

## Mineral concentrations in plasma of young and adult red deer

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### ABSTRACT

Eastern Croatia (Kopački rit) is one of the most famous habitat of red deer (*Cervus elaphus*). Knowledge of normal electrolyte and mineral concentrations, together with other hematological and biochemical values, are essential for appropriate diagnosis of diseases and health control. The aim of this study was to determine mineral and electrolyte concentrations in young red deer population and compare them with adults. Serum total calcium (tCa), inorganic phosphorus (iP), total magnesium (tMg), total protein and albumin were determined by spectrophotometric analysis; and ionized calcium ( $Ca^{2+}$ ), magnesium ( $Mg^{2+}$ ), sodium ( $Na^+$ ), potassium ( $K^+$ ) and chloride ( $Cl^-$ ) concentrations were determined by ion-selective electrodes (ISE). Young animals showed significantly higher values of  $K^+$ , tCa,  $Ca^{2+}$ ,  $Mg^{2+}$ , iP and albumin, while  $Na^+$ ,  $Cl^-$  and protein concentrations were lower. There was no significant difference in the tMg concentration and  $Ca^{2+}/Mg^{2+}$  ratio in relation to age. In wild animals, these laboratory analyses are still in their very beginning due to difficulties in collecting data and lack of equipment in veterinary practice. Electrolyte and mineral values determined in this study can be considered reference data for health control and disease diagnosis.

**Key words:** mineral, electrolyte, ionized calcium, red deer, *Cervus elaphus*

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## Introduction

Red deer (*Cervus elaphus*), one of the largest deer species, was once found from Europe through northern Africa, Asia, and North America. Eastern Croatia (Kopački rit) is one of the most famous habitat of red deer. The intensity of cervid management in Croatia is increasing as more animals are being held in captivity for commercial and recreational purposes.

Knowledge of normal electrolyte and mineral concentrations, together with other hematological and biochemical values, are essential for appropriate diagnosis of diseases and health control. Fluid and electrolyte balance are involved in regulation of acid-base balance (GIUNDICE et al., 1994). The evaluation of acid-base status provides important information, especially in relation to the urinary, respiratory, digestive and endocrine systems (BOUDA et al., 2000).

Alterations of electrolyte levels and acid-base balance in deer may directly affect rates of enzymatic reactions, and therefore, a variety of biological processes (CARLSON, 1997). Laboratory testing and interpretation of laboratory data can provide significant insights regarding diseases and therapeutic approaches (THRALL, 2004). To the authors' knowledge, there has been little study on the relationship between age and the electrolyte and mineral status of red deer and no adequate reference intervals are available (BOUDA et al., 2000; BARIĆ RAFAJ et al., 2011).

Specific reference intervals are needed for each animal species for appropriate interpretation of hematological and serum biochemical results. Less often, a distinct reference value is needed for an analyte from a specific animal age or breed. Many values vary with the age of the animal, with major changes occurring before puberty (MEYER and HARVEY, 2004). Consequently, some analytes require different reference intervals for different age groups. The aim of this study was to determinate mineral and electrolyte concentrations in young red deer population and compare them with adults.

## Materials and methods

Serum electrolytes and minerals were determined in samples from 34 physically restrained, healthy young red deer (*Cervus elaphus*), of both sexes, aged from 6 to 14 months. The results were compared to values obtained from 11 adult animals, of both sexes, aged from 2 to 3 years. The deer habitat was in Baranja, at an altitude of 100 m. All animals were kept on free pasture and sampled once, in August. The blood samples were taken from 07-09 hours. The deer were sampled by jugular venipuncture without sedation. Assessment of health status was confirmed by hematological and biochemical findings (BARIĆ RAFAJ, 2011). For serum analyses blood was collected in plain vials with clot activator and separation gel (Vacutainer blood collection system; Becton, Dickinson and Co., Rutherford, New Jersey 07070, USA) and centrifuged (1200g, 10 minutes).

For ionized electrolyte determination blood was collected in a heparinized syringe. The air bubbles were expelled from the syringe and the needle was inserted into a rubber stopper to prevent exposure of the blood to the air. After collection, the closed syringe was immersed in a mixture of ice and water.

Serum total calcium (tCa), inorganic phosphorus (iP), total magnesium (tMg), total protein and albumin were determined by spectrophotometric analysis using Olympus AU 600 analyzer (Olympus Diagnostica GMBH, Hamburg, Germany) and original reagents from manufacturer. The ionized calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ), sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ) and chloride ( $\text{Cl}^-$ ) concentrations were determined by ion-selective electrodes (ISE), using an acid-base analyzer Stat Profile CCX (Nova Biomedical, Waltham, Ma, USA).

