DOI: 10.1111/j.1439-0396.2007.00790.x

ORIGINAL ARTICLE

Daily pattern of some fatty acids in the athletic horse

G. Piccione, A. Assenza, M. Borruso, F. Fazio and G. Caola

Department of Morphology, Biochemistry, Physiology and Animal Production, Veterinary Chronophysiology Laboratory, Section of Veterinary Physiology, Faculty of Veterinary Medicine, University of Messina, Messina, Italy

Keywords
daily rhythm, fatty acids, horse

Correspondence
Prof. Giuseppe Piccione, Department of Morphology, Biochemistry, Physiology and Animal Production, Veterinary Chronophysiology Laboratory, Section of Veterinary Physiology, Faculty of Veterinary Medicine, University of Messina, Polo Universitario dell'Annunziata, 98168 Messina, Italy. Tel: +39 090 350 35 84; Fax: +39 090 350 39 75; E-mail: giuseppe.piccione@unime.it

Received: 24 January 2006;
accepted: 1 March 2007
First published online: 13 March 2008

Summary

In the sport field, non-esterified fatty acids (NEFA) are important for the physical performance during the aerobic exercise of short intensity and long duration. In man, rat, goat and in the sedentary horse studies on the chronometabolism showed the presence of a circadian rhythm of the plasmatic concentration of NEFA while data for the athletic horse are lacking. To define a chronogram helpful for a specific planning and the differentiation of the training programmme in the athletic horse, the circadian pattern of some fatty acids (NEFA, palmitic, stearic, oleic, linoleic and linolenic acids) was studied in five Sella Italiana horses. These horses trained following a daily model of activity consisting of walk, trot, gallop and jump of obstacles of different heights. Blood samples were collected from the jugular vein every 4 h, starting at 08:00 hours, for 2 days to assess the concentrations of total NEFA (by spectrophotometry), palmitic, stearic, oleic, linoleic and linolenic acids (by gas chromatography). ANOVA for repeated measures showed a statistical significant effect of the time of the day in NEFA, oleic and linolenic acids. The application of the periodic model showed the periodic pattern of NEFA, oleic, linoleic and linolenic acids. Acrophases were in the afternoon for all parameters. The results obtained showed a different trend of the circadian pattern of the studied parameters in the athletic horse than in the sedentary one because the physical activity and the post-prandial metabolism acted as zeitgebers.

Introduction

Non-esterified fatty acids (NEFA) are metabolites with a high energetic capacity that competes with glucose because of the similar metabolic use. During the day, there is a 'turning point' of the energetic exchange, when the use of fatty acids is increased and that of carbohydrates is inhibited. The factors that regulate the energetic homeostasis influence the mobilization and the use of NEFA. In the sport field, NEFA are important for the physical performance during aerobic exercise of low intensity and long duration. Several studies on chronometabolism in men (Morgan et al., 1999; Heptulla et al., 2001), rats (de Gomez Dumm et al., 1984) and goats (Alila-Johansson et al., 2004) showed the presence of a circadian rhythm of the plasma concentration of NEFA. A circadian rhythm of NEFA was shown also in sedentary horses with an acrophase recorded in the morning at about 07:00 hours (Orme et al., 1994). Furthermore, in men, diurnal variations in the ability to metabolize NEFA at the muscle and heart level were demonstrated (Stavinoha et al., 2004). This information is useful for the identification, in men, of an athletic chronoperformance able to optimize the levels of the physical performance and training. Also, the athletic horse presents daily variations of some haematological and haematochemical

parameters (Piccione et al., 2001, 2004a,b, 2005a,b). The identification of a daily variation in the level of fatty acids will be useful for establishing a correlation between the rhythmic pattern and possible episodes of 'poor performance'. This could help in generating a chronogram helpful for specific planning and characterization of the training programme in the athletic horse. For this reason, we aimed to establish the circadian pattern of NEFA, palmitic, stearic, oleic, linoleic and linolenic acids in the athletic horse.

