INTRODUCTION
During their agonistic activities, horses would undergo a range of potential psychophysical stressors (i.e., confinement and exposition to new environmental stimuli, restraining or punitive methods, transport, social interaction with conspecifics and human beings, etc.). Even if these welfare problems are well recognized and several studies have been carried out to determine the parameters most involved and indicative of the physiological response to physical exercise in the horse (Seren and Venturini, 1972; Ferlazzo et al, 1984; Li and Chen, 1986; Church et al, 1987; Wilson et al, 1991; MacCarthy et al, 1991), still conflicting data exist. During competition, some additional stressors may exacerbate the physiological stress response related to physical effort in trotters. Psychological and conditioned components of such a response may be highlighted comparing endocrine-metabolic responses due to physical exercise with those elicited by competition.

AIM OF THE STUDY
To investigate and compare the endocrine-metabolic changes elicited by a training session and a sporting competition in trotters.

MATERIALS AND METHODS
Animals
The experiment was carried out on a group of 8 trotters, aged 2-3 years, already trained and participating to competitions. The horses were stalled in individual boxes (4 m x 4 m). Every subject, three times a week, was subjected to a training section, and occasionally was left grazing in an external paddock (about 1000 m2). They were twice a day fed with a ration composed by hay and pellet feed.

Treatments
The 8 animals were randomly subjected to two different treatments: a standard training (ST) and a sporting competition (COMP). The “standard” training (ST) consisted in: after a warm-up horses went at steady trot for 2 laps (1600 m). The sporting competition (COMP) consisted in: after a warm-up horses went at trotting race for 2 laps (1600 m).

Laboratory Analysis
From each subject, at every ST and C, blood samples were collected from the jugular vein:
• immediately after the end of ST and COMP (Tpost);
• 60 minutes after ST and C (T60m);
• 120 minutes after ST and C (T120m);
• 24 hours after the end of the ST and C (T24h).

At each time, 15 ml blood samples were collected and divided into two evacuated tubes with anticoagulant (K-ETDA), 5 ml also added with 0.05 ml of aprotinine for avoiding protein denaturation (for the β-endorphin assay). Samples were centrifuged at 2500 rpm and stored at −20°C
until analysis. Plasma were assayed for β-endorphin (Immunoassay, kit EIA Peninsula Laboratories), cortisol (Kit RIA Cortisol Orthoclinical Diagnostics), packed cell volume (PCV) by micromethod and total proteins, blood urea nitrogen (BUN), glucose, Ca, P, Fe, Mg, Cl and lactate deidrogenase (LDH) by colorimetric method (Analyser Express Plus 560, Bayer Diagnostics).

Statistics
Repeated-measures GLM was used to analyse the data for sampling time and treatments (ST vs COMP). Least square means were evaluated by t-test. Adrenal cortex and opioid responses were further evaluated by Correlation Analysis (MINITAB® Release 14).

RESULTS AND DISCUSSION
Sampling time significantly affected plasma β-endorphin, cortisol, PCV, glucose, total proteins and P concentrations (Graph 1-5, Table 1). These parameters always decreased to return to pre-exercise baseline values within T60m after ST, whereas some of them lasted elevated up to T120m (cortisol) (Graph 3) or T24h (β-endorphin) (Graph. 1) after COMP (Table 1). Higher plasma β-endorphin, cortisol, PCV, total proteins, LDH and Ca concentrations were recorded after COMP compared with ST (P<0.001). In particular, β-endorphin values recorded after COMP was remarkable greater (7.22 times) the corresponding values recorded after ST (Table 1). Such a large difference in the opioid response seems to suggest that these peptides may play an important role as indicators of the psychological component of stress.

The greater adrenal gland response, as well as the greater mobilisation of the metabolic resources (glucose, Graph 4) and the prolonged plasma LDH activity (Graph. 6) seem to confirm there is an effect of such additional psycho-physical stressors during competition, which enhance the physiological response to stress in trotters.

No correlation between plasma cortisol and β-endorphin concentrations was found, probably because of a delay of the cortisol response compared to the β-endorphin one.

CONCLUSIONS
The Standard Training acted as a mild stress to the trotters, always inducing a transient but significant increase of the circulating cortisol and β-endorphin. However, such a physiological response to stress was enhanced after sporting competition in trotters. Plasma β-endorphin, cortisol, glucose and LDH concentration increases were significantly greater after COMP than after ST.

The opioid response showed a dramatic raise after COMP, suggesting that these neuropeptides could be used as a key indicator for assessing the effect of the psycho-physical stressors acting during competition.

REFERENCES


ACKNOWLEDGEMENTS
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Graph 1. Mean plasma β-endorphin concentrations for treatments and sampling time

Graph 2. Mean PCV values for treatments and sampling time

Graph 3. Mean plasma cortisol concentrations for treatments and sampling time

Graph 4. Mean plasma glucose concentrations for treatments and sampling time

Graph 5. Mean plasma total protein concentrations for treatments and sampling time

Graph 6. Mean plasma LDH activity for treatments and sampling time
<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Tpost</th>
<th>T60m</th>
<th>T120m</th>
<th>T24h</th>
<th>Time</th>
<th>ST vs HST</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-endorphin (ng/ml)</td>
<td>1.04±1.14A</td>
<td>7.48±1.14AB</td>
<td>0.65±1.14B</td>
<td>0.61±1.14</td>
<td></td>
<td>ST &lt;</td>
</tr>
<tr>
<td>Cortisol (μg/d)</td>
<td>17.29±2.15A</td>
<td>20.19±2.15AA</td>
<td>13.56±2.15AA</td>
<td>21.69±2.15AB</td>
<td>*</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>52±1.83A A</td>
<td>66±3.66AB</td>
<td>43±1.83B</td>
<td>43±3.66B</td>
<td>30±5.17CA</td>
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</tr>
<tr>
<td>Tot. proteins (g/l)</td>
<td>64.12±1.78AA</td>
<td>70.82±2.26AB</td>
<td>6130±1.15b</td>
<td>61.64±1.45B</td>
<td>56.68±1.60CA</td>
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<tr>
<td>BUN (mmol/l)</td>
<td>5.76±0.62</td>
<td>5.79±0.78</td>
<td>5.81±0.63</td>
<td>5.85±0.79</td>
<td>5.81±0.57</td>
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<tr>
<td>Glucose (mmol/l)</td>
<td>3.01±0.50A</td>
<td>5.85±0.99AB</td>
<td>2.23±0.42</td>
<td>3.28±0.83B</td>
<td>2.26±0.20</td>
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<td>Ca (mmol/l)</td>
<td>2.54±0.06A</td>
<td>2.90±0.07B</td>
<td>2.72±0.13</td>
<td>2.88±0.16</td>
<td>2.69±0.10</td>
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<tr>
<td>P (mmol/l)</td>
<td>1.53±0.11A</td>
<td>1.58±0.11A</td>
<td>1.22±0.11B</td>
<td>1.11±0.11B</td>
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<tr>
<td>Fe (μmol/l)</td>
<td>46.52±12.98</td>
<td>65.26±16.44</td>
<td>46.04±12.56</td>
<td>53.60±15.89</td>
<td>44.53±15.19</td>
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<td>Mg (mmol/l)</td>
<td>1.93±0.08</td>
<td>2.10±0.10</td>
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<td>Cl (mmol/l)</td>
<td>105.19±1.04</td>
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<td>LDH (IU/l)</td>
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<td>1111±183.86Ra</td>
<td>954±77.25</td>
<td>1110±97.71</td>
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