

Acid-base status, hematology and serum biochemistry values for a zoo population of Grant's zebra (*Equus quagga boehmi*)

Diana Brozić^{1*}, Lana Paden², Zvonko Stojević², Marcela Šperanda³, Mislav Đidara³, Jadranko Boras⁴, Jasna Stošić⁵, Kristina Starčević⁶, and Tomislav Mašek¹

¹*Department of Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

²*Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

³*Department of Animal Science, Faculty of Agriculture, University of Osijek, Osijek, Croatia*

⁴*Osijek ZOO Garden, Osijek, Croatia*

⁵*Zagreb ZOO Garden, Zagreb, Croatia*

⁶*Department of Animal Husbandry, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

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ABSTRACT

The study was carried out on 14 female and 12 male Grant's zebras to investigate the acid-base status, hematological and serum biochemistry values of a captive population. Sampling was performed during the years 2010 and 2011. The examined parameters included: acid-base parameters, hematology, serum biochemistry, enzyme activity and serum minerals. The most noticeable differences from zebras in other studies were higher MCHC and MCH values and lower MCV values. In comparison to domestic and wild horses, the zebras had very similar values for hematological parameters. Hematological values did not differ between males and females. Determination of acid-base status revealed similar values to domestic and wild horses, with high variations only in chloride values. Zebras had similar values to healthy horses for total protein, triglycerides, total cholesterol, ALP, ALT, AST and albumin/globulin ratio, while their GGT was higher. We found significant differences between male and female zebras for five biochemical parameters. Female zebras had higher values for ALT, albumin, albumin/globulin ratio and triglycerides than males, while males had significantly higher values for GGT and AST. The findings of this study may be useful to veterinary clinicians or biologists working

*Corresponding author:

Diana Brozić, Department of Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, Heinzelova 55, 10000 Zagreb, Croatia, Phone: +385 1 2390 274; Fax: +385 1 2441 390; E-mail: diana.brozic@vef.hr

with captive or free-ranging zebras. Future studies should focus on the link between diet and blood metabolites, and stress hormones in Grant's zebra and similar animals.

Key words: Grant's zebra; serum biochemistry; acid-base balance; hematology

Introduction

The plains zebra is the most abundant and widespread species of zebra, inhabiting tropical grasslands from East Africa to South Africa (HACK and RUBENSTEIN, 1998). There are six subspecies of the plains zebra (*Equus quagga*) and it is estimated that the Grant's subspecies comprises over 75% of the total plains zebra population (GROVES and BELL, 2004). According to the IUCN Red List of Threatened Species, the plains zebra population is currently stable, with a decline in populations reported in certain areas.

Blood analyses on acid-base status, hematology and serum biochemistry values could be of interest in assessing disease, nutritional status, habitat quality, and environmental stressors (MAŠEK et al., 2009), as well as in prediction of the success of reintroduction and translocation programs (MATHEWS and al., 2006). Establishment of reference values in wild animals is complicated by factors such as gender, age or habitat, with any handling also potentially contributing to increased levels of stress, which will consequently affect blood biochemical parameters. The small samples sizes and different methodologies followed by researchers in previous studies also complicate the interpretation of data, resulting in reference values that are of little or no use to veterinarians or zoo technicians.

The aim of this study was therefore to determine the acid-base status, and hematological and biochemical values for a small population of captive Grant's zebras, and to compare these values with others present in the literature. The values reported in this study may potentially contribute to improve decision-making processes, to ensure the success of reintroduction and translocation programs used for Grant's zebras as well as for diagnostic purposes.

Materials and methods

The study was carried out on 14 female and 12 male Grant's zebras at Zagreb (45°48'51"N, 15°58'40"E) and Osijek Zoos (45°33'03"N, 18°41'38"E) where temperature averages range from -0.1 °C (January) to 20.8 °C (July). During routine veterinary examination, five milliliter blood samples were taken from each animal from the v. jugularis, into a sterile blood tube without additives for serum analyses, and an ethylene diamine tetra-acetic acid (EDTA) treated tube for hematology. Sampling was performed 4 times during the years 2011 and 2012 (in January, April, July, and September).

