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Influence of L-Carnitine on fitness and oxidative stress parameters in Trotter Horses subjected to Laval's test

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ABSTRACT

In the last few years, in addition to grain, the high energy requirements of racehorses have been met with dietary supplements of vegetable oil, which may, however, represent an easily oxidisable substrate. Carnitine can be used to improve lipid metabolism. We evaluated the changes in performance and oxidative stress parameters measured in 4 trotters receiving a diet containing soybean oil and L-Carnitine and subjected to two Standardized Exercise Tests (SET) according to Laval's protocol (3 hits at increasing speed) at an interval of 30 days. Blood samples were taken at rest, just after each of the three hits, and at 10, 20 and 40 min after each test to determine lactic acid, glucose, Non-Esterified Fatty Acid (NEFA), β-hydroxybutyrate, Reactive Oxygen metabolites (ROMs), Glutathione Peroxidase (GSH-Px), and Superoxide Dismutase (SOD). L-Carnitine influenced ROMs and SOD and resulted in a reduction in the oxidative stress parameters. Some indices of the fitness status also improved.

Key words: Trotter, L-Carnitine, Oxidative stress, Laval's SET

RIASSUNTO

INFLUENZA DELLA L-CARNITINA SULLA *FITNESS* E SUI PARAMETRI INDICATORI DELLO STRESS OSSIDATIVO IN TROTTATORI SOTTOPOSTI AL TEST STANDARDIZZATO SECONDO LAVAL

Nell'alimentazione del cavallo trottatore in attività agonistica negli ultimi anni si è diffuso sempre di più l'impiego di grassi vegetali, in aggiunta ai cereali, per coprire i fabbisogni energetici di un'attività muscolare che può essere considerata mista aerobico-anaerobico. I grassi vegetali tuttavia sono un substrato facilmente ossidabile che, se non correttamente utilizzato, può incrementare lo stress ossidativo dell'individuo. Per contrastare questo evento si è voluto testare l'effetto della somministrazione di L-Carnitina che, com'è noto, migliora il metabolismo dei lipidi a livello cellulare. Sono stati pertanto presi in esame i cambiamenti delle performances e dei parametri indicatori dello stress ossidativo in 4 cavalli trottatori, in attività agonistica, sottoposti ad un test standardizzato secondo Laval (3 ripetizioni a velocità crescente) che ricevevano diete contenenti olio e L-Carnitina. In 2 successivi test effettuati a distanza di 30 giorni sono stati valutati, su campioni di sangue prelevati a riposo, alla fine di ogni ripetizione (Hit) e 10, 20 e 40 min dopo la fine dell'esercizio, acido lattico, glucosio, NEFA, β-idrossibutirrato, ROMs, GSH-Px e SOD. Le variazioni riscontrate indicano un effetto positivo imputabile alla somministrazione della L-Carnitina che ha indotto variazioni dei livelli di ROMs e SOD indicative di un minor stress ossidativo e miglioramento della condizione di fitness.

Parole chiave: *Cavallo trottatore, L-Carnitina, Stress ossidativo, Laval's SET*

Introduction

The great knowledge of the biochemistry of exercise-related metabolism achieved over the last few years has allowed breeders and trainers to optimize nutrition and training techniques to the type of activity performed by racehorses. A horse's athletic performance can be enhanced by ergogenic substances favoring the utilization by muscle of available energy sources. The effects of the administration of substances such as sodium bicarbonate, Carnitine, and branched-chain amino acids on performances have been studied extensively (Quintavalla *et al.*, 1994). In particular, Carnitine is an endogenous substance that can also be used as a dietary supplement to improve lipid metabolism and favor fatty acid transfer through cell membranes (Falaschini and Trombetta, 1994; Harris and Harris, 1998). The energy used during athletic performance can result from the combination of aerobic and anaerobic metabolism and these mechanisms are activated by the intensity and duration of exercise.

The increased breathing rate and oxygen uptake that occur during an intense effort may trigger the production of reactive substances that can damage cell walls and increase muscle fatigue. Threshold situations are reached when the energy demand exceeds the aerobic metabolic capacity (Mills *et al.*, 1996), resulting in the production of reactive oxygen metabolites (ROMs). Vegetable and/or fish oil dietary supplements could increase the oxidisable substrate, favoring peroxidation (Gramenzi *et al.*, 2001). Based on these considerations, we studied the effect of dietary oil and L-Carnitine on performance and oxidative stress parameters in trotters training for the racing season.

