THE EFFECT OF AGE AND GENDER ON BLOOD HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS IN ŽEMAITUKAI HORSES

Zoja Miknienė[1], Kęstutis Maslauskas[1], Sigita Kerzienė[2], Jūratė Kučinskienė[3], Audrius Kučinskas[1]

[1]*Department of Non-infectious Diseases*
[2]*Department of Social Sciences*
[3]*Department of Food Safety and Quality*
Veterinary Academy, Lithuanian University of Health Sciences
Tilžės 18, LT-47181 Kaunas, Lithuania; e-mail: mikniene@lva.lt

Abstract. The aim of the present study was to investigate and show the differences in blood parameters related with age and sex of Žemaitukai horse breed in Lithuania. According to the blood parameters, they may be assigned to a particular blood type.

Haematological parameters [(red blood cells (RBC), white blood cells (WBC), hemoglobin concentration (Hb), hematocrit (HCT), lymphocytes (LYM), platelet (PLT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC)] and biochemical parameters [total protein (TP), albumin (Alb), calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), urea (Urea), glucose (Glu), creatinine (Crea) concentration, heart (CK-MB) and total (CK-NAC) creatinekinase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamaglutamiltransferase (GGT)] activity were determined for 173 (92 mares and 81 stallions) clinically healthy Žemaitukai horses. It was found that the RBC and WBC were significantly reduced in the blood with increasing age. Statistically significant differences between sexes were established: Hb ($p < 0.001$), HCT ($p < 0.001$), RBC ($p < 0.01$), TP ($p < 0.05$), Alb ($p < 0.01$), Urea ($p < 0.05$), P ($p < 0.01$), and Ca ($p < 0.05$) concentrations. It was found that in foals, the average of WBC, RBC, LYM, PLT, P, ALT and ALP values are significantly higher than in adult blood group values. Average of RBC values in Žemaitukai stallions and mares correspond to the lower limit of Thoroughbred horses. The quantity of RBC in Žemaitukai mares is closest to Murgese breed mares RBC value and value of WBC range in Thoroughbred mares WBC volume limits. However, the value of WBC in Žemaitukai stallions corresponds to normal WBC range in Kathiawari (India) horses. The morphological parameters of Žemaitukai - can be attributed to warm-blooded equine type. The results of the morphological and biochemical blood parameters of Žemaitukai horses can be classified as "warm-blooded" horse blood type, which can be helpful in future research basing the use of Žemaitukai.

Keywords: Žemaitukai, blood, value.

Introduction. Haematological and serum biochemical parameters in horses are used for the clinical diagnosis of organic, infectious and parasitic diseases. Interpretation of diagnostic parameters is important to determine the specific breed of horse, and the relevant age group of some limits of physiological blood parameters. They are also used for recovery monitoring and treating the diseases. It is important in postoperative patients and it is used to assess the metabolic condition of a single animal. It is used in sport horses to plan specific physical exercise (Altinsaat, 2008; Gurgoze et al., 2010).

The blood profile of horses can be influenced by their temperament, which classifies horses as „hot-blooded“ (HB) – fast and tough horses (Arabians and Thoroughbred), „warm-blooded“ (WB) – derived from the crossing of horses between HB and CB, (this group is the biggest), and „cold-blooded“ (CB) or heavy horses – the heavy horse breed, adapted to the heavy work and part of the horse like pony class (Orsini, 2003; Robinson, 2003; Feldman et al., 2006; Knottelbelt, 2006).

In focus of the present study is on Žemaitukai horses. This is one of the oldest breeds in Europe. It was mentioned for the first time in the 6–7th centuries. This breed became especially famous in the 14th century. Žemaitukai were recognized as excellent warhorses during the battles against Crusaders. This is a particularly strong horse, highly manoeuvrable, enduring and fast. Žemaitukai are universal horses, belonging to the pony class due to their size and type. Their height at the withers range from 128 to 142 cm, oblique body length is 136–148 cm, weight is 360–420 kg, and chest girth is 154–187 cm. Žemaitukai have a rather weighty body but thin yet strong legs and energetic temperament; these traits provide them with agility and manoeuvring abilities (Macijauskienė, 2002).

Blood haematology and serum biochemistry of the European and American horse breeds are well established and described by many scientists and details can be found in various articles and text books (Čebuli-Kaudune ir kt. 2002; Mohri, 2004; Knottenbelt, 2006; Lacerda, 2006; Altinsaat, 2008; Satue ir kt., 2012). The information about the ancient Lithuanian breeds (such as Žemaitukai) is limited for the moment. Žemaitukai horse exterior features are described as the pony class horses, but their blood type is unknown so far.

