

INFLUENCE OF A CALMATIVE ON SELECTED BLOOD PARAMETERS IN HORSES UNDER STRESSFUL CONDITIONS

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The results of few studies on tryptophan supplementation conducted in horses are contradictory and none of these studies have shown that tryptophan is an effective calmative in horses. The objective of the present study was to determine changes in haematological parameters, plasma lactate, serum cortisol and biochemical profile, as well as the behaviour of untreated and calmative treated horses under stressful conditions in order to determine the effects of the applied calmative. The study also aims to confirm the importance of a 24 hour rest after work.

The same six horses were on duty twice, Trial I and Trial II, under stressful conditions. Blood samples were collected three times, at pre-transport, post-stress and resting phase. Horses' behaviour was observed carefully by the riders.

The study showed that most changes of the haematological and biochemical profiles reflect the response to exercise as horses were physically active while being on duty. The effect of the calmative is mainly reflected by a minor extent of changes of measured parameters, as determined by comparison at individual sampling phases. According to the observations of horses' behaviour, we may conclude that the use of calmatives did calm the horses. The study confirmed that horses need at least 24 hours of rest after working.

Key words: equine calmative, stress, behaviour, blood parameters

INTRODUCTION

Stress has been defined, in general, as an abnormal or extreme adjustment in the physiology of the animal as to cope with adverse effects of its environment or management (Muir, 2004; Dienstbier, 1989). Horses are exposed to an excess of stress-inducing situations. In fact, many horses experience stressful events on a routine basis. Most of these are related to routine training and management practices. Stressors can include environmental changes, transport, exposure to

novelty, weaning, fear due to restraint, contacts with people and training, but also injury or disease. Stress not only directly affects the well-being of the horse and its performance, but more importantly, its health condition, too (Art and Lekeux, 2005; Christensen *et al.*, 2005; Ott, 2005; Stull *et al.*, 2004; Shanahan, 2003; Williams *et al.*, 2002; Grandin, 1997; Smith *et al.*, 1996).

The stress response is as an adaptive pattern of behavioural, neural, endocrine, immune, haematological, and metabolic changes directed toward the restoration of homeostasis (Muir, 2004). Horses are very individualistic and as such respond to stress in different ways. In addition to physiological responses, such as increased concentrations of cortisol and lactate, increased heart rate and corticosteroid-binding globulin capacity, some horses respond in observable way, displaying behaviour changes and extreme nervousness (Christensen *et al.*, 2005; Stull and Rodiek, 2000; Alexander and Irvine, 1998; Bagshaw *et al.*, 1994). They can develop stereotypic and/or redirected behaviours (Waters *et al.*, 2002; Nicol, 1999), as well.

Being naturally fearful and flight animals, the horse's behaviour becomes increasingly important when being ridden in environments beyond the rider's control. It is, therefore, not surprising that there are several equine calmative preparations (Equivit B-quiet, Cavalor Calm, etc) available commercially. Do these supplements that are aimed at 'calming' horses, really work? Veterinarians are faced with real issue when asked to provide advice in this area (Harris, 2005).

These 'calming' products commonly include ingredients such as magnesium, B vitamins, lecithins, various herbs, as well as L-tryptophan, the amino acid precursor for serotonin, a neurotransmitter implicated in sedation, inhibition of aggression, fear and stress, in various animal species and humans (Grimmett and Sillence, 2005; Davis, 2000). The effects of tryptophan on behaviour are species dependent, as well being subject to the influence of diet, exercise, age, gender, breed, social status, and level of arousal (Grimmett and Sillence, 2005).

The results of few studies on tryptophan supplementation conducted in horses are contradictory and none has produced direct evidence that tryptophan is an effective calmative in horses, nor that it had effect on exercise performance in horses (Vervuert *et al.*, 2005; Farris *et al.*, 1998; Bagshaw *et al.*, 1994; Paradis *et al.*, 1991). Nevertheless, supplements containing tryptophan are continuously used to calm horses prior to transportation, competition or working. Therefore, the objective of the present study was to determine changes in haematological, plasma lactate, serum cortisol and serum biochemistry profile, as well as behaviour of calmative treated and untreated horses under stressful conditions in order to determine the effects of the calmative. The study also aims to confirm the importance of 24 hours of rest after working.

