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RESEARCH ARTICLE

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Biochemical and haematological reference intervals for 3–4-week-old kiwi chicks (*Apteryx mantelli*) reared in captivity

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ABSTRACT

Aims: To establish biochemical and haematological 95% reference intervals (RI) for juvenile North Island brown kiwi (*Apteryx mantelli*) chicks using parametric analysis.

Methods: Blood samples were collected from healthy 3–4-week-old, captive-reared North Island brown kiwi chicks (n = 32; male = 19, female = 13). Concentrations of total plasma protein (TPP), uric acid, glucose, phosphorus, calcium, albumin, globulin, potassium, and sodium and activities of aspartate aminotransferase and creatine kinase (CK) were measured using an automated VetScan VS2 laboratory analyser and the Abaxis Avian-Reptilian Profile Plus. Total and differential white blood cell counts were determined manually on blood smears prepared with a modified Wright-Giemsa stain. TPP was also measured manually using a refractometer, and packed cell volume (PCV) was measured using microhaematocrit tubes. RI were calculated using parametric and non-parametric methods depending on the distribution of the data. Confidence intervals (90%) around upper and lower bounds of the RI were calculated to assess certainty of the intervals.

Results: Biochemical (n = 28; male = 17; female = 11) and haematological (n = 22; male = 14, female = 8) 95% RI were generated for juvenile North Island brown kiwi. The 90% CI of the upper or lower limits of the majority of the RI generated were > 0.2 times the RI, reflecting the relatively small sample size. There was no evidence of a difference in mean biochemical values collected from sedated *vs*. un-sedated, and male *vs*. female chicks. However, the concentration of phosphorus was higher (p = 0.034) in samples that were analysed > 1 hour after collection (2.097 (SD 0.202) mmol/L) compared to samples analysed < 1 hour after collection (1.856 (SD 0.349) mmol/L).

Conclusions: This data provides the first comprehensive biochemical and haematological RI generated for *Apteryx* spp. chicks carried out under uniform collection and sample handling protocols, making the results robust and applicable to other captive-reared kiwi chicks.

Clinical relevance: In comparison to published RI from adult kiwi (Doneley 2006; Morgan 2008), kiwi chicks in this study had lower PCV, TPP and uric acid concentrations, and higher CK activities, white cell counts and lymphocyte counts. These RI are of value to wildlife veterinarians and conservation managers assessing the health of individual kiwi chicks, and for population-level comparison of birds of different ages and living in different managed or wild habitats.

Abbreviations: CK: Creatine kinase; ONE: Operation Nest Egg; PCV: Packed cell volume; RI: Reference interval; TPP: Total plasma protein; WCC: White cell count

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Kiwi; reference interval; *Apteryx* spp; haematology; biochemistry

Introduction

Kiwi (*Apteryx* spp.) populations have been in decline for decades, primarily due to predation from introduced mammalian predators (Taborsky 1988; McLennan and Potter 1992; McLennan *et al.* 1996, 2004). As a species of high conservation value, intervention is ongoing to attempt to conserve all five species of kiwi, both in captivity, by captive-breeding and rearing, and in the wild, through predator control and habitat protection (Colbourne *et al.* 2005; Robertson *et al.* 2011; Germano *et al.* 2018). Operation Nest Egg (ONE) is a captive-rearing programme in which eggs are removed from the wild, and chicks are hatched in captivity and grown to a predator-proof weight, at which point they are released, usually back to the location they came from (Colbourne *et al.* 2005). Several hundred chicks are reared through this programme annually, with a proportion of these requiring veterinary care for various reasons. Despite kiwi forming a substantial proportion of native birds treated at wildlife hospitals every year, there are no published haematology or biochemistry data for kiwi chicks or juveniles.

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Diagnostic blood analyses are routinely used in many veterinary species, including wildlife, to identify and monitor subclinical or clinical disease and for routine monitoring of animal health. These diagnostic tests can be particularly beneficial in avian species where signs of disease may be subtle and difficult to detect (Harr 2002; Sabater and Forbes 2014; Scope and Schwendenwein 2020). Diagnostic assays also have the potential to provide information on the metabolic and nutritional status of animals (Ferrer 1990; McDonald *et al.* 2010), which can be particularly valuable for monitoring wildlife held in captivity for extended periods.

