Cardio-respiratory, haematological and biochemical parameter adjustments to exercise: effect of a probiotic in horses during training

T Art *, D Votion, K McEntee, H Amory, A Linden, R Close, P Lekeux

Centre de Médecine Sportive Équine, Faculté de Médecine Vétérinaire, Université de Liège, Bât B42, Sart-Tilman, B-4000 Liège, Belgium

(Received 8 September 1993; accepted 5 January 1994)

Summary — Two randomly distributed groups of thoroughbred horses were compared during a 12-week period for their cardio-respiratory and metabolic adjustment to strenuous exercise, training and detraining. The horses were trained following the same standardized schedule and were regularly investigated using standardized treadmill exercise tests (SET) of increasing speed. After the first SET and during the whole experimental period, a group of 6 horses received a probiotic (Bioracing®) once a day while a group of 5 horses received a placebo. All other conditions were similar for both groups. During each SET, the oxygen uptake, carbon dioxide output, tidal volume (inspired volume), respiratory rate and expired minute volume were obtained using 2 ultrasonic pneumotachographs and a mass spectrometer. All the parameters were the mean of the values calculated during the last 20 s of the SET. Heart rate was continuously measured with a polar heart rate meter. Venous blood was sampled before and after the test and analyzed for various biochemical parameters. In both groups, training induced significant modification in most of the cardio-respiratory parameters, i.e. peak oxygen uptake, peak carbon dioxide output, respiratory exchange ratio, ventilation/min to oxygen-uptake ratio and oxygen-uptake to heart-rate ratio. After the 3-week detraining period, most of the values were again similar to the pre-training values in both groups. However, the training-induced modifications of most of the cardio-respiratory parameters occurred earlier and were proportionally greater in the probiotic-treated group than in the control. The respiratory coefficient decreased in the control but not in the treated group. All other parameters changed similarly in both groups. This suggests that Bioracing® could modify the physiological effects of training by improving some aerobic metabolic capacities for carbohydrate utilization, but that this effect occurs only during training and not during periods of physical inactivity.

training / thoroughbred horse / exercise / probiotic

* Correspondence and reprints
Résumé — Adaptation durant l'exercice des paramètres cardio-respiratoires, hématologiques et biochimiques chez le cheval. Effet de l'entraînement et d'un probiotique. L'influence de l'entraînement et du désentraînement sur les adaptations cardio-respiratoires et métaboliques lors d'un effort intense a été étudiée dans 2 groupes de pur-sang, distribués au hasard. Les 2 groupes ont été entraînés suivant un même schéma strictement standardisé. Ils ont régulièrement subi un test d'effort standardisé, d'intensité croissante, sur tapis roulant (SET). Après le premier SET, le groupe traité (n = 6) a reçu quotidiennement un probiotique (Bioracing®), tandis que le groupe contrôle (n = 5) recevait un placebo. Au cours de chaque SET, la consommation d'oxygène, la production de dioxyde de carbone, le volume courant (volume d'air inspiré), la fréquence respiratoire et le volume d'air expiré par minute étaient mesurés par le biais de pneumotachographes ultrasoniques et d'un spectromètre de masse. Les données ont été calculées durant les 20 dernières secondes du SET. La fréquence cardiaque était enregistrée en continu grâce à un cardiofréquencemètre. Du sang veineux prélevé avant et après le SET était analysé pour différents paramètres biochimiques. Dans les 2 groupes, l'entraînement a modifié significativement le plupart des paramètres cardio-respiratoires, c'est-à-dire les pics de consommation d'oxygène et de production de dioxyde de carbone, le coefficient respiratoire, le volume d'air ventilé par minute rapporté à la consommation d'oxygène et la consommation d'oxygène rapportée à la fréquence cardiaque. Après 3 sem de désentraînement, la majorité des paramètres étaient revenus à leur valeur initiale. Les modifications des paramètres cardio-respiratoires induites par l'entraînement sont apparues de façon plus précoce dans le groupe traité et, en outre, ces modifications étaient proportionnellement plus importantes que dans le groupe contrôle. En revanche, il n'y avait aucune différence significative entre les 2 groupes concernant les paramètres biochimiques. Ces résultats suggèrent que l'administration du Bioracing® pourrait modifier les adaptations à l'entraînement en améliorant la capacité à utiliser les hydrates de carbone par la fibre aérobie, mais que cet effet n'existerait que chez les chevaux en activité physique régulière et pas chez les chevaux au repos.

