Haematological and serum biochemical reference values in free-ranging red deer (*Cervus elaphus atlanticus*)

Olav Rosef[1], Håvard L. Nystøyl[2], Tore Solenes[2] & Jon M. Arnemo[2,3]

[1] Department of Environmental and Health Studies, Telemark University College, N-3800 Bø i Telemark, Norway (olav.rosef@hit.no).
[2] Section of Arctic Veterinary Medicine, Department of Food Safety and Infection Biology, The Norwegian School of Veterinary Science, P.O. Box 6204, N-9292 Tromsø, Norway.
[3] Department of Forestry and Wilderness Management, Hedmark University College, N-2480 Koppang, Norway.

Abstract: Analyses of haematological and biochemical constituents were carried out on the Norwegian subspecies of free-ranging red deer (*Cervus elaphus atlanticus*). All animals were captured from January to March by using a mixture of xylazine and tiletamin-zolazepam. Immobilisation was performed with plastic projectile syringes fired from a dart gun. Fourteen haematological parameters were analysed. There were no differences in the values between hinds and stags and between adults and calves ($P > 0.01$). Of the 22 biochemical compounds investigated there was a significant difference ($P < 0.01$) between calves and adults for lactate dehydrogenase (LD), globulin, beta globulin, gamma globulin, and the minerals Na, K, Mg, Zn, Ca, and P. Differences ($P < 0.01$) between hinds and stags were found in cholesterol, gamma glutamyl transferase (GGT), alpha-1 globulin, alpha-2 globulin and Cu. The blood values determined in this study can be used as reference values for this red deer subspecies immobilised with a mixture of xylazine-tiletamin-zolazepam for health control and diagnosis of diseases.

Key words: chemical immobilisation, haematology, minerals, serum biochemistry.

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Introduction

Considerable information on haematology and serum biochemistry for farmed red deer (*Cervus elaphus*) is available from New Zealand (Wilson & Pauli, 1982; Wilson & Pauli, 1983; Cross *et al*., 1988), Australia (Agar & Godwin, 1992) and Europe (Zomborszky *et al*., 1996; Peinado *et al*., 1999). The establishment of deer farming and the concomitant recognition of illness in individuals and of production-limiting health have highlighted the need for an evaluation of blood constituents as an aid for diagnosis. This requirement was emphasised in a review of trace elements deficiencies in red deer (Bonniwell, 1988) in which the author specifically commented on the diagnostic difficulties arising from the lack of appropriate reference data. Red deer farming in Norway is based on the subspecies *Cervus elaphus atlanticus* and originates from wild captures. Copper deficiency has been described among farmed red deer in Norway (Rosef *et al.,* 2001). There is one report of haematological parameters in the *Cervus elaphus atlanticus* subspecies, but the number of animals examined was low (Arnemo *et al*., 1994). In general, few reports exist on blood reference values of red deer (Knox *et al.,* 1988). Most of the data is from deer that have been anaes-

Table 1. Haematological values in free-ranging red deer (*Cervus elaphus atlanticus*) immobilised with xylazine-tiletamin-zolazepam.

Parameter	Constituent unit	*n*	Mean	Standard deviation	Confidence Limit (95%)
White blood cells (WBC)	x10⁹/L	29	2.2	0.6	2.0-2.4
Red blood cells (RBC)	x10¹²/L	29	8.8	0.9	8.4-9.1
Haemoglobin (Hb)	G/L	29	131.6	11.6	27.4-135.8
Packed cell volume (PCV)	L/L	29	0.35	0.03	0.33-0.36
Mean corpuscular volume (MCV)	F/L	29	39.7	4.1	38.2-41.2
Mean corpuscular haemoglobin concentration (MCHC)	g/L	29	381.3	8.5	378.2-384.4
Red cell distribution width (RDW)	%	29	18.9	1.5	18.3-19.4
Trombocytes (PLT)	x10⁹/L	26	328.0	73.4	299.8-356.2
Neutrophils	x10⁹/L	29	0.93	0.49	0.75-1.11
Lymphocytes	x10⁹/L	29	1.08	0.30	0.97-1.19
Monocytes	x10⁹/L	29	0.04	0.06	0.02-0.06
Eosinophils	x10⁹/L	29	0.14	0.10	0.10-0.17
Basophils	x10⁹/L	29	0.003	0.019	0.000-0.010
Large unstained cells (LUC)	x10⁹/L	29	0.0	0.0	0-0