In conservation-managed wildlife species such as kiwi, blood samples are routinely taken for pre-release or translocation health screening, or as a component of diagnostic assessment for individual health issues. However, as is the case for many wildlife species, comprehensive and reliable published reference data on 'normal' veterinary diagnostic physiological parameters in kiwi are limited. Where species-specific reference data are available for kiwi, sample sizes are generally small (Morgan 2008; Bansal 2020) or not stated (Doneley 2006), and the analytical and statistical methods used to generate intervals are often unreported. The health status of the sample population is often unknown, and this can cause issues in the generation of reference intervals (RI): the inclusion of non-healthy individuals can increase the width of RI, which, in clinical practice, may lead to false negatives in diagnosis (Horn *et al.* 2001). Furthermore, the effect of bird demographics, such as age/life stage, which are known to influence blood biochemistry and haematology in many avian species including ratites (e.g. emus and ostriches), may be unclear (Fair *et al.* 2007; Sabater and Forbes 2014).

The objective of this study was to generate biochemical and haematological 95% RI for healthy 3–4-week-old North Island brown kiwi (*Apteryx mantelli*) chicks. This data will provide a useful tool for clinicians and conservation managers assessing kiwi chick health and suitability for release, as well as potentially other aspects of wildlife health, such as the metabolic/nutritional status of captive birds.

Materials and methods

Venous blood samples were collected from unmedicated, clinically healthy kiwi chicks prior to a study of the safety and pharmacokinetics of anticoccidial drugs in kiwi. This study was carried out under Conservation Wildlife Act Authority permit (DOC 100547-CAP) with all procedures approved by the Massey University Animal Ethics committee (AEC 22/33).

Birds

Kiwi chicks were all ONE chicks, originating from the Forest Lifeforce Restoration Trust's kiwi project at Maungataniwha native forest (Hawkes Bay; 38° 49′00"S 176°48′01"E) via the National Kiwi Hatchery Aotearoa (Rainbow Springs, Rotorua). All chicks had regained their hatch weight (minimum 320 g), were eating well, gaining weight, and were ready to be moved from brooder boxes to outdoor pens as per standard ONE protocols (Bassett 2012). All birds were examined physically prior to blood collection, and any with detected medical or behavioural concerns – as assessed by experienced captive kiwi practitioners or a wildlife veterinarian – were excluded from this study. The birds were all fed a standard captive kiwi diet, comprising of a mix of beef mince, ox heart, cat biscuits, fruit/vegetables, and vitamin/mineral additives (C. Dean[1] pers. comm.), and kept outdoors in naturalistic enclosures for the duration of the study. After completing captive-rearing, these birds were released back to the same location from which they were lifted as eggs.

Sample collection (pre-analytical methods)

Kiwi chicks were fasted for 9–10 hours prior to blood collection. Chicks were physically restrained and up to 0.4 mL of blood was collected via either an indwelling 22–24 ga IV catheter placed in the medial metatarsal vein with or without sedation (0.8–1 mg/kg midazolam (Mylan, Auckland, NZ) IM in the caudal thigh) or by direct venipuncture if a catheter could not be placed or catheterisation was not deemed appropriate for that bird. Venipuncture was carried out with a 25 ga needle and 1 mL syringe from the medial metatarsal vein using a standard aseptic protocol. Blood was transferred immediately to a 0.5 mL lithium heparin microtainer (Knight-Benedikt, Seven Hills, NSW, Australia) and agitated gently to heparinise the sample. A small amount of unheparinised blood, taken directly from the collection syringe, was used to make multiple blood smears using the cover-slip method immediately after collection from each bird (Hume 1995; Fudge 2000). Whole blood samples were stored chilled (in a cooler protected from direct ice contact) until analysis. The time of collection was recorded.

Biochemical analysis

Plasma biochemistry values were measured from 100 µL of whole blood using the VetScan VS2 Chemistry Analyser with the Abaxis Avian-Reptilian Profile Plus (Zoetis, Parsippany, NJ, USA), which analyses 12

[1]C. Dean, National Kiwi Hatchery Aotearoa, Rotorua, NZ.

biochemical analytes from plasma. The VS2 is self-calibrating with an internal quality control system, eliminating the need for external controls. The machine was maintained regularly as per standard protocol and run within recommended operating temperatures (15–32°C).