Materials and methods

Five Sella Italiana mares, all 8 years old, weighing 565 ± 45 kg, were used. For 30 days prior to the study, the animals underwent the same pattern of daily activity. They were housed in individual stalls (3.5 × 3.5 m), under a natural photoperiod (sunrise at 05:00 hours and sunset at 20:00 hours) and natural indoor temperature (19–21 °C). The horses were fed traditional rations, based hay (first cut meadow hay, sun cured, late cut, on average 8 kg/horse/day) and a mixture of cereals (oats and barley, 50% each, approximately 3.5 kg/horse/day), three times daily (at 08:00, 12:00 and 17:00 hours). Water was available *ad libitum*. From the composition of the feedstuffs administered, it can be computed that the diet contained less than 2% of lipids (approximately 1.37% on dry matter basis), mainly from oats (4.5% ether extract). Then, the horses were given 8.98 kg of dry matter/day and 123 g of ether extract /day. On the basis of the feed composition, the average daily intakes of palmitic, stearic, oleic, linoleic and alfa-linolenic acids were very low, 1.72 g, 0.08 g, 3.33 g, 4.33 g and 0.64 g respectively. Ether extract was determined by Soxhlet extraction. Fatty acids were determined by capillary gas chromatography after lipid extraction and esterification using sodium methoxide as catalyst. The horses were trained from 15:00 to 16:00 hours. Training included 15 min warming up [5-min walking, 5-min trotting, 5-min galloping, six fences of different height (1–1.4 m)] and a show jumping competition on a distance of 350 m with six oxers, seven simple fences, one double combination (vertical and vertical) and one triple combination (oxer, vertical and vertical). The maximal height of the fences was to compute the workload, the heart rate of horses, all mounted by the same rider, was recorded by a heart rate monitor (Polar S610, Polar Electro Oy, Kempele, Finland). The heart rate electrodes were applied before entering the course (the positive electrode on the left side under the saddle near the withers, the negative electrode near the heart, fastening it under the tummy by means of a rubber belt). The heart rate during the warming up phase and the show jumping competition were recorded by a Polar S610 receiver, fastened at the rider's wrist. Then, the logged data were transferred to a PC via an infrared interface and visualized through specific software (POLAR HORSE TRAINER, Polar Electro Oy, Kempele, Finland). Blood sample were collected via an intravenous cannula inserted into the external jugular vein, at rest and after the show jumping competition and the lactate level of the whole blood was determined promptly with a portable blood lactate analyser (Accusport; Boehringer-Mannheim, Germany). Heart rate and lactate levels were used to quantify the workload. In fact, horses presented an average heart rate of 210 ± 15 bpm and an average blood lactate of 5.0 ± 0.5 mmol/l. Both values qualify the show jumper competition as aerobic/anaerobic work (Clayton, 1994).

After 30 days of pre-conditioning, blood samples were collected at 4-h intervals over a 48-h period (starting at 08:00 hours on day 1 and finishing at 04:00 hours on day 2) Blood plasma was prepared by centrifugation (3000 *g*, 10 min) from heparinized blood. Samples were stored at −20 °C. Individual plasma samples were assessed for the concentrations of NEFA spectrophotometrically (model DU-40; Beckman Instruments, Fullerton, CA, USA), using a standard assay-kit (Randox, London, UK). Subsequently, the concentrations of the palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids were determined according to the method illustrated by Hernández-Pérez et al. (2002), using a gas chromatography–mass spectrometer (HP-5970- MSD; AGIVENT TECHNOLOGY, Ringoes, NJ, USA).

All the results were expressed as mean ± SD. One-way repeated-measures analysis of variance (ANOVA) was used to determine significant differences. p values < 0.05 were considered statistically significant. *Statistical Newman-Keuls* (SNK) test was applied for post-hoc comparisons. The acrophase of a rhythm was determined by an iterative curve-fitting procedure based on the single cosinor procedure (Nelson et al., 1979). For each variable and for each animal, a cosine wave was fitted to the data points according to the function $Y_t = A + M \cos(\theta_t + \varphi)$, where Y_t denotes each data point in the time series, M is the mean level of the rhythm, A is the amplitude, θ_t is the trigonometric angle (in degrees) corresponding to time t and φ is the angle displacement for the acrophase. The value of φ was determined by iteration:

the true value of φ was considered to be the one that produced the smallest sum of squares of the deviations between iterated cosine functions and the raw data.

