UNIVERSITI PUTRA MALAYSIA

REFERENCE INTERVAL VALUES FOR HAEMATOLOGY AND SERUM BIOCHEMISTRY PARAMETERS OF BORNEAN SUN BEARS

STEPHANIE LAVANIA PETRUS

REFERENCE INTERVAL VALUES FOR HAEMATOLOGY AND SERUM BIOCHEMISTRY PARAMETERS OF BORNEAN SUN BEARS

STEPHANIE LAVANIA PETRUS

A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfillment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia,

Serdang, Selangor Darul Ehsan.

MARCH 2016

CERTIFICATION

It is hereby certified that we have read this project paper entitled "Reference Interval Values for Haematology and Serum Biochemistry Parameters of Bornean Sun Bears", by Stephanie Lavania Petrus and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project

DR HAZILAWATI HAMZAH

DVM (UPM), MVSc (UPM), PhD (Murdoch)

Senior Lecturer

Department of Veterinary Pathology & Microbiology

Faculty of Veterinary Medicine, Universiti Putra Malaysia

(Supervisor)

MR WONG SIEW TE, D.J.N.

MSc (Montana)

Bornean Sun Bear Conservation Centre (BSBCC)

Founder & Chief Executive of BSBCC

(Co-Supervisor)

PROF DR MOHD ARIFF OMAR
BSc (Lousiana), MSc (Oklahoma), PhD (Texas)
Professor
Department of Veterinary Pre Clinical Science
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

DR. REUBEN SUNIL KUMAR SHARMA
DVM (UPM), MSc (UPM), PhD (Cambridge)
Senior Lecturer
Department of Veterinary Pre Clinical Science
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

PROF. DR. NOORDIN MOHAMED MUSTAPHA
DVM (UPM), M.Sc. (UPM), Ph.D. (Murdoch)
Professor
Department of Veterinary Pathology & Microbiology
Faculty of Veterinary Medicine, Universiti Putra Malaysia
(Co-Supervisor)

DR. LAURA BENEDICT
DVM (UPM)
Wildlife Veterinarian,
Wildlife Rescue Unit,
Sabah Wildlife Department
(Co-Supervisor)

DEDICATIONS

This project paper is dedicated to God Almighty, through whom all things are possible,

To my parents, who are the reason I am where I am with endless love and support,

To all my teachers who have committed themselves towards the noble cause of education.

ACKNOWLEDGEMENTS

It is with deepest appreciation and gratitude that I thank God for making this project possible.

I would like to thank my project supervisor, Dr. Hazilawati Hamzah from the bottom of my heart for the round-the-clock dedication, time, wisdom and unfailing patience that she had granted me throughout the duration of this project.

To my co-supervisors, Prof. Dr. Mohd Ariff Omar, Dr Reuben Sunil Kumar Sharma and Prof. Dr. Noordin Mohamed Mustapha and Dr Laura Benedict for their unceasing support and guidance to improve the project.

My deepest gratitude to Mr Wong Siew Te, without whom this project would have not materialized. Thank you for the sea of information and providing all the materials I needed for this project.

Special thanks to Dr Pakeeyaraj Nagalingam, Tee Thy Lim and Wong Lin May for their enthusiastic assistance and providing essential information.

A special thank you to all my classmates of DVM 2016 who assisted me directly or indirectly in this project, especially Ayesha Shafinaz Azlan, without whom I would not have been able to do this project.

Last but not least, my most heartfelt gratitude to my family and friends for their relentless love and support throughout my final year project.

Content

List of Tables

List of Figures

List of Appendices

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

SELANG RUJUKAN UNTUK PARAMETER HEMATOLOGI DAN SERUM BIOKIMIA BERUANG MATAHARI BORNEO

Oleh

Stephanie Lavania Petrus

2016

Penyelia: Dr. Hazilawati Hamzah

Penyelia bersama: Prof. Dr. Mohamed Ariff Omar, Dr. Reuben Sharma, Dr. Laura Benedict, Prof. Dr. Noordin Mohamed Mustapha

Beruang matahari (*Helarctos malayanus euryspilus*) yang terdapat di Borneo adalah berbeza daripada beruang-beruang di tanah besar Asia dan Sumatera (*Helarctos malayanus malayanus*), yang mewakili bentuk biasa. Nilai-nilai rujukan parameter darah yang kini digunakan tidak khusus kepada subspesies *Helarctos malayanus euryspilus* di Borneo. Oleh itu, terdapat keperluan untuk mewujudkan nilai-nilai rujukan untuk hematologi dan biokimia serum untuk *Helarctos malayanus euryspilus*, yang berguna dalam diagnosis penyakit dan penilaian status kesihatan Borneo beruang matahari. Sebanyak 65 keputusan hematologi dan serum biokimia untuk beruang matahari yang sihat dari segi klinikal berdasarkan rekod yang terkumpul antara tahun 2006-2015 di Pusat Konservasi Beruang Matahari Borneo (BSBCC) Sepilok, Sandakan, Sabah

(5˚51'47.9 "N 117˚5'57.8" E) telah digunakan untuk kajian ini. Perbandingan statistik antara jantina, kumpulan umur (neonat, juvana, remaja, dan dewasa), puasa dan tidak puasa, serta keadaan hidup beruang '(dalam kurungan atau liar) telah dilaksanakan. Bagi mewujudkan selang rujukan, had rujukan terendah dan teratas telah dikira dengan min ± 2 sisihan piawai untuk data bertaburan Gaussian, dan nilai-nilai yang paling ekstrem rendah dan tinggi menjadi had rujukan bagi data tidak bertaburan Gaussian. Keputusan menunjukkan nilai lipoprotein berketumpatan tinggi (HDL) dalam neonat lebih tinggi ($p<0.05$) daripada kumpulan remaja. Kreatinin dalam neonat adalah jauh lebih rendah ($p<0.05$) daripada sub dewasa. Kiraan platelet dan tahap fosfat berkurangan dengan meningkatnya umur, manakala protein penuh dan globulin meningkat secara ketara dengan usia. Fosfatase alkali (ALP) dalam neonat adalah lebih tinggi berbanding kumpulan umur lain. Keputusan juga menunjukkan beruang dalam kurungan mempunyai jumlah kolesterol dan ALP yang lebih tinggi ($p<0.05$) daripada beruang liar. Keputusan kajian ini adalah sedikit berbeza dengan nilai-nilai Rujukan Selang Fisiologi ISIS 2013, yang diwujudkan untuk *Helarctos malayanus*.

Kata kunci: Hematologi, biokimia serum, beruang matahari Malaya, *Helarctos malayanus euryspilus*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 – Project.

REFERENCE INTERVAL VALUES FOR HAEMATOLOGY AND SERUM BIOCHEMISTRY PARAMETERS OF BORNEAN SUN BEARS

By

Stephanie Lavania Petrus

2016

Supervisor: Dr Hazilawati Hamzah

Co-Supervisors: Prof Dr Mohamed Ariff Omar, Dr. Reuben Sharma, Dr Laura Benedict and Prof Dr Noordin Mohamed Mustapha

Sun bears in Borneo (*Helarctos malayanus euryspilus*) are different from those in the Asian mainland and Sumatra (*Helarctos malayanus malayanus*) which represent the typical form. Blood parameter reference values currently in use are not specific to the *Helarctos malayanus euryspilus* subspecies in Borneo. Therefore, there is a need to establish reference values for haematology and serum biochemistry parameters for *Helarctos malayanus euryspilus*, which will be useful in the diagnosis of disease and assessment of health status of Bornean sun bears. A total of 65 haematology and serum biochemistry results of 44 sun bears that were clinically healthy based on the records between 2006 to 2015 from the Sepilok Bornean Sun Bear Conservation Centre (BSBCC) in Sandakan, Sabah (5˚51'47.9"N 117˚5'57.8"E) were used in this study. Statistical comparison between sex (male, female), age group (neonate, juvenile, sub adult and adult), state of fasting (fasting and non-fasting) and the bears' life condition (captive or wild) was also performed. The lower and upper reference limits for parameters with Gaussian distribution were set at mean ± 2 standard deviations and for parameters that were not normally distributed most extreme lower and upper values were used. Results showed high-density lipoprotein (HDL) levels in neonates are higher ($p<0.05$) than sub adults. Creatinine level in neonates was significantly lower than sub adults. Platelet count and phosphate level decreased ($p<0.05$) with age, while total protein and globulin significantly increased with age. Alkaline phosphatase (ALP) in neonates was higher ($p<0.05$) than the other age groups. Result also showed captive bears had higher ($p<0.05$) total cholesterol and ALP levels than wild bears. Results of this study were slightly different than ISIS Physiological Reference Intervals 2013 established for *Helarctos malayanus*.