Payne's formula was used to obtain corrected calcium values (PAYNE, 1973):  
Corrected  $\text{Ca}^{2+1}$  (mmol/L) = tCa (mmol/L) + 0.025 × (40 - albumin (g/L))

Alternative formula referring to protein instead albumin concentrations was proposed by PFITZENMEYER et al. (2007): Corrected  $\text{Ca}^{2+2}$  (mmol/L) = 0.592 - 0.00449 × protein (g/L) + 0.410 × tCa (mmol/L)

Multiple regression derived formula for ionized calcium: Corrected  $\text{Ca}^{2+3}$  (mmol/L) = 0.9296 - 0.00199 × protein (g/L) + 0.1778 × tCa (mmol/L)

The concentration of globulin (glo) was calculated as the difference between total serum protein and albumin. In addition, we determined A/G ratio (albumin/globulin ratio) and  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ratio to estimate potential use and impact of these parameters on the health of the animals.

All data were reported as mean ± standard deviation (SD) and the corresponding minimum and maximum values, respectively. Student's *t*-test was used to determine statistical differences, and significance was set at  $P < 0.05$ . All statistical analysis were performed with the statistical software program Statistica (Statistica 8 for Windows, StatSoft Inc.).

## Results

Young animals showed significantly higher values of  $\text{K}^+$ , tCa,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and iP, while  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations were lower. There was no difference in the tMg concentration and  $\text{Ca}^{2+}/\text{Mg}^{2+}$  in relation to age.

There was a significantly higher level of plasma protein in older than in young deer, with slightly lower albumin levels. Young deers had significantly lower globulin levels than older, resulting in a significantly higher A/G ratio. All data are shown in the Table 1.

Multiple regression analysis provided formula for ionized calcium: Corrected  $\text{Ca}^{2+3}$  (mmol/L) = 0.9296 - 0.00199 × protein (g/L) + 0.1778 × tCa (mmol/L).

Table 1. Statistical analyses of mineral concentration in plasma of young (n = 34) and adult (n = 11) red deer (*Cervus elaphus*)

Parameter <sup>a</sup>	Unit	Young red deer, n = 34		Adult red deer, n = 11		P-values <sup>b</sup>
		Mean ± SD	Range (min-max)	Mean ± SD	Range (min-max)	
Na <sup>+</sup>	mmol/L	139.03 ± 1.02	136.80-141.50	147.09 ± 1.55	144.90-150.30	0.000
K <sup>+</sup>	mmol/L	5.34 ± 0.44	4.61-6.46	4.49 ± 0.70	3.67-5.76	0.000
Cl <sup>-</sup>	mmol/L	101.76 ± 1.57	99.50-104.9	107.11 ± 2.33	102.80-111.20	0.000
tCa	mmol/L	2.84 ± 0.15	2.37-3.17	2.15 ± 0.17	1.93-2.48	0.000
Ca <sup>2+</sup>	mmol/L	1.31 ± 0.04	1.22-1.37	1.13 ± 0.05	0.98-1.18	0.000
tMg	mmol/L	0.85 ± 0.14	0.41-1.03	0.86 ± 0.17	0.71-1.26	0.854
Mg <sup>2+</sup>	mmol/L	0.47 ± 0.02	0.43-0.53	0.42 ± 0.04	0.38-0.51	0.000
iP	mmol/L	3.20 ± 0.24	2.74-3.86	1.48 ± 0.37	0.82-2.16	0.000
Ca <sup>2+</sup> /tCa		0.46 ± 0.02	0.43-0.52	0.52 ± 0.03	0.48-0.57	0.000
Mg <sup>2+</sup> /tMg		0.57 ± 0.13	0.47-1.10	0.50 ± 0.05	0.40-0.56	0.062
Ca <sup>2+</sup> /Mg <sup>2+</sup>		2.81 ± 0.14	2.50-3.00	2.72 ± 0.30	1.90-3.10	0.176
Ca <sup>2+</sup> corr1	mmol/L	3.12 ± 0.12	3.18-3.36	2.52 ± 0.15	2.30-2.82	0.000
Ca <sup>2+</sup> corr2	mmol/L	1.47 ± 0.12	1.32-1.60	1.05 ± 0.07	0.92-1.18	0.000
Ca <sup>2+</sup> corr3	mmol/L	1.31 ± 0.02	1.24-1.36	1.13 ± 0.03	1.07-1.18	0.000
Prot	g/L	62.85 ± 3.36	55-68	93.90 ± 6.20	87-106	0.000
Alb	g/L	28.75 ± 2.33	20.40-33.26	25.39 ± 1.87	22.83-28.71	0.000
Glob	g/L	34.12 ± 2.64	28.00-39.93	68.51 ± 7.02	60.86-82.08	0.000
A/G ratio		0.85 ± 0.10	0.59-1.04	0.38 ± 0.06	0.29-0.46	0.000

