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RESEARCH ON THE EFFECT OF SOME STRESS FACTORS CONSEQUENTLY CHANGING THE LIFE REGIME ON SOME HEMATOLOGICAL PARAMETERS IN HORSES

CERCETĂRI ASUPRA EFECTULUI UNOR FACTORI DE STRES CARE MODIFICĂ REGIMUL DE VIAȚĂ AL CAILOR ASUPRA UNOR PARAMETRI HEMATOLOGICI

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ABSTRACT | REZUMAT

General Adaptation Syndrome in animals is an intense topic addressed by researchers in the field of veterinary medicine. It is known that the general adaptation syndrome, produced by short exposure to stressors of low or medium intensity, generally has a positive effect, inducing stress, and improving the body's adaptive capacity. Our research aimed to evaluate the stressful effect of changes in the diet and maintenance of horses, by evaluating consecutive haematological changes. In our experiment, we used 15 traction horses (12 females and 3 males) from the Romanian semi-heavy breed. The 15 horses were aged between 4-8 years and were divided into 3 groups. The sample of animals was uniform in terms of maintenance. Blood samples were collected from the jugular vein in 5 ml heparinized tubes. Blood samples were used to assess haemoglobin, haematocrit, red blood cell count, leukocyte count, neutrophil, and lymphocyte counts. Haematological parameters were determined using a ProCyte Dx Hematology Analyzer. The data obtained were statistically analysed, calculating the Media and Standard Deviation, using the Microsoft Excel application. During the spring we identified a significant increase in the level of leukocytes in the blood (42.4%).We showed a significant increase in the number of red blood cells (18.42%), haematocrit (17.85%), and the level of haemoglobin in the blood (13.26%). In case of all animals in the experimental groups, the change in life and work during the spring season, led to the installation of specific manifestations of stress, finding a significant increase in red blood cells, haematocrit, haemoglobin, le-ukocytes, and neutrophil / lymphocyte ratio.

Keywords: stress, red blood cells, haematocrit, haemoglobin, leukocytes, traction horses

Sindromul general de adaptare, la animale reprezintă un subiect intens abordat de cercetătorii din domeniul medicinii veterinare. Este cunoscut faptul că sindromul general de adaptare, produs în urma expunerii scurte la factori stresanți cu intensitate scăzută sau medie, are în general un efect pozitiv, inducând eustres, și ameliorarea capacitaților adaptative ale organismului. Scopul cercetărilor noastre a fost acela de a evalua efectul stresant al schimbărilor regimului de hrănire și întreținere la caii de muncă, prin evaluarea modificărilor hematologice consecutive. În experimentul nostru am utilizat 15 cai de tracțiune (12 femele și 3 masculi) din rasa semigreu românesc. Cei 15 cai au avut vârste cuprinse între 4 și 8 ani și au fost împărțiți în 3 loturi. Eșantionul de animale a fost uniform din punct de vedere al stării de întreținere. Parametrii hematologici au fost determinați cu ajutorul unui analizor ProCyte Dx Hematology Analyzer. Datele obținute au fost analizate statistic, calculându-se Media și Deviația Standard (SD), folosind aplicația Microsoft Excel. In perioada de primăvară am identificat o creștere semnificativă a nivelului leucocitelor din sânge (42,4%). Totodată în această perioadă am evidențiat o amplificare semnificativă a numărului de hematii (18,42%), hematocritului (17,85%) și nivelului de hemoglobină din sânge (13,26 %). În cazul tuturor animalelor din cele trei loturi experimentale modificarea regimului de viață și muncă pe parcursul sezonului de primăvară, a condus la instalarea manifestărilor specifice stresului, constatându-se amplificarea nivelului hematiilor, hematocritului, hemoglobinei, leucocitelor și a raportului neutrofile / limfocite.

Cuvinte cheie: stres, globule roșii, hematocrit, hemoglobină, leucocite, cai de tracțiune

It is known that the general adaptation syndrome, produced by short exposure to stressors of low or medium intensity, generally has a positive effect, inducing eustress, and improving the body's adaptive capacity (2, 6, 15, 19, 20). It is also known that the ex-posure of animals to strong stressors for a long period has as a consequence the installation of distress, with its negative implications that can lead to a pathological evolution (15, 19, 20). Various researchers have been concerned with identifying practical and non-invasive ways to assess stress in animals in general and in horses in particular. Laboratory methods based on the dosing of cortisol in plasma, saliva, or faeces have been experimented with. Also, clinical evaluations capture the effect of stress on cardiac activity and blood pressure.

