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VARIABILITY OF SELECTED CLINICAL AND HAEMATOLOGICAL INDICES IN YOUNG STALLIONS DURING 100-DAY PERFORMANCE TEST

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Abstract

The study was conducted on 20 clinically healthy stallions, aged 3-4 years. The evaluation of the degree of adaptation of stallions to exercise loads in the course of a 100-d training programme was conducted three times: (I) - on the 1st d of training, (II) - on the 46th d of training, and (III) - on the 97th d of training. Rectal temperature (T) and number of heart and respiratory rates were measured. Blood was sampled before exercise (1), after exercise (2), and 30 min after rest (3). The following indices were determined in the blood: concentration of haemoglobin, haematocrit level, number of red blood cells, white blood cells, lymphocytes, neutrophils, eosinophils, basophils, monocytes, and platelets. Post-exertion changes in the evaluated indicators showed moderate intensity to the applied loads with respect to functional potential of tested horses. The effect of the standard 100-d training was to stimulate the body's adaptation to functional tasks, which was evidenced, among others, by lower variability of post-exertion T and the number of red blood cells. The highest T increase was found at the beginning of the programme (about 2.1%), whereas during the subsequent studies (II and III) it was significantly ($P<0.05$) lower (0.98% and 0.84%, respectively). The variability in red blood cell count in the course of training showed a downward trend (16.4%, 13.7%, and 11.0%, respectively). The results confirm the relatively low exercise loads and good health of young stallions during the 100-d performance test.

Key words: horse, physical effort, clinical indices, haematology.

The research conducted in the recent years on the physiology of horses' physical effort has enabled to demonstrate a correlation between clinical and haematological parameters, which can be used in the evaluation of physiological functions of horses and their adaptability to exercise loads (13, 15, 30). A proper preparation of horses for implementation, often involving an exhausting exercise, is very important, because in many cases (especially in the final stages of training and during competition), the level of such an exercise reaches the maximum physiological capacity of these animals. Post-exertion changes of many indicators of blood are generally very clear, but usually disappear quickly. Too intense exercise results in long maintenance of these changes and may be a sign of overtraining of horses (8).

Despite widely available literature data on the changes of blood parameters during exercise of horses, not much attention was paid to clinical and haematological indices in young stallions during a performance test. Meanwhile, 100-d training is one of the methods of selection and evaluation of young stallions in the breeding of sport horses. Assessing the degree of adaptation of these animals to the effort in the context of their future use is of a crucial importance.

The aim of the study was to assess the variability of selected clinical and haematological indices in young stallions during the 100-d performance test.

Material and Methods

The study was conducted on 20 stallions of various breeds (half-breed - seven, Wielkopolska - five, Hanoverian - four, Holstein - two, and Oldenburg - two) aged 3-4 years, during 100-d performance test. The horses staying in training station in Biały Bór (Poland) were owned by the state horse studs (four stallions) and private breeders (16 stallions). The choice and number of horses used in the study were determined by the consent of the owners and organisational aspects. Data

from the interview prior to the test procedure, clinical examinations of horses, and obtained *ex post* results of blood tests confirmed the good health of the animals. The horses were regularly subjected to preventive treatments, including deworming and vaccinations against influenza and tetanus.

Feed was balanced in terms of energy, proteins, vitamins, and minerals considering the specific requirements connected with standards of animal feeding (4). The main feed rations were hay (6 kg), crushed oat grain (4.5 kg), and Heidelberg Haferfrei stuff as muesli (1.5 kg). Animals were housed in stables that meet the conditions of welfare, with a continuous access to water and mineral blocks.

The intended purpose of the study was carried out on the basis of exercise loads, whose form and intensity resulted from the assumptions of 100-d stallion performance test. In the first 2 weeks, training lasted about 30 min (tarsus alternating with trot - 10 min each, short gallops - 2-3 min). Between the 3rd and 7th week, total time of the training session was about 25 min (tarsus - 15 min, trot - 7 min, gallop - 3 min, 3-4 jumping over low obstacles with a height up to 40 cm). In the 6th - 7th week, an additional preparation for cross (trot in the field - 15 min) was introduced. The training from 7th to 14th week was extended to 45 min and also included several jumps by more complicated obstacles with a height of 70-120 cm and prolonged gallop up to 2 km at a rate of 500 m/min. Interference in the realisation of this programme was limited to minimum and only to clinical trials and blood sampling.