<sup>a</sup> Na<sup>+</sup>-sodium, K<sup>+</sup>-potassium, Cl<sup>-</sup>-chloride, Ca<sup>2+</sup>-ionized calcium, Mg<sup>2+</sup>-ionized magnesium, tCa-total serum calcium, iP-inorganic phosphorus, tMg-total magnesium, Prot-total proteins, Alb-albumins, Glob-globulins, Ca<sup>2+</sup>corr1-corrective value for the ionized calcium using first corrective formula, Ca<sup>2+</sup>corr2-corrective value for the ionized calcium using alternative corrective formula, Ca<sup>2+</sup>corr3-our corrective formula, A/G ratio-albumin/globulin ratio. <sup>b</sup> Significance was set at P<0.05.

Reference ranges for young and adult red deer are calculated as mean ± 2 SD, and given in the Table 2.

Table 2. Reference values for red deer (*Cervus elaphus*)

Parameter <sup>a</sup>	Unit	Reference values for young red deer	Reference values for adult red deer
Na <sup>+</sup>	mmol/L	136.96 - 141.11	143.84 - 150.34
K <sup>+</sup>	mmol/L	4.43 - 6.24	3.01 - 5.96
Cl <sup>-</sup>	mmol/L	98.58 - 104.95	102.22 - 112.0
tCa	mmol/L	2.54 - 3.14	1.80 - 2.50
Ca <sup>2+</sup>	mmol/L	1.24 - 1.38	1.01 - 1.24
tMg	mmol/L	0.56 - 1.13	0.51 - 1.21
Mg <sup>2+</sup>	mmol/L	0.42 - 0.52	0.34 - 0.50
iP	mmol/L	2.71 - 3.70	0.69 - 2.27
Ca <sup>2+</sup> /tCa		0.42 - 0.51	0.46 - 0.58
Mg <sup>2+</sup> /tMg		0.31 - 0.83	0.40 - 0.59
Ca <sup>2+</sup> /Mg <sup>2+</sup>		2.53 - 3.09	2.09 - 3.35
Prot	g/L	56.03 - 69.68	80.82 - 106.98
Alb	g/L	24.02 - 33.47	21.45 - 29.33
Glob	g/L	28.84 - 39.40	54.47 - 82.55
A/G ratio	Unit	0.65 - 1.05	0.26 - 0.50

<sup>a</sup> Na<sup>+</sup>-sodium, K<sup>+</sup>-potassium, Cl<sup>-</sup>-chloride, Ca<sup>2+</sup>-ionized calcium, Mg<sup>2+</sup>-ionized magnesium, tCa-total serum calcium, iP-inorganic phosphorus, tMg-total magnesium, Prot-total proteins, Alb-albumins, Glob-globulins, A/G ratio-albumin/globulin ratio.

### Discussion

The manifestations of electrolytes disbalance can differ among animals of different ages, gender and species. As clinical symptoms are rarely disease-specific, the diagnosis of a electrolyte-related disease is usually accomplished with laboratory biochemical analysis (PULS, 1994; UNDERWOOD and SUTTLE, 1999; JOHNSON et al., 2007). To the authors' knowledge, this is the first report outlining reference electrolyte values age-dependent for red deer (*Cervus elaphus*).

Mean serum levels of Na<sup>+</sup> showed increase with age, while mean K<sup>+</sup> levels showed decrease. The reference values for K<sup>+</sup> determined by BOUDA et al. (2000) were 7.03 mmol/L, by ENGLISH and LEPHERD (1981) were 7.58 mmol/L for fawns and 7.62 mmol/L for adults, and by KNOX et al. (1988) 12.8 mmol/L. These values were considerably higher than in other domestic ruminants. We found lower value which agrees more with other ruminants like cattle and sheep, and our minimum and maximum references (young 4.61-6.46; adults 3.67-5.76 mmol/L) are not so wide as those found by KNOX et al. (1988) (2.1-21.4 mmol/L).

Our mean serum Cl<sup>-</sup> concentration in young population was 101.76 mmol/L, which is similar to findings by BOUDA et al. (2000) (100.5 mmol/L). For adults, mean Cl<sup>-</sup> concentration was 107.11 mmol/L, which is similar to the value reported by HARGREAVES and MATTHEWS (1995) (106.0 mmol/L). These results indicate a need for different age-dependent reference values.