The levels of metabolites (total protein, albumin, triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol), enzyme activities (gamma-glutamyltransferase-

GGT, alkaline phosphatase-ALP, aspartate amino transferase-AST, and alanine aminotransferase-ALT) were assayed by an automatic analyzer (SABA-18, Analyzer Medical System, Roma, 00153, Italy). Serum globulin concentrations were calculated by subtracting the albumin values from total protein values. Enzyme assays were done at 37 °C, and alkaline phosphatase activity was measured at pH 10.5. Reagents for all analyses were obtained from Randox Laboratories (Ardmore, Antrim, 03440, UK). The number of erythrocytes, leukocytes, platelets, levels of hemoglobin and hematocrit were established using an automatic counter (pocH-100iV DIFF, Sysmex Europe GmbH, Hamburg, 21149, Germany). Blood smears were prepared and stained according to Pappenheim, and evaluated under a microscope to obtain the differential blood count. The relative ratio of individual leukocytes is given in percentages in relation to their total number. The acid-base status was determined by RapidLab 348 (Bayer, Leverkusen, 51373, Germany) on the ion selective electrode principle. This included the blood pH, gases ($p\text{CO}_2$, $p\text{O}_2$), electrolytes (Na^+ , K^+ , HCO_3^-), base excess of extracellular fluid (BE_{ecf}), and oxygen saturation (O_2 SAT). Analyses were performed immediately after collection of blood.

Data were analyzed using the Statistica software (STATISTICA 2010 program, Tulsa, OK, USA). We calculated mean, minimum, maximum, median and discarded outliers (values more than 3 standard deviations from the mean). The Gaussian type of distribution was verified with skewness and kurtosis tests, and Shapiro and Wilks tests. All data with non-parametric distribution were log-transformed prior to further statistical analyses. Reference intervals were calculated as the data lying between the 2.5th and 97.5th percentiles (SOLBERG, 1999). Effects of season and sex were tested by two-way ANOVA. Multivariate Principal Component Analyses (PCA) was used to examine differences between the male and female populations. P values <0.05 were considered to be statistically significant.

Results

The average hematological, acid-base and blood biochemistry values are shown in Table 1. Sex significantly influenced the values for ALT, Albumin, Albumin/globulin ratio and triglycerides, which were higher in female zebras, and GGT which was higher in male zebras (Table 2).

We did not find a significant influence of season and, therefore, only the influence of sex is presented and discussed. The principal component analyses score plot revealed sex differences in biochemical parameters (Fig. 1A), and the most important are marked in the PCA loadings plot (Fig. 1B).

Table 1. Acid-base status, serum chemistry and hematologic values for adult Grant's Zebra (*Equus quagga boehmi*)

	n	Min	Max	Mean	Median	Reference range*
Hematology						
Erythrocytes (10 ¹² /L)	20	9.4	14.6	11.2	10.9	9.4-14.4
Hemoglobin (g/dL)	20	125.0	197.8	158.8	157.0	125.4-196.9
Hematocrit (%)	20	0.4	0.6	0.5	0.5	0.4-0.5
MCV (fL)	20	36.2	44.6	40.8	41.3	36.3-44.6
MCH (pg)	20	11.8	15.4	13.6	13.8	11.8-15.4
MCHC (g/dL)	20	315.2	352.4	334.0	334.0	315.6-352.0
Leukocytes (10 ⁹ /L)	20	6.2	8.3	7.1	7.0	6.2-8.3
Neutrophils (%)	20	48.0	89.0	66.9	65.8	48.7-88.5
Neutrophil band (%)	20	0.0	4.0	1.8	2.0	0.0-3.9
Lymphocytes (%)	20	7.0	48.0	29.0	29.0	7.5-47.4
Eosinophils (%)	20	0.0	2.0	0.6	0.5	0.0-2.0
Monocytes (%)	20	0.0	3.0	1.5	1.5	0.0-3.0
Basophils (%)	20	0.0	1.0	0.1	0.0	0.0-1.0
Platelet count (10 ⁹ /L)	20	123.0	1618.8	677.7	307.0	128.5-1611.1
Acid-base status						
Na (mmol/L)	18	126.0	140.3	133.0	133.0	126.1-140.2
K (mmol/L)	18	2.8	3.9	3.3	3.3	2.8-3.9
Cl (mmol/L)	18	89.2	102.1	95.3	95.0	89.5-102.0
pH	18	7.35	7.47	7.41	7.41	7.35-7.47
pCO ₂ (kPa)	18	5.5	7.5	6.8	6.8	5.5-7.5
pO ₂ (kPa)	18	4.7	27.3	9.1	6.3	4.7-26.6
O ₂ SAT (%)	18	65.6	99.5	79.2	77.2	65.9-99.5
ctCO ₂ (mmol/L)	18	29.9	34.1	32.0	32.2	29.9-34.1
HCO ₃ act (mmol/L)	18	28.9	32.4	30.6	30.6	28.9-32.4
HCO ₃ std (mmol/L)	18	26.4	30.4	28.7	29.0	26.5-30.4
BEeef (mmol/L)	18	4.4	58.0	10.3	6.3	4.4-53.9
BEb (mmol/L)	18	3.0	6.1	4.8	4.9	3.0-6.1
Anion Gap	18	3.8	13.3	10.0	10.2	4.1-13.3
Serum chemistry						
Total protein (g/L)	20	57.2	72.0	62.9	63.5	57.3-71.5
Albumin (g/L)	20	28.8	41.0	35.3	36.1	28.9-40.9
Globulin (g/L)	20	22.3	43.2	27.5	27.2	22.5-42.0
Albumin/globulin	20	0.7	1.7	1.3	1.4	0.7-1.7

