ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXVIII(1) SECTIO E 2013

Laboratory of Reproductive Biology, Department of Animal and Environmental Hygiene,
University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin,
e-mail: agnieszka.rowicka@up.lublin.pl

AGNIESZKA ROWICKA

Exercise-induced physiological response to effort and its effect on the welfare of sport horses

Indukowana treningiem odpowiedź fizjologiczna na wysiłek i jego wpływ na dobrostan koni sportowych

Summary. The study was conducted on 18 half-blood, clinically healthy horses. Blood for tests was collected twice: at the beginning of March, when the indoor season was approaching an end (smaller number of participations), and at the end of May, when an effort was particularly intensified due to the continuous training and the beginning of the open season (more starts). In the whole blood, the following determinants of well-being were determined: the total number of erythrocytes (RBC), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), the total number of leukocytes (WBC), lymphocytes, monocytes, and granulocytes. The study was performed using a hematology analyzer MS9. Mean values were compared between the collections (stallions and mares), and in I and II collections the animals of both sexes were compared among each other. The study showed that the growing physical effort of sport horses caused an increase in parameters such as: RBC, Ht, MCV, WBC and in the number of granulocytes. The decrease was noted in the Hb, the number of lymphocytes and MCHC. The results showed that the welfare of horses was maintained.

Key words: horses, welfare, hematological indices

INTRODUCTION

Animal welfare is the term concerning the physical and psychological well-being of animals and is described as the quality of life as perceived by the animal itself [Ohl and van der Staay 2012]. It includes five freedoms [FAWC 1979], (1) freedom from thirst, hunger or malnutrition, (2) freedom from lack of appropriate comfort and shelter (freedom from thermal and physical discomfort), (3) freedom from rapid diagnosis and treatment of pain, injury, parasites (freedom from injury and disease), (4) freedom to display most normal patterns of behaviour and (5) freedom from fear and stress

[Bartussek 1999]. Equine welfare is an increasingly relevant concern for horse industry, participants and horse owners in recent years, in terms of developing an appreciation of horse well-being and proper horsemanship [Ohl and van der Staay 2012, Visser and van Wijk-Jansen 2012]. Horses as unique athletes, are designed to high level physical exercise and performance. Unfortunately, even in horses strenuous exercise and the chronic realization of exhausting effort, may induce stress that may have deleterious effects [Art and Lekeux 2005, Bis-Wencel 2011]. Exercise, recognized as a stressful factor that can alter the horse's immunity response and increase its susceptibility to diseases, can influence the systemic immunity during strenuous effort, or be the cause of overtraining. On the other hand, moderate training can have beneficial effect on defense mechanisms [Hines *et al.* 1996, Raidal *et al.* 2000, Art and Lekeux 2005]. The image of those metabolic and immune changes is the level of hematological indices of blood. Many studies show that different processes in the organism are manifested by changes in the values of the clinical and hematological indices, and can be triggered by exercise. Different intensity, kind and type of training to which horses are subjected affect specific hematological parameters [Bis-Wencel *et al.* 2011]. It should not be forgotten, that the social environmental conditions to which a horse is exposed may affect its ability to respond to challenging situations, altering its behavior, physiology and compromising its welfare [Rivera *et al.* 2002]. The aim of the study was to assess the influence of exercise on the well-being of horses.

MATERIAL AND METHODS

The study was conducted on 18 half-blood horses, in the spring-summer season. The horses were divided into two groups, depending on the gender (8 stallions and 10 mares). All horses were kept in individual stalls in the stables with constant access to running water, and were competing in show jumping discipline. The animals were subjected in a daily training program, which included 1 hour of carousel, 10 min. walking, 10 min. of relaxed trot, 5 min. of working trot and 5 to7 min. of gallop. During the competitions, horses participated in an additional 1h lunging and 1h of riding under saddle. Animals were under constant veterinary supervision during the experiment. Blood samples were collected from the external jugular vein, into dry tubes in early March (a smaller number of starts) and in late May (intensified effort due to the continuous training and more competitions). All collections were performed in the morning, before feeding and watering the horses. The determined parameters were: the total number of erythrocytes (RBC); hematocrit (Ht); hemoglobin (Hb); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); the total number of leukocytes (WBC), lymphocytes, monocytes and granulocytes. Tests were performed on the MS9 analyzer and the results are presented as arithmetic means ($\bar{x}$) and standard deviations (SD), and subjected to statistical mathematical analysis. The significance of differences between mean values were calculated using Student's t test for dependent variables, Microsoft Office Excel 2007, taking the statistical significance limit at $p \leq 0.05$ and $p \leq 0.01$.

