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HAEMATOLOGY AND SERUM BIOCHEMISTRY OF CAPTIVE UNSEDATED CHITAL DEER (*AXIS AXIS*) IN AUSTRALIA

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ABSTRACT: Haematological and serum biochemical values were measured in blood samples collected over a 12-mo period from 37 unsedated chital deer (*Axis axis*). Stags and hinds, ranging in age from birth to maturity, were sampled. Haemoglobin, total erythrocyte and haematocrit values were low at birth and sex differences were not apparent in neonates and juveniles, but were in adults. Chital stags had higher erythrocyte parameters ($P < 0.001$) and lower erythrocyte indices than hinds, and the total leucocyte count was higher in stags ($P < 0.01$). Some parameters (erythrocytes, muscle enzymes, glucose, cortisol) decreased over successive serial sampling. The differential leucocyte count of older stags decreased during the initial handling period. The major rutting period in February and March was characterised by changes in the differential leucocyte count, elevations in serum muscle enzymes, and lower serum cortisol levels. Alkaline phosphatase activity in serum reflected the annual antler cycle of chital stags. Serial sampling over many weeks, either weekly or tri-weekly, produced haematological and biochemical changes in successive samples which may have reflected a reduction in stress and excitement associated with restraint.

Key words: Chital deer, *Axis axis*, haematology, serum biochemistry, adaptation to handling, captive study.

INTRODUCTION

The farming of chital deer in Australia has created a need for haematological and serum biochemical reference data. The only published values are for haematology of single blood samples from 41 chital deer in zoological gardens in India (Naik et al., 1964), England (Hawkey, 1975; Hawkey and Hart, 1985) and California (Karesh et al., 1986) and there is no available data on serum biochemistry. The populations of chital deer sampled in those studies may not be representative of the population of chital deer in Australia and their data do not distinguish between the sexes. Furthermore, blood samples were collected from deer unaccustomed to restraint and handling. Excitement and stress during restraint have been found to affect haematological values in many species of deer (Presidente, 1979; Wesson et al., 1979; Mautz et al., 1980; Rehbinder and Edquist, 1981; Wilson and Pauli, 1982). Frequent handling of animals permits adaptation to restraint for blood sampling and associated haematological changes have been reported in cattle (Gartner et al., 1969) and wild bighorn sheep (Franzmann and Thorne, 1970). Chemical restraint was used by Hawkey (1975) and Hawkey and Hart (1985) during blood sampling from chital and this form of restraint has been shown to depress some blood parameters in cervids, when compared with the unsedated state (Seal et al., 1972a; Presidente et al., 1973; Wesson et al., 1979; Kocan et al., 1981).

The aim of the present study was to assess the affects of restraint and handling of unsedated chital deer on haematological and serum biochemical values. The samples were collected over a 12 mo period from stags and hinds, aged from birth to maturity.

MATERIALS AND METHODS

Animals

Blood samples were collected from 10 hinds, at least 2-yr-old, on alternate days, three times a week for 7 wk in 1987 and for 6 wk in 1988, in February and March (only nine hinds were sampled in 1988 due to one death). These hinds were joined with a stag at the end of March 1987, and 3 mo later were sampled weekly for 3 wk. Blood from one 18-mo and one 2-yr-old chital stag was collected fortnightly for 3 mo

TABLE 1. Haematology values expressed as means (SD) of chital deer from birth to maturity.

Group	Hb g/L	RBC $\times 10^{12}$/L	WBC $\times 10^{9}$/L	HCT L/L	MCV fl	MCH pg	MCHC g/L
Hinds	144.6[a]	11.64[a]	3.92[ac]	0.39[a]	33.68[a]	12.47[a]	368[a]
$n = 10$	(6.6)	(0.72)	(0.44)	(0.02)	(2.58)	(0.91)	(7)
Pregnant hinds	164.3[b]	13.28[b]	4.87[b]	0.47[bc]	35.45[b]	12.43[a]	351[b]
$n = 10$	(5.3)	(0.80)	(1.08))	(0.01)	(2.61)	(0.88)	(7)
Juvenile hind	164.8[b]	14.25[c]	4.30[bcd]	0.46[bc]	30.85[ac]	11.47[abd]	358[b]
$n = 1$							
Stags	165.4[bc]	14.27[c]	4.41[d]	0.46[c]	31.97[c]	11.62[b]	363[a]
$n = 7$	(6.5)	(0.34)	(0.44)	(0.01)	(1.46)	(0.64)	(15)
Juvenile stag	171.2[c]	16.48[d]	4.52[bd]	0.46[bc]	27.70[d]	10.38[e]	374[a]
$n = 1$							
Neonates	109.0[d]	10.22[e]	8.40[e]	0.34[d]	33.15[a]	10.94[de]	327[c]
$n = 8$	(11.3)	(1.60)	(2.60)	(0.04)	(4.60)	(1.40)	(9)

[a,b,c,d,e]: Means with the same superscript did not differ significantly at the 5% level.

and then weekly for 9 mo, from May 1986 to May 1987. Another 18-mo-old stag died after 6 mo of sampling, and a fourth stag was sampled only occasionally due to handling difficulty. Three stags aged 5, 11 and 13 mo were included during the last 6 mo of weekly blood sampling. A hand-reared hind was sampled monthly from 7 to 10 mo of age and a hand-reared stag was sampled six times when 13- to 18-mo-old. Serum was collected for biochemical assay from a separate population of 10 stags ranging in age from 1- to 2-yr, at a nearby abattoir. Single blood samples were also taken from eight neonates (five females and three males) when they were caught for weighing and ear-tagging at 1- to 2-days-old.

