

## AGE AND SEX COMPARISON IN DETERMINING BASELINE BLOOD AND COAGULATION PROFILES IN SEMI-EXTENSIVE RUSA DEER (*RUSA TIMORENSIS*)

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**ABSTRACT.** The objective of the study was to establish the baseline values for blood and coagulation parameters in normal and healthy rusa deer (*Rusa timorensis*) of different ages and sexes. The sample population consists of 40 rusa deer, divided into four groups of (i) juvenile males (ii) juvenile females (iii) adult males and (iv) adult females. The findings showed significant ( $p < 0.05$ ) higher values in erythrocyte count, calcium concentration and prothrombin time in the adult males compared to adult female rusa deer. On the other hand, the total protein concentration was significantly higher in adult females than adult male deer. No significant differences in blood or coagulation parameters were observed between sexes in the juvenile deer. Between age group, the adult deer had significantly higher mean cell volume, plasma protein and globulin concentration than juvenile rusa deer. Thus, it is necessary to take into account the age and sex of the rusa deer when using blood reference values for the diagnosis of diseases or health assessment.

**Keywords:** rusa deer, haematology, serum biochemistry, blood coagulation, sex, age

## INTRODUCTION

Deer in general is not only important as an alternative source of meat, but also valuable for its fur and velvet. The deer farming industry has increased in many regions with modern deer-farming techniques. It was initiated by New Zealand and till now continues to lead in that area (Chardonnet *et al.*, 2002). On the other hand, deer farming has also served to replace game venison from illegal hunting of endangered wild deer species (Dahlan, 2009). In Malaysia, deer meat (venison) is becoming popular due to its fine and unique taste (Dahlan, 2000). The population of captive deer in Malaysia for the year 2003 and 2004 were 8,077 and 8,402 heads respectively, an increment of 3.9% in a year (Nurulaini *et al.*, 2007). Updated data indicated that the population of captive deer was 13,136 in 2012 (Wahid, 2013). According to the International Union for Conservation of Nature (IUCN) Red List, rusa deer is categorised as a vulnerable species that is facing a high risk of extinction estimated at less than 10,000 mature population in their native geographical area in Indonesia and with a decreasing population trend (Hedges *et al.*, 2015). Deer are also susceptible to

diseases such as malignant catarrhal fever (Sutherland *et al.*, 1987) and a recent case was reported in Malaysia in 2011 (Suhaila *et al.*, 2011). Other diseases incidences that were reported in rusa deer in Malaysia include epizootic haemorrhagic disease in 2003 in Perak (Maizan *et al.*, 2003) and trypanosomiasis in Lenggong, Perak in 2006 (Adrian *et al.*, 2009).

The increasing deer population will definitely require veterinary care where the blood profiles are fundamental for the determination of the health status of these animals (Poljicak *et al.*, 2003). Haematological analyses are frequently used to diagnose and evaluate the progress and status of certain diseases (Szabo *et al.*, 2005). To date, there are several reports on blood parameters of rusa deer. However, their focus were limited to the haemograms and biochemical parameters of different sexes and physiological status of rusa deer (Audigé, 1992a, 1992b; Tomkins and Jonsson, 2005) and blood morphology (Salakij *et al.*, 1998). The recent focus was on adults and mixed sexes (Zawida *et al.*, 2012). An earlier study was also conducted on the sambar deer (*Cervus unicolor*) at University Agriculture Park, Universiti Putra Malaysia (Siti Norzubaidah, 2010). In that study, the blood profile of sambar deer were determined during the adaptation period after transported to a new environment. Besides rusa deer, the blood profiles of other species of deer, namely red deer (Cross *et al.*, 1994; Marco and Lavin, 1999), chital deer (Chapple *et al.*, 1991; Gupta *et al.*, 2007), sambar deer (Siti Norzubaidah, 2010) and barking deer (Gupta *et al.*, 2007) were also available.

There are very limited studies also dealing with blood coagulation parameters in clinically healthy deer as reported in intensively farming and game fallow deer and red deer (Sutherland *et al.*, 1985; Siroka *et al.*, 2011). In diseased deer, previous reports focused on the changes of coagulation parameters, particularly in prothrombin time (PT) and activated partial thromboplastin time (APTT) in epizootic haemorrhagic diseases virus and malignant catarrhal fever. In these deer, a prolongation of PT and APTT were reported (Sutherland *et al.*, 1987).