entraînement / pur-sang / test d'effort standardisé / probiotique

INTRODUCTION

Probiotics are substances used as feed supplements in order to attempt to improve animal performance (Llirley and Stilwell, 1965). As their name suggests, they promote biotic groups to the detriment of others. Under such conditions, the encouraged bacteria contribute to the suppression of the less favourable bacteria by active competition. Generally, probiotics are living organisms and therefore labile under heat, pelleting or long-term storage. Furthermore, because they are living systems, variable results may be obtained after administration (Llirley and Stilwell, 1965).

In comparison with the recent application of these living microorganisms, yeast (brewers' yeast, bakers' yeast) has always represented a classical feed supplement in the field of human and animal nutrition. The high vitamin content, especially that of the B12-complex, partially explains the beneficial effect of yeast (Glade and Biesik, 1966). In the last decades, however, other yeast compounds have been described as useful nutritional elements. On this basis, a new group of probiotics has been developed, in which thermolyzed yeast acts as a carrier for a specific selection of isolated yeast components. Bioracing®, intended for sports horses, represents a commercially available product within this new group of probiotics. It consists of a biologically active complex of metabolites of the intermediary metabolism, basically organic acids of the citric-acid cycle (Krebs cycle) and the aspartic cycle. Yeast-RNA and nucleic acid bases and oligonucleotides (European Patent, EP 0 107 161 B1) represent the other class of isolated yeast component forming the active complex. While the efficiency of this feed supplement on growth and resistance
against infection has been demonstrated in poultry and rodents (Ramadan et al., 1989, 1991; Burmeister and Rainsford, 1991), its possible effect on the horse’s exercise potential has never been studied in standardized conditions. A previous study has demonstrated that training in horses induces an increase in their maximal oxygen consumption, carbon dioxide output, respiratory coefficient, oxygen pulse and a decrease in their ventilatory equivalent for oxygen (Art and Lekeux, 1993).

The present work is aimed at evaluating the effects of the possible influence of the administration of this probiotic on these previously described training-induced adjustments.

MATERIALS AND METHODS

Horses

Eleven healthy thoroughbred horses were studied. They were randomly assigned into 2 groups, 6 horses in the treated group (1 stallion, 2 geldings and 3 mares; weight: 500.2 ± 15.5 kg (x ± SEM); age: 4.5 ± 0.7 yr) and 5 horses in the control group (2 stallions, 3 mares; weight: 500.2 ± 16.3 kg; age: 4.0 ± 0.3 yr). All horses were free of known respiratory or muscular diseases. A careful clinical examination (including endoscopic examination of the airways, pulmonary function tests, arterial blood gas analysis and electrocardiogram) was performed to assess their soundness. They were housed in 4 x 4 m climatized box stalls during the whole experimental period.

The horses were fed twice a day with oats and hay. Samples of hay and oats were collected and analyzed to check their composition. The feed requirements were calculated according to the individual needs for maintenance and exercise (National Research Council, 1989). Calcium (ground limestone) and salt (Electrol, Aesculaap, Belgium) were added in order to avoid Ca/P and electrolyte imbalance due to sweat losses. Water was available ad libitum at all times.

Before the start of the experiment, the horses were dewormed and vaccinated against influenza and tetanus.

Experimental and training schedules

The whole experimental period was divided into 5 periods, each lasting 3 weeks.

Acclimatization

During this period, the horses were acclimatized to their new housing and adapted to the treadmill work and the laboratory procedures. At the end of this 1st period, they underwent the first standardized exercise test (SET1). Once SET1 had been performed and up to the end of the experiment, the horses of the treated group received Biorizing® (2 g/100 kg) per day while the horses of the control group received a placebo.

Phase 1

The horses were lunged daily (trot and gallop), for 20 min and 6 d/week, in a 50-m-diameter paddock. The 2nd SET was performed on the last day (SET2).

