Original Article

Hematologic and serum biochemical reference intervals for Formosan Sika Deer (*Cervus nippon taiouanus*) from a semi-free-ranging population in Taiwan

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Abstract

The Formosan sika deer (*Cervus nippon taiouanus*), an endemic Taiwanese subspecies, holds a pivotal role in local conservation. This study addresses a significant knowledge gap by establishing population-specific reference intervals (RIs) for this species, as existing RIs are based on limited captive populations and may not accurately reflect the health of semi-free-ranging cohorts. We analyzed 632 sampling events from 232 healthy adult deer within Kenting National Park over 12 years (2013–2024). This study adhered to ASVCP guidelines, establishing instrument-specific RIs and employing linear mixed-effects models (LMMs) to evaluate the complex interactions among analytical instruments, sex, and body weight. Our results confirmed significant inter-instrument variations and showed that both sex and body weight significantly influence numerous hematologic and biochemical parameters. This study provides crucial, long-term RIs and a robust analytical framework, offering direct and practical implications for the future health management and conservation of this endemic subspecies.

Keywords: Formosan sika deer, hematology, reference intervals, serum biochemistry, wildlife medicine

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Introduction

Hematologic and serum biochemical parameters are indispensable tools for assessing the physiological status and diagnosing diseases in animals (Drevemo *et al*., 1974; Lepitzki and Woolf, 1991). In wildlife health management, the establishment of species- and population-specific reference intervals (RIs) is critical for effective health surveillance, clinical interpretation, and conservation interventions (Harr 2002; Chaffin *et al*., 2008).

The Formosan sika deer (*Cervus nippon taiouanus*) is an endemic subspecies of Taiwan (Liang *et al*., 2020). However, existing hematologic and biochemical RIs for this subspecies are primarily based on data from just 34 captive individuals at the Taipei Zoo (Kang *et al*., 2015). Directly applying these captive-derived RIs to wild or semi-free-ranging populations poses significant limitations and a high potential for misinterpreting health status. Wild individuals contend with environmental stressors like food scarcity and predation, which directly influence critical biochemical indicators such as cortisol and glucose (Turko *et al*., 2023). Moreover, their diverse seasonal diets, starkly contrasting with the consistent feed provided in captivity, significantly affect blood glucose, blood lipids, and hepatic and renal function (Turko *et al*., 2023). For example, research on Mediterranean pond turtles has revealed higher monocyte, aspartate aminotransferase (AST), and creatine kinase values in wild individuals, underscoring the profound impact of environmental and captive conditions on physiological parameters (Marques *et al*., 2025).

In recent years, the expansion of the deer population and its increased overlap with human-dominated landscapes have led to a higher frequency of health assessments, traumatic injuries, free-ranging dog attacks, and human-wildlife conflicts (Yen *et al*., 2015; Liang *et al*., 2020). Consequently, the need for robust veterinary care and reliable diagnostic parameters for this population has escalated. Developing population-specific hematologic and biochemical RIs is crucial for evidence-based clinical decision-making in veterinary care and for strengthening the scientific rigor of conservation management. Relying on RIs from captive animals can lead to serious misjudgments. It might cause unnecessary medical intervention in healthy individuals due to misinterpreting their physiological norms, or conversely, lead to early-stage diseases being overlooked, missing vital intervention opportunities and risking disease progression or delayed recovery. Such discrepancies are well-documented. For example, hematological reference ranges in captive African Grey parrots vary significantly due to differences in morphology, feeding ecology, habitat, and migratory behaviors (Gaspar *et al*., 2021). Similarly, captive Black-Faced Ibises show distinct hematologic and biochemical profiles compared to related species, including higher leukocytes and certain enzyme levels (Silva *et al*., 2020). These cases highlight how species-specific and environmental factors necessitate tailored reference data for accurate health assessments and effective wildlife management.

The establishment of accurate and reliable RIs requires stringent adherence to criteria for subject selection, standardized analytical methodologies, and appropriate statistical analyses. As recommended by the American Society for Veterinary Clinical Pathology (ASVCP) guidelines, RIs should ideally represent the central 95% distribution of values obtained from a well-defined, healthy reference population, and should include 90% or 95% confidence intervals (CIs) for both the lower and upper reference limits. Furthermore, the interpretation of RIs must consider factors such as species, sex, age, environmental conditions, nutritional status, and physiological state (Geffre *et al*., 2009; Friedrichs *et al*., 2012).

The objectives of this study were to: (1) establish *de novo* hematologic and serum biochemical RIs for Formosan sika deer within a semi-free-ranging environment, using ASVCP-compliant methodology; and (2) investigate the influence of analytical instruments, sex, and body weight on the measured hematologic and biochemical parameters. The ultimate goal of this research is to improve the accuracy of clinical laboratory data interpretation and contribute to the evidence-based health management and long-term conservation of this valuable endemic species.

Materials and Methods

Study Area: This study was conducted within the Sheding Formosan Sika Deer Restoration Area of Kenting National Park (21°57'50" N, 120°49'37" E), an approximately 120-hectare area situated at an elevation of 152 meters. The area experiences a tropical climate with hot, humid summers (mean July: 28.3°C) and mild, dry winters (mean January: 20.5°C). Annual relative humidity typically ranges from 73% to 87%. The deer population is managed under semi-free-ranging conditions, receiving supplemental feed and water only when holding area resources are insufficient.

Sample collection: Data for this study, including hematologic and serum biochemical values, were retrospectively obtained from electronic health records generated during routine annual health monitoring of the core sika deer population from 2013 to 2024. This population, located in the Kenting Sika Deer Reintroduction Area, has been confirmed through genetic sequencing and analysis as the purest Formosan sika deer population. Each sika deer was assigned a unique individual identification number. A total of 632 sampling events were conducted on 232 unique adult Formosan sika deer (102 males, 130 females), with some individuals providing multiple samples over the twelve-year study period. This figure represents the total sampling events, and the dataset includes repeated samples from the same deer across different years. Because different hematological and serum biochemical instruments may have been used across various sampling years, and while we recognize that repeated sampling introduces statistical dependence (a common characteristic of longitudinal data), we treated each sampling event as an independent observation for analysis. This approach was taken because the study's aim was to establish

population-level RIs and to capture the overall variability of the population across different years, rather than focusing on individual longitudinal trends.