The growing deer farming industry have enhanced continuous and improved research on animal health and veterinary issues. Although there are some information available on the blood profiles in rusa deer, this is an initial study reporting the extensive blood and coagulation baseline profiles of rusa deer being determined in Malaysia in semi-captive management, which have taken into consideration the differences of sex and age.

## MATERIALS AND METHOD

### Study area and management

This study was conducted in the deer unit, University Agriculture Park, Universiti Putra Malaysia (UPM). There were a total of 130 rusa deer in the unit. These deer were kept in a group of mixed sex and age. These animals were fed chopped Napier (*Pennisetum purpureum*) and Guinea grass (*Panicum maximum*) and supplemented with the small ruminant pellet. Feeding was done once a day in the morning and water was available *ad libitum*. Herd health programmes were

conducted in this animal unit which includes vaccination for foot and mouth disease (FMD) vaccine every six months and received preventive medicine for anthelmintics with albendazole. Two months prior to sampling, they were screened for tuberculosis (TB) by using the commercial TB test kit and single cervical tuberculin skin test. All deer were non-reactive towards both tests.

### Animal sampling

Forty apparently healthy rusa deer of different sexes and ages with normal appetite and behaviour were randomly selected for blood collection. The deer were divided into four groups: thirteen juvenile ( $\leq 1$ -year-old) males, five juvenile females, thirteen adult males and nine adult females. The deer were herded into a dark room to minimise stress levels. They were then individually selected and moved to the next dark room for handling and blood collection. The deer were physically restrained by a well-trained keeper. The eyes were covered to ensure they remained calm. Approximately 5 ml of blood was collected via jugular acupuncture into EDTA tube, plain tube and sodium citrate tube for haematology, serum biochemistry and for the determination of PT and APTT, respectively. (Becton Dickinson and Company, NJ, USA) All tubes were transported in an ice box and analysed within 4 hours of sampling at the Hematology and Clinical Biochemistry Laboratory of the Faculty of Veterinary Medicine, UPM.

### Erythrocyte analysis

The hematological parameters were determined with the EDTA-anticoagulated blood. The haematology analyser (Cell-Dyn<sup>®</sup> 3700, Abbott Diagnostics Division, IL, USA) was used to determine the erythrocyte and leucocyte numbers and haemoglobin concentrations. The packed cell volume (PCV) was determined by the microhaematocrit centrifuge method using non-heparinised microhaematocrit capillary tubes. The PCV values was read using a micro-haematocrit reader. The mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) were determined using Eq. (1) and Eq. (2).

$$MCV = \frac{PCV (L/L)}{\text{erythrocyte count } (x10^{12})} \times 1000 \text{ fL} \quad (1)$$

$$MCHC = \frac{\text{haemoglobin (g/L)}}{PCV (L/L)} \text{ g/L} \quad (2)$$

### Leucocyte analysis

A smooth, thin and tongue-shaped blood smear was made from the EDTA blood for the differential count. The smear was stained with Wright stain (Sigma-Aldrich<sup>®</sup>, St. Louis, USA) according to procedures described by the manufacturer. Differential leucocyte count was done under light microscope using the 'Battlement method'. A hundred leucocytes were counted and the percentages of neutrophils, lymphocytes, monocytes, eosinophils and basophils from total leucocytes were determined and converted to absolute values.

### Serum biochemistry analysis

Plasma sample was obtained by cutting the capillary tube containing whole blood just above the buffy coat using a diamond pen. The protein concentration of the plasma samples were determined using the refractometer. One drop of plasma was transferred onto the prism and read on S.P. g/% scale. The protein concentration was expressed as g/L. Icterus index was also determined by observing the plasma compared with icteric index standards (potassium dichromate solutions).