thetised, tranquillised or shot prior to blood collection; procedures that result in lower measurements for haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell numbers (RBC), blood protein and fibrinogen, and some blood enzymes (Marco & Lavín, 1999). A further difficulty in the establishment of a reference range of values for blood constituents from stress-susceptible animals such as deer is that handling and sampling alone may alter some parameters. Limited data suggests that Hb and PCV are elevated following stress during sampling (Kocan *et al.*, 1981). It is therefore desirable that reference values for blood components are collected by uniform procedures that are commonly employed, and that all individuals included in a study are sampled under similar management. Data on haematology is of importance in comparative physiology for health monitoring purposes. The aim of the study was to establish reference ranges for haematological and serum biochemical values in free-ranging red deer as baseline values.

Material and methods

Blood was collected from chemically immobilised free-ranging red deer at winterfeeding sites using a mixture of xylazine-tiletamin-zolazepam (Rompun® dry powder, Bayer AG, Leverkusen, Germany and Zoletil forte® dry powder, Virbac International, Carros Cedex, France). This mixture is the standard drug combination for the immobilisation of red deer in Norway. The drugs were dissolved in 5 ml sterile water and transferred to plastic darts (Dan-Inject ApS, Børkop, Denmark) with 1.2 mm x 40 mm needles (Dan-Inject). The darts were shot at 10-15 metres distance using a blowgun (Dan-Inject) while the animals were feeding. The dosages used were approximately 250 mg of xylazine and 250 mg of tiletamin and zolazepam per 100 kg body mass (Arnemo *et al.*, 2002) and the time from darting until samples were taken was about 30 minutes. All the samples were collected in winter from January through March. All animals were apparently healthy and were released or brought to red deer farms after immobilisation.

Blood samples (9 mL) for haematological analysis were obtained from the jugular vein, using the Venoject® II EDTA (K_2) and 0.8 x 40 mm needles (Terumo Europe N.V. 3001 Lauven, Belgium) and brought to the laboratory within 48 hours. For biochemical and element analyses 9 mL was collected in a Venoject® II Autosep® Gel+Clot Act, left to clot at room temperature for 1-2 hours and centrifuged at 3000 RPM for 5 minutes. The serum was removed and kept frozen at –80 °C for 8-12 months before analyses.

White blood cells (WBC), red blood cells (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean capsular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), trombocytes (PLT), neutrophiles, lymphocytes, monocytes, eosinophils,

Table 2. Serum biochemical values in free-ranging red deer (*Cervus elaphus atlanticus*) immobilised with xylazine-tiletamin-zolazepam.

Parameter	Constituent unit	*n*	Mean	Standard deviation	Confidence Limit (95%)
Aspartate aminotransferase (AST)	U/L	81	59.1	19.1	55.0-63.3
Alanine amino transferase (ALT)	U/L	81	54.2	11.7	51.7-56.7
Alkaline phosphatase (AP)	U/L	81	223.8	129.9	195.5-252.1
Gamma glutamyl transferase (GGT)	U/L	78	20.4	9.1	18.4-22.4
Glutamate dehydrogenase (GD)	U/L	78	2.0	1.3	1.7-2.3
Creatine kinase (CK)	U/L	81	265.8	252.9	210.7-320.9
Lactate dehydrogenase (LD)	U/L	81	706.9	157.6	672.5-741.2
Total protein (TP)	g/L	81	64.8	6.1	63.6-66.3
Albumin	g/L	81	36.6	4.3	35.7-37.5
Globulin	g/L	81	28.4	5.4	27.2-29.5
Globulin alpha-1	g/L	81	4.14	0.82	4.0-4.3
Globulin alpha-2	g/L	81	6.4	1.1	6.1-6.6
Globulin beta	g/L	81	5.3	2.1	4.8-5.7
Globulin gamma	g/L	81	12.6	3.9	11.8-13.5
Urea	mmol/L	81	5.6	2.2	5.1-6.0
Creatinine	µmol/L	81	153.5	27.1	147.6-159.4
Total bilirubin	µmol/L	76	1.46	0.99	1.24-1.68
Cholesterol	mmol/L	81	1.20	0.51	1.08-1.31
Triglycerides	mmol/L	81	0.10	0.08	0.08-0.12
Free fatty acids	mmol/L	81	0.15	0.16	0.11-0.18
ß-hydroxybutyrate (B-HBA)	mmol/L	81	0.26	0.08	0.24-0.28
Glucose	mmol/L	81	9.2	3.0	8.5-9.7

basophiles and large unstained cells (LUC) were measured. These 14 haematological tests performed were assayed by the Central Laboratory, Norwegian School of Veterinary Science (http://sentrallaboratoriet.no/) with an Advia® 120 Hematology System using Advia 120 MultiSpecies ™ System Software (Bayer Corporation, Tarrytown, N. Y., USA). The system consists of laser detection in a flow cytometer to measure size and internal complexity of cells based on light scatter at different angles, providing an automated CBC and a 5-part differential WBC count in each sample.