Results

The results obtained during the experimental period indicated the existence of daily rhythms of NEFA, oleic, linoleic and linolenic acids (Figs 1 and 2), while palmitic and stearic acids have not shown a daily rhythm during the 2 days of monitoring (Fig. 3). ANOVA indicated a highly significant effect of time on NEFA, oleic and linolenic acids, at both days, as follows: NEFA, $F_{(11,44)}=32.23$ $p < 0.001$; oleic acid $F_{(11,44)}=3.65$ $p < 0.001$; linolenic acid $F_{(11,44)}=33.77$ $p < 0.001$. The post-hoc multiple comparison SNK has shown statistically significant differences ($p < 0.001$) if we compare all the 4-h time intervals. On the other hand, there was no significant effect of time during the 2 days of monitoring for linoleic, palmitic and stearic acid: linoleic acid $F_{(11,44)}=0.76$ $p = 0.67$; palmitic acid $F_{(11,44)}=1.11$ $p = 0.38$; stearic acid $F_{(11,44)}=1.04$ $p = 0.43$. For NEFA, oleic, linoleic and linolenic acids, the application of the periodic model and the statistical analysis of the cosinor procedure enabled us to define the periodic parameters and their acrophases (expressed in hours) during the 2 days of monitoring. These parameters showed diurnal acrophases, as follows: NEFA at 16:04 hours (12:32–19:36 hours) for the 1st day and at 17:32 hours (13:36–21:28 hours) for the

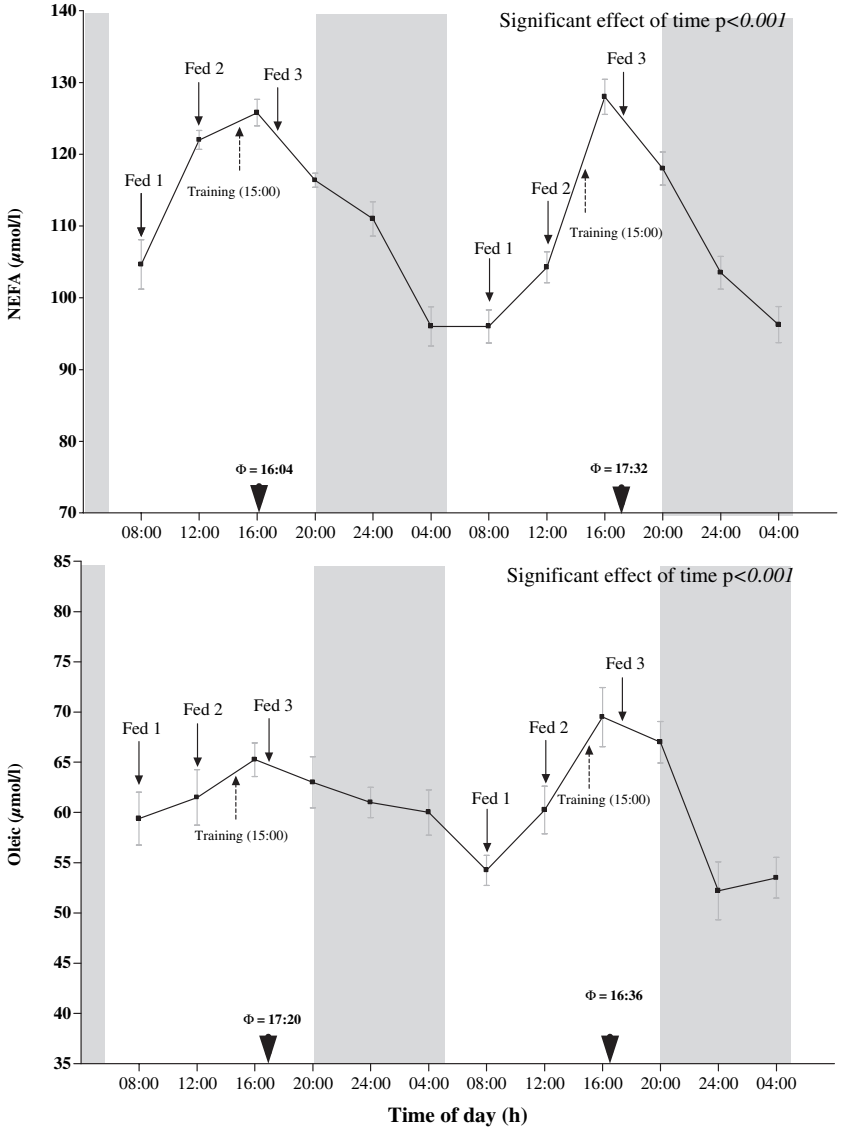

Fig. 1 Daily rhythms of NEFA (Non-esterificated fatty acids) and oleic acid in athletic horses. Each point represents the mean (SD) (n = 5) of NEFA and oleic acid. The grey bar indicates the dark phase of the 48-h light and dark (LD) cycle. The arrowheads indicate the acrophases.