MATERIAL AND METHODS

Animals and study protocol

The present study was conducted on six mature male horses of different breed, aging from 10 to 14 years (average 12.43 years). Horses belong to the

mounted police troops, therefore, were subjected to the same training programme and diet. None of the horses had ever showed any of stereotypic or redirected behaviours. The horses were determined to be clinically healthy before the study, based on physical and blood analytical examination. They were housed individually in boxes and fed hay approximately 1.5-2 % of bodyweight divided in three rations per day and oats approximately 0.7- 0.8 % of bodyweight divided in two rations per day. Water was available *ad libitum*. The diet was supplemented with minerals and vitamins.

The same horses were on the same duty twice, Trial I and Trial II, under stressful conditions that included transport and exposure to a roaring crowd of people at a basketball game. While during Trial I, horses remained untreated, serving as the control, during Trial II horses received equine calmativie, Cavalor Calm[®] (Nutriquine, Vitamex, Drongen, Belgium), three times per day (3x12.5 g, cca 3.75 mg L-tryptophan/kg), three days prior to the basketball event the interval between the trials was one month. Each trial was regarded as a 2-day follow-up study.

A standard 2-horse, tandem axle, forward facing horse trailer was used for 5 kilometres transportation to the hall where the basketball game took place. At each trial horses were on duty at the basketball event approximately five hours, transported back, unloaded and housed individually in boxes for 24 hours of resting.

Blood sampling and analysis

Blood samples were collected three times. Pre-transport samples were obtained in the afternoon, when the horses were relaxed in their boxes, immediately before the transport (pre-transport phase). Post-stress samples were obtained immediately after the horses were transported back and unloaded after being on duty (post-stress phase). Resting samples were collected 24 hours after the second ones (resting phase). During the resting phase horses were kept in their boxes, resting.

Blood for complete blood count (CBC) and white cell differential count (WCDC) was collected into tubes containing EDTA (Greiner Bio-One, Kremsmuenster, Austria) and analysed immediately after the arrival to the lab. Blood for plasma glucose and lactate determination was collected into tubes containing lithium iodacetate and heparin (Greiner Bio-One, Kremsmuenster, Austria) and blood for serum cortisol and biochemical profile determination into plain tubes (Greiner Bio-One, Kremsmuenster, Austria). Serum and plasma samples were kept frozen at -20°C until analysed.

CBC and WCDC were determined by an automated laser haematology analyser H*1 with species-specific software (Bayer - Siemens, Leverkusen, Germany). CBC included white blood cells (WBC), red blood cells (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT). WCDC represented a six-part differential: neutrophils (NEUT), lymphocytes (LYMP), monocytes (MONO), eosinophils (EOS), basophils (BASO) and large unstained cells (LUC).

The LUC category consists of a heterogeneous population of all large cells that fail to exhibit any peroxidase activity (atypical lymphocytes, immature granulocytes and blasts).

Serum biochemical profile included electrolytes (sodium (Na), potassium (K), chloride (Cl)), creatinine, urea, total protein, albumin, magnesium, alkaline phosphatase (AP), creatine kinase (CK), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Plasma lactate and serum biochemical profile, with the exception of electrolytes, were determined by automated chemistry analyser RA-XT (Bayer - Siemens, Leverkusen, Germany). Electrolytes were determined by electrolyte analyser Ilyte Na/K/Cl (Instrumentation Laboratory, Lexington, MA, USA).

Serum cortisol concentrations were measured using commercial enzyme immunoassay (Active Cortisol EIA, DPC, Los Angeles, USA). All samples were analysed within a single run, with variability coefficients (CV) of 2.05% and 5.29% for low ($\bar{X} = 4.23 \mu\text{g/dL}$) and high ($\bar{X} = 25.16 \mu\text{g/dL}$) values, respectively.

Horses' behaviour

During both trials, horses' behaviour was observed carefully by riders that were 'blind' to the treatment the horses had received.

Statistical analysis

Data were analysed by the use of commercial software (SPSS, Chicago, Illinois, USA). Within each trial changes of measured parameters were assessed by the use of repeated measures ANOVA. The values at the post-stress phase and resting phase were compared with values at pre-transport phase at both trials, as the latter served as basal values. Differences in measured parameters between trials were determined by paired t-test at each sampling time. A value of $p < 0.05$ was considered significant.

RESULTS

During both trials horses remained clinically healthy.