The 22 biochemical constituents, aspartate amino transferase (AST), alanine amino transferase (ALT), alkalin phosphatase (AP), gamma glutamyl transferase (GGT), glutamate dehydrogenase GD, creatine kinase (CK), lactate dehydrogenase (LD), total serum protein concentration (TP), albumin, globulin, alpha-1 globulin, alpha-2 globulin, beta globulin, gamma globulin, urea, creatinine, total bilirubin, cholesterol, triglycerides, free fatty acids and ß-hydroxybutyrate (B-HBA) were measured by the Central Laboratory, Norwegian School of Veterinary Science and assessed on an Advia® 1650 System (Bayer Corporation, Tarrytown, N. Y., USA) (Tryland *et al.*, 2002).

Analysis of the elements Mg, Cu, Zn and Ca was performed

Table 3. Serum mineral levels in free-ranging red deer (*Cervus elaphus atlanticus*).

Parameter	Constituent unit	*n*	Mean	Standard deviation	Confidence Limit (95%)
Na	mmol/L	183	139.4	19.2	136.7-142.2
K	mmol/L	183	5.9	2.9	5.5-6.3
Mg	mmol/L	183	0.48	0.11	0.46-0.50
Cu	µmol/L	183	13.0	3.6	12.5-12.5
Zn	µmol/L	183	8.23	1.79	8.0-8.5
Ca	mmol/L	183	1.94	0.37	1.89-1.99
P	mmol/L	48	1.24	0.42	1.12-1.36

Table 4. Differences in serum biochemical parameters between adults and calves ($P < 0.01$). Mean values are shown with standard deviation.

Parameter		*n*	Adults	*n*	Calves
Lactate dehydrogenase (LD)	U/L	37	640.6 (116.8)	44	762.6 (166.9)
Globulin	g/L	37	30.8 (5.7)	44	26.3 (4.2)
Globulin beta	g/L	37	6.4 (2.6)	44	4.3 (0.8)
Globulin gamma	g/L	37	14.3 (3.5)	44	11.2 (3.7)
Na	mmol/L	37	136.8 (13.4)	44	144.7 (25.7)
K	mmol/L	37	5.5 (2.1)	44	6.8 (3.7)
Mg	mmol/L	37	0.46 (0.07)	44	0.51 (0.15)
Zn	µmol/L	37	7.8 (1.3)	44	9.0 (2.0)
Ca	mmol/L	37	1.84 (0.29)	44	2.08 (0.43)
P	mmol/L	23	0.99 (0.26)	25	1.41 (0.47)

by atomic absorption spectrometry (Perkin Elmer® AAS 3100) according to the NS (Norwegian Standard) 4773, NS 4776 and NS 4781 and by atomic emission according to an internal standard for Na and K (Telemark University College). For P analysis a spectrometric method described in NS 4724 with Perkin-Elmer UV/VIS Lambda 20 spectrometer was performed.

Values from both young and adults and the different genders were compared using the Student`s unpaired *t*-test. *P*-values less than 0.01 were considered significant.

Results

Means, standard deviations, confidence limits (95%) and sample sizes of the 14 haematological parameters are given in Table 1. There were no differences in the haematological values between hinds and stags and between adults and calves ($P > 0.01$). Results from serum biochemical and mineral analyses are given in Tables 2 and 3. A significant difference ($P < 0.01$) between adults and calves was found for LD, globulin, beta globulin, and gamma globulin concentrations, and for the minerals Na, K, Mg, Zn, Ca and P (Table 4). Differences between hinds and stags ($P < 0.01$) in the blood levels of cholesterol, GGT, alpha-1 globulin, alpha-2 globulin and Cu are shown in Table 5.