Phosphorus is an important mineral element which is an essential component of buffer systems in blood and other body fluids and crucial to energy metabolism. The amount of phosphorus absorbed by the animal depends on the source of phosphorus, the amount of intake, the calcium-phosphorus ratio, intestinal pH, disease and parasites, environment, the age of the animal and dietary levels of minerals. SCHARFE et al. (1998) found inorganic phosphorus concentration 2.4 mmol/l in newborn fallow deer. In our study mean concentration of inorganic phosphorus for young population was 3.20 mmol/L, while for adult red deer we found mean concentration of 1.48 mmol/L. These decrease with age is understandable considering that phosphorus requirements are higher during early growth.

Investigation conducted by CHAPMAN et al. (1980) showed that electrophoretic pattern of globulins fraction in fallow deer differ significantly compared to red deer. We found differences in serum protein concentrations and A/G ratio related to age. Mean total protein concentration in young population was 62.85 g/L. SCHARFE et al. (1998) found total protein concentration 54.0 g/L in newborn fallow deer, while SLAVICA et al. (2000) reported values from 51.4 to 70.5 g/L in different deer species, and BOUDA et al. (2000) found 66 g/L in the red deer population. For adult population we found significantly higher values (93.90 g/L). This could be explained by seasonal changes in plasmatic proteins. Plasmatic proteins showed minimum values during spring and summer and a maximum at the peak of the mating period (GASPAR-LÓPEZ et al., 2010). Young population had slightly higher albumin levels than adults. Considering these findings, young population had much higher A/G ratio than adult population of red deer (0.85 vs. 0.38). Therefore, age and state of development influences the concentration of blood proteins.

Calcium (the ionized form) is an essential component in the production of enzymes and hormones that regulate digestion, energy and fat metabolism. Ionized calcium is a vital component in ion transport, muscle contractions, blood clotting systems, maintaining cells and connective tissues, nerve transmission, blood pressure control and wound healing (SOMER, 1995; WHITNEY and ROLFES, 1996; SIZER and WHITNEY, 1997). Ca<sup>2+</sup> represents free fraction and 45% of the total calcium in the plasma is accepted as its physiologically active form. In the majority of laboratories, only total calcium is routinely measured and ionized calcium concentration is calculated based on calcium, protein or albumin concentrations (GIDENNE et al., 2003). Ionized calcium is the best measure of active serum calcium (SCHENCK and CHEW, 2008) but most of laboratories do not have Ca<sup>2+</sup> analyzers so most of clinicians in human medicine use Payne's formula to obtain

corrected calcium values (PAYNE et al., 1973). In human medicine, in elderly patients with protein or energetic malnutrition and very low concentrations of albumin, correction with Payne's formula usually gives false hypercalcemic results, so hypocalcemia may be seriously underdiagnosed. Since we found distinct total protein concentrations and low albumin concentrations in red deer population, use of Payne's formula provide higher, obviously false results. Then we used alternative formula referring to protein instead albumin concentrations, proposed by PFITZENMEYER et al. (2007). Values corrected with this alternative formula showed more adequate results for ionized calcium, but still statistically different than those values obtained by measurement of ionized calcium. Since these two formulas are not satisfying, we used multiple regression to find out new formula for ionized calcium. Values corrected with our formula showed adequate and precise results, so we propose an alternative to direct measurement of  $\text{Ca}^{2+}$  with this simple formula useful in veterinary units, which are often deprived of high-performance equipment.

$\text{Mg}^{2+}$  is a physiologically active form and reflect better the intracellular concentration participating in many enzymatic reactions (REIS et al., 2008). Measuring the ionized Mg concentration is uncommon, but interpretation of tMg is complicated by magnesium binding to albumin. That means that a low tMg level may be seen with a normal ionized concentration in animals with hypoalbuminemia.  $\text{Mg}^{2+}$  concentration varies according to the extent of protein-binding, acid-base balance and metabolic derangement. While tMg concentration was similar in young and adult population, young population had higher  $\text{Mg}^{2+}$  concentration (0.47 vs 0.42 mmol/L). That difference is a result of a different albumin and protein levels. It is known that magnesium concentration decreases with age as a consequence of stress susceptibility, defective membrane functions and disruption of intracellular calcium metabolism, inflammation, cardiovascular diseases, including atherosclerosis and ischaemic injury, diabetes, fibrosis, immune dysfunction and other diseases associated with aging (RAYSSIGUIER et al., 1993).

