VETERINAYRY SCIENCE

Maksymovych I., Slivinska L., Winiarczyk S., Buczek K., Staniec M. Hematological and serum biochemical reference values in healthy working horses Hutsul breed

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Abstract. The purpose of this study was to investigate and establish the differences in blood parameters in horses Hutsul breed in the Transcarpathian region. In accordance with established haematological and serum biochemical values, they can be used to assess the metabolic state of the animal, monitoring treatment of animals.

It was found that the performance of PCV, MCV, MCH, MCHC Hutsul breed horses were within physiological fluctuations were similar with the literature data. The highest values were total bilirubin, glucose, AST and potassium.

Investigated hematological and biochemical blood parameters in horses Hutsul breed that will use data from a study of healthy and sick animals.

Keywords: horse Hutsul breed, morphology, biochemistry, blood parameters

Introduction. Hutsul horse – aboriginal rock domesticated horses that spread in the Carpathians and in many Eastern European countries, is one of the world's gene pool. Its breeding in Ukraine is concentrated in the mountainous and foothill areas of Zakarpattia, Lviv, Ivano-Frankivsk, Chernivtsi regions [1]. In Europe Hutsul breed horses bred in Poland, the Czech Republic, Hungary, Slovakia, Austria, Romania, Germany [2].

Horses Hutsul breed formed under the influence of mountain climate, in a depleted feed, as characterized by good health, high labor productivity, efficient use of food, fitness and endurance. In the current population of horses Hutsul breed characterized by typical mountain horses grounds. Unique and biological quality of the horses to ensure their effective use in diverse economy [3]. In particular, Hutsul breed of horses used in breeding, sport, hippotherapy, agrotourism, agricultural work. They are well suited for use in the mountains. For mountain biking trails horses of this breed with packs on their backs weighing 100 pounds or more are up to 100 km per day [4].

Population Hutsul horses evolved over the centuries and came from people in ancient times (beginning of 5- to 9 th century) and so the researchers of this breed differently interpret it and have common views on the origin and formation of its features. The first information about mountain horses were still in the early XVII century. Species bred by crossing local mountain horses Galicia and Bukovina noritskogo type stallions and horses eastern type, including Arabian breed. However, it is believed that Hutsul horses – are the direct descendants of the tarpan, who moved to the Carpathians yore [5].

The main type of Hutsul horses – harness-onerary. Body type its massive, rounded body shape, strong constitution. The trunk is wide, deep, long, medium size head with a wide forehead, straight profile, thick neck is short, low withers long, steep shoulder, short limbs, well put. Hutsul horses of small stature (height at the withers for

horses is 135–145 cm for mares – 132–143 cm). They are hardy, undemanding, are highly resistant to diseases, fertility, longevity. The color usually bay, gray and red, rarely crow, dun, cream-colored with dark shades [6].

Our study aimed to establish reference values for blood parameters Hutsul breed horses and their comparison with accepted values for this species. Horses that do the hard work, especially in mountainous areas, the physiological limits of blood parameters may differ from other horses [7]. Although it is not clear whether these differences result physiological adaptation of animals or the result of work in difficult conditions. Such research will improve the welfare of horses [8].

Hematological and biochemical blood parameters in horses used for early diagnosis of non-contagious, infectious and parasitic diseases. They are also used to monitor disease, recovery and treatment of animals. This is important in postoperative patients. Such studies are used to assess the metabolic state of the animal in sport horses for planning specific physical activities, as well as with the scientific purpose [9].

Hematological and biochemical parameters of blood horses of various breeds and productivity trends are well known and are described by many scientists and can be covered in various articles and books [10, 11]. Research concerning the Hutsul breed horses limited to specific publications and describe only some blood parameters [12–15].

The aim of this study was research the haematological and biochemical blood parameters in horses Hutsul breed that will allow to use the data in the study as clinically healthy and sick animals.

Materials and methods. In total, 24 clinically healthy horses Hutsul breed aged 3 to 7 years from two farms in the Transcarpathian region were used in this research.

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 24 animals in October 2014, and they were handled with care to minimize stress-induced effects. To reduce circadian variations, all samples were collected before the morning feeding. The diet included horse hay, oats, bran, and did not receive supplements and minerals. The water was used ad libitum.

Blood samples were taken from the jugular vein using a 16-gauge needle into the vacuum blood tubes, 10 ml (Vacutest, Italy). Tubes of etilendiaminotetraacetic acid (EDTA) were used to study the blood haematology and the tubes without EDTA were used for blood biochemical examination. The haematological and biochemical studies of the horses Hutsul breed blood were carried out at the Laboratory Department of Internal Diseases and Clinical Diagnostics of the Lviv National University of Veterinary Medicine and Biotechnologies.

