific mycoplasma DNA vaccines.

Bacterin 1 and bacterin 2 vaccines, of poultry mycoplasma origin, were tested for the ability to produce an immune response in ostriches. A group of 88 six-week old ostriches was divided into three groups. Group 1 (28 birds) received a single vaccination (1 ml) of bacterin 1, group 2 (28 birds) received a single vaccination (1ml) of bacterin 2 and group 3 served as control and was not vaccinated. Blood was drawn on days 0, 7, 14 and 21 and all sera tested for both ostrich anti-bacterin 1 and anti-bacterin 2 antibodies. Both group 1 and 2 elicited immune responses compared to the control group. However, group 1 reached a maximum titre at day 21, with only 10% of the birds having a titre value above 0.2. In group 2, on the other hand, 93% of the birds had a titre value above 0.2 by day 21. Whether or not these immune responses will result in protection against ostrich mycoplasma infections will be determined in upcoming challenge trials.

The first step in DNA vaccine development is the identification of suitable candidate genes. The pathogenicity of mycoplasmas is dependant on their ability to attach to the host cell and therefore attachment protein genes were targeted as vaccine candidates. *Ms01* DNA was isolated from mycoplasma cultures and the whole genome (~700 kbp) sequenced using the Roche GS20 system. NCBI and CLCBio Blast searches for attachment protein gene analogues were therefore conducted on the contiguous sequences obtained from the whole genome sequencing of *Ms01*. This revealed that *Ms01* has a five-gene *opp* operon structure similar to that of *M. hominis*. Sequence alignments indicated significant sequence homology between the operons with the exception of the first gene, *P100*, which in *M. hominis* is responsible for host attachment. Assuming that the *P100* homologue of *Ms01* is also involved in attachment to the ostrich host, this gene may be an ideal candidate gene for the development of DNA vaccines for use in ostriches. No significant homology to attachment protein genes in other mycoplasmas could be found. The *P100* gene of *Ms01* will now be amplified and cloned into a suitable plasmid vaccine vector after which efficacy trials will be launched.

PRELIMINARY RESULTS ON THE EFFECT OF GENOTYPE ON EMBRYONIC POSITION IN DEAD-IN-SHELL OSTRICH EGGS

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The unexpectedly high level of embryonic deaths in the South African Black x Zimbabwean Blue combination is a cause of concern, especially since the best hatchability results in absolute terms were achieved in the reciprocal cross (Brand *et al.* 2007). Embryonic mortality as a result of genetic incompatibility can negatively influence hatchability. The experimental population used for the study (2006) was the commercial, pair-bred flock of both pure South African blacks and Zimbabwean blues ostriches and different combinations of these strains maintained at the Oudtshoorn Research Farm in the Klein Karoo region of South Africa. Dead-in-shell eggs, with embryos between days 35 and 42 of incubation, were opened to record the position of embryos. The normal position of the embryo before pipping was used as a reference to identify embryos in malposition (Deeming 1995). Chi square procedures were used to assess the effects of genotype (Van Ark 1990). No significant difference could be found between genotypes for the proportion of correct positions or embryos in malposition (Table 1). It appears that effects other than genotype influence the position of dead-in-shell embryos in the last week of incubation.

Table 1. Proportions of overall and classified dead-in-shell eggs displaying normal embryonic positions in relation to genotype

Genotype	N	Embryos in the normal position (%)	Embryos in normal position in respect to body part (%)		
			Head	Beak	Foot
Overall	769	41.6	45.3	41.6	39.6
Black ♂ x Black ♀	543	41.4	44.2	40.6	38.8
Blue ♂ x Blue ♀	18	33.3	38.9	38.9	33.3
Blue ♂ x Black ♀	111	36.9	42.7	38.2	36.0
Black ♂ x Blue ♀	40	55.0	65.0	60.0	52.5
Back crosses	57	45.6	48.2	45.6	47.4
Chi ²		4.84	7.30	6.75	5.26

Critical Chi² (P = 0.05) for 4 d.f. = 9.488.

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GEOMETRIC LIMB SIMILARITY BETWEEN TWO FLIGHTLESS BIRDS: AN
EXTINCT TERROR BIRD (PHORUSRHACINAE GEN.) V. THE OSTRICH
(*STRUTHIO CAMELUS*)

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An example of the extinct terror bird, *Phorusrhacinae gen.*, evolved in South America from the Middle Palaeocene to the Pliocene-Pleistocene eras. It was unable to fly and engaged in terrestrial locomotion. The ostrich evolved from the Early Miocene period onwards. The ostrich is mostly a forager and when necessary will run to escape danger, charge or perform territorial or courtship displays. The ostrich prefers grounded running at intermediate speeds and the selection thereof over walking results in the minimisation of the metabolic cost of locomotion. The aim was to propose a comparative model of limb strength supporting b.wt. during defence and predation in the terror bird and ostrich.

We calculated the pressures exerted in kg.cm⁻² of an ostrich (120 kg b.wt.) and terror bird (350 kg b.wt., *Titanis walleri*) (maximum b.wt. and age of 18 mth. assumed).

Using the formula: Force (N) = Pressure (kg.cm⁻²) x Area (m²), the area of impact of an ostrich kick was calculated. The point of impact was: Assumed area impact terror

bird (m²) = ostrich area impact (m²) x $\frac{TBb.wt.}{Ostrichb.wt.}$. Relative acceleration of kicks

from each bird was calculated using: Force (N) = mass (kg) x acceleration (m.s⁻²).