While the monitoring program included various procedures, the data used for establishing RIs and comparisons were limited to samples from individuals clinically determined as healthy by a veterinarian. These individuals showed no behavioral abnormalities and tested negative for hemoparasites via routine blood smear. Clinical molecular detection for filarial worms, utilizing *Nem18S*-F (5′-CGCGAATRGCTCATTACAACAGC-3′) and *Nem18S*-R (5′-GGGCGGTATCTGATCGCC-3′) primers with a 58°C annealing temperature, yielding a 900 bp amplicon (Floyd *et al*., 2005), also yielded negative results. Routine monitoring and sample collection procedures were conducted in collaboration with the wildlife staff of the Kenting National Park Headquarters and under the Headquarters' approval, strictly adhering to animal welfare guidelines.

Sample collection consistently took place during the cooler early morning hours to minimize heat stress. Chemical immobilization was achieved via intramuscular dart administration using a combination of ketamine (3 mg/kg) and xylazine (1.5 mg/kg). Blood samples were drawn within 10 min of immobilization via jugular venipuncture using an 18G × 1.5" needle and a 25 mL syringe. Body weight was consistently measured using a calibrated digital scale.

For hematologic analyses, whole blood was immediately transferred into 10 mL EDTA tubes (BD Vacutainer®, Becton, Dickinson and Company, USA). Serum samples were collected into additive-free 10 mL tubes of the same brand. All samples were transported to the laboratory within 4 h of collection. Serum was separated by centrifugation at 3000 × g for 15 min, and serum biochemical analyses commenced immediately thereafter. Hematologic analyses and blood smear evaluations were completed within 6–12 h of sample collection.

Hematologic and Biochemical Analysis: Hematologic parameters measured included red blood cell count (RBC), hemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), platelet count (PLT), total plasma protein (TPP), and fibrinogen concentration (Fib). Analyses for the period 2013–2021 (n=510; 238 males, 272 females) were performed using the Sysmex F-820 semi-automated hematology analyzer (TOA Medical Electronics Co., Ltd, Kobe, Japan). Subsequent samples collected from 2022 to 2024 (n=122; 46 males, 76 females) were processed with the Exigo EOS Vet hematology analyze (Boule Medical AB, Spanga, Sweden).

Serum biochemical parameters measured included glucose (GLU), AST, alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (CRE), lactate dehydrogenase (LDH), and gamma-glutamyltransferase (GGT). Analyses were conducted using three different analyzers over the study period. From 2013 to 2017, 251 samples were analyzed using the Kodak Ektachem DT60 (Eastman Kodak Co., Rochester, New York, USA). The AmiShield VCA-TC-100 (AmShield Biomed, Taoyuan, Taiwan) processed 259 samples collected between 2018 and 2021, and analyses for the 122 samples from 2022 to 2024 were performed using the SPOTCHEM™ EZ SP-4430 (ARKRAY, Inc., Kyoto, Japan).

Hematologic parameters measured included RBC, Hb concentration, PCV, MCV, MCH, MCHC, WBC, PLT, TPP, and Fib concentration. From 2013–2021 (n=510; 238 males, 272 females), analyses were performed using a Sysmex F-820 semi-automated hematology analyzer (TOA Medical Electronics Co., Ltd, Kobe, Japan). This analyzer underwent standardized annual calibration by Sysmex Taiwan Co., Ltd. Subsequent samples collected from 2022 to 2024 (n=122; 46 males, 76 females) were processed with an Exigo EOS Vet hematology analyzer (Boule Medical AB, Spanga, Sweden), also receiving standardized annual calibration by Syngen Biotech Co., Ltd.

Serum biochemical parameters measured included GLU, AST, ALT, BUN, CRE, LDH, and GGT. These analyses utilized three different analyzers over the study period. From 2013 to 2017, 251 samples were analyzed using the Kodak Ektachem DT60 (Eastman Kodak Co., Rochester, New York, USA), which underwent standardized annual calibration by Johnson & Johnson Taiwan Ltd. The AmiShield VCA-TC-100 (AmShield Biomed, Taoyuan, Taiwan) processed 259 samples from 2018 to 2021 and was calibrated annually by ProtectLife International Biomedical Inc. Analyses for the 122 samples from 2022 to 2024 were performed using the SPOTCHEM™ EZ SP-4430 (ARKRAY, Inc., Kyoto, Japan). This instrument was calibrated annually by Syngen Biotech Co., Ltd.; notably, each batch of its reagent strips included a unique calibration code for numerical correction, ensuring accuracy across different lots. Regular internal quality control measures were performed for all analyzers used in this study, adhering to manufacturer guidelines, to ensure consistency and reliability of results throughout the entire study period.

It is important to note that the comparison of analytical instruments was conducted using distinct cohorts of samples collected across different time periods, rather than matched samples from the same animals analyzed concurrently on multiple instruments. For hematology, samples processed by the Sysmex F-820 (2013–2021) were temporally distinct from those by the Exigo EOS Vet (2022–2024). Similarly, biochemistry samples analyzed by the Kodak Ektachem DT60 (2013–2017), AmiShield VCA-TC-100 (2018–2021), and SPOTCHEM™ EZ SP-4430 (2022–2024) belonged to different time strata.

We acknowledge that this temporal separation, even with individual deer identification, means biological variability inherent to the animals across years (e.g., age-related changes, seasonal or environmental factors) could contribute to observed instrument differences alongside true analytical variations. Our primary mitigation strategy involved establishing instrument-specific RIs, which inherently account for the characteristics of samples processed by each analyzer during its operational period. However, direct head-to-head instrument comparisons using

concurrently analyzed, paired samples would offer a more precise assessment of analytical bias. This limitation is further discussed in the Discussion section.

Statistical analysis: All statistical analyses were conducted using Microsoft Excel and SAS software (version 9.4). Outliers were identified and excluded using Tukey's method (k=3) to prevent skewing of RIs. Data distribution for each parameter was assessed for normality using the Shapiro-Wilk test (for n<50) or the Kolmogorov-Smirnov test (for n≥50). Appropriate statistical tests were then selected based on sample size and distribution.

Reference intervals (RIs) were calculated using the Reference Value Advisor Excel add-in, adhering to ASVCP guidelines. The central 95% RIs were determined, and 90% CIs for both lower and upper limits were calculated to indicate precision. Data distribution and any applied transformations were recorded for each parameter.

To evaluate the effects of analyzer, sex, and body weight on hematologic and biochemical variables, homogeneity of variances was assessed using Bartlett's test (for normally distributed data) or Levene's test (for non-normally distributed data). Group comparisons were subsequently performed using Student's t-test, Welch's t-test, or the Mann-Whitney U test, based on data distribution and variance equality. Linear regression analysis was used to assess the relationship between body weight and hematologic or biochemical parameters. For consistent body weight measurements, the same scale and assistants were used throughout data collection.