Haematological parameters

Haematological parameters measured at both trials are presented in Table 1. At Trial I, only EOS decreased significantly at the post-stress phase. Additionally, a slight increase of WBC, RBC, HGB, HCT and NEUT was determined at post-stress phase. Opposite to Trial I, a significant decrease of WBC, RBC and HCT, and a slight decrease in HGB were determined at post-stress phase of Trial II. After 24 hours of rest, these parameters almost reached pre-transport values at both trials. Other haematological parameters showed only minor changes.

In comparison with Trial I, significantly lower MCV and significantly higher MCHC values were determined at all sampling times of Trial II. There were also significant differences in HGB and MONO at pre-transport phase and in HCT at

post-stress phase between the trials. Despite lower PLT values at all three phases of Trial II, PLT values differ significantly from Trial I only at resting phase.

Table 1. Haematological parameters at Trial I and Trial II (mean \pm SD)

| Measured parameters | Trial | Pre-transport phase | Post-stress phase | Resting phase |
|----------------------------|----------|---------------------|--------------------|-------------------|
| WBC ($\times 10^9/L$) | Trial I | 6.180 \pm 0.95 | 6.400 \pm 1.18 | 6.210 \pm 1.15 |
| | Trial II | 7.160 \pm 0.97 | 6.530 \pm 1.05* | 7.340 \pm 0.55 |
| RBC ($\times 10^{12}/L$) | Trial I | 7.530 \pm 0.57 | 7.940 \pm 0.63 | 7.740 \pm 0.35 |
| | Trial II | 7.960 \pm 0.76 | 7.280 \pm 0.63* | 7.960 \pm 0.63 |
| HCT (L/L) | Trial I | 0.363 \pm 0.025 | 0.383 \pm 0.010# | 0.370 \pm 0.011 |
| | Trial II | 0.363 \pm 0.029 | 0.333 \pm 0.019* | 0.370 \pm 0.014 |
| HGB (g/L) | Trial I | 136.30 \pm 8.7# | 140.70 \pm 2.7 | 136.00 \pm 4.2 |
| | Trial II | 146.70 \pm 7.0 | 136.20 \pm 6.1 | 143.00 \pm 6.8 |
| MCV (fL) | Trial I | 48.800 \pm 3.2# | 48.500 \pm 3.2# | 48.100 \pm 3.0# |
| | Trial II | 45.900 \pm 3.2 | 46.200 \pm 3.9 | 46.800 \pm 3.6 |
| MCH (pg) | Trial I | 18.100 \pm 1.2 | 17.800 \pm 1.1 | 17.600 \pm 1.0# |
| | Trial II | 18.500 \pm 1.3 | 18.800 \pm 2.0 | 18.100 \pm 1.3 |
| MCHC (g/L) | Trial I | 374.00 \pm 6.8# | 367.20 \pm 6.4# | 366.50 \pm 3.1# |
| | Trial II | 404.80 \pm 27.4 | 407.50 \pm 24.4 | 386.30 \pm 6.8 |
| PLT ($\times 10^9$) | Trial I | 346.00 \pm 231 | 273.00 \pm 112 | 200.00 \pm 89# |
| | Trial II | 164.00 \pm 116 | 194.00 \pm 102 | 153.00 \pm 86 |
| NEUT (%) | Trial I | 58.200 \pm 4.3 | 59.600 \pm 5.0 | 55.900 \pm 3.9 |
| | Trial II | 48.300 \pm 10.6 | 56.500 \pm 6.6 | 59.600 \pm 11.0 |
| LYMPH (%) | Trial I | 32.300 \pm 3.4 | 32.100 \pm 4.2 | 36.000 \pm 2.5 |
| | Trial II | 41.800 \pm 11.8 | 35.300 \pm 5.9 | 31.300 \pm 10.4 |
| MONO (%) | Trial I | 1.100 \pm 0.4# | 1.200 \pm 0.6# | 1.100 \pm 0.1 |
| | Trial II | 3.600 \pm 1.7 | 2.800 \pm 1.4 | 2.500 \pm 1.6 |
| EOS (%) | Trial I | 3.200 \pm 2.3 | 2.600 \pm 2.1* | 2.550 \pm 1.8 |
| | Trial II | 1.400 \pm 0.6 | 1.600 \pm 1.3 | 1.900 \pm 1.1 |
| BASO (%) | Trial I | 0.300 \pm 0.1 | 0.400 \pm 0.1 | 0.300 \pm 0.2# |
| | Trial II | 0.400 \pm 0.6 | 0.350 \pm 0.1 | 2.500 \pm 1.7 |
| LUC (%) | Trial I | 5.000 \pm 1.3 | 4.200 \pm 0.5 | 4.800 \pm 1.0 |
| | Trial II | 4.500 \pm 1.7 | 3.400 \pm 1.7 | 3.600 \pm 1.3 |

