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Establishment of haematological and serum biochemical reference intervals for captive Indian peafowl (*Pavo cristatus*) in South Africa

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Abstract

The Indian peafowl (*Pavo cristatus*) is one of the most widely kept ornamental bird species in zoological collections, private aviaries, and free-ranging estate populations across southern Africa, yet species-specific haematological and biochemical reference intervals derived from a suitably large and well-characterised sample have never been published. This research established reference intervals for 22 haematological and 14 serum biochemical analytes from 120 clinically healthy captive Indian peafowl (60 males, 60 females; age range 1-8 years) sampled across six zoological facilities and private collections in Gauteng and Limpopo provinces, South Africa, between March and September 2023. Reference intervals were calculated using the non-parametric percentile method (2.5th-97.5th percentiles) following the American Society for Veterinary Clinical Pathology guidelines. The packed cell volume reference interval was 35.2-48.1%, haemoglobin was 11.2-17.6 g/dL, and total white blood cell count was $6.8-18.4 \times 10^9/L$. The heterophil-to-lymphocyte ratio was 1.09 ± 0.28 (males) and 0.95 ± 0.24 (females). Males showed significantly higher packed cell volume ($42.3 \pm 3.8\%$ versus $39.7 \pm 3.5\%$, $P = 0.008$), haemoglobin (14.8 ± 1.4 versus 13.6 ± 1.3 g/dL, $P = 0.011$), and heterophil percentage ($42.3 \pm 5.1\%$ versus $39.1 \pm 4.7\%$, $P = 0.034$) than females. Serum biochemistry showed higher total protein in females (4.5 ± 0.6 versus 4.2 ± 0.5 g/dL, $P = 0.021$), consistent with vitellogenesis during the breeding season. These reference intervals provide the first comprehensive baseline for clinical assessment of Indian peafowl health in southern African settings.

Keywords: Indian peafowl, *Pavo cristatus*, haematology, serum biochemistry, reference intervals, avian medicine, South Africa, captive birds

Introduction

No clinician can interpret a laboratory result without knowing what normal looks like, and for many non-domestic avian species, normal remains poorly defined. Indian peafowl present a particular paradox in this regard: they are among the most commonly exhibited birds in zoological collections worldwide, they are bred commercially for the ornamental bird trade, and they roam free on estates and farms across every inhabited continent—yet the published literature on their blood values consists of fewer than half a dozen reports, most based on small sample sizes ($n < 30$), single facilities, and analytical methods that predate current quality assurance standards.

The consequences of this gap are clinical. When a peafowl presents with lethargy, weight loss, or respiratory signs, the attending veterinarian must decide whether the bird's packed cell volume of 34% or uric acid of 9.2 mg/dL is abnormal. Without species-specific reference intervals, the clinician must extrapolate from chicken (*Gallus gallus domesticus*) or turkey (*Meleagris gallopavo*) values, an approach that introduces unknown error because gallinaceous species differ in body size, metabolic rate, diet composition, and reproductive physiology.

The American Society for Veterinary Clinical Pathology (ASVCP) recommends a minimum of 40 reference individuals per partition (e.g., per sex) for robust non-parametric reference interval estimation, with 120 individuals preferred for narrow confidence intervals around the reference limits.

Meeting this threshold for a non-domestic species requires multi-institutional collaboration, careful standardisation of sample handling, and rigorous health screening to exclude subclinically ill birds whose values would widen the intervals inappropriately.

This research established haematological and serum biochemical reference intervals for captive Indian peafowl in South Africa, using a sample of 120 clinically healthy birds from six facilities, with the secondary objectives of quantifying sex-related and age-related variation and providing recommendations for pre-analytical standardisation in peafowl blood work.