To account for repeated measures within individual deer and to evaluate the combined effects of sex, body weight, and instrument on hematologic and serum biochemical parameters, linear mixed-effects models (LMMs) were applied. Each deer (Deer_ID) was included as a random effect to control for intra-individual correlation, while sex, body weight, and instrument were specified as fixed effects. To facilitate the evaluation of body weight intervals, male and female deer body weights were categorized into quartiles (Q1, Q2, Q3, Q4) for assessment:

Q1 (lightest 25%): Male ≤37.75 kg; Female ≤27.75 kg.

Q2 (26%-50% lightest): Male ≤45 kg; Female ≤31 kg.

Q3 (51%-75% heaviest): Male ≤55 kg; Female ≤35 kg.

Q4 (heaviest 25%): Male >55 kg; Female >35 kg.

Models were evaluated for convergence and normality of residuals. Parameters for which models failed to converge or yielded unstable estimates were excluded from further interpretation.

Results

Establishment of hematologic and serum biochemical RIs: RIs for hematologic and serum biochemical parameters were established based on 632 samples collected between 2013 and 2024, following ASVCP guidelines. Detailed sample information is provided in Supplementary Table 1. To account for analytical and temporal variability, data were stratified by instrument type. Most parameters exhibited significant inter-instrument differences, except for PLT, which did not differ significantly between the Sysmex F-820 and Exigo EOS-VET analyzers (P = 0.485). Therefore, instrument-specific RIs were generated (Tables 5–9), with instrument comparisons shown in Tables 1–4.

Table 1 Comparison of hematologic parameters between analyzers in Formosan Sika Deer (Sysmex F-820 Semi-Automated Hematology Analyzer vs. Exigo EOS Vet Hematology Analyze).

Variables	Unit	Sysmex-F820		Exigo EOS-VET		Method	*P* -Value
		N	Mean ± SD	N	Mean ± SD		
PCV	(%)	510	33.48±32.75	122	26.48±27.10	t-test	< 0.001 ***
RBC	(10^6/μL)	510	8.59±8.49	122	7.71±7.850	t-test	< 0.001 ***
Hb	(g/dL)	510	10.13±10.00	122	10.53±10.50	t-test	0.011 *
MCV	(fL)	510	39.98±39.15	122	34.76±35.00	t-test	< 0.001 ***
MCH	(pg)	510	12.02±11.80	122	13.54±13.70	U-test	< 0.001 ***
MCHC	(g/dL)	510	30.51±39.35	122	39.09±39.10	U-test	< 0.001 ***
WBC	(10^3/μL)	146	7.05±5.68	122	3.45±3.20	U-test	< 0.001 ***
PLT	(10^3/μL)	364	240.40±65.50	122	358.90±329.50	U-test	0.485
TP	(g/dL)	445	327.10±7.50	122	180.70±177.50	U-test	< 0.001 ***
Fib	(mg/dL)	508	101.71±54.00	120	90.30±67.50	U-test	< 0.001 ***

Sysmex-F820: Sysmex F-820 semi-automated hematology analyzer, 2013-2021; Exigo EOS-VET: Exigo EOS Vet hematology analyze, 2022-2024; PCV: packed cell volume; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell; PLT: platelet; TP: total plasma protein; Fib: fibrinogen; Welch's t: Welch's t-test: t-test: Independent samples t-test; U-test: Mann–Whitney U test; *: P < 0.05; **: P < 0.01, ***: P < 0.001.

Table 2 Comparison of serum biochemical parameters between analyzers (Kodak Ektachem DT 60 vs. AmiShield VCA-TC-100) in Formosan Sika Deer.

Variables	Unit	Kodak Ektachem DT 60		AmiShield VCA-TC-100		Method	*P* -Value
		N	Mean ± SD	N	Mean ± SD		
GLU	(mg/dL)	51	201.88±188.00	259	54.44±53.00	U-test	< 0.001 ***
AST	(U/L)	251	82.60±66.00	259	30.20±29.00	U-test	< 0.001 ***
ALT	(U/L)	50	49.80±47.00	259	2.03±1.90	U-test	< 0.001 ***
BUN	(mg/dL)	251	26.97±21.70	259	7.75±7.80	U-test	< 0.001 ***
CRE	(mg/dL)	251	1.70±1.30	-	-	-	-
LDH	(U/L)	198	472.30±426.00	-	-	-	-
GGT	(U/L)	200	41.90±34.00	-	-	-	-

Kodak Ektachem DT 60 (2013-2017); AmiShield VCA-TC-100 (2018-2021); GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$.

Table 3 Comparison of serum biochemical parameters between analyzers (Kodak Ektachem DT 60 vs. SPOTCHEM™ EZ SP-4430) in Formosan Sika Deer.

Variables	Unit	Kodak Ektachem DT 60		SPOTCHEM™ EZ SP-4430		Method	*P* -Value
		N	Mean ± SD	N	Mean ± SD		
GLU	(mg/dL)	51	201.88±188.00	122	67.40±62.00	U-test	< 0.001 ***
AST	(U/L)	251	82.60±66.00	122	37.10±35.00	U-test	< 0.001 ***
ALT	(U/L)	50	49.80±47.00	122	1.65±1.60	U-test	< 0.001 ***
BUN	(mg/dL)	251	26.97±21.70	122	7.03±7.10	U-test	< 0.001 ***
CRE	(mg/dL)	251	1.70±1.30	122	167.90±118.00	U-test	< 0.001 ***
LDH	(U/L)	198	472.30±426.00	-	-	-	-
GGT	(U/L)	200	41.90±34.00	-	-	-	-

Kodak Ektachem DT 60 (2013-2017); SPOTCHEM™ EZ SP-4430 (2022-2024); GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$.

Table 4 Comparison of serum biochemical parameters between analyzers (AmiShield VCA-TC-100 vs. SPOTCHEM™ EZ SP-4430) in Formosan Sika Deer.