*P<0.05 in comparison with pre-transport values (Repeated measures ANOVA)

#P<0.05 – paired t-test at each sampling time

Trial I – untreated horses

Trial II – calmative-treated horses

Plasma lactate, serum cortisol and serum biochemical profile

Changes of plasma lactate, serum cortisol and serum biochemical profile at both trials are presented in Table 2.

Table 2. Plasma lactate, serum cortisol and biochemical profile at Trial I and Trial II (mean \pm SD)

| Measured parameters | Trial | Pre-transport phase | Post-stress phase | Resting phase |
|---------------------------|----------|-------------------------------|--------------------------------|--------------------------------|
| Urea (mmol/L) | Trial I | 5.53 \pm 0.83 | 5.77 \pm 0.58 | 5.67 \pm 0.54 |
| | Trial II | 5.65 \pm 0.92 | 5.72 \pm 0.85 | 5.33 \pm 0.90 |
| Creatinine (μ mol/L) | Trial I | 149.00 \pm 17.8 | 165.80 \pm 21.2 | 150.20 \pm 8.9 |
| | Trial II | 144.20 \pm 19.6 | 155.10 \pm 20.2 | 156.70 \pm 30.8 |
| Glucose (mmol/L) | Trial I | 5.75 \pm 0.26 [#] | 5.68 \pm 0.44 | 5.50 \pm 0.41 |
| | Trial II | 5.38 \pm 0.15 | 5.52 \pm 0.64 | 5.40 \pm 0.44 |
| Na (mmol/L) | Trial I | 138.50 \pm 0.7 [#] | 140.60 \pm 2.0 [#] | 137.50 \pm 1.1 [#] |
| | Trial II | 136.30 \pm 0.8 | 138.30 \pm 1.5 | 134.60 \pm 1.0 |
| K (mmol/L) | Trial I | 3.73 \pm 0.32 [#] | 2.48 \pm 0.40 [*] | 3.75 \pm 0.48 [#] |
| | Trial II | 4.44 \pm 0.26 | 2.84 \pm 0.33 [*] | 4.50 \pm 0.50 |
| Cl (mmol/L) | Trial I | 103.00 \pm 0.6 [#] | 104.00 \pm 1.1 [#] | 102.60 \pm 1.1 [#] |
| | Trial II | 100.70 \pm 0.9 | 101.50 \pm 2.1 | 99.10 \pm 2.0 |
| Mg (mmol/L) | Trial I | 0.90 \pm 0.06 [#] | 0.92 \pm 0.04 | 0.88 \pm 0.15 |
| | Trial II | 1.03 \pm 0.05 | 0.97 \pm 0.05 | 1.02 \pm 0.10 |
| Proteins (g/L) | Trial I | 64.90 \pm 2.6 | 69.60 \pm 2.2 [*] | 65.60 \pm 3.4 [#] |
| | Trial II | 66.10 \pm 3.0 | 68.30 \pm 3.7 | 67.20 \pm 3.6 |
| Albumins (g/L) | Trial I | 38.80 \pm 1.9 | 40.80 \pm 2.1 [*] | 38.20 \pm 2.8 |
| | Trial II | 38.80 \pm 2.5 | 40.70 \pm 2.3 | 38.50 \pm 2.3 |
| AP (U/L) | Trial I | 99.70 \pm 26.8 | 105.90 \pm 26.1 [*] | 99.40 \pm 24.1 |
| | Trial II | 79.70 \pm 19.2 | 85.70 \pm 15.0 | 82.40 \pm 13.2 |
| ALT (U/L) | Trial I | 8.67 \pm 2.36 [#] | 11.93 \pm 2.45 [#] | 8.53 \pm 1.88 [#] |
| | Trial II | 27.70 \pm 4.14 | 23.82 \pm 2.53 | 24.32 \pm 2.33 |
| AST (U/L) | Trial I | 268.10 \pm 31.1 | 292.20 \pm 33.6 [*] | 269.40 \pm 33.3 [#] |
| | Trial II | 285.50 \pm 33.6 | 294.70 \pm 24.7 | 281.30 \pm 28.8 |
| CK (U/L) | Trial I | 130.50 \pm 8.3 | 149.40 \pm 13.7 [*] | 162.70 \pm 52.9 |
| | Trial II | 285.10 \pm 324.0 | 210.30 \pm 113.7 | 141.90 \pm 21.8 |
| Lactate (mmol/L) | Trial I | 0.65 \pm 0.22 | 0.62 \pm 0.21 | 0.66 \pm 0.21 |
| | Trial II | 0.66 \pm 0.26 | 0.50 \pm 0.22 | 0.84 \pm 0.20 |
| Cortisol (μ g/dL) | Trial I | 14.30 \pm 11.2 | 5.40 \pm 4.9 | 3.50 \pm 1.6 |
| | Trial II | 4.20 \pm 1.5 | 5.40 \pm 2.6 | 2.10 \pm 1.3 [*] |