Animal handling

Prior to the period of handling of animals in a cradle designed for restraint and collection of blood samples, "training" and "acclimatisation" to yarding by routine movement of the deer, as described by Harthoorn (1979, 1981a, b), was carried out on several occasions. Each animal was regarded as "untrained" for the first 3 to 4 wk (hinds) and 7 to 8 wk (stags) of handling and sampling in the restraint cradle, but thereafter were regarded as "trained." Hinds were bled from 0700–0800 hours, and stags from 0900–1100 hours. The deer were run a few hundred metres from their paddock into yards immediately prior to bleeding, and restrained in a drop-floor cradle for no more than 2 min. The time elapsed from yarding to release after blood collection was <1 hr for each group of animals. One stag was sampled only four times due to handling difficulty. The neonates were sampled while held in the paddock.

Blood collection and analysis

Blood was collected with an 18-ga needle by jugular venipuncture, using the Vacutainer blood collection system (Becton, Dickinson and Co., Rutherford, New Jersey 07070, USA). The samples were collected into a plain tube for serum and EDTA tube for haematology. Blood samples were centrifuged within 2 hr of collection, and serum harvested for biochemical analysis. Serum fractions were stored at −20 C until used for cortisol analysis. Samples for haematology were refrigerated and analysis was completed within 36 hr. Standard manual haematological techniques were used, with the exception of total cell counts, which were measured on a coulter ZBi counter (Coulter Electronics, Dunstable LU63HT, England). Settings for RBC were Aperature Current Setting (ACS) 0.177, Amplitude (AMP) 1/8 and Threshold Level (TL) 10. White blood cell settings were ACS 0.25, AMP 1/2 and TL 12.5. A 100 μm diameter orifice tube was used throughout. Biochemical profiles were compiled by the use of a Technicon SMA 12/60 autoanalyser (Technicon, Sydney, 2113, Australia) calibrated using commercial standards (American Dade, Miami, Florida 33152, USA). Electrophoretic patterns were determined with cellulose acetate strips using the Gelman sepratek system (Gelman Clemco, Sydney, 2064, Australia) and scanned on a Gelman R scanner and recorder for levels of albumin, globulins and albumin to globulin ratios (A:G). Total plasma protein was measured on an American Optics refractometer (American Optics, Buffalo, New York 14215, USA).

Cortisol assay

Total cortisol was measured in 25 μl of serum using an (^{125}I) radioimmunoassay kit (Diagnostic

TABLE 2. Differential leucocyte counts expressed as $\bar{x}$ (SD) of chital deer from birth to maturity.

	Leucocytes (absolute)					
	Neutrophils					
	Banded	Segmented	Lymphocytes	Monocytes	Eosinophils	Basophils
Hinds	0.028	1.673[a]	1.946[a]	0.136[ab]	0.181	0.003
n = 10	(0.037)	(0.335)	(0.377)	(0.060)	(0.086)	(0.008)
Pregnant hinds	0.002	1.766[a]	2.741[b]	0.168[a]	0.162	0.001
n = 10	(0.009)	(0.265)	(0.307)	(0.073)	(0.109)	(0.008)
Juvenile hind	0.000	1.780[a]	2.252[ab]	0.117[ab]	0.134	0.000
n = 1						
Stags	0.005	1.738[a]	2.385[a]	0.085[b]	0.127	0.001
n = 7	(0.023)	(0.548)	(0.594)	(0.020)	(0.042)	(0.006)
Juvenile stag	0.000	1.373[a]	3.119[b]	0.098[bc]	0.104	0.006
n = 1						
Neonates	0.000	5.810[b]	2.320[ab]	0.174[ac]	0.085	0.000
n = 8	(0.000)	(1.740)	(1.290)	(0.088)	(0.103)	(0.000)

[a,b,c,d,e]: Means with the same superscript did not differ significantly at the 5% level.

Products Corporation, Los Angeles, California 90045, USA). High and low concentration control samples were included in duplicate in each assay. The inter-assay coefficients of variation were 6.4% and 7.5% for the high and low samples respectively. The lower limit of reading for the assay was 20 nmol/L, and the upper limit 2,000 nmol/L.

Statistical analysis

Means and standard deviations were calculated for animals grouped by age, sex and pregnancy status: (1) non-pregnant hinds, (2) pregnant hinds, (3) adult stags (>12-mo-old), (4) the juvenile hind (<12-mo-old), (5) the juvenile stag, (6) abattoir stags, and (7) neonates. Groups were compared by one-way analysis of variance at the 5% level significance. Regressions were performed on parameters over time. Blood samples from the non-pregnant adult hinds in 1987 and 1988 were compared. Regressions were applied to mean serum cortisol levels in the hinds on each day that samples were assayed.

RESULTS

Haematology

Tables 1 and 2 present means of haematological parameters for each group of animals. Red cell parameters, comprised of haemoglobin (Hb), total red blood cells (RBC) and haematocrit (HCT), increased 40 to 60% from birth to 10-mo-old, and values at maturity were 15 to 50% higher than at birth. Sex differences were not apparent in neonates or juveniles, but were significant in adults. Red cell parameters were 15 to 22% higher in adult stags than hinds ($P < 0.001$), and the total white blood cell (WBC) count was significantly greater in stags ($P < 0.01$). The WBC count of neonates was significantly greater than that of juveniles and adults ($P < 0.001$).

Mean corpuscular haemoglobin concentration (MCHC) varied significantly ($P < 0.05$) between pregnant and non-pregnant hinds, and between the same non-pregnant hinds sampled in different years. During pregnancy there was a significant increase in Hb, RBC and HCT ($P < 0.001$) and mean corpuscular volume (MCV) ($P < 0.01$), and a decrease in MCHC ($P < 0.001$). There was also an increase in the WBC count ($P < 0.001$) due to an increase in the number of lymphocytes ($P < 0.001$).

Lymphocytes predominated in the differential white cell counts for all animals except neonates, which had a neutrophil to lymphocyte ratio of 2:1. The number of neutrophils was higher in stags during the initial 2 mo of sampling but lymphocytes predominated for the remaining 10 mo. The differential white cell count in hinds was variable throughout the sampling periods but during pregnancy the number of lymphocytes measured was always higher than the number of neutrophils.