Table 1. Acid-base status, serum chemistry and hematologic values for adult Grant's Zebra (*Equus quagga boehmi*) (continued)

	n	Min	Max	Mean	Median	Reference range*
Triglycerides (mmol/L)	20	0.2	0.5	0.3	0.3	0.2-0.5
Total cholesterol (mmol/L)	20	2.4	4.6	3.0	3.0	2.4-4.5
Gamma-glutamyl transferase (U/L)	20	14.1	33.9	22.7	21.5	14.3-33.6
Alanine aminotransferase (U/L)	20	1.9	8.4	5.6	6.3	2.0-8.3
Aspartate aminotransferase (U/L)	20	215.0	407.2	312.4	310.5	219.3-404.7

*2.5th to 97.5th percentile

Table 2. Values of select parameters that differed significantly between male and female zebra

Parameter	Sex		P value
	Male	Female	
Alanine aminotransferase (U/L)	3.40	7.49	***
Gamma-glutamyl transferase (U/L)	25.94	19.59	*
Albumin (g/L)	31.80	38.13	***
Albumin/globulin	1.14	1.46	**
Triglycerides (mmol/L)	0.26	0.37	*

* P<0.05; ** P>0.01; *** P>0.001

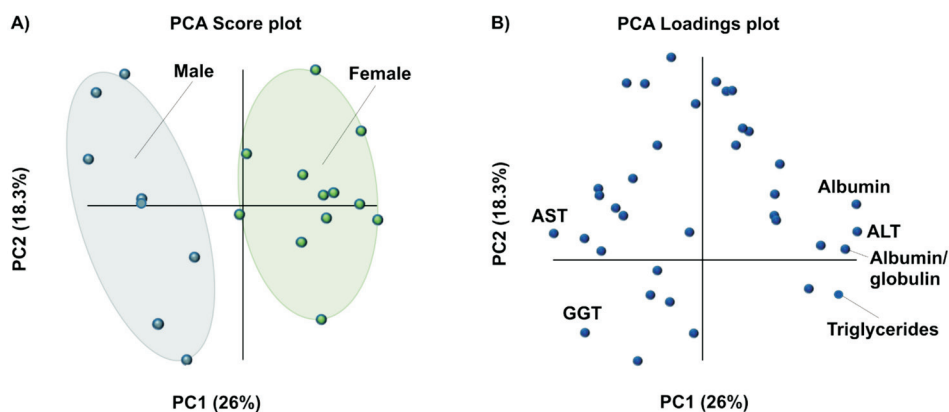


Fig. 1. A) Principal component analyses score plot (cases) showing two clusters corresponding to male and female zebras, B) Principal component analyses loadings plot (variables). Variables significantly higher in males (AST, GGT) and in females (albumin, ALT, albumin/globulin, triglycerides) are marked.