Material and methods

Four trotter horses (average age 4 years; average body weight: 401.2 ± 14.3 kg) were studied for two months during the racing season. Training sessions were 3-4/week, the workload was planned for racing on average twice a month. The subjects' fitness status before the study was evaluated by means of a standard exercise test (SET_0) with rising speed according to Laval's protocol (Demonceau and Auvinet, 1992) as follows:

1. warming-up for 10 min at a small trot;
2. 3 trot hits of 3 min run at a trot at constant speed with 1 min rest intervals;
3. speed increased at each hit.

Speed and heart rate were continuously measured and recorded using a Speed Puls Equus - R (Bauman & Haldi, CH) fitted to the sulky. Recordings were downloaded on a PC using the FITSOFT EQUUS 2 software, which simultaneously shows speed and heart rate (Figure 1).

This SET evaluation indicated that the horses

Figure 1. Heart and speed registration during a SET. Upper line is heart, the lower is speed.

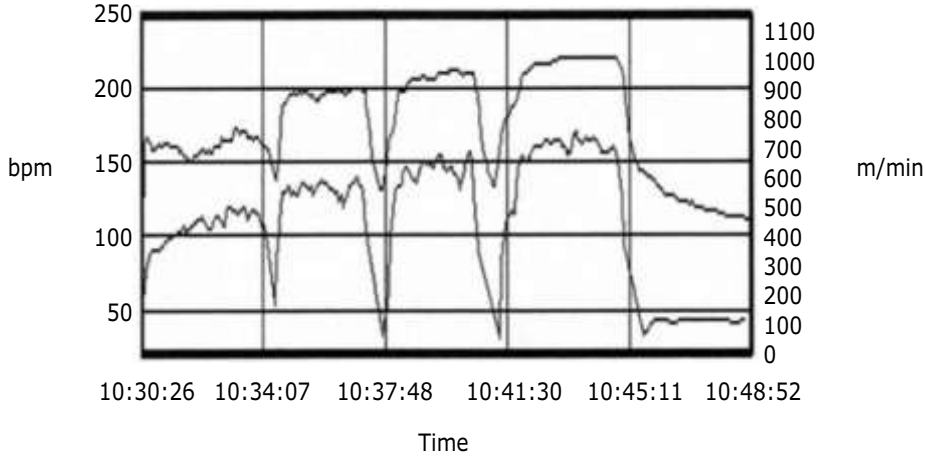

Table 1. Feed content, chemical analysis of concentrate, and daily supply of nutrients.

Feed	%	Chemical composition		%
Barley	25.0	Moisture		12.10
Corn	24.0	Crude protein		15.80
Wheat middlings	10.0	Ether extract		6.80
Soybean meal 44	9.5	Crude fiber		6.70
Cornflakes	7.0	Phosphorus		0.67
Linseed meal	6.0	Calcium		0.61
Dehydrated alfalfa meal	5.0	Ash		7.00
Oil	4.0			
Molasses	4.0	Nutrients supply		
NaCl	1.0			
NaH_2PO_4	1.0	Protein	kg/d	1.489
$CaCO_3$	1.0	Fat	"	0.480
IMV	1.0	ED	MJ/d	122.9
Yeast	0.5			

were in a satisfactory fitness condition and provided the reference data for speed. The experimental phase began with the administration of a control diet (see Table 1) consisting of 7 kg grass hay, 2 kg rolled oat, 4 kg specific mixed feed and 250 ml soybean oil. Another test (SET_1) was performed after 30 days. The diet was thereupon supplemented with L-Carnitine, providing 10 g/d of the active principle. The last test (SET_2) was conducted 30 days into the L-Carnitine diet.