TABLE 3. Serum protein fractions expressed as $\bar{x}$ (SD) in chital deer.

	Protein g/L	Albumin g/L	Globulins 1 g/L	2 g/L	β_1 g/L	β_2 g/L	Gamma g/L	A:G[f] g/L
Hinds	70.2[a]	36.8[a]	2.4[a]	4.6[a]	5.6[a]	4.1[a]	15.5[a]	1.18[a]
n = 10	(3.9)	(1.5)	(0.3)	(0.6)	(0.8)	(0.3)	(3.1)	(0.18)
Pregnant hinds	69.8[a]	38.3[c]	2.2[ab]	3.9[b]	7.0[b]	4.2[a]	14.4[a]	1.24[a]
n = 10	(5.4)	(1.5)	(0.4)	(0.6)	(1.1)	(0.7)	(3.6)	(0.22)
Juvenile hind	56.3[d]	33.7[d]	2.2[ab]	5.0[ac]	5.7[a]	3.0[c]	7.3[bc]	1.35[ab]
n = 1								
Stags (A)	63.6[c]	37.9[a]	2.5[ab]	4.5[a]	5.8[a]	3.2[bc]	9.7[bc]	1.55[b]
n = 7	(3.4)	(2.3)	(0.5)	(0.5)	(0.5)	(0.5)	(2.6)	(0.36)
Stags (B)	67.2[b]	32.9[d]	2.7[a]	5.7[c]	10.7[c]	3.9[ab]	11.6[c]	0.97[c]
n = 10	(4.2)	(3.2)	(0.5)	(0.9)	(1.5)	(0.6)	(1.9)	(0.19)
Juvenile stag	62.8[c]	39.8[a]	2.0[b]	5.1[ac]	6.3[ab]	3.0[c]	6.8[d]	1.73[d]
n = 1								
Neonates	61.1[cd]	27.5[c]	2.2[ab]	3.2[b]	4.3[a]	8.0[d]	12.2[c]	1.63[d]
n = 8	(14.9)	(1.9)	(0.9)	(1.3)	(2.6)	(7.8)	(9.1)	(1.53)

[a,b,c,d,e]: Means with the same superscript did not differ significantly at the 5% level.
Stags (A), stags from research farm; (B), stags from abattoir.
[f] Albumin to globulin ratio.

TABLE 4. Elevated ("untrained") and baseline ("trained") means (SD) for aspartate aminotransferase (AST) and creatine kinase (CK) in serum of chital deer.

	Untrained mean		Trained mean	
	AST	CK	AST	CK
Hinds 1987	286	2,129	79[a]	508[a]
n = 10	(265)	(1,478)	(21)	(201)
Hinds 1988	165	1,623	84[a]	372[a]
n = 9	(25)	(914)	(22)	(180)
Pregnant hinds	—	—	62[b]	317[b]
n = 10			(10)	(63)
Juvenile hind	2,040	7,400	109[abc]	434[ab]
n = 1				
Stags (A)[f]	308	2,850	74[ad]	326[a]
n = 7	(176)	(1,801)	(19)	(86)
Stags (B)[f]	76	4,977	—	—
n = 10	(82)	(1,351)		
Juvenile stag	—	—	66[bd]	294[b]
n = 1				
Neonates	125	838	—	—
n = 8	(59)	(682)		

[a,b,c,d,e]: Superscripts are for untrained and trained means combined. Means with the same superscript did not differ significantly at the 5% level.
[f]Stags (A), stags from research farm; (B), stags from abattoir.

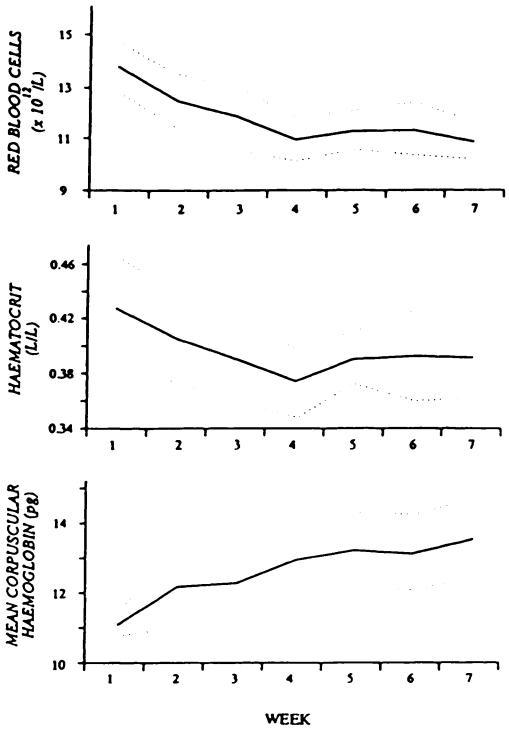

FIGURE 1. Weekly changes in red blood cell parameters (red blood cell count and haematocrit) and the mean corpuscular haemoglobin ($\bar{x}$ and SD) of chital hinds over the first 7 wk of tri-weekly blood sampling.

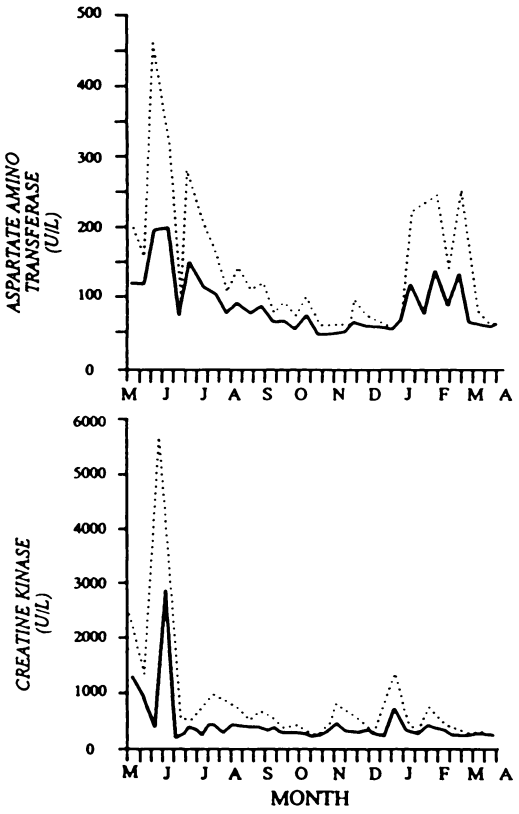

FIGURE 2. Serum levels (U/L) of aspartate aminotransferase and creatinine kinase ($\bar{x}$ and SD) measured in weekly blood samples from 7 chital stags over 12 mo.

