

Review Articles

THE DETERMINATION OF BLOOD PROFILE IN CAPTIVE WILD UNGULATES WITH FOCUS ON ELAND (*TAUROTRAGUS ORYX*)

SILBEROVÁ P.¹, LUKEŠOVÁ D.¹, PINC L.²

¹*Institute of Tropics and Subtropics, Czech University of Life Sciences, Prague, Czech Republic*

²*Faculty of Agrobiological, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic*

Abstract

*There is a lack of information about wild animals concerning physiological parameters and reference values, which is necessary knowledge for the successful breeding and management of these animals, especially for the species kept for meat production, such as eland (*Taurotragus oryx*). Three phases of laboratory examination – pre analytic, analytic, and post analytic, lead to determination of blood profile in wild ungulates and establishing their physiological values. However results can be influenced by many factors, including species specificity, method applied, age and sex of the animal, diet, breeding conditions, environmental factors, stress, handling procedures and method of sedation therefore cautious interpretation of results is advisable. There have been published only six studies, regarding eland, on various blood parameters, however under different conditions, using different testing methods and on very limited number of animals.*

Key words: blood profile, reference ranges, eland, *Taurotragus oryx*, wild ungulates, laboratory analyses, blood sampling

INTRODUCTION

Animals in wilderness live in natural habitats to which they are fully adapted, while captivity can result in severe stress. They fully depend on care provided by humans that are responsible for their welfare, living space, nutrition and veterinary care. Quality of living conditions in captivity is a crucial factor influencing health and reproduction.

Unfortunately there is lack of information about wild animals concerning physiological parameters (reference values), which is necessary knowledge for the successful breeding and management of these animals, especially for the species kept for the meat production, such as eland (*Taurotragus oryx*) that has been recommended by FAO as a wild animal suitable for domestication (Woodford, 2000). Eland is one of the largest species of antelopes, its conformation morphological and physiological features resemble cattle. Utilization of these animals for meat and milk production could replace cattle damaging significantly natural habitats in south and east Africa.

To determine the blood profile in wild ungulates and establish their physiological values, we must go through three phases of laboratory examination – pre analytic, analytic and post analytic (Doubek et al., 2005; Kraft and Dür, 2001).

Pre analytic phase

It is defined as procedures and operations from indication of examinations till the sample analysis, i.e. getting animal ready for the collection of biological samples and its subsequent processing, transport and storage. This phase contribute to the quality of final results with 50–60% (Doubek et al., 2005).

Results can be significantly influenced by the day time of blood sampling, position of the patient during collection, type of specimen tube as well as collection techniques applied (Cork and Halliwell, 2002; Masopust, 1990), in wild animals by the tranquillizers used and by the stress the animals sustained (Vahala et al., 1989). In ungulates the blood for hematological analyses is usually taken from *vena jugularis* into sterile vials with some anticoagulant (usually lithium heparin or EDTA (ethylenediaminetetraacetic acid)) (Zima et al., 2007; CYMEDICA s.r.o., 2008).

For the separation of blood elements from blood serum (plasma) it is advisable to centrifuge the sample by 1000–1500 revolutions for 10 minutes under room temperature. Plasma or serum should be separated from blood as soon as possible, but not later than 2 hours after the collection (IDEXX Laboratories Inc., 2010; Zima et al., 2007). The samples must be delivered as fast as possible, and handled

with care in adequate temperature and light conditions as folic acid and bilirubin are not stable if exposed to direct light (Cork and Halliwell, 2002). Provided the sample is processed within 24–48 hours after the collection, for the most parameters the storage temperature of 4°C is sufficient. For prolonged storage temperature should not exceed –18°C. During the storage the samples should be sealed properly to prevent evaporation, microbiological contamination, light exposure, gasses diffusion and metabolism of blood elements (Zima et al., 2007).

Analytic phase

This phase comprises qualitative and quantitative determination, i.e. determination of the amount and quality of the examined substance (Doubek et al., 2005).

Laboratory examination of blood samples:

- a) hematological – red and white blood picture, hemoglobin content, hematocrit and blood coagulation (Zima et al., 2007; Laboma Písek, 2007),
- b) biochemical – determination of organic or inorganic substances of blood, e.g. protein, urea, cholesterol, glucose, enzymes, hormones, electrolytes, minerals and vitamins; and determination of acid-base balance (Cork and Halliwell, 2002),
- c) microbiological – bacteria, fungi and viruses,
- d) serological – determination of antibodies presence and quantity in blood serum (Masopust, 1990).