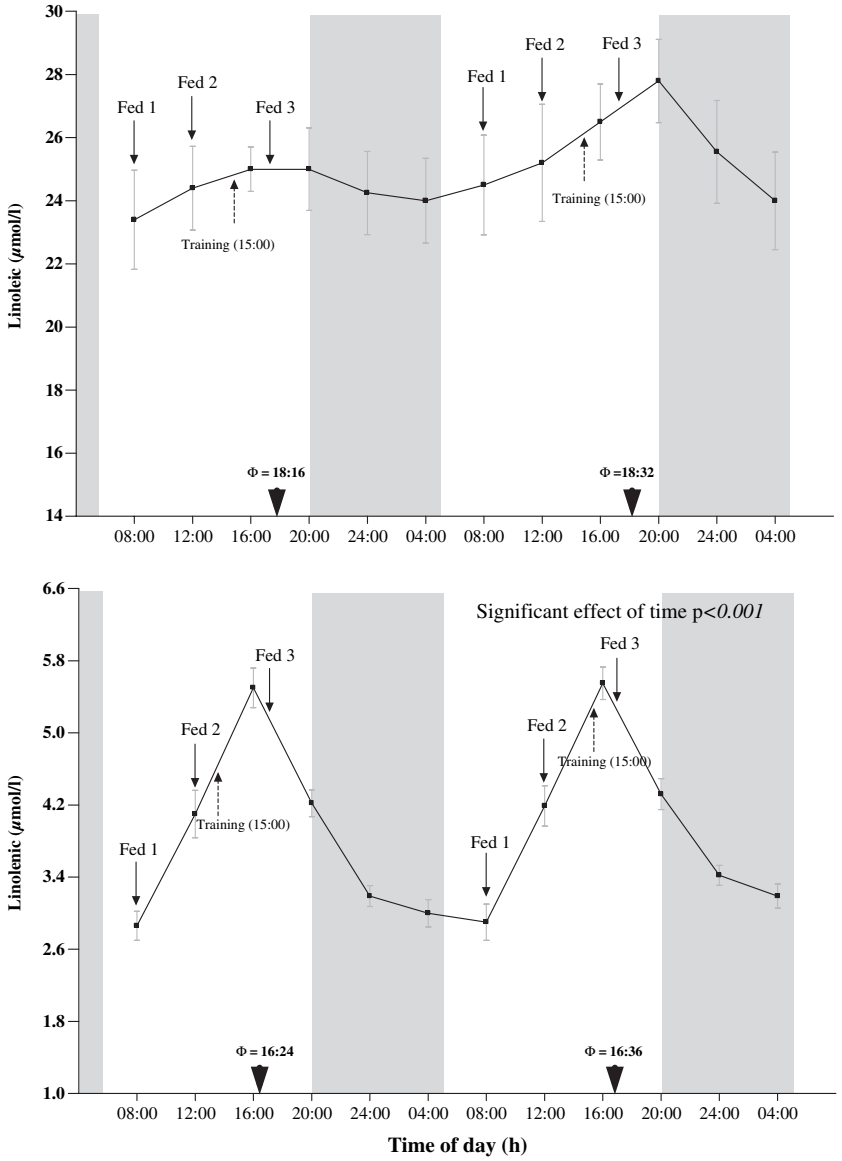

Fig. 2 Daily rhythms of linoleic and linolenic acids in athletic horses. Each point represents the mean (SD) (n = 5) of linoleic and linolenic acids. The grey bar indicates the dark phase of the 48-h light and dark (LD) cycle. The arrowheads indicate the acrophases.

2nd day; oleic acid at 17:20 hours (12:36–22:04 hours) for the 1st day and at 16:36 hours (12:00–21:12 hours) for the 2nd day; linoleic acid at 18:16 hours (14:24–22:08 hours) for the 1st day and at 18:32 hours (14:12–22:52 hours) for the 2nd day; linolenic acid at 16:24 hours (11:44–21:04 hours) for the 1st day and at 16:36 hours (11:08–22:12 hours) for the 2nd day. Table 1 shows the midline estimating statistic of rhythm (MESOR), with the fiducial limits (FL) at 95%, the amplitude, expressed in the same unit as the relative MESOR, the acrophase, calculated using the singular cosinor method and expressed in hours, together with the confidence interval at 95% for the periodic plasma parameters NEFA, oleic, linoleic and linolenic acids.