The hinds which were handled three times a week in 1987 had decreases in the Hb, RBC and HCT parameters over time, with values from initial samples being similar to those measured in stags. The stags had no consistent changes in Hb, RBC and HCT, but decreases in the RBC count ($P < 0.05$) and HCT of hinds occurred (Fig. 1). The mean corpuscular haemoglobin (MCH) increased over time ($P < 0.01$) (Fig. 1), and MCV ($P < 0.001$) and MCHC followed similar trends. These consistent changes over time did not occur when the hinds were sampled again 1 yr later, when mean values for Hb, RBC and HCT were lower and of similar levels to those measured in the last weeks of the first year. Haemoglobin concentration did not fall consistently over time in either year of sampling.

Electrophoresis

Results from the electrophoresis of proteins are shown in Table 3. There were no seasonal fluctuations in blood protein levels in stags over 12 mo. Hinds had significantly higher levels of gammaglobulins ($P < 0.001$) and total protein ($P < 0.01$) than stags, while stags had a higher A:G ratio ($P < 0.01$). Alpha-2 globulins were signif-

TABLE 5. Serum biochemistry values expressed as $\bar{x}$ (SD) of chital deer.

	AP U/L	LDH U/L	ALT U/L	Bilirubin μmol/L	UN mmol/L	Glucose mmol/L	P mmol/L	Ca mmol/L
Hinds	118[a]	453[a]	51[a]	4.5[a]	7.5[a]	7.9[a]	2.2[a]	2.3[a]
$n = 10$	(38)	(45)	(10)	(0.8)	(0.8)	(1.2)	(0.1)	(0.0)
Pregnant hinds	149[b]	331[b]	26[c]	5.1[a]	9.0[b]	7.3[a]	2.2[a]	2.3[a]
$n = 10$	(52)	(43)	(4)	(1.3)	(0.8)	(1.2)	(0.2)	(0.1)
Juvenile hind	319[c]	1,017[c]	119[de]	6.2[a]	11.7[cd]	6.7[ab]	2.6[ab]	2.5[bc]
$n = 1$								
Stags	219[cd]	486[a]	57[a]	5.3[a]	9.7[bc]	8.2[a]	2.4[a]	2.4[ab]
$n = 7$	(31)	(24)	(4)	(2.5)	(0.7)	(1.6)	(0.2)	(0.1)
Abattoir stags	196[d]	775[c]	127[d]	3.8[ab]	11.2[d]	5.9[b]	2.2[a]	2.4[ab]
$n = 10$	(64)	(82)	(31)	(1.1)	(1.5)	(1.3)	(0.6)	(0.1)
Juvenile stag	271[c]	569[d]	63[ae]	3.1[b]	9.6[bd]	7.5[a]	2.8[b]	2.7[c]
$n = 1$								
Neonates	2,493[e]	978[c]	55[abe]	13.0[c]	7.7[abc]	5.1[b]	3.1[b]	2.7[c]
$n = 8$	(975)	(987)	(39)	(4.1)	(2.4)	(2.2)	(0.6)	(0.1)

[a,b,c,d,e]: Means with the same superscript did not differ significantly at the 5% level.

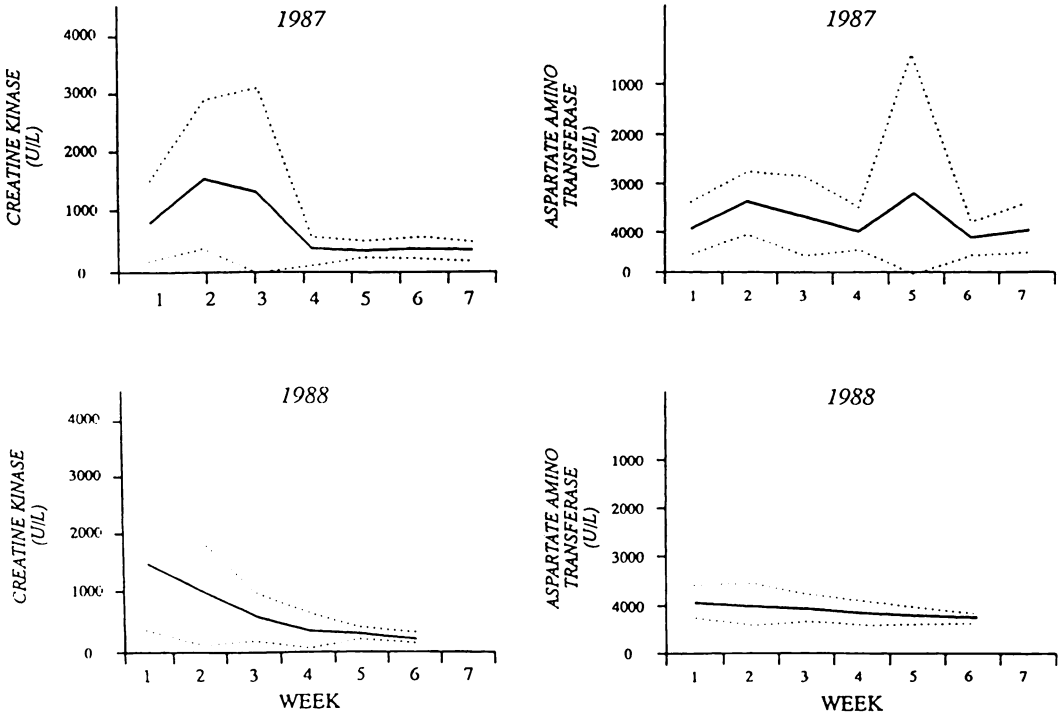

FIGURE 3. Serum enzyme levels (U/L) ($\bar{x}$ and SD) in 10 chital hinds during tri-weekly blood sampling.

icantly higher ($P < 0.05$) in the hinds in 1987 (4.9 g/L, SD = 0.6) compared with 1988 (4.2 g/L, SD = 0.4). The population of stags from the abattoir showed some differences in protein levels from other stags.