Blood was collected from the jugular vein into vacuum tubes at rest (AR), just after each Hit (Hit_1, Hit_2, Hit_3), and at 10, 20 and 40 min after the end of each SET (AE_{10}, AE_{20}, AE_{40}). The tubes (containing EDTA fluoride) were immediately centrifuged (15 min at 3,500 rpm); plasma was frozen at -20°C and afterwards analyzed for lactic acid, glucose, Non-Esterified Fatty Acid (NEFA), β-hydroxybutyrate (all using Du Pont's Dimension system autoanalyzer at 37°C), and ROMs. Whole blood, sampled with silicon-coated vacuum tubes, was analyzed for glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD); all oxidative stress parameters were analyzed with commercial kits at 37°C.

Lactic acid was determined using an adaptation of Marbach and Weil's method that utilized oxidation of lactic acid in piruvic acid and glucose method is an adaptation of the hexokinase glucose-6-phospate dehydrogenase method presented as a clinical laboratory method by Kunst *et al.* (1983).

The β-hydroxybutyrate reagents (SIGMA Diagnostics) are for quantitative enzymatic determination obtained from the method described by Williamson. NEFA QUICK "BMY" is an enzymatic test for non-esterified fatty acids using Acyl-CoA synthetase and Acyl-CoA oxidase.

GSH-Px was investigated using UV (RANSEL) at 37°C based on the method of Paglia and Valentine.

SOD, whose role is to accelerate the dismutation of the toxic superoxide radical (O_2^-) produced during oxidative energy processes, was investigated using RANSOD.

The ROMs test measures the ability of transition metals to catalyse a Fenton reaction.

We calculated the consumption of O_2 (ml/kg/min) and energy (kcal/min) that occurred in the course of the two SETs according to McConaghy (1994). Using the Cap.Aero software (developed by Dr. Gloria) we obtained from lactate and heart rate values V_{200} (speed corresponding to 200 beats/min), V_2 (speed corresponding to 2 mmol of lactic acid), and V_4 (speed corresponding to 4 mmol of lactic acid). The Heart Scores for each test and hit were calculated according to Courouce and Auvinet (1993). Data were subject-

ed to mathematical-statistical analysis by ANOVA (Wilkinson Leland, 1989) considering sample (AR, H_1, H_2, H_3, AE_{10}, AE_{20}, AE_{40}) and diet (Control and Carnitine) factors.

Results and discussion

Feed composition and the nutrients content of the daily ration (the latter being in line with the indications of Kohnke, 1992) are reported in Table 1. The second phase of the study envisaged supplementation with 10 g/d L-Carnitine.

Oxygen and energy consumption during the tests, calculated according to the formula of McConaghy (1994), are reported in Table 2 as are Heart Score (HS) values at V_2, V_4 and V_{200}.

In the final SET, conducted 30 days into the L-Carnitine diet, O_2 consumption was considerably higher (86.4 *vs*. 78.3 ml/kg/min) than in SET_1, whereas the energy requirement was slightly higher (1,180 *vs*. 1,146 kcal/min). At the speeds corresponding to 2 and 4 mmol lactic acid and a rate of 200 beats/min, the heart scores improved from SET_1 to SET_2.

The speed values recorded in each hit are reported in Table 3. A significant increase attributable to the diet (P=0.043) occurred in the sole case of Hit_1.

At Rest Time, the means of the energy-related parameters (glucose 4.8 mmol/l; NEFA 89.6 μEq/l; β-HBA 0.459 mmol/l), the oxidative stress indices (GSH-Px 149.5 U/gHb; ROMs 17.4 UCarr; SOD 91.0 U/gHb) and lactic acid (0.651 mmol/l) were in the normal range; GSH-Px was higher than the value reported by McMeniman and Hintz (1992) in resting ponies.

The means of the energy parameters and of lactic acid (Table 4) did not change significantly from SET_1 to SET_2. The higher values of lactic acid seen in SET_2 (+ L-Carnitine) depended on the higher speed sustained by the horses.

As regards the mean values of the oxidative stress parameters (Table 4), ROMs values were significantly lower (P=0.0096) after L-Carnitine supplementation.

In SET_2 subjects performed better (657 *vs*. 601 m/min), experienced less oxidative stress, and produced a lower amount of these end catabolites (ROMs). The other two parameters did not change significantly, even though GSH-Px values were lower after SET_2; SOD values were higher than those recorded with the Control diet.