RESULTS AND DISCUSSION

The reduced level of welfare in horses is related to management factors such as low forage feeding or short feeding time, social isolation and lack of planned and unrestrained exercise. It is obvious that welfare problems can be reduced or partly prevented by improving the knowledge of horse enthusiasts. It is known that exercise-induced stress may have a dual effect on the immune system according to the intensity and chronicity of the effort [Hines *et al.* 1996, Raidal *et al.* 2000]. Because of relative exercise and training-induced immunosuppression, bacterial and viral agents may find a favorable growth habitat in young sports horses [Art and Lekeux 2005].

Mean values and standard deviations of determined blood indicators are shown in Table 1 and tendencies are shown in Figures 1–7. Mean values were compared between the collections, and the both sexes were compared among themselves.

The red blood cells (RBC) values in stallions stayed at the same level in I and II collection (8.78 T/l). In mares the values varied from 8.69 T/l in I collection to 8.98 T/l in II collection, and a light increase between the collections was noted 8.73 T/l ±9.88 T/l (Fig. 1). In terms of hemoglobin (Hb) the decrease was noted in stallions (8.37mmol/l ±8.20 mmol/l) and mares (8.60 mmol/l ±8.59 mmol/l), as well as between the collections (8.50 mmol/l ±8.42 mmol/l). Those results are probably the consequence of the effort and intensive exercise, as it is said by many researches [Krumrych 2007, Muñoz *et al.* 2008,] and was confirmed in present study (Fig. 2).

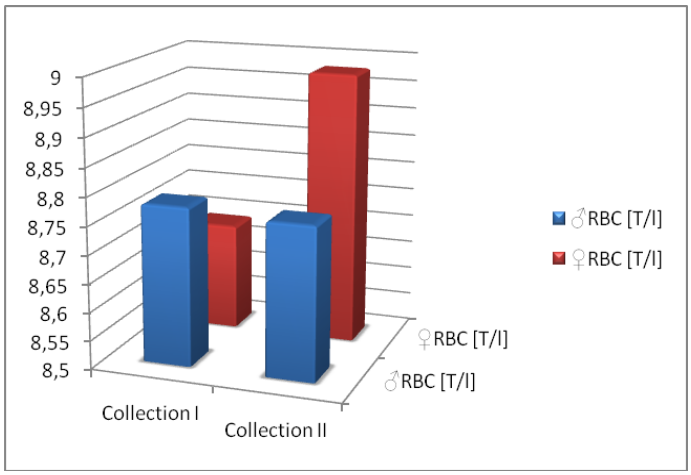

Fig. 1. Mean RBC values
Rys. 1. Średnie wartości liczby erytrocytów

Table 1. Mean hematological indices of sport horses in first and second collection ($\bar{x}$ ±SD)
Tabela 1. Średnie wartości parametrów hematologicznych krwi koni sportowych w I i II pobraniu ($\bar{x}$ ±SD)