We proposed a model, thereafter, to predict the ecological existence of the terror bird

and ostrich: $\frac{TBb.wt.}{Ostrichb.wt.} = \frac{TBvolume}{Ostrichvolume} = k^3$. The arbitrary constant (k³)

defined the ratios of b.wt. of the terror bird and ostrich. The ratio of respective heights:

$\frac{TBHeight}{OstrichHeight} = k$, and the ground reaction force/b.wt. = $\frac{2,355}{Forceofkick}$.

We propose that the terror bird was both an efficient predator and a scavenger, and swifter during locomotion than the ostrich. We suggest that the correlations between the terror bird and the ostrich calculated in this study can be used to predict the limb morphology and locomotion paleo-biology of future skeletal discoveries, even if in part. The principles thereof can also be used to compare the locomotion characteristics of the living flightless birds (ostrich, emu, rhea, cassowary and kiwi) and recently extinct flightless ancestors (moa, dodo and elephant bird). The ostrich retains its browsing characteristics and speed of locomotion. Defensive characteristics are commonly displayed during protection of chicks and eggs, the force exerted by its kick being formidable.

THE EFFECT OF DIETARY PROBIOTIC SUPPLEMENTATION ON PRODUCTION PERFORMANCE OF OSTRICH CHICKS

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Poor growth performance of ostrich chicks in Iran prompted an investigation of the benefits of using probiotics; a microbial feed supplement that may benefit ostriches by improving intestinal microflora balance. Probiotics has been used to enable newly hatched chicks establish normal microflora (Fuller 1989). The supplement contains live lactic acid bacteria and other microbial products such as *Bacillus* and yeasts (Fox, 1988). Arslan and Saatci (2004) found a positive response on live weight, feed consumption and feed efficiency in quail supplemented (via feed and water) with *Lactobacillus bulgaricus*. However, *Saccharomyces cerevisiae* supplementation in the quail ration did not affect growth (Yalcin *et al.* 2000).

In this study 180 one-day old ostrich chicks were randomly allocated to 9 dietary treatments in a 3 x 3 factorial experiment with 4 replicates and 5 birds per replicate. One group served as the control, while the other groups were the treatment groups fed Yeast (*Saccharomyces cerevisiae*), Bioplus2-B[®] (containing *Bacillus subtilis* and *Bacillus licheniformis*) and combination of Yeast and Bioplus2-B[®]. Each experimental group was fed *ad libitum* for 9 weeks. All birds were kept in an enclosed house until 28 days of age and then moved to outdoor pens equipped with a shelter. Starter diet (0-28 days) contained corn (530 g/kg), alfalfa (50 g/kg), soybean meal (370 g/kg) and premix. This diet was formulated to contain 213 g/kg CP, 11.5 g/kg lysine, 4.2 g/kg methionine and 8.7 g/kg total sulfur amino acids. Grower diet (29-63 days) contained corn (510 g/kg), alfalfa (100 g/kg), soybean meal (340 g/kg) and premix. This diet was formulated to contain 205 g/kg CP, 11 g/kg lysine, 4g/kg methionine and 8 g/kg total sulfur amino acids. The nitrogen-corrected true metabolizable energy (TMEn) in the both diets was 11.3 MJ/kg.

Ostrich chicks supplemented with 0.04% Bioplus2-B[®] had higher live weight, lower feed consumption and better feed conversion than chicks on other levels and types of supplements the exception being 0.08%Bioplus-2B[®] (Table 1). This level and type of probiotic thus appears suitable for inclusion in ostrich chick diets.

Table 1. Mean liveweight (kg), total feed consumption (kg) and FCR (kg/kg BW) of 63-day-old ostrich chicks

Groups	Liveweight	Feed consumption	FCR
Control	5.21 ^c	13.97 ^{ab}	2.68 ^a
0.04% Bioplus-2B [®]	7.37 ^a	13.24 ^b	1.80 ^d
0.08% Bioplus-2B [®]	6.66 ^{ab}	15.24 ^{ab}	2.29 ^c
0.1% Yeast	6.10 ^{bc}	14.78 ^{ab}	2.42 ^{bc}
0.1% Yeast + 0.04% Bioplus-2B [®]	5.92 ^{bc}	15.49 ^a	2.62 ^{ab}
0.1% Yeast + 0.08% Bioplus-2B [®]	5.84 ^{bc}	15.63 ^a	2.67 ^{ab}
0.2% Yeast	6.27 ^{bc}	14.25 ^{ab}	2.28 ^c
0.2% Yeast + 0.04% Bioplus-2B [®]	6.25 ^{bc}	15.52 ^a	2.48 ^{abc}
0.2% Yeast + 0.08% Bioplus-2B [®]	5.91 ^{bc}	15.55 ^a	2.63 ^{ab}
s.e.m.	0.338	0.650	0.084

Means in columns with different superscripts differ significantly ($P<0.05$).