Discussion

By use of the MESOR (intended as the arithmetic mean for equidistant data covering an integral number of cycles), it was possible to show a daily variation in the concentration of the analyzed fatty acids, mainly of oleic acid followed by linoleic, stearic, palmitic and linolenic acids, which, combined, represent approximately 90% of total seric NEFA. Previous studies (Shorland et al., 1952; Hambleton et al., 1980; Luther et al., 1981; Rose and Sampson, 1982; Orme et al., 1994) reported some rather contrasting data, showing that the percentage of single fatty acids reflects the fatty acid composition of the diets (Hallebeek and Beynen, 2002). The energetic

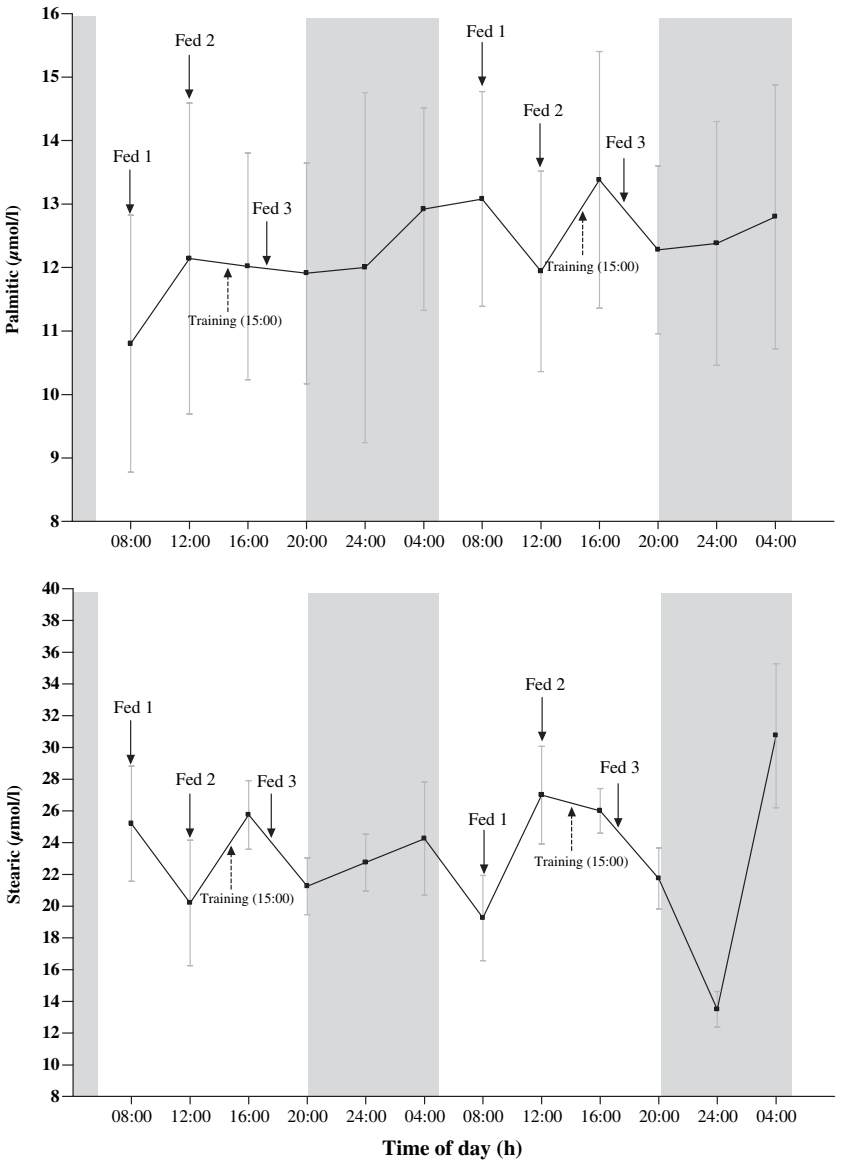

Fig. 3 Variation in plasma palmitic and stearic acids (μmol/l, mean ± SD) over 48 h in five horses.

integration of the athletic horse daily requirements, with polyunsaturated fatty acids adequately balanced by antioxidant factors like vitamin E (Bergero, 2004), is nowadays a consolidated practice. In particular, the linolenic acid favours the aerobic and anaerobic sportive performance, because it supplies energy without provoking exercise-induced dismetabolic myopathies. Omega 3 stabilizes cellular membranes, improving the permeability and the exchange of substrates involved in energetic metabolism (Gibney and Bolton-Smith, 1988) and increases the deformability of red cells, improving the oxygen transport at the peripheral level (Van der Brug et al., 1995). The long term administration of the lipidic substrate, together with an appropriate training, induces a 'biochemical adaptation' characterized by a higher availability and use of NEFA from muscular tissue and a consequent improvement of the performance (Dunnett et al., 2002; Sloet van Oldruitenborgh-Oosterbaan et al., 2002). ANOVA showed a significant effect of time in some of the analyzed parameters. The application of the trigonometrical statistical model for the periodic analysis indicated the existence of periodic patterns for NEFA, oleic, linoleic and linolenic acids, which presented diurnal post-meridian acrophases between 16:04 hours and 8:32 hours. These times corresponded to those of training and food consumption. In the athletic horse,

