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INFLUENCE OF 8-HOUR ROAD TRANSPORTATION ON SELECTED PHYSIOLOGICAL PARAMETERS IN HORSES

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Abstract

The objective of this study was to assess the physiological responses in mature healthy horses prior to an 8 h transport, as well as immediately and 24 h after transportation in a horse truck, under autumn conditions typical for central Poland. The study was conducted on a group of 60 horses of different breed. Horses ranged from 4 to 10 years of age. Venous blood was taken 3 times: I - directly before loading the horses to the trailer, II - directly after unloading the horses from the trailer, III – after period of 24 h resting. There was no change in the concentrations of urea, cholesterol, creatinine, and magnesium and in the activity of alkaline phosphatase during the three subsequent samplings. Albumin, total protein, alanine aminotransferase, aspartate aminotransferase, total bilirubin, creatine kinase, and triglycerides were mostly influenced by the transport. The obtained results indicate that the transportation of horses alters concentrations of physiological variables of their metabolism.

Key words: horses, road transport, truck, blood biochemistry

Racing and show competitions, breeding, leisure activities, sale, or slaughter are the most common reasons for horses' transportation (24, 25). Studies on stress and well-being during transportation of farm animals have primarily been directed toward the cattle and swine industries, and little information on the effects of long-distance transportation on the physiological responses of horses is available (13, 27). Transportation of horses designed for slaughter is very different than the transport of other animals because they often undergo very long distance transport (>24 h compared to other species), and the trade takes place during the whole year. Transport by road is more uncomfortable for animals than by rail or air (16). Moreover, because of the obvious influence of increased transit time and additional stressors, there is ample evidence demonstrating that longer periods of road transport have a greater impact on welfare than shorter periods of transport under the same conditions (10, 11). For example, during transport, horses are forced to maintain unnatural body postures for long periods. Combined with the additional stress of being placed in an unfamiliar environment, it is likely to have a detrimental effect on the welfare and performance of some horses. In the available literature, the effect of transportation on blood biochemical and haematological parameters of horses during the hot summer months has been cited (25). Serum concentrations of lactate, activity of creatine kinase and aspartate aminotransferase, as well as cortisol and neutrophil:lymphocyte ratio is increased during transport and in the early post transit period. Other studies also report an increase in red blood cell count, packed cell volume, haemoglobin, and plasma proteins (22, 25).

Due to the fact, that a number of horses being transported to slaughter houses in the European Community are still high, about 130,000 per year, it seems reasonable to conduct studies in this area (18). The welfare of animals during transportation should be assessed using a range of behavioural, physiological, and carcass quality measures. In addition, health is an important part of welfare so the extent of any disease, injury, or mortality resulting from, or exacerbated by, transport should be measured. The objective of this study was to assess the health status and physiological responses in mature healthy horses prior to an 8-h transport, immediately and 24 h after the transport, using a horse truck and under the autumn conditions typical for central Poland.

Material and Methods

The tests involved 60 Polish cold-blooded horses earmarked for slaughter, aged between 4 and 10 years, including 29 males and 32 mares. Their average weight was 680 ±50 kg. The study was conducted in November 2007, and all transports were made at the same starting time and with the same transporter, transport conditions, and driver. On the morning of each journey, the animals were loaded at 9.00 am into a truck and transported by road. Every time, the floor of the transporter was bedded with cereal straw. The animals had access to hay, but water was not available during transit.

Jugular blood samples were collected three times: I – directly before transport to the horse abattoir (at a time when the animals were still in their stalls), II - directly after unloading the animals from the transport semi-trailer, after a journey lasting approx. 8 h, III - after a 24-h rest at the stable of the horse abattoir. Blood samples were obtained by slow suction using a 20 ml syringe and 20 G needle. The sample was transferred through the needle into two 10 ml serum separating tubes without anticoagulant (Sarstedt, Numbrecht, Germany). The samples were centrifuged for 10 min at 5,000 g within 1 h after collection; serum was separated from packed cells and then stored in a -80˚C freezer for a maximum of 10 d before analysis. Analysis of the serum samples was carried out using the automated diagnostic analyser (Konelab Prime 30 ISE, Thermo Scientific, Waltham, USA), following the method validated and warranted by Thermo Fisher Scientific. Blood samples were analysed for concentrations of urea, albumin, total protein, total bilirubin, triglycerides, cholesterol, creatinine, and magnesium. The activities of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and creatine kinase were also measured. Quality control was made at least once a day, using two level controls, normal and abnormal (Nortrol, code 981043, Abtrol, code 981044, Konelab T Series, Thermo Fisher Scientific Oy, Finland). None of the collected blood samples showed signs of haemolysis, which was additionally confirmed by laboratory tests.