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INFLUENCE OF DIFFERENT PROCESSING METHODS ON NUTRITIONAL ASPECTS OF OSTRICH (*STRUTHIO CAMELUS*) MEAT

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This study aimed at evaluating processing losses (PL) due to cooking, roasting, or grilling compared to fresh meat. Estimates of tenderness (shear force - SF), measurements of water holding capacity (WHC), and an evaluation of processing methods on nutritional composition (NC) were made. The experiment was carried out at the Food Technology Laboratory of Dom Bosco Catholic University, Campo Grande-MS, Brazil. Four male African Black ostriches were slaughtered between 18 and 24 months of age. The muscle *iliofibularis* was removed for quality assessment. Cooked meat lost more weight (44.6%) compared to grilled (37.0%) or roasted (35.4%) meat. There was no influence ($P>0.05$) of processing method on SF except for when fresh meat was evaluated. The WHC averaged 27.9% and was not significantly influenced ($P>0.05$) by processing methods. Dry matter (DM) of fresh meat was 23.5%, which was not significantly different ($P>0.05$) from roasted (35.9%), cooked (34.6%), or grilled (34.4%) meat. There was no difference ($P>0.05$) in crude protein, ether extract, and mineral matter content for the different processing methods.

Table 1. Shear force (Kgf/cm²) of ostrich meat using different processing methods

Processing method	Tenderness (Kgf/cm ²)
Fresh meat	0.40 ^b
Roasted	2.96 ^a
Cooked	2.69 ^a
Grilled	2.46 ^a

Means followed by the same letter do not differ according to Tukey's test ($P>0.05$).

Table 2. Weight loss of ostrich meat with different processing methods

Processing method	Time (min)	Weight before (g)	Weight after (g)	Loss (%)
Roasted	28	200.66	133.00	35.4 ^b
Cooked	10	163.21	93.79	44.6 ^a
Grilled	20	168.29	105.88	37.0 ^b

Means in the last column followed by the same letter do not differ according to Tukey's test ($P>0.05$).

Table 3. Dry matter (%), crude protein (%), ether extract (%) and mineral matter (%) content for ostrich meat using different processing methods

Processing method	Dry matter	Crude protein	Ether extract	Mineral matter
Cooked	34.59 ^a	84.41 ^a	2.80 ^a	6.41 ^a
Roasted	35.90 ^a	81.34 ^a	2.63 ^a	6.41 ^a
Fresh meat	23.50 ^b	85.51 ^a	2.64 ^a	6.95 ^a
Grilled	34.42 ^a	81.60 ^a	2.67 ^a	6.89 ^a

Means followed by the same letters within rows do not differ according to Tukey's test ($P>0.05$).

It was concluded that different processing methods can be used for ostrich meat, with no negative impacts on its NC, WHC or tenderness.

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A METAPOPOPULATION MODELLING APPROACH FOR WILD GREATER RHEAS IN CENTRAL ARGENTINA

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In Argentina, the progressive conversion of native grasslands into croplands has lead Greater Rheas (*Rhea americana*) to exist in a smaller number of relatively isolated populations with occasional exchange of individuals. A stochastic non-structured and spatially explicit metapopulation model was developed for a 4000-km² agro-ecosystem of central Argentina. The population parameters included in the model were: 1) carrying capacity for each suitable habitat; 2) average and maximum dispersion distance of the species; and 3) finite rate of population increase. Thirty-six simulations of the model were run with 1000 replicates each lasting 100 years. The main scenario consisted of a variable habitat loss, and different levels of correlation between population dynamics. Models with and without dispersion were employed, assuming logistic growth with a “scramble” density-dependence. Three scenarios were tested: a) equal loss (1 bird/year) in all habitats; b) higher loss in of the largest habitat (2 birds/year) compared to the rest (1 bird/year); and c) considerable loss on the largest habitat (8 birds/year), intermediate loss in medium-sized habitats (bird/year), and lower loss (1 bird/year) in small habitats.

In the first scenario, a 40% decrease in the abundance of the metapopulation was observed; in the second, the abundance decreased 47%; while extinction occurred at approximately year 83 in the third, before the simulation period was completed. Models without dispersion but with different levels of correlation among populations showed an equivalent decrease in population abundance (21-28%). However, the highest risk of extinction was observed when the populations had high synchronisation in their dynamics. The models with dispersion and different levels of correlation showed a higher decrease in the metapopulation abundance (84-86%). Therefore, the risk of extinction of these populations increases as the habitat loss and dispersion rate augment and decreases when correlation among populations and dispersal rate are lower.

PHYSIOLOGICAL STRESS RESPONSE IN CAPTIVE GREATER RHEAS (*RHEA AMERICANA*)

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An important constraint to farming Greater rheas is the high mortality rate of chicks. Stress is thought to contribute to the development of diseases and disorders, resulting in reduced fitness and survival of rhea chicks, but there is no information about stress biology of the species. One indicator of physiological stress is increased secretion of glucocorticoids, a steroid that helps cope with life threatening situations. However, repeated exposure to stress could result in a long-term increases in blood concentrations of glucocorticoid and this can lead to reduced fitness.

We studied the effects of a challenge with adrenocorticotrophic hormone (ACTH) to test adrenal function and characterize corticosterone concentrations in captive Greater rheas. Six 10-month-old birds (three sub-adults of each sex) reared in captivity were injected intravenously with ACTH (5 IU/Kg) and blood was sampled at 0 min (baseline level, before injection), at 15, 30 and 60 min, and at 24 and 48 h after injection. Plasma corticosterone was measured with an ¹²⁵I-based radioimmunoassay (RIA) that was validated for specificity (parallelism of the RIA standard and the rhea plasma curves, $y = -0.07x + 166.75$ and $y = -0.09x + 171.61$, respectively), accuracy ($y = 1.11x + 19.72$), and precision (intra- and inter-assay variation <10%). Data were analysed by repeated measures ANOVA. Corticosterone concentrations (mean $\pm$ SEM; ng/mL) increased significantly from 3.98 ± 1.04 (0 min) to 89.83 ± 12.42 (15 min), 141.6 ± 21.98 (30 min) and 166.54 ± 16.01 (60 min). The samples taken at 30 and 60 min did not differ. No effect of sex or the interaction of time and sex were detected. The delay from ACTH injection to the peak concentration was similar to that found in other avian species (Ludders et al., 1998), but the response in rheas was approximately four to ten times greater.