Variables	Unit	AmiShield VCA-TC-100		SPOTCHEM™ EZ SP-4430		Method	*P* -Value
		N	Mean ± SD	N	Mean ± SD		
GLU	(mg/dL)	259	54.44±53.00	122	67.40±62.00	U-test	< 0.001 ***
AST	(U/L)	259	30.20±29.00	122	37.10±35.00	U-test	< 0.001 ***
ALT	(U/L)	259	2.03±1.90	122	1.65±1.60	U-test	< 0.001 ***
BUN	(mg/dL)	259	7.75±7.80	122	7.03±7.10	U-test	< 0.001 ***
CRE	(mg/dL)	-	-	122	167.90±118.00	-	-
LDH	(U/L)	-	-	-	-	-	-
GGT	(U/L)	-	-	-	-	-	-

AmiShield VCA-TC-100 (2018-2021); SPOTCHEM™ EZ SP-4430 (2022-2024); GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$.

Table 5 Descriptive statistics and reference intervals for hematologic parameters in Formosan Sika Deer (2013–2021, Sysmex F-820 Semi-Automated Hematology Analyzer).

Parameter	Unit	Descriptive statistics						Central 95% RIs			
		N	Mean	SD	Median	Min	Max	Lower limit (with 90% CI)	Upper limit (with 90% CI)	Data Distribution	Method
PCV	(%)	510	33.48	32.75	7.37	10.30	76.90	18.99 (*17.73-20.18*)	47.98 (*46.24-49.75*)	G	UT-S
RBC	(10^6/μL)	510	8.59	8.49	1.87	2.49	17.54	4.92 (*4.66-5.16*)	12.26 (*11.92-12.63*)	G	UT-S
Hb	(g/dL)	510	10.13	10.00	1.95	1.60	19.00	6.30 (*6.00-6.59*)	13.96 (*13.64-14.27*)	G	UT-S
MCV	(fL)	510	39.98	39.15	7.28	4.30	93.40	25.66 (*23.77-28.07*)	54.30 (*51.24-56.86*)	G	UT-S
MCH	(pg)	510	12.02	11.80	1.88	7.90	21.30	8.94 (*8.79-9.11*)	16.31 (*15.94-16.69*)	NG	Box-Cox-S
MCHC	(g/dL)	510	30.51	29.35	5.24	16.90	87.20	23.22 (*22.70-23.81*)	41.96 (*40.19-43.58*)	NG	Box-Cox-S
WBC	(10^3/μL)	146	7.05	5.68	4.40	2.60	30.30	3.07 (*2.92-3.25*)	19.52 (*15.73-24.42*)	NG	Box-Cox-S
PLT	(10^3/μL)	364	240.40	65.50	362.33	12.40	2891.00	24.25 (*20.00-28.00*)	1060.75 (*972.00-542.00*)	NG	NP
TP	(g/dL)	445	327.10	7.50	789.64	5.50	8763.00	6.00 (*5.80-6.00*)	1708.70(*1396.00-2601.00*)	NG	NP
Fib	(mg/dL)	508	101.71	54.00	110.26	0	383.00	0.00 (*0.00-0.00*)	291.65 (*286.00-318.00*)	NG	NP

PCV: packed cell volume; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell; PLT: platelet; TP: total plasma protein; Fib: fibrinogen; CI: confidence interval; NG: Non-Gaussian; G: Gaussian; Untransformed: UT; Box-Cox: Box-Cox transformed data; NP: non-parametric; R: robust; S: standard.

Table 6 Descriptive statistics and reference intervals for hematologic parameters in Formosan Sika Deer (2022–2024, Exigo EOS-VET Hematology Analyzer).

Parameter	Unit	Descriptive statistics						Central 95% RIs			
		N	Mean	SD	Median	Min	Max	Lower limit (with 90% CI)	Upper limit (with 90% CI)	Data Distribution	Method
PCV	(%)	122	26.48	27.10	4.57	9.00	38.10	17.85 (16.32-19.51)	36.14 (34.43-37.80)	G	UT-R
RBC	(10^6/μL)	122	7.71	7.85	1.54	2.59	11.30	4.73 (4.29-5.21)	10.87 (10.34-11.32)	G	UT-R
Hb	(g/dL)	122	10.53	10.50	3.02	3.50	36.70	4.52 (2.06-7.09)	16.53 (13.65-19.84)	G	UT-S
MCV	(fL)	122	34.76	35.00	3.50	26.30	44.90	27.81 (26.87-28.80)	41.75 (40.87-42.63)	G	UT-R
MCH	(pg)	122	13.54	13.70	1.12	10.90	16.90	11.32 (11.08-11.60)	15.76 (15.46-16.03)	G	UT-S
MCHC	(g/dL)	122	39.09	39.10	1.33	35.30	42.40	36.44 (36.12-36.79)	41.74 (41.36-42.08)	G	UT-S
WBC	(10^3/μL)	122	3.45	3.20	1.31	1.30	8.00	1.55 (1.43-1.69)	6.80 (6.14-7.46)	NG	Box-Cox-S
PLT	(10^3/μL)	122	358.90	329.50	218.20	25.00	1098.00	47.10 (25.00-85.00)	1017.20 (822.00-1098.00)	G	NP
TP	(g/dL)	122	180.70	177.50	66.30	20.00	381.00	45.50 (28.30-64.70)	309.50 (291.40-328.80)	G	UT-R
Fib	(mg/dL)	120	90.30	67.50	89.30	20.00	738.00	25.50 (23.30-28.00)	307.70 (236.70-402.90)	NG	Box-Cox-S

PCV: packed cell volume; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell; PLT: platelet; TP: total plasma protein; Fib: fibrinogen; CI: confidence interval; NG: Non-Gaussian; G: Gaussian; Untransformed: UT; Box-Cox: Box-Cox transformed; NP: non-parametric; R: robust; S: standard.

Table 7 Descriptive statistics and reference intervals for serum biochemical parameters in Formosan Sika Deer (2013–2017, Kodak Ektachem DT 60).

Analyte	Unit	Descriptive statistics						Central 95% RIs			
		N	Mean	SD	Median	Min	Max	Lower limit (with *90% CI*)	Upper limit (with *90% CI*)	Data Distribution	Method
GLU	(mg/dL)	51	201.88	188.00	76.97	4.10	417.00	35.87 (4.10-120.00)	408.90 (326.00-417.00)	NG	NP
AST	(U/L)	251	82.60	66.00	70.60	3.00	733.00	22.60 (17.10-29.60)	235.80 (176.40-316.60)	NG	Box-Cox-S
ALT	(U/L)	50	49.80	47.00	14.20	23.00	95.00	21.00 (16.30-26.62)	78.60 (68.50-88.40)	G	UT-S
BUN	(mg/dL)	251	26.97	21.70	58.21	1.50	934.00	6.82 (4.89-10.93)	101.77 (50.80-192.77)	NG	Box-Cox-S
CRE	(mg/dL)	251	1.70	1.30	2.78	0.30	40.20	0.63 (0.55-0.72)	4.49 (3.38-6.18)	NG	Box-Cox-S
LDH	(U/L)	198	472.30	426.00	235.70	197.00	2900.00	241.90 (229.50-255.80)	997.60 (892.00-1117.90)	NG	Box-Cox-S
GGT	(U/L)	200	41.90	34.00	44.60	8.00	566.00	16.70 (15.00-18.70)	110.80 (88.40-143.10)	NG	Box-Cox-S

GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; CI: confidence interval; NG: Non-Gaussian; G: Gaussian; Untransformed: UT; Box-Cox: Box-Cox transformed; NP: non-parametric; R: robust; S: standard.

