Original article

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

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 horse tester. 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, *ie* 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

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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 ultra-soniques 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 la 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 filière 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 (Lilley 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 (Lilley 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, 1986). 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 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, *ie* 6 horses in the treated group (1 stallion, 2 geldings and 3 mares; weight: 500.2 ± 15.5 kg ($x \pm$ 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 Bioracing® (2 g/100 kg) *po* daily 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 meter, 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 lungeing 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 10m/s and 2 bouts of 660 m at 11 m/min with a 6° slope (3 d week). A 2-min walk period alternated with the heats. 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.

Detraining

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 (Satö, 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 external diameter, 10 cm long) (British Patent application 8608906, 1986). Each pneumotachometer had a full scale range of -60 to $+60$ $l \cdot s^{-1}$ (giving a total peak flow range of -120 to $+120$ $l \cdot s^{-1}$). 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 (Woakes *et al*, 1987). The mask and 2 flow tubes together weighed 0.7 kg. Tidal volume (V_T) 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 (KDG Flowmeters, Burgess Hill, UK).

Oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide output ($\dot{V}_{CO_2}$)

A mass spectromer (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 continually measured O_2 and CO_2 concentrations in the expired respiratory gases on a breath-by-breath basis. Airflow signals from each nostril and respiratory O_2 and CO_2 concentrations signals underwent analog-to-digital conversion and were recorded using a data acquisition system. Tidal volume, expired minute volume (V_E(body temperature and pressure, saturated with water vapour)) $\dot{V}_{O_2}$ (standard temperature and pressure, dry) and $\dot{V}_{CO_2}$ (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 monoiodoacetate, 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 corticol (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 2–6 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 (Merck).

Total serum protein was measured by refractometry (Refractometer Analis, Namur, Belgium). Blood glucose and LA were determined by colorimetric methods (Merck and Boeringher, respectively). Cortisol was obtained using a radioimmunoassay (RIA) method (gamma coat [^{125}I] cortisol radioimmunoassay kit; Baxter Travenol Diagn Inc). The assay detection limit was 6.6 nmol/l. Haemoglobin determination was performed by a blood gas analyser (AVL950Hb, VEL, Louvain, Belgium).

Other measurements

The horses were weighed before each SET. The rectal temperature ($T°$) 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 step of the SET (11 m/s). Because some of the horses did not reach their $\dot{V}_{O_2max}$ 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 values 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, *ie* 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; $\dot{V}_E/\dot{V}_{O_2}$ 33.7 ± 1.6 and 31.0 ± 3.3 l/l in the treated and control groups, respectively; and $\dot{V}_{O_2}$/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, $\dot{V}_E/\dot{V}_{O_2}$, $\dot{V}_{O_2}$/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 $\dot{V}_{O_2}$/HR; while their R remained unchanged; their $\dot{V}_E/\dot{V}_{O_2}$ decreased faster than in the control group (figs 1 and 2).