Parameters Wskaźniki	Collection I – Próba I				Collection II – Próba II			
	♂		♀		♂		♀	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
RBC (T/l)	8.78	1.27	8.69	0.98	8.78	1.65	8.98	1.62
Total – Razem	$\bar{x}$: 8.73; SD : 1.08				$\bar{x}$: 9.88; SD : 1.59			
Hb (mmol/l)	8.37	0.97	8.60	0.86	8.20	0.98	8.59	1.07
Total – Razem	$\bar{x}$: 8.5; SD : 0.89				$\bar{x}$: 8.42; SD : 1.02			
Ht (l/l)	0.42	0.06	0.43	0.05	0.45	0.05	0.48	0.06
Total – Razem	$\bar{x}$: 0.43^{a}; SD : 0.05				$\bar{x}$: 0.47^{b}; SD : 0.06			
MCH (fmol)	0.96	0.05	0.99	0.04	0.96	0.23	0.99	0.15
Total – Razem	$\bar{x}$: 0.97; SD : 0.04				$\bar{x}$: 0.97; SD : 0.18			
MCHC (mmol/l)	19.6^{A,a}	0.8	19.64^{A,a}	0.38	17.97^{B,b}	1.01	18.02^{B,b}	0.76
Total – Razem	$\bar{x}$: 19.62^{A,a}; SD : 0.58				$\bar{x}$: 18.00^{B,b}; SD : 0.85			
MCV (fl)	48.82	2.43	50.51	1.91	52.7	9.41	54.17	7.73
Total – Razem	$\bar{x}$: 49.76^{a}; SD : 2.25				$\bar{x}$: 53.51^{b}; SD : 8.28			
WBC (G/l)	5.71^{a}	0.90	6.04	0.87	6.30^{b}	1.15	6.47	1.44
Total – Razem	$\bar{x}$: 5.89; SD : 0.88				$\bar{x}$: 6.39; SD : 1.28			
Lim (%)	30.24	3.49	31.18^{a}	4.68	28.52	4.60	28.20^{b}	7.14
Total – Razem	$\bar{x}$: 30.76^{a}; SD : 4.1				$\bar{x}$: 28.34^{b}; SD : 5.97			
Mon (%)	4.41	2.11	3.65	1.48	4.04	1.51	3.77	1.45
Total – Razem	$\bar{x}$: 3.98; SD : 1.77				$\bar{x}$: 3.88; SD : 1.44			
Gra (%)	65.35	3.86	65.14	5.63	67.35	5.04	67.4	7.68
Total – Razem	$\bar{x}$:65.23^{a}; SD : 4.78				$\bar{x}$: 67.37^{b}; SD : 6.46			

A, B, C – mean values marked with big letters are significantly different at $p \leq 0,01$; a, b, c – mean values marked with small letters are significantly different at $p \leq 0,05$; RBC – red blood cell; Hb – hemoglobin; Ht – hematocrit; MCH – mean cell hemoglobin; MCHC – mean cell hemoglobin concentration; MCV – mean cell volume; WBC – number of leukocytes; Lim – number of lymphocytes, Mon – number of monocytes; Gran – number of granulocytes

A, B, C – średnie oznaczone dużymi literami różnią się istotnie przy $p \leq 0,01$; a, b, c – średnie oznaczone małymi literami różnią się istotnie przy $p \leq 0,05$; RBC – liczba erytrocytów; Hb – hemoglobina; Ht - hematokryt; MCH – średnia masa hemoglobiny w krwince; MCHC – średnie stężenie hemoglobiny w krwince; MCV – średnia objętość krwinek czerwonych; WBC – liczba leukocytów; Lim – liczba limfocytów; Mon – liczba monocytów; Gran – liczba granulocytów

One of the consequences of intensive effort is also the decrease in floating blood amount, because of displacement of water from plasma beyond the vascular system (to muscles), what causes the increase of Ht level [Muñoz *et al.* 2008, Krumrych 2009]. Hematocrit values (Ht) in present study increased in stallions (0.42 l/l ±0.45 l/l), mares (I collection 0.43 l/l ±0.48 l/l). The increase between the collections (0.43 l/l ±0.47 l/l) was statistically significant at $p \leq 0.05$ (Fig. 3). Those

results confirm the spleen mobilization and the improvement of the oxygen transport in blood. The values mentioned above are the direct reflection of homeostasis in the organism. They show that the effort causes fluctuations of the physiological indices, but those fluctuations do not bring any harm for the horse's body.