Nowadays automatic analyzers are widely used and unlike classical test tube methods they can speed up the procedures and eliminate mistakes (Kraft and Dürr, 2001). For example, in our project aimed at the determination of blood profile in captive Eland (*Taurotragus oryx*), in the Institute of Tropics and Subtropics we use portable blood analyzers from IDEXX Laboratories Inc. These automatic portable analyzers have many advantages. Rechargeable battery pack allows independent operation in the field for up to 4 hours (IDEXX Laboratories Inc., 2010). E.g. IDEXX VetTest® Chemistry Analyzer provides reliable chemistry results in several minutes. It utilizes dry-slide technology – special layer provides accurate results even on samples compromised by icterus, hemolysis or lipemia (CYMEDICA s.r.o., 2008). It can measure 26 biochemical parameters from a very little sample (40 µl of sample to run one test) (IDEXX Laboratories Inc., 2010). For hematological examination can be used IDEXX VetAutoread™ Hematology Analyzer, which offers thorough screening, presurgical testing, and general health checkups in-house. It works on the principle of the cell nucleus fluorescence and measures all red and white blood cells characteristics (CYMEDICA s.r.o., 2008). The electrolytes, acid-base balance (anion gap, tCO₂ and bicarbonate), blood gasses, ionized calcium,

glucose and hemoglobin could be examined by VetStat® Electrolyte and Blood Gas Analyzer (IDEXX Laboratories Inc., 2010). An anaerobic collection and processing within 30 minutes should be observed to receive correct results (Kraft and Dürr, 2001).

Post analytic phase

The phase is realized by mutual cooperation between the laboratory and a veterinarian. It includes computation, recording of values, archiving, dispatch and utilization by a veterinarian. The most important part of the post analytic phase is correct interpretation of the results with regard to physiological values (reference values), results of other examinations, and clinical picture of patients (Doubek et al., 2005).

Reference ranges

Reference ranges are set of laboratory examination values majority (95%) of the given population falls within (Doubek et al., 2005). If there are enough data to compute reference values for healthy individuals of certain species, it is possible to stratify them in accordance with the category of animals (age, sex etc.) (Kraft and Dürr, 2001). To determine reference values none of the sampled animals should show any clinical signs of a disease (Farver, 2008). Two methods how to determine reference ranges are currently in use: nonparametric and parametric (Doubek et al., 2005).

a) Nonparametric method: The results are aligned upwardly according to values and at the both ends some results are excluded (usually 2.5%). Reference range is then determined using 95% of the results. The advantage of this method is that the type of distribution (symmetric, asymmetric) is not taken into account. The disadvantage is that high numbers of results are needed so as the exclusion of 2.5% at both ends would be reliable enough (Farver, 2008; Doubek et al., 2005).

b) Parametric method (classical reference range) is used when the tested value has symmetrical distribution (Gaussian distribution). However most of the biological or medical values are not distributed symmetrically. In such cases the data are mathematically transformed, so as the distribution would be more Gaussian, and then tested again for the symmetrical distribution (Thrall, 2006; Kraft and Dürr, 2001). In case the data cannot be transformed the nonparametric method is used (Doubek et al., 2005). Statistically defined reference interval comprises values within the limits of the mean ± 2 standard deviations. Values beyond these ranges therefore should not be considered as pathological but as very low or very high because the ranges can be found in healthy selected reference population with the probability of 95% or pos-

sibly 99% defined in advance (Zima et al., 2007; Doubek et al., 2005). While using this method less numbers of data are needed.

No laboratory values should be considered constant. The age, sex of the animal as well as the method used ought to be taken into consideration (Thrall, 2006; Doubek et al., 2005). It is therefore necessary to describe accurately criteria applied to obtain the reference range (Kraft and Dürr, 2001).

Blood profile of wild ungulates with special regard to captive Eland (*Taurotragus oryx*)

Studies focused on blood and metabolic profile of wild animals and subsequent reference values of various species are not numerous. Papers that have been published are more focused on farm animals mainly cattle and sheep (Whitaker et al., 1999; Kida, 2003; Peinado et al., 1995), cervids (Poljicak-Milas et al., 2004), various exotic mammals (giraffes, elephants, lamas and carnivores and also on some antelopes (Rietkerk et al., 1994; Vassart and Greth, 1991; Vahala and Kase, 1993a). There have been published only six studies, regarding eland, on various blood parameters, however under different conditions, using different testing methods and on very limited numbers of animals.