Assessment of the response of stallions to the applied load in the course of the whole training programme was conducted three times: (I) - on the 1st d of training (4 d after the initial classification of stallions), (II) - on the 46th d of training (half of the training programme), and (III) - on the 97th d of training (2 d before the test ending the whole training programme).

Training of stallions during all tests was carried out from June to September. The average ambient temperature and humidity during all these tests were as follows: 28, 25, and 24°C and 44%, 58%, and 73%, respectively.

Clinical studies, as well as the blood sampling on test days (I, II, III) were performed three times: before exercise (in the stable, before saddling), immediately after exercise, and after 30 min of rest. Clinical studies of stallions included the measurement of rectal temperature (T) and the assessment of heart rate (HR) and respiratory rate (RR).

Blood for laboratory tests was drawn from the external jugular vein into tubes with dipotassium ethylene diamine tetraacetic acid (K_2EDTA) using a closed vacuum system Vacuette®. In order to reduce the impact of the circadian rhythm, the first blood samples were collected at fixed time (8:00 ±30 min). The recovered material was stored at 4°C. Haematological examination was performed not later than 8 h after collection. The following indices were determined in the blood: red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (Ht), white blood cell count (WBC), number of lymphocytes (L), neutrophils (N), eosinophils (E), basophils (B), monocytes (M), and platelets (PLT). The determination was performed using a semi-automatic method with the help of ABC Vet haematology analyser (HORIBA ABX, France). The percentage and number of leukocytes were calculated on blood smears stained with May-Grünwald-Giemsa method.

The results were presented as the arithmetic mean, standard deviation (SD), and expressed as a percentage increase or decrease in the analysed variables (+%, -%). One-way analysis of variance and the Tukey test for distribution of medium for homogeneous groups were used. The correlation between variables was assessed by the use of Pearson correlation coefficients (r). All analyses were performed using the statistical package StatSoft v. 6.0.

Results

The average values of determined clinical parameters are shown in Table 1. Rectal temperature (T) before the exercise loads was characterised by a relatively high stability. The analysis of the results registered immediately after the exercise showed a statistically significant increase ($P<0.05$) in the T in all three tests. The highest increase was found at the beginning of the programme (about 2.1%). Post-exertion growth of T during subsequent tests (II and III) was significantly lower ($P<0.05$) and was 0.98% and 0.84%, respectively. Exercise-induced increase in the value was temporary, because after 30 min of restitution, the T decreased to the resting level. Average T of stallions after rest differed significantly ($P<0.05$) from the value before the exercise in the first test and during the subsequent tests (II and III); however, the difference was not confirmed statistically.

Mean values of HR at rest during all three series of tests were 38.00 /min., while RR values ranged: from 14.00 to 15.00/min. In all tests, measurements carried out immediately after exercise demonstrated a significant ($P<0.05$) increase in these values (79.00-82.00/min, 58.00-62.00/min, respectively). After a 30-min rest, stallions displayed a clear decline in the values of HR and RR.

Pre-exertion mean values of RBC, Hb, and Ht revealed a relatively low diversity (Table 2). Comparison of the percentage resting variability of these indices showed a statistically significant difference ($P<0.05$) between Ht indices in II and III test. Immediately after the exercise, an increase in the values in all three tests was confirmed. The greatest variability was found in the RBC, wherein its dynamics in the course of training showed a decreasing tendency (about 16.4%, 13.7%, and 11.0%, respectively). A statistically significant ($P<0.05$) difference between the results recorded in the first and last series of the tests confirmed this phenomenon. After 30-min restitution, a significant decline of RBC parameters to the rest level was registered.

Table 1
Mean clinical indicators in the course of physical effort of stallions

Test		T (°C)	HR (/min)	RR (/min)
I	1	$38.08^{aA} \pm 0.28$	$38.0^{aA} \pm 4.52$	$15.0^{aA} \pm 2.38$
	2	$38.88^{b} \pm 0.17$	$82.0^{b} \pm 4.42$	$62.0^{b} \pm 4.2$
	3	$38.44^{c} \pm 0.18$	$44.0^{c} \pm 3.59$	$17.0^{a} \pm 1.99$
II	1	$37.99^{aA} \pm 0.28$	$38.0^{aA} \pm 3.80$	$14.0^{aA} \pm 2.1$
	2	$38.36^{b} \pm 0.25$	$79.0^{b} \pm 3.24$	$59.0^{b} \pm 3.85$
	3	$38.12^{a} \pm 0.26$	$41.0^{a} \pm 3.66$	$16.0^{a} \pm 2.21$
III	1	$38.05^{aA} \pm 0.26$	$38.0^{aA} \pm 2.33$	$15.0^{aA} \pm 1.98$
	2	$38.37^{b} \pm 0.22$	$79.0^{b} \pm 3.08$	$58.0^{b} \pm 3.43$
	3	$38.15^{ab} \pm 0.22$	$39.0^{a} \pm 2.09$	$15.0^{a} \pm 1.77$