Keywords: Haematology, serum biochemistry, Malayan sun bear, *Helarctos malayanus euryspilus*

1.0 INTRODUCTION

Sun bears in Borneo (*Helarctos malayanus euryspilus*) are different from those in the Asian mainland and Sumatra, which represent the typical form (*Helarctos malayanus malayanus*), and warrant a sub species differentiation (Meijaard, 2004). The Bornean species is smaller, almost half the size of the Asian mainland and Sumatran sun bear and there are craniometrics differences between the two subspecies.

Although quantitative data on population size or trend are lacking, it is suspected that the global population of sun bears has declined by more than 30% over the past 30 years, equivalent to three bear generations. Deforestation has reduced the area of occupancy and extent of occurrence of sun bears, and has also reduced their habitat quality in the remaining forests. This animal species is currently listed as a 'vulnerable' according to the International Union for Conservation of Nature (IUCN) (IUCN, 2008). Tropical evergreen rainforest is the sun bears' main habitat in Borneo, Sumatra, and Peninsular Malaysia (Chauhan, 2006; Gong and Harris, 2006).

Retrospective data obtained from the Bornean Sun Bear Conservation Centre (BSBCC), which is the only sun bear conservation centre in the world, were used in this study. The BSBCC was founded in Sabah, Malaysia in 2008 with the aims to provide care and rehabilitation of rescued sun bears, and to increase awareness of sun bears internationally. The Centre is currently home to 37 rescued sun bears. All bears at this centre are orphaned and/or ex-captive bears.

Sun bears are the least researched bear species in the world. On-going research is key to increasing this species protection. Haematological and serum biochemistry profiles of this animal are becoming increasingly important as tools for physiological studies in this species (BSBCC, 2015).

Blood parameter reference values currently used are based on the International Species Information System (ISIS) and not specific to the *Helarctos malayanus euryspilus* subspecies in Borneo (Teare, 2013), and there is very little information on Malayan sun bear haematology and serum biochemistry values. The data presented in this study will provide baseline reference values that are useful in the diagnosis of diseases and assessment of health status of Malayan sun bears.

The objective of this study is to establish reference values for haematology and serum biochemistry profiles for the Bornean sun bear (*Helarctos malayanus euryspilus).* This study was conducted to assess variation of the normal haematology and serum biochemistry ranges of *Helarctos malayanus* from *Helarctos malayanus euryspilus.*

2.0 LITERATURE REVIEW

2.1 Population and distribution

Helarctos malayanus sun bears can be found in Laos, Peninsular Malaysia, Thailand, Myanmar (Burma), Kampuchea (Cambodia), Sumatra, Borneo, Vietnam, and Bangladesh (Mills and Servheen, 1991). Malayan sun bear numbers are declining drastically due to habitat destruction and poaching for bear parts used in exotic foods, medicines, or aphrodisiacs (Mills and Servheen, 1991). Japan and Korea have demand for bear gall bladders for medicinal uses which may be factoring the threatened status of this species (Payne and Andau, 1991). The exact number of individuals in the wild is unknown. However, *H. malayanus* is listed as vulnerable according to the International Union on the Conservation of Nature and Natural Resources (Corbet and Hill, 1992) under Appendix I species of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

2.2 Ecology and behaviour

In Thailand, *H. malayanus* inhabits dense forests up to 2,500 m. In Borneo, it occurs up to 2,300 m (Corbet and Hill, 1992) and in Indonesia, up to 2,800 m (Santiapillai, 1988). In Sabah on the Borneo island, *H. malayanus* is predominantly found in dipterocarp forests but can also be located in lower montane and swamp forests (Payne and Andau, 1991). *Helarctos malayanus* is the most arboreal of the ursids and an opportunistic omnivore. It feeds on fruits, small vertebrates, and honey (Ewer, 1973). *H. malayanus* uses its claws to tear open hollow trees to obtain honey and bee larvae (Cranbrook, 1991). *H. malayanus* is opportunistic (Servheen, 1993) and will eat almost anything, including crops such as manioc and sweet potatoes (Harrisson, 1949). Malayan sun bears also eat the heart of coconut palms (Fitzgerald *et al*., 2002).

The Malayan sun bear in the wild is solitary (Ortolani and Caro, 1996) and largely nocturnal (Lekagul and McNeely, 1977), but its activity could be a response to human perturbations. It was found that the bears were largely diurnal in an unperturbed area and strictly nocturnal at a heavily travelled site (Griffiths and van Schaik, 1993).

2.3 Species differentiation

The Malayan sun bear is the smallest of the eight living bear species. Adults are about 120–150 cm long and weigh 27–65 kg (Stirling, 1993). Pocock (1941) stated that based on measurements, due to the smaller size of the Bornean type of sun bear it is accurate to regard it as a distinct sub-species.

Craniometric differences have been evaluated in Malayan sun bear skulls. Skull of the Bornean sun bear was significantly smaller than those from Sumatra and the Asian mainland, while its relatively large maxillary teeth further distinguished the Bornean from the Sumatran form (Meijaard, 2004). This showed that Bornean specimens stood out compared to Sumatran specimens because of their relatively long upper tooth row in relation to skull length. The Bornean specimens of both sexes were significantly smaller than the Asian and Sumatran specimens while Sumatran and Asian/Peninsular Malaysian specimens were similar in size (Meijaard, 2004). This is suggestive of the divergence of the Bornean sun bears from the other two geographic groups before they diverged from each other. The Bornean environment is possibly a cause for the difference between them and from that in Sumatra, mainland Asia, and the Malay Peninsula, which could have resulted in the morphological differentiation of the Bornean form from the rest (Meijaard, 2004).

2.4 Haematology and Serum Biochemistry

Monitoring the clinical pathology parameters is a very basic way of evaluating and monitoring the health status of any animal. Haematology and serum biochemical analyses could be used to assess health, disease status, nutritional status, habitat quality and stress levels of a wild population (Hanks, 1981). Blood parameters can also be used to predict survivability in reintroduction and translocation programmes (Mathews *et al.,* 2006). Previous studies have shown that elevation, climate, species, sex, age, and reproductive status affected the haematological values of bears (Huber *et al*., 1997).

3.0 MATERIALS AND METHODS

3.1 Study Area and Animals

This study utilized 65 haematology and serum biochemistry results of 44 clinically healthy sun bears between 2006 to 2015 obtained from Bornean Sun Bear Conservation Centre (BSBCC) in Sepilok, Sandakan, Sabah (5˚51'47.9"N 117˚5'57.8"E). There are two bear houses in the BSBCC which can accommodate up to 43 bears and it has eight large fenced forested enclosures. As of February 2016, the centre houses 37 bears. All bears at the Centre are orphaned or ex-captive bears. Bears are maintained on a diet of local fruits, corn, sweet potato and rice porridge. In this study these bears represented the captive sample group and the wild bears were captured in Ulu Segama Forest Reserve. All bears sampled appeared physically healthy, with normal behavioural responses, and were clinically healthy during examination at the time of blood collection, as determined by body temperature, hydration, heart and respiration rate, and a detailed external physical examination by a wildlife veterinarian. Furthermore, all bears were also pretested and were found negative for malaria.

3.2 Blood Collection

Captured bears were immobilized with Zoletil (tiletamine HCl/zolazepam HCl) at a dose of 5 mg/kg of estimated body mass (Virbac Laboratories, Carros, France), delivered with a jab stick on unsuspecting bears, thus causing minimal excitation during the procedure. Blood was collected from the cephalic vein using a 18 or 21-gauge sterile indwelling catheter, depending on the size of the animal, and a 10 mL syringe and was transferred to blood vacutainers (Becton Dickinson, Franklin Lakes, New Jersey, USA), with and without ethylene diamine tetraacetic acid (EDTA) for hematology and serum biochemistry analyses, respectively. Samples were immediately stored on cool packs at 4–8°C and transported to the Pathology & Clinical Laboratory (M) Sdn. Bhd, Sandakan.