Vahala and Kase (1993b) proved significant influence of age to some blood serum parameters of 92 clinically healthy Lechwe waterbucks (*Kobus lechwe*). In neonates the highest values have been noted in cholesterol, alkaline phosphatase, calcium and phosphorus; and the lowest values of total proteins, glucose, creatinine, urea, AST and ALT. In juvenile females there were lower values of cholesterol and higher values of glucose, urea, alkaline phosphatase and ALT than in young males. Vahala and Kase (1993b) recorded higher levels of urea and cholesterol and lower levels of glucose, triglycerides, and sodium in adult females of Lechwe waterbucks compared with males. To draw comparisons there were 1345 blood samples collected from Holstein cows at the age of 1–6 years in Louisiana. Concentrations of creatin kinase, alkaline phosphokinase, phosphorus, calcium and glucose were decreasing together with age while concentrations of cholesterol, urea nitrogen, total protein and globulin were increasing (Roussel et al., 1982). Hematological and biochemical values have been reported in American bison (*Bison bison*), pronghorn antelope (*Antilocapra americana*), and white tailed deer (*Odocoileus virginianus*). There were significantly higher concentrations of selenium in full blood samples of pronghorns than in white tailed deer or bison. Differential white blood cells count was distinctly different in white tailed deer than

Tab. 1: Normal blood parameters of cattle and horse (Cork and Halliwell, 2002) and some hematological (Pospisil et al., 1984a) and biochemical parameters of captive eland (*Taurotragus oryx*) (Vahala et al., 1989)

Blood parameters	Cow	Horse	Eland		
			(male/female)	mean	ranges
Red blood cells ($\times 10^{12}/l$)	5–10	6–13	0/5	8.46	6.80–10.18
Hematocrit (%)	24–46	30–52	0/5	41.6	33–47
Mean hemoglobin (g/l)	80–150	110–190	0/5	128	109.6–150.0
White blood cells ($\times 10^9/l$)	4–12	5–13	0/5	4.64	2.4–6.5
Lymphocytes (%)	45–75	25–70	0/5	37.4	31–49
Monocytes (%)	2–7	2–7	0/5	46	1–8
Neutrophils (%)	15–45	30–65	0/5	54	50–60
Eosinophils (%)	2–20	0–11	0/5	3.4	0–15
Basophils (%)	0–2	0–3	0/5	0.6	0–2
ALT (alanine amino-transferase) (iu/l)	11–40	3–23	5/5	0.31	0.13–1.23
Alkaline phosphatase (iu/l)	35–350	95–233	5/5	2.47	0.5–6.25
AST (aspartamine aminotransferase) (iu/l)	60–150	200–400	5/5	0.61	0.45–0.86
Creatinine ($\mu\text{mol}/l$)	90–240	110–170	5/5	240.2	185–277
Urea (mmol/l)	2–10	3–7	5/5	8.58	5.6–13.5
Glucose (mmol/l)	2–3	3.3–5.6	5/5	10.31	3.51–12.83
Sodium (mmol/l)	132–152	132–150	4/4	114	90–164
Potassium (mmol/l)	3.8–5.8	3–5	4/4	5.86	5.39–6.25
Chloride (mmol/l)	95–110	98–110	5/5	84.5	62–101

Tab. 2: Some blood parameters of wild eland (*Taurotragus oryx*) from different sources

Source:	Drevemo et al. (1974)		Hawkey (1975)		Kitchen (1978)	
Number of animals	10 (drug-immobilized)	10 (shot)	7		not given	
Blood parameters	Mean \pm SD	Mean \pm SD	Mean	Ranges	Mean	Ranges
Red blood cells ($\times 10^{12}/l$)	8.57 \pm 1.46	8.11 \pm 0.9	7.1	5.6–8.2	7.8	7.2–8.3
Mean hemoglobin (g/l)	129 \pm 15	144 \pm 29	127	110–151	140	121–158
Hematocrit (%)			36	29–44	41	34.5–46.5
White blood cells ($\times 10^9/l$)	6.69 \pm 5.59	5.85 \pm 1.61	5.7	4.4–8.5	6.9	5.9–7.8
Neutrophils (%)			70	56–82	25	15.9–34.5
Lymphocytes (%)			23	11–29	75	63–86
Monocytes (%)			3	1–6	0	
Eosinophils (%)			3	1–5	0	
Calcium (mg/100 ml)	11.5 \pm 0.7	12.6 \pm 2.2				
Magnesium (mg/100 ml)	1.9 \pm 0.3	2.3 \pm 0.3				
Phosphorus (mg/100ml)	4.0 \pm 1.0	5.9 \pm 1.1				
Cuprum (ppm)	0.89 \pm 0.13	0.89 \pm 0.11				

in pronghorn and bison. Markedly higher values of cortisol and T_3 were found in bison (Clemens et al., 1987). Biochemical parameters were determined from the blood serum of clinically healthy males and females antelopes of the subfamilies *Tragelaphinae*, *Hippotraginae*, and *Aepycerotinae* older than one year, kept in Dvůr Králové nad Labem ZOO (Vahala et al., 1989). The results obtained from elands are shown in Table 1. Most values are within limits which can be found in cattle.