Table 1 MESOR (M), fiducial limits (FL) at 95%, amplitude (*A*) and acrophase (Φ), expressed in hours, with confidence interval (CI) at 95%, of non-esterificated fatty acids (NEFA), oleic, linoleic and linolenic acids during the 2 days of study

	Parameter				
	M	95% FL	*A*	Φ	95% CI
NEFA					
Day 1	112.62	108.21–117.03	13.74	16:04	12:32–19:36
Day 2	107.66	102.15–113.17	15.63	17:32	13:36–21:28
Oleic acid					
Day 1	61.69	60.66–62.71	2.59	17:20	12:36–22:04
Day 2	59.44	55.81–63.07	8.90	16:36	12:00–21:12
Linoleic acid					
Day 1	24.34	24.08–24.60	0.75	18:16	14:24–22:08
Day 2	25.59	24.99–26.19	1.69	18:32	14:12–22:52
Linolenic acid					
Day 1	3.81	3.34–4.27	1.22	16:24	11:44–21:04
Day 2	3.92	3.44–4.40	1.17	16:36	11:08–22:12

the effect of physical exercise on the haematic concentration of NEFA is well documented (Dunnett et al., 2002; Margotta et al., 2002). Non-esterified fatty acids are highly energetic metabolites whose metabolic contribution is inversely proportional to the work intensity and directly proportional to its duration (Hambitzer and Bent, 1988; Caola and Assenza, 2001). In a submaximal exercise NEFA as energetic substrate is needed in order to limit the muscle glycogen consumption and delay the fatigue, supporting the long-term physical performance. Furthermore, the onset of physical activity activates the sympatic nervous system that promotes the lipomobilization of NEFA with a greater secretion of catecholamines. This insures an adequate contribution of energy to the skeletal muscle and myocardium, optimizing the metabolic response to the physical exercise (Cappa, 1999). Physical training induces a metabolic adaptation to the stress induced by physical exercise reducing the catecholaminergic response (Baragli et al., 2002). The planned physical exercise induces great morphological, biochemical and physiological modifications to guarantee the homeostasis during the sportive performance. At the muscle level, training increases the capillarization, the energetic metabolism and the recovery ability (Hambitzer and Bent, 1988; Caola and Assenza, 2001), improving the metabolization of NEFA. Together with physical exercise, fasting increases the concentration of NEFA (Rose and Sampson, 1982). Sticker et al. (1995) showed the increase of NEFA as a result of the physical activity both in normally fed subject and fasted ones. This increase in fasted subjects was followed by a sudden decrease, below the basal (pre-exercise) values, showing a greater use of NEFA for energetic scopes. On the other hand, with feeding, the concentration of NEFA decreased about 1 h after the meal (Jose-Cunilleras et al., 2002), because of an increase of serum insulin. Thus, the administration of carbohydrates before the physical exercise leads to a reduction of NEFA oxidation (Pagan and Harris, 1999; Jose-Cunilleras et al., 2002) with negative effects on the performance (Duren et al., 1999). On the other hand, a reduced food amount, before the trial, does not compromise the performance, assuring the motor activity of the gastrointestinal tract (Pagan and Harris, 1999). A study on the circadian variations of serum concentrations of NEFA in the sedentary horse, (Orme et al., 1994), showed a diurnal acrophase at 07:00 hours and for oleic acid between 04:00 hours and 10:00 hours, while the acrophase of stearic and linoleic acid occurred at 17:00 hours. Contrary to what observed in sedentary horses (Orme et al., 1994), the presence of a daily rhythm for the parameters here investigated might be related to the regular training undertaken by the athletic horses. Physical exercise, regularly undertaken in the early afternoon, might be the exogenous zeitgebers that trigger the daily rhythm of these parameters. Furthermore, the effects of exercise, training in particular, on several physiological–metabolic parameters in men (Reilly et al., 1997) and horses (Piccione et al., 2005a,b; Fazio et al., 2006) are well known. For example, we showed in our previous research the existence of the circadian rhythm of tryptophan and serotonin in sedentary and athletic horses. It is interesting that both parameters changed their acrophases in athletic horses trained in the afternoon (Piccione et al., 2005a; Fazio et al., 2006). The detection of the factors able to determine and influence the periodicity of the energetic parameters is of fundamental importance to establish a chronogram or reference for the objective assessment of the sport performance and the planning of training programmes. In fact, given the periodic variations of the functional systems, this information will allow a better planning of submaximal and maximal workloads for the athletic horse.