Small volumes of blood may limit full biochemical analysis and in this situation, using a hand-held refractometer to measure total plasma protein (TPP) is often more accessible, and feasible. Therefore, to measure TPP manually, the plasma portion resulting from packed cell volume (PCV) measurement (described below) was dispensed onto the stage of a handheld refractometer (CETI Digit-012 clinical hand-held analogue refractometer; Medline Scientific, Rotherham, UK).

Haematological analysis

Approximately 15–60 µL of whole blood was loaded into a heparinised microhaematocrit tube and centrifuged at 5,000 rpm for 5 minutes to measure PCV.

Slides were fixed with methanol and stored for up to 9 months before staining. Slides were prepared with a commercial modified Wright-Giemsa stain (Sigma-Aldrich, St Louis, MO, USA). Blood smears were manually reviewed by a wildlife veterinarian (BG) for enumeration of the estimated total and differential white blood cell counts (WCC) (Hume 1995; Fudge 2000). All leukocytes in 10 fields of view at 400 x magnification, in areas of the slides where cells are evenly distributed, were counted on a single microscope (Olympus CX41; Tokyo, Japan). Then the total WCC in 10^9/L blood was calculated with the following equation (Walberg 2001):

$$\mathrm{WCC} = \frac{\text{Total number of leucocytes in 10 fields of view}}{10} \times 2$$

All haematology parameters were expressed as absolute (x 10^9/L) and relative (%) counts. All reviewed slides of marginal quality were excluded from further analysis. If more than one good quality slide was available from an individual bird, one was randomly selected to be included in the generation of reference intervals.

Data analysis

For each parameter, descriptive statistics (mean, median) were generated in R software (R Foundation for Statistical Computing, Vienna, Austria). A histogram and QQ-plots were generated to check for normality of distribution. The Shapiro–Wilk test for normality was carried out on all samples (normal = p-value > 0.05). Any non-normal data sets were transformed using Box–Cox transformation to attempt to reduce skewness and kurtosis, and normality was retested.

Reference intervals (95%) were calculated using Reference Value Advisor software (version 2.1; Geffré *et al.* 2011) and determined following American Society for Veterinary Clinical Pathology guidelines (Friedrichs *et al.* 2012). RI for normally distributed data (before or after transformation) were determined using the parametric method. However, if normality could not be established, the robust method was used instead. Occasionally the software generated more than one valid RI depending upon the assumptions made about the native data distribution. In these cases, the interval that was tighter was selected to try and reduce the chance of false negative results (Horn *et al.* 1998; Higgins *et al.* 2019). Dixon and Tukey (Horn's algorithm) tests were used to identify outliers, which were consequently excluded.

Confidence intervals (90%) around upper and lower bounds of the RI were calculated in Reference Value Advisor using a parametric bootstrapping method. The width of these CI is considered an indicator of the certainty of intervals, with narrower CI indicating a more precise RI. If the CI is $\geq$ 0.2 width of the RI, it is generally recommended that more samples should be assessed (Friedrichs *et al.* 2012).

The effect of time to analysis (> or < 1 hour from collection), sedation status (sedated or not sedated), and sex was assessed using t-tests allowing for unequal variance between groups.

Results

A total of 32 (male = 19, female = 13) healthy, juvenile, captive-hatched North Island brown kiwi chicks were included in this study over the 2022–2023 kiwi breeding season (from November 2022 to April 2023). All kiwi chicks were in good body condition, and no evidence of ill health was noted upon physical examination. The mean body weight was 374 (SD 33.4; min 326, max 438) g and the mean age was 24.5 (SD 2.6; min 19, max 32) days. Blood samples were collected in the morning between 08:15 and 10:45. Twenty-five of the 32 birds were sedated with midazolam for catheter placement. Plasma biochemistry analysis was carried out 20–90 (mean 59.44; SD 16.9) minutes after collection. Good quality blood smears were collected from 26/32 sampled birds.

Following analysis of samples, datasets from six birds were omitted from the calculation of RI. Three birds were omitted from the study due to observation of abnormal white blood cell morphology suggestive of an inflammatory response. Biochemical values for two birds were omitted due to sample haemolysis. Haematological values were omitted from one bird due to an over-representation of basophils, likely due to an abnormality in the staining of the smear. In addition, several individual outliers were identified and removed prior to generation of biochemical

intervals for TPP (manual n = 1, VS2 n = 1), globulin (n = 1), and creatine kinase (CK; n = 1). Four values for PCV were omitted from analysis as blood clots were observed in the sample prior to analysis (n = 28). Calculation of RI was then carried out as described, omitting the outlier/s. The concentration of bile acids was below the level of detection for the VetScan analyser, so these are reported as < 35 µmol/L, and no RI was generated.