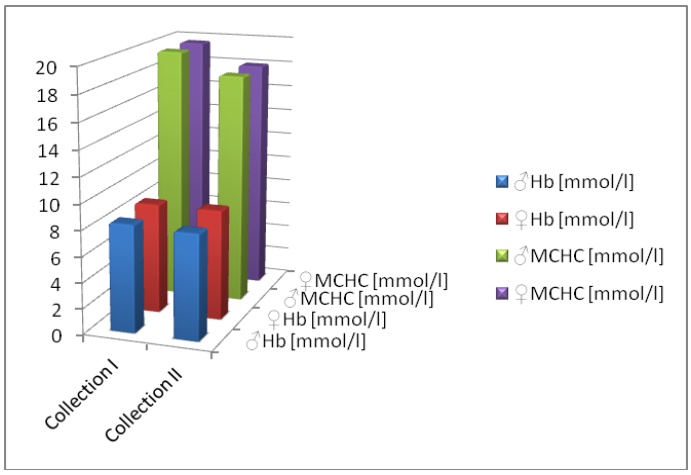

Fig. 2. Mean Hb and MCHC values
Rys. 2. Średnie wartości hemoglobiny i MCHC

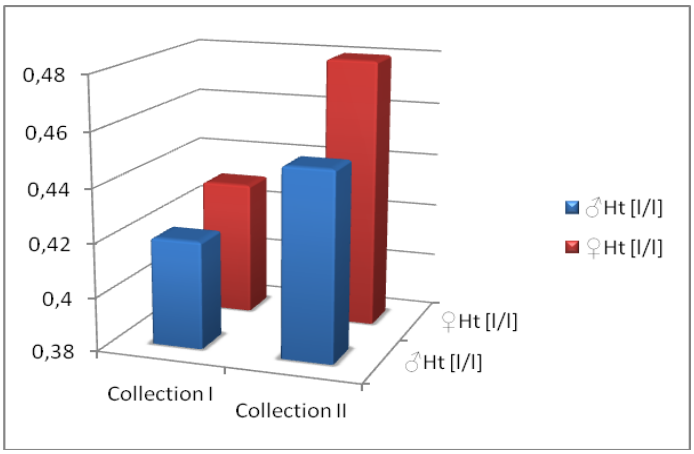

Fig. 3. Mean hematocrit values
Rys. 3. Średnie wartości hematokrytu

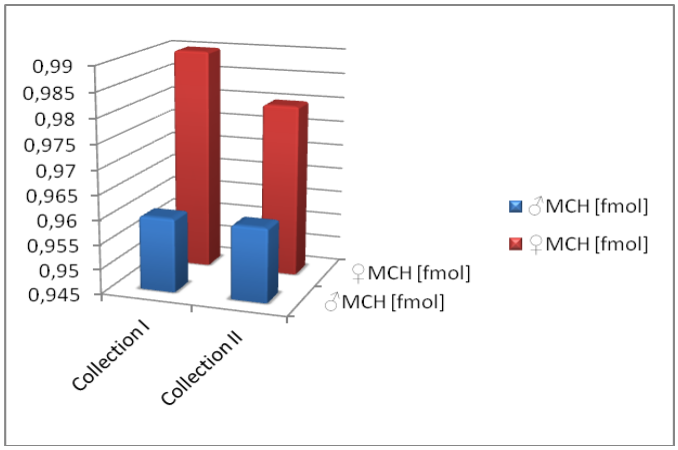

Fig. 4. Mean MCH values
Rys. 4. Średnie wartości MCH

The values of MCH were in range of the reference values [Krumrych 2007] and did not change (Fig. 4) during the experiment (stallions 0.96 fmol, mares 0.99 fmol and 0.97 fmol between collections). As for the MCHC, this parameter placed oneself below the reference values [Krumrych 2007] and decreased between the collections. In stallions from 19.60 to 17.97 mmol/l, in mares from 19.64 to 18.02 mmol/l, and between collections from 19.62 to 18.00 mmol/l (Fig. 2). The differences in values obtained from stallion and mares were statistically significant at the significance level $p \leq 0.05$ and $p \leq 0.01$, as well as the differences between the collections. The above mentioned situation may be due to anemia, caused by lack of iron as a result of intense exercise and effort below the horses efficiency.

