

# Serum biochemical parameters in clinically healthy adult Bosnian mountain horse



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## Abstract

The Bosnian mountain horse, the only indigenous horse breed in Bosnia and Herzegovina, is an internationally recognized ancient breed and represents an important animal resource of this country. It belongs to the Warmblood horses. No data is available in the literature for serum biochemical parameters in the Bosnian mountain horse breed. Therefore, the aim of this study was to investigate the mean values of certain biochemical parameters in Bosnian mountain horse, and to compare the obtained values with reference intervals for adult horses in general, adult Warmblood horses and values previously reported for other horse breeds. Blood samples were collected from 30 clinically healthy adult horses of both sexes at the Borike stud farm. Samples for biochemical analysis were processed using standard procedures with the Catalyst One™ Chemistry Analyzer. The following mean values were comparable to previously published reference intervals in horses: glucose  $4.96 \pm 1.30$  mmol/L; urea  $7.83 \pm 2.18$  mmol/L; creatinine  $112.05 \pm 29.75$

$\mu\text{mol/L}$ ; phosphorus  $0.91 \pm 0.22$  mmol/L; calcium  $2.93 \pm 0.18$  mmol/L; total protein  $72.45 \pm 5.75$  g/L; albumin  $28.91 \pm 3.27$  g/L; globulin  $43.50 \pm 5.49$  g/L; alkaline phosphatase  $170.90 \pm 54.70$  U/L; total bilirubin  $22.00 \pm 11.01$   $\mu\text{mol/L}$ ; cholesterol  $2.54 \pm 0.28$  mmol/L and amylase  $33.50 \pm 12.77$  U/L. The obtained mean concentrations of urea and total protein were higher than those reported in any other Warmblood horses. Also, a higher mean concentration of globulin was found. However, all mean values were within the reference intervals for adult horses in general, adult Warmblood horses and previously published values for other horse breeds. Amylase activity was much lower than the recommended reference intervals for adult horses in general. The results obtained in this study indicate the need for further study to establish reference values of serum biochemical parameters in this Bosnian and Herzegovinian mountain horse breed.

**Key words:** *biochemistry; blood serum; Bosnian mountain horse*

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## Introduction

Investigation of serum for a biochemical profile is a routine part of the diagnostic evaluation of equine health status, along with other laboratory diagnostic procedures, pertinent patient history information and the physical examination (Stockham, 1995). Values of biochemical parameters in horses are used for clinical diagnosis of organic, infectious and parasitic diseases, and for monitoring recovery during treatment, postoperative monitoring and in the assessment of metabolic conditions of an individual or an entire herd (Gurgoze and Icen, 2010; Mikniene et al., 2014).

Generally, pathological processes are not the only cause of changes to serum biochemical values. Values may differ due to genetic factors and various environmental factors, e.g. nutritional quality, availability of water, parasites and climate (Čebulj-Kadunc et al., 2003; Pritchard et al., 2009; Pađen et al., 2014). Differences in sample collection and handling, using varying laboratory instruments, methods and reagents can also affect the values of biochemical parameters (Gul et al., 2007). Reference ranges of serum biochemical parameters found in textbooks and established by veterinary laboratories are often based on pleasure horses living under good husbandry conditions (Pritchard et al., 2009).

Consequently, serum biochemical values obtained abroad may not be fully applicable to horses living under local conditions, due to the influence of multiple factors on these values (Čebulj-Kadunc et al., 2003; Gul et al., 2007). The Bosnian mountain horse (Bosnian and Herzegovinian mountain horse, Bosnian pony), the only indigenous horse breed in Bosnia and Herzegovina, is an internationally recognized ancient breed that belongs to the Warmblood (WB) horses (Dekić et al., 2014).

It was created by crossing the Tarpan (*Equus caballus gmelini*) and the Asian wild horse (*Equus caballus przewalskii*) with the Arabian breed (Žiga and Telalbašić, 2009). This breed belongs to the group of the largest ponies and is frequently used for light farm work, light draft, pack and riding (Dekić et al., 2014).

Bosnian mountain horses are adapted to specific environmental and climatic conditions of the region and have characteristics that might be reflected in their serum biochemical parameters (Dekić et al., 2014).

Biochemical parameters in various horse breeds have been investigated and published by many authors (Gupta et al., 2002; Čebulj-Kadunc et al., 2003; Mohri et al., 2005; Lacerda et al., 2006; Pritchard et al., 2009; Simenew et al., 2011; Ebge-Nwiyi et al., 2012; Jagrič-Munih et al., 2012; Niedźwiedz et al., 2013; Takasu et al., 2013; Mikniene et al., 2014; Pađen et al., 2014; Cywińska et al., 2015; Freeman and Klenner, 2015).