Descriptive data for biochemistry measurands from 26–28 chicks and haematology measurands from 22–28 chicks are reported in Tables 1 and 2, respectively, and the corresponding RI are reported in Tables 3 and 4. Of the 28 chicks whose data were used to calculate the biochemistry RI, 17 were male and 11 were female. Of the 22 chicks whose data were used to calculate the haematology RI (other than PCV), 14 were male and 8 were female. Histograms for each RI and an abbreviated clinic-friendly version of these intervals are presented in the Supplementary Information. The width of the 90% CI as a proportion of the total interval is also presented for each parameter. The 90% CI of the upper or lower limits of 9/12 biochemical parameters, and 10/12 haematological parameters generated were > 0.2 times the RI. Reference limits where the width of the CI exceed 0.2 times the RI indicate a less accurate estimate of the reference limits (Friedrichs *et al.* 2012).

There was no evidence of a difference in the mean biochemical values collected from sedated *vs* unsedated, and male *vs* female chicks. However, the concentration of phosphorus was higher (Welch's t-test, p = 0.034) in samples that were analysed > 1 hour after collection (mean 2.097 (SD 0.202) mmol/L; n = 13) compared to samples analysed < 1 hour after collection (mean 1.856 (SD 0.349) mmol/L; n = 19). However, considering the small sample size, intervals were not partitioned.

Table 1. Descriptive statistics for blood biochemical measurands for 3–4-week-old North Island brown kiwi (*Apteryx mantelli*) chicks.

Measurand[a]	N	Mean	SD	Median	Min	Max
Sodium (mmol/L)	27	140.22	2.76	140	136	147
Potassium (mmol/L)	27	5.28	0.60	5.1	4.2	6.7
Calcium (mmol/L)	27	2.60	0.13	2.6	2.33	2.84
Phosphorus (mmol/L)	27	1.95	0.29	2	1.27	2.4
Uric acid (mmol/L)	27	200.30	73.40	188	78	366
AST (U/L)	27	108.04	18.68	106	75	147
CK (U/L)	26	1,513.92	412.62	1,412.5	948	2,526
Glucose (mmol/L)	27	7.69	0.62	7.7	6.4	9.1
Total protein g/L						
VS2	26	37.19	3.10	37	30	43
Manual	28	39.96	3.82	40	31	46
Albumin (g/L)	27	25.33	2.06	25	22	30
Globulin (g/L)	26	12.27	2.15	12	8	15

[a]All measurements made on a Vetscan VS2 chemistry analyser with Abaxis Avian-Reptilian Profile Plus reagent rotor (Zoetis, Parsippany, NJ, USA), except manual measurement of total protein, which was measured with a refractometer. Bile acid data is not reported here as these were consistently below the level of detection (< 35 µmol/L).
AST = aspartate aminotransferase; CK = creatine kinase.

Discussion

This article presents the first published biochemistry and haematology RI for North Island brown kiwi chicks. The sample size used to generate RI in this study is less than the American Society for Veterinary Clinical Pathology guideline of 40 individuals (Friedrichs *et al.* 2012), and this is reflected in relatively wide 90% CI for most reference limits. However, this is the largest dataset to date from a group of healthy kiwi of the same age, kept under consistent husbandry conditions and sampled in a controlled manner.

Several biochemical and haematological parameters generated in this study varied from existing published reference intervals for kiwi reported by Doneley (2006), Bansal (2020), and Robertson (2006). The most notable biochemical differences were for TPP (measured by both refractometer and automated analyser), PCV, uric acid and CK. The RI for TPP, PCV and uric acid were all lower than previously published intervals in kiwi, and while PCV and uric acid intervals overlapped the lower limit for adult kiwi, the TPP interval generated for kiwi chicks was below the adult interval. CK activities were higher in chicks compared with adults, though the interval overlapped the upper limits previously reported for adult kiwi. Haematology RI for kiwi chicks showed a wider range of WCC, as well as higher lymphocyte values (both % and counts) and lower heterophil values (both % and counts) than other reported kiwi and ratite RI (Smith 2003; Robertson 2006; Uhart *et al.* 2006). The kiwi chick RI for WCC, lymphocytes and heterophils covered previously reported adult intervals, but were much wider.