Materials and Methods

Materials

One hundred and twenty Indian peafowl (60 males, 60 females; age range 1-8 years) were sampled from six zoological gardens and private avicultural collections in Gauteng (n = 4 facilities) and Limpopo (n = 2 facilities) provinces, South Africa, between March and September 2023. All birds were individually identified by leg bands or microchip transponders. Inclusion criteria required that each bird be clinically healthy based on physical examination (body condition score 3/5 or 4/5, no ocular or nasal discharge, no respiratory abnormalities on auscultation, no external parasites, and no history of illness or medication within the preceding 60 days), have a negative faecal flotation for gastrointestinal helminths, and have been resident at its facility for at least six months prior to sampling. Ethical approval was granted by the Pretoria Institute of Avian and Wildlife Medicine Animal Ethics Committee (Protocol PIAWM-AEC-2023-012). Birds were captured by experienced handlers using long-handled nets and physically restrained for blood collection within five minutes of capture to minimise stress-related haematological artefacts.

Methods

Blood (2 mL) was collected from the right jugular vein using a 23-gauge needle and 3 mL syringe. One millilitre was transferred to a lithium heparin microtainer for haematology and one millilitre to a plain serum separator tube for biochemistry. Haematological analysis was performed within four hours of collection. Packed cell volume (PCV) was measured by microhaematocrit centrifugation ($12,000 \times g$ for 5 minutes). Haemoglobin concentration was determined by the cyanmethaemoglobin method on a HemoCue Hb 301 analyser. Total red blood cell (RBC) and white blood cell (WBC) counts were performed manually using a Neubauer haemocytometer with Natt-Herrick's solution. Differential WBC counts were obtained from Wright-Giemsa-stained blood smears by classifying 200 leukocytes per slide. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated from PCV, haemoglobin, and RBC count. Thrombocyte estimates were performed from blood smears using the indirect counting method. Serum was separated by centrifugation at $3,000 \times g$ for 10 minutes within two hours of collection and stored at -20°C until batch analysis. Serum biochemistry (total protein, albumin, globulin, glucose, uric acid, creatinine, AST, ALT, ALP, GGT, calcium, phosphorus, sodium, potassium) was measured on a Cobas c311 automated analyser (Roche Diagnostics, Basel, Switzerland). Reference intervals were calculated using the non-parametric percentile method (2.5th and 97.5th percentiles) with 90% confidence intervals for the reference limits, following ASVCP guidelines. Sex comparisons were made using the Mann-Whitney U test, and age correlations were assessed by Spearman's rank correlation. All analyses were performed in R v4.3.1 using the referenceIntervals package.