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NEST POPULATION DYNAMICS IN COLONY BREEDING OSTRICHES

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Ostriches have a complex breeding system in which a male can breed with one or multiple females. Eggs laid in a nest of a territory holding male can come from within or outside his breeding unit (Kimwelle and Graves 2003). While the number of territory holding males suggests the number of active nests, some males may also attract a larger number of females than other males. We tested the hypothesis that the number of established nests in the ostrich colony will be similar to the number of males, and that egg production in the ostrich colony will bias towards nests of certain males. The study was carried out in a colony of 125 birds (90 females and 35 males), which were maintained in a 13.74-hectare paddock on a commercial farm in the southwest of Western Australia. Eggs were collected daily from beginning of July to end of March during the 2003/2004 breeding season.

Over the season, a total of 59 nests were excavated and 3618 eggs were laid. The mean ($\pm$ SEM) number of eggs laid per nest was 72 ± 13 , over the mean nest activity period of 132 ± 11 days, with a lay rate of 0.7 ± 0.1 eggs/day/nest. Nine nests did not have any eggs deposited in them and 416 eggs (11%) were found outside nests. The most active nest contributed 432 eggs over 259 days, with an accumulation rate of 1.7 eggs per day. The most productive nest resulted in 151 eggs over a period of just 65 days, with an accumulation rate of 2.3 eggs per day. Nine of the 50 active nests (18%) contributed 53% (1910) of the total number of eggs at a combined lay rate of 1.07 eggs per day. The combined rate of lay for the 9 most productive nests was 8.4 eggs per day. These results suggest that elements of individual performance and social interaction among birds may be responsible for variable egg production in nests and also imply that some males would be re-nesting. Identification of individuals and produced chicks would help to understand the dynamic nature of the ostrich colony and help eliminating poor performers.

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EMU OIL: A POTENTIAL TREATMENT FOR CHEMOTHERAPY INDUCED MUCOSITIS?

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Mucositis is a side effect of chemotherapy, resulting in severe inflammation and ulceration of the alimentary tract. Symptoms, including abdominal pain, bloating and diarrhoea can be so debilitating that they limit chemotherapy dose, reducing the likelihood of successful remission. Current treatments for mucositis are often ineffective, thus presenting a need for the development of new therapeutic strategies. Emu Oil has been demonstrated to have a beneficial effect on some forms of arthritis and other inflammatory conditions (Snowden and Whitehouse, 1997). A pilot study was conducted investigating the effects of orally administered Emu Oil on histological and biochemical indicators of 5-fluorouracil (5-FU) induced mucositis in rats. We aimed to investigate the potential of Emu Oil to protect, or promote healing of a damaged intestine.

The experiment consisted of four treatment groups, including a saline/water control group, a 5-FU/ water control group, and two Emu Oil treatment groups, which received either 0.5 ml or 1 mL of Emu Oil daily. Emu Oil was administered via oro-gastric gavage, and 5-FU (150 mg/kg) was administered via a single intraperitoneal injection at $t=0$ hours. All animals were gavaged with Emu Oil or water for 5 days (from $t=-120$ to $t=0$) prior to 5-FU administration, and continued to receive Emu Oil/water up to, but not including the day of cull ($t=48, 72$ or 96). Myeloperoxidase, an enzyme present in neutrophils, was used as an indicator of acute inflammation. At 96 hours, myeloperoxidase activity was lower in animals from both Emu Oil-treated groups compared to 5-FU-treated control animals. This effect was observed in jejunum-ileum junction and ileum sections of the small intestine (Fig. 1) and was supported by improvements in villus and crypt integrity.

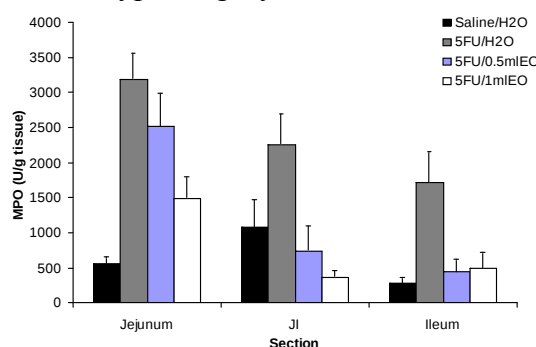


Fig. 1. Level of MPO in the Jejunum, Jejunum-Ileum Junction (JI) and Ileum, 96 hours post 5-FU administration. Means ($\pm$ s.e.m.) with different superscript within section differ significantly ($P<0.05$).

In this preliminary study, Emu Oil decreased parameters of acute inflammation in the 5-FU-damaged small intestine, and improved villus and crypt structure in the intestinal mucosa. These promising findings suggest potential of Emu Oil as a therapeutic strategy for mucositis. Further studies are required to determine its mode of action and the effects of dose escalation and inter-batch variation.