Age, sex, reproductive status, nutritional condition, and environmental factors can all influence avian blood biochemistry and haematology (Ferrer 1990; Walton 2001; Fair *et al.* 2007). Many of the observed differences, particularly the lower concentration of TPP and PCV and higher concentration of lymphocytes, have been documented in juveniles of a wide range of avian species, including ratites (e.g. Palomeque *et al.*

Table 2. Descriptive statistics for haematology measurands for 3–4-week-old North Island brown kiwi (*Apteryx mantelli*) chicks.

Measurand[a]	N	Mean	SD	Median	Min	Max
Total WBC (x 10^9/L)	22	15.63	5.89	15.4	8	31.4
Heterophil (%)	22	37.99	14.44	37.5	15	65
Heterophil (x 10^9/L)	22	5.84	2.90	5.29	1.89	12.48
Lymphocyte (%)	22	53.27	13.87	54	31	75
Lymphocyte (x 10^9/L)	22	8.48	4.44	7.215	3.2	18.15
Monocyte (%)	22	3.19	2.03	2.9	0	7
Monocyte (x 10^9/L)	22	0.50	0.37	0.45	0	1.26
Eosinophil (%)	22	4.23	2.76	4.1	0	9
Eosinophil (x 10^9/L)	22	0.59	0.40	0.55	0	1.52
Basophil (%)	22	1.57	1.49	1	0	5.4
Basophil (x 10^9/L)	22	0.28	0.30	0.145	0	1.2
PCV (%)	28	37.82	3.6035	38	28.2	44

[a]Counts generated by manual counting of Wright-Giemsa stained blood smears.
PCV = packed cell volume; WBC = white blood cell.

Table 3. Calculated 95% reference intervals (with 90% CI) for blood biochemical measurands for 3–4-week-old North Island brown kiwi (*Apteryx mantelli*) chicks.

Measurand[a]	N	Method[b]	Lower limit		Upper limit	
			Limit (90% CI)	Width CI/width RI[c]	Limit (90% CI)	Width CI/width RI[c]
Sodium (mmol/L)	27	P	134.44 (133.03–135.94)	0.25	146.01 (144.43–147.52)	0.27
Potassium (mmol/L)	27	PT	4.33 (4.16–4.52)	0.14	6.90 (6.24–7.67)	0.55
Calcium (mmol/L)	27	P	2.33 (2.26–2.40)	0.25	2.87 (2.79–2.94)	0.27
Phosphorus (mmol/L)	27	P	1.34 (1.19–1.50)	0.25	2.57 (2.40–2.73)	0.27
Uric acid (mmol/L)	27	PT	72.44 (54.61–98.83)	0.15	371.68 (319.21–428.91)	0.37
AST (U/L)	27	P	68.93 (59.44–79.10)	0.25	147.14 (136.45–157.38)	0.27
CK (U/L)	26	PT	916.46 (824.01–1,017.86)	0.11	2,624.70 (2,173.06–3,117.27)	0.55
Glucose (mmol/L)	27	R	6.43 (6.13–6.79)	0.25	9.01 (8.55–9.50)	0.37
Total protein g/L						
VS2	26	P	30.69 (29.08–32.41)	0.26	43.70 (41.88–45.43)	0.27
Manual	28	P	31.98 (30.07–34.02)	0.25	47.95 (45.81–50.00)	0.26
Albumin (g/L)	27	P	21.03 (19.98–22.15)	0.25	29.64 (28.46–30.77)	0.27
Globulin (g/L)	26	R	7.14 (ND)	ND	16.42 (ND)	ND

[a]All measurements made on a Vetscan VS2 chemistry analyser with Abaxis Avian-Reptilian Profile Plus reagent rotor (Zoetis, Parsippany, NJ, USA), except manual measurement of total protein, which was measured with a refractometer.
[b]Method used to establish reference interval: parametric (P) where distribution was Gaussian, parametric-transformed (PT) where distribution was Gaussian following Box-Cox transformation, or robust (R) where distribution was non-Gaussian, as determined by the Shapiro-Wilk normality test.
[c]Reference intervals where the width of the CI exceed 0.2 times the interval indicate a less accurate estimate of the reference limits.
AST = aspartate aminotransferase; CK = creatine kinase; ND = parameter could not be calculated.