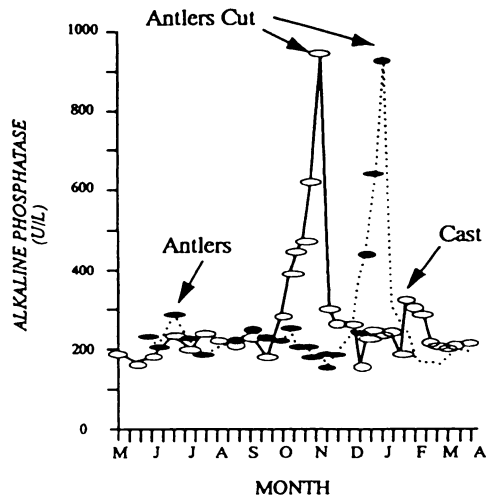

FIGURE 4. Serum levels (U/L) of alkaline phosphatase throughout the annual antler cycle of an 18- (O) and 24-mo-old (●) chital stag.

Levels of gammaglobulins were significantly lower in the juveniles, and the juvenile hind also had significantly lower total protein and beta-2 globulin levels than adult hinds ($P < 0.001$). Protein levels in neonates also differed from older animals (Table 3). Gammaglobulins in neonates ranged from 1 to 21 g/L, and levels of plasma proteins were increased in August and September compared with other months ($P < 0.01$).

During pregnancy, levels of albumin ($P < 0.05$) and beta-1 globulins ($P < 0.01$) were elevated, and alpha-2 globulins were low ($P < 0.05$). In addition, there were significant weekly fluctuations in levels of alpha-1 globulins in hinds ($P < 0.01$) which followed no trend.

Serum biochemistry

Serum concentrations of muscle enzymes aspartate amino transferase (AST) and creatine kinase (CK) were at least doubled in most "untrained" animals compared with "trained" animals (Table 4).

The maximum serum CK level in the stags was 7,400 U/L. Stabilized enzyme levels ("trained" levels) were reached after approximately eight fortnightly samples from the adult stags (Fig. 2). Elevated enzyme levels, measured 10 mo after sampling began (Fig. 2), were present only in one 2-yr-old stag, and were elevated for 4 wk from February to March 1987. There were fewer values from "untrained" animals, and mean enzyme concentrations were generally lower in the hinds in the second year of sampling (Fig. 4). The AST concentration was elevated in 14 samples (of total 68) in 1987 compared with four (of total 54) elevated levels in 1988, and CK concentrations were elevated in 16 samples in 1987 compared with 12 samples in 1988. Changes were not noticeable over time in means of other serum biochemical values (Table 5). Neonates had high concentrations of lactate dehydrogenase (LDH), ranging from 413 to 920 U/L, with the exception of one value which reached 3,187 U/L. Alanine amino transferase (ALT) concentrations were higher in the hinds in 1987 than in 1988 ($P < 0.001$).

Alkaline phosphatase (AP) activity in stags reflected changes in the annual antler cycles (Fig. 4). The mean serum level of AP in Table 5 is for stabilized concentrations in the four older stags. Growing antler, referred to as "velvet antler," was surgically removed 2 cm above the pedicle after 60 days of growth. At this stage AP reached a peak of 960 U/L (SD = 36 U/L) and then fell, returning to the initial level within 1 wk. The AP activity fell gradually during growth of the first antler pedicle in stags from 11- and 13-mo-old. Values fell from approximately 300 U/L to reach a level of 200 U/L (SD = 34 U/L) after their antlers had been cut and stopped growing. The AP was elevated in neonates, being higher than any levels reached in older animals, and there was variation between neonates. Concentrations of AP in adult hinds ranged from 61 to 220 U/L, remaining constant for each hind over time and were lower than the stabilized level in adult stags (219, SD = 31 U/L). The AP levels increased to a mean of 149 U/L (SD = 52 U/L) during pregnancy ($P < 0.05$).

Total bilirubin did not change seasonally in the stags, and neonates had a higher mean concentration of bilirubin than all other groups.

Urea nitrogen concentrations (UN) in the stags fluctuated non-seasonally over 12 mo, and all stags exhibited the same pattern. Non-pregnant hinds had lower UN levels than the stags ($P < 0.001$), which increased during pregnancy ($P < 0.01$). In 1987 the hinds had lower UN than in 1988 ($P < 0.001$). Serum glucose levels decreased significantly in the hinds ($P < 0.01$) over successive blood samples in 1987 and levels also decreased over time in the stags. The mean glucose level was lower in the hinds in the second year ($P < 0.05$), being similar to the levels measured during the final weeks of sampling in the first year. During pregnancy, glucose levels were similar to the lower levels measured in non-pregnant hinds. Serum glucose levels were low in the neonates. Phosphorous and calcium levels did not change seasonally, and were higher in the juveniles and neonates.

Serum cortisol

There were no significant sex differences in cortisol concentration. A circannual rhythm in serum cortisol concentration was apparent in the oldest chital stags (range 70 to 195 nmol/L), and appeared to reflect inversely serum enzyme levels. Cortisol concentrations were highest during antler growth phases, and lowest from January to March.