The aim of this study was to determine the haematological and biochemical parameters of Žemaitukai horses by revealing the differences related to age and gender. In accordance with these findings, the Žemaitukai horses may be assigned to specific type of horse blood.

Materials and methods. In total, 173 clinically healthy Žemaitukai horses (old type Žemaitukai horses) from seven farms in Lithuania were used in this trial. By

determining the effect of the age and gender of the animal on the normal ranges of blood haematological and serum biochemical parameters, all horses were grouped by gender, stallions; n=81 and mares, n=92, and were divided into six age groups. The distribution of the Žemaitukai horses, assigned to the six different age groups, on the basis of the farms they were obtained from is shown in Table 1. The first group (group A) consisted of foals from birth to 1 year old (11 months) (n=12), the second (Group B) –the youngster horses over 1 year (12 months) to 3 years (35 months) (n=19), the third (Group C) – young stock horses over 3 years (36 months) to 6 years (71 months) (n=48), the fourth (group D) - adult horses aged 6 years (72 months) to 10 years (119 months) (n=48), the fifth group (Group E) – older adults horses aged 10 years (120 months) to 14 years (167 months) (n=35), and the sixth group (group F) geriatric horses – aged 14 years (168 months) and older (n=11). All horses were healthy and did not show any signs of abnormality during the study period. The mares were not pregnant and were not lactating during the trial. The blood samples were collected from 173 animals between March-May in 2010, and they were handled with care to minimize stress-induced effects. To reduce circadian variations, all samples were collected before the morning feeding. Horses were fed three times a day with hay, they once received oat meal, and did not receive supplements and minerals. The water was used *ad libitum*. Before taking blood samples, horses did not have any physical exercise.

Blood samples were taken from the Jugular vein using a 21-gauge needle into the vacuum blood tubes, 5ml (BD Vacutiner, United Kingdom). Tubes of etilendiaminotetraacetic acid (EDTA) were used to study the blood haematology and the tubes without EDTA were ued for blood biochemical examination. The haematological and biochemical studies of the Žemaitukai horse blood were carried out at the Large Animal Clinic Clinical Research Laboratory of the Veterinary Academy, Lithuanian University of Health Sciences .

The blood haematology was analysed using a haematological analyzer Abacus Junior Vet (Diatron Messtechnik GmbH, Austria, 2006). The red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (Hb) and mean cell volume (MCV) were measured directly; packet cell volume (PCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentrations (MCHC) were calculated automatically.

After collection of blood samples into the vacutainer tube without EDTA they were centrifuged at 3000 U/min for 10 min. to obtain plasma (Hettich Universal, UK) and fractionated blood separated serum was evaluated. Concentrations of serum total protein (TP), albumin (Alb), calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), urea (Urea), glucose (Glu), creatinine (Crea), heart (CK-MB) and total (CK-NAC) creatine kinase levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and the gamaglutamiltransferase (GGT) activities were measured using an automated blood chemistry analyzer Hitachi 705 (Hitachi, Japan) by using the DIAS (Diagnostic Systems GmbH, Germany) reagents.

All data were analyzed using SPSS statistical package (SPSS for Windows 15.0, SPSS Inc., Chicago, IL, USA, 2006) and Microsoft Excel (2003) software. Blood morphology and serum biochemical parameters were compared by using ANOVA model with sex (mares and stallions) and age (6 groups) factors. LSD test (α=5%) used to determine significant differences among the treatment groups. Analysis of the data is statistically significant at $p < 0.05$. Research carried out in accordance with the 1997 11 06 Republic of Lithuania on animal care, maintenance and use of the Law. 8-500 (Valstybės Žinios, 1997 11 28, No. 108).

Results. With the increasing age of horses, the concentrations of the RBC, WBC, LYM, Hb and PLT decrease with statistically significant determination. The MCH concentration increases accordingly. Statistically significant differences between mares and stallions were found for RBC, HCT, Hb ($p<0.01$, $p<0.001$ and $p<0.05$) and MCHC ($p<0.05$).

When the age of the horses increases, TP, GGT and CK-MB concentrations in serum increase with statistically significant determination, while Glu, ALP, P, and K concentrations decrease. The means Alb ($p<0.001$), AST ($p<0.05$), Ca ($p<0.05$), P ($p<0.01$) and Na ($p<0.001$) in all mares of different age were lower with statistical significance. The CK-NAC ($p<0.001$), CK-MB ($p<0.001$) and Mg ($p<0.05$) were higher than those of stallions in different age groupss.