Pospisil et al. (1984a, b) presented comparison of erythrocyte count, hematocrit, hemoglobin content, mean corpuscular volume of the erythrocyte and white blood picture from 21 species of antelopes including eland. Data were collected at zoo in Dvůr Králové nad Labem during the period of 6 years, these results are shown in Table 1. The authors paid special attention to the direct relationship of hematocrit and hemoglobin content and inverse relationship of erythrocyte count and mean corpuscular volume of erythrocytes. They also dealt with the significance of an increased total surface area for O_2 exchange. Other values of blood picture of domestic animals (cattle, goat and sheep) were compared with reference values obtained from other resources (Hawkey and Hart, 1984; Hawkey, 1975; Kitchen, 1978; Drevemo et al., 1974), which you can find in Table 2. A comparison of 21 species of antelopes shows that most of the values of elands were close to the values of greater kudu, white tailed gnu, gemsbok, and bongo. Results were compared also with normal human range and in most cases they were in good accord with them. In terms of eland, most of the parameters almost agreed with literature (in Table 2) safe for the slightly elevated leucocytes found in

Dvůr Králové ZOO (Pospisil et al., 1984a). The hematological values found in eland are reported in Table 1.

Results from another research show values of erythrocyte count, hemoglobin content and hematocrit significantly higher in 10 species of drug-immobilized animals of African ruminants in Kenya than in shot ones (Drevemo et al., 1974). However inorganic phosphorus was higher in shot elands and wildebeest. In this study 10 blood samples from shot elands were compared with 10 drug-immobilized elands. Most of the values were within limits that can be found in cattle.

Drug immobilization techniques enable collection of blood samples from wild animals. Drevemo and Karstad (1974); Furley (1986) a Grootenhuis et al. (1976) dealt with the influence of drugs used for immobilization on some blood parameters. To immobilize animals, veterinary drugs containing xylazine or a combination of xylazine-etorphin-acepromazine were used. Blood samples from impalas and elands were collected each 15 minutes together with measuring rectal temperature and heart rate beat since the immobilizing agents were applied. The results showed that over the progressive immobilization the bodily temperature was decreasing as well as heart rate beat, counts of erythrocytes, leucocytes, hemoglobin, and also packed cell volumes in circulating blood. The authors therefore recommend to collect blood immediately after the immobilization of the animal (Drevemo and Karstad, 1974). To immobilize animals in captivity, xylazine alone proved to be satisfactory in most instances Grootenhuis et al., (1976).

Blood gas and acid base analysis in bongo (*Tragelaphus eurycerus*) and eland (*Tragelaphus oryx*) antelopes

were tested on blood levels of pH, oxygen and carbon dioxide by Boyd et al. (2000). They indicated metabolic acidosis ($\text{pH} < 7.35$ and $\text{HCO}_3^- < 20$) in all manually restrained animals. Normal acid base values were observed in 42% of the immobilized animals, nevertheless the values obtained were compared only with the reference ranges of cattle, without any knowledge of normal values in bongo and eland. There were no differences mentioned between those two species.

CONCLUSION

Results that have been obtained so far are sometimes confusing and discrepant. It might be caused by many factors, including species specificity, diet, breeding conditions, environmental factors, stress, handling procedures and methods of sedation. Thus the results often do not permit clear conclusions. Therefore there is a research program currently in progress at the Institute Tropics and Subtropics, Czech University of Life Sciences Prague, aimed at the determination of blood profile and reference ranges for the individual categories, selected by age and sex, of eland (*Taurotragus oryx*). The research is realized on the farmed elands bred for the meat production at the university farm in Lány and in the selected zoological gardens in the Czech Republic. The effect of the nutrition and environments on the health status and welfare of the animals are also studied. Results could be an important part of veterinary care of these animals, because it seems that utilization of eland (*Taurotragus oryx*) for meat and milk production could replace cattle damaging significantly natural habitats in South and East Africa.

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Corresponding author:

Ing. Petra Silberová, Ph.D.
Czech University of Life Sciences Prague
Institute of Tropics and Subtropics
Kamýcká 129
165 20 Prague 6-Suchbát
Czech Republic
e-mail: silberova@its.czu.cz