3.3 Statistical Analysis

Data was categorized by sex (male or female), lifestyle (captive or wild), state of fasting (fasting or non-fasting) and age groups (neonate, juvenile, sub adult or adult). Neonates are less than one year old, juveniles are one to three years old, sub adults are between ages three to five and adults are categorised as more than five years old. There were multiple entries for a single animal as it moved between age groups over the years. Statistical analyses were performed using SPSS version 20.

Outliers were removed by Horn's algorithm using Tukey's interquartile fences (Horn and Pesce 2003; Horn and Pesce 2005) with values exceeding interquartile (IQ) fences set at Q1 - 1.5 x IQR and Q3 + 1.5 x IQR (IQR = interquartile range, Q1 and Q3 are the 25th and 75th percentiles, respectively). Each distribution was tested for normality using the Shapiro-Wilk statistic.

Basic statistics including mean, median, standard deviation, minimum and maximum values were determined for each variable at 95% and 90% confidence interval of means for normally distributed and non-normally distributed date, respectively. The Levene's test for equality of variances was used to test for homogeneity of variance for each variable. For variables satisfying the assumptions of normality and homogeneity of variance, comparisons were made either using a t-test (two groups) or a one-way analysis of variance with the post hoc Tukey's HSD test. For non-normally distributed data, Mann-Whitney test and Kruskal-Wallis tests were performed. To establish reference intervals, lower and upper reference limits were calculated as mean ± 2 standard deviations. For comparisons between the means of this study and ISIS, one sample t-test was used for each variable.

4.0 RESULTS

The mean, standard deviation, reference interval, and minimum and maximum value were calculated and tabulated for 14 haematological and 24 serum biochemical parameters in sun bears (Table 4.1).

Table 4.1: Established reference intervals for haematology and serum biochemistry parameters of sun bears (*Helarctos malayanus euryspilus*)

Parameters	*n*	Mean	SD	Min	Max	Reference Interval
Haemoglobin (g/L)	64	141	17.01	103	187	107 – 175
RBC (10^{x12}/L)	64	5.85	0.686	4.35	7.19	4.48 – 7.22
MCV (fL)	64	71.3	4.55	62	83	62.2 – 80.5
PCV (L/L)	64	0.417	0.0473	0.34	0.55	0.323 – 0.512
MCH (pg)	64	24.1	1.609	21	29	20.91 – 27.34
MCHC (g/L)	62	338	13.6	290	367	311 – 366
RDW(%)	62	15.24	1.223	12.8	19.2	12.80 – 17.69
WBC (10^{x9}/L)	62	13.10	3.7454	6.40	22.60	5.62 – 20.6
Neutrophils(10^{x9}/L)	60	9.01	3.1550	3.90	16.60	2.7 – 15.32
Lymphocytes (10^{x9}/L)	60	2.80	1.0470	0.70	5.60	0.71 – 4.90
Monocytes (10^{x9}/L)	58	0.71	0.375	0	1.70	0 – 1.46
Eosinophils (10^{x9}/L)	60	0.25	0.331	0	1.39	0 – 0.91
Basophils (10^{x9}/L)	63	0.02	0.109	0	0.70	0 – 0.24
Platelets (10^{x9}/L)	59	345	130.7	104	646	81 – 609
Glucose (mmol/L)	57	5.2	1.200	2.3	7.2	2.8 – 7.6
Total Cholesterol (mmol/L)	62	8.36	1.780	4.40	13.80	4.81 – 11.93
Triglyceride(mmol/L)	62	2.89	0.676	1.30	4.55	1.54 – 4.24
HDL Cholesterol (mmol/L)	62	4.74	0.795	2.00	6.65	3.15 – 6.33
LDL Cholesterol (mmol/L)	59	2.14	0.853	0.35	4.49	1.43 – 3.84
Total Cholesterol:HDL Ratio	62	1.7	0.27	1.3	3.3	1.2 – 2.3

Table 4.1: Established reference intervals for haematology and serum biochemistry parameters of sun bears (*Helarctos malayanus euryspilus*) - continued

Parameters	*n*	Mean	SD	Min value	Max value	Reference Interval
Sodium (mmol/L)	57	136	2.55	130	143	131 – 141
Potassium (mmol/L)	59	5.3	0.43	4.5	6.4	4.4 – 6.1
Chloride (mmol/L)	60	103	3.8	90	109	96 – 111
Urea (mmol/L)	61	2.8	0.97	1.0	5.6	0.8 – 4.7
Creatinine (umol/L)	64	108	42.5	24	268	22 – 193
Uric Acid (umol/L)	65	0.09	0.036	0.05	0.30	0.02 – 0.16
Calcium (mmol/L)	61	2.18	0.131	1.82	2.52	1.92 – 2.44
Corrected Calcium (mmol/L)	55	2.38	0.112	1.98	2.68	2.16 – 2.60
Phosphate (mmol/L)	62	1.77	0.367	1.20	2.61	1.04 – 2.51
Total Protein (g/L)	65	70	6.2	57	86	58 – 83
Albumin (g/L)	65	29	3.3	21	38	22– 35
Globulin (g/L)	65	41	5.7	29	57	30 – 53
A:G Ratio	64	0.7	0.13	0.4	1.0	0.4 – 0.9
Alkaline Phosphatase (U/L)	63	69.9	42.41	5.0	186.0	0 – 154.7
Total Bilirubin (mmol/L)	64	1.9	0.07	1.7	2.0	0 – 2.0
GGT (U/L)	63	16	9.4	0	45	0 – 35
AST (U/L)	65	114	28.9	65	187	56 – 172
ALT (U/L)	65	36	10.1	13	59	15 – 56

Haematology and serum biochemistry values varies between the values established in this study for *Helarctos malayanus euryspilus* and the values established for *Helarctos malayanus* by ISIS. WBC and other inflammatory cells, platelets, total cholesterol, triglycerides, potassium, uric acid, GGT and ALT have significantly different mean values (Table 4.2). MCH, MCHC, sodium, chloride, creatinine, calcium, phosphate, albumin, globulin, total bilirubin, AST have statistically significant mean differences (Table 4.2). However, the means and the reference interval vary slightly and are not significant clinically. Therefore they are not clinically significant. The other parameters did not have statistically significant difference between both means.

Table 4.2: Comparison of haematology and serum biochemistry parameters between *Helarctos malayanus euryspilus* and *Helarctos malayanus* (ISIS).

Parameters	*Helarctos malayanus euryspilus*			*Helarctos malayanus* ISIS		
	n	**Mean**	**Reference Interval**	***n***	**Mean**	**Reference Interval**
Haemoglobin (g/L)	64	141	107 – 175	279	145	105 – 191
RBC (10^{x12}/L)	64	5.85	4.48 – 7.22	249	5.97	4.42 – 7.88
MCV (fL)	64	71.3 ^{a}	62.2 – 80.5	240	70.0 ^{b}	61.0 – 81.1
PCV (L/L)	64	0.417	0.323 – 0.512	343	0.413	0.308 – 0.539
MCH (pg)	62	24.1 ^{a}	20.9 – 27.3	233	24.6 ^{b}	21.4 – 27.2
MCHC (g/L)	64	338 ^{a}	311 – 366	271	350 ^{b}	280 – 398
WBC (10^{x9}/L)	62	13.10 ^{a}	5.62 – 20.6	308	10.52 ^{b}	5.84 – 17.43
Neutrophils(10^{x9}/L)	60	9.01 ^{a}	2.7 – 15.32	307	7.45 ^{b}	3.47 – 13.74
Lymphocytes (10^{x9}/L)	60	2.80 ^{a}	0.71 – 4.90	308	1.92 ^{b}	0.44 – 4.49
Monocytes (10^{x9}/L)	58	0.710 ^{a}	0 – 1.46	281	0.503 ^{b}	0.091 – 1.384
Eosinophils (10^{x9}/L)	60	0.256 ^{a}	0 – 0.91	250	0.549 ^{b}	0.069 – 1.877
Platelets (10^{x9}/L)	59	345 ^{a}	81 – 609	90	518 ^{b}	167 – 871
Glucose (mmol/L)	57	5.26	2.86 – 7.66	341	5.21	3.05 – 8.69
Total Cholesterol (mmol/L)	62	8.36 ^{a}	4.81 – 11.93	304	6.31 ^{b}	3.51 – 9.74
Triglyceride(mmol/L)	62	2.89 ^{a}	1.54 – 4.24	127	1.53 ^{b}	0.29 – 3.25
Sodium (mmol/L)	57	136 ^{a}	131 – 141	318	139 ^{b}	132 – 148
Potassium (mmol/L)	59	5.3 ^{a}	4.4 – 6.1	321	4.6 ^{b}	3.8 – 5.4
Chloride (mmol/L)	60	103 ^{a}	96 – 111	301	108 ^{b}	101 – 117
Uric Acid (umol/L)	65	0.096 ^{a}	0.024 – 0.169	111	0.064 ^{b}	0.008 – 0.12
Creatinine (umol/L)	64	108 ^{a}	22 – 193	338	124 ^{b}	64 – 201
Calcium (mmol/L)	61	2.18 ^{a}	1.92 – 2.44	326	2.33 ^{b}	2.05 – 2.67
Phosphate (mmol/L)	62	1.77	1.04 – 2.51	310	1.72	1.23 – 2.49
Total Protein (g/L)	65	70	58– 83	301	72	62 – 82
Albumin (g/L)	65	29 ^{a}	22 – 35	285	33 ^{b}	26 – 43
Globulin (g/L)	65	41 ^{a}	30 – 53	280	39 ^{b}	28 – 50
Alkaline Phosphatase (U/L)	63	69	0 – 154	334	69	21 – 175
Total Bilirubin (mmol/L)	64	1.9 ^{a}	0 – 2.0	289	2.9 ^{b}	0.0 – 8.4
GGT (U/L)	63	16.76 ^{a}	0 – 35.648	207	53 ^{b}	9 – 188
AST (U/L)	65	114 ^{a}	56 – 172	308	105 ^{b}	49 – 205
ALT (U/L)	65	36 ^{a}	15 – 56	312	54 ^{b}	20 – 115