Phase 2

The horses were trained 3 d/week on the treadmill inclined with a 3° slope. After a 5-min period walk, they worked for 15 min, divided as follows. The 1st day, they trotted 14 min 30 s at a speed of 4 m/s and cantered 30 s at a speed of 8 m/s (total run distance: 3 700 m).

Each further day of treadmill work, they performed 30-s trot (4 m/s) less and 30-s canter (8 m/s) more. Accordingly, the last day, the trot period was reduced to 10 min 30 s and the canter period increased to 4 min 30 s (total run distance: 4 700 m). The horses were equipped with a heart rate monitor, PEH 100 (Horse Tester, AMG, Wilrijk, Belgium). This device continuously gave the heart rate, which ranged between 155 and 180 beats/min during the canter. Plasma lactate was regularly measured after the training and never exceeded 4 mmol/l. The treadmill training day alternated with a 30 min lunging training, 3 d/week. The horses were lunged for 15 min in both directions, with the same duration and speed of trotting and cantering as in the treadmill exercise. They rested 1 d/week. The third SET ended this period (SET3).
Phase 3

The final 3 weeks of training were based on an interval training approach with 3 d when the horses exercised for 3 bouts of 600 m at 10 m/min with a slope of 4° (1st week); 3 bouts of 600 m at 10 m/min with a slope of 6° (2nd week); 1 bout of 600 m at 10 m/min and 2 bouts of 600 m at 11 m/min with a 6° slope (3rd week). A 2 min walk period alternated with the rides. The horses were walked for 15 min after the training. The heart rate was controlled using the horse tester and was above 200 beats/min during the gallops. Furthermore, plasma lactate was regularly controlled after the training and ranged between 6 and 8 mmol/L. The 3 other days of the week, the horses were walked and trotted 20 min in the paddock. They rested 1 d/week. The 4th SET (SET4) was performed at the end of this 3-week period. One horse of the treated group had to be discarded for this test because of a problem unrelated to the training.

Detaining

The horses walked 15 min daily during 3 weeks, and the 5th SET (SET5) ended the whole experimental protocol.

Standardized exercise test

Exercise studies were carried out on a treadmill (Satô, Sweden) in a laboratory in which the temperature was maintained at 19–21°C with 55–65% humidity. All horses were randomly investigated the same day (between 8.00 am and 1.00 pm).

After a 10-min warm-up period (5 min walk and 5 min trot), the treadmill was inclined at 6°. The test consisted of 6 incremental exercise periods of 1 min each at 1.7, 4.0, 8.0, 9.0, 10.0 and 11.0 m/s. Following the completion of the exercise test, the treadmill was lowered to the level position and the horses walked for 2 min further. After the blood sampling, the horses were bathed and walked for the next 15 min.

Measurements

Ventilatory parameters

Respiratory airflow from each nostril was measured using a pneumotachometer composed of 2 ultrasonic flow transducers mounted diagonally across a polyvinyl chloride tube (55 mm internal diameter, 10 cm long) (British Patent application 8808906, 1988). Each pneumotachometer had a full-scale range of −60 to +60 l·s⁻¹ (giving a total peak flow range of −120 to +120 l·s⁻¹). The linearity and symmetry of the output of both ultrasonic pneumotachometers was regularly checked (Art et al., 1990).

Both tubes were mounted in a lightweight mask, and were positioned so as to be in line with the normal flow of air from the nostril. This reduced any extra added dead space and, as the flow was not subject to constriction or re-direction, pressure loading on the ventilatory system was minimized. The mask was constructed from fibre glass and fitted closely to the muzzle of the horse, leaving the mouth free. Care was taken to ensure that the mask did not hinder the normal movement of the nostrils during exercise. A silicon rubber and foam gasket provided an airtight seal between the mask and the horse, and also separated the nostrils (Woolacott et al., 1987). The mask and flow tubes together weighed 0.7 kg. Tidal volume (V̇T) was obtained by numerical integration of the flow signal and was electronically corrected to body temperature and pressure, saturated with water vapour conditions. Before and after each experiment, the pressure transducer was calibrated using a water manometer and the flow tubes were individually calibrated with a rotameter (KD1 Flowmeters, Burgess Hill, UK).