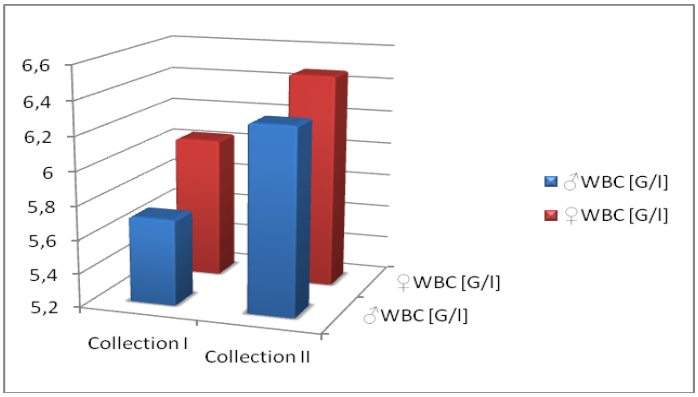

Fig. 5. Mean MCV values
Rys. 5. Średnie wartości MCV

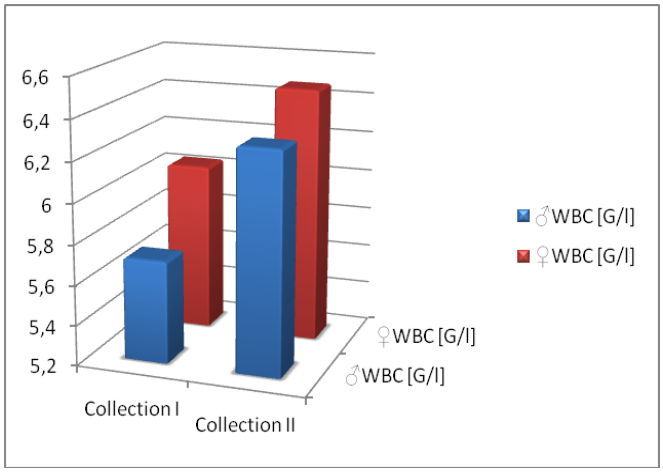

Fig. 6. Mean WBC values
Rys. 6. Średnie wartości liczby białych krwinek

The increase was reported regarding the mean corpuscular volume (MCV), what was confirmed by other authors [Muñoz *et al.* 2008]. In stallions (48.82 fl ±52.70 fl), mares (50.51 fl ±54.17 fl) and also between collections (49.76 fl ±53.51 fl), while the differences between the collections were statistically significant at $p \leq 0.05$ (Fig. 5). The obtained results may be due to anemia.

The change of white blood cells count is one of the body's reactions on the physical effort. Hard and violent efforts cause the increase of the WBC, while after effort the values decrease. This leucocystosis is not connected with the production of new cells, but with the release of current amount of cells to floating blood from the spleen [Krumrych 2007]. The increase of white blood cells count was also noted in the study concerning cross country events [Muñoz *et al.* 2008]. The WBC values increased from 7.44 G/l to 8.56 G/l. In the present study, mean white blood cell values in both collections ranged from 5.89 ±6.39 G/L. There was an increase in white blood cells (Fig. 6) in stallions and mares in collection II compared to collection I (stallions: 5.71 ±6.30 G/L, mares: 6.04 ±6.47 G/l). The differences in those values for stallions were statistically significant at the significance level $p \leq 0.05$. Lymphocytes constitute the 30% of the whole WBC count. Muñoz [Muñoz *et al.* 2008] observed the increase of Lim during the effort (Fig. 7). That increase was reflected by the release of huge lymphocytes amount to blood, due to the activation of erythrocyte reservoir in the spleen [Bis-Wencel 2011]. The average level of lymphocytes in the study was 30.76% in the first and 28.34% in the second collection. The slight decrease in the level of lymphocytes included both stallions 30.24 ±28.52% and mares 31.18 ±28.20%, although those differences were statistically significant only in mares and between the collections (at $p \leq 0.05$).

Monocytes are connected with phagocytosis and are able to dismiss the substances that regulate the inflammatory processes. They resident one or two days in blood and then move to tissues, where they differentiate to macrophages [Krumrych 2007]. The

average number of monocytes in horses was 3.98% in the I and 3.88% in the II collection (stallions 4.41 ±4.04, mares 3.65 ±3.77). The differences were not statistically significant (Fig. 7).The increase in the number of granulocytes was noted both between the collections, as well as among stallions and mares in the second collection in comparison to first. The increase was respectively 65.23 ±67.37% between the collections. The same tendency was characteristic for stallions respectively 65.35 ±67.35%, and mares 65.14 ±67.40% (Fig. 7). The differences were statistically significant only between the collections ($p \leq 0.05$), and the increase in the number of granulocytes was probably correlated with leucocytosis caused by effort.