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ARTIFICIAL INSEMINATION OF FEMALE OSTRICHES USING VOLUNTARY CROUCH

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In the ostrich, the artificial insemination technique is based on restraining the female in a specially constructed crash to which the female needs to be brought in after being caught in a paddock (von Reutenfeld 1977). Variation to this technique may include a gentle restraint inside the paddock blindfolding the female or not depending on her temperament. While such procedure may be successful for semen deposition into the oviduct it can be labour intensive and stressful to the birds and handlers. Moreover, the procedure needs to be repeated at regular intervals to maintain female fertility at maximum and this may lead to repeated stress. An alternative approach is where female ostriches could be induced to crouch voluntarily and allow access to cloaca at their own will, an approach that is successfully used in the emu (*Dromaius novaehollandiae*) for artificial insemination (Malecki and Martin 2004). In the ostrich, spontaneous crouch has rarely been reported although crouching females have been used to successfully train male ostriches for semen collection (Rybnik et al. 2007). This suggested to us that it should be feasible to develop the artificial insemination technique for female ostriches using a voluntary crouch.

We present a preliminary report on development of this new technique. The female is followed until she voluntarily assumes crouching position. Then the inseminator lowers himself behind the female and places his hands on her back to provide additional stimulation. The female ostrich usually responds by either bending her neck or keeping it upright, or by putting her neck on the ground and rocking it from left to right, clapping her beak. The tail is raised to expose the vent. Passing the female phallus (*phallus phemininus*), a hand is then introduced into the proctodeum and the fingers are directed to the left through urodeum and left to the oviduct opening. The hand and fingers then guide the insemination straw into the vagina and semen is deposited at a desired depth. Occasionally, assistance from a second person is required by applying pressure on the female's back to maintain her in a crouching position. Such approach appears to be stress-free for the female and requires only 1–2 people. This technique could be adopted by the ostrich industry but factors contributing to voluntary crouch and female receptivity need to be identified in future studies.

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CROUCHING BEHAVIOUR AND OVIPOSITION RATE IN FEMALE OSTRICHES REARED WITHOUT MALES

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In ostrich farming, the female behaviour facilitating stress free artificial insemination and the effect of male presence on ovulation rate are factors that still need to be addressed if a viable AI protocol is to be developed. It has been shown that female emus crouching voluntarily into the mating position can be artificially inseminated by one person without any restraint (Malecki and Martin 2004). In the ostrich, the adoption of such an approach has not been attempted.

We reared 2004 hatch female ostriches in a female only flock from 12 months of age and studied crouching behaviour when 2 and 3 years old. In May 2006, a month before the peak of the breeding season, randomly chosen females ($n=15$) were scored for voluntary crouch by entering the paddock every 4 h, 3 times a day, for 3 days ($n=9$ observations). Female response to human approach was recorded (1 – crouch; 0 – no crouch). Seven females did not score any point and were excluded. The mean score for crouching females was 6.3 ± 3.7 (s.d.). The voluntary crouch scoring was repeated a month later (peak of the season) for 6 days ($n=18$ observations) with the remaining eight females. The mean score was 17.3 ± 1.1 (s.d.). The females were then kept in one paddock until the next breeding season, excepting two that were taken to a breeding flock. The group ($n=6$) commenced laying in March 2007 and it was in April divided at random into two groups of three and maintained in neighbouring paddocks for the rest of the season. Each group was on one side neighbouring a trio (1 male and two females). In the 2007 breeding season, the females laid eggs at the same rate as the females maintained with males (0.20 ± 0.2 v. 0.19 ± 0.01 eggs/female/day, respectively). The females crouched reliably when approached by a human and the artificial insemination technique based on voluntary crouch was developed (Malecki and Rybnik 2008). Rearing female ostriches without males and selection for a voluntary crouch can be a useful strategy for establishing a flock that can be artificially inseminated without physical restraint and that can produce eggs without males being present.

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BONE AND EGG QUALITY OF BREEDER OSTRICHES FED A MAINTENANCE DIET (LOW CALCIUM) AND A LAYER DIET (HIGH CALCIUM)

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It is known that during the life of the bird, particularly in poultry, the bone system is influenced by endogenous and exogenous factors, such as hormone secretion, egg production, nutrition, and temperature, and its mass may change according to body requirements. Calcium deficiency leads to incomplete calcification of the produced organic matrix. This deficiency may be due to lack of calcium in feed ingredients, or of the pro-hormone Vitamin D, which is essential for the absorption of Ca^{2+} and $(\text{PO}_4)^{3-}$ ions by the small intestine (Junqueira and Carneiro 2004). Bone tissue density results from the deposition of calcium and phosphorus in the form of hydroxyapatite during the process of bone mineralization. Both minerals comprise 70% of the bone, and the remaining 30% consist of organic matter, particularly collagen (Kalebo and Strid 1988; Field 1999).

This study aimed at evaluating bone variation in ostriches, and determining the relationship between bone and egg quality. Ostrich female breeders (5–7 years of age) were used to evaluate egg production, egg weight, eggshell quality, bone strength, bone dry matter and total mineral content of three birds fed a maintenance diet (low calcium, 0.93% Ca) and 3 birds fed a layer diet (high calcium, 3.83%) for 8 weeks.

Females were slaughtered to determine bone strength, dry matter, ash, as well as calcium and phosphorus bone levels in the tibia and the femur. The proximal epiphysis of the right tibia and the distal epiphysis of the right femur bones were analysed immediately after slaughter by clinical radiological procedures (65kVp x 3mAs, with a 90 cm clearance). Bone strength was measured in an EMIC DL 10000 apparatus set with a 10 cm clearance.