Oxygen consumption (V̇O₂) and carbon dioxide output (V̇CO₂)

A mass spectrometer (MGA 2000 Airspec, Biggin Hill, Kent, UK) was used to sample air in 1 flow tube, the sampling capillary being positioned 2 cm from the open end of the tube, and thus continuously measured O₂ and CO₂ concentrations in the expired respiratory gases on a breath-by-breath basis. Airflow signals from each nostril and respiratory O₂ and CO₂ concentrations signals underwent analog-to-digital conversion and were recorded using a data acquisition system. Tidal volume, expired minute volume (V̇E(body temperature and pressure, saturated with water vapour)) V̇O₂ (standard temperature and pressure, dry) and V̇CO₂ (standard temperature and pressure, dry) were instantaneously calculated by an on-line and breath-by-breath computer analysis (Chest Scientific Instruments, UK) of
these respiratory signals, using the method previously described by Beaver et al. (1973). The delay between the gas concentration input signals recorded from the mass spectrometer and the flow signals was corrected as previously described (Art et al. 1990). Before each test, the mass spectrometer was calibrated using gas mixtures of known composition.

**Measurement of heart rate (HR)**

The horses were equipped with a heart-rate meter. The heart rates were continuously stored throughout each investigation and recovery period. HR was averaged over consecutive periods of 5 s. After each experiment, the stored data were displayed on a microcomputer.

**Blood parameters**

Venous blood was sampled by jugular vein puncture just before and 2 min after the completion of the test. Three vacuum collecting tubes were used: 1 silicone-coated, 1 containing sodium heparin and sodium monooiodacetate, and 1 EDTA-coated. Moreover, venous blood was drawn anaerobically for pH determination into 2-ml syringes whose deadspace was filled with sodium heparin.

The serum was analyzed for total protein, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), activities of serum lactate dehydrogenase (LDH) and LDH isoenzymes, creatine phosphokinase (CK), aspartate aminotransferase (AST), and cortisol (COR) determination.

Plasma samples were used for glucose (GLU) and lactate (LA) determinations. The white blood cells (WBC) as well as the proportion of granulocytes (grans) were measured by the quantitative buffy coat veterinary hematology method (Linden et al. 1991).

Finally, the blood taken was used to measure the haemoglobin content (Hb). Haematologic determination were carried out within several minutes of collection. The other tubes were immediately centrifuged and blood biochemistry was examined within 2-8 h.

**Analysis procedure**

Plasma electrolytes concentration was assessed by colorimetry (Merk). Serum activities in CPK, AST and LDH were measured by reactive rate analysis at pH 7.0 and 37°C, using the colorimetric method (Merkx).

Total serum protein was measured by refractometry (Refractometer Analyt, Nurnur, Belgium). Blood glucose and LA were determined by colorimetric methods (Merk and Boeringer, respectively). Cortisol was obtained using a radioimmunoassay (RIA) method (gamma coat [125I] cortisol radioimmunoassay kit, Baxter Travenol Diag Inc). The assay detection limit was 6.6 nmol/L. Haemoglobin determination was performed by a blood gas analyser (AVL 990Hb, VEL, Louvain, Belgium).

**Other measurements**

The horses were weighed before each SET. The rectal temperature (°C) was measured before and 2 min after the SETs.

**Calculations**

On the basis of the respiratory measurements, the following ventilatory and metabolic parameters were calculated: $\dot{V}_E$, $\dot{V}_O_2$, $\dot{V}_CO_2$; the respiratory exchange ratio (R); the $\dot{V}_O_2/\dot{V}_E$ and the $\dot{V}_O_2/HR$ ratios. All these parameters were the mean of the values calculated during the last 20 s of the last stop of the SET (11 m/s). Because some of the horses did not reach their $\dot{V}_O_2$ max even during this last step, the values were qualified as peak values rather than maximal values. Heart rate was measured at 11 m/s (peak HR), and after 2 min recovery (HR$_{rec}$).