The mean values of blood haematological and biochemical parameters in Žemaitukai horses according to age and gender are shown in tables 2–5.

Discussion. The haematological and serum biochemical parameters are widely used in diagnosis of diseases, and their prognosis. It is also used to monitor the treatment of disease, and to evaluate the animal's metabolic status, and in herd monitoring programs (Čebuli-Kaudune et al., 2003).

Table 1. **The distribution of six different age groups of Žemaitukai horses** (group A, B, C, D, E, F) (n=173)

Age group	Nr. 1	Nr. 2	Nr. 3	Nr. 4	Nr. 5	Nr. 6	Nr. 7
Group A (1-11 month)	5	2	-	1	1	1	2
Group B (12-35 month)	2	4	-	-	3	5	5
Group C (36-71 mointh)	3	2	20	3	3	10	7
Group D (72-119 month)	-	2	17	12	6	10	1
Group E (120-167 month)	8	1	6	6	3	8	3
Group F (168 month)	1	3	-	4	-	-	3

Table 2. **Mean values with standard deviation** (±SD) **of blood morphological parameters in Žemaitukai horses**

Parameter	Group A	Group B	Group C	Group D	Group E	Group F
n	12	19	48	48	35	11
RBC, $x10^{12}$/l	9.80±1.18 ab	8.69±1.11 bc	8.39±1.08 c	7.83±0.91 de	7.45±1.03 e	6.72±0.87 f
WBC, $x10^{9}$/l	10.48±2.70 abc	11.08±3.57 b	9.79±1.89 cf	8.68±1.50 def	8.64±1.46 ef	8.75±1.46 f
LYM, $x10^{9}$/l	3.46±1.26 abc	3.98±1.57 b	2.83±1.27 c	2.19±0.92 def	1.76±0.72 ef	1.79±0.85 f
Hb, g/l	139.00±19.64	140.74±17.98	142.25±21.33 a	139.75±19.15	135.71±22.30	127.00±16.40 b
PCT, %	30.62±4.37 abef	30.39±3.51 bef	33.36±4.36 cd	33.29±3.97 de	31.54±3.91 ef	29.51±4.19 f
MCV, fl	31.25±2.49 a	35.16±3.00 b	39.94±2.57 c	42.58±2.66 def	42.51±2.63 ef	44.09±2.47 f
MCH, pg	14.20±1.25 a	16.23±0.98 bc	16.99±1.46 c	17.91±1.73 de	18.24±1.59 ef	18.95±1.03 f
MCHC, g/l	454.67±28.16abdef	463±20.18 bf	417.42±77.46cdef	422.21±50.18 def	430.09±39.94 ef	430.55±10.34 f
PLT, $x10^{9}$/l	186.75±77.06 a	149.16±49.73	135.53±58.34 bc	152.81±64.61	148.26±60.99	124.91±46.21 c

a, b, c, d e – Means with different superscript in columns are significantly different ($p<0.05$)

Table 3. **Mean values with standard deviation** (±SD) **of blood morphological parameters in Žemaitukai horse mares and stallions**

Parameter	Stallions	Mares
n	81	92
RBC, $x10^{12}$/l	7.74±0.15 ***	8.36±0.13
WBC, $x10^{9}$/l	9.48±0.26	9.29±0.21
LYM, $x10^{9}$/l	2.72±0.14	2.38±0.14
Hb, g/l	135.57±2.05 *	141.78±2.24
PCT, %	30.84±0.41 ***	33.42±0.45
MCV, fl	40.33±0.49	40.33±0.45
MCH, pg	17.69±0.20	17.04±0.20 *
MCHC, g/l	440.06±3.52 *	424.99±5.68
PLT, $x10^{9}$/l	150.59±7.09	144.54±6.27

*- $p<0.05$; ** -$p<0.01$; *** - $p<0.001$

Some horses with a different age, sex, breed temperament and blood type have a different range of blood morphological and biochemical parameters (Robinson, 2003; Feldman et al., 2006; Knottenbelt, 2006; Rubino et al., 2006; Kaneko et al., 2008; Gurgoze et al., 2010; Satue et al., 2012). As, for example, in hot-blooded (or Thoroughbred) horses blood RBC value, PCT and hemoglobin concentration as well as serum creatinine (Lumsden, 1980; Robinson, 2003; Kaneko et al., 2008) are higher than in cold-blooded (or heavy horses) horses, but the total protein, phosphorus, and some enzyme activity values are below (Robinson, 2003; Feldman et al., 2006). MCV in HB horses is lower than in the heavy horses. Some horses inherited the minor differences of Hb, MCH and MCHC levels (Satue et al., 2012). Haematological and biochemistry parameters of the blood in youngster and young adult horses are generally higher than in the older individuals (Robinson, 2003; Feldman et al., 2006; Reed et al., 2010; Satue et al., 2012).