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Relatively recent research has highlighted the effects of stress on haematological parameters, which has increased the range in order to assess the intensity of the general adaptation syndrome. Thus, stress induces changes in the hemogram, by increasing the number of red blood cells, haematocrit, and haemoglobin (3, 4, 5, 7, 11, 12).

Stress also causes changes in the leukogram, by increasing the number of leukocytes and neutrophils and decreasing the number of lymphocytes (5, 7, 12, 16). Hence the specific influence of stress on the Neutrophil/Lymphocyte ratio, which during the General Adaptation Syndrome suffers an obvious rise. In general, the evaluation of the Neutrophil/Lymphocyte ratio has become a recognized method as effective in assessing stress in mammals and is also easy to implement (13, 16, 17). In particular, the issue of stress has increased relevance in the case of the horse species, characterized by a specific temperament, which gives it a greater sensitivity to the action of stressors (1, 9, 10, 13). Moreover, workhorses in particular, through specific activities, are more intensely subjected to potentially stressful factors. In Romania, in certain mountain areas, where forestry activities have a significant share and where the access to mechanized means of transport is limited by the rugged terrain, the use of work horses to transport wooden material is a practice often applied. Our research aimed to evaluate the stressful effect of changes in the diet and maintenance of horses, by evaluating consecutive haemato-logical changes. The experiments were performed in Valea Doftanei locality from Prahova County, a mountainous area of Romania, where there are multiple forest exploitations and where workhorses are used intensively.

MATERIALS AND METHODS

In our experiment, we used 15 traction horses (12 females and 3 males) from the Romanian semi-heavy breed. The 15 horses were between 4-8 years old. All animals at the clinical examination were considered clinically healthy. The sample of animals was uniform in terms of maintenance. These animals were raised and exploited in the Doftana Valley, located in a mountainous area of Prahova County. This locality is located at an altitude of 800m (above sea level). The animals were subjected to experimental procedures in 2 successive experimental periods: winter and spring. For this study, the authors received the approval of the Ethics Committee of USAMV Bucharest. The experimental procedures were performed with the explicit written consent of the animals' owners. The experimental procedures, by their nature, did not affect the horses' welfare. Thus, the only intervention of the experimenters was the collection of blood samples, an action that was done in strict accordance with the rules of protection of the welfare of horses. During the experimental spring period, the horses were treated in different ways. Group 1 was represented by 5 horses of those selected who were subjected to a daily schedule change, by putting to work in moderate physical exertion, in cultivated agricultural areas. Outside the working hours, the 5 horses in question were kept in their shelters in the owners' households. Under these conditions, the animals were fed ad libitum. The food consisted of green mass, hay, and concentrate supplements. The water was provided at will. Group 2 consisted of 5 horses from those selected who were moved to areas of forestry, placed at an altitude of about 1000m, where they were subjected to specific activities, in a regime of intense physical effort. Under these conditions, the horses were housed in makeshift sheds, being fed with a green meal, hay, and concentrate supplements. The water was provided at will. Group 3 was represented by 5 horses of those selected who were moved to alpine grazing areas, at an altitude of 1200m, not being used for physical activities. Under these conditions, the horses had daily access to the pasture, throughout the period.

Blood samples were collected as follows: the first sampling, during the winter (January 15-20); the second sampling, during the spring, in the first week of the modification of the life and maintenance regime (April 20-27); the third sampling, during the spring, in the third week of the modification of the life and maintenance regime (May 10-15).

Blood samples were aseptically collected from the jugular vein, in 5 ml heparinized tubes, using disposable needles (25 mm, 0.8 mm). All samples were transported in an ice-cold box to the laboratory of the pathophysiology discipline of the Faculty of Veterinary Medicine Bucharest. Blood samples were transported for immediate processing, with an average of 2 hr between blood collection and laboratory analysis, and were used to assess haemoglobin, haematocrit, red blood cell count, leukocyte count, neutrophil, and lymphocyte weight. The above-mentioned haematological parameters were determined using a ProCyte Dx Hematology Analyzer. The data obtained were statistically analysed, calculating the Media and Standard Deviation (SD), using the Microsoft Excel application. At the same time, the statistical significance of the differences between the groups was calculated using the t-test (Student), using the Microsoft Excel application. The experimental protocol to which the animals from the three groups were subjected (Table 1).