However, to date there have been no attempts to determine the mean values of biochemical parameters in the Bosnian mountain horse breed. Therefore, this study was conducted to investigate the mean values of certain biochemical parameters in clinically healthy adult Bosnian mountain horses, and to compare the obtained values with reference intervals for adult horses in general, adult WB horses, and with values previously reported for other horse breeds.

## Materials and methods

Blood samples were collected in May 2014 from 30 adult horses of both gender (16 females and 14 males), aged between 2 and 23 years, at the Borike stud farm. No mares were pregnant.

Standard clinical procedures (anamnesis and physical examination)

**Table 1.** Average, minimal and maximal values of biochemical variables in Bosnian mountain horses (average  $\pm$  SD, min, max) and reference intervals for adult horses in general and adult WB horses

Variables	Average $\pm$ SD	Min.	Max.	Reference intervals for adult horses in general <sup>†</sup>	Reference intervals for adult WB horses <sup>*</sup>
GLU (mmol/L)	4.96 $\pm$ 1.30	1.39	6.91	2.80-5.50	3.05-5.27
UREA (mmol/L)	7.83 $\pm$ 2.18	4.20	11.10	2.50-8.30	3.3-8.8
CREA ( $\mu$ mol/L)	112.05 $\pm$ 29.75	62.00	177.00	40-150	<176.8
P (mmol/L)	0.91 $\pm$ 0.22	0.56	1.36	0.8-1.8	0.5-1.3
Ca (mmol/L)	2.93 $\pm$ 0.18	2.66	3.33	2.6-3.3	2.0-3.2
TP (g/L)	72.45 $\pm$ 5.75	59.00	82.00	58-75	55-75
ALB (g/L)	28.91 $\pm$ 3.27	23.00	36.00	23-35	-
GLOB (g/L)	43.50 $\pm$ 5.49	32.00	53.00	30-50	-
ALP (U/L)	170.90 $\pm$ 54.70	94.00	295.00	84-150	<350
TBIL ( $\mu$ mol/L)	22.00 $\pm$ 11.01	10.00	43.00	17-34	11.97-53.01
CHOL (mmol/L)	2.54 $\pm$ 0.28	1.86	2.94	-	1.04-3.11
AMYL (U/L)	33.50 $\pm$ 12.77	0.00	65.00	75-150 **	-

<sup>†</sup>Freeman and Klenner (2015)

<sup>\*</sup>Paden et al. (2014)

GLU: glucose. UREA; urea. CREA: creatinine. P: phosphor. Ca: calcium. TP: total protein. ALB: albumin. GLOB: globulin. ALP: alkaline phosphatase. TBIL: total bilirubin. CHOL: cholesterol. AMYL: amylase.

were performed before sampling. Trias (temperature, pulse and respiratory frequency), adsppection, palpation, auscultation and percussion were performed, and no clinical symptoms were noted. Mares were kept outdoors on pastures of a mountain plateau. The pastures provided sufficient nutrition with addition of grain once a day. Stallions were housed in individual box stalls under natural outdoor conditions in terms of temperature and photoperiod. Diet of stallions is based on hay with the addition of grain once a day. Drinking water was available *ad libitum*.

Blood samples were collected in the morning before feeding and watering. Blood was drawn from the jugular vein (*vena jugularis externa*) in heparin tubes. The tubes were kept in the refrigerator (4 °C) during transport to the laboratory. Blood serum was separated by

centrifugation at 1600 g for 20 minutes. The serum was aspirated and stored at -20 °C until further analysis.

Concentrations of biochemical parameters were measured using standard procedures with the Catalyst One™ Chemistry Analyzer (IDEXX Laboratories, Netherlands). Data were processed using the GLM procedure of statistical software Minitab 17. All parameters were reported as a mean, standard deviation (SD), minimum and maximum. All animals were treated in accordance to Internal Ethical Committee by respecting animal welfare and well-being.

## Results

The values of the analysed parameters and reference intervals for adult horses in general and adult WB horses are shown in Table 1.

## Discussion

The results of influence of gender on biochemical parameters in Bosnian mountain horse breed from the Borike Stud farm was studied by Rukavina et al. (2018), who found that gender significantly affected urea, phosphorus, total bilirubin and amylase levels. Values of urea, total bilirubin and amylase were significantly higher in males, while phosphorus was significantly higher in females. Creatinine concentrations were higher in stallions, while the activity of alkaline phosphatase was higher in mares. However, mean values of these biochemical parameters have not previously been published.