Table 4. **Mean values with standard deviation** (±SD) **of blood biochemical parameters in Žemaitukai horse**

Parameter	Group A	Group B	Group C	Group D	Group E	Group F
n	12	19	48	48	35	11
TP, g/l	62.61±9.56abcef	65.33±8.58 bcdef	62.40±10.92 cf	68.96±9.16 def	67.11±8.08 ef	66.04±8.50 f
Alb, g/l	29.03±4.84	28.96±3.74	30.03±4.86	30.84±4.96 a	29.22±4.95	27.54±5.12 b
Glu, mmol/l	4.85±1.38 ae	3.19±1.50 bcdef	3.40±1.28 cdef	3.33±1.16 def	3.89±2.55 ef	3.35±1.05 f
Urea, mmol/l	5.29±0.84	5.29±1.11	5.16±1.15	5.53±1.70	5.12±1.12	5.99±1.24
Crea, mmol/l	0.98±0.17	0.94±0.13	1.04±0.20	1.00±0.18	0.96±0.22	0.92±0.19
AST, TV/l	311.11±67.31	305.86±66.93	311.10±106.86	297.06±89.23	283.64±82.49	299.09±76.17
ALT, TV/l	14.61±4.25	14.70±4.62	14.20±5.94	13.20±3.58	12.57±3.77	12.76±4.05
ALP, TV/l	599.25±361.42a	402.79±142.69bcdef	357.21±158.52 cdef	338.67±114.99def	370.60±166.28ef	442.55±236.37f
GGT, TV/l	30.00±6.78	27.75±5.64	27.24±11.72 abc	26.83±13.70 bc	25.58±12.93 c	40.57±30.39 e
CK-NAC,U/l	314.92±215.83	357.47±137.13	354.11±143.82	361.57±167.80	401.77±197.98	352.91±126.66
CK-MB, U/l	477.80±249.26a	619.40±242.31	583.70±236.51	603.83±284.3	652.53±264.26b	585.94±204.35
Ca, mmol/l	2.72±0.38	2.68±0.36	2.65±0.47	2.75±0.32	2.69±0.34	2.67±0.47
P, mmol/l	1.33±0.42 a	0.94±0.30 bcde	0.93±0.29 cde	0.88±0.20 de	0.82±0.26 ef	0.71±0.15 f
Mg, mmol/l	0.74±0.10abdef	0.71±0.13 bcdef	0.65±0.11 c	0.74±0.12 def	0.71±0.12 ef	0.76±0.16 f
K, mmol/l	5.12±0.59acdef	4.52±0.37 bf	5.16±0.85 cdef	5.14±0.72 def	4.99±0.60 ef	5.00±0.71 f
Na, mmol/l	151.63±9.72	152.13±5.71	154.51±4.34	153.69±4.72	153.80±4.42	152.64±5.50

a, b, c, d, e, f – Means with different superscript in columns are significantly different ($p<0.05$)

Table 5. **Mean value with standard deviation** (±SD) **of blood biochemical parameters in Žemaitukai horse mares and stallions**

Parameter	Stallions	Mares
n	92	81
TP, g/l	65.55±1.04	65.95±1.04
Alb, g/l	31.25±0.50	28.06±0.50 ***
Glu, mmol/l	3.58±0.14	3.53±0.22
Urea, mmol/l	5.26±0.14	5.40±0.14
Crea, mmol/l	1.04±0.02	0.93±0.02
AST, TV/l	318.78±8.48	286.38±9.30 *
ALT, TV/l	13.99±0.51	13.16±0.48
ALP, TV/l	348.73±12.21	400.42±20.78
GGT, TV/l	26.78±1.02	28.59±2.30
CK-NAC, U/l	314.48±12.84	419.18±21.27 ***
CK-MB, U/l	532.31±22.14	677.00±31.37 ***
Ca, mmol/l	2.76±0.04	2.63±0.04 *
P, mmol/l	0.98±0.03	0.83±0.03 ***
Mg, mmol/l	0.69±0.01	0.73±0.01 *
K, mmol/l	5.14±0.08	4.92±0.07
Na, mmol/l	154.57±0.43	152.40±0.68 **

* – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$

In this study, Žemaitukai horses were used to compare with the horses of the following breeds: HB Arabian, Thoroughbred, and Akhal-Teke horses (Gavazzi et al., 2002; Robinson, 2003; Mohri et al., 2004; Feldman et al., 2006; Lacerda, 2006; Altinsaat, 2008; Kaneko et al., 2008), CB - Clysdale, Pakistan, Haflinger, Percheron horses (Feldman et al., 2006; Petruse et al., 2007; Pritchard et al., 2009) and WB - Quater, Appaloosa, Criollo, Murgese, Lipizzan, Trakhener, Hannoveraner, Kathiawari, BH, Standardbred horses (Kästner et al., 1999, Tateo, 2008 ; Čebuli-Kaudune et al., 2002, Gupta et al., 2002; Feldman et al., 2006; Lacerda, 2006; Rubino et al., 2006; Gul et al., 2007; Petruse et al., 2007; Piccione et al., 2010).

The mean RBC values in Žemaitukai mares and stallions are located at the lower end of the normal Thoroughbred horses (Feldman et al., 2006), but in the middle of the normal range for cold-blooded (Feldman et al., 2006; Lacerda, 2006) and Arabian horses (Altinsaat, 2008). However compared with Kathiawari, Trakhener and Hannoveraner horse breeds, the RBC values reache the upper limit. The RBC values in Žemaitukai mares and stallions are similar to RBC values in Murgese horses (Rubino et al., 2010). In spite of the fact that Žemaitukai horse RBC levels are more similar to the values characteristic of warm-blooded horses, the mean PCV in Žemaitukai is by 17.2–22.7% lower than in warm-blooded horses (Lacerda, 2006; Feldman et al., 2006; Altinsaat, 2008), except Murgeze horses (Rubino, 2006). Comparison of our results with some breeds of warm-blooded horses reveals that the means of RBC in Žemaitukai foals are by 8.0–12.0% higher (Čebuli-Kaudune et al., 2002; Feldman et al., 2006; Tateo et al., 2008), but in young stocks, adults and older adult horses they are similar with those in Arabian horses (Altinsaat, 2008).

The mean WBC values in Žemaitukai mares and stallions were by 8.7–27.6% higher than in warm-blooded horses belonging to Lipizzan, Quater and Standardbred breeds (Čebuli-Kaudune ir kt., 2002; Feldman ir kt., 2006; Kästner ir kt., 2008) and range for warm-blooded horses WBC within specified limits (Lacerda, 2006; Feldman et a., 2006; Atinsaat 2008). The WBC values in Žemaitukai stallions are similar to WBC concentrations in Kathiawari horses (Gupta et al., 2002), and in mares they are similar to the values in Murgese horses (Rubino et al., 2010).

The mean hemoglobin concentration, MCH and MCHC in Žemaitukai horses are similar to the commonly cited values for warm-blooded horses (Čebuli-Kaudune et al., 2002; Feldman et al., 2006; Lacerda, 2006; Knottenbelt, 2006; Radostits et al., 2010) and reaches the highest of their limit, but MCV values are lower. The means of the MCH and MCHC values were by 3.0–14.5% higher than in Quater (Kästner et al., 2008), Appaloosa (Piccione et al., 2010), Lipizaan (Čebuli-Kaudune et al., 2002) horses. It can be explained by higher RBC and haemoglobin concentrations.

In Žemaitukai youngsters, the mean RBC, WBC, PCV, MCH, and Hb concentrations and MCHC values were by 8.5–12.0% higher than cited in adult warm-blooded horses, but MCV values were by 13.7% lower than in foals (Kästner, 1999; Čebuli-Kaudune et a., 2002; Gul et al., 2007; Altisaat, 2008; Härtlova et al., 2010). Feldman et al., Satue et al. and Čebule-Kaudune considered that RBC, Hb and PCV mean values were significantly higher in stallions than in mares of warm-blooded horses (Čebuli-Kaudune et al., 2002; Feldman et al., 2006; Satue et al., 2012), however our results indicate the contrary. Similarly, the mean WBC values in mares were by 2% lower than in stallions. Some authors submit that this may be due to androgen effect on erythropoiesis (Čebuli-Kaudune et al., 2002; Feldman et al., 2006; Altinsaat, 2008; Satue et al., 2009).

In Žemaitukai foals, the means of RBC, WBC, HGB, PCV and MCHC values were higher than in older horses, as cited for other warm-blooded foals (Feldman et al, 2006).The lower RBC values could have been compensated by means of an increased erythrocyte size, which caused higher MCH values (Satue, 2009). A similar decline in RBC and WBC values with age has also been reported for other warm-blooded horses (Knottenbelt, 2006; Fieldman, 2006).