Serum cortisol levels in the non-pregnant hinds ranged from 68.6 nmol/L to 194.0 nmol/L, with an overall mean of 130.1 nmol/L (SD = 22.3 nmol/L, n = 58). Over the 6 wk tri-weekly sampling period there was a significant decrease in mean serum cortisol levels in the hinds (r = 0.44, $P < 0.05$). After weeks without handling, pregnant hinds had serum cortisol levels higher than levels measured in

non-pregnant hinds during early weeks of sampling ($\bar{x}$ = 146.9, SD = 25.2 nmol/L).

DISCUSSION

Some haematological and serum biochemical parameters decreased over successive samples (Figs. 1–3), which may have reflected a reduction in stress with adaptation to restraint and handling. Similar changes to muscle enzymes have been described for wild ungulates subjected to graded exercise (Harthoorn, 1977), where it was shown that enzyme levels declined over a period of 2 to 3 wk post-capture. In the present study Hb, RBC and HCT values rose 10 to 20% in chital hinds during early restraint periods (Fig. 1), and similar effects of excitement or stress on haematological parameters have been found in other species of deer (Presidente, 1979; Rehbinder and Edquist, 1981; Wilson and Pauli, 1982). The observed haematological changes suggest that hinds adapted to handling within 4 wk of tri-weekly blood sampling.

Haematological parameters appeared to differ between the sexes in response to handling and restraint. Stags did not show distinct erythron changes over time with handling, but had an altered differential leucocyte count during initial sampling periods. The annual male reproductive cycle of chital deer (Chapple, 1989) may have confounded changes in the blood associated with adaptation of the stags to handling. In that study increased excitement and activity of chital stags during the major rutting period in the early months of the year were characterised by altered differential leucocyte counts, increased serum enzyme levels and lowered serum cortisol levels. Leucocyte changes also have been found in caribou during the rut (McEwan and Whitehead, 1969). Seasonal changes in cortisol may therefore have refleced a seasonal change in the response to handling, or may simply have been due to circannual changes associated with increased rutting activity.

Serum cortisol concentrations also decreased over time in the hinds, possibly indicating reduced stress (Griffin and Hibma, 1991) with regular handling. However, significant changes in haematological and serum biochemical values over time were not reflected in serum cortisol levels, suggesting that these values may be more sensitive indicators of handling stress than serum cortisol. The relationship between serum levels of cortisol and muscle enzymes varies between studies. In the present study, levels appeared to be inversely related, while Rehbinder and Edquist (1981) found levels in reindeer were directly related.

Elevations to serum muscle enzyme concentrations in the present study were probably the result of fear or excitement of deer not used to handling. As animals adapted to regular handling, enzyme concentrations were reduced. Greater increases in CK and AST in stags compared with hinds (Figs. 2, 3) suggested that the stags reacted more vigorously to handling. Higher serum levels of muscle enzymes in males compared with females have also been found in wild red deer (Kent et al., 1980). Wilson and Pauli (1982) did not find this difference between farmed red deer stags and hinds, a finding that may have reflected the lower excitability of farmed red deer. The elevated enzyme concentrations may reflect the greater susceptibility of chital stags than hinds to stress-related death (capture myopathy) after handling (Chapple, 1989).

Changes in serum enzymes in the present study do not imply severe muscle damage, since red deer with known clinical histories of severe muscle damage were reported by Wilson and Pauli (1982) to have serum levels of CK from 35,000 to 47,500 U/L, which are significantly higher than the elevated levels measured in the chital stags (Table 4). Duncan and Prasse (1986) reported that serum levels of CK and AST were more sensitive indicators of muscle damage than LDH and ALT, which has been confirmed in the present study. Muscle enzymes have been shown to in-

crease in animals during the excitement and stress of restraint, due to increased cell permeability and cell damage (Duncan and Prasse, 1986). In the present study increases in serum enzyme levels (CK and AST) during initial sampling periods (Fig. 3) were of similar magnitude to those found in other deer species after capture (Bubenik, 1982; Morris and Bubenik, 1983; Wilson and Pauli, 1983).

Higher WBC counts in stags than hinds may be due to greater activity by the stags, including aggressive interactions, since increased muscular activity increases blood and lymph circulation, sequestering leucocytes in capillary beds into large-vessel blood (Schalm et al., 1975). Declining serum glucose levels in chital hinds over subsequent sampling periods may also be a response to decreased stress concurrent with adaptation to handling, since elevated glucose levels during the excitement of capture have been demonstrated in many species (Seal and Erickson, 1969; Franzmann and Thorne, 1970; Seal et al., 1972a; Pearson and Mellor, 1976; Karns and Crichton, 1978; Presidente, 1979; Kock et al., 1987).

Alkaline phosphatase is active in the proliferation of cellular areas of the antler, concentrations of which are correlated with antler growth (Graham et al., 1962; Morris and Bubenik, 1983; West and Nordan, 1976; Bubenik et al., 1987) and antler size (Sempere et al., 1986; Bubenik et al., 1987). In the present study AP concentrations peaked before the antlers began to calcify and then fell precipitously (Fig. 4). A similar pattern of seasonal change in serum AP levels coinciding with the annual antler cycle has been demonstrated in males of other deer species (Graham et al., 1962; West and Nordan, 1976; Brown et al., 1978; Morris and Bubenik, 1983). However, the changes did not occur until growth of the second set of antlers, and prior to this, levels were higher than the baseline in older stags, which has also been found in other deer species (LeResche et al., 1974). Elevated serum AP levels in chital neonates (Table 5) are probably the result of active bone metabolism and growth.

Differences were apparent between the present results (Table 1) and those from earlier studies on chital deer haematology (Naik et al., 1964; Hawkey, 1975; Hawkey and Hart, 1985; Karesh et al., 1986), which may be attributed to age, sex, and methods of restraint used for blood collection. The present study is the first to report haematology and serum biochemistry values for manually restrained, unsedated adult and juvenile chital deer, and is directly applicable to deer managed on farms.