Egg production was not affected by the diet ($P>0.05$). The egg weight was higher on breeder than on a maintenance diet ($1748 \text{ g} \pm 180$ v. $1529 \text{ g} \pm 148$). The eggshell percentage was lower while eggshell calcium content was higher ($P<0.05$) in birds fed the breeder diet (15.3 ± 1.5 v. $16.1 \pm 1.7\%$ and 396.9 ± 41 v. $375.8 \pm 39 \text{ mg/g}$, respectively). Phosphorus eggshell content was not influenced ($P>0.05$) by Ca level in the feed. Tibia and femur bone strength was superior ($P<0.05$) in birds fed the breeder diet.

It was concluded that while feeding ostriches with a low calcium diet resulted in reduced bone strength, egg production was not affected for the 8-week period of the experiment. Presumably, the mobilisation of calcium and phosphorus reserves in the bones is sufficient to supply the birds' requirements in the short term. However, the long term feeding of such diets could lead to impairment of egg production.

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THE FORMULATION OF A NEAR-NATURAL DIET FOR CAPTIVE KIWI

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Population numbers of North Island brown kiwi (*Apteryx mantelli*) are estimated to have almost halved in the last decade. Despite some success of captive breeding, captive bred kiwi suffer higher embryonic and adult mortality rates, smaller eggs (Department of Conservation, 2004) and lower hatching rates than wild kiwi (McLennan *et al.*, 1996). A major contributor to these problems is likely to be diet. The current diet fed to kiwi in captivity was formulated over 30 years ago with no reference to the nutrient requirements of the birds. This study aims to reformulate the captive diet so that it more closely matches the nutrient composition of the natural diet.

The nutrient composition of the average diet of wild North Island brown kiwi was sourced from the literature (Kleinpaste, 1990) and used as the basis for the formulation of the near-natural diet. The nutrient composition of the near-natural diet closely matched that of the natural diet. Predicted values were validated by analysis of the formulated near-natural diet.

Table 1. Comparison of the natural diet, the near-natural diet as calculated and the near-natural diet as analysed (dry matter basis).

Diet	Ash (g/kg)	Crude protein (g/kg)	Gross energy (kJ/kg)	Organic matter (g/kg)	Fat (g/kg)	Total fatty acids (g/kg)	Carbohydrate (g/kg)
Near-natural diet (calculated)	0.6	5.6	2.4	9.5	1.8	1.5	2.2
Near-natural diet (analysed)	0.8	5.3	2.4	9.2	1.6	1.5	2.3
Natural diet*	0.8	5.2	2.3	9.4	1.7	1.3	2.4

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PRESENT STATUS OF EMU FARMING IN INDIA

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There are currently more than 10,000 emu farms in India with capacities ranging from 4– 10,000 birds, with an average of 60 birds. Management, feeding, breeding and hatching procedures for emu rearing are standardized for Indian conditions. They are able to achieve the technical standards shown in Table 1. Farmers rear breeding emus in pairs, trios (two females and one male) or flocks and the egg production is comparable in all these three systems (Ramamurthy 2006; Reddy 2006; Maini 2007). The chick mortality is lower, hatchability higher and other traits are comparable, with the earlier reports of Reddy (2006) and Maini (2007). Specialised emu incubators are fabricated locally, which are giving hatchability up to 80%. Due to high prices and demand for emu oil, the processing of emu has started as per HACCP standards. The skin, feathers and meat are also fetching remunerative prices. Based on this demand, many farms will expand and new farms will emerge in India, as alternative to chicken farming.

Table 1. Technical standards observed in emu farms in India.

Sex ratio/mating type	Pairs, trios or flock mating
Laying season	August–February
Daily feed intake/adult bird	800–1000 g
Annual egg production	10–65 eggs (average 30 eggs)
Hatchability	60–80%
Incubation period	50–60 days
Egg weight	400–700 g
Chick weight at hatch	330–550g
Mortality during first month	5%
Age at slaughter	16–20 months
Dressed meat yield obtained	40% of liveweight
Oil obtained from bird	5–6 L
Skin yield	10–12 % of liveweight
Floor space (breeding)	50–100 m ² /pair
Floor space (finishing)	5–10 m ² /bird

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SEMEN CHARACTERISTICS OF 2- AND 3-YEAR-OLD MALE OSTRICHES

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Ostrich males reach sexual maturity between 2 and 3 years of age. Egg fertility of females mated to 2-year-old males is reported to be poorer than for those mated to 3-year-old males, suggesting that the output of semen and spermatozoa could be related to age.

In the middle of two breeding seasons (2006 and 2007) on the commercial ostrich farm in Poland, we collected ejaculates from the same male ostriches ($n=4$) using a dummy method (Rybnik et al. 2007), first when males were 2 years old and then when 3 years old. Semen volume (mL), concentration ($10^9/\text{mL}$), total number of spermatozoa (10^9) and motility (0–5 score of mass movement) were determined as reported previously by Rybnik et al. (2007). Ejaculate volume and total number of spermatozoa did not differ between 2-year-old and 3-year-old males. The concentration of spermatozoa was higher in 2- than in 3-year-olds while motility was higher in 3- than 2-year-old males (Table 1).

Table 1. Ejaculate characteristics of 2- and 3-year-old ostriches (mean $\pm$ s.e.m).