Discussion

Both physical and chemical methods are used in the capture of deer. The chosen methods depend on individual circumstances and have been reviewed by various authors (Jones, 1984; Giacometti, 1994; Kreeger *et al.*, 2002). Among the chemical capturing methods, a xylazine-ketamine mixture is most often used for deer (Haigh & Hudson, 1993). In this study we used a mixture of xylazine-tiletamin-zolazepam because of the cost of the chemicals and the positive results already achieved with this immobilisation regime. When reference blood parameters are to be established in wild animal species it is essential to take into account different methods of capture, as significant differences occur depending on the method used (Marco & Lavín, 1999). Some authors have even suggested that two ranges of reference blood values should be established according to the method of capture (Cross *et al.,* 1988). Red deer spleen has red cell storage as an important function (Hartwig & Hartwig, 1985). A major contribution to the observed changes in RBC, PCV and Hb may be because the spleen contracts due to cathecholamine release during physical restraint. The increase in the blood cell count during capture by physical means has been estimated to be as much as 40% (Cross *et al.*, 1988). Cathecholamines can also cause a transient leucocytosis with raised levels of neutrophils, monocytes and lymphocytes. Marco & Lavín (1999) found higher blood cell values in red deer captured by physical means than those captured using a mixture of xylazine and ketamine. In this study the animals were tranquillised before sampling. The serum values are in the same range though lower for animals captured chemically (Marco & Lavín 1999), and lower also than those described by Cross *et al.* (1988). Alpha-2 adrenergic agonists as used in the present study initially increase blood pressure, followed by long-term hypotension and the entrance of interstitial fluid into the circulatory system to stabilise blood pressure (Wolkers *et al.*, 1994) and can give lower blood values. Using alpha-2 adrenergic agonists before handling the animals is our common procedure and we suggest that the blood values found in this study can be used as reference values for *Cervus elaphus atlanticus* subspecies.

An increase in total protein and constituent electrophoretic fractions during physical capture has been described in some species of wild ungulates as a result of haemoconcentration (English & Lepherd, 1981). However, the use of anaesthetic substances may alter capillary permeability and cause a certain degree of haemodilution, such as loss of proteins, particularly albumin. In this study the concentration

Table 5. Differences in serum biochemical parameters between hinds and stags ($P < 0.01$). Mean values are shown with standard deviation.

Parameter		*n*	Hinds	*n*	Stags
Cholesterol	U/L	31	1.18 (0.31)	6	0.70 (0.17)
Gamma glutamyl transferase (GGT)	U/L	31	16.8 (6.9)	6	30.7 (15.5)
Globulin alpha-1	g/L	31	3.73 (0.71)	6	5.08 (1.27)
Globulin alpha-2	g/L	31	5.82 (0.57)	6	7.6 (3.29)
Cu	µmol/L	31	12.4 (3.3)	6	16.1 (4.3)

of total proteins, albumin and alpha-2 globulin were similar to those reported by Kent *et al.* (1980), Reid & Towers (1985), Wolkers *et al.* (1994) and Marco & Lavín (1999). However, we found significantly higher levels of alpha-1 and alpha-2 globulin among stags than hinds (Table 5). Stress can cause a decrease in total serum protein and albumin, and often an increase in alpha-2 globulin. Similar findings are observed in crushing injuries, bone fractures and as a result of surgery. Tissue repair requires protein reserves, and the increased protein turnover results in increased alpha-2 globulin (Kaneko, 1997). In particular, stag fights during the rut can provoke damages. Stress and tissue repair among the stags can partly explain the differences. Total serum protein has been found to be higher among adults than calves and young animals (Reid & Towers, 1985; Wilson & Pauli, 1982). In the present study we found significantly lower values in the globulin fraction among calves (Table 4). There is a general increase in total protein, a decrease in albumin, and an increase in globulins with advancing age. Hormone effects on serum proteins can be either anabolic or catabolic. Testosterone and estrogens are generally anabolic and the general increase with age can explain the higher serum levels among adult deer (Kaneko, 1997). Higher values among calves than adults were found for LD. LD activities are high in various tissues of the body and are not organ specific (Cardinet, 1997). A possible explanation for the higher level among calves can be the high metabolism by young growing animals.

Copper deficiency is common in farmed red deer livestock. It can be manifested as an ill-thrift condition or disease in the absence of specific clinical signs or with typical clinical signs as enzootic ataxia. This has been ruled out in the *Cervus elaphus atlanticus* subspecies in Norway where wild living red deer had a higher serum copper concentration than farmed individuals (Rosef *et al.*, 2001). The values in that study are in accordance with the values found in this study and can be referred to as reference values. The copper levels, however, are lowest among females and highest among stags, possibly because females excrete copper in the milk. Padilla *et al.* (2000), however, found low serum Cu (9.86 µmol/L) values in young red deer kept on pasture. Values less than 8 µmol/L are below the critical level for Cu deficiency (Mackintosh *et al.*, 1987). Our study showed a mean value of 13.0 µmol/L.