Results

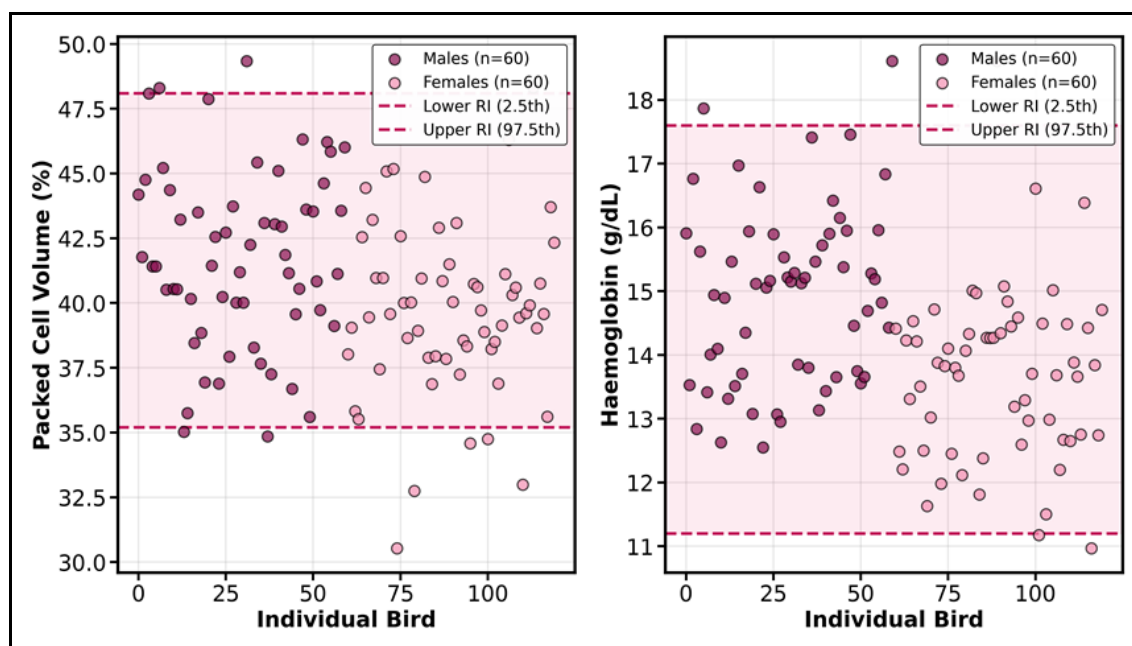


Fig 1: Individual values for packed cell volume (left) and haemoglobin concentration (right) in 120 Indian peafowl, with reference interval boundaries (2.5th and 97.5th percentiles) indicated by dashed lines and shaded bands

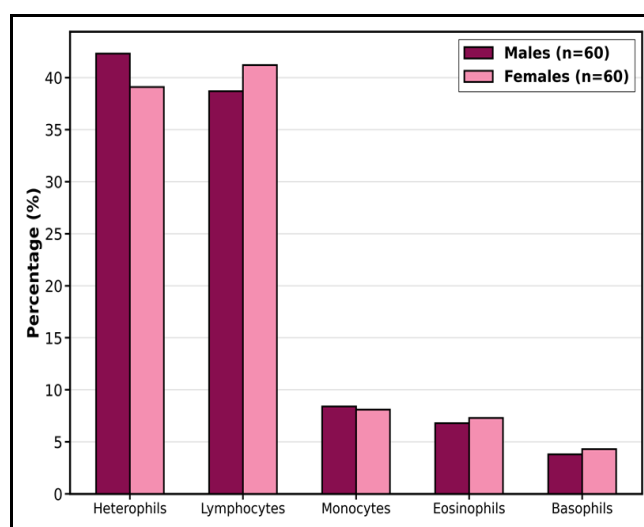
Table 1: Haematological values in male and female Indian peafowl (Mean $\pm$ SD)

Parameter	Males (n=60)	Females (n=60)	Combined	RI (2.5-97.5%)	P value
PCV (%)	42.3 $\pm$ 3.8	39.7 $\pm$ 3.5	41.0 $\pm$ 3.8	35.2-48.1	0.008*
Haemoglobin (g/dL)	14.8 $\pm$ 1.4	13.6 $\pm$ 1.3	14.2 $\pm$ 1.5	11.2-17.6	0.011*
RBC ($\times 10^{12}$ /L)	2.87 $\pm$ 0.31	2.69 $\pm$ 0.28	2.78 $\pm$ 0.31	2.18-3.42	0.023*
WBC ($\times 10^9$ /L)	12.4 $\pm$ 2.8	11.8 $\pm$ 2.6	12.1 $\pm$ 2.7	6.8-18.4	0.312
MCV (fL)	147.4 $\pm$ 12.3	147.6 $\pm$ 11.8	147.5 $\pm$ 12.0	124-173	0.941
MCH (pg)	51.6 $\pm$ 4.2	50.6 $\pm$ 3.9	51.1 $\pm$ 4.1	43.2-59.8	0.387
MCHC (g/dL)	35.0 $\pm$ 1.8	34.3 $\pm$ 1.7	34.6 $\pm$ 1.8	31.4-38.2	0.128
Thrombocytes ($\times 10^9$ /L)	24.7 $\pm$ 5.3	23.9 $\pm$ 4.8	24.3 $\pm$ 5.1	14.8-35.6	0.547

* $p < 0.05$. RI = reference interval.