^{ab}Means within row with different superscript letters are significantly different at p<0.05.

There were no significant differences between male and female and the state of fasting and non-fasting for each blood variable. The values for means and standard errors of haematology and serum biochemistry parameters between sexes and the state of fasting (fasting or non-fasting) is shown in Appendix 1 and Appendix 2.

There were statistically significant differences between captive and wild bears for mean corpuscular haemoglobin concentration (MCHC) at 340 ± 2.0 (SE) and 324 ± 7.4 (SE), and alanine aminotransferase (ALT) at 36 ± 1.2 (SE) and 46 ± 6.3 (SE), respectively (Table 4.3). However, the values were close and both fall within the established reference ranges and therefore have been categorized as clinically insignificant. Alkaline phosphatase (ALP) levels are significantly different with wild bears having a much lower value compared to captive bears at 45 ± 6.3 (SE) and 35 ± 1.2 (SE) respectively (Table 4.3 and Figure 4.1). There was a significant difference for the total cholesterol level at 8.50 ± 0.235 (SE) and 6.86 ± 0.415 (SE) for captive and wild bears, respectively (Table 4.3 and Figure 4.2).

Table 4.3: Means and standard errors of haematology and serum biochemistry parameters between captive and wild sun bears.

Parameters	Captive		Wild	
	n	**Mean ± SE**	***n***	**Mean ± SE**
MCHC (g/L)	59	340 ± 2.0	5	324 ± 7.4
Total Cholesterol (mmol/L)	57	8.50 ± 0.235	5	6.86 ± 0.415
Total Cholesterol HDL Ratio	57	1.7 ± 0.02	5	1.8 ± 0.36
A:G Ratio	60	0.7 ± 0.02	5	0.5 ± 0.09
Alkaline Phosphatase (U/L)	60	80.0 ± 6.394	5	14.8 ± 1.56
ALT (U/L)	60	35 ± 1.2	5	45 ± 6.3

[ab] Means within row with different superscript letters are significantly different at $p<0.05$.

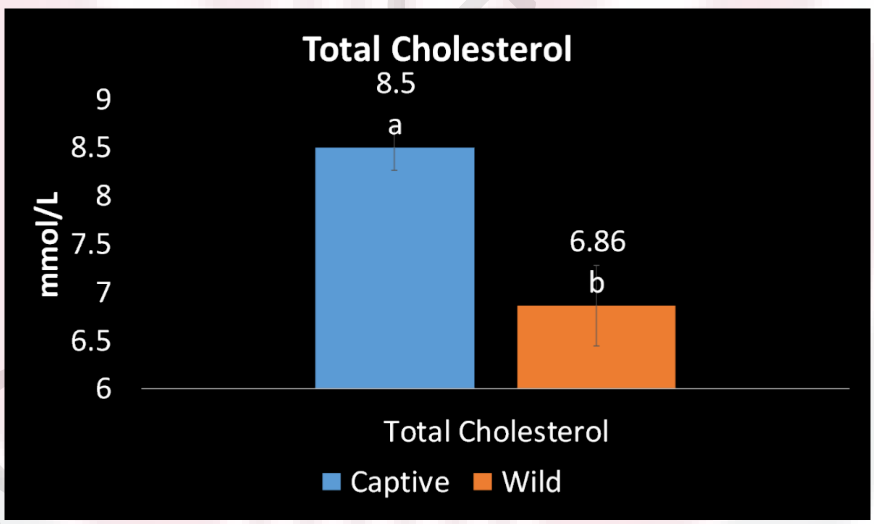

Figure 4.1: Concentrations of total serum cholesterol in captive and wild sun bears.

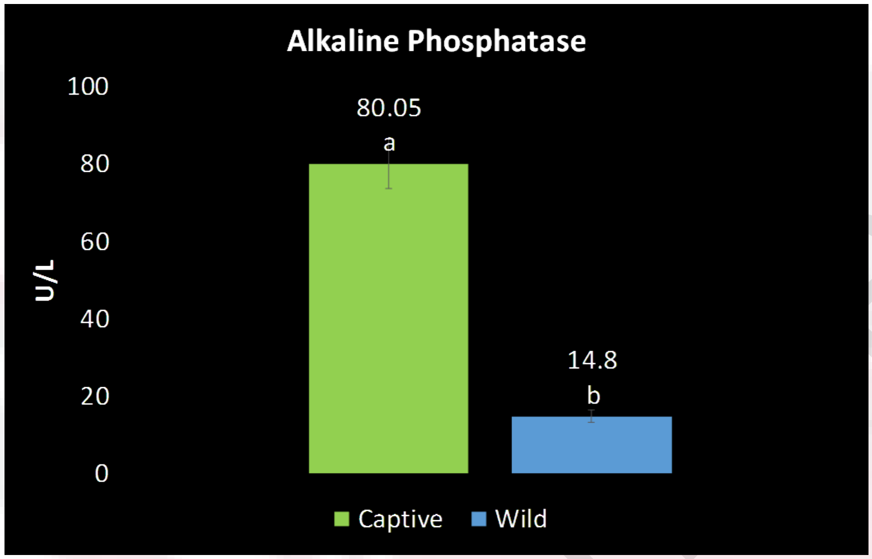

Figure 4.2: Levels of serum alkaline phosphatase (ALP) in captive and wild sun bears.

There was a significant difference between the levels of creatinine in neonate and sub adult groups where the level in sub adults was much higher which is seen in Table 4.4 and Figure 4.3. According to Table 4.4, high density lipoprotein (HDL) level in neonates was significantly higher than the sub adult group (Figure 4.4). Albumin: globulin (A:G) ratio are significantly different (Table 4.4). However, the A:G ratio should not be used as a mean to diagnose a disease on its own. Total protein and albumin levels should first be evaluated. Mean corpuscular haemoglobin values are significantly lower in neonates compared to other age groups (Table 4.4). In Table 4.4, platelet counts of all groups significantly differ from each other and there is a trend of a decrease as the animal ages (Figure 4.5). The level of phosphate was significantly different between all age groups with neonates having the highest value and it decreases as the animal ages (Table 4.4 and Figure 4.6). There were significant differences between all age groups for total protein and globulin (Table 4.4). The trend is increasing as the animal gets older (Figure 4.7). Alkaline phosphatase (ALP) level in neonates was significantly higher from other age groups as seen in Table 4.4 and Figure 4.8.