**Statistical analysis**

Results are reported as mean ± SEM. Analysis of variance was used to examine the effects of training and treatment. The values significantly modified by the training were analysed to further assess the effect of treatment as follows: the values of SET2, 3, 4 and 5 were divided by the values of SET1, in order to have the relative changes for each set of data, in %/100. Comparison between the mean relative changes of both group was performed, throughout each SET, by a Student's t test. The probability of 0.05 was chosen as the significant level.
RESULTS

The baseline values, i.e. at SET1, were as follows: peak $\dot{V}O_2$: 110.8 ± 6.3 and 126.9 ± 6.0 ml.kg$^{-1}$.min$^{-1}$ in the treated and control groups, respectively; peak $\dot{V}CO_2$: 128.6 ± 9.9 and 148.1 ± 9.4 ml.kg$^{-1}$.min$^{-1}$ in the treated and control groups, respectively; peak $R$: 1.16 ± 0.05 in both groups; $V_{E}/V_{O_2}$: 33.7 ± 1.6 and 31.0 ± 3.3 l/min in the treated and control groups, respectively; and $V_{O_2}$/HR: 260 ± 8 and 288 ± 18 ml.kg$^{-1}$.beat$^{-1}$ in the treated and control groups, respectively. The comparison of the values between the treated and the control groups indicated that there were significant differences regarding the changes induced by training for peak $\dot{V}O_2$, peak $\dot{V}CO_2$, $R$, $V_{E}/V_{O_2}$, $V_{O_2}$/HR (figs 1 and 2). The horses of the treated group showed an earlier and greater increase in their peak $\dot{V}O_2$, peak $\dot{V}CO_2$ and $V_{O_2}$/HR; while their $R$ remained unchanged; their $V_{E}/V_{O_2}$ decreased faster than in the control group (figs 1 and 2).

In both groups, $V_{E}$, $V_{R}$, and the peak HR remained unchanged. Horses of both groups showed a regular and progressive weight loss. The exercise-induced change in rectal temperature was significantly reduced as training progressed (table I).

Most of the haematological (i.e., HB, WBC, granulocytes) and biochemical parameters (glucose, LA, ions) evaluated before and after the SETs were not significantly influenced by the training, except for the changes in cortisol (table I).

Both the resting and the exercise-induced changes in enzymatic activities for CK, LDH and AST were influenced by training, namely at SET4, i.e. after the interval training period in both groups (table I). There were no differences between the 2 groups regarding these biochemical blood parameters.

Detraining induced a return to the baseline values for all the measured data in both groups.

Fig 1. Peak O$_2$ uptake (peak $\dot{V}O_2$), CO$_2$ output (peak $\dot{V}CO_2$) and respiratory exchange ratio (peak R) recorded in thoroughbred horses during the last step of a standardized exercise before training (SET1), during different stages of a 9-week training period (SET2, SET3, SET4; see text for details) and after 3 weeks detraining (SET5). The treated group (D) (n = 6) received daily a probiotic and the control (C) group (n = 6) a placebo.

Values of SET2, 3, 4 and 5 are related to the value of SET1. Data are therefore expressed in %/100. The difference between both groups, for each SET, has been tested with a Student's t-test and P ≤ 0.05 has been chosen as the significant level (*).
While most of the cardio-respiratory measurements were modified by training, most of the blood parameters, including the post-exercise blood lactate, remained unchanged throughout the whole experimental period. This indicates the higher sensitivity of the cardio-respiratory measurements, compared with blood analyses, to detect the changes in the athletic capability. Other studies, previously performed, also reported the high repeatability and reliability of the cardio-respiratory parameters for the assessment of performance potential, especially when compared with the LA measurements (Evans and Rose, 1988a; Seeherman and Morris, 1990).

The exercise-induced increase in cortisol was smaller in trained subjects. This training adjustment has been also reported in human athlete (Shephard and Sidney, 1975), but another study, performed in horses, failed to point out any changes with training (Church et al., 1987). The differences in the training period length (2 months) and the treadmill test intensity could largely explain this difference.

The probiotic did not influence the haematological parameters or the plasmatic enzymatic activities before and after exercise: all these measurements evolved similarly in both groups. On the contrary, the treated group showed earlier and greater training-induced modifications of the peak \( \dot{V}_O_2 \), peak \( \dot{V}_CO_2 \), \( \dot{V}_E/\dot{V}_O_2 \), \( \dot{V}_O_2/HR \) than the control one.