We describe reference values which are in agreement with other ruminants like cattle and sheep (KANEKO et al., 1997; DIAS et al., 2008). A study on cattle (MOHRI et al., 2007) reported that age had no effect on sodium, potassium and chloride levels, but did on calcium, phosphorus and magnesium levels. Significant age related changes were also seen for the amounts of serum total protein, albumin and globulin.

The calcium to magnesium ratio is important in the absorption, use, and excretion of these minerals (SOMER, 1995). Magnesium and calcium act in conjunction, help the regulation of nerve and muscle tone. Magnesium competes with calcium, preventing it from triggering certain physiological events (nerve message relay or muscle contraction).  $\text{Mg}^{2+}$  modulates and controls cell  $\text{Ca}^{2+}$  entry and  $\text{Ca}^{2+}$  release from sarcoplasmic and endoplasmic reticular membranes. In human medicine, the  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ratio appears to be an important guide for signs of peripheral vasoconstriction, ischemia or spasm and

possibly atherogenesis (McCARRON et al., 1987; ALTURA and ALTURA, 1996). A high  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ratio also predisposes to arterial spasms, and increases catecholamine release (SHEEHAN and SEELIG, 1984). We found no difference related to age for the  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ratio.

In wild animals, these laboratory analysis are still in their very beginning due to difficulties in collecting data and lack of high-performance equipment in veterinary practice. This explains the deficiency of reference values which compromises the reliability of diagnosis made in red deer. Some of the values obtained were comparable to those previously reported in literature, but differences found demonstrate the need for age related reference intervals. Electrolyte and mineral values determined in this study can be considered reference data for health control and disease diagnosis.

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**SAŽETAK**

Istočni dio Hrvatske (osobito područje Parka prirode Kopački rit) značajno je stanište jelena običnog (*Cervus elaphus*). Utvrđivanje referentnih vrijednosti za laboratorijske pokazatelje kod brojnih vrsta divljih životinja, pa tako i ove, nije još u potpunosti provedeno. Razlog tome su poteškoće u prikupljanju uzoraka i relativno pomanjkanje opreme u područnim laboratorijima. Utvrđivanje referentnih raspona za serumske odnosno plazmatske koncentracije minerala i elektrolita, uz ostale hematološke i biokemijske pokazatelje predstavlja temelj za objektivno procjenjivanje zdravstvenog stanja. Pri tome je posebno važno precizno utvrditi referentne vrijednosti za svaku pojedinu dobnu kategoriju, kako zbog nepoznavanja fizioloških razlika ne bi došlo do pogrešnog tumačenja laboratorijskih nalaza. Cilj ovog istraživanja bio je odrediti i usporediti serumsku odnosno plazmatsku koncentraciju minerala i elektrolita kod mladih i odraslih jedinki u običnog jelena te utvrditi postoje li značajne razlike ili se mogu koristiti iste referentne vrijednosti za ove pokazatelje u obje dobne skupine. Serumaska razina ukupnog kalcija (tCa), anorganskog fosfora (iP), ukupnog magnezija (tMg) te albumina i ukupnih proteina određena je spektrofotometrijski, a plazmatska koncentracija ioniziranog kalcija ( $\text{Ca}^{2+}$ ), magnezija ( $\text{Mg}^{2+}$ ), natrija ( $\text{Na}^+$ ), kalija ( $\text{K}^+$ ) i klorida ( $\text{Cl}^-$ ) određena ion-selektivnim elektrodama (ISE). U mladih jedinki nađene su signifikantno više vrijednosti  $\text{K}^+$ , tCa,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , iP i albumina, dok su vrijednosti  $\text{Na}^+$ ,  $\text{Cl}^-$  i proteina bile niže u odnosu na odrasle jelene. Za tMg i omjer  $\text{Ca}^{2+}/\text{Mg}^{2+}$  nije ustanovljena značajna razlika kod različitih dobnih kategorija. Dobiveni rezultati mogu se smatrati referentnim za koncentraciju istraživanih minerala i elektrolita u jelena običnog (*Cervus elaphus*) te biti oslonac u procjeni i praćenju zdravstvenog stanja ove divljači. Pri tome treba istaknuti da je za neke pokazatelje utvrđena fiziološka razlika kod različitih dobnih kategorija pa je za pravilno tumačenje nalaza od najvećeg značenja rabiti referentne vrijednosti dobivene od primjerene dobne skupine.

**Ključne riječi:** minerali, elektroliti, ionizirani kalcij, jelen obični, *Cervus elaphus*

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