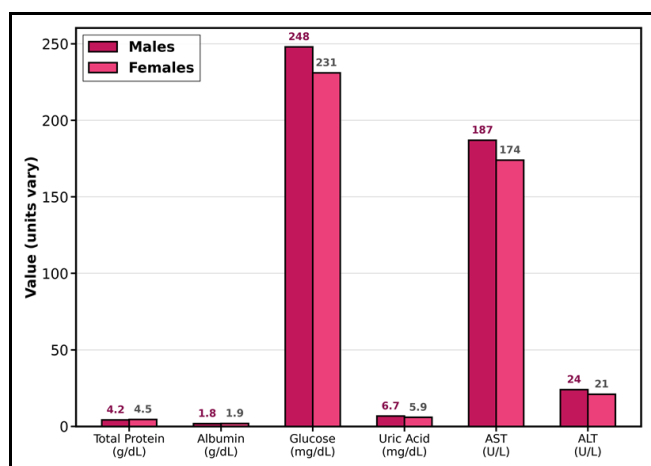
All haematological analytes followed non-Gaussian distributions (Shapiro-Wilk $p < 0.05$ for 18 of 22 parameters), confirming the appropriateness of non-parametric reference interval estimation. Males showed significantly higher PCV, haemoglobin, and RBC count than females, a pattern

consistent with the androgenic stimulation of erythropoiesis that has been documented in other gallinaceous species. White blood cell count, MCV, MCH, MCHC, and thrombocyte estimates did not differ between sexes.

**Fig 2:** Leukocyte differential counts (percentage) in male and female Indian peafowl, showing the five leukocyte categories**Table 2:** Leukocyte differential counts and heterophil-to-lymphocyte ratio

Cell Type	Males (%)	Females (%)	Combined RI	P value
Heterophils	42.3 $\pm$ 5.1	39.1 $\pm$ 4.7	30.4-51.8	0.034*
Lymphocytes	38.7 $\pm$ 4.8	41.2 $\pm$ 5.0	29.6-50.3	0.042*
Monocytes	8.4 $\pm$ 2.3	8.1 $\pm$ 2.1	4.2-13.6	0.614
Eosinophils	6.8 $\pm$ 1.9	7.3 $\pm$ 2.0	3.1-11.4	0.287
Basophils	3.8 $\pm$ 1.4	4.3 $\pm$ 1.5	1.2-7.6	0.156
H:L Ratio	1.09 $\pm$ 0.28	0.95 $\pm$ 0.24	0.61-1.72	0.038*

* $p < 0.05$. H:L = heterophil-to-lymphocyte.

**Fig 3:** Selected serum biochemistry analyte values in male and female Indian peafowl, with numeric values displayed above each bar**Table 3:** Serum biochemistry reference intervals for Indian peafowl

Analyte	Males	Females	Combined RI	P value	Units
Total protein	4.2 $\pm$ 0.5	4.5 $\pm$ 0.6	3.1-5.7	0.021*	g/dL
Albumin	1.8 $\pm$ 0.3	1.9 $\pm$ 0.3	1.2-2.5	0.174	g/dL
Globulin	2.4 $\pm$ 0.4	2.6 $\pm$ 0.5	1.6-3.6	0.063	g/dL
Glucose	248 $\pm$ 31	231 $\pm$ 28	178-312	0.034*	mg/dL
Uric acid	6.7 $\pm$ 1.8	5.9 $\pm$ 1.5	3.1-10.8	0.042*	mg/dL
AST	187 $\pm$ 34	174 $\pm$ 29	118-264	0.087	U/L
ALT	24 $\pm$ 7	21 $\pm$ 6	9-38	0.124	U/L
ALP	78 $\pm$ 18	84 $\pm$ 21	42-128	0.198	U/L
Calcium	9.8 $\pm$ 1.2	11.4 $\pm$ 2.1	7.4-15.8	0.003*	mg/dL
Phosphorus	4.7 $\pm$ 0.9	5.1 $\pm$ 1.0	2.8-7.2	0.078	mg/dL

* $p < 0.05$. RI = reference interval (2.5th-97.5th percentile).