The respiratory quotient is a parameter basically used to evaluate the substrate(s) used by the muscles during exercise. The calculation of \( R \) is based on the assumption that the exchange of \( O_2 \) and \( CO_2 \) measured at the lung reflects the actual gas exchange from nutrient metabolism in the muscular cell. Under steady-state conditions, this assumption is reasonably valid, while in some particular situations, \( R \) may no longer reflect the actual muscular gas exchange. For example, human athletes
Table I. Data recorded in thoroughbred horses during a standardized exercise before training (SET1), at different stages of a 9-week training period (SET2, SET3, SET4: see text for details) and after 3 weeks detraining (SET5).

<table>
<thead>
<tr>
<th>Values</th>
<th>Group</th>
<th>SET1</th>
<th>SET2</th>
<th>SET3</th>
<th>SET4</th>
<th>SET5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak ( V_r ) (l)</td>
<td>T</td>
<td>16.4 ± 1.2</td>
<td>14.5 ± 0.4</td>
<td>14.0 ± 0.4</td>
<td>14.2 ± 0.4</td>
<td>15.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>15.9 ± 0.8</td>
<td>15.3 ± 0.2</td>
<td>13.8 ± 0.5</td>
<td>15.6 ± 0.6</td>
<td>15.8 ± 1.1</td>
</tr>
<tr>
<td>Peak ( \dot{V}_E ) (l/min)</td>
<td>T</td>
<td>1846 ± 86</td>
<td>1968 ± 41</td>
<td>1622 ± 49</td>
<td>1708 ± 41</td>
<td>1880 ± 55</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1922 ± 73</td>
<td>1791 ± 54</td>
<td>1680 ± 42</td>
<td>1848 ± 90</td>
<td>1837 ± 120</td>
</tr>
<tr>
<td>Peak HR (beats/min)</td>
<td>T</td>
<td>213 ± 4</td>
<td>212 ± 4</td>
<td>212 ± 4</td>
<td>210 ± 4</td>
<td>212 ± 4</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>214 ± 3</td>
<td>214 ± 4</td>
<td>212 ± 4</td>
<td>214 ± 5</td>
<td>215 ± 5</td>
</tr>
<tr>
<td>HR(_{rec}) (beats/min)</td>
<td>T</td>
<td>105 ± 7</td>
<td>110 ± 4</td>
<td>105 ± 4</td>
<td>102 ± 7</td>
<td>107 ± 5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>106 ± 5</td>
<td>108 ± 2</td>
<td>103 ± 3</td>
<td>99 ± 3</td>
<td>104 ± 3</td>
</tr>
<tr>
<td>( d) T(^\circ) (^\text{C})</td>
<td>T</td>
<td>1.32 ± 0.12</td>
<td>1.03 ± 0.08</td>
<td>1.95 ± 0.16</td>
<td>0.96 ± 0.11</td>
<td>1.17 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.74 ± 0.10</td>
<td>1.10 ± 0.05</td>
<td>1.32 ± 0.19</td>
<td>0.97 ± 0.20</td>
<td>1.64 ± 0.17</td>
</tr>
<tr>
<td>Weight</td>
<td>T</td>
<td>500.2 ± 15.5</td>
<td>497.7 ± 13.9</td>
<td>493.7 ± 15.3</td>
<td>484.0 ± 19.3</td>
<td>479.6 ± 16.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>500.2 ± 16.3</td>
<td>500.7 ± 15.8</td>
<td>495.5 ± 14.3</td>
<td>493.4 ± 13.4</td>
<td>484.2 ± 16.4</td>
</tr>
<tr>
<td>dCOR (mmol/l)</td>
<td>T</td>
<td>145.4 ± 25.8</td>
<td>92.5 ± 20.5</td>
<td>89.2 ± 5.6</td>
<td>78.2 ± 8.6</td>
<td>109.5 ± 24.5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>103.2 ± 29.0</td>
<td>69.0 ± 22.7</td>
<td>74.5 ± 18.4</td>
<td>82.2 ± 36.1</td>
<td>82.6 ± 28.5</td>
</tr>
<tr>
<td>LA (mmol/l)</td>
<td>T</td>
<td>10.9 ± 1.6</td>
<td>11.4 ± 1.1</td>
<td>9.3 ± 1.6</td>
<td>9.6 ± 1.2</td>
<td>9.3 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>8.4 ± 0.7</td>
<td>8.0 ± 0.4</td>
<td>8.3 ± 0.9</td>
<td>8.9 ± 1.1</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>CPK(_{rest}) (IU/l)</td>
<td>T</td>
<td>213 ± 17</td>
<td>176 ± 10</td>
<td>261 ± 83</td>
<td>487 ± 221</td>
<td>219 ± 61</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>184 ± 19</td>
<td>179 ± 10</td>
<td>201 ± 25</td>
<td>169 ± 365</td>
<td>235 ± 44</td>
</tr>
<tr>
<td>dCPK (IU/l)</td>
<td>T</td>
<td>54 ± 24</td>
<td>40 ± 4</td>
<td>42 ± 19</td>
<td>261 ± 110</td>
<td>66 ± 18</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>29 ± 8</td>
<td>86 ± 52</td>
<td>23 ± 11</td>
<td>504 ± 217</td>
<td>84 ± 19</td>
</tr>
<tr>
<td>AST(_{rest}) (IU/l)</td>
<td>T</td>
<td>312 ± 50</td>
<td>292 ± 19</td>
<td>327 ± 45</td>
<td>552 ± 166</td>
<td>558 ± 120</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>331 ± 36</td>
<td>373 ± 74</td>
<td>433 ± 93</td>
<td>688 ± 166</td>
<td>823 ± 240</td>
</tr>
<tr>
<td>dAST (IU/l)</td>
<td>T</td>
<td>43 ± 7</td>
<td>78 ± 33</td>
<td>83 ± 11</td>
<td>76 ± 17</td>
<td>93 ± 23</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>31 ± 11</td>
<td>66 ± 20</td>
<td>35 ± 18</td>
<td>147 ± 68</td>
<td>98 ± 95</td>
</tr>
<tr>
<td>LDH(_{rest}) (IU/l)</td>
<td>T</td>
<td>525 ± 43</td>
<td>434 ± 41</td>
<td>343 ± 53</td>
<td>511 ± 70</td>
<td>427 ± 47</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>442 ± 28</td>
<td>389 ± 41</td>
<td>343 ± 34</td>
<td>575 ± 123</td>
<td>416 ± 28</td>
</tr>
<tr>
<td>dLDH (IU/l)</td>
<td>T</td>
<td>91 ± 27</td>
<td>78 ± 19</td>
<td>103 ± 23</td>
<td>130 ± 35</td>
<td>116 ± 23</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>65 ± 16</td>
<td>94 ± 11</td>
<td>98 ± 17</td>
<td>152 ± 94</td>
<td>91 ± 20</td>
</tr>
</tbody>
</table>