In the present study, the mean values of twelve biochemical parameters were determined and results evaluated based on the reference intervals for adult horses in general, adult WB horses and previously published values for other horse breeds. The values of the serum biochemical parameters in Table 1 showed that most parameters (all but one) were within the reference ranges established for adult horses in general and adult WB horses (Freeman and Klenner, 2015).

The parameter showing deviation was serum amylase activity, which was lower in this study than the lowest reference value limit (Freeman and Klenner, 2015). Considerably low values for serum amylase activity have been published for the Posavina and Croatian Coldblood horse breeds (Pađen et al., 2014). The animals in our study were clinically healthy and it is believed that the obtained values were not a reflection of abnormal processes in the body. According to Tennat and Center (2008), the rate of amylase synthesis is influenced by diet. It can be several times higher in animals fed a high-carbohydrate diet compared to those fed a high-protein diet. The nutrition of the examined horses on pastures with slow digestible carbohydrates and the addition of whole

grains may be a reason for the low serum amylase activity.

The mean urea concentration in this study was lower than in working equids of Ethiopia (Simenew et al., 2011), but higher than previously reported for other horse breeds (Čebulj-Kadunc et al., 2003; Lacerda et al., 2006; Jagrič-Munih et al., 2012; Ebge-Nwiyi et al., 2012; Niedźwiedź et al., 2013; Takasu et al., 2013; Mikniene et al., 2014; Cywińska et al., 2015). Our results of mean urea concentration were similar to those obtained for Posavina and Croatian Coldblood horses (Pađen et al., 2014).

According to Pađen et al. (2014), urea concentrations may be elevated due to a high protein diet and increased protein catabolism, which would additionally explain the lower amylase activity measured in this study.

The mean total protein concentration found in the present study, was at the upper limit of the recommended reference intervals for adult horses in general and adult WB horses (Freeman and Klenner, 2015). Albumin concentrations in Bosnian mountain horses were similar to previously reported albumin concentrations in different horse breeds (Gupta et al., 2002; Lacerda et al., 2006; Jagrič-Munih et al., 2012; Mikniene et al., 2014; Pađen et al., 2014). Higher albumin concentrations were reported for Turkmen horses (Mohri et al., 2005). The mean globulin concentration in this study was in agreement with previously reported concentration by Lacerda et al. (2006) for the Criollo horse breed, and was in the upper limit of the reference intervals for adult horses in general (Freeman and Klenner, 2015). According to Gurgoze and Icen (2010) differences in total protein, albumin and globulin concentrations, are likely associated with animal breed and rearing conditions. This may be also related to the immune condition of the investigated breed that is well adapted to its environmental and climatic conditions.

The mean creatinine concentration in this study was higher than in the Hucul horse breed (Cywińska et al., 2015) and lower than in Polish Konik (Niedźwiedz et al., 2013). The mean creatinine concentration in Bosnian mountain horses was similar to values previously cited for other horse breeds (Čebulj-Kadunc et al., 2003; Jagrič-Munih et al., 2012; Mikniene et al., 2014; Pađen et al., 2014). Differences in creatinine concentrations reflect different nutritional conditions due to the connection between creatinine and creatine metabolism. The level of creatinine depends on the total body content of creatine, which in turn depends on dietary intake and muscle mass (Gurgoze and Icen, 2010). The increase in creatinine concentration is in relation to muscular activity (Piccione et al., 2008).

The mean glucose concentration in Bosnian mountain horses was similar to glucose concentrations in Criollo (Lacerda et al., 2006), Standardbred (Jagrič-Munih et al., 2012) and Posavina and Croatian Coldblood horses (Pađen et al., 2014). In Brasileiro de Hipisimo and Thoroughbred horses (Lacerda et al., 2006), glucose concentrations were higher than the value obtained here. Mikniene et al. (2014) reported lower glucose concentrations in Žemaitukai horses. Variations in glucose concentrations may arise from breed, environmental conditions, nutrition and timing of blood sampling.

The alkaline phosphatase activity recorded in this study was consistent with previously reported values for Pakistan (Pritchard et al., 2009) and Standardbred horses (Jagrič-Munih et al., 2012). In other studies, higher values of alkaline phosphatase activity were recorded (Mohri et al., 2005; Gul et al., 2007; Takasu et al., 2013; Mikniene et al., 2014; Pađen et al., 2014).

Hasković et al. (2011) noticed the effect of seasonal fluctuation on the enzyme

activity of Bosnian mountain horses living under natural conditions due to the temperature influence on metabolism. They recorded lower enzyme activity in spring than in autumn due to the influence of lower autumn temperatures and the resulting need for greater energy metabolism. These seasonal fluctuations may be the cause of the lower alkaline phosphatase activity found in this study, as blood sampling was performed during the spring period.