To ensure conformity of the variable distributions with the normal distribution, conformity was checked using the Kolmogorv-Smirnov test. All variables were judged as compatible, or logarithmically compatible with the normal distribution. Parametric comparisons were made with multifactorial analysis of variance (MANOVA) in combination with the Tukey test. A paired t-test was used to compare values between the respective samplings (I, II, III), while an unpaired t-test was used to compare basal values between the three groups. Differences with a significance level at $P<0.05$ were considered significant.

Table 1

Mean serum analytes before (I) and after 8 h of transportation (II) and 24 h resting (III)

Marker (unit)	I $_{(n=60)}$		II$_{(n=60)}$		III$_{(n=60)}$	
Albumin (g/L)[a]	31	max 38 min 18 SD 4.36	35,8	max 44 min 25 SD 4.1	36,3	max 43 min 22 SD 4.19
ALT (U/L)[b]	21	max 108 min 5 SD 17.5	29	max 202 min 8 SD 30.9	24	max 186 min 9 SD 42
AST (U/L)[c]	303	max 1457 min 160 SD 187	375	max 1416 min 27 SD 226	410	max 1961 min 16 SD 306
Total protein (g/L)[d]	57.4	max 86 min 41 SD 9.33	67.9	max 97 min 47 SD 10.4	67.8	max 104 min 43 SD 10
Total bilirubin (µmol/L)[e]	18.92	max 63.7 min 6.2 SD 12.68	31.68	max 90.8 min 11.1 SD 18.84	48.23	max 104.1 min 13.2 SD 22.83
Urea (mmol/L)$_{NS}$	4.64	max 8.27 min 1.54 SD 1.36	5.22	max 9.8 min 2.94 SD 1.54	5.38	max 7.99 min 2.66 SD 1.36
Cholesterol (mmol/L)$_{NS}$	2.36	max 4.5 min 1.06 SD 0.72	2.84	max 5.35 min 1.38 SD 0.83	3.07	max 6.79 min 1.62 SD 0.94
AP (U/L)$_{NS}$	207	max 546 min 49 SD 112.7	235	max 505 min 66 SD 112	249	max 663 min 54 SD 133
CK (U/L)[f]	270	max 1163 min 57 SD 241	373	max 1383 min 51 SD 273	367	max 1309 min 80 SD 271
Creatinine (µmol/L)$_{NS}$	103	max 178 min 66 SD 23	124	max 439 min 79 SD 47	115	max 260 min 78 SD 27
Magnesium (mmol/L)$_{NS}$	0.59	max 0.92 min 0.39 SD 0.11	0.65	max 0.88 min 0.4 SD 0.09	0.65	max 0.98 min 0.45 SD 0.1
Triglycerides (mmol/L)[g]	0.18	max 3.55 min 0.1 SD 0.46	0.11	max 0.7 min 0.1 SD 0.12	0.3	max 4.64 min 0.1 SD 0.67

Statistically significant ($P<0.05$) differences: [a] albumin: I-II, I-III, [b] ALT: I-II, II-III, [c] AST: I-II, I-III, II-III, [d] total protein: I-II, I-III, [e] total bilirubin: I-II, I-III, II-III, [f] CK: I-II, I-III, [g] triglycerides: I-III, II-III.

Results

Consolidated data and statistical effects relating to equine blood chemistry are presented in Table 1. There was no statistical difference between horses in regard to the activity of ALT, AST, AP, and CK and concentrations of albumins, total protein, total bilirubin, urea, cholesterol, creatinine, Mg, and triglycerides regarding age, sex, and weight. There was no change ($P>0.05$) in the concentrations of urea, cholesterol, creatinine, and Mg and activity of AP during the three subsequent samplings. The average activity of the CK and concentration of albumin and total protein relative to time increased during the three subsequent samplings ($P<0.05$); statistically significant differences were found between the samplings I and II, as well as I and III ($P<0.05$) (Table 1). Examination of the changes in the average ALT and AST activity and concentration of total bilirubin revealed a statistically significant increase ($P<0.05$) relative to time. Moreover, analysis of the respective samplings compared to each other revealed statistically significant differences between all the samplings ($P<0.05$), (Table 1). Analyses of the changes in the average triglycerides concentration revealed statistically significant differences between the respective samplings. There was a significant increase in triglyceride concentration between samplings I and III, and between II and III (Table 1).

