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Changes of serum free amino acids in eventing horses at rest and during exercise in response to dietary protein

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ABSTRACT

Although there are published expertise about serum free amino acids, to the knowledge of the authors no data were reported on eventing horses. In this way, it is important to study the concentration of dietary protein and serum free amino acid during exercise in order to improve nutritional strategies. The aim of this study was to investigate the concentration of the serum free lysine (Lys), threonine (Thr), leucine (Leu), isoleucine (Ile) and valine (Val) of eventing horses fed diets with different levels of protein at before and during exercise. Twenty-four Brazilian Sport Horses trained for eventing were used in a randomized block design with 4 diets (7.5%, 9.0%, 11.0% and 13.0% of CP) and 6 repetitions (horses). Horses were blocked according to their experience in competitions. The test protocol consisted of a warm up of walking and trotting and then a gallop starting at 6.0 m/s with increases in speed of 1 m/s every minute up to 10 m/s. Venous blood samples were collected before the test and during exercise. Concentration of Lys, Thr, Leu, Ile and Val from diets and serum free amino acids were determined by HPLC analysis. Total intake values of Lys, Thr, Leu, Ile and Val were affected by dietary protein levels. Differences on the serum free concentrations of Lys, Leu, Ile and Val as a function of the sampling time were observed. The Lys requirement for athletic horses appear to be lower than what is currently proposed. Moreover, the effect of exercise on serum free Lys, Leu, Ile and Val concentration, may be interpreted as an indicator of these amino acids metabolic response.

1. Introduction

In athletic horses, dietary protein:amino acid, especially lysine and threonine, ratios have an important role in the nitrogen balance, acid-base balance and muscle protein metabolism (Graham-Thiers and Kronfeld, 2005). Moreover, the serum free branched chain amino acids (BCAA) play a critical role during and after exercise on muscle recovery in horses (Matsui et al., 2006).

The concentrations of free amino acids in horses' serum or plasma can vary under different conditions, in particular with different nutritional programs, type and quality of food (Bergero et al., 2005; Graham-Thiers and Bowen, 2011; Urschel and Lawrence, 2013). Furthermore, AA were previously examined mostly in growing horses, rather than in athletic/exercising horses.

Therefore, it is important to study the concentrations of dietary protein and serum free amino acids during exercise in order to improve nutritional strategies. In addition, lysine is essential to horses in the composition and growth of muscle, and leucine, isoleucine and valine play a role in the muscle tissue metabolism.

Dietary crude protein can change horses serum free amino acid concentrations during exercise. To our knowledge, these effects need to be more investigated considering intense exercising horses. The aim of this study was to investigate the concentration of serum free lysine, threonine, leucine, isoleucine and valine of eventing horses fed diets with different levels of crude protein, before and during exercise.

2. Material and methods

The study was conducted at the Equine Performance Evaluation Laboratory at the Brazilian Army Cavalry School, Rio de Janeiro, Brazil. The chemical analyses were performed at the Equine Health Laboratory and at the Mycological and Mycotoxicologic Research Center both at the Universidade Federal Rural do Rio de Janeiro.

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3. Management of horses

Twenty-four Brazilian Sport Horses, 16 males and 8 females, aged between 8 and 15 yr, mean 488 kg BW (432–562 kg) and mean 5.3 BCS (5.0–5.5 BCS) (Henneke et al., 1983) were used. Horses were randomly distributed in individual 4 × 4 m box stalls, with a water dispenser, a feeder, and wood shaving bedding. Diet adaptation lasted 21d.

All horses were trained according to the Brazilian Army Cavalry School´s protocol for eventing. The yearly program comprised 10 months of training divided into three cycles (initial, intermediate and final) of three months each. The study began at the middle of the intermediate cycle (day 130) and finished at the end of the final cycle (day 270). The weekly workload consisted of 60 min of daily training (6 d per week), including 30% walking, 30% trotting, 10% galloping, and 30% jumps on dirt and grass tracks. The training program targeted the eventing modality and was considered as intense physical activity (NRC, 2007). On the 24 h that preceded the exercise test, horses were not submitted to intense activities, but to flexing exercises (light exercise consisting of walking and trotting flexing exercises and slow canter).

4. Experimental design

Twenty-four horses were used in a randomized block design with 4 diets (protein levels) and 6 repetitions (horses). Horses were blocked according to their experience in competitions (eventing experience: level 1 and 1*).