T - rectal temperature, HR - heart rate, RR - respiratory rate, I - first training day, II - 46th training day, III - 97th training day, 1, 2, 3 - blood sampling (before exercise, immediately after exercise, and 30 min after rest, respectively) $^{a\ b\ c}$ - means marked with different letters differ significantly at $P<0.05$ (for differences between the 1, 2, 3) in subsequent tests, $^{A\ B}$ - means marked with different letters differ significantly at $P<0.05$ (for differences between mean values of resting); ± SD

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Table 2
Mean red blood cell counts in the course of physical efforts of stallions

Test		RBC (T/L)	Hb (mmol/L)	Ht (L/L)
I	1	$9.52^{aA} \pm 0.75$	$8.10^{ab} \pm 0.57$	$0.4175^{aA} \pm 0.03$
	2	$11.08^{b} \pm 0.86$	$8.95^{b} \pm 0.58$	$0.4574^{b} \pm 0.03$
	3	$9.77^{a} \pm 0.92$	$8.54^{ab} \pm 0.63$	$0.4230^{a} \pm 0.03$
II	1	$9.50^{aA} \pm 0.69$	$8.25^{aA} \pm 0.77$	$0.4019^{aA} \pm 0.03$
	2	$10.80^{b} \pm 0.92$	$9.09^{b} \pm 0.86$	$0.4430^{b} \pm 0.04$
	3	$9.65^{a} \pm 0.87$	$8.36^{a} \pm 0.81$	$0.4106^{a} \pm 0.03$
III	1	$9.61^{aA} \pm 0.68$	$8.32^{aA} \pm 0.41$	$0.4214^{aA} \pm 0.03$
	2	$10.67^{b} \pm 0.73$	$9.14^{b} \pm 0.45$	$0.4620^{b} \pm 0.04$
	3	$9.71^{a} \pm 0.74$	$8.55^{ab} \pm 0.50$	$0.4309^{a} \pm 0.02$

RBC - red blood cells, Ht - haematocrit, Hb - haemoglobin; symbols I, II, III, 1, 2, 3, $^{a\ b\ c\ A\ B}$ - as in Table 1; ± SD

As in the case of erythrocyte parameters, there was no difference between the mean resting values of WBC, L, N, E, B, and M in the subsequent tests (Table 3). In the case of lymphocytes, a significant increase in their number in the subsequent tests (approximately by 28.7%, 23.7%, and 24.4%) was registered. The statistically confirmed increase in L and E counts (by 24.4% and 38.1%, respectively) was found only in blood samples taken after the exercise. Exercise-induced increase in the number of these cells was transient and the results recorded after rest did not differ significantly from the pre-shipment values.

There were no differences in the number of resting platelets (PLT) (Table 3). The stability of a large number of these cells in the course of training of stallions was confirmed by the lack of significant difference in the percentage variation in the tests II and III on the background of the initial test results. Significantly higher mean values of this parameter were found in the blood samples collected in subsequent tests, immediately after exercise (an increase by approximately 21%, 18% and 35%). Despite the high dynamics of the PLT, it seems that the interindividual differences prevented statistical confirmation. This phenomenon may be confirmed by the decrease in the average PLT after 30-min rest of stallions in all three tests.

Values of haematological indices were in direct positive correlation with the results of measurements of T, HR, and RR (Table 4) and, in addition, the results of measurements of HR and RR correlated with much more marked blood indices than T. No significant association of these two clinical parameters with other blood parameters was observed only in the case of B. The internal temperature of the tested stallions was correlated at the least level with the values of WBC indices (excluding E) and PLT.