Table 8 Descriptive statistics and reference intervals for serum biochemical parameters in Formosan Sika Deer (2018–2021, AmiShield VCA-TC-100).

Analyte	Unit	Descriptive statistics						Central 95% RIs			
		N	Mean	SD	Median	Min	Max	Lower limit (with *90% CI*)	Upper limit (with *90% CI*)	Data Distribution	Method
GLU	(mg/dL)	259	54.44	53.00	14.39	14.00	150.00	26.04 (21.69-29.85)	82.84 (77.69-88.91)	G	UT-S
AST	(U/L)	259	30.20	29.00	7.39	14.00	64.00	18.08 (17.28-18.93)	47.10 (45.11-49.07)	NG	Box-Cox-S
ALT	(U/L)	259	2.03	1.90	0.53	1.10	5.20	1.30 (1.20-1.30)	3.35 (3.00-3.70)	NG	NP
BUN	(mg/dL)	259	7.75	7.80	1.17	3.70	10.00	5.45 (5.23-5.67)	10.06 (9.89-10.22)	G	UT-S
CRE	(mg/dL)	-	-	-	-	-	-	-	-	-	-
LDH	(U/L)	-	-	-	-	-	-	-	-	-	-
GGT	(U/L)	-	-	-	-	-	-	-	-	-	-

GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; CI: confidence interval; NG: Non-Gaussian; G: Gaussian; Untransformed: UT; Box-Cox: Box-Cox transformed; NP: non-parametric; R: robust; S: standard.

Table 9 Descriptive Statistics and Reference Intervals for Serum Biochemical Parameters in Formosan Sika Deer (2022–2024, SPOTCHEMTM EZ SP-4430).

Analyte	Unit	Descriptive statistics						Central 95% RIs			
		N	Mean	SD	Median	Min	Max	Lower limit (with *90% CI*)	Upper limit (with *90% CI*)	Data Distribution	Method
GLU	(mg/dL)	122	67.40	62.00	30.80	15.00	224.00	25.80 (23.10-28.90)	145.40 (129.90-161.70)	NG	Box-Cox-S
AST	(U/L)	122	37.10	35.00	14.30	15.00	83.00	16.10 (14.70-17.60)	71.00 (65.10-76.50)	NG	Box-Cox-S
ALT	(U/L)	122	1.65	1.60	0.49	0.80	3.20	0.85 (0.78-0.92)	2.79 (2.60-2.96)	NG	Box-Cox-S
BUN	(mg/dL)	122	7.03	7.10	0.72	5.20	8.80	5.60 (5.42-5.80)	8.46 (8.26-8.65)	G	UT-S
CRE	(mg/dL)	122	167.90	118.00	188.50	50.00	1500.00	50.00 (50.00-50.00)	655.20 (374.00-1500.00)	NG	NP
LDH	(U/L)	-	-	-	-	-	-	-	-	-	-
GGT	(U/L)	-	-	-	-	-	-	-	-	-	-

GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; CI: confidence interval; NG: Non-Gaussian; G: Gaussian; Untransformed: UT; Box-Cox: Box-Cox transformed; NP: non-parametric; R: robust; S: standard.

Differences in Hematologic and Serum Biochemical Parameters Between Sexes: Several parameters showed sex-related differences, with results varying across instruments and years. In hematologic data, female deer had significantly higher TPP (P = 0.011, 2013–2021, Sysmex F-820) and PLT (P = 0.007, 2022–2024, Exigo EOS-VET), while males exhibited higher Fib levels (P = 0.029, 2022–2024, Exigo EOS-VET). However, the observed difference in PLT could not be confirmed in the mixed-effects analysis due to convergence failure.

Among biochemical parameters, Kodak data (2013–2017) revealed sex differences in GLU (P < 0.001), AST (P < 0.001), ALT (P = 0.038), LDH (P = 0.019), and CRE (P = 0.007), with all but GLU being higher in females. Of these, only the LDH difference was supported by the mixed model. AmiShield data (2018–2021) showed higher values in females for GLU (P < 0.001), ALT (P < 0.001), and BUN (P < 0.001), though the ALT result could not be evaluated in the mixed model. Similarly, SPOTCHEM EZ data (2022–2024) showed differences in ALT (P = 0.012), BUN (P = 0.004), and CRE (P < 0.001); ALT again was excluded from model-based interpretation. Details are presented in Tables 10–14.

Table 10 Sex differences in hematologic parameters of Formosan Sika Deer (2013–2021, Sysmex F-820 Semi-Automated Hematology Analyzer).

Variables	Unit	Male		Female		Method	*P* -Value
		N	Mean ± SD	N	Mean ± SD		
PCV	(%)	238	32.77±7.12	272	34.10±7.54	Welch's t	0.467
RBC	(10^6/μL)	238	8.44±1.72	272	8.72±1.98	Welch's t	0.850
Hb	(g/dL)	238	9.88±1.69	272	10.34±3.13	Welch's t	0.955
MCV	(fL)	238	39.82±8.36	272	40.11±6.21	Welch's t	0.700
MCH	(pg)	238	12.01±1.95	272	12.03±1.81	U-test	0.408
MCHC	(g/dL)	238	30.81±6.04	272	30.24±4.41	U-test	0.596
WBC	(10^3/μL)	66	6.83±4.32	80	7.23±4.48	U-test	0.937
PLT	(10^3/μL)	172	254.19±377.53	192	228.05±348.68	U-test	0.317
TP	(g/dL)	210	322.22±834.91	235	331.45±748.65	U-test	0.011 *
Fib	(mg/dL)	238	104.33±113.88	270	99.41±107.12	U-test	0.150

PCV: packed cell volume; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell; PLT: platelet; TP: total plasma protein; Fib: fibrinogen; Welch's t: Welch's t-test: t-test: Independent samples t-test; U-test: Mann–Whitney U test; *: P < 0.05; **: P < 0.01, ***: P < 0.001.