The concentration of calcium obtained in the present study was similar to calcium concentrations in previous studies on horses (Čebulj-Kadunc et al., 2003; Mohri et al., 2005; Lacerda et al., 2006; Pritchard et al., 2009; Jagrič-Munih et al., 2012; Mikniene et al., 2014; Pađen et al., 2014). Simenew et al. (2011) reported lower calcium concentrations in working equide of Ethiopia. Calcium levels are significantly affected by sweating during prolonged exertion (Weiss et al., 2002). Also, the serum level of calcium is influenced by nutrition. A high protein diet may interfere with calcium absorption. For example, proteins in soybean meal used as a protein supplement contain phytic acid, which reduces the efficiency of calcium absorption (Schryver et al., 1987). Lower calcium levels can occur in herbivores, whose nutrition is based on plants containing high amounts of potassium oxalate (Pađen et al., 2014).

The mean total bilirubin concentration obtained in this study was higher than the literature values for Polish Konik horses (Niedźwiedz et al., 2013) and Posavina and Croatian Coldblood horses (Pađen et al., 2014). Healthy horses have higher total bilirubin concentrations than any other domestic species (Hornbuckle et al., 2008).

Variations detected in the values of observed serum biochemical parameters in studies of different researchers could be due to breed, geographical, season and

climate conditions of sampled horses, as well as nutritional factors, management and sample size. Also, different methodologies and equipment used by the laboratory might be reflected on the values of the examined biochemical parameters (Lacerda et al., 2006; Pritchard et al., 2009; Mikniene et al., 2014).

## Conclusions

Serum amylase activity was lower than the recommended reference interval for adult horses in general. Mean urea and globulin concentrations were at the upper limit or were higher than values reported in the literature for other horse breeds, but were within the reference intervals for adult horses in general and adult Warmblood horses. The results obtained in this study confirm the need for further study to establish reference values of serum biochemical parameters in the Bosnian mountain horse breed.

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## Serumski biokemijski pokazatelji odraslih klinički zdravih bosanskih brdskih konja

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Bosanski brdski konj, jedina autohtona pasmina konja u Bosni i Hercegovini, je međunarodno priznata drevna pasmina i predstavlja važan animalni resurs Bosne i Hercegovine. Spada u toplokrvne konje. U literaturi nisu dostupni podatci o vrijednostima biokemijskih pokazatelja za Bosanskog brdskog konja. Iz tog razloga, cilj ovog rada je bio istražiti srednje vrijednosti nekih biokemijskih pokazatelja Bosanskog brdskog konja, usporediti dobivene vrijednosti s referentnim vrijednostima za odrasle konje općenito, toplokrvne konje te s vrijednostima objavljenim za druge pasmine konja. U istraživanje je bilo uključeno 30 klinički zdravih odraslih konja oba spola od kojih je uzorkovana krv na ergeli „Borike“. Uzorci su obrađeni standardnim postupkom koristeći The Catalyst One™ Chemistry Analyzer. Navedene srednje vrijednosti su uspoređene s prethodno objavljenim referentnim intervalima za konje: glukoza  $4,96 \pm 1,30$  mmol/L; urea  $7,83 \pm 2,18$  mmol/L; kreatinin  $112,05 \pm 29,75$   $\mu$ mol/L; fosfor  $0,91 \pm$

$0,22$  mmol/L; kalcij  $2,93 \pm 0,18$  mmol/L; ukupni protein  $72,45 \pm 5,75$  g/L; albumin  $28,91 \pm 3,27$  g/L; globulin  $43,50 \pm 5,49$  g/L; alkalna fosfataza  $170,90 \pm 54,70$  U/L; ukupni bilirubin  $22,00 \pm 11,01$   $\mu$ mol/L; kolesterol  $2,54 \pm 0,28$  mmol/L i amilaza  $33,50 \pm 12,77$  U/L. Srednje vrijednosti ureje i ukupnih proteina su bile više u odnosu na vrijednosti nađene u drugim istraživanjima rađenim na toplokrvnim konjima. Utvrđena je i viša srednja vrijednost globulina. Međutim, sve srednje vrijednosti su bile unutar referentnih vrijednosti objavljenih za odrasle konje općenito, odrasle toplokrvne konje, kao i ranije objavljene vrijednosti za druge pasmine konja. Vrijednosti aktivnosti amilaze su bile značajno niže u odnosu na preporučene referentne vrijednosti za odrasle konje općenito. Dobiveni rezultati ukazuju na potrebu za daljim istraživanjima kako bi se ustvrdile referentne vrijednosti serum biokemijskih pokazatelja Bosanskog brdskog konja.

**Gljučne riječi:** *biokemija, krvni serum, bosanski brdski konj*