Blood in plain tubes were centrifuged at  $250\times g$  for 1 min to obtain the serum. The serum samples were evaluated for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), creatine kinase (CK), blood urea nitrogen (BUN), creatinine, total protein (TP), albumin (A), bilirubin, calcium ( $\text{Ca}^{++}$ ), inorganic phosphate, sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^-$ ), chloride ( $\text{Cl}^-$ ), glucose and cholesterol using the chemistry analyser Hitachi 902 (Roche Diagnostics, IN, USA). The globulin (G) concentration and A/G ratios were calculated using Eq. (3) and Eq. (4).

$$\text{Globulin (g/L)} = \text{TP} - \text{A (g/L)} \quad (3)$$

$$\text{A/G} = \frac{\text{A (g/L)}}{\text{TP} - \text{A (g/L)}} \quad (4)$$

### Blood coagulation analysis

Blood samples in 0.109M sodium citrate tube were centrifuged at  $250\times g$  for 10 min to separate the plasma. The plasma were

then used to determine PT and APTT by the Coagulation Analyser (Diagnostica Stago, Start 4, NJ, USA) according to manufacturer's instructions.

### Data analysis

Mean and standard deviation of the blood and coagulation parameters from each sex and age group were calculated using SPSS Statistical Package ver.16.0 (IBM Corp., New York, USA). SPSS was also used to determine the significant difference between groups at 95% significant levels ( $p < 0.05$ ) using one way ANOVA method.

## RESULTS AND DISCUSSION

The blood parameters of the rusa deer with respect to sex and age are presented in Table 1. The erythrocyte count was significantly higher in adult males compared to adult females. Inversely the MCV was significantly greater in adult females than males. On the other hand, plasma protein concentration was significantly higher in the adults compared to than juvenile rusa deer. However, no significant differences in plasma protein were observed between sexes of adult deer, although adult females had a higher mean plasma protein concentration than adult male rusa deer.

There were no significant difference between the values of differential leucocyte count. In general, each sample had least basophils and band neutrophils, followed by eosinophils, monocytes, lymphocytes, and the highest number were the segmented neutrophils.

**Table 1.** Blood parameters of rusa deer in comparison with age and sex.

Parameter	Unit	Values (mean ± S.D.)			
		Juvenile (≤1 year-old)		Adult (>1 year-old)	
		Male (n=13)	Female (n=5)	Male (n=13)	Female (n=9)
Erythrocytes	×10 <sup>12</sup> /L	11.52 <sup>a,b</sup> ± 0.57	11.64 <sup>a,b</sup> ± 0.23	11.79 <sup>a</sup> ± 0.63	10.67 <sup>b</sup> ± 1.11
Haemoglobin	g/L	152.54 ± 13.48	165.60 ± 10.41	165.31 ± 10.65	157.78 ± 14.72
PCV <sup>c</sup>	L/L	47.00 ± 4.06	47.40 ± 3.21	49.65 ± 4.12	45.89 ± 5.33
MCV <sup>d</sup>	fL	34.34 <sup>a</sup> ± 1.46	35.37 <sup>a,b</sup> ± 1.43	35.95 <sup>a,b</sup> ± 3.17	38.92 <sup>b</sup> ± 4.72
MCHC <sup>e</sup>	g/dL	386.40 ± 37.50	402.94 ± 32.26	391.37 ± 16.28	384.73 ± 39.00
Plasma protein	g/L	63.85 <sup>a</sup> ± 3.34	64.80 <sup>a</sup> ± 5.22	70.15 <sup>a,b</sup> ± 3.41	74.89 <sup>b</sup> ± 9.71
Leucocytes	×10 <sup>9</sup> /L	5.62 ± 1.91	4.08 ± 1.43	5.12 ± 2.90	5.49 ± 1.10
Band Neutrophils	×10 <sup>9</sup> /L	0.04 ± 0.04	0.03 ± 0.03	0.03 ± 0.04	0.01 ± 0.04
Seg. Neutrophils	×10 <sup>9</sup> /L	3.01 ± 1.31	1.86 ± 0.80	2.56 ± 2.00	2.88 ± 0.66
Lymphocytes	×10 <sup>9</sup> /L	2.04 ± 0.70	1.68 ± 0.56	1.97 ± 1.11	1.82 ± 0.50
Monocytes	×10 <sup>9</sup> /L	0.39 ± 0.20	0.36 ± 0.14	0.38 ± 0.15	0.47 ± 0.20
Eosinophils	×10 <sup>9</sup> /L	0.12 ± 0.08	0.14 ± 0.13	0.14 ± 0.11	0.27 ± 0.20
Basophils	×10 <sup>9</sup> /L	0.03 ± 0.04	0.01 ± 0.02	0.03 ± 0.05	0.04 ± 0.09

a, b Values bearing different superscript within a row differ significantly ( $p < 0.05$ ).