Table 11 Sex differences in hematologic parameters of Formosan Sika Deer (2022–2024, Exigo EOS-VET Hematology Analyzer).

Variables	Unit	Male		Female		Method	*P* -Value
		N	Mean ± SD	N	Mean ± SD		
PCV	(%)	46	25.70±4.87	76	26.95±4.34	t-test	0.289
RBC	(10^6/μL)	46	7.48±1.63	76	7.85±1.48	Welch's t	0.914
Hb	(g/dL)	46	10.02±1.95	76	10.83±3.49	Welch's t	0.918
MCV	(fL)	46	34.67±3.14	76	34.81±3.72	t-test	0.987
MCH	(pg)	46	13.52±1.07	76	13.55±1.15	Welch's t	0.998
MCHC	(g/dL)	46	39.05±1.37	76	39.12±1.32	t-test	0.855
WBC	(10^3/μL)	46	3.46±1.23	76	3.44±1.36	U-test	0.565
PLT	(10^3/μL)	45	76.27±59.37	76	98.65±102.62	t-test	0.007 **
TP	(g/dL)	46	399.87±226.99	76	334.03±210.41	U-test	0.052
Fib	(mg/dL)	46	195.22±58.73	76	171.92±69.39	t-test	0.029 *

PCV: packed cell volume; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell; PLT: platelet; TP: total plasma protein; Fib: fibrinogen; Welch's t: Welch's t-test: t-test: Independent samples t-test; U-test: Mann–Whitney U test; *: P < 0.05; **: P < 0.01, ***: P < 0.001.

Table 12 Sex differences in serum biochemical parameters of Formosan Sika Deer (2013–2017, Kodak Ektachem DT 60).

Variables	Unit	Male		Female		Method	*P* -Value
		N	Mean ± SD	N	Mean ± SD		
GLU	(mg/dL)	27	217.15±88.66	24	184.71±58.41	Welch's t	< 0.001***
AST	(U/L)	120	69.18±66.54	131	94.91±72.13	U-test	< 0.001***
ALT	(U/L)	27	47.33±14.14	23	52.61±14.03	t-test	0.038*
BUN	(mg/dL)	120	29.73±83.64	131	24.45±9.98	U-test	0.074
CRE	(mg/dL)	120	1.58±0.63	131	18.20±3.81	U-test	0.007**
LDH	(U/L)	91	457.85±286.24	107	484.64±182.64	t-test	0.019*
GGT	(U/L)	93	48.15±62.44	107	36.37±16.96	U-test	0.528

GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; Welch's t: Welch's t-test: t-test: Independent samples t-test; U-test: Mann–Whitney U test; *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$.

Table 13 Sex differences in serum biochemical parameters of Formosan Sika Deer (2018–2021, AmiShield VCA-TC-100).

Variables	Unit	Male		Female		Method	*P* -Value
		N	Mean ± SD	N	Mean ± SD		
GLU	(mg/dL)	118	53.06±14.45	141	55.60±14.29	Welch's t	< 0.001***
AST	(U/L)	118	30.24±7.60	141	30.16±7.23	U-test	0.930
ALT	(U/L)	118	2.13±0.50	141	1.95±0.55	U-test	< 0.001***
BUN	(mg/dL)	118	7.91±1.20	141	7.61±1.13	t-test	< 0.001***
CRE	(mg/dL)	-	-	-	-	-	-
LDH	(U/L)	-	-	-	-	-	-
GGT	(U/L)	-	-	-	-	-	-

GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; Welch's t: Welch's t-test: t-test: Independent samples t-test; U-test: Mann–Whitney U test; *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$.

Table 14 Sex differences in serum biochemical parameters of Formosan Sika Deer (2022–2024, SPOTCHEM EZ SP-4430).

Variables	Unit	Male		Female		Method	*P* -Value
		N	Mean ± SD	N	Mean ± SD		
GLU	(mg/dL)	46	62.48±27.47	76	70.38±32.49	U-test	0.076
AST	(U/L)	46	38.67±15.65	76	36.08±13.42	U-test	0.277
ALT	(U/L)	46	1.83±0.55	76	1.54±0.43	U-test	0.012 *
BUN	(mg/dL)	46	7.01±0.74	76	7.04±0.71	t-test	0.004 **
CRE	(mg/dL)	46	252.52±273.47	76	116.61±73.30	U-test	< 0.001 ***
LDH	(U/L)	-	-	-	-	-	-
GGT	(U/L)	-	-	-	-	-	-

GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; Welch's t: Welch's t-test: t-test: Independent samples t-test; U-test: Mann–Whitney U test; *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$.

Influence of Body Weight on Hematologic and Serum Biochemical Parameters: Linear regression identified several parameters that were positively correlated with body weight (Tables 15-16). RBC, Hb, and PCV showed strong associations, especially RBC in the 2022–2024 Exigo EOS-VET dataset ($R^2 = 0.208$). In the Sysmex F-820 data (2013–2021), all evaluated hematologic parameters were significantly associated with body weight ($P < 0.001$). In biochemical parameters, GLU and ALT displayed positive trends across datasets. TPP was significantly associated with weight only in the 2022–2024 data ($R^2 = 0.016$, $P < 0.001$). CRE showed significant correlations in the 2013–2017 and 2022–2024 datasets, and BUN had a weak but significant correlation ($R^2 < 0.08$).

Combined Effects of Sex and Body Weight, and Analyzer on Hematologic and Serum Biochemical Parameters: Linear mixed-effects models (LMMs) were employed to assess the influences of sex, body weight, and analytical instruments on various hematological and serum biochemical parameters in deer. Individual deer and sampling year were included as random effects to account for repeated measurements and temporal variability. Table 17 summarizes the estimated variances for these random effects and the *P* -values from the Type 3 Tests of Fixed Effects. Further model details are in Supplementary Table 2.

The LMMs analysis revealed several significant fixed effects. While sex alone significantly influenced only WBC counts ($P = 0.012$), a more pronounced effect was observed through the highly significant interaction between body weight and sex for RBC ($P < 0.001$), Hb ($P < 0.001$), PCV ($P < 0.001$), MCV ($P < 0.001$), MCH ($P < 0.001$), and Fib ($P < 0.001$). For these parameters, increasing body weight correlated with a decrease in values for both sexes, but the decline was distinctly steeper in females (e.g., RBC: -0.09369 for females vs. -0.06528 for males). This highlights the sex-specific impact of body weight on these indicators.