* P<0.05 in comparison with pre-transport values (Repeated measures ANOVA)

P<0.05 – paired t-test at each sampling time

Trial I – untreated horses

Trial II – calmative-treated

Though not significantly, plasma lactate concentrations slightly decrease at the post-stress phase in both trials. There were no significant changes in plasma lactate between trials.

Serum cortisol concentrations ranged from $14.3 \pm 11.2 \mu\text{g/dL}$ at the pre-transport phase of Trial I to $2.1 \pm 1.3 \mu\text{g/dL}$ at the resting phase of Trial II. No significant changes of serum cortisol were observed at Trial I, while at Trial II serum cortisol decreased significantly during the resting phase. There were no significant changes in cortisol between trials.

Within biochemical profile, potassium showed the most evident change at both trials. At Trial I potassium decreased from 3.73 ± 0.32 to 2.48 ± 0.40 mmol/L (by 52.9 ± 26.2 %) and after 24 hours of rest returned to 3.75 ± 0.48 mmol/L. At Trial II, potassium decreased from 4.44 ± 0.26 to 2.84 ± 0.33 mmol/L (by 58.3 ± 21.3 %) and returned to 4.50 ± 0.50 mmol/L at the resting phase.

An increase in urea, creatinine, sodium, chloride, proteins, albumins, AP and AST was observed at the post-stress phase in both trials. Among these parameters, AP, AST, proteins and albumins increased significantly at Trial I. At the resting phase almost all parameters returned to pre-transport values. CK showed uncommon changes at Trial II due to high SD values at the pre-transport phase. At Trial I CK increased significantly at post-stress phase and decreased in Trial II.

Paired t-test showed significant differences in sodium, chloride and ALT at all three sampling phases. In addition, there were significant differences in Mg and glucose at pre-transport phase and in proteins and AST at resting phase. Potassium differed significantly between trials at the pre-transport and resting phases.

Behaviour changes

During both trials, horses behaviour was observed carefully by riders. According to their observations, calmativ treated horses at Trial II showed no behavioural changes that could signal stress throughout the study. On the contrary, untreated horses at Trial I displayed nervousness and showed behavioural changes, such as tail swishing, pawing, snorting, neighing, shaking, rearing and bucking, especially when exposed to stress.

DISCUSSION

Tryptophan supplementation appears to be effective in reducing aggression and possibly fearfulness in some species, but its impact on hyperreactivity and stress are questionable. The response is species-dependent and there are no scientific publications that confirm the efficacy of tryptophan as a calmativ in excitable horses (Grimmett and Sillence, 2005). Nevertheless, tryptophan supplements continue to be used as an equine calmativ.

Police horses used in the present study were on the same duty twice, Trial I and Trial II, under stressful conditions that included transport and exposure to a roaring crowd of people at a basketball game. At trial I horses remained untreated, while at Trial II received the equine calmativ. In order to determine calmativ effects the changes in haematological parameters, plasma lactate, serum cortisol and serum biochemistry profile, as well as behaviour were determined.