Table 4.4: Means and standard errors of haematology and serum biochemistry parameters between age groups of sun bears

Parameters	Neonate		Juvenile		Sub Adult		Adult	
	n	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE
MCH (pg)	11	23.0 ± 0.51 ^{a}	14	23.7 ± 0.33 ^{ab}	13	23.9 ± 0.28 ^{ab}	26	24.8 ± 0.33 ^{ab}
Platelets (10^{x9}/L)	9	426 ± 54.4 ^{a}	12	390 ± 41.5	13	340 ± 24.7	25	296 ± 22.7 ^{a}
HDL Cholesterol (mmol/L)	11	5.33 ± 0.200 ^{a}	13	4.66 ± 0.152 ^{ab}	12	4.43 ± 0.271 ^{b}	26	4.67 ± 0.154 ^{ab}
Creatinine (umol/L)	12	78 ± 19.4 ^{a}	14	111 ± 9.4 ^{ab}	13	135 ± 12.2 ^{b}	26	111 ± 5.5 ^{ab}
Phosphate (mmol/L)	11	2.20 ± 0.091 ^{a}	13	1.90 ± 0.090 ^{b}	12	1.64 ± 0.074 ^{bc}	26	1.59 ± 0.055 ^{c}
Total Protein (g/L)	12	63.58 ± 1.270 ^{a}	14	68.64 ± 1.087 ^{b}	13	71.46 ± 1.708 ^{bc}	26	74.73 ± 0.909 ^{c}
Globulin (g/L)	12	35.08 ± 0.925 ^{a}	14	40.07 ± 0.848 ^{b}	13	42.08 ± 1.425 ^{bc}	26	45.12 ± 1.051 ^{c}
A:G Ratio	12	0.8 ± 0.03 ^{a}	14	0.7 ± 0.06	13	0.7 ± 0.03	26	0.6 ± 0.02 ^{a}
Alkaline Phosphatase (U/L)	12	140.3 ±13.54 ^{a}	14	71.6 ± 8.17 ^{b}	13	48 ± 5.85 ^{b}	26	60.2 ± 9.29 ^{b}

^{abc}Means within row with different superscript letters are significant different at p<0.05.

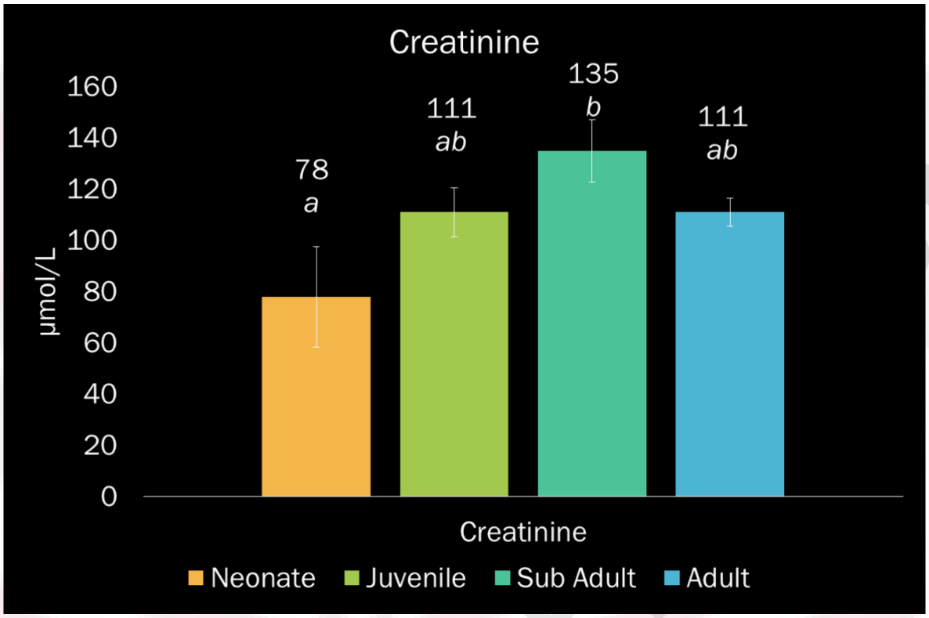

Figure 4.3: Levels of serum creatinine in sun bears at different age groups.

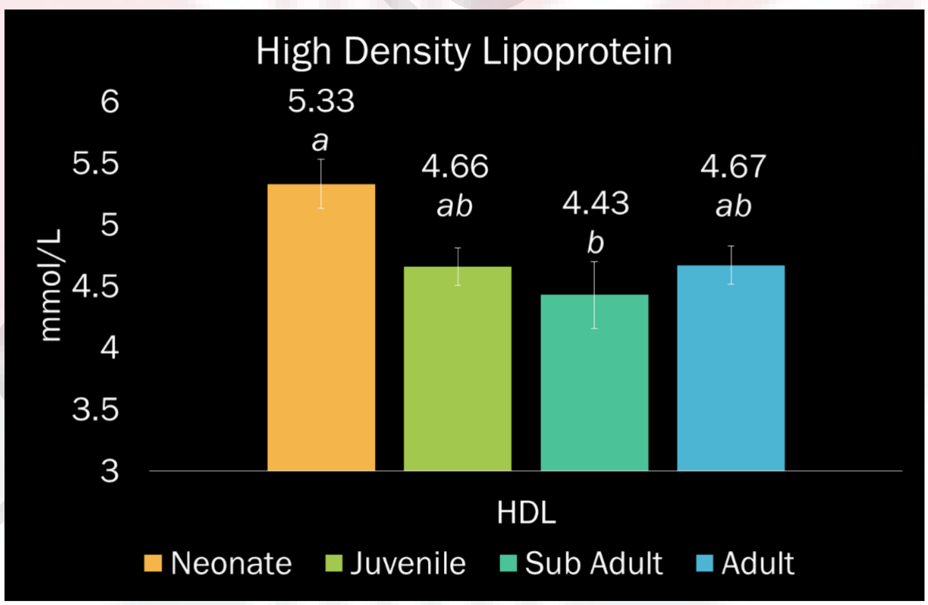

Figure 4.4: Levels of high density lipoprotein (HDL) in sun bears at different ages.

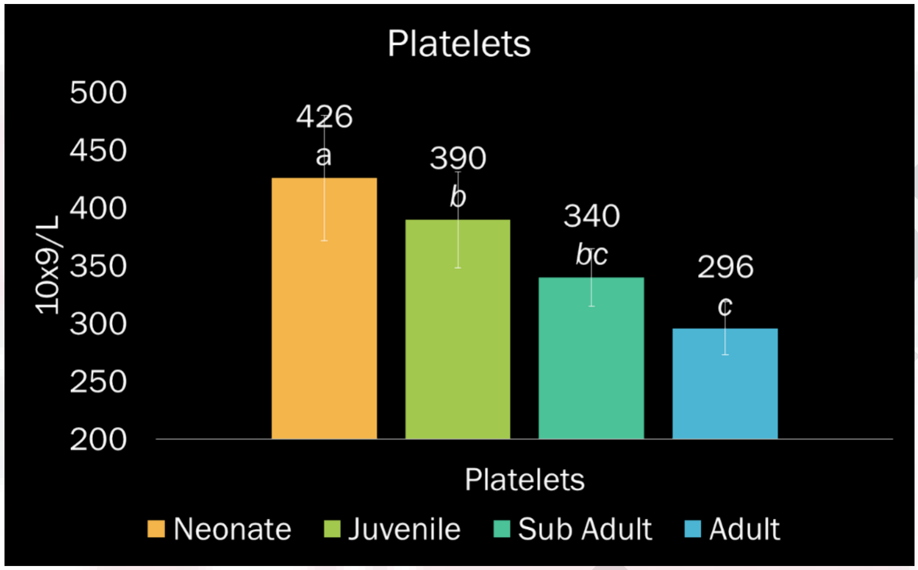

Figure 4.5: Numbers of platelet in bears with different age groups.

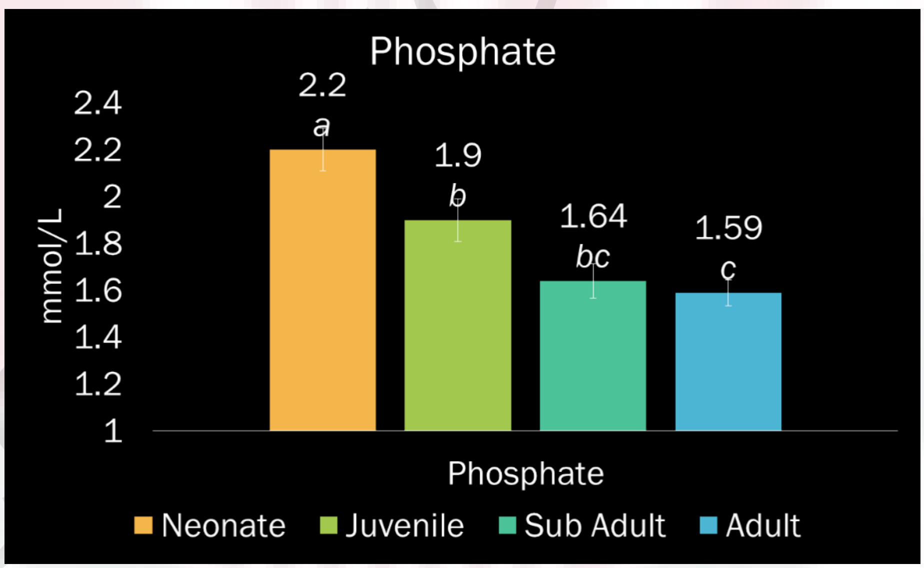

Figure 4.6: Levels of serum phosphate in sun bears at different age groups.