Table 3
Mean white blood cell count and number of platelets in the course of exercise of stallions

Test		WBC (G/L)	L (G/L)	N (G/L)	E (G/L)	B (G/L)	M (G/L)	PLT (G/L)
I	1	8.28^{A} ±1.41	1.92^{aA} ± 0.52	5.69^{A} ± 0.93	0.21^{A} ± 0.06	0.03^{A} ± 0.02	0.43^{A} ± 0.11	183.75^{A} ± 75.87
	2	9.55 ± 1.5	2.47^{b} ± 0.6	6.29 ± 1.13	0.23 ± 0.06	0.04 ± 0.02	0.54 ± 0.13	222.15 ± 95.32
	3	8.73 ± 1.28	2.11^{ab} ± 0.45	5.86 ± 1.22	0.22 ± 0.08	0.04 ± 0.02	0.51 ± 0.1	184.85 ± 79.03
II	1	8.35^{A} ± 1.01	2.07^{aA} ± 0.5	5.6^{A} ± 0.7	0.19^{A} ± 0.07	0.04^{A} ± 0.02	0.45^{A} ± 0.23	182.75^{A} ± 92.47
	2	9.69 ± 1.7	2.56^{b} ± 0.61	6.26 ± 1.21	0.23 ± 0.06	0.04 ± 0.02	0.59 ± 0.13	215.25 ±115.44
	3	8.98 ± 1.44	2.36^{ab} ± 0.48	5.88 ± 1.04	0.21 ± 0.05	0.04 ± 0.02	0.5 ± 0.13	193.1 ± 88.87
III	1	8.61^{aA} ± 1.24	2.01^{aA} ± 0.36	5.89^{A} ± 1.09	0.21^{aA} ± 0.1	0.03^{A} ± 0.02	0.47^{A} ± 0.12	183.75^{A} ± 90.9
	2	10.16^{b} ± 1.49	2.5^{b} ± 0.42	6.75 ± 1.22	0.29^{b} ± 0.06	0.04 ± 0.02	0.59 ± 0.16	247.3 ± 131.23
	3	9.08^{ab} ± 1.63	2.24^{ab} ± 0.43	5.98 ± 1.29	0.21^{a} ± 0.07	0.04 ± 0.02	0.57 ± 0.16	202.25 ± 101.27

WBC - white blood cells, L - lymphocytes, N - neutrophils, E - eosinophils, B - basophils, M - monocytes, PLT - platelet, symbols I, II, III, 1, 2, 3, $^{a\ b\ c\ A\ B}$ - as in Table 2. ± SD

Table 4
Statistically significant values (P≤0.05) of correlation coefficients (*r*) between the results of clinical tests and haematological indices of tested stallions

	T	HR	RR
RBC	0.3887	0.5785	0.5724
Hb	0.2088	0.4619	0.4503
Ht	0.3247	0.4898	0.4794
WBC	-	0.3796	0.3456
L	-	0.4037	0.3643
N	-	0.2667	0.2442
E	0.1466	0.2571	0.2786
B	-	-	-
M	-	0.3070	0.2647
PLT	-	0.1897	0.1981

Discussion

Mean values of clinical pre-exertion test of the examined horses were within the set of physiological norms for the species (28). An increase in body temperature resulting from exercise loads of all stallions was consistent with the results of other tests (31). It has been shown that during an intense work of horses' muscles, the body temperature may increase by more than 3°C (6). The reaction of the tested animals was characterised by a much lower intensity (an increase by 0.32-0.80°C), despite a relatively high ambient temperature and elevated temperatures, particularly during the last test. The result, coupled with a significant decline of T after a rest, suggests a moderate loading of stallions in relation to their functional potential.

An increase in the internal temperature of the body induces a variety of responses, which prevent its further growth. The most important of these is increased heart rate and breathing, as a result of arousal centres of the circulatory and respiratory systems (8). Mean values of these indicators were increased significantly in tested horses because of used exercise loads, which exceeded the resting values two- and four times, respectively. The results of other studies suggest that the post-exertion growth of both parameters remains linear and directly proportional according to the intensity of exercise (26, 28). The magnitude of these changes is, therefore, a good exponent of physical load, although the conditions in this case are multifactorial (19, 26). The factors, which also have a great influence on the tested parameters include the type of exercise, time and frequency of its duration, and individual responsiveness to a given dose of physical activity (1, 18). Measurements of both parameters, carried out after the rest, were consistent with the results of other tests (26) and point out a good preparation of stallions regarding their function in relation to the applied loads.

The results of haematological tests conducted before and after exercise were within the physiological norms for healthy horses (16, 33). The increased demand of working muscles for oxygen, evidenced by a rise in the number of post-exertion HR and RR values, resulted in an increase in red blood cell parameters (RBC, Hb and Ht). These results correspond with the results of other studies, which demonstrated a similar relationship (10, 13, 17). Exercise-induced increase in the size of these indicators in stallions was relatively moderate and similar to reactions reported during standardised exercise tests of young racehorses (10) and daily training cycle of sport horses (15, 20, 21). A much greater increase in erythrocyte count was presented to be a result of heavy and long-term loads (2, 24, 29, 30).