The study showed significant changes of some of the haematological parameters at both trials, though the values remained within the normal reference

range of our laboratory and agreed with those published in the literature (Eades and Bounous, 1997; Lumsden *et al.*, 1980). More interestingly, the study showed an opposite pattern of WBC, RBC, HCT and HGB changes at the post-stress phase. At Trial I, an increase in WBC, RBC, HCT and HGB indicate a possible haematological response to exercise (Kingston, 2004), as horses were physically active for five hours while being on duty. At Trial II, a significant decrease in WBC, RBC and HCT, and slight decrease in HGB might indicate a possible effect of the treatment.

MCV values differed significantly between the trials at all sampling times. Significantly lower values at Trial II might be ascribed to calmative effects. Namely, reflex splenic contractions occur in horses in response to fright, excitement, and exercise, thus increasing the numbers of young macrocytic erythrocytes in the circulating pool (Ricketts, 2004). This might explain significantly higher MCV values in untreated horses.

The study confirmed the importance of 24 hours of rest after working, as haematological parameters that changed at post-stress phase reach almost pre-transport values at resting phase.

On the contrary to haematological parameters, plasma lactate and most of the biochemical profile parameters showed the same trend of changes at both trials.

Plasma lactate was measured as an indicator of muscle fatigue and fitness (Ricketts, 2004; Rainger *et al.*, 1995). At both trials plasma lactate concentrations remained within reference ranges reported in literature (Stull and Rodiek, 2000; Rainger *et al.*, 1995; Lumsden *et al.*, 1980) at all three sampling phases, which indicates no muscular fatigue due to transport and work.

Common physiological measures of stress are cortisol, beta endorphin and heart rate (Christensen *et al.*, 2005; Stull and Rodiek, 2000; Alexander and Irvine, 1998; Grandin, 1997). In the present study, measurement of serum cortisol was used as a physiological indicator of stress. Serum cortisol did not exceed reported reference ranges for horses at both trials, at any of three sampling phases (Stull and Rodiek, 2000; Eades and Bounous, 1997). At Trial I serum cortisol showed a wide range at pre-transport ($14.3 \pm 11.2 \mu\text{g/dL}$) and post-stress phases ($5.4 \pm 4.9 \mu\text{g/dL}$). Though, there was quite a decrease at post-stress phase, no significant changes were observed. Despite obvious differences in serum cortisol between trials at the pre-transport phase, the difference was not significant, probably due to a high SD value at Trial I ($14.3 \pm 11.2 \mu\text{g/dL}$ versus 4.2 ± 1.5). At both trials changes in serum cortisol concentrations may be attributed to normal diurnal variations (Stull and Rodiek, 2000; Irvine and Alexander, 1994). Albeit, we may speculate that a more narrow spread of cortisol concentration at Trial II and significant a decrease at resting phase may reflect the effects of the calmative.

Though, some of the parameters of the biochemical profile changed significantly at both trials, all parameters, with the exception of ALT at Trial II, remained within normal reference values (Eades and Bounous, 1997; Kaneko *et al.*, 1997). The most striking change observed at both trials was a significant decrease in potassium at the post-stress phase. At Trial I potassium decreased by $52.9 \pm 26.2 \%$ and at Trial II by $58.3 \pm 21.3\%$. After 24 hours of rest, values

returned to pre-transport values at both trials. The sharp fall at the post-stress phase is probably a result of a re-entry of potassium into the intracellular compartment as it is known that soon after the beginning of activity potassium is released from muscle fibres (Coenen, 2005). There was a significantly higher potassium concentration at the pre-transport and resting phases of Trial II, which might indicate treatment effects.

Many biochemical parameters, such as urea, creatinine, sodium, potassium, chloride, proteins, albumins, AP and AST, showed the same trend of changes at both trials at post-stress phase. While being on duty horses were physically active for approximately five hours, thus changes in biochemical profile reflect exercise induced processes (Kingston, 2004). In comparison with Trial II, some of these parameters, like AP, AST, proteins and albumins, changed significantly at Trial I indicating more pronounced changes probably due to the absence of the calmative agent. Changes of sodium, chloride proteins and albumins indicate mild dehydration due to physical activity (Coenen, 2005; Kingston, 2004).

Two serum enzymes with high activity in skeletal muscles and are elevated clinically in horses with muscular diseases are CK and AST (Kingston, 2004). At all sampling times, the activities of AST and CK remained within the normal reference ranges (Eades and Bounous, 1997). An increase in AST at both trials, and CK at Trial I at the post-stress phase, indicates minimal muscular insults, as horses were physically active. AST returned to pre-transport values after 24 hours of rest. Serum CK values at Trial II showed uncommon changes due to a high SD value at pre-transport phase and reflect the horses individual response to physical activity.