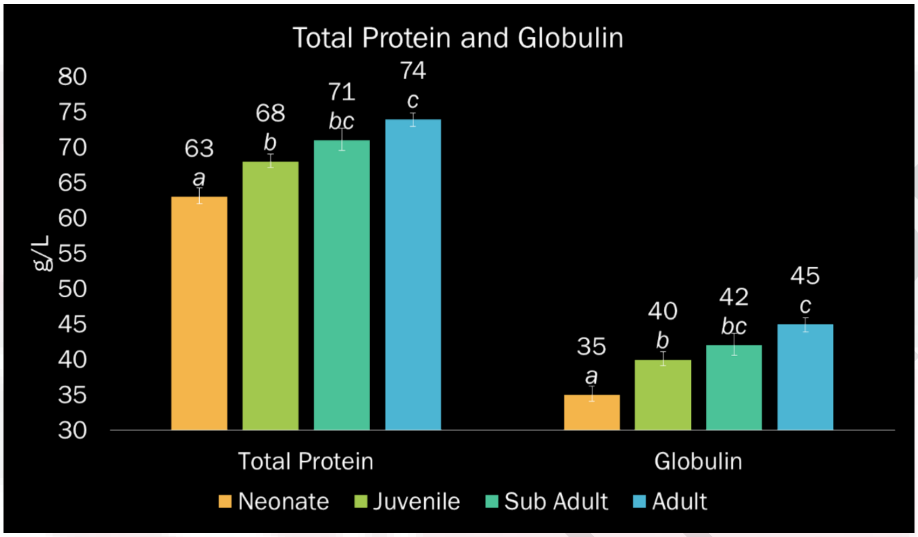

Figure 4.7: Concentrations of total serum protein and globulin in sun bears with different age groups.

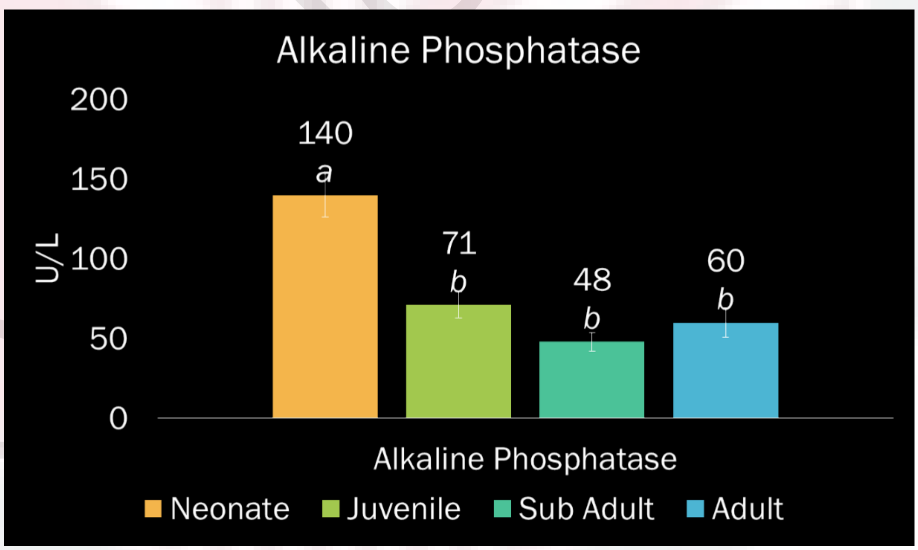

Figure 4.8: Levels of serum alkaline phosphatase (ALP) in sun bears at different age groups

5.0 DISCUSSION

Creatinine is a chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Creatinine excretion and total body protein mass increase with age, as there is an increase in muscle cell mass with growth (Proctor *et al*, 1999). As the animal grows, its muscle mass increases and therefore there is more creatinine generation.

High density lipoprotein decreases as the animal ages. The most striking decreases, attributable to hormonal changes due to testosterone and estradiol. Sun bears undergo puberty at ages three to four years of age (White, 2005), which is the sub adult stage, where the decrease in HDL is most likely due to the effects of hormones. HDL cholesterol in growing males, particularly, is shown to decrease with age as the level of testosterone increases (Van Pottelbergh, 2002).

Decrease in platelet number is correlated to the decrease in the thrombopoietin levels occurring from birth to adulthood (Ishiguro *et al*, 1999). Platelets decrease may also be due to reduced haematopoietic stem cell reserve in aging individuals (Segel, 2006). In humans, the number of platelets decreases quickly in childhood, stabilizes in adulthood, and further decreases in old age (Biino, 2013).

Higher phosphorous level in young Andean bears relative to adults was mainly due to skeletal development and bone ossification in juveniles (Castellanos *et al*., 2010). Phosphorus absorption correlates positively with calcium concentrations and is important in bone metabolism. Growth hormone has stimulatory effects on osteoblast and chondrocyte activity to promote bone growth (DiGirolamo *et al*, 2007). Therefore, phosphorus levels may be elevated in young due to the increased growth rate.

Elevated globulin could reflect antigenic stimulation due to an unknown pathogen encountered by wild animals as the animal ages (Weber *et al., 2002).* With age, the total serum protein tends to increase, resulting in decrease in albumin as a result of progressive increase in globulins (Kaneko, 2008). Total globulins and gammaglobulins are associated with infections. As both albumin and globulin play roles in maintaining blood osmotic pressure, increase in gammaglobulin will result in decreasing albumin to maintain the osmotic balance (Payne and Payne, 1987).

The bone ALP isoenzyme is most likely the cause of the elevated AP levels. Bone AP increase with osteoblast hyperactivity and bone remodelling (Castellanos *et al*., 2010). Osteoblastic activity is highest when the animal is young and growing compared to when it is an adult.

The total cholesterol level differences between captive and wild bears could be attributed to the activity level of the bears (Figure 4.7). Even though the captive bears receive enrichment training and free-roaming time, they are more sedentary than their wild counterparts. Mean daily movement distance of wild bears is 1454.5 m. In wild bears, 50% of the day is spent active, mainly with foraging and food handling activities (Paisley and Garshelis, 2006). This is in contrast to daytime activity of 30–35% in sun bears observed in a zoo, whereof foraging made up 6–8% of the overall activity budget despite daily feeding enrichment (Riese, unpublished data). A study conducted on 29 captive Malayan sun bears and Asiatic black bears showed the animals were active during 36% of the day, half of which was attributed to stereotypic behaviour (Vickery and Mason, 2004). In humans, individuals subjected to hard exercise had significantly lower total cholesterol than age-matched non-active control individuals (Aires *et al*., 2002).

When animal is fasted, decrease in the ALP level could be attributed to the decrease in intestinal and hepatic (liver 1 and liver 2) secretion of ALP isoenzyme due to decreased food intake (Neafsey and Schwartz, 1977). Wild boars fed energy deficit feed for 24 weeks showed greatly reduce in serum ALP level (Wolkers *et al.*, 1994). Intestinal AP (IAP) is regulated by dietary macronutrients and fasting. In a study where mice were fasted for two days, significant decrease in IAP expressions, which was related to the decreased LPS-dephosphorylating activity, was observed. The re-introduction of food in these mice resulted in the recovery of IAP expression, as an adaptive response of IAP to food availability (Goldberg, 2008). This could mean that the bears in the wild are undergoing starvation in contrast to the captive bears that have sufficient food at regular intervals.