References

Alila-Johansson, A.; Eriksson, L.; Soveri, T.; Laakso, M. L., 2004: Daily and annual variations of free fatty acid, glycerol and leptin plasma concentrations in goats (capra hircus) under different photoperiods. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **13**, 119–131.

Baragli, P.; Tedeschi, D.; Mazzantini, A. F.; Casini, L.; Leotta, R.; Martelli, F.; Sighieri, C., 2002: Variazioni della cinetica delle catecolamine plasmatiche in risposta all'allenamento nel cavallo *Proceeding New Finding in Equine Practice Congress* **4**, 231–236.

Bergero, D., 2004: New insights related to the nutritional management of endurance horses. *Proceedings European Equine Health & Nutrition Congress* **2**, 41–50.

Caola, G.; Assenza, A., 2001: Energetica muscolare processi bioenergetici e utilizzazione dei substrati metabolici durante esercizio fisico. In: G. Caola (ed.), *Fisiologia dell'esercizio fisico del cavallo*. Edagricole, Bologna, pp. 23–43.

Cappa, M., 1999: Regolazione dell'omeostasi endocrina in corso dell'esercizio fisico. In: M. Cappa (ed.), *Endocrinologia dell'esercizio fisico*. UTET, Torino, pp. 3–17.

Clayton, K. M., 1994: Training show jumper. In: D. R. Hodgson, R. J. Rose (eds), *The Athletic Horse*. W.B. Saunders Company, Philadelphia, pp. 429–438.

Dunnett, L. E.; Marlin, D. J.; Harris, R. C., 2002: Effect of dietary lipid on response to exercise: relationship to metabolic adaptation. *Equine Veterinary Journal. Supplement* **34**, 75–80.

Duren, S. E.; Pagan, J. D.; Harris, P. A.; Crandall, K. G., 1999: Time of feeding and fat supplementation affect plasma concentrations of insulin and metabolites during exercise. *Equine Veterinary Journal. Supplement.* **30**, 479–484.

Fazio, F.; Assenza, A.; Crisafulli, G.; Piccione, G.; Caola, G., 2006: The influence of exercise on daily rhythm of serum homocysteine in horses. *Journal of Physiology Science* **56**, 455–458.

Gibney, M. J.; Bolton-Smith, C., 1988: The effect of dietary supplement of n-3 polyunsaturated fat on platelet lipid composition, platelet function and platelet plasma membrane fluidity in healthy volunteers. *British Journal of Nutrition* **60**, 5–12.

de Gomez Dumm, I. N.; de Alaniz, M. J.; Brenner, R. R., 1984: Daily variations of the biosynthesis and compositions of fatty acids in rats fed on complete and fat-free diets. *Lipids* **19**, 91–95.

Hallebeek, J. M.; Beynen, A. C., 2002: The plasma level of triacylglicerols in horses fed high-fat diets containing either soybean oil or palm oil. *Journal of Animal Physiology and Animal Nutrition* **86**, 111–116.

Hambitzer, R.; Bent, E., 1988: Effect of long distance exercise on free fatty acids in blood plasma of Arab horses. *Journal of Veterinary Medicine A* **35**, 622–625.

Hambleton, P. L.; Slade, L. M.; Hamar, D. W.; Kienholz, E. W.; Lewis, L. D., 1980: Dietary fat and exercise conditioning effect on metabolic parameters in the horse. *Animal Science* **51**, 1330–1339.

Heptulla, R.; Smitten, A.; Teague, B.; Tamborlane, W. V.; Yong-Zhan, M. A.; Caprio, S., 2001: Temporal patterns of circulating leptin levels in lean and obese adolescents: relationships to insulin, growth hormone and free fatty acids rhythmicity. *The Journal of clinical endocrinology and metabolism* **80**, 90–96.

Hernández-Pérez, J. M.; Cabré, E.; Fluvià, L.; Motos, A.; Pastor, C.; Corominas, A.; Gassull, M. A., 2002: Improved Method for Gas Chromatographic–Mass Spectrometric Analysis of 1-^{13}C-labeled Long-Chain Fatty Acids in Plasma Samples. *Clinical Chemistry* **48**, 906–912.