The values of the energy indices, lactic acid and oxidative stress parameters in the three hits, independently of the diet, are reported in Table 5. Differences were significant only for glucose (P=0.0338) and lactic acid values (P=0.001), which increased throughout.

Table 2. O_2 and energy consumption and Heart Score in SET_1 and SET_2.

		SET_1 (Control diet)	SET_2 (+ L-Carnitine)
O_2 consumption	ml/kg/min	78.3 ± 11.8	86.4 ± 7.0
Energy	kcal/min	1,146 ± 166	1,180 ± 348
HS V_2	m/beat	3.00 ± 0.28	3.03 ± 0.34
HS V_4	"	3.06 ± 0.27	3.12 ± 0.29
HS V_{200}	"	2.99 ± 0.32	3.06 ± 0.39

Table 3. Mean speed in SET_1 and SET_2 (m/min).

	SET_1 (Control diet)	SET_2 (+ L-Carnitine)
Hit_1	524	583
Hit_2	609	643
Hit_3	674	700

The mean values of the parameters measured in the recovery phase were analyzed in terms of the diet factor (Table 6). Differences were significant for lactic acid (P=0.0515), whose mean level was lower - witnessing a faster return to baseline - when the subjects had been receiving L-Carnitine.

Significant differences with the diet factor were also found for ROMs (P=0.0465) and SOD (P=0.0008): the former were lower (15 *vs.* 20 UCarr) and the latter higher (154.2 *vs.* 47.2 U/g Hb) with the L-Carnitine supplementation.

This could indicate that administration of L-Carnitine shifted the utilization of the oxidisable substrate, lipids in the case of the present work, towards a better metabolization for energy purposes.

Table 4. Mean values of energy, lactic acid and oxidative stress parameters with the two diets.

		SET_1 (Control diet)	SET_2 (+ L-Carnitine)	P
Glucose	mmol/l	4.8	5.4	ns
NEFA	μEq/l	155.2	137.5	ns
β-HBA	mmol/l	0.397	0.412	ns
Lactic acid	"	4.2	7.1	ns
GSH-Px	U/gHb	156.3	146.5	ns
ROMs	Ucarr	21.2	16.3	0.0096
SOD	U/gHb	122.2	240.3	ns

Table 5. Mean values of energy, lactic acid and oxidative stress parameters according to Hit.

		Hit_1	Hit_2	Hit_3	P
Glucose	mmol/l	4.5	4.9	5.8	0.0338
NEFA	μEq/l	153.2	150.7	135.2	ns
β-HBA	mmol/l	0.407	0.407	0.397	ns
Lactic acid	"	1.9	4.5	10.5	0.001
GSH-Px	U/gHb	149.1	153.2	152.0	ns
ROMs	Ucarr	19.0	17.0	19.3	ns
SOD	U/gHb	205.5	137.2	201.2	ns

Table 6. Mean values of energy, lactic acid and oxidative stress parameters according to diet.

		Control diet	Carnitine diet	P
Glucose	mmol/l	2.7	3.3	ns
NEFA	μEq/l	320.2	228.2	ns
β-HBA	mmol/l	0.482	0.415	ns
Lactic acid	"	5.8	5.1	0.0515
GSH-Px	U/gHb	168.3	156.9	ns
ROMs	Ucarr	20.9	15.3	0.0465
SOD	U/gHb	47.2	154.2	0.0008

ns: not significant

By contrast, GSH-Px was similar in the two phases. Analysis of the samples collected in the recovery phase (AE_{10}, AE_{20}, AE_{40}) independently of the diet showed the absence of significant differences. The sole lactate concentration exhibited a significant reduction in this phase, as reported in previous works (Falaschini and Trombetta, 1994, 2001; McMeniman and Hintz, 1992) ROMs and SOD values showed the same trend.

Conclusions

Our results lend further support to the positive effect of L-Carnitine on trotters fed oil-containing diets. Besides improved performance (in terms of speed and heart scores), we noted a faster return to basal values of lactic acid, as previously reported (Falaschini and Trombetta, 2001). The availability of a greater amount of easily oxidisable substrate did not increase ROMs production while subjects were receiving Carnitine. This allows us to hypothesize that the energy substrate provided by oil was metabolized by the muscle fibers for use as energy source.

The paper must be attributed equally to the authors

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