During both trials, the behaviour was observed carefully by riders that are excellent observers of changes in their horses' behaviour. Police horses used in the study experience stressful events on a routine basis. Though, the police horses used are trained to react calmly, not to be fearful, in potentially frightening or stressful situations and are used to contacts with people and exposure to novelty, the study showed that horses are still fearful and flight animals. Compared to calmative-treated horses at Trial II, horses at Trial I showed changes in behaviour that can signal stress. In horses, fear is additionally problematic due to fear reactions that can cause serious injury to both the horse and rider. Christensen *et al.* (2005) showed that exposure to novelty is reflected in changes in horse's behaviour and an increase in heart rate.

In conclusion, the study showed that most changes of haematological and biochemical profile parameters reflect the response to exercise as horses were physically active while being on duty. The effect of calmative is mainly reflected by a minor extent of changes of measured parameters as determined by comparison at individual sampling phases. On the other hand, the study showed that equine calmative did calm horses according to riders' observation. Though, further investigations are needed to make conclusions concerning the calmative effects and effects on physiological parameters of tryptophan supplements in horses in general in larger groups of horses under different stressors. The study confirmed that horses need at least 24 hours of rest after work.

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REFERENCES

1. Alexander SL, Irvine CHG, 1998, The effect of social stress on adrenal axis activity in horses: the importance of monitoring corticosteroid-binding globulin capacity, *J Endocrinol*, 157, 425-32.
2. Art T, Lekeux P, 2005, Exercise-induced physiological adjustments to stressful conditions in sports horses, *Livest Prod Sci*, 92, 101-11.
3. Bagshaw CS, Ralston SL, Fisher H, 1994, Behavioural and physiological effects of orally administered tryptophan on horses subjected to acute isolation stress, *Appl Anim Behav Sci*, 40, 1-12.
4. Christensen JW, Kelling LJ, Lindestrøm Nielsen B, 2005, Responses of horses to novel visual, olfactory and auditory stimuli, *Appl Anim Behav Sci*, 93, 53-65.
5. Coenen M, 2005, Exercise and stress: impact on adaptive process involving water and electrolytes, *Livestock Prod Sci*, 92, 131-45.
6. Davis JM, 2000, Serotonin and central nervous system fatigue: nutritional considerations, *Am J Clin Nutr*, 72(suppl), 573S-578S.
7. Dienstbier RA, 1989, Arousal and physiological toughness: implications for mental and physical health, *Psychol Rev*, 96, 84-100.
8. Eades SC, Bounous DI, 1997, Significance of laboratory tests, In: Pratt PW, editor, 1st ed, St. Louis: Mosby-Year Book, 1-31.
9. Farris JW, Hinchecliff KW, McKeever KH, Lamb DR, Thompson DL, 1998, Effect of tryptophan and of glucose on exercise capacity of horses, *J Appl Physiol*, 85, 807-86.
10. Fraser SB, Richie JSD, Fraser AF, 1975, The term 'stress' in the veterinary context, *Br Vet J*, 131, 653-62.
11. Grandin T, 1997, Assessment of stress during handling and transport, *J Anim Sci*, 75, 249-57.
12. Grimmer A, Sillence MN, 2005, Calmatives for the excitable horse: A review of tryptophan, *Vet J*, 170, 24-32.
13. Harris P, 2005, Nutrition, behaviour and the role of supplements for calming horses: The veterinarian's dilemma, *Vet J*, 170, 10-1.
14. Irvine CH, Alexander SL, 1994, Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse, *Domest Anim Endocrinol*, 11, 227-38.
15. Kaneko JJ, Harvey JW, Bruss L, 1997, Appendixes, In: Kaneko JJ, Harvey JW, Bruss L, editors, 5th ed, *Clinical biochemistry of domestic animals*, San Diego: Academic Press, 885-905.
16. Kingston JK, 2004, Hematologic and serum biochemical responses to exercise and training, In: Hinchliff KW, Kaneps AJ, and Geor RJ, editors, *Equine sports medicine and surgery*, 1st ed, Edinburgh: Saunders, 939-48.
17. Lumsden JH, Rowe R, Mullen K, 1980, Hematology and biochemistry reference values for the light horse, *Can J Comp Med*, 44, 32-42.
18. Muir W, 2004, Recognizing and treating pain in horses, In: Reed SM, Bayly WM, Sellon DC, *Equine Internal Medicine*, 2nd ed, St Louis: Saunders, 1529-41.
19. Nicol C, 1999, Understanding equine stereotypies, *Equine Vet J Suppl*, 28, 20-5.
20. Ott EA, 2005, Influence of temperature stress on the energy and protein metabolism and requirements of the working horse, *Livestock Prod Sci*, 92, 123-30.
21. Paradis MR, Breeze RG, Bayly WM, Counts DF, Leagreid WW, 1991, Acute haemolytic anemia after oral administration of L-tryptophan in ponies, *Am J Vet Res*, 52, 742-7.
22. Rainger JE, Evans DL, Hodgson DR, Rose RJ, 1995, Distribution of lactate in plasma and erythrocytes during and after exercise in horses, *Br Vet J*, 151, 299-310.