6.0 CONCLUSION

There are variations between the haematology and serum biochemistry ranges of *Helarctos malayanus* and *Helarctos malayanus euryspilus*. All mean values for *Helarctos malayanus euryspilus* fall within the reference intervals established by ISIS for *Helarctos malayanus*. All reference interval values established for *Helarctos malayanus euryspilus* are unique to the values established by ISIS. There are differences in certain blood parameters between lifestyle conditions and age groups of Malayan sun bears.

7.0 RECOMMENDATIONS

The sample size could be increased for more accurate data. Sampling area should be increased to more parts of Borneo to include variation in location and diet. Also, the establishment of reference intervals for more haematology and serum biochemistry values such as thyroid function assays should be conducted.

REFERENCES

Aires, N., Selmer, R. & Thelle, D. (2003). The validity of self-reported leisure time physical activity, and its relationship to serum cholesterol, blood pressure and body mass index. A population based study of 332,182 men and women aged 40–42 years. *European Journal of Epidemiology*, 18(6), 479-485.

Biino, G., Santimone, I., Minelli, C., Sorice, R., Frongia, B., Traglia, M., ... & Francavilla, M. (2013). Age-and sex-related variations in platelet count in Italy: Aa proposal of reference ranges based on 40987 subjects' data. *PLoS One*, 8(1), e54289.

BSBCC. (2015). Sun bear facts. Available at: http://www.bsbcc.org.my. (Accessed: 10 October 2015)

Castellanos, A., Arias, L., Jackson, D. & Castellanos, R. (2010). Hematological and serum biochemical values of Andean bears in Ecuador. *Ursus*, *21*(1), 115-120.

Chauhan, N. S. (2006). The status of Malayan sun bears in India. In *Understanding Asian bears to secure their future*, Japan Bear Network, Ibaraki, Japan. pp. 50-56.

Corbet, G. B. & Hill, J. E. (1992). *The mammals of the Indomalayan region: a systematic review*. Oxford University Press, Oxford. pp. 488.

Cranbrook, E. A. R. L. O. F. (1991). Mammals of South-East Asia. *Oxford University Press, New York, NY (USA).*

DiGirolamo, D. J., Mukherjee, A., Fulzele, K., Gan, Y., Cao, X., Frank, S. J. & Clemens, T. L. (2007). Mode of growth hormone action in osteoblasts. *Journal of Biological Chemistry*, *282*(43), 31666-31674.

Ewer, R. F. (1973). *The carnivores*. Cornell University Press, Cornell. pp. 170.

Fetherstonhaugh, A. H. (1940). Some notes on Malayan bears. *Journal of the Malayan Nature Society*, 1, 15-22.

Fitzgerald, C. S. & Krausman, P. R. (2002). Helarctos malayanus.*Mammalian Species*, 696(1), 1-5.

Goldberg, R. F., Austen, W.G., Zhang, X., Munene, G., Mostafa, G., Biswas, S., McCormack, M., Eberlin, K. R., Nguyen, J. T., Tatlidede, H. S., et al. (2008). Intestinal alkaline phosphatase is a gut mucosal defense factor maintained by enteral nutrition. *Proceedings of the National Academy of Sciences of the USA*,105, 3551–3556.

Gong, J. & Harris, R. B. (2006). The status of bears in China. In *Understanding Asian bears to secure their future*, pp. 50-56. Japan Bear Network, Ibaraki, Japan. pp. 50-56.

Griffiths, M., & Van Schaik, C. P. (1993). Camera-trapping: a new tool for the study of elusive rain forest animals. *Tropical Biodiversity*, *1*(2), 131-135.

Hanks, J. (1981). Characterization of population condition. In C. W. Fowler & T. D. Smith (eds.) *Dynamics of large mammal populations*. John Wiley & Sons, New York. pp. 47-73.

Harrison, R. G., & Weiner, J. S. (1949). Vascular patterns of the mammalian testis and their functional significance. *Journal of Experimental Biology*, *26*(3), 304-316.

Horn, P. S. & , Pesce AJ. (2003). Reference intervals: an update. *Clinica Chimica Acta*, 2003, 334, 5-23.

Horn, P. S. & Pesce, A. J. (2005). *Reference intervals: A user's guide*. AACC Press, Washington DC.

Huber, J. D., Egleton, R. D. & Davis, T. P. (2001). Molecular physiology and pathophysiology of tight junctions in the blood–brain barrier. *Trends in Neurosciences*, *24*(12), 719-725.

Ishiguro, A., Nakahata, T., Matsubara, K., Hayashi, Y., Kato, T., Suzuki, Y. & Shimbo, T. (1999). Age-related changes in thrombopoietin in children: Reference interval for serum thrombopoietin levels. *British Journal of Haematology*, 106(4), 884-888.

Kaneko, J. J., Harvey, J. W. & Bruss, M. L. (2008). *Clinical Biochemistry of Domestic Animals*. Elsevier, California. pp. 904.

Khalil, A., Jay-Gerin, J.P. & Fülöp. T. (1998). Age-related increased susceptibility of high-density lipoproteins (HDL) to in vitro oxidation induced by γ-radiolysis of water. *FEBS Letters*, 435(2–3), 153-158.

Kirkland RT, Keenan BS, Probstfield JL, et al. (1987). Decrease in Plasma High-Density Lipoprotein Cholesterol Levels at Puberty in Boys With Delayed Adolescence: Correlation With Plasma Testosterone Levels. *Journal of the American Medical Association*, 257(4), 502-507.

Kirkland, R. T., Keenan, B. S., Probstfield, J L., et al. (1987). Decrease in Plasma High-Density Lipoprotein Cholesterol Levels at Puberty in Boys With Delayed Adolescence: Correlation With Plasma Testosterone Levels. *Journal of the American Medical Association*, 257(4), 502-507.

Lekagul, B., & McNeely, J. A. (1977). Mammals of Thailand. *Mammals of Thailand.*

Matthews, S. M., Beecham, J. J., Quigley, H., Greenleaf, S. S. & Leithead, H. M. (2006). Activity patterns of American black bears in Yosemite National Park. *Ursus*, *17*(1), 30-40.

Meijaard, E. (2004). Craniometric differences among Malayan sun bears (*Ursus malayanus*): Evolutionary and taxonomic implications. *Raffles Bulletin of Zoology*, 52, 665-672.

Mills, J. A. & Servheen, C. (1991). The Asian trade in bears and bear parts. TRAFFIC USA/WWF. Washington D. C. pp. 131.

Ortolani, A. & Caro, T. M. (1996). The adaptive significance of color patterns in carnivores: Phylogenetic tests of classic hypotheses. *Carnivore Behavior, Ecology, and Evolution*, *2*, 132-188.

Paisley, S. & Garshelis, D. L. (2006). Activity patterns and time budgets of Andean bears (*Tremarctos ornatus*) in the Apolobamba Range of Bolivia. *Journal of Zoology*, *268*(1), 25-34.

Payne, J. M. & Payne, S. (1987). *The metabolic profile test*. Oxford University Press, Oxford. pp 3-12.

Payne, J. U. N. A. I. D. I. & Andau, M. (1991). Large mammals in Sabah. In R. Kiew (ed.) *The state of nature conservation in Malaysia.* Malayan Nature Society, United Selangor Press, Kuala Lumpur, Malaysia. pp 177-183.

Pocock, R. I., (1941). The fauna of British India, including Ceylon and Burma. Mammalia Vol. II. In *Carnivora (suborders Aeluroidae (part) and Arctoidae)*. Taylor & Francis , Ltd., London, United Kingdom. pp. 503.

Proctor, D. N., O'brien, P. C., Atkinson, E. J. & Nair, K. S. (1999). Comparison of techniques to estimate total body skeletal muscle mass in people of different age groups. *American Journal of Physiology-Endocrinology and Metabolism*, *277*(3), E489-E495.

Santiapillai, C. & Ashby, K. R. (1988). The clouded leopard in Sumatra. *Oryx*, *22*(01), 44-45.

Segal, J. B. & Moliterno, A. R. (2006). Platelet counts differ by sex, ethnicity, and age in the United States. *Annals of Epidemiology*, 16(2),123–30.

Stirling, I. (1993). *Bears: Majestic creatures of the world*. Rodale Press, Emmaus, USA. 240 pp.

Teare, J. A. (2013). *"Helarctos _malayanus*. No selection by gender. All ages combined Standard International Units 2013 CD. html" *in* ISIS Physiological Reference Intervals for Captive Wildlife: A CD-ROM Resource., International Species Information System, Eagan, MN.