RESULTS AND DISCUSSIONS

The level of red blood cells in all 3 groups was higher between April 20-27, compared to that recorded during the winter (January 15-20), the differences being statistically significant ($P < 0.05$). In the case of groups 2 and 3, the erythrocyte values from the samples collected between May 10-15, compared to those recorded during the winter interval (January 15-20), the differences being statistically significant ($P < 0.05$) (Table 2). In all 3 groups, the level of leukocytes in the blood was higher between April 20-27, compared to that recorded during the winter (January 15-20), the differences being statistically significant ($P < 0.05$).

In the case of group 2, the leukocyte values of sample 3 (May 10-15) compared with sample 1 (January 15-20) corresponding to this group, show statistically significant differences ($P < 0.05$). The level of neutrophils in the blood in all 3 groups was higher between April 20-27, compared to that recorded during the winter (January 15-20), the differences being statistically significant ($P < 0.05$). In the case of Group 2, the neutrophil values of sample 3 (May 10-15) compared

Table 1

The experimental protocol to which the animals from the three groups were subjected

Blood sampling	Sampling schedule	Group 1 (n=5)	Group 2 (n=5)	Group 3 (n=5)
1	January 15-20	Stabulation	Stabulation	Stabulation
2	April 20-27	Moderate physical exertion	Intense physical effort (forest regime)	Reduced physical effort (grazing regime)
3	May 10-15	Moderate physical exertion	Intense physical effort (forest regime)	Reduced physical effort (grazing regime)

with sample 1 (January 15-20) corresponding to this Group, indicate statistically significant differences (P<0.05).

The level of lymphocytes in the blood, in all 3 groups, in the case of samples 2 (April 20-27) of the three experimental groups, compared with samples 1 (January 15-20) corresponding to each group, shows significant differences in terms of statistical (P <0.05).

In the case of group 2, the lymphocyte values in the case of sample 3 (May 10-15) analysed in comparison with sample 1 (January 15-20) corresponding to this group, indicate statistically significant differences (P <0.05).

In all 3 groups, the Neutrophil/Lymphocytes ratio in samples 2 (April 20-27) compared to samples 1 (January 15 -20) corresponding to each group, shows statistically significant differences (P<0.05).

In the case of Group 2, the Neutrophil/Lymphocyte ratio in the case of sample 3 (May 10- 15), compared to sample 1 (January 15-20) corresponding to this Group, indicates statistically significant differences (P <0.05) (Table 3).

In the case of group 1, the level of red blood cell count was higher by 7.65% between April 20-27, compared to that recorded during the winter (January 15-20), the differences being significant. In the case of group 2, the same parameter was higher by 12.29% between April 20-27, compared to the values recorded during the winter, the differences being significant. In the case of group 3, the level of red blood cell count was higher by 11.46% between April 20-27, compared to the one recorded in winter (January 15-20), the differences being significant.

These results derive from the development of an adaptive response to the stress induced by the change in work and life regime. These conditions induced, through an adrenergic mechanism, the splenic mobilization of red blood cells and were implicitly accompanied by the amplification of this parameter. The evolutions mentioned above correspond to the theoretical foundations of the general adaptation syndrome described by several authors (2, 5, 7, 14).

Also, our results are in line with those published by Campbell and Sandoe (2015) and Popescu et al. (2014, 2019), who noticed similar effects in the conditions of the general adaptation syndrome to work horses (1, 17,18).

Comparing the red blood cells values from the samples collected between May 10-15, in the case of Groups 2 and 3, with the samples taken from the same Groups in the winter interval, we find significant differences, the first being higher by: 7.66% (Group 2) and 2.37% (Group 3). In the case of animals in Groups 2 and 3, which climbed to high altitudes in spring (1000 m in the case of logging and 1200 m in the case of pasture), the stress involved in changing the lifestyle was doubled by hypoxic stress induced by subjecting the animals to high altitudes, with a low partial pressure of oxygen in the inspired air. This condition led to the maintenance of elevated red blood cell values in the 3rd week after the change of work and life regime.