Ejaculate parameters		Volume (mL)	Sperm concentration ($\times 10^9/\text{mL}$)	Number of spermatozoa ($\times 10^9$)	Motility
Age (years)	2 ($n=19$)	0.7 ± 0.1	$4.0 \pm 0.4^{**}$	3.3 ± 0.6	$3.8 \pm 0.2^{***}$
	3 ($n=47$)	1.1 ± 0.1	3.2 ± 0.1	3.5 ± 0.4	4.8 ± 0.1

n , number of ejaculates; **, $P<0.01$; ***, $P<0.001$ (ANOVA, Fisher l.s.d.)

We conclude that the ejaculate output of 2- and 3-year-old males is comparable. In three males, the ejaculate did not change with age while in one male it increased. The between-male variation in the output of semen and spermatozoa may be responsible for variation in egg fertility as some males do not appear to achieve their reproductive potential at 2 years of age.

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CHARACTERISTICS OF OSTRICH EJACULATE IN THE SECOND HALF OF THE BREEDING SEASON

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High output of semen and spermatozoa during the period of egg production is crucial for the implementation of AI technology. The main constraint to semen production in ostriches is seasonality. Ostriches are spring breeders in general but the breeding season can last 6–8 months, beginning at the end of winter and finishing in early autumn. To study seasonal changes, we collected ejaculates in July, August (summer) and September (end of summer/early autumn) on a commercial ostrich farm in Poland. Collections were carried out daily for 10 days in each month, at about 20-day intervals, from 3-year-old male ostriches ($n = 4$) using a dummy method (Rybnik et al. 2007). Semen volume (mL), concentration ($10^9/\text{mL}$), total number of spermatozoa (10^9) and motility (0–5 score of mass movement) were determined as reported previously by Rybnik et al. (2007).

Table 1. Ejaculate characteristics of ostriches during the breeding season (mean $\pm$ s.e.m).

Month	Ejaculate volume (mL)	Sperm concentration ($\times 10^9/\text{mL}$)	Total number of spermatozoa ($\times 10^9$)	Motility
July ($n=34$)	1.0 ± 0.1^a	3.7 ± 0.2^a	4.2 ± 0.7^a	4.7 ± 0.1
August ($n=40$)	1.3 ± 0.1^a	2.8 ± 0.1^b	3.5 ± 0.4^a	4.9 ± 0.1
September ($n=13$)	2.1 ± 0.2^b	4.4 ± 0.3^c	9.1 ± 1.3^b	4.5 ± 0.1

n , number of ejaculates; means within columns with same superscript differ significantly ($P < 0.05$, ANOVA and l.s.d.)

In September, two males lost libido and ejaculates could not be collected from them. The lowest volume was recorded in July (Table 1) and the highest in September. The concentration of spermatozoa was lower in August than in July or September. The total output of spermatozoa was higher in September than in July or August. Motility was not affected. Collection day had no effect on ejaculate parameters in July and August. Ejaculate volume tended to decline in September. Although the anticipated seasonal decline in ejaculate parameters is not evident from ejaculate values, the loss of libido is indicative of the end of the season. Some males maintained libido and sperm output for longer than others, suggesting that the effect of season on egg fertility could be minimized through selection.

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HAEMATOLOGY AND SERUM PROFILE IN GROWING OSTRICHES

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The health status of ostriches, like other avian species, can be determined from their blood parameters. Blood profiling, which was initially used to detect sub clinical metabolic disorders, has recently been applied to evaluate the effects of different treatments on metabolic, nutritional and animal welfare conditions. Blood samples were collected from the brachial vein of 12 juvenile ostriches (aged 12–16 weeks) using 21G needle. Samples were collected in sodium citrated tubes for hematological studies and serum for serological tests. The birds were provided with feed containing 20 to 21 % CP and a ME of 2225 to 2205 Kcal/kg. Laboratory analysis of the blood samples was carried out within four hours of collection. Blood samples revealed the following haematological parameters.

Table 1. Haematological parameters of juvenile ostriches in India

Parameters	Unit	Min	Max	s.d.	Mean (± s.e.)
Total erythrocyte count	$\times 10^{12}/L$	1.59	1.78	0.06	1.68 ± 0.02
Haemoglobin concentration	g/L	100	120	8.21	112.50 ± 3.35
Packed cell Volume	%	31	36	1.91	34.16 ± 0.79
Total leukocyte count	$\times 10^9/L$	6.5	9.8	1.29	7.95 ± 0.53
Erythrocyte sedimentation rate	mm/h	1.3	2.5	0.42	1.68 ± 0.17
Heterophils	%	59	66	2.43	62.17 ± 0.94
Lymphocytes	%	29	34	1.88	31.17 ± 0.79
Eosinophils	%	1	2	0.51	1.33 ± 0.21
Monocytes	%	1	2	0.55	1.50 ± 0.22
Basophils	%	3	5	0.75	3.83 ± 0.31

Biochemical parameters revealed mean values of Alkaline Phosphatase (KA units), SGOT (IU/dL), glucose (mg/dL), total protein (g/dL), albumin (g/dL), globulin (mg/dL) and cholesterol (mg/dL) as 15.4, 17.3, 185.2, 3.8, 2.2, 1.5 and 54.6, respectively. Results of haematological parameters should be correctly interpreted because they are necessary for making correct diagnosis with good anamnesis and physical examination. The values of PCV, Hb and total RBC values belonging to the juveniles are lower than the values in adult ostriches (Raukar and Simpraga 2005). Similar results have also been observed in the domestic fowl (Oyewale 1987). Total protein and haematology might provide an immediate indication of health status. Low values of total protein at young age could be associated with high incidence of leg deformities and poor weight gain, given that in this phase growing ostriches require high protein content in the diet. Alkaline Phosphatase values tend to fall with increase in age and stabilize in adults. Periodical testing of blood could give a better picture of the bodily condition of ostriches.