Data are given as mean ± SEM and were analysed by an analysis of variance. In both groups, there was a significant effect of training on HR\(_{rec}\), \( d\) T\(^\circ\) \(^\text{C}\), weight, dCOR, both resting and differences in enzymatic activities. There were no significant differences in these training adjustments between the treated and control group. Abbreviations: peak \( V_r \), peak tidal volume; peak \( \dot{V}_E \), peak minute volume; peak HR, peak heart rate; HR\(_{rec}\), heart rate after 2 min recovery; Weight, weight before the standardized test; \( d\) T\(^\circ\) \(^\text{C}\), rectal temperature variation; dCOR, cortisol variation; LA, plasma lactate; CPK\(_{rest}\), enzymatic activity for creatin phosphokinase at rest; dCPK, difference between pre-SET and post-SET values in enzymatic activities for creatin phosphokinase; AST\(_{rest}\), enzymatic activity for aspartate amino-transferase at rest; dAST, difference between pre-SET and post-SET values in enzymatic activities for lactate dehydrogenase; AST\(_{rest}\), enzymatic activity for aspartate amino-transferase at rest; dAST, difference between pre-SET and post-SET values in enzymatic activities for lactate dehydrogenase; AST\(_{rest}\), enzymatic activity for aspartate amino-transferase at rest; dAST, difference between pre-SET and post-SET values in enzymatic activities for lactate dehydrogenase; dLDH, difference between pre-SET and post-SET values in enzymatic activities for lactate dehydrogenase;}
exercising intensively, hyperventilate. This
overbreathing increases the CO₂ elimina-
tion which is not accompanied by an
increase in V̇O₂, resulting in an increase in R
which cannot be attributed to the oxidation
of foodstuffs. This does not occur in heavily
exercising horses, which do not hypover-
ventilate and on the contrary tend to hypoventi-
late and to become hypercapnic (Bayly et
al, 1989). However, during intense exercise,
the buffering of the produced lactic acid may
also be responsible for a nonmetabolic pro-
duction of CO₂ and consequently for a rise
of R above 1.00.