In both groups, $\dot{V}_E$ V_T, 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 (*ie* 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 CPK, LDH and AST were influenced by training, namely at SET4, *ie* 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.
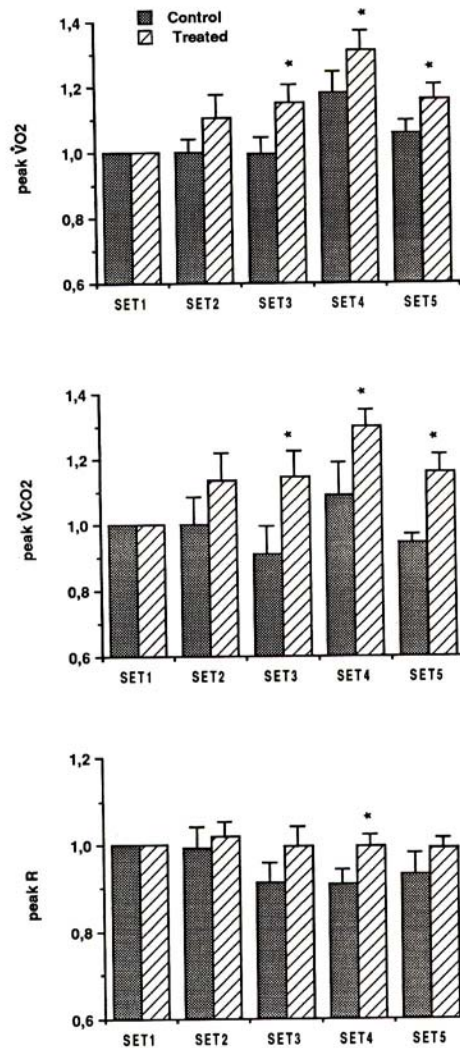
Fig 1. Peak O$_2$ uptake (peak $\dot{V}_{O_2}$), CO$_2$ output (peak $\dot{V}_{CO_2}$) and respiratory exchange ratio (peak R) recorded in thoroughbred horses during the last step of a standardized exercise before training (SET1), during different stages of a 9-week training period (SET2, SET3, SET4; see text for details) and after 3 weeks detraining (SET5). The treated group (▨) (*n* = 6) received daily a probiotic and the control (▨) group (*n* = 5) 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 $P \leq 0.05$ has been chosen as the significant level (*).
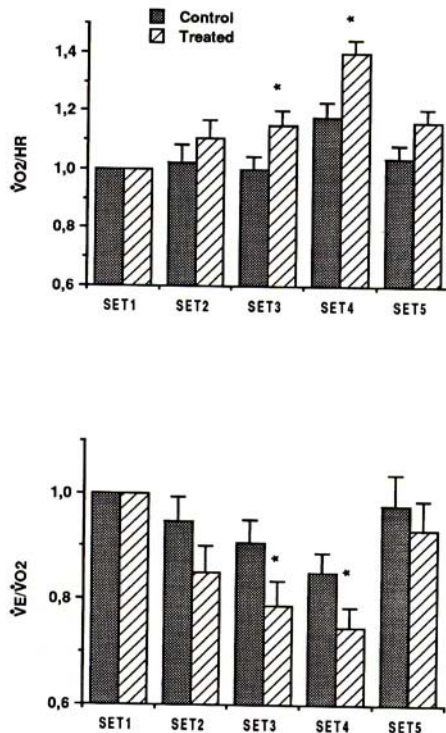
Fig 2. $\dot{V}_E/\dot{V}_{O_2}$, $\dot{V}_{O_2}/HR$ recorded in thoroughbred horses during the last step of a standardized exercise before training (SET1), during different stages of a 9-week training period (SET2, SET3, SET4; see text for details) and after 3 weeks detraining (SET5). The treated group ($\boxtimes$) ($n = 6$) received daily a probiotic and the control group ($\blacksquare$) ($n = 5$) 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 $P \leq 0.05$ has been chosen as the significant level (*).

DISCUSSION

The effect of training on the cardio-respiratory and ventilatory parameters has been previously studied (Art and Lekeux, 1993). The present experiment was carried out to attempt to assess the possible influence of a probiotic supplement on some physiological adjustments to training in horses.

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, peformed 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

T Art *et al*

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).