23. Ricketts SW, 2004, Hematologic and biochemical abnormalities in athletic horses, In: Hinchliff KW, Kaneps AJ, Geor RJ, *Equine sports medicine and surgery*, 1st ed, Edinburgh: Saunders, 950-66.
24. Shanahan S, 2003, Trailer loading stress in horses: behavioural and physiological effects of nonaversive training (TTEAM), *J Appl Anim Welf Sci*, 6, 263-74.
25. Smith BL, Jones JH, Hornof WJ, Miles JA, Longworth KE, Willits NH, 1996, Effects of road transport on indices of stress in horse, *Equine vet J*, 28, 446-54.
26. Stull CL, Rodiek AV, 2000, Physiological responses of horses to transportation using commercial van during summer conditions, *J Anim Sci*, 78, 1458-66.
27. Stull CL, Spier SJ, Aldridge BM, Blanchard M, Stott JL, 2004, Immunological response to long-term transport stress in mature horses and effects of adaptogenic dietary supplementation as an immunomodulator, *Equine Vet J*, 36, 583-89.
28. Vervuert I, Coenen M, Watermülder E, 2005, Metabolic responses to oral tryptophan supplementation before exercise in horses, *J Animal Physiol Anim Nutr* 89, 140-5.
29. Waters AJ, Nicol CJ, French NP, 2002, Factors influencing the development of stereotypic and redirected behaviours in young horses: findings of a four year prospective epidemiological study, *Equine Vet J*, 34, 572-9.
30. Williams RJ, Marlin DJ, Smith N, Harris RC, Haresign W, Davies Morel MC, 2002, Effects of cool and humid environmental conditions on neuroendocrine response of horses to treadmill exercise, *Vet J*, 164, 54-63.

UTICAJ SREDSTAVA ZA UMIRENJE NA ODABRANE PARAMETRE KRVNE SLIKE KONJA IZLOŽENIH STRESU

NEMEC SVETE ALENKA, ČEBULJ-KADUNC NINA I KRULJC P

SADRŽAJ

Nekoliko studija koje su imale za cilj da dokažu da li suplementacija obroka triptofanom ima umirujuće efekte na konje rezultiralo je kontradiktornim nalazima i nijedna od njih nije potvrdila ovu hipotezu. Cilj naših ispitivanja je bio da analiziramo promene u vrednostima hematoloških i biohemijskih parametara, koncentraciji laktata i kortizola i promene u ponašanju konja u uslovima stresa sa i bez dodatka sredstava za umirenje (Cavalor Calm® - Nutriquine, Vitamex, Drongen, Belgium). Ispitivanja su takođe imala za cilj da potvrde značaj jednodnevnog odmora konja posle izlaganja opterećenju.

Ista grupa od 6 konja je bila dva puta izložena opterećenju pod uslovima stresa. Uzorci krvi su uzimani tri puta: pre transporta, posle stresa i u fazi odmora. Ponašanje konja su pažljivo pratili jahači.

Naši rezultati su ukazali da su zapažene promene u vrednostima hematoloških i biohemijskih parametara bile rezultat izlaganja opterećenju. Uticaj sredstava za umirenje na ove vrednosti je bio minimalan. Međutim, bile su zapažene promene u ponašanju konja u smislu njihovog smirivanja. Takođe smo potvrdili da je konjima posle većih napora neophodno bar 24 časa odmora.