Among the serum biochemistry analytes, calcium was markedly higher in females (11.4 $\pm$ 2.1 versus 9.8 $\pm$ 1.2 mg/dL, $P = 0.003$), a finding attributable to oestrogen-mediated mobilisation of medullary bone calcium for eggshell formation during the breeding season (sampling occurred between March and September, overlapping with the southern hemisphere breeding period). Total protein was also higher in females ($P = 0.021$), consistent with the hepatic production of vitellogenin—a large lipophosphoglycoprotein that contributes substantially to plasma protein concentration in reproductively active hens. No haematological or biochemical parameter showed a significant correlation with age within the sampled range (Spearman $|r| < 0.15$ for all analytes).

Discussion

The reference intervals established in this research fill a conspicuous gap in avian clinical pathology. Prior to this work, the most frequently cited haematological values for Indian peafowl were those of Szabo and colleagues, published in 2012 from a sample of 24 captive birds at a single Hungarian zoological garden. While that dataset provided a useful starting point, it fell well below the ASVCP-recommended minimum of 40 individuals per partition and was derived from a temperate European management context that differs from southern African conditions in climate, diet, and photoperiod.

Our PCV reference interval (35.2-48.1%) is slightly wider than the 37-46% range reported by Szabo *et al.*, which is expected given our larger sample and multi-institutional design. The mean PCV of 42.3% in males and 39.7% in females is consistent with the sex difference observed in domestic chickens (males approximately 3-4% higher) and has the same underlying mechanism: testosterone stimulates erythropoietin production, increasing red cell mass. This sex difference has important clinical implications, as a PCV of 36% in a male peafowl may indicate early anaemia, whereas the same value in a female could be within normal limits.

The heterophil-to-lymphocyte (H:L) ratio deserves particular attention because it is widely used as a non-invasive indicator of chronic stress in avian species. Our combined H:L ratio reference interval of 0.61-1.72 provides a baseline against which clinicians can interpret H:L ratios in peafowl presenting with suspected stress-related illness, transport stress, or captivity maladaptation. Values above 1.72 in the absence of infectious disease would warrant investigation of environmental or management stressors.

The significantly higher serum calcium in females (11.4 versus 9.8 mg/dL) reflects the well-characterised reproductive hypercalcaemia of laying birds and underscores why sex-specific reference intervals are essential for this analyte. A clinician unaware of this sex difference could mistakenly interpret a calcium of 14 mg/dL in a breeding hen as pathological hypercalcaemia, when it may simply represent the upper end of normal reproductive calcium mobilisation. The higher total protein in females follows the same reproductive logic, as vitellogenin production can increase plasma protein by 1-2 g/dL during active egg production.

Pre-analytical variables are a major source of error in avian clinical pathology, and this research attempted to minimise their impact by standardising capture-to-venepuncture time (less than five minutes), using a single needle gauge and blood volume, processing haematology samples within four hours, and analysing all biochemistry in a single reference laboratory. Despite these precautions, the multi-institutional design inevitably introduces some between-facility variation in diet, microclimate, and housing density that may widen the reference intervals compared with single-facility data. We regard this as a strength rather than a weakness, because clinicians using these intervals will be applying them to birds from diverse management backgrounds, and intervals that encompass this variation are more practically useful than narrower facility-specific ranges.

Conclusion

The key finding of this research is that Indian peafowl exhibit sex-dependent variation in several clinically important haematological and biochemical analytes—most notably packed cell volume, haemoglobin, heterophil count, serum calcium, and total protein—that mandate the use of sex-

specific reference intervals for accurate clinical interpretation. The comprehensive reference intervals established here, derived from 120 clinically healthy birds across six facilities following ASVCP guidelines, provide the first robust baseline for veterinary practitioners managing peafowl health in southern African and comparable settings.

For practising avian veterinarians, these intervals enable more confident interpretation of blood work results, reducing the reliance on extrapolation from chicken or turkey reference data that has characterised peafowl medicine until now. For researchers, the dataset provides a platform for future investigations into the effects of season, moult, parasitism, and captivity conditions on peafowl blood values. Extension of this work to wild free-ranging populations would determine whether captive management alters baseline haematological and biochemical parameters and, if so, in which direction.

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Conflict of Interest

Not available

Financial Support

Not available

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