The blood haematology was analysed using an automated hematology analyzer Mythic 18 (Orphee S.A., Switzerland) by using the PZ Cormay S.A. (Poland) reagents. The red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), differential leukocyte count, 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 fractionated blood separated serum was evaluated. Concentrations of serum total protein (TP), albumin (Alb), total bilirubin (TBIL), glucose (Glu), urea (Urea), creatinine (Crea), calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and the gamaglutam-

iltransferase (GGT) activities were measured using an automated blood chemistry analyzer BS-120 (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., P.R. China) by using the PZ Cormay S.A. (Poland) reagents.

The results of hematological and biochemical studies compared the results established for horses [16, 17]. All data were analyzed using Microsoft Excel (2010) software.

Results and discussion. When interpreting diagnostic blood parameters in horses is necessary to consider the breed, age, sex, physical activity, as some physiological limits blood may differ [18, 19].

The mean values of blood haematological and biochemical parameters in healthy working horses Hutsul breed are shown in tables 1–2.

Number of red blood cells healthy animals fairly constant, so setting change their number has diagnostic value. However, keep in mind that their number can vary depending on time of day study, age, sex, performance, exercise animals. Analysis of RBC and HGB in sport horses used to establish the degree of fitness and ability of exercise training. Exercise horses requires an increase in blood oxygenation, and specific response to this stress is to increase the number of red blood cells and hemoglobin [18]. The average values of RBC and HGB horses Hutsul breed (Table 1) were within the physiological oscillations identified for this species [3, 16, 17].

In a number of diseases in animals can be modified erythrocyte hemoglobin saturation that is adjusted with the analysis of red blood indices. Indicators PCV, MCV, MCH, MCHC used for differential diagnosis of anemia in animals [20]. Horses Hutsul breed PCV is within physiological fluctuations, but it was the lower limit of abstract rules [16, 17]. The mean MCV, MCH, MCHC horses were similar with the literature data.

Table 1. Mean values with standard deviation (M±m) of blood morphological parameters for healthy working horses Hutsul breed

Parameter	Blood morphological parameters for horses Hutsul breed		Reference indices from the literature	
	M±m	lim	Winnicka A., 2008 [16]	Влізло В.В., 2014 [17]
RBC, $10^{12}/l$	7,5±0,12	6,7–7,9	5,5-10,0	6,0–9,0
HGB, g/l	116,6±2,38	102,0–126,0	80,0-180,0	90,0-140,0
PCV, %	31,1±0,63	27,0–34,3	24,0-52,0	35,0-45,0
MCV, fl	41,6±0,60	37,0–44,4	35,0-58,0	37,0-58,0
MCH, pg	15,6±0,21	14,1–16,7	10,0-20,0	16,0-20,0
MCHC, g/dl	37,5±0,12	36,9–38,2	31,0-37,0	31,0-36,0
WBC, $10^9/1$	8,5±0,50	5,1–13,1	5,5–12,0	6,0-11,0
LYM, $10^9/1$	4,4±0,36	2,1–7,1	1,5–7,7*	_
$MON, 10^9/l$	0,20±0,023	0,10-0,30	0-1,5*	_
GRA, 10 ⁹ /l	4,4±0,21	2,9–5,6	2,3-9,5*	_
PLT, 10 ⁹ /l	106,0±8,27	62,0–151,0	150,0-400,0	200,0-600,0

^{* -} Andriichuk A., 2012

White blood cells in the body do primarily protective. Depending on the type they are involved in phagocytosis, making interferon, lysozyme, histamine and other biologically active substances. Lymphocytes play a major role in specific defense reactions – the formation of cellular and humoral immunity. The average values of WBC horses Hutsul breed physiologically experienced considerable fluctuations. The increase in the number of leukocytes was saline, which was due to exercise. In the analysis of leukocyte subpopulations stipulated difference compared with prescribed standards for horses [16–18].

The main role of platelets in the body - participated in the initial homeostasis. Mean blood PLT Hutsul breed horses was lower than that given in the literature data [16, 17]. However, in the literature, we found a message show limits fluctuations platelets in sport horses, which is consistent with the results of our research [18].

To assess the status of the organism, the establishment of functional and morphological state of individual organs or systems, as well as identifying pathological condition, it is recommended to perform basic laboratory tests. In their activity is common veterinary specialist with situations where deviations in laboratory tests are fundamental in diagnosis [17, 20].

Reduction of total protein in serum develops when the body is insufficient protein, liver and kidneys. Less common increase in its concentration [21]. The mean of total protein and albumin in horses hutsul breed is within the limits set for this type of animal (Table 2).