Altinsaat and Harvey report that the value of Hb in foal decreased rapidly within two weeks following the birth and the Hb at this low level went on until one year old. Our result indicated that the values of Hb in foals were within the limits reported for warm-blooded breeds including Arabian horses (Altinsaat, 2008). It is suggested that lower haemoglobin concentrations in foals may result from the fact that the life span of erythrocytes is shorter in young animals. This was confirmed in this study by lower Hb concentration and PCV in the foal and they were similar in Lipizzan foals (Čebuli-Kaudune et al., 2002) but lower than in Thoroughbred foals (Feldman et al., 2006).

According to blood haematology of the present study,

Žemaitukai horses can be classified as warm-blooded horses.

The mean of total protein values in Žemaitukai mares and stallions are located at the upper limit for hot-blooded horses (Lacerda, 2006; Kaneko et al., 2008, Gurgoze et al., 2010) and vary within the range of mean TP values in Criollo, Kathawari, Brazileiro, Standardbreds and other warm-blooded horse breeds (Gupta et al., 2002; Orsini, 2003; Lacerda, 2006; Knottenbelt, 2006; Tateo et al., 2008; Reed et al., 2010). Our result indicated that the values of TP in mares, foals and young stock horses were similar to those in Murgese horses (Rubino et al., 2010).

The means of albumin (Alb) in Žemaitukai mares and stallions were by 14.5–21.0% lower than in hot-blooded horses, and were similar to the commonly cited values for warm-blooded horses (Gupta, 2002; Lacerda, 2006). Roubies et al (2006) showed that serum glucose (Glu) levels were higher in lambs compared with ewes (Roubies et al., 2006). Similarly, some authors reported that Glu levels in calves were higher than the reference range for adult cattle (Egli et al., 1998; Knowles et al., 2000; Mohri et al, 2007b; Zanker et al., 2001). Similarly, Zinkl et al. (1990) reported that Glu levels of older donkeys were significantly lower than those of younger animals (Zinkl et al., 1990). A similar age-dependent change has also been observed in cats (Levy et al., 2006). The results of the present study showed that serum Glu levels in foal and youngsters of Žemaitukai horses were significantly different as compared with its levels in adult mares and stallions. In our study, the serum Glu and urea (Urea) levels in Žematukai mares and stallions were within the reference range for warm-blooded horses (Gupta ir kt., 2002; Knottenbelt, 2006; Reed ir kt., 2010). The serum Glu reference value in Žematukai horses is similar to its levels in Kathiawari (Gupta et al., 2002) horses, and the serum Urea levels vary within thereference levels for adult Thoroughbred, Criollo and Brazilero horses (Lacerda, 2006). The variations in the reports of different researchers are considered that have been arisen from species, geographical and nutritional factors, timing of blood sampling, and the methodology and equipment used by laboratories.

In Žemaitukai stallions and mares, the creatinine (Crea) concentration values range with age; similarly as in some other warm-blooded breeds, the highest Crea values are higher in adult horses than in geriatric horses (Feldman et al., 2006; Gupta et al., 2002; Rubino et al., 2010). In Žemaitukai stallions, Crea values were by 10.6% lower than in mares of all ages. Mohri et al. (2007) reported that the value of Crea in foal are higher than in adult horses, and reache the limit amount at one year of age (Mohri et al. , 2007a, 2007b), but in Žemaitukai horses the situation different. In our study, the Crea values in adult horses were by 2.0–5.8% higher than in foals and geriatric horses; similarly in Kathiawari and Murgese horses (Gupta et al., 2002; Rubino et al., 2010). Feldman et al. (2006) and Levy et al. (2006) also established that the Crea concentration is lower in foals and kitten than in adult horses and cats (Feldman et al., 2006; Levy et al., 2006). This may depend on body composition, nutrient intake, muscle development (Morag, 2002; Kaneko et al., 2008; Gurgoze et al., 2010).

Enzymes are used in equine medicine to assess muscle, liver and heart function (Kaneko et al., 2008). In the present study, there were no significant differences in enzyme values between age groups, except CK-MB levels, but they were between genders. The CK-NAC and CK-MB activity levels in mares were significantly higher than in stallions, but AST was reverse. The CK-MB activity was for the first time evaluated in Žemaitukai horses, what helps to accurately assess the capacity of the heart muscle contractions during endurance exercises. The results of the present study showed that serum CK-MB activity levels in foals were significantly different as compared with its levels in older adult mares and stallions. The CK-MB and CK-NAC activities in foals were lower compared with its level in adult horses.