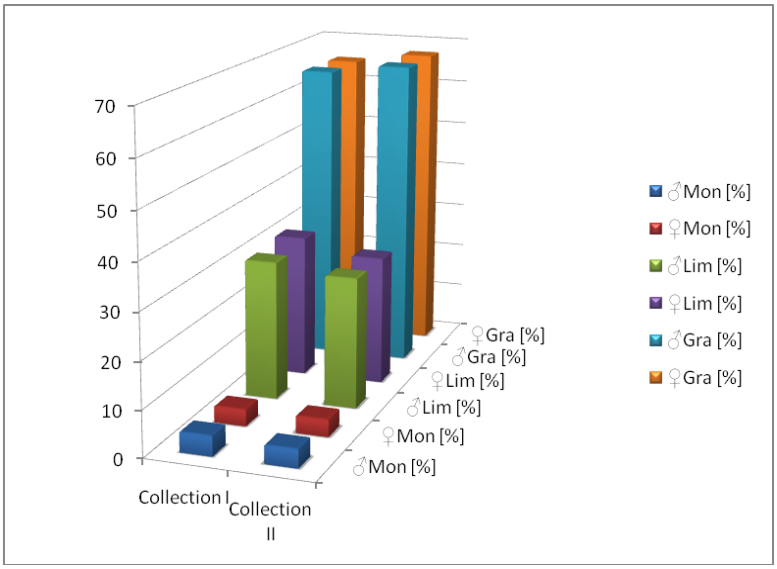

Fig. 7. Mean leucogram values
Rys. 7. Średnie wartości parametrów białokrwinkowych

Large numbers of horses worldwide are used in sport, but the impact of riding itself on the horses' welfare has received comparatively little attention. Given the high potential for welfare implications due to different riding techniques (i.e. the use of force, as opposed to gradual training, to achieve certain postures and movements in the horse), devices commonly used during riding and loads of effort [von Borstel *et al.* 2009]. The psychological stress measure is highly confounded with physical exertion [Perna *et al.* 1997], that is why it is so difficult to control, even during the investigations involving riding, and can give rise to serious welfare problems [von Borstel *et al.* 2009]. The conducted experiment confirmed that the long-term exercise has a very strong effect on the horses' well-being. However, to assess the whole spectrum further tests must be performed.

CONCLUSION

In practice, interpretation of welfare status and its translation into the active management of perceived welfare issues are strongly needed, because horse training is to a large extent based on tradition [van Weeren 2008]. In contrast to the training of other animals, the application of modern learning theory in equitation is relatively new [McGreevy and McLean 2007] and influenced by context, and especially by cultural and social values. In assessing whether or not a given welfare status is morally acceptable, animal welfare scientists must be aware that scientifically based, operational definitions of animal welfare will necessarily be influenced strongly by a given society's moral comprehension [Jones and McGreevy 2010, Ohl and van der Staay 2012,]. There is an increasing understanding that training should be based on reinforcement of correct behavioral responses, but the knowledge about the kind of reinforcement that works best is low [Ladewig 2007]. Unfortunately, most trainers want a short learning period to obtain rapid results, despite the implications this might have for the horse. That is why recent research proved to be useful and is mandatory for obtaining information necessary for the best animal welfare possible [Hendriksen *et al.* 2011]. A thorough knowledge of the stresses, the origins and mechanisms, will help the owners and trainers to minimize their occurrence by appropriate training and competition programs, to sustain the animal welfare protection [Art and Lekeux 2005].

REFERENCES

Art T., Lekeux P., 2005. Exercise – induced physiological adjustments to stressful conditions in sports horses. Livest. Prod. Sci. 92, 101–111.

Bartussek H., 1999. A revive of the animal needs index (ANI) for the assessment of animals' well-being in the housing systems for Autrian proprietary products an legislation. Livest. Prod. Sci. 61, 179–192.

Bis-Wencel H., 2011. Long–term exercise and its effect on selected haematological indices of blood in jumping horses. Zesz. Nauk. UP we Wrocławiu 583, 79–87.