1991; Costa *et al.* 1993; Uhart *et al.* 2006). This may be a result of, for example, changes in the production (haematopoiesis) and lifespan/longevity of red blood cells (Kaminski *et al.* 2014), differential expression of the immune system through different developmental stages (Alonso *et al.* 1990), or changes in diet, environment or management as birds age (Howlett *et al.* 1998). Some effects may be specific to captive-reared kiwi; for example, the movement of kiwi chicks from sterile indoor brooders to outdoor naturalistic pens at the age of sampling for this study could have resulted in an immune response, and elevation of lymphocytes, with exposure to novel pathogens and toxins in the outdoor environment (Howlett *et al.* 1998).

Handling stress may also have affected the measured parameters, particularly CK, which was higher in kiwi chicks in this study as compared to previously reported intervals. CK is typically the most sensitive indicator of muscle damage in birds (Franson *et al.* 1985; Lumeij *et al.* 1988). It appears likely that this elevation was a physiologic response to additional handling for blood collection, which can result in skeletal muscle damage and leakage of enzymes into the bloodstream (Bollinger *et al.* 1989; Scope *et al.* 2002). In comparison to the standard procedure to collect blood from wild kiwi, the effects of capture, handling and stress on the kiwi chicks in this study, while difficult to account for, were presumably higher and more prolonged due to the nature of the sample collection protocol, which included IM administration of sedative and use of an IV catheter (Aktas *et al.* 1995). While some blood samples in this study (n = 7) were collected via direct venipuncture rather than by sedation and placement of an IV catheter (n = 25), there was no observed significant difference in mean CK activities between these two groups of birds. Sampling at < 10 minutes after beginning the handling procedure, such as was documented by Bailey *et al.* (1997), may be recommended for RI generation to

Table 4. Calculated 95% reference intervals (with 90% CI) for haematology measurands for 3–4-week-old North Island brown kiwi (*Apteryx mantelli*) chicks.

Measurand[a]	N	Method[b]	Lower limit		Upper limit	
			Limit (90% CI)[c]	Width CI/width RI[d]	Limit (90% CI)	Width CI/width RI[d]
Total WBC (x 10^9/L)	22	R	7.14 (6.36–8.83[d])	0.10	32.95 (25.88–40.25)	0.56
Heterophil (%)	22	P	7.29 (0–16.14)	0.26	68.69 (59.38–77.59)	0.30
Heterophil (x 10^9/L)	22	PT	1.44 (ND–2.21)	ND	13.20 (10.53–16.27)	0.49
Lymphocyte (%)	22	P	23.78 (15.94–32.29)	0.28	82.77 (73.82–91.32)	0.30
Lymphocyte (x 10^9/L)	22	R	2.95 (2.56–3.60[d])	0.05	21.77 (15.46–29.27)	0.73
Monocyte (%)	22	P	0 (−2.27 to −0.12)	0.32	7.50 (6.19–8.75)	0.34
Monocyte (x 10^9/L)	22	P	0 (−0.49 to −0.06)	0.34	1.28 (1.05–1.51)	0.36
Eosinophil (%)	22	P	0 (−3.2 to 0.06)	0.32	10.10 (8.32–11.80)	0.34
Eosinophil (x 10^9/L)	22	P	0 (−0.50 to −0.02)	0.33	1.45 (1.19–1.70)	0.35
Basophil (%)	22	R	0 (−3.25 to −1.00)	0.49	4.58 (3.08–5.70)	0.57
Basophil (x 10^9/L)	22	PT	0 (ND to −0.01)	ND	1.13 (0.76–1.55)	0.30
PCV (%)	28	P	30.29 (28.5–32.21)	0.25	45.34 (43.32–47.28)	0.26

[a]Counts generated by manual counting of Wright-Giemsa stained blood smears.
[b]Method used to establish reference interval: parametric (P) where distribution was Gaussian, parametric transformed (PT) where distribution was Gaussian following Box-Cox transformation, or robust (R) where distribution was non-Gaussian, as determined by the Shapiro-Wilk normality test.
[c]Negative values for lower reference limits have been replaced with zero.
[d]Reference intervals where the width of the CI exceeds 0.2 times the interval indicate a less accurate estimate of the reference limits.
ND = parameter could not be calculated; PCV = packed cell volume; WBC = white blood cell.

minimise increases in CK activity. However, considering the likely application of these intervals in-clinic, the intervals reported here may encompass increases in CK activity observed in chicks under typical handling stress.