Throughout the study, the differences in calcium, phosphorus, magnesium, sodium and potassium concentrations in Žematukai horses did not exceed the ones for warm-blooded horses and were similar to the Kathiawary horses (Lacerda, 2006; Gurgoze et al., 2010; Gupta et al., 2002). It was demonstrated that phosphorus concentration decreased significantly in Žemaitukai horses with age. Similar results were also reported by some authors for donkeys and cats (Zinkl et al., 1990; Levy et al., 2006). It suggests that this age-related decrease is probably reflected by decreased bone metabolism as animals grow older. Feldman (2006) has provened that younger animals absorb dietary calcium more efficiently and achieve much higher maximum rates of absorption for both calcium and phosphorus in comparison with older animals (Feldman et al., 2006).

The present study shows that age and sex significantly influence blood haematological and serum biochemical parameters in Žemaitukai horses. The haematological and serum biochemical values determined in the present study serve as reference values for Žemaitukai horses and could be used in diagnosing diseases and determining their prognosis, as well as in preventive programs.

Conclusions

1. It was established that, the RBC, Hb, PCV, TP, Alb, Crea, Ca, P, and AST levels in Žemaitukai mares are significantly higher, and the MCH, MCHC, CK-NAC, CK-MB and Mg levels in Žemaitukai mares are significantly lower than in stallions.

2. It was established that, the mean WBC, RBC, LYM, PLT, P, ALT and ALP values in foals are significantly higher than in adult individuals.

3. In accordance with the blood haematological and serum biochemical data of this study, Žemaitukai horses are classified as warm-blooded horses.

Acknowledgements. The present study was partially supported by LUHS Academy of Science Fund.

References

1. Altinsaat C. The Effects of Age and Gender on Blood Parameters in Arabian Horses. Kafkas Universitesi veteriner fakultesi dergisie. 2008. 14 (2).

P. 173–178.

2. Čebuli-Kaudune N., Božic M., Kosec M. and Cestnik V. The influence of age and gender on haematological parameters in Lipizzan horses. Journal of veterinary medicine. 2002. V. 49. P. 217–221.

3. Egli CP, Blum JW. Clinical, haematological, metabolic and endocrine traits during the first three months of life of suckling Simmentaler calves held in a cow-calf operation. J Vet Med A 1998. 45. P. 99–118.

4. Feldman F. B., Zinkl G. J., Jain N. C. Schalm's veterinary hematology. Blackwell publishing, Ltd/. 2006. 5th ed. P. 1232.

5. Gavazza A., Delgadillo A. J., Gugliucci B., Pasquini A. nnd Lubas G... Haematological Alterations Observed in Equine Routine Complete Blood Counts. A Retrospective Investigation. Comparative Clinical Pathoogy. 2002. 11. P. 131–139.

6. Gupta A. K., Kumar S. and Pal Y. Biochemical, heaematological and thyroid hormone profile in healthy Indian Kathiawari horse. Asian-Australasian journal of animal science. 2002. 15(8). P. 1215–1230.

7. Gul S. T., Ahmad M., Khan A. and Hussain I. Haemato-biochemical observations in apparently healthy equine species. Pakistan veterinary journal. 2007. 27(4). P. 155–158.

8. Gurgoze S. Y. and Icen H. The influence of age on clinical biochemical parameters in pure-bred Arabian mares. Journal of equine veterinary science. 2010. 30(10). P. 569–574.

9. Härtlova H., Řehark D., Sedmikova M., Mendlik J., Kralova J. Effect of extruded fodder on biochemical and haematological parameters of Standardbred horses under training conditions. Turkmen Journal of Veterinary Animal Science. 2010. 34(4). P. 365–372.

10. Kästner S. B. R., Feige K., Weishaupt M.A., Auer J. A. Heart rate and haematological responses of Quarter horses to a reining competition. Journal of equine veterinary science. 1999. 19(2). P. 127–131.

11. Kaneko, J.J., Harvey, J.W., Bruss, M.Clinical Biochemistry of Domestic Animals. 6th Ed. Harcourt Bruce and Co. Asia PTE Ltd., Singapore. 2008. P. 619–680.

12. Knottenbelt D. Vital signs, normal values, Saunders Equine Formulary, W.B. Saunders Co., Philadelphia. 2006. P. 7–22.