The concentration of total bilirubin though not extend beyond physiological fluctuations, but was close to the maximum value [16]. 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 [22].

In the study of glucose found that the level of Hutsul breed horses also had higher mean values for this species, which we believe was due to stress during the selection of animal blood [17].

In modern laboratory diagnostics monitoring urea and creatinine are used to diagnose kidney disease. In our study, the serum urea levels in horses Hutsul breed were within the reference range for the species of animal [16, 17]. Contents creatinine may depend on body composition, nutrient intake, muscle development [22]. In the studied animals is at the lower limit of the physiological limits.

Enzymes are used in equine medicine to assess muscle, liver and heart function [17, 22]. As a result of studies found that serum AST activity Hutsul horse breed was slightly higher compared with the average animal of this species [3, 22]. However, there are reports that these values may be acceptable. ALT and GGT performance horses Hutsul breed did not differ from those established by other authors [16, 17].

Table 2. Mean values with standard deviation (M±m) of blood biochemical parameters for healthy working horses Hutsul breed

Parameter	Blood biochemical parameters for horses Hutsul breed		Reference indices from the literature	
	M±m	lim	Winnicka A., 2008 [16]	Влізло В.В., 2014 [17]
TP, g/l	67,2±0,75	62,5–72,5	60,0-78,0	65,0-80,0
Alb, g/l	36,5±0,32	34,8–38,2	29,0-59,0	_
TBIL, μmol/l	17,8±0,75	12,1-23,1	13,7–25,6	7,0–17,0
Glu, mmol/l	4,9±0,10	4,3–5,3	3,1-6,2	3,0-5,0
Urea, mmol/l	5,2±0,15	4,3-6,0	4,1-7,4	3,5-6,0
Crea, µmol/l	101,0±1,74	90,3-108,8	106,1–167,9	100,0–160,0
AST, U/l	451,2±14,04	358,0–574,0	205,0-555,0	50,0-200,0
ALT, U/l	8,5±0,73	6,0-13,0	3,0-25,0	5,0-15,0
ALP, U/l	176,5±12,58	44,0-304,0	109,0-315,0	100,0-250,0
GGT, U/l	13,7±0,70	10,0-17,0	12,0-45,0	20,0-40,0
Ca, mmol/l	2,90±0,024	2,74-2,98	2,25-3,12	2,5–3,5
P, mmol/l	1,36±0,047	1,16-1,61	1,13-1,90	1,2-1,8
Mg, mmol/l	0,80±0,015	0,70-0,86	0,70-1,15	0,7-1,0
Na, mmol/l	144,2±1,76	134,0-150,8	139,1–156,5	135,0-145,0
K, mmol/l	5,2±0,17	4,5–5,9	3,5–4,7	2,8–4,8

ALP activity depends on age and physiological state of the organism. Research ALP used primarily to diagnose diseases of the skeletal system [17]. In our study, ALP varies widely, but did not exceed specified limits [16, 22].

Throughout the study, the differences in calcium, phosphorus, magnesium and sodium concentrations in horses Hutsul breed did not exceed the ones for horses and were similar to the literature searches. The difference found in the contents of potassium, since the figure was 23 % higher than to the commonly cited values for horses [16, 17]. This increase may be due to both climatic factors feeding conditions and breed features [17, 22].

The haematological and serum biochemical values determined in the present study serve as reference values

for horses Hutsul breed and could be used in diagnosing diseases and determining their prognosis, as well as in preventive measures.

Conclusions

- 1. It was found that the performance of PCV, MCV, MCH, MCHC Hutsul breed horses were within physiological fluctuations were similar with the literature data. The highest values were total bilirubin, glucose, AST, and potassium.
- 2. Investigated hematological and biochemical blood parameters in horses Hutsul breed that will use data from a study of healthy and sick animals.

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Максимович И., Сливинская Л., Вынярчик С., Бучек К., Станец М. Гематологические и биохимические показатели крови здоровых рабочих лошадей гуцульской породы

Аннотация. Целью данного исследования было провести исследования, анализ и установить референтные значения крови лошадей гуцульской породы. Установленные гематологические и биохимические показатели крови могут быть использованы для оценки метаболического состояния лошадей, диагностики заболеваний, мониторинга эффективности лечения больного животного.

Было установлено, что показатели гематокрита, MCV, MCH, MCHC в лошадей гуцульской породы находились в пределах физиологических колебаний и соглашались с литературными данными. Самые высокие значения были установлены по общему билирубину, глюкозе, AcAT и калии.

Исследование гематологических и биохимических показателей крови у лошадей гуцульской породы будут использоваться при исследования здоровых и больных животных.

Ключевые слова: лошади, гуцульская порода, морфология, биохимия, нормы