At the same time, in the case of group 1, the differences between the red blood cells level measured during the winter and that of those evaluated between May 10-15, were insignificant, indicating an adaptation of the animals to the lifestyle specific to spring work activities.

Results obtained by us in the evaluation of haematocrit and haemoglobin, in the conditions of our experiment, were in line with those obtained by us in evaluating the number of red blood cells. Thus, we recorded evolutions of these parameters that closely followed the fluctuations in the number

Table 2

Hemogram values for the three experimental groups

Group	The average number of red blood cells (M/mL) Average ±SD			The average haematocrit (%) Average ±SD			Medium haemoglobin (g/dl) Average ±SD		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
1	9.4±0.85	10.12*±0.60	9.48±0.81	38.2±2.80	41*±1.52	39.4±2.72	13.2±0.32	15.8*±0.23	13.4±0.54
2	9.92±0.80	11.14*±0.25	10.68*±0.12	38.6±1.61	43*±1.81	41*±2.62	13.4±0.34	17.6*±0.62	17.6*±0.37
3	10.12±0.72	11.28*±0.52	10.36*±0.32	42.8±3.43	47.8*±3.32	43.4±1.81	13.8±0.53	17.4*±0.43	14.4±0.82

*P<0.05

Table 3

Leukogram values in the case of the three experimental groups

Group	Leukocyte (K/mL) Average ±SD			Neutrophile (%) Average ±SD			Lymphocyte (%) Average ±SD			Neutrophile/Lymphocyte Ratio Average ±SD		
	Sample			Sample			Sample			Sample		
	1	2	3	1	2	3	1	2	3	1	2	3
1	7.4±0.60	10.8*±0.38	8±0.32	52.8±2.85	69.2*±2.72	59.6±2.52	39.8±1.82	29*±2.32	35.8±2.12	1.32±0.22	2.3*±0.21	1.66±0.14
2	8.4±0.52	10.8*±0.82	9.8*±0.84	58.2±3.65	73.6*±1.93	70.6*±6.93	30.4±1.62	24.4*±1.72	24.4*±2.32	1.91±0.12	3.01*±0.13	2.89*±0.12
3	8±0.21	10.2*±0.45	8.4±0.42	60.4±3.55	71.2*±4.62	66.6±3.62	44.2±1.74	35.2*±2.62	42.2±2.36	1.36±0.13	2.02*±0.14	1.57±0.11

*P<0.05

of red blood cells. We can emphasize that the rise of haematocrit and haemoglobin, due to stress induced by lifestyle changes, but also induced by hypoxic stress, was achieved through a mechanism based mainly on the numerical amplification of red blood cells and less on the increase in red blood cell volume or their haemoglobin load.

Comparing the values of leukocytes from samples 2 (April 20-27) of the three experimental groups with samples 1 (January 15-20) corresponding to each group, we find significant differences, the former being higher by: 45.94% (in the case of Group 1), 28.57 % (in the case of Group 2) and 27.5% (in the case of Group 3). These results derive from the development of an adaptive response to the stress induced by the change in work and life regime. This result may be an effect of increasing the blood level of glucocorticoid hormones (2, 5, 8, 21). Indeed, these hormones induce the centralization of neutrophils, respectively the inhibition of their adhesion to the vascular endothelium and the delay of diapedesis (5, 7, 21). Hence the increase in the total number of leukocytes in the blood fluid. Our results are in line with those published by Campbell and Sandoe (2015), Hausberger et al. (2020), and Popescu et al. (2014, 2019), who noticed similar effects in the general syndrome of adaptation to work paths (1, 10, 17, 18).

Comparing the leukocyte values from sample 3 (May 10-15) of Group 2 with sample 1 (January 15-20) corresponding to this Group, there are significant differences, the former being higher with 16.66%. The maintenance of elevated leukocyte values in the 3rd week after the change in work and life regime indicates that the level of stress to which the horses in this group were subjected was more intense, with a persistent character, being induced by the difficult regime working in forestry. Comparing the share of neutrophils in the leukocyte formula, from samples 2 (April 20-27) of the three experimental groups, with samples 1 (January 15-20) corresponding to each group, there are significant differences, the former being higher by 31.06% (for Group 1), 26.46% (for Group 2), and 17.88% (for Group 3).