The analytical instrument also significantly influenced several parameters. The hematology analyzer affected RBC, PCV, MCV, MCHC, and WBC (all $P < 0.05$). Similarly, the serum chemistry analyzer significantly impacted GLU, AST, ALT, BUN, and CRE (all $P < 0.001$). For LDH and GGT, the serum chemistry analyzer's effect was not estimable (NA). No significant effects were detected for TPP and PLT counts. These findings collectively underscore the complex interplay of biological and technical factors on deer hematological and biochemical profiles.

Table 15 Results of linear regression analysis: hematologic variables and body weight in Formosan Sika Deer.

Models	Variables	N	R^2	ANOVA *P* -value	Body Weight Coefficient (β)	*P* -value
Sysmex-F820 (2013-2021)	PCV	509	0.038	< 0.001***	47.967	< 0.001***
	RBC	509	0.078	< 0.001***	52.485	< 0.001***
	Hb	509	0.043	< 0.001***	50.295	< 0.001***
	MCV	509	0.007	0.064	32.689	< 0.001***
	MCH	509	0.018	0.002**	28.186	< 0.001***
	MCHC	509	< 0.001	0.657	39.228	< 0.001***
	WBC	145	0.030	0.038*	40.794	< 0.001***
	Plt	363	0.003	0.295	38.534	< 0.001***
	TPP	444	< 0.001	0.648	38.266	< 0.001***
	Fib.	507	0.023	< 0.001***	36.335	< 0.001***
Exigo EOS-VET (2022-2024)	PCV	121	0.064	0.003**	53.511	< 0.001***
	RBC	121	0.208	< 0.001***	62.025	< 0.001***
	Hb	121	0.034	0.044 *	42.826	< 0.001***
	MCV	121	0.167	< 0.001***	-12.094	0.218
	MCH	121	0.181	< 0.001***	-24.913	0.037 *
	MCHC	121	0.043	0.023 *	106.638	< 0.001 ***
	WBC	121	0.014	0.199	31.724	< 0.001***
	Plt	119	< 0.001	0.785	34.985	< 0.001***
	TPP	121	0.016	0.161	37.829	< 0.001***
	Fib.	121	0.029	0.060	29.892	< 0.001***

Sysmex-F820: Sysmex F-820 semi-automated hematology analyze, 2013-2021; Exigo EOS-VET: Exigo EOS-VET hematology analyzer, 2022-2024; PCV: packed cell volume; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell; PLT: platelet; TP: total plasma protein; Fib: fibrinogen; ***:** $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$.

Table 16 Results of linear regression analysis: serum biochemical variables and body weight in Formosan Sika Deer

Models	Variables	N	R^2	ANOVA *P* -value	Body Weight Coefficient (β)	*P* -value
Kodak Ektachem DT 60 (2013-2017)	GLU	50	0.179	0.002***	24.200	< 0.001***
	AST	250	0.007	0.198	38.635	< 0.001***
	ALT	49	0.009	0.513	42.443	< 0.001***
	BUN	250	0.002	0.510	37.278	< 0.001***
	CRE	250	0.000	0.937	37.380	< 0.001***
	LDH	197	< 0.001	0.764	36.717	< 0.001***
	GGT	199	0.036	0.007**	35.109	< 0.001***
AmiShield VCA-TC-100 (2018-2021)	GLU	258	0.118	< 0.001***	52.325	< 0.001***
	AST	258	0.002	0.452	40.359	< 0.001***
	ALT	258	0.104	< 0.001***	25.022	< 0.001***
	BUN	258	0.077	< 0.001***	18.388	< 0.001***
	CRE	N/A	N/A	N/A	N/A	N/A
	LDH	N/A	N/A	N/A	N/A	N/A
	GGT	N/A	N/A	N/A	N/A	N/A
SPOTCHEM™ EZ SP-4430 (2022-2024)	GLU	121	0.013	0.215	38.274	< 0.001***
	AST	121	0.030	0.058	30.111	< 0.001***
	ALT	121	0.186	< 0.001***	18.552	< 0.001***
	BUN	121	0.007	0.378	26.070	0.015 *
	CRE	121	0.120	< 0.001***	31.768	< 0.001***
	LDH	N/A	N/A	N/A	N/A	N/A
	GGT	N/A	N/A	N/A	N/A	N/A

Kodak Ektachem DT 60: 2013-2017; AmiShield VCA-TC-100: 2018-2021; SPOTCHEM™ EZ SP-4430: 2022-2024; GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; N/A: Not applicable; *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$

Table 17 Random effects variance estimates and fixed effects test results from linear mixed-effects models for hematological and biochemical parameters.

Random Effects				Fixed Effects		
Analyzed Parameters	**Deer ID**	**Year**	**Sex effect (*P* -value)**	**Body Weight*Sex effect (*P* -value)**	**Hematology Alyzer effect (*P* -value)**	**Serum Chemistry Alyzer effect (*P* -value)**
RBC	0.219	0.422	0.592	< 0.001***	0.046 *	N/A
Hb	0.186	0.751	0.204	< 0.001 ***	0.633	N/A
PCV	3.565	6.280	0.905	< 0.001***	0.002 **	N/A
MCV	0	3.177	0.551	< 0.001***	0.003 **	N/A
MCH	0.003	1.474	0.547	< 0.001***	0.073	N/A
MCHC	0.383	10.716	0.502	0.796	0.003**	N/A
WBC	5.101	1.148	0.443	0.247	0.012 *	N/A
TPP	0	114143	0.082	0.213	0.736	N/A
PLT	1356.690	69605	0.409	0.086	0.396	N/A
Fib	267.460	9331.650	0.729	< 0.001***	0.178	N/A
GLU	23.818	101.080	0.790	0.981	N/A	<0.001***
AST	0	36.304	0.950	0.774	N/A	<0.001***
ALT	0	92.015	0.853	0.809	N/A	<0.001***
BUN	436.660	0	0.747	0.758	N/A	<0.001***
CRE	0	408.250	0.829	0.053	N/A	<0.001***
LDH	0	1713.980	0.738	0.949	N/A	N/A
GGT	97.764	130.610	0.746	0.511	N/A	N/A

PCV: packed cell volume; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell; PLT: platelet; TP: total plasma protein; Fib: fibrinogen; GLU: Glucose; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: Lactate Dehydrogenase; GGT: Gamma-Glutamyl transferase; N/A: Not applicable; *: $P < 0.05$; **: $P < 0.01$, ***: $P < 0.001$

Discussion

This study retrospectively analyzed hematological and serum biochemical results from 632 adult Formosan sika deer. We collected these data over twelve years (2013–2024) from a semi-captive deer population within the Formosan sika deer conservation area of Kenting National Park. Our primary aim was to establish population-specific hematologic and serum biochemistry RIs for this group, adhering to guidelines from the American Society for Veterinary Clinical Pathology (ASVCP) and recommendations from the literature (Geffre *et al*., 2009; Friedrichs *et al*., 2012).