Discussion

The most noticeable differences from zebras in other studies are that MCHC and MCH values were higher and MCV values were lower, although the ranges for these values partially overlapped (HAWKEY, 1975; JONES, 1976). Values for erythrocytes were higher than the higher reference limit in comparison to the values of other authors, while hematocrit values were within reference ranges (HAWKEY, 1975; JONES, 1976; POSPISIL et al., 1985; SEAL et al., 1977). Normal hematocrit values exclude partial dehydration or high excitement during handling. In comparison to domestic and wild horses, the zebras in our trial had similar values for hematological parameters (PLOTKA et al., 1988; LUMSDEN et al., 1980). The relative differential revealed a predominance of neutrophils, which was also noted in other investigations on zebra hematology (HAWKEY, 1975; JONES, 1976; POSPISIL et al. 1985; ISIS, 2002). The similar values in zebras and domestic horses could lead to the conclusion that they are well adapted to the zoo environment, and that the stimuli that usually change hematological values in wild animals, such as catecholamine action, are not present to a high degree. Hematological values did not differ between males and females.

To the best of our knowledge this is the first detailed report on acid-base status in zebras. Studies of acid-base status are becoming more important in veterinary medicine because there are numerous diseases and conditions that are accompanied by changes in acid-base status. Our values for potassium and sodium were similar to those reported for horses (KANEKO et al., 2008; BORCHARD et al., 1982a), while chloride values were higher or lower compared to values reported for horses. The observed pH values fell within a narrow range (7.36 and 7.47) and were very similar to values for horses (7.32-7.44) (ISIS, 2002).

While the zebras in our study had similar values to healthy horses for total protein, triglycerides, total cholesterol, ALP, ALT, AST and albumin/globulin ratio, their GGT was higher (KANEKO et al., 2008; BORCHARD et al., 1982b). They also had higher values for albumin/globulin ratio and AST activity and lower values for total protein, globulin and GGT activity than those in ISIS (ISIS, 2002). We found significant differences between male and female zebras for six biochemical parameters. Female zebras had higher values for ALT, albumin, albumin/globulin ratio and triglycerides than males, while males had significantly higher values for GGT and AST (Fig. 1).

Conclusions

Due to the relatively small number of individuals in this study and the lack of comparative published data, these values should be regarded primarily as an aid to the establishment of biochemical reference values for zebras. This study forms the basis for

studies on *ex situ* management, and its effect of stress hormones of Grant's zebras and similar animals. Therefore, future studies should focus on the link between diet and blood metabolites, and stress hormones.

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SAŽETAK

Istraživanje je provedeno na 14 ženskih i 12 muških Grantovih zebri kako bi se utvrdile vrijednosti za acidobazni status te hematološke i biokemijske vrijednosti kod populacije u zoološkim vrtovima. Uzorci seruma uzeti su tijekom 2011. i 2012. te su pretraženi na: 14 pokazatelja acidobaznog statusa, 13 hematoloških, 10 metabolita te 4 enzima i 3 minerala. Najveća odstupanja u odnosu na druge studije bile su veće MCHC i MCH vrijednosti i niže vrijednosti MCV. U usporedbi s domaćim i divljim konjima, zebre su imale vrlo slične vrijednosti hematoloških pokazatelja. Hematološke vrijednosti se nisu značajno razlikovale ovisno o spolu. Određivanje acidobaznog statusa pokazalo je slične vrijednosti prema domaćim i divljim konjima s izuzetkom vrijednosti klora. Zebre su imale slične vrijednosti za ukupne proteine, trigliceride, ukupni kolesterol, ALP, ALT, AST i omjer albumin/globulin kao zdravi konji, dok je GGT vrijednost bila veća. Ustanovljene su značajne razlike između mužjaka i ženki za pet biokemijskih parametara. Ženke su imale više vrijednosti za trigliceride, omjer albumina i globulina, albumine i ALT u odnosu na mužjake, dok su mužjaci imali značajno više vrijednosti za GGT i AST. Nalazi ovog istraživanja mogu biti korisni za veterinare i biologe koji rade sa zebrama u divljini ili u zoološkim vrtovima. Buduća istraživanja treba usmjeriti na vezu između prehrane i krvnih metabolita te hormona stresa od Grantovih zebri i ostalih divljih ekvida.

Ključne riječi: Grantova zebra, serum, biokemijski pokazatelji, acidobazni status, hematološki pokazatelji