Post-exertion increase in basic parameters of RBC is primarily conditioned by the release of erythrocytes from the spleen into the peripheral blood. In horses their number may be even doubled in the peripheral blood, as a result of the release of erythrocyte-rich blood stored in the spleen (especially during a high load exercise). An increase in Hb, directly related to the increase in RBC levels is a response to the increased demand for oxygen by working tissue. An increase in RBC indices in the course of physical exercise is also conditioned by the reduction in blood volume due to the movement of water from plasma, mainly to working muscles. Much of the water is lost with sweat and exhaled air, increasing the blood density, and thus the apparent increase in the number of morphotic components in this area. Less visible growth of these indicators resulting from contraction of the spleen was also observed in stressful situations such as: going to the racetrack, waiting for the start, and blood collection (8, 13, 15).

A clear decrease in RBC parameters recorded 30 min after the end of each exercise session was consistent with the results of previously conducted studies (5, 19, 24, 25). After the exercise, erythrocytes are generally rapidly eliminated from the circulation, and then stored in the spleen (17). This reaction constitutes one of the signs of adaptation to the applied exercise loads. The longer persistence of post-exercise changes may indicate that very high loads, disproportionate to the degree of fitness of the organism, were used (29).

Resting values of erythrocyte parameters before the next training sessions during the 100-d observation of stallions are contradictory to the results of several studies indicating their growth during the several months of horse training programmes (5, 11). It is believed that this highly desirable manifestation of the body's adaptation to effort, directly affecting its efficiency and achievements in sport is especially obtained during endurance training of horses (30). Other authors emphasise the fact that this effect is manifested in the highest degree in the animals, characterised by a relatively small number of RBCs at the start of the training programme (27). The average value of this parameter for stallions was relatively high (about 9.5 T/L) comparing to the physiological ranges reported in the literature (16, 33) for all horses (5-10 T/L), which could partly explain the lack of the response in the tested animals.

The observed post-exertion (and temporary) increase in the number of lymphocytes and eosinophils, evoking small leukocytosis, stayed in agreement with the results of other studies (22, 25), but it is worth stressing, that at the same time, the growth of WBC depended on the intensity and duration of exercise (14). The results of the study are consistent with the results obtained by other authors (13, 25) investigating the course of moderate and standard physical effort of horses used for different sports. An additional confirmation of the relatively small load of stallions was quick (after only 30 min) return of these indicators to the resting values. The increase in the WBC count in the blood is one of the most frequently studied responses of the body to exercise. This observation should be called pseudoleukocytosis, because it is not related to the production of new cells, but is connected with the release of lymphocytes from the spleen, lymph nodes, and bone marrow into peripheral blood. Reaction of exercise-induced release of lymphocytes to peripheral blood is caused by increased levels of catecholamines, especially epinephrine (7). Lack of the effect of 100-d training programme on the resting values of leukocytes is consistent with previous observations indicating that in comparison to the erythrocytes, a regular exercise does not cause significant changes in the remaining values of the WBCs count and their subpopulation (9, 13).

Platelets have been the object of relatively few studies carried out in the course of exercise of horses, and the obtained results have a great diversity (9, 22, 23, 32, 34). According to Kestin *et al.* (12) variability in the number of platelets can be determined by varying intensity of exercise loads. An intense exercise can cause an increase in PLT in the range of 18%-80% as a result of their release from the spleen, bone marrow, and pulmonary circulation (3). Temporary, post-exertion growth of these cells was also found in the examined horses.

Analysis of correlation of measured parameters, namely: T, HR, and RR together with the determination of blood indices showed a significant association between majority of these parameters. Since the direction of changes of the values of these indicators is directly proportional to the intensity of exercise, it is not surprising that they are among the most commonly used indices in the assessment of exercise loads and endurance of horses (19, 26, 30).

Summing up, the conducted studies demonstrated a significant variation of clinical and haematological indices in young stallions during the 100-d performance test. Analysis of the results registered immediately after the exercise showed a statistically significant increase in the clinical values (T, HR, RR) and the parameters concerning erythrocytes (RBC, Hb, Ht) and leukocytes (L, E). Post-exertion growth of these indicators was temporary because 30 min after the end of each exercise session, there was a clear decline in the values to the resting levels. The obtained results confirm the relatively low exercise load and good health of young stallions during the performance test.

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