Jose-Cunilleras, E.; Hinchcliff, K. W.; Sams, R. A.; Devor, S. T.; Linderman, J. K., 2002: Glycemic index of a meal fed before exercise alters substrate use and glucose flux in exercising horses. *Journal of Applied Physiology* **92**, 117–128.

Luther, D. G.; Cox, H. U.; Dimopoullos, G. T., 1981: Fatty acid composition of equine plasma. *American Journal of Veterinary Research* **42**, 91–93.

Margotta, A.; Guidi, L.; Zicarelli, F.; Infascelli, F., 2002: Influenza dell'aggiunta di olio di mais alla dieta sulla lattacidemia di cavalli sottoposti a gare di endurance *Proceeding New Finding in Equine Practice Congress* **4**, 189–196.

Morgan, L. M.; Apostolakov, F.; Wright, J.; Gama, R., 1999: Diurnal variations in peripheral insulin resistance and plasma non-esterified fatty acid concentrations: a possible link? *Annual Clinical Biochemistry* **36**, 447–450.

Nelson, W.; Tong, Y. L.; Lee, J. K.; Halberg, F., 1979: Methods for cosinor-rhythmometry. *Chronobiologia* **6**, 305–323.

Orme, C. E.; Dunnett, M.; Harris, R. C., 1994: Variation in the concentration of long chain free fatty acids in equine plasma over 24 hours. *British Veterinary Journal* **150**, 339–347.

Pagan, J. D.; Harris, P. A., 1999: The effects of timing and amount of forage and grain on exercise response in thoroughbred horses. *Equine Veterinary Journal. Supplement* **30**, 451–457.

Piccione, G.; Assenza, A.; Fazio, F.; Giudice, E.; Caola, G., 2001: Different periodicities of some haematological parameters in exercise-loaded athletic horses and sedentary horses. *Journal of Equine Science* **12**, 17–23.

Piccione, G.; Caola, G.; Refinetti, R., 2004a: Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* **21**, 1–19.

Piccione, G.; Assenza, A.; Grasso, F., 2004b: Daily rhythm of circulating fat soluble vitamin concentration (A, D, E and K) in the horse. *Journal of Circadian Rhythm* **2**, 1–4.

Piccione, G.; Assenza, A.; Fazio, F.; Percipalle, M.; Caola, G., 2005a: Central fatigue and nycthemeral change of serum tryptophan and serotonin in the athletic horse. *Journal of Circadian Rhythm* **3**, 1–4.

Piccione, G.; Fazio, F.; Giudice, E.; Grasso, F.; Morgante, M., 2005b: Nycthemeral change of some haematological parameters in horses. *Journal of Applied Biomedicine* **3**, 123–128.

Reilly, T.; Atkinson, G.; Waterhouse, J., 1997: Circadian rhythms in sport performance. In: T. Reilly, G. Atkinson, J. Waterhouse (eds), *Biological Rhythms & Exercise*. Oxford University press, Oxford, pp. 38–62.

Rose, R. J.; Sampson, D., 1982: Changes in certain metabolic parameters in horses associated with food deprivation and endurance exercise. *Research Veterinary Science* **32**, 198–202.

Shorland, F. B.; Bruce, L. W.; Jessop, A. W., 1952: The component fatty acids of lipids from fatty tissues, muscle and liver. *Biochemical Journal* **52**, 400–407.

Sloet van Oldruitenborgh-Oosterbaan, M. M.; Annee, M. P.; Verdegaal, E. J.; Lemmens, A. G.; Beynen, A. C., 2002: Exercise- and metabolism-associated blood variables in Standardbreds fed either a low- or a high-fat diet. *Equine Veterinary Journal* **34**, 29–32.

Stavinoha, M. A.; Rayspellicy, J. W.; Hart-sailors, M. L.; Mersmann, H. J.; Bray, M. S.; Young, M. E., 2004: Diurnal variations in the responsiveness of cardiac and skeletal muscle of fatty acids. *American Journal of Physiology Endocrinology Metabolism* **287**, 878–887.

Sticker, L. S.; Thompson, D. L.; Bunting, L. D.; Fernandez, J. M.; De Pew, C. L.; Nadal, M. R., 1995: Feed deprivation of mares: plasma metabolite and hormonal concentrations and response to exercise. *Journal of Animal Science* **73**, 3696–3704.

van der Brug, G. E.; Peters, H. P. F.; Handerman, M. R.; Shep, G.; Mosterd, W. L., 1995: Hemorheological response to prolonged exercise-no effects of different kinds of feedings. *International Journal of Sports Medicine* **16**, 231–237.