Values	Group	SET1	SET2	SET3	SET4	SET5
Peak V_T (l)	T	16.4 ± 1.2	14.5 ± 0.4	14.0 ± 0.4	14.2 ± 0.4	15.9 ± 0.5
	C	15.9 ± 0.8	15.3 ± 0.2	13.8 ± 0.5	15.6 ± 0.6	15.8 ± 1.1
Peak $\dot{V}_E$ (l/min)	T	1 846 ± 86	1 686 ± 41	1 622 ± 49	1 708 ± 41	1 880 ± 55
	C	1 922 ± 73	1 791 ± 54	1 680 ± 42	1 848 ± 90	1 837 ± 120
Peak HR (beats/min)	T	213 ± 4	212 ± 4	212 ± 4	210 ± 4	212 ± 4
	C	214 ± 3	214 ± 4	212 ± 4	214 ± 5	215 ± 5
HR_{rec} (beats/min)	T	105 ± 7	110 ± 4	106 ± 4	102 ± 7	107 ± 5
	C	106 ± 5	108 ± 2	103 ± 3	99 ± 3	104 ± 3
d T° (°C)	T	1.32 ± 0.12	1.03 ± 0.08	1.05 ± 0.16	0.88 ± 0.11	1.17 ± 0.28
	C	1.74 ± 0.10	1.10 ± 0.05	1.08 ± 0.19	0.97 ± 0.20	1.64 ± 0.17
Weight	T	500.2 ± 15.5	497.7 ± 13.9	493.7 ± 15.3	484.0 ± 19.3	479.8 ± 16.1
	C	500.2 ± 16.3	500.7 ± 15.8	495.5 ± 14.3	493.4 ± 13.4	484.2 ± 16.4
dCOR (nmol/l)	T	145.4 ± 25.8	92.5 ± 20.5	89.2 ± 5.6	76.2 ± 8.6	109.5 ± 24.5
	C	103.2 ± 29.0	69.0 ± 22.7	74.5 ± 18.4	82.2 ± 36.1	82.8 ± 28.5
LA (mmol/l)	T	10.9 ± 1.6	11.4 ± 1.1	9.3 ± 1.6	9.6 ± 1.2	9.3 ± 1.4
	C	8.4 ± 0.7	9.0 ± 0.4	8.3 ± 0.9	8.9 ± 1.1	8.0 ± 0.7
CPK_{rest} (IU/l)	T	213 ± 17	176 ± 10	281 ± 83	487 ± 221	219 ± 61
	C	184 ± 19	179 ± 10	201 ± 25	189 ± 365	235 ± 44
dCPK (IU/l)	T	54 ± 24	40 ± 4	42 ± 19	261 ± 110	66 ± 18
	C	29 ± 8	86 ± 52	23 ± 11	504 ± 217	84 ± 19
AST_{rest} (IU/l)	T	312 ± 50	292 ± 19	327 ± 45	552 ± 168	558 ± 120
	C	331 ± 36	373 ± 74	433 ± 93	688 ± 166	823 ± 240
dAST (IU/l)	T	43 ± 7	78 ± 33	82 ± 11	76 ± 17	93 ± 23
	C	31 ± 11	66 ± 20	35 ± 18	147 ± 68	98 ± 95
LDH_{rest} (IU/l)	T	525 ± 43	434 ± 41	343 ± 53	511 ± 70	427 ± 47
	C	442 ± 26	389 ± 41	343 ± 34	576 ± 123	416 ± 28
dLDH (IU/l)	T	91 ± 27	78 ± 19	103 ± 23	130 ± 35	116 ± 23
	C	65 ± 18	94 ± 11	98 ± 17	152 ± 34	81 ± 20

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}, dT°, 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_T: peak tidal volume; peak $\dot{V}_E$: expired minute volume; peak HR: peak heart rate; HR_{rec}: heart rate after 2 min recovery; Weight: weight before the standardized test; dT°: rectal temperature variation; dCOR: cortisol variation; LA: plasma lactate; CPK_{rest}: enzymatic acitivity for creatin phosphokinase at rest; dCPK: difference between pre-SET and post-SET values in enzymatic activities for creatin phosphokinase; LDH_{rest}: enzymatic activity for lactate dehydrogenase at rest; dLDH: difference between pre-SET and post-SET values in enzymatic activities for lactate dehydrogenase; AST_{rest}: enzymatic acitivity for aspartate amino-transferase at rest; dAST: difference between pre-SET and post-SET values in enzymatic activities for aspartate amino-transferase; the treated group (T; $n = 6$) received daily a probiotic and the control group (C; $n = 5$) a placebo.

exercising intensively, hyperventilate. This overbreathing increases the CO_2 elimination which is not accompanied by an increase in $\dot{V}_{O_2}$, 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 hyperventilate and on the contrary tend to hypoventilate 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 production of CO_2 and consequently for a rise of R above 1.00.

Training enhances free fatty acid utilization, 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 acidotic than the horses of the control group, this suggests that in the treated horses, the carbohydrates remained the main substrate for energy, even after training. This suggested that the possible effects of the probiotic are mainly related to an improvement, either in the carbohydrate aerobic enzymatic capacity, and/or in the carbohydrate 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. Consequently, the attempts to explain the differences 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 indirect 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 Buren et al, 1985; Kulkarni et al, 1986). Yeast by itself has been shown to increase feed efficiency and increase nitrogen retention in horses by a still unexplained mechanism (Glade, 1984; Glade and Biesik, 1986). 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 resorption of these organic acids, they could directly enter the Krebs' cycle either as substrates, or as intermediary acids, consequently 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 glycogenolytic aerobic capacity seemed to be more enhanced proportionally 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, ie 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 capacities, but that this effect seems to occur only during training and not during periods of physical inactivity.

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