Calves showed significantly higher levels of Na, K, Zn, Mg, P and Ca than adults. This can be explained by the high activity of osteoblast and osteoclast in calves for remodelling bone tissue. Stress and physical exertion lead to an increase in the concentration of potassium in the blood of wild ungulates (Kock *et al.*, 1987). The mean value of potassium is higher in this study than reported by Wilson & Pauli (1983) and Arnemo *et al.* (1994). Physically captured deer have a higher level of these minerals than those captured by use of tranquillisers (Marco & Lavín, 1999). In general, the calves in this study were very calm when captured at the feeding places. The mean values of calcium, sodium, magnesium and potassium were similar to the concentrations described by Marco & Lavín (1999) but lower than those published by Wilson & Pauli (1983).

Blood serum urea concentration was lower in this study than that determined by Wilson & Pauli (1983) and Knox *et al.* (1988). This parameter reflects the intake of effective rumen-degradable protein, food nitrogen content and its balance with fermentable metabolic energy. Increased levels of blood serum urea may be associated with high protein food catabolism and with protein in the diet. The blood samples in this study were collected in winter when no high quality protein foods were available, which may explain the lower values.

The cholesterol concentration is normally strictly regulated, varying only slightly due to diet and time of year (Bartley, 1989). We found the same concentration as Marco & Lavín (1999), who found higher levels in deer captured by physical means compared to chemically immobilized animals. We found, however, a significantly higher level among hinds than stags. Peinado *et al.* (1999) postulated that cholesterol values are dependent on the nutritional situation. Our samples were collected some months after the rut when the stags were still in poorer condition than the hinds.

GGT was significantly higher among stags than hinds (Table 5) though the difference is difficult to explain. Serum GGT levels were in the same range as described by Reid & Towers (1985). Elevated serum GGT levels were found on a red deer farm coinciding with an outbreak of facial eczema, a mycotoxicosis known to cause liver damage and to elevate serum GGT concentrations (Towers & Stratton, 1978). Increased activity of GGT is a useful index of hepatic disorder.

We found lower values of triglycerides and glucose among the chemically immobilised animals than Marco & Lavín (1999). Some authors, however, describe an increase in glucose concentrations in animals immobilised by the use of alpha-2 adrenergic drugs which inhibit the release of insulin and increase glucose output from the liver, resulting in an increase in the concentration of blood glucose (Jalanka, 1988; Arnemo *et al.,* 1994; Arnemo & Ranheim, 1999).

The biochemical values in this study were overall lower than those reported by Marco & Lavín (1999) for animals captured by chemical immobilisation. However, we stored the samples frozen for 8-12 months. This can influence the enzyme values that can decrease during storage (Hunter & Madin, 1978; Thoresen *et al.*, 1995; Tryland & Brun, 2001; Tryland *et al*, 2002) which could partly explain the lower values observed in this study.

We have used the xylazine-tiletamin-zolazepam mixture for immobilisation for blood sampling. This mixture is also normally used when immobilising farmed red deer. Blood values determined in this study can thus be used as reference values for the *Cervus elaphus atlanticus* subspecies immobilised with a mixture of xylazine, tiletamin and zolazepam for health control and diagnosis of diseases.

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Abstract in Norwegian /Sammendrag:

Hematologiske og biokjemiske parametere er analysert på norsk frittlevende hjort (*Cervus elaphus atlanticus*). Hjorten ble immobilisert i tidsrommet januar til mars ved hjelp av et spesialgevær ladet med plast kanyler som inneholdt en blanding av xylazin og tiletamin-zolazepam. Det var ingen forskjeller i de14 undersøkte hematologiske verdiene mellom hinder, kalver og bukker (*P*>0,01). Av de 22 biokjemiske parametrene som ble undersøkt var det en signifikant forskjell mellom kalver og voksne (*P*<0,01) når det gjelder laktat dehydrogenase, globulin, beta globulin, gamma globulin og mineralene Na, K, Mg, Zn, Ca og P. Det var en signifikant forskjell mellom hinder og bukker (*P*<0.01) på parametrene kolesterol, gamma glutamyl transferase, alfa-1 globulin, alfa-2 globulin og Cu. Blodverdiene som ble målt i dette studiet kan bli brukt som referanseverdier for norsk hjort som er immobilisert med blandingen xylazin-tiletamin-zolazepam for helsekontroll og for diagnostisering av sykdommer.