Increases in red cell parameters during pregnancy may have assisted the foetus, with similar changes found in white-tailed does (Seal et al., 1972b). The increase in WBC count during pregnancy in chital hinds ($P < 0.01$) was primarily due to a rise in lymphocyte count ($P < 0.001$), which may have been an adaptation to increase foetal immunity. Serum glucose levels during pregnancy were lower than those measured in non-pregnant hinds during the first year of sampling (Table 5), and this may be due to foetal demands for energy causing maternal levels to fall (Russel et al., 1967).

Although no evidence was found in the present study, other studies have found that serum proteins and gammaglobulins alter in some species after stressful handling (Selye, 1946; Gartner et al., 1965, 1969; LeResche et al., 1974; English and Lepherd, 1981), possibly due to haemaconcentration. Protein increases with age (Table 3) have also been found in white-tailed deer (Youatt et al., 1965; Seal and Erickson, 1969), and adult protein levels did not appear to be reached before 10 mo of age. The wide range in gamma globulin levels in the neonates may indicate differences in the time and amount of milk ingested. The higher albumin : globulin ratio in neonates probably reflected the amount of colostrum ingested.

Levels of UN may be directly related to nutritional status in some cervids (LeResche et al., 1974), and the differences in

UN levels between groups of chital deer (Table 5) may be explained by levels of nutrition and different pastures grazed (Chapple, 1989). However, much higher levels of UN in chital neonates than adults may be related to an inability to adequately handle porphyrin breakdown products (Bush et al., 1983).

In conclusion, this study found variation in blood composition of chital deer with age and sex, and confirmed previous reports of changes in haematological and serum biochemical values with regular handling of animals. The stressful effect of restraint and handling must therefore be considered when measuring blood parameters. The most pronounced changes over time were decreases in red cell parameters and serum levels of muscle enzymes and glucose, suggesting that values found in initial blood samples were elevated above normal levels.

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LITERATURE CITED

BROWN, R. D., R. L. COWAN, AND J. F. KAVANAUGH. 1978. Effect of pinealectomy on seasonal androgen titers, antler growth and feed intake in white-tailed deer. Journal of Animal Science 47: 435–440.

BUBENIK, G. A. 1982. Chemical immobilization of captive white-tailed deer and the use of automatic blood samplers. *In* Chemical immobilization of wildlife, L. Nielsen, J. C. Haigh, and M. E. Fowler (eds.). Wisconsin Humane Society, Milwaukee, Wisconsin, pp. 335–354.

———, D. SCHAMS, AND G. COENEN. 1987. The effect of artificial photoperiodicity and antiandrogen treatment on the antler growth and plasma levels of LH, FSH, testosterone, prolactin and alkaline phosphatase in the male white-tailed deer. Comparative Biochemistry and Physiology A 87: 551–559.

BUSH, M., R. S. CUSTER, J. C. WHITLA, AND R. J. MONTALI. 1983. Haematologic and serum chemistry values of captive scimitar-horned oryx (*Oryx tao*): Variations with age and sex. Journal of Zoo Animal Medicine 14: 51–55.

CHAPPLE, R. S. 1989. The biology and behaviour of chital deer *Axis axis* in captivity. Ph.D. Thesis. The University of Sydney, Sydney, Australia, 496 pp.

DUNCAN, J. R., AND K. W. PRASSE. 1986. Veterinary laboratory medicine—Clinical pathology. Iowa State University, Ames, Iowa, 285 pp.

ENGLISH, A. W., AND E. E. LEPHERD. 1981. The haematology and serum biochemistry of wild fallow deer (*Dama dama*) in New South Wales. Journal of Wildlife Diseases 17: 289–295.

FRANZMANN, A. W., AND E. T. THORNE. 1970. Physiologic values in wild bighorn sheep (*Ovis canadensis canadensis*) at capture, after handling and after captivity. Journal of the American Veterinary Medical Association 157: 647–650.

GARTNER, R. J. W., J. W. RYLER, AND A. W. BEATTIE. 1965. The influence of degree of excitation on certain blood constituents in beef cattle. Australian Journal of Experimental Biological and Medical Science 43: 713–724.

———, L. L. CALLOW, K. G. CORINNE, AND P. M. PEPPER. 1969. Variations in the concentration of blood constituents in relation to the handling of cattle. Research in Veterinary Science 10: 7–12.

GRAHAM, E. A., R. RAINEY, R. E. KUHLMAN, E. H. HOUGHTON, AND C. A. MOYER. 1962. Biochemical investigations of deer antler growth: I. Alterations of deer blood chemistry resulting from antlerogenesis. Journal of Bone and Joint Surgery 44A: 482–488.

GRIFFIN, J. F. T., AND M. HIBMA. 1991. The impact of domestication on red deer immunity and disease resistance. *In* The biology of deer, R. D. Brown (ed.). Springer Verlag, New York, New York. In Press.

HARTHOORN, A. M. 1977. Problems relating to capture. Animal Regulation Studies 1: 23–46.

———. 1979. The use of corals to capture and train wild ungulates prior to relocation. The Veterinary Record 104: 349.

———. 1981a. Comparison of two methods of capture of wild animals. The Veterinary Record 108: 37.

———. 1981b. Mechanism and prevention of capture myopathy. *In* Wildlife diseases of the Pacific Basin and other countries, M. E. Fowler (ed.). University of California, Davis, California, pp. 248–252.

HAWKEY, C. M. 1975. Comparative mammalian haematology. Heinemann, London, England, 310 pp.

———, AND M. G. HART. 1985. Normal haematological values of axis deer (*Axis axis*), Pere David's deer (*Elaphurus davidianus*) and barasingha (*Cervus duvauceli*). Research in Veterinary Science 39: 247–248.

KARESH, W. B., D. L. JANSSEN, AND J. E. OOSTERHUIS. 1986. Neonatal haematology of selected species of cervidae and bovidae. Journal of Zoo Animal Medicine 17: 138–146.