Discussion

The presented study showed that 8-h road transportation of horses affected physiological parameters of their metabolism. The biological measures, which were most sensitive to the transport, were albumin, total protein, ALT, AST, total bilirubin, CK, and triglycerides. During the study, a statistically significant increase in the concentration of total protein and albumin was noted. An increase in the concentration of these parameters is most often associated with transient dehydration of the animal due to the transportation (8-10). However, there was no evidence of dehydration and electrolyte disturbances. Taking into account that after unloading the horses had constant access to water, we can hypothesise that significantly higher levels of these two parameters can be related to the antioxidant properties of blood proteins, particularly albumin, as a result of increased production of ROS. These proteins are characterised by strong antioxidant properties, *i.e.* surface binding of a number of ligands, such as fatty acids and copper. Albumin binds copper and iron ions, which assist in the prevention of erythrocyte peroxidation by limiting the Fenton reaction (12). Another aspect of antioxidant activity of albumin may come from its capacity to bind homocysteine, a sulfur-containing amino acid, which results from the catabolism of methionine residue. Elevated plasma homocysteine is a well-known risk factor for atherosclerosis and may act through oxidation of LDL (28). Properties of albumin have been confirmed in numerous *in vitro* studies, using a model of oxidative damage to low-density lipoprotein (LDL) induced by copper ions (4, 17, 28). These findings correspond with research made by Yang *et al*. (5, 6), who noted an increased synthesis of albumin in the liver after roughly 3 h post the start of exercise. It seems that the obtained results can be associated with increased production of free radicals in liver cells. Albumin, the most abundant circulating protein in the plasma, exerts important antioxidant activities. The molecule acts through its multiple-binding sites and free radical-trapping properties. Under physiological or pathological conditions, function associated with changes in the redox status, the albumin structure, and its beneficial antioxidant properties can be altered. In general, albumin constitutes the major plasma protein target of oxidant stress (17).

During the study, the concentration of total bilirubin was also determined. Bilirubin, in low concentrations according to the reference values, has potent antioxidant properties, as it is involved in removal of free radicals. By analysing the concentration of bilirubin, a statistically significant increase in the concentration of this parameter was observed; however, at all times this result remained between the reference ranges. Furthermore, the analyses of the activity of liver enzymes (AST, ALT) showed that there was no damage to the liver in any of the animals tested, and considering continuous access to hay during transport and after unloading the animals, it can be assumed that the increase in total bilirubin is not associated with liver damage or lack of access to feed. It seems that an increase in total bilirubin concentration was probably caused by a reaction to increased production of free radicals caused by stress factors. These observations are consistent with results of other authors; who studied the impact of road transport on selected blood parameters in sheep and horses and found increased levels of bilirubin after the transport (7, 15).

Two serum enzymes with high activity in skeletal muscle are CK and AST. Physiologically an increase in CK is associated with moderate exercise or initiation of a training programme. However, myopathies such as exertional rhabdomyolysis are diagnosed with activities from several to hundreds of thousands of IU/L (1). In this study, the CK peaked at 373 ± 273 IU/L following transport. This value is slightly increased in comparison to the reference range. The activity of AST was also mildly increased, but it remained in the reference range. The limited increase in the two serum muscle enzymes indicates moderate muscular insult due to road transport. Moreover, several authors have reported a mild to moderate increase in plasma CK activity after severe physical stress or exercise (2, 3, 20). However, rats and turkeys show a significant increase in CK activity even with mild stress or exercise (19, 26).

It has been known for a long time that prolonged stress plays a major role in increasing the blood levels of triglycerides in animals, as well as in humans (14, 21). A human study concerning psychological and mental stress showed that these cause triglycerides to stay in the bloodstream for longer, contributing to cardiovascular health problems (23). Our results revealed higher concentrations of triglycerides in the third sampling. In comparison to the pre-transport sampling there was no significant drop in concentration immediately after transport in sample two. Although the mechanism for stress-induced lipid reactivity has not been articulated, one hypothesis is that acute stress reduces the clearance of triglycerides. The current study provides the evidence that short-term stress can reduce plasma triglyceride clearance in horses, as well as its increase. Future studies must determine the extent to which such diminished clearance rates are related to, and may explain the transient elevations in triglycerides during stress, and the clinical relevance of diminished stress-induced triglyceride clearance rates (9, 13, 25).

Results of many studies clearly demonstrated that biochemical alterations after training exercise are common in race horses. Nonetheless, most of the parameters in liver, renal and lipid profile showed minor increases in their levels, with a statistically significant difference observed only for ALT, total protein, and albumin. Rarely, a mild increase in plasma glucose concentration and a decrease in triglyceride level were seen in race horses after the races (2, 3, 5). Similar to this observation, a significant increase in total protein and albumin concentrations has been reported in humans after a marathon running, and is probably a secondary reaction to the effect of dehydration after the races. In conclusion, we have determined that transportation of horses alters concentrations of physiological variables of their metabolism. The metabolite response appeared to be minimal compared to those in moderately exercised horses and was limited to the period of transport, as well as 24 h after the transport.

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