c Packed Cell Volume; d Mean Cell Volume; e Mean Cell Haemoglobin Concentration.

The biochemical data (Table 2) also revealed a significant difference in blood Cl<sup>-</sup>, Ca<sup>++</sup>, AST, CK, and globulin concentration, where Cl<sup>-</sup> concentration in juvenile females was significantly higher than adult females. Adult females also had the lowest values of Ca<sup>++</sup> concentration. As for the serum enzymes, a huge range of values were observed especially for ALP and CK, in which the juvenile males reported the highest concentrations and the adult females the lowest. The adult females significantly ( $p < 0.05$ ) had the highest total protein and globulin concentrations than the other groups of deer. Albumin values, on the other hand, were fairly constant among groups of

deer. Consequently, a reduction in albumin to globulin ratio with age were observed.

The coagulation parameter, PT was significantly longer in adult males in comparison with the other groups. While APTT showed no difference between the groups of deer (Table 3).

## DISCUSSION

The erythrocyte count, haemoglobin concentration, MCV and MCHC values were similar to reports on sambar deer (Siti Norzubaidah, 2010), chital deer (Chapple *et al.*, 1991) and red deer (Marco and Lavin, 1999). In Chapple *et al.* (1991)

**Table 2.** Biochemical parameters of rusa deer in comparison with age and sex.

Parameter	Unit	Values (mean $\pm$ S.D.)			
		Juvenile ( $\leq 1$ year-old)		Adult ( $>1$ year-old)	
		Male (n=13)	Female (n=5)	Male (n=13)	Female (n=9)
Na <sup>c</sup>	mmol/L	147.25 $\pm$ 4.52	148.80 $\pm$ 4.91	144.21 $\pm$ 6.14	146.24 $\pm$ 4.89
K <sup>d</sup>	mmol/L	5.97 $\pm$ 0.59	6.28 $\pm$ 1.01	5.78 $\pm$ 0.87	5.63 $\pm$ 1.11
Cl <sup>e</sup>	mmol/L	97.13 <sup>a,b</sup> $\pm$ 2.21	98.78 <sup>b</sup> $\pm$ 2.42	96.03 <sup>a,b</sup> $\pm$ 2.58	94.79 <sup>a</sup> $\pm$ 2.19
Ca <sup>f</sup>	mmol/L	2.57 <sup>a,b</sup> $\pm$ 0.09	2.56 <sup>a,b</sup> $\pm$ 0.18	2.63 <sup>a</sup> $\pm$ 0.11	2.44 <sup>b</sup> $\pm$ 0.22
Phosphate	mmol/L	2.30 $\pm$ 0.68	2.43 $\pm$ 0.61	1.93 $\pm$ 0.48	2.50 $\pm$ 0.28
Urea	mmol/L	8.61 $\pm$ 0.72	8.40 $\pm$ 1.00	8.85 $\pm$ 0.88	8.24 $\pm$ 0.76
Creatinine	mmol/L	172.7 $\pm$ 18.3	174.4 $\pm$ 19.7	186.6 $\pm$ 19.1	165.9 $\pm$ 15.6
Glucose	mmol/L	5.12 $\pm$ 0.83	4.78 $\pm$ 0.39	5.05 $\pm$ 1.16	3.73 $\pm$ 1.80
Cholesterol	mmol/L	1.96 $\pm$ 0.27	1.77 $\pm$ 0.23	2.12 $\pm$ 0.36	1.96 $\pm$ 0.37
T. Bilirubin	$\mu$ mol/L	0.75 $\pm$ 1.21	1.10 $\pm$ 0.23	2.88 $\pm$ 6.01	1.80 $\pm$ 1.48
ALT <sup>g</sup>	U/L	75.28 $\pm$ 10.00	67.64 $\pm$ 9.00	73.49 $\pm$ 9.15	77.14 $\pm$ 16.78
ALP <sup>h</sup>	U/L	161.2 $\pm$ 45.6	156.8 $\pm$ 48.8	152.4 $\pm$ 30.1	112.4 $\pm$ 52.0
AST <sup>i</sup>	U/L	73.58 <sup>b</sup> $\pm$ 21.50	63.24 <sup>a,b</sup> $\pm$ 32.62	50.72 <sup>a,b</sup> $\pm$ 12.13	49.06 <sup>a</sup> $\pm$ 6.25
CK <sup>j</sup>	mmol/L	1655.5 <sup>b</sup> $\pm$ 1363.7	626.8 <sup>a,b</sup> $\pm$ 231.0	651.0 <sup>a,b</sup> $\pm$ 637.1	399.8 <sup>a</sup> $\pm$ 253.2
Total protein	g/L	69.34 <sup>a</sup> $\pm$ 1.18	69.10 <sup>a</sup> $\pm$ 5.12	74.32 <sup>a</sup> $\pm$ 4.07	83.86 <sup>b</sup> $\pm$ 12.25
Albumin	g/L	39.28 $\pm$ 5.18	38.84 $\pm$ 8.03	41.49 $\pm$ 3.78	44.48 $\pm$ 5.39
Globulin	g/L	30.05 <sup>a</sup> $\pm$ 5.31	30.26 <sup>a</sup> $\pm$ 4.03	32.82 <sup>a,b</sup> $\pm$ 4.77	39.38 <sup>b</sup> $\pm$ 8.37
A/G Ratio <sup>k</sup>		1.38 $\pm$ 0.45	1.34 $\pm$ 0.49	1.30 $\pm$ 0.32	1.16 $\pm$ 0.21