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PRODUCTION PERFORMANCE OF OSTRICHES IN INDIA

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Ostrich farming in India commenced in April 2000 with the main objective of observing ostrich behaviour, studying their adaptability to the Indian climate and measuring growth rate, breeding capacity and feed efficiency. One hundred ostrich chicks were imported from Malaysia and kept indoors for the first 8 weeks and later maintained in a 60-acre paddock until the birds could be sexed. Twenty four birds that survived till the breeding age were fed with chick diet from 0-3 months, grower I and II diets from 3-12 months, conditioner diet from 12-18 months, and a breeder ration during laying (>18 months). The CP for the diets fed during the chick to adult stage ranged from 19-20% and ME from 2100-2450 Kcal/kg. Chicks from breeding birds commenced hatching from the year 6 onwards. In year 6, of the 487 eggs produced 24.2% were fertile with a hatchability of 13.9 % for all eggs set. Livability of chicks was 58.8% from 0-18 months with 29.4% of deaths recorded over the first two months of age. Omphalitis was the main cause of death in chicks. The rearing used for birds was similar to their parents except for their feed, which was supplemented with an imported feed premix. The diet specifications provided to year 6 birds are in Table 1.

Table1. Feed specifications for birds hatched in India.

Nutrients	Chick (0-3 months)	Grower I (3-6 months)	Grower II (6-12 months)	Conditioner (12-18 months)	Breeder (>18 months)
C.P. (%)	20.86	20.9	19.28	19.1	20.03
ME (Kcal/kg)	2225	2205	2191	2103	2428
Lysine (%)	1.13	1.12	0.93	0.96	1.13
Methionine (%)	0.38	0.39	0.37	0.37	0.39
Calcium (%)	0.53	0.63	0.67	0.7	3.45
Phosphorus (%)	0.15	0.16	0.75	0.15	0.71

The body weight, feed conversion ratio and daily feed consumption of the progeny at one month was 3.7 ± 0.2 kg, 1.67 and 240 g/ bird, respectively. At 18 months, body weight, feed conversion ratio and daily feed consumption was 117.3 ± 2.40 kg, 6.30 and 2720 g/bird, respectively. Highest weight gain was observed over 3-4-month period. It was observed that birds spent 70-75% of their time in feeding, ingestion of feed and forage related activities. Smith *et al* (1995) observed that feed conversion ratio in early stages of growing may be 2:1, but the ratio deteriorates to 5:1 when the birds attain the weight of 70 kg. Feeding of ostriches is the major part of the expense and also the most critical one.

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REPRODUCTIVE AND HATCHING PERFORMANCE OF OSTRICHES IN INDIA

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Ostriches are usually seasonal breeders, but they are also known to be opportunistic breeders. As a pilot project, ostrich farming commenced in India during the year 2000 with the import of one hundred chicks. Due to the paucity of knowledge on the basic biology of ostrich, there has been a slow growth of this industry worldwide. Among the various reasons, reproduction and hatching is one of main reason for retarded growth of the industry.

Out of 100 ostriches, 24 (9 males and 15 females) adapted to the conditions were sexually separated at 9 months of age. Seven males and 14 females were housed as trios (1:2) and one in pair (1:1) in eight breeding paddocks. One male was kept as a reserve. Each bird was provided with an area of 500 m². The mean age of sexual maturity observed among the females was 787.8 ± 29.9 days. The minimum and maximum age of sexual maturity in observed was 593 days and 1398 days, respectively. During the 5-year period, 2221 eggs were laid. The results revealed that the main laying season in India was from October to March when it accounted for 57.2 % of total egg production (1270 eggs). The egg production during the five year period was 224, 562, 516, 431 and 488 with an average of 18.6 ± 2.9, 35.1 ± 4.9, 34.4 ± 5.8, 28.7 ± 4.6 and 32.5 ± 5.0 per female per year. There seems to be a wide difference between the minimum and maximum number of eggs laid per bird per year in the flock. Egg weight showed significant difference ($P \leq 0.01$ using matrix test) between the years of production. There was a significant increase in the egg weight from first (1161.1 ± 30.9) to fifth year (1597 ± 31.9) of production. Although the fertility was observed from second year onwards, peak fertility and hatchability was observed during the sixth year of production. Out of the 487 settable eggs that were laid during the sixth year, 118 eggs were fertile and 369 eggs were infertile. Out of the 118 fertile eggs, 68 chicks hatched, 32 were dead germs and 18 were dead in shells. Fertility rate was 24.2% and the hatchability was 15.4 ± 2.2% (on total egg set) and 56.6 ± 7.1% (on fertile eggs). In the incubator, during the first 38 days, a temperature of 36.3–36.4 °C and a relative humidity of 20–25% were provided and from 39th day onwards until hatching, the temperature was reduced to 96.8–97.1 °F and a RH of 30–35% was provided (Wilson *et al* 2000).

Though maximum egg production was recorded during the second year of production, the same was not reflected in hatching performance. The maximum hatching performance was recorded only during the sixth year of production. Breeding efficiency can be further efficiently enhanced by individual observation of the birds.

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