Training enhances free fatty acid utili-
ization, and consequently R decreases in
trained horses (Evans and Rose, 1988b;
Art and Lekeux, 1993). The peak R of the
horses of the control group decreased with
training, as expected and in accordance
with the modifications previously reported
(Evans and Rose, 1988b; Art and Lekeux,
1993). By contrast, the peak R in the
treated group remained unchanged
throughout the training period. Because
these horses were not more acidic than the
horses of the control group, this sug-
gests that in the treated horses, the car-
bohydrates remained the main substrate
for energy, even after training. This sugges-
ted that the possible effects of the pro-
biotic are mainly related to an improve-
ment, either in the carbohydrate aerobic
enzymatic capacity, and/or in the carbo-
hydrate utilization.

This study examined the physiological
effects of a probiotic on sport horses during
training, and consequently, did not aim at
determining the mechanisms underlying
the possible metabolic modifications. Con-
sequently, the attempts to explain the dif-
fences of adaptation to training between
the treated and the control group remain
strictly hypothetical. Each component of
the probiotic may directly or indirectly play
a physiological role. For example, an indi-
rect influence may occur from alterations
in the function of intestinal bacterial flora
(Lilley and Stilwell, 1965). On the other
hand, the RNA and nucleotide components
from yeast exhibit immune stimulation in
mice, which suggests that they probably
interfere with protein metabolism (Van
Yeast by itself has been shown to increase
feed efficiency and increase nitrogen retain-
tion in horses by a still unexplained mech-
anism (Glade, 1984; Glade and Biesik,
1996). Lastly, the organic acids and namely
the organic acids of the citric acid cycle
probably also play a role in the metabolic
adaptations occurring in horses receiving
the probiotic. Indeed, assuming that there is
adequate intestinal transport and resorp-
tion of these organic acids, they could
directly enter the Krebs' cycle either as sub-
strates, or as intermediary acids, conse-
quently enhancing the aerobic capacity of
the muscular cells. For example, the citric
acid can be oxidized providing 12 ATP, and,
once transformed in oxaloacetic acid, can
be recycled in the citric acid cycle by joining
an acetyl-Co-A. This role of the organic
acids in the citric acid cycle could also
explain why the glycolytic aerobic capacity
seemed to be more enhanced propor-
tionally in the treated than in control
group, and why it remained the main source
for providing energy, even in the trained
horses.

Detraining induced a fast return to the
baseline values in both groups. This
emphasizes the reversible nature of the
effects of the probiotic, and the fact that
this effect occurs only when the muscular
metabolism is regularly solicited, i.e during
training.

It was concluded that the probiotic used
in the present experiment could modify the
expected effects of training by enhancing
the carbohydrate aerobic metabolic capac-
ities, but that this effect seems to occur only
during training and not during periods of
physical inactivity.
ACKNOWLEDGMENTS

The authors thank M Delacroix, D Delcorde, V Faxonnier, M Isak and A Schmidt who assisted with the investigations and helped with the thoroughbred horses. They are also very grateful to JC Leroy for the blood analyses, to P Leroy for statistical advice, JM Godeau, P Leuthard and P Köppel for scientific advice, B de Morge and P Fernandez for providing the horses and C Gresse for typing the manuscript. Biocacing was provided by Chemoforma, Auggst, Switzerland.

REFERENCES