a,b Values bearing different superscript within a row differ significantly ( $p < 0.05$ ). c Sodium; d Potassium; e Chloride; f Calcium; g Alanine Transaminase; h Alkaline Phosphatase; i Aspartate Transaminase; j Creatine Kinase; k Albumin/Globulin ratio.

**Table 3.** Blood coagulation parameters of rusa deer in comparison with age and sex.

Parameter	Unit	Values (mean $\pm$ S.D.)			
		Juvenile ( $\leq 1$ year-old)		Adult ( $>1$ year-old)	
		Male (n=13)	Female (n=5)	Male (n=13)	Female (n=9)
PT <sup>c</sup>	mmol/L	25.84 <sup>a</sup> $\pm$ 1.88	25.00 <sup>a</sup> $\pm$ 1.86	29.91 <sup>b</sup> $\pm$ 3.24	24.36 <sup>a</sup> $\pm$ 3.35
APTT <sup>d</sup>	mmol/L	35.82 $\pm$ 42.95	42.06 $\pm$ 42.04	28.65 $\pm$ 6.65	25.52 $\pm$ 6.26

a,b Values bearing different superscript within a row differ significantly ( $p < 0.05$ ).

c Prothrombin Time; d Activated Partial Thromboplastin Time.

study, the erythrocyte count, haemoglobin concentration and PCV were significantly different between adult male and female deer. However, in this study, only the erythrocyte count was significantly higher in the adult males in comparison with the female rusa deer. This differences observed in the erythrocyte count may be correlated with body size, erythrocyte size and number (Hawkey and Hart, 1985), as the body size of rusa deer are relatively smaller than other breeds of deer.

The differential leucocyte count of the rusa deer was similar to that of the adult sambar deer (Siti Norzubaidah, 2010) and no significance difference in leucocyte counts among deer of different ages or sexes. In chital deer, the lymphocytes were found to be predominant in the adults rather than in the juveniles. However, in this present study, the neutrophils were more predominant when compared to the others. The differences in the differential leucocyte count among the deer could have been caused by the response to handling and restraint of the animals during sampling (Chapple *et al.*, 1991).