The results are consistent with those of leukocytes in the blood, suggesting that this parameter has been amplified in particular by increasing the number of neutrophils. They probably derive from an adaptive response to the stress induced by the change in work and life regime. This result may be an effect of increased plasma levels of glucocorticoids. Under these conditions, there is a decrease in endothelial adhesion of neutrophils, which leads to prolonged circulation time and their movement from the marginal sector to the circulatory (2, 5, 7, 21).

Comparing the share of neutrophils in the leukocyte formula, in the case of sample 3 (10-15 May) of Group 2 with sample 1 (January) corresponding to this Group, significant differences are found, the first being higher by 21.30%.

The maintenance, in the case of group 2, of increased leukocyte values in the 3rd week after the change in work and life regime, indicates that the level of stress to which the horses in this group were subjected was intense and persistent, being induced by the difficult working regime in logging. Comparing the share of lymphocytes in the leukocyte formula, in the case of samples 2 (April 20-27) of the three experimental groups, with samples 1 (January 15-20) corresponding to each group, we find significant differences, the former being lower by: 27.13% (in case of Group 1), 19.73% (in case of Group 2) and 20.36% (in case of Group 3). These results probably derive from the development of the same adaptive response to the stress induced by the change in work and life regime. The result may be an effect of increasing the blood level of glucocorticoid hormones, which have among their specific effects that of inducing tissue sequestration of lymphocytes (5, 6, 15, 16, 17). Comparing the share of lymphocytes in the leukocyte formula, in the case of sample 3 (10-15 May) of Group 2 with sample 1 (15-20 January) corresponding to this Group, significant differences are found, the first being lower by 19.73%.

The maintenance of low lymphocyte counts in the leukocyte formula in the 3rd week after the change in work and life, in the case of group 2, indicates that the level of stress to which the horses in this group were subjected was more intense, with a persistent character, being induced by the difficult regime of work in the forest exploitation. Comparing the Neutrophil / Lymphocyte ratio from samples 2 (April 20-27) of the three experimental groups, with samples 1 (January 15-20) corresponding to each group, we find significant differences, the former being higher by: 74.24% (in case of Group 1), 57.59% (in case of Group 2), and 48.52% (in case of Group 3). Comparing the Neutrophil / Lymphocyte ratio in the case of sample 3 (May 10-15) of Group 2 with sample 1 (January) corresponding to this Group, we find significant differences, the first being higher by 51.30%.

This result is a consequence of the variation in the number of neutrophils and lymphocytes in the leukocyte formula. The Neutrophil/Lymphocyte ratio has proven to be an effective way to assess stress. Under stress, increased levels of glucocorticoid hormones and adrenaline induce major changes in the leukocyte profile in mammals (12, 16, 18, 21). The maintenance of the high values of the Neutrophil / Lymphocyte ratio, in the case of group II, in the 3rd week

after the change of work and life regime, indicates that the level of stress to which the horses in this group were subjected was more intense, having a persistent character, induced by the difficult working regime in forestry.

CONCLUSIONS

In the case of all animals in the three experimental groups, the change in life and work during the spring season, led to the installation of specific manifestations of stress, finding a significant increase in red blood cells, haematocrit, haemoglobin, leukocytes, and Neutrophil / Lymphocyte ratio. In the case of horses from group I, subjected to intense physical exertion in the household, specific manifestations of stress occurred for a short period of time. Thus, three weeks after the change in lifestyle and work, the parameters followed returned to normal values. In the case of horses from group II, subjected to intense physical exertion in the forest regime, due to the demanding work regime, but also to the exposure to a high altitude (hypoxic stress), the specific manifestations of stress manifested over a long period (over 3 weeks), during this period the parameters mentioned above are maintained at modified values. In the case of horses from groups III, subject to a maintenance regime specific to grazing, the specific manifestations of stress manifested themselves for a shorter period. Three weeks after the change in lifestyle and work, the evaluated parameters tended to return to normal values, except for the number of red blood cells that remained high for a longer period of time. Our research confirms again that evaluating the Neutrophil / Lymphocyte ratio is an effective way to assess stress in workhorses, being easy to apply and providing relevant results. The specific activities of working in the forest regime, at high altitudes, prove to be an intense stress factor, to which the animals are subjected. This effect is amplified in the context of the relatively sudden transition, from the permanent housing regime to the forest work regime. Our results suggest that, in the case of these animals, the change of maintenance and work regime should be done gradually, in order to avoid the possible negative effects that can be induced by the intense stress.

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