Bis-Wencel H., Lutnicki K., Rowicka A. Z., Bryl M. 2011. Long-term exercise and its effect on selected hematological parameters of blood in horses. Medycyna Wet. 67 (6), 418–421.

von Borstel U.U., Duncan I.J.H., Shoveller A.K., Merkies K., Keeling L.J., Millman S.T., 2009. Impact of riding in a coercively obtained Rollkur posture on welfare and fear of performance horses. App. Anim. Behav. Sci. 116, 228–236.

FAWC, Farm Animal Welfare Council, 1979. First Press Notice, 5(12), MAFF, London.

Hendriksen P., Elmgreen K., Ladewig J., 2011. Trailer-loading of horses: Is there a difference between positive and negative reinforcement concering effectiveness and stress-related signs? J. Vet. Behav. 6, 261–266.

Hines M.T., Schott II H.C., Bayly W.M., Leroux A.J., 1996. Exercise and immunity: a revive with emphasis on the horse. J. Vet. Inter. Med. 10, 280–289.

Jones B., McGreevy P.D., 2010. Ethical equitation: Applying a cost-benefit approach. J. Vet. Behav. 5, 196–202.

Krumrych W., 2007. Wskaźniki laboratoryjne krwi koni – wartości referencyjne i interpretacja. PIWet. Puławy.

Krumrych W., 2009. Wpływ standardowego wysiłku fizycznego koni na wartości wybranych wkaźników klinicznych i hematologicznych. Medycyna Wet. 65(6), 399–403.

Ladewig J., 2007. Clever Hans till whinnying with us. Behav. Proc. 76, 20–21.

McGreevy P., McLean A., 2007. Roles of learning in equitation. J. Vet. Behav. 2, 109–118.
Muñoz A., Riber C., Trigo P., 2008. Erythrocyte indices in relation to hydration and electrolytes in horses performing exercises of different intensity. Comp. Clin. Pathol. 17, 213–220.
Ohl F., van der Staay F.J., 2012. Animal welfare: At the interface between science and society. Vet. J. 192, 13–19.
Perna F.M., Schneiderman N., LaParierre A., 1997. Psychological stress, exercise and immunity. Int. J. Sports Med. Suppl. 18, 78–83.
Rivera E., Benjamin S., Nielsen B., Shelle J., Zanella A.J., 2002. Behavioral and physiological responses of horses to initial training: the comparison between pastured versus stalled horses. App. Anim. Behav. Sci. 78, 235–252.
Raidal S.L, Love D.N., Bailey G.D., Rose R.J., 2000. Effort of single bouts of moderate and high intensity exercise and training on equine peripheral blood neutrophil function. Res. Vet. Sci. 68, 141–146.
Visser E.K., van Wijk-Jansen E.E.C., 2012. Diversity in horse enthusiast with respect to horse welfare An explorative study. J. Vet. Behav. 7, 295–304.
van Weeren P.R., 2008. How long will equestrian traditionalism resist science? Vet. J. 175, 289–290.

Streszczenie. Badania przeprowadzono na grupie 18 klinicznie zdrowych koni szlachetnej półkrwi. Krew pobrano dwa razy, na początku marca (mniejszy wysiłek) i pod koniec maja (zintensyfikowany wysiłek ze względu na ciągły trening i uczestnictwo w zawodach). We krwi pełnej oznaczono parametry będące wyznacznikami dobrostanu: ogólną liczbę erytrocytów (RBC), hematokryt (Ht), stężenie hemoglobiny (Hb), średnią objętość krwinek czerwonych (MCV), średnią zawartość hemoglobiny (MCH), średnie stężenie hemoglobiny w krwince (MCHC), ogólną liczbę leukocytów (WBC), limfocytów, monocytów i granulocytów. Badania przeprowadzono przy użyciu analizatora hematologicznego MS9. Średnie wartości zostały porównane pomiędzy pobraniami oraz pomiędzy płciami w obrębie pobrań. Badania wykazały, że zwiększający się wysiłek koni sportowych spowodował wzrost parametrów, takich jak RBC, Ht, MCV, WBC oraz liczba granulocytów. Spadek zanotowano w przypadku hemoglobiny, liczby limfocytów i MCHC. Powyższe wyniki wykazały, że dobrostan koni został zachowany.

Słowa kluczowe: konie, dobrostan, parametry hematologiczne