The analytical sensitivity of the method used to detect analytes in blood may have also influenced the observed differences in these intervals as compared to previously reported intervals. The choice of analytical method for some tests reflects the accessibility of testing methods to veterinary or wildlife practitioners working with kiwi. For example, manual enumeration of blood smears (Fudge 2000) does not require special equipment (other than a microscope), is less time-consuming, more economical, and widely accessible to veterinary clinics, rehabilitation centres, and researchers (Ammersbach *et al.* 2015; Carisch *et al.* 2019), and is also the standard approach for collection of blood for haemoanalysis from kiwi chicks, usually during pre-release screening. However, this is not the current method of choice for obtaining the most accurate total leukocyte count in birds (Walberg 2001; Campbell 2015). One of the major limitations of using blood smears for WCC is that there is greater inherent variation in slide preparation and observer cell counts. To minimise variation in the generation of these RI, only a single experienced wildlife veterinarian carried out counts in the present study. However, it is worth noting that values generated by other practitioners may vary from those presented here. In addition, while it is generally recommended that blood smears are stained as soon as possible after preparation (Walberg 2001), due to study logistics, the smears in this study were fixed, but then not stained, for up to 9 months after preparation. Future studies would be required to determine what effect, if any, this delay in staining may have had on the resulting blood cell counts. In this study, we also report intervals for the concentration of TPP made using both a handheld refractometer and the more accurate, but less accessible, biuret method, employed by the VetScan Avian-Reptilian profile (Lumeij and Maclean 1996; Harr 2002), so that the most appropriate comparison can be made, depending on laboratory resources. Also, while avian albumin and globulin concentrations are known to be poorly represented by the method used to measure these analytes by the VS2 (bromcreosol green dye-binding), intervals are reported here for completeness (Rosenthal 2000; Greenacre *et al.* 2008). Interpretation of biochemical data generated by other laboratory biochemical analysers using these intervals should be done with care, as values for some biochemical parameters can vary considerably between different models of analyser (Greenacre *et al.* 2008; Ruiz-Jimenez *et al.* 2022) as well as between individual machines, particularly in-clinic point-of-care analysers that are not usually operated or maintained under the same highly controlled conditions as analysers within reference laboratories (Rishniw *et al.* 2012).

Most avian blood biochemical parameters have been found to provide consistent values when analysed up to 3 hours after collection on the VetScan VS2 (Hoppes *et al.* 2015). However, elevations in CK, phosphorus, and potassium can occur with increasing time to analysis and/or sample haemolysis (and release of intracellular components into the blood), as observed in this study, despite samples being processed within 2 hours of collection (Fudge 2000; Hrubec *et al.* 2002). In this study, there was an observed difference in potassium concentrations in samples processed > 1 hour following collection; however, the data were not partitioned because of the small sample size. The risk of generating RI from artefactually elevated values is that the resulting interval is more likely to support false negatives; that is, birds display symptoms of disease but are considered diagnostically normal (Horn *et al.* 2001). Future studies could consider collecting and processing samples in < 1 hour to tighten the RI for potassium presented here. Alternatively, where samples are not able to be processed immediately, analysis of plasma separated soon after collection, instead of whole blood, would reduce the artefactual increases in blood parameters due to cellular leakage. However, as the VS2 analyses a set sample volume (100 µL) regardless of sample type, this limits the feasibility of analysing plasma in avifauna where blood sample sizes are usually very small, and was not possible in this study. Clinicians should endeavour to process blood chemistry samples as quickly as possible (within < 2 hours) for a true comparison to the values reported here.

Clinicians and wildlife managers working with kiwi chicks can use these RI as a starting point for the evaluation of clinical data to help characterise the physiological state of individual birds. Further studies to determine accurate RI from larger cohorts of juvenile and adult kiwi, with known age and medical and fasting status, using the same analytical techniques, and held under the same controlled conditions (time of day, season, enclosure environment) would be beneficial to confirm the patterns seen in this study, and to draw conclusions about the physiological differences between adult and neonatal kiwi. From a captive-rearing perspective, it would be interesting to compare healthy wild and captive chicks of the same age to see if factors of captivity, such as diet or husbandry conditions, have any influence on blood biochemical and haematological health indicators, and to measure the response of normal parameters to pathological conditions, which could further assist in the diagnosis and treatment of clinical cases.

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