13. Knowles TG, Edwards N, Butterworth A, Warriss PD. Changes in the blood biochemical and haematological profile of neonatal calves with age. Veterinary Research 2000. 147. P. 593–598

14. Lacerda L. Hematological and biochemical parameters in three high performance horse breeds from Southern Brazil. Archives of Veterinary Science. 2006. 11(2). P. 40–44.

15. Levy T. K., Crawford P. C. Werner L.L. Effect of age оп reference intervals of serum biochemical values in kittens. Journal of American Veterinary Medicine Association. 2006. 228. P. 1033–1037.

16. Lumsden J., Rowe R., Mullen K. Hematology and biochemistry references value for the light horse. Canadian Journal. 1980. 44. P. 32–42.

17. Macijauskienė G. V. Žemaitukai: history, research, conservation (in Lithuanian). Monograph. Saulės delta. Šiaulai. 2002. P. 123

18. Mohri M., Sardari K. and Faryaneh N. Serum biochemistry of Iranian Turkmen (Akhal-Teke) horses. Comparative Clinical Pathology. 2004. 13. P. 128-131.

19. Mohri M., Allahyari L., Sardari K. Effects of common anticoagulants on Routine plasma biochemistry of horse and comparison with serum. Journal of equine veterinary science. 2007a. 27(7). P. 313–316.

20. Mohri M, Sharifi K, Eidi S. Hematology and serum biochemistry of Holstein dairy calves: age-related changes and comparison with blood composition in adults. Research in veterinary science, 2007b. 83. P. 30–39.

21. Morag G. K. Veterinary laboratory medicine. 2002. P. 368.

22. Orsini J. A. And Divers T.. Manual of equine emergencies: treatment and procedures. 2 ed. 2003. P. 755–766.

23. Piccione G., Casella S., Giannetto C., Messina V., Monteverde V., Caola G., Guttadauro S. Haematological and haematochemical responses to training and competition in Standardbred horses. Comparative Clinical Pathology. 2010. 19(1). P. 95–101.

24. Petruse C.,Falcä C., Kakucs B. Researches regarding the hematological profile of Haflinger and Lipizzan horses. Lucräri stiintifice medicinä veterinarä. 2007. XL. P. 557–560.

25. Pritchard J. C., Burn C. C., Barr A. R. S., Whay H. R. Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. Research in veterinary science. 2009. 87. P. 389–395.

26. Reed S., Bayly W., Sellon D. Equine internal medicine. 2010, Sauners, 3rd.ed. P. 1466.

27. Radostits O., Gay C. C., Hinchcliff K. W., Constable Veterinary medicine. -10th ed. London. 2010. P. 2165.

28. Robinson N. E., Sprayberry K. A. Current therapy in equine medicine, 5th ed.. 2003, Elsevier Sauders. P. 956–977.

29. Roubies N, Panousis N, Fytianou A, Katsoulos D, Giadinis N, Karatzias H. Effects of age and reproductive stage on certain serum biochemical parameters of Chios sheep under Greek rearing conditions. Journal of Veterinary Medicine Association. 2006. 53. P. 277–281.

30. Rubino G., Cito A. M., Lacinio R., Bramante G., Caroli A., Pieragostini E. and Petazzi F. Hematology and some blood chemical parameters as a function of Tick-bone disease (TBD) sing in horses. Journal of equine veterinary science. 2006. 26(10). P. 475–480.

31. Satue K., Blanco O., Munoz A. Age-related differences in the hematological profile of Andalusian brood mares of Carthusian strain. Veterinarni Medicina. 2009. 54. P. 175–182.

32. Satue K., Hernandez A. and Muñoz A. Physiological Factors in the Interpretation of Equine Hematological Profile. From: Hematology - Science and Practice, Dr. Charles Lawrie (Ed.). 2012. P. 573–596.

33. Tateo A., Valle E., Padalino B., Centoducati P., Bergero D. Change in some physiologic variable induced by Italian traditional conditioning in Standardbred yearling. Journal of equine veterinary science. 2008. 28(12). P. 743–750.

34. Zanker IA, Hammon HM, Blum JV. Delayed feeding of first colostrums: are there prolonged effects on haematological, metabolic and endocrine parameters and on growth performance in calves? Journal of Animal Physiology Animal Nutrition. 2001. 85. P. 53–66.

35. Zinkl JG, Mae D, Guzman MP, Farver TB, Humble JA. Reference ranges and the influence of age and sex on hematologic and serum biochemical values in donkeys (Equus asinus). American Journal of Veterinary Research. 1990. 51. P. 408–413.

Received 14 August 2012
Accepted 9 January 2014