However, interpreting these RIs is subject to several limitations, primarily stemming from the retrospective nature of data collection and the sequential use of multiple analytical instruments across the extended study period. Equipment upgrades necessitated using different analyzers, which led to observed inter-instrument differences likely attributable to variations in calibration, reagents, or technique. Compounding this, the retrospective design precluded direct method comparison studies to quantify analytical bias, as comparisons were inherently made using distinct sample cohorts collected across different time periods rather than concurrently analyzed, paired samples. This limitation directly contrasts with the rigorous methodology for method comparison emphasized in guidelines for establishing RIs (Friedrichs *et al*., 2012). This temporal separation, even with individual deer identification, means that inherent biological variability in the animals across years (e.g., age-related changes, seasonal influences, or other environmental factors) could also contribute to the observed instrument differences, alongside true analytical variations (Yochem *et al*., 2008; Graesli *et al*., 2015). Furthermore, while including repeated samples from individuals across multiple years was essential for capturing long-term population variability, it inherently introduces a degree of statistical dependence, a common characteristic of longitudinal data that, if ignored, can lead to biased estimates and invalid statistical inferences (Shott, 2011). Despite these significant analytical and statistical challenges, we adhered to ASVCP guidelines by generating separate RIs for each analyzer used for each parameter (Geffre *et al*., 2009; Friedrichs *et al*., 2012). Future research should prioritize developing conversion equations to enhance the comparability of data across different instruments and the clinical utility of these RIs.

Results indicated significant sex-based differences in several parameters. Females exhibited higher total TPP (2013–2021, Sysmex F-820) and PLT count (2022–2024, Exigo EOS-VET), while males displayed higher fibrinogen (Fib) levels (2022–2024, Exigo EOS-VET). These findings may reflect physiological changes related to coagulation and protein metabolism during reproductive periods, consistent with trends in wild and captive cervids and other ungulates (Chapple, 1989; Cross *et al*., 1994; Nie *et al*., 2023).

Serum biochemical analysis also revealed sex-based differences in AST, ALT, LDH, and CRE concentrations depending on the analyzer and period. While females exhibited higher concentrations of AST, ALT, LDH, and CRE only with the Kodak Ektachem DT 60 (2013–2017 data), some parameters showed the opposite trend with other analyzers. These patterns partially align with cervid studies showing elevated enzyme activities in reproductive females compared to males, although results varied depending on the specific analyzer and time period (Topal *et al*., 2010; Nie *et al*., 2020).

Body weight correlated positively with erythrocytic indices RBC, Hb, PCV, GLU, and ALT (Tables 15–16). These positive correlations suggest increased oxygen-carrying capacity and metabolic

demands in heavier individuals, potentially linked to age, nutrition, or dominance, consistent with similar associations reported in farmed deer and other wildlife (Karpinski *et al*., 2023). CRE correlated significantly with body weight in both 2013–2017 and 2022–2024 subsets, while BUN, despite some significance, had low explanatory power ($R^2 < 0.08$). This suggests a weak association between urea metabolism and body mass in this population.

Stress-related effects on GLU and ALT warrant careful consideration, even with chemical immobilization. Acute capture and handling stress can activate the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system, leading to catecholamine and cortisol surges (Sapolsky *et al*., 2000). Furthermore, ALT increases might reflect muscle damage from capture exertion rather than solely a hepatic origin (Paterson, 2014). Although the ketamine-xylazine protocol minimizes handling stress, pre-anesthesia agitation or struggling during darting could still transiently influence these parameters in some individuals. Therefore, the potential for stress-induced elevations in GLU and ALT should be acknowledged when interpreting these values in captured wild animals.

Moreover, our mixed-effects model revealed negative associations between body weight and certain hematologic variables, including RBC, Hb, and PCV (Supplementary Table 2). While these parameters typically increase with metabolic demands and larger body mass, such inverse correlations may reflect acute physiological responses under field immobilization. Lighter individuals, potentially more vulnerable or less conditioned, may experience heightened sympathetic-adrenal responses during darting and capture. This can trigger splenic contraction and lead to transient elevations in circulating erythrocyte indices (Cross *et al*., 1988; Potocnik and Wintour, 1996; Gupta *et al*., 2007; Pernett *et al*., 2021). In cervids, particularly under stress, splenic reservoirs play a critical role in modulating peripheral red cell counts, often confounding baseline hematologic interpretations in field conditions (Mentaberre *et al*., 2010; Johns, 2018).

Therefore, in semi-captive settings where chemical restraint is necessary, body weight not only reflects general health status but may also serve as a proxy for individual stress susceptibility. The observed inverse trends may thus represent complex interactions between physiological condition, capture stress, and sex-based behavioral responses. These findings underscore the importance of contextualizing hematologic data with respect to capture methodology and individual-level variation when evaluating wildlife health metrics.

The associations identified between body weight and parameters like RBC, Hb, TPP, and GLU reinforce their value as indirect markers of nutritional or metabolic status and overall physical condition in wildlife health assessments. In resource-limited field settings without extensive individual histories, using blood-based metrics alongside body weight provides valuable insight into population-level health trends, nutritional plane, and potential responses to environmental changes. These data are essential for informing conservation management decisions, including population monitoring, reintroduction success evaluation, and disease surveillance.

In summary, this study provides the first large-sample, analyzer-stratified hematologic and biochemical RIs for semi-free-ranging Formosan sika deer in Kenting. It highlights the significant influence of the analytical platform, sex, and body weight on these parameters. The findings provide crucial diagnostic benchmarks and robust reference data for veterinarians and wildlife managers, supporting health monitoring, improved diagnostic accuracy, and informed conservation decisions, while also emphasizing considerations for interpreting data collected across different instruments and time.

Data Availability Statement: Supplementary tables 1 and 2 are available on request from the corresponding author.

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