KARNS, P. D., AND V. F. J. CRICHTON. 1978. Effects of handling and physical restraint on blood parameters in woodland caribou. The Journal of Wildlife Management 42: 904–908.

KENT, J. E., D. I. CHAPMAN, AND N. G. CHAPMAN. 1980. Serum constituents of red deer (*Cervus elaphus*). Research in Veterinary Science 28: 55–57.

KOCAN, A. A., B. L. GLEEN, T. R. THEDFORD, R. DOYLE, K. WALDRUP, G. KUBAT, AND M. G. SHAW. Effects of chemical immobilization on hematologic and serum chemical values in captive white-tailed deer. Journal of the American Veterinary Medical Association 179: 1153–1156.

KOCK, M. D., D. A. JESSOP, R. K. CLARK, AND C. E. FRANTI. 1987. Effects of capture on biological parameters in free ranging bighorn sheep (*Ovis canadensis*): Evaluation of normal, stressed and mortality outcomes and documentation of postcapture survival. Journal of Wildlife Diseases 23: 652–662.

LERESCHE, R. E., U. S. SEAL, P. D. KARNS, AND A. W. FRANZMANN. 1974. A review of blood chemistry of moose and other cervidae with emphasis on nutritional assessment. Naturaliste Canada 101: 263–290.

MAUTZ, W. W., U. S. SEAL, AND C. B. BOARDMAN. 1980. Blood serum analysis of chemically and physically restrained white-tailed deer. The Journal of Wildlife Management 44: 343–351.

MCEWAN, E. H., AND P. E. WHITEHEAD. 1969. Changes in the blood constituents of reindeer and caribou occurring with age. Canadian Journal of Zoology 47: 557–562.

MORRIS, J. M., AND G. A. BUBENIK. 1983. Seasonal levels of minerals, enzymes, nutrients and metabolic products in plasma of intact and castrated adult male white-tailed deer (*Odocoileus virginianus*). Comparative Biochemistry and Physiology 74A: 21–28.

NAIK, S. H., H. M. BHATIA, A. J. BAXI, AND P. V. NAIK. 1964. Hematological study of Indian spotted deer (axis deer). Journal of Experimental Zoology 155: 231–236.

PEARSON, R. A., AND D. J. MELLOR. 1976. Some behavioural and physiological changes in pregnant goats and sheep during adaptation to laboratory conditions. Research in Veterinary Science 20: 215–217.

PRESIDENTE, P. J. A., J. H. LUMSDEN, K. R. PRESNELL, W. A. RAPLEY, AND B. M. MCCRAW. 1973. Combination of etorphine and xylazine in captive white-tailed deer. II. Effects on hematologic, serum, biochemical and blood gas values. Journal of Wildlife Diseases 9: 342–348.

———. 1979. Haematology and serum biochemistry of deer in Australia. *In* Deer refresher course 49, The Post-Graduate Committee in Veterinary Science, The University of Sydney, New South Wales, Australia, pp. 205–226.

REHBINDER, C., AND L. E. EDQUIST. 1981. Influence of stress on some blood constituents in reindeer (*Rangiferi tarandus* L.). Acta Veterinary Science Scandinavia 22: 480–492.

RUSSEL, A. J. F., J. M. DONEY, AND R. L. REID. 1967. The use of biochemical parameters in controlling nutritional state in pregnant ewes, and the effect of undernourishment during pregnancy on lamb birthweight. Journal of Agricultural Science, Cambridge 68: 351–358.

SCHALM, O. W., N. C. JAIN, AND E. J. CARROLL. 1975. Veterinary haematology, 3rd ed. Lea and Felsiger, Philadelphia, Pennsylvania, 807 pp.

SEAL, U. S., AND A. W. ERICKSON. 1969. Hematology, blood chemistry and protein polymorphisms in the white-tailed deer (*Odocoileus virginianus*). Comparative Biochemistry and Physiology 30: 695–713.

———, J. J. OZOGA, A. W. ERICKSON, AND L. J. VERME. 1972a. Effects of immobilization on blood analyses of white-tailed deer. The Journal of Wildlife Management 36: 1034–1040.

———, L. J. VERME, J. J. OZOGA, AND A. W. ERICKSON. 1972b. Nutritional effects on thyroid activity and blood of white-tailed deer. The Journal of Wildlife Management 36: 1041–1052.

SELYE, H. 1946. The general adaptation syndrome and the disease of adaptation. Journal of Clinical Endocrinology 6: 117.

SEMPERE, A. J., G. A. BUBENIK, AND J. H. SMITH. 1986. Antler cycle and thermolabile and thermostable alkaline phosphatase in white-tailed deer: Circannual and circadian rhythms and variation after thyroxine, dexamethasone and ACTH administration. Acta Endocrinology 111: 133–159.

WESSON, J. A., III, P. F. SCANLON, R. L. KIRKPATRICK, AND H. S. MOSBY. 1979. Influence of chemical immobilization and physical restraint on packed cell volume, total protein, glucose and blood urea nitrogen in blood of white-tailed deer. Canadian Journal of Zoology 57: 756–767.

WEST, N. O., AND H. C. NORDAN. 1976. Hormonal regulation of reproduction and the antler cycle in the male Columbian black-tailed deer (*Odocoileus hemionus columbianus*). Part II. The effects of methallibure and hormone treatment. Canadian Journal of Zoology 54: 1637–1656.

WILSON, P. R., AND J. V. PAULI. 1982. Blood constituents of farmed red deer (*Cervus elaphus*). I. Haematological values. New Zealand Veterinary Journal 30: 174–176.

———. 1983. Blood constituents of farmed red deer (*Cervus elaphus*). II. Biochemical values. New Zealand Veterinary Journal 31: 1–3.

YOUATT, W. G., C. J. VERME, AND D. E. ULLREY. 1965. Composition of milk and blood in nursing white-tailed does and blood composition of their fawns. The Journal of Wildlife Management 29: 79–84.

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