Van Pottelbergh, I., Braeckman, L., De Bacquer, D., De Backer, G. & Kaufman, J. M. (2003). Differential contribution of testosterone and estradiol in the determination of cholesterol and lipoprotein profile in healthy middle-aged men. *Atherosclerosis*, 166(1), 95-102.

Vickery, S. & Mason, G. (2004). Stereotypic behavior in Asiatic black and Malayan sun bears. *Zoo Biology*, 23(5), 409-430.

Weber, D.K., Danielson, K., Wright, S. & Foley, E. (2002). Hematology and serum biochemistry of Duskyfooted Wood Rat *(Neotoma juscipes). Journal of Wildlife Disease*, 38: 576-582.

White, Jr, D., Berardinelli, J. G. & Aune, K. E. (2005). Age variation in gross and histological characteristics of the testis and epididymis in grizzly bears. *Ursus*, *16*(2), 190-197.

White Jr, D., Berardinelli, J. G. & Aune, K. E. (2005). Seasonal differences in spermatogenesis, testicular mass and serum testosterone concentrations in the grizzly bear. *Ursus*, *16*(2), 198-207.

Wolkers, J., Wensing, T., Bruinderink, G. G. & Schonewille, J. T. (1994). The effect of

undernutrition on haematological and serum biochemical variables in wild boar (Sus scrofa). *Comparative Biochemistry and Physiology Part A: Physiology*, *108*(2), 431-437.

Wong, S. T., Servheen C. & Ambu, L. (2004). Home range, movement and activity patterns, and bedding sites of Malayan sun bears (*Helarctos malayanus*) in the Rainforest of Borneo. *Biological Conservation*, 119, 169-181.

APPENDIX

Appendix 1: Means and standard errors of haematology and serum biochemistry parameters between sexes.

Parameters	**Male**			**Female**		
	n	**Mean**	**SE**	***n***	**Mean**	**SE**
Haemoglobin (g/L)	21	138.33	4.230	43	142.63	2.406
RBC (10^{x12}/L)	21	5.73	0.139	43	5.91	0.108
PCV (L/L)	21	0.41	0.011	43	0.42	0.007
MCV (fL)	21	70.71	1.113	43	71.72	0.654
MCH (pg)	21	24.14	0.373	43	24.12	0.240
MCHC (g/L)	21	24.14	0.373	43	24.12	0.240
RDW-CV (%)	21	14.96	0.287	43	15.57	0.212
WBC (10^{x9}/L)	20	12.58	0.820	42	13.36	0.586
Neutrophils(10^{x9}/L)	19	8.93	0.750	41	9.05	0.491
Lymphocytes (10^{x9}/L)	20	2.87	0.259	42	2.93	0.185
Monocytes (10^{x9}/L)	19	0.74	0.104	42	0.80	0.075
Eosinophils (10^{x9}/L)	20	0.28	0.074	41	0.28	0.061
Basophils (10^{x9}/L)	20	0.02	0.020	43	0.03	0.018
Platelets (10^{x9}/L)	20	326.20	36.384	39	354.97	17.937
Glucose (mmol/L)	17	5.55	0.216	40	5.14	0.206
Total Cholesterol (mmol/L)	20	8.80	0.458	42	8.16	0.251
Triglyceride(mmol/L)	20	2.96	0.148	42	2.87	0.106
HDL Cholesterol (mmol/L)	20	4.89	0.158	42	4.67	0.129
LDL Cholesterol (mmol/L)	20	2.55	0.293	41	2.09	0.122
Total Cholesterol HDL Ratio	20	1.79	0.053	42	1.76	0.045
Sodium (mmol/L)	18	134.56 [a]	1.329	41	136.76 [b]	0.544
Potassium (mmol/L)	18	5.24	0.108	42	5.37	0.073
Chloride (mmol/L)	18	102.28 [a]	1.057	42	104.45 [b]	0.520
Urea (mmol/L)	20	2.88	0.222	44	2.98	0.165
Creatinine (umol/L)	21	127.81	11.811	44	101.53	5.733
Uric Acid (umol/L)	21	0.10	0.008	44	0.09	0.005
Calcium (mmol/L)	20	2.18	0.032	42	2.18	0.021
Corrected Calcium (mmol/L)	17	2.41	0.033	40	2.35	0.023
Phosphate (mmol/L)	20	1.73	0.081	42	1.80	0.057
Total Protein (g/L)	21	71.00	1.255	44	70.57	0.990

Albumin (g/L)	21	28.52	0.824	44	29.41	0.484
Globulin (g/L)	21	42.43	1.279	44	41.16	0.871
A:G Ratio	21	0.74	0.052	44	0.73	0.020
Alkaline Phosphatase (U/L)	21	73.62	12.193	44	75.71	7.340
Total Bilirubin (mmol/L)	20	1.97	0.021	44	1.99	0.010
GGT (U/L)	20	16.75	2.022	44	17.32	1.551
AST (U/L)	21	108.38	6.174	44	117.20	4.395
ALT (U/L)	21	35.43	2.042	44	36.61	1.599

[ab] Means within row with different superscript letters are significantly different at $p<0.05$.

Appendix 2: Means and standard errors of haematology and serum biochemistry parameters between state of fasting (fasting and non-fasting).

Parameters	Fasting			Non-Fasting		
	n	Mean	SE	*n*	Mean	SE
Haemoglobin (g/L)	39	141.41	2.666	25	140.92	3.581
RBC (10^{x12}/L)	39	5.88	0.111	25	5.82	0.138
PCV (L/L)	39	0.42	0.008	25	0.42	0.009
MCV (fL)	39	70.87	0.731	25	72.20	0.902
MCH (pg)	39	24.10	0.274	25	24.16	0.293
MCHC (g/L)	39	340.26	2.320	25	336.72	3.617
RDW-CV (%)	39	15.42	0.237	25	15.29	0.251
WBC (10^{x9}/L)	37	12.57	0.570	25	13.91	0.812
Neutrophils(10^{x9}/L)	36	8.45	0.463	24	9.85	0.723
Lymphocytes (10^{x9}/L)	37	3.14 [a]	0.188	22	2.44 [b]	0.251
Monocytes (10^{x9}/L)	37	0.79	0.071	24	0.76	0.109
Eosinophils (10^{x9}/L)	37	0.25	0.057	22	0.35	0.088
Basophils (10^{x9}/L)	33	0.04	0.141	24	0	-
Platelets (10^{x9}/L)	35	335.54	20.278	24	359.33	29.897
Glucose (mmol/L)	37	5.44	0.184	20	4.95	1.311
Total Cholesterol (mmol/L)	37	8.51	0.261	25	8.15	0.410
Triglyceride(mmol/L)	37	2.98	0.096	25	2.77	0.158
HDL Cholesterol (mmol/L)	37	4.87	0.115	25	4.56	0.181
LDL Cholesterol (mmol/L)	37	2.28	0.149	24	2.18	0.234
Total Cholesterol HDL Ratio	37	1.75	0.026	25	1.80	0.079
Sodium (mmol/L)	40	136.48	0.659	20	135.35	1.047
Potassium (mmol/L)	40	5.30	0.063	20	5.39	0.134
Chloride (mmol/L)	40	104.30	0.578	20	102.80	0.914
Urea (mmol/L)	39	2.82	0.181	25	3.14	0.184
Creatinine (umol/L)	40	113.05	7.603	25	105.18	8.110
Uric Acid (umol/L)	40	0.08 [a]	0.003	25	0.11 [b]	0.010
Calcium (mmol/L)	37	2.19	0.020	25	2.16	0.032
Corrected Calcium (mmol/L)	37	2.38	0.022	20	2.35	0.038
Phosphate (mmol/L)	37	1.80	0.061	25	1.75	0.073
Total Protein (g/L)	40	70.83	0.974	25	70.52	1.314
Albumin (g/L)	40	29.58	0.544	25	28.40	0.653
Globulin (g/L)	40	41.25	0.802	25	42.08	1.373
A:G Ratio	40	0.73	0.019	25	0.74	0.047
Alkaline Phosphatase (U/L)	40	78.08	7.492	25	70.16	11.249
Total Bilirubin (mmol/L)	40	2.00	-	24	1.95	0.023
GGT (U/L)	39	16.44	1.373	25	18.24	2.333

AST (U/L)	40	111.00	4.242	25	119.72	6.381
ALT (U/L)	40	35.70	1.444	25	37.08	2.355

[ab] Means within row with different superscript letters are significantly different at $p<0.05$.