The plasma protein, which consists of albumin, globulin and fibrinogen, showed a significant difference between age groups. The adults were observed to have higher plasma protein concentration compared to the juvenile deer. This could be attributed to the fact that young animals are not being fully immunocompetent. On the other hand their maternal immunity will also wane with time (Chapple *et al.*, 1991). Limited reports are available on the blood sodium, potassium, chloride, calcium and phosphate values. In the current study, the chloride and calcium

values were reported to be the lowest in the adult females. The low concentrations of these electrolytes in the adult females could be due to the effect of diet, water intake, nutritional status and nursing of the young. The deer in this study also showed lower blood urea nitrogen, creatinine, glucose, and total bilirubin concentrations in comparison with the fallow, sambar, red and axis deer (Peinado *et al.*, 1999). Blood urea nitrogen concentration is highly dependent on the diet and nutritional status. Thus, comparison of this parameter between animals may not be appropriate unless management or husbandry conditions of the animals are standardised. The higher blood creatinine concentration observed in sambar deer than in rusa deer may be a consequence of larger muscle mass in sambar as compared to the rusa deer (DelGiudice *et al.*, 1992).

The mean blood glucose concentration is lower in adults than the young deer. The lower blood glucose might be due to lower energy needs of the adult deer (Gill, 1990), or to a more developed ruminating system in which the monosaccharides in the food are degraded to volatile fatty acid in the fore-stomachs (Wolk and Josefczak, 1988). On the other hand, the increase in total bilirubin in blood concentration is reported to be associated with the decrease in body condition of the animals (Berger, 1956).

The serum enzyme values obtained in this study are similar to the values obtained in sambar deer (Siti Norzubaidah, 2010). Juvenile deer generally have higher serum ALP concentration than the adults. This was shown in our study and also in the previous findings on the axis and red deer (Peinado *et al.*, 1999). ALP activity in blood is associated

with the osteoblastic activity and also an index of skeletal and antler growth. Thus young animals are expected to show higher levels than the older ones (Kie *et al.*, 1983). When comparison were done between sex, the serum AST and CK concentrations were recorded highest in juvenile males. Since these enzymes are sensitive indicators of muscle damage, it may also be associated with fear or excitement during handling (Duncan and Prasse, 1986). The results suggested that the juvenile males react more vigorously to handling than the juvenile females or older deer.

The total protein, albumin, and globulin concentrations in the rusa deer in this study are similar to the data obtained from chital deer (Chapple *et al.*, 1991). In this study, adult deer, irrespective of sex had higher protein concentration than juvenile deer. Since the blood protein concentration is mainly the sum of albumin and globulin concentration, and also a reflection of the nutritional status of an animal, this suggests that the adult deer are more likely to adapt at obtaining nutritious food compared to the young deer. The adult deer also have greater exposures to diseases that may result in the mounting of immune response levels, which is reflected in the increase in blood globulin concentration.

Deer are easily excitable, thus physical restraining with minimal excitation of the animal is important in the sampling of blood for analysis. Studies have shown that poor physical restraining techniques will increase the values of erythrocyte and leucocyte counts, PCV, haemoglobin, ALT, AST and CK concentrations (Maede *et al.*, 1990; Marco and Lavin, 1999). However, if the animal is

conditioned and used to frequent handling, the effect on these values can be minimised (English and Lepherd, 1981).

The information for the normal range of coagulation parameters such as PT and APTT in deer were limited. We observed a significant increase in PT in the adult males but the reason was unknown. However, since these values were normally distributed, it can be used as preliminary base-line studies in the future. Values reported by other researchers vary considerably making it very hard to establish them as reference ranges. For instance, Sutherland *et al.* (1985) found PT to be 16.3 seconds, 20.9 seconds by Siroka *et al.* (2011) while our results were more prolonged within 24.3-29.9 seconds. An assumption of increased the PT could be due to the possible stress associated with handling of these animals but yet needs to be proved.

## CONCLUSION

The objective of the study was achieved by generating a blood and coagulation baseline profiles of rusa deer in Malaysia in semi-captive management. It is necessary to take into account the age and sex factors when using these blood reference values for monitoring the health status or diagnosis of diseases in the rusa deer.

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