5. Experimental diets

The horses were fed experimental diets from day 130–270. The intake was calculated for 1.8% of BW. Diets were formulated for intense exercising horses, according to NRC (2007) with 4 different levels of CP: 7.5%, 9.0%, 11.0%, and 13.0%, at a concentrate and hay (50:50) on a DM basis (Table 1). The concentrate was fed 3 times daily in equal amounts at 0400, 1300, and 2000 h, and the roughage was fed 2 times daily in equal amounts at 1100 and 1600 h. Soybean oil, previously weighed and calculated individually, was added directly to the concentrate 3 times daily in equal amounts.

Table 1
Ingredients and nutrients composition of total diets, concentrate and hay (50:50) on a DM basis.

Item	Protein diet level (%)			
	7.5	9.0	11.0	13.0
Soybean meal (%)	1.5	7.0	12.5	17.5
Rolled oats (%)	28.3	23.0	19.5	15.8
Commercial concentrate (%)	10.0	10.0	8.0	7.0
Soybean oil (%)	7.0	7.0	7.0	6.7
Mineral supplement (%)[a]	2.0	2.0	2.0	2.0
Calcium carbonate (%)	1.0	1.0	1.0	1.0
L-lysine (%)	0.15	–	–	–
Cynodon dactylon hay (%)	50	50	50	50
Total	100	100	100	100
Dry matter (%)	88.1	88.0	87.9	87.9
Crude protein (%)	7.5	9.0	11.0	13.0
Lysine (%)	0.35	0.36	0.50	0.62
Digestible energy (Mcal/kgDM)	2.8	2.8	2.8	2.8
Total amino acids[b]				
Lysine (%)	0.35	0.36	0.49	0.62
Threonine (%)	0.29	0.32	0.58	0.64
Leucine (%)	0.75	0.87	0.90	1.04
Isoleucine (%)	0.99	1.02	1.13	1.35
Valine (%)	0.37	0.39	0.43	0.61

[a] Omolene-fós: Ca (Max) 150 g, P (Min) 70 g, S 10 g, Mg 10 g, Na 150 g, Fe 2.500 mg, Cu 820 mg, Zn 2500 mg, Mn 2124 mg,I 20 mg, Se 12,5 mg, Co 20 mg, Cr 6 mg.

[b] The concentration of amino acid of diets was achieved after extracting the solution with total amino acid of ingredient sample by HPLC according to Antonie et al. (1999).

6. Exercise test

The exercise test was carried out on the 262nd day of the training period, at the end of final cycle, on a high-speed treadmill (Galloper 5500 - Sahinco, São Paulo, Brazil). The protocol consisted of a warm up of walking and trotting for 6 min and then a gallop starting at 6.0 m/s with increases in speed of 1 m/s every minute up to 10 m/s. The treadmill inclination was set to 4% during the test. The total test time was 11 min, covering 3.400 m, followed by 6 min of trot and walk to cool-down, without slope.

7. Blood samples

Venous blood samples were collected from the left jugular vein of each horse on the following times: before the test (6 h after meal) and during exercise (last 15 s of the last gallop). Blood was collected into silicone tubes without anticoagulant and was processed immediately. The serum was transferred to a 2 mL Eppendorf and samples were kept at −18 °C until analyses of the free amino acids.

8. Amino acid analysis

Total concentration of lysine (Lys), threonine (Thr), leucine (Leu), isoleucine (Ile) and valine (Val) from diets (concentrate and coast-cross hay) and blood serum free amino acids were determined by high-performance liquid chromatography (HPLC). All analytical methods were compared with the reference amino acid external standard in triplicate evaluation. The standard curve for calibration method of AA evaluated was obtained with values of 100–800 μmol/L with correlation levels of ≥99% (Helrich, 1990). Diet and serum amino acid concentrations were determined using reverse-phase HPLC of phenylisothiocyanate derivatives. The chromatographic conditions mobile phase (methanol:acetonitrile:MilliQ water 10:20:70); the HPLC equipment was composed of a binary pump equipped with micro vacuum degasser, manual injection (30 μL injected), column compartment and fluorescence detector; for analysis use a Hypersil ODS(C18) column. The extraction of the solution with total amino acid of diet samples were performed according to Antonie et al. (1999) and the extraction of the solution with serum free amino acid, according to Hill et al. (1979). After extraction, the tubes were cooled and the samples was filtered using HPLC 13-mm syringe filters (0.45 μm, 30 mm)[5]; the filtrate was diluted with mobile phase (1:2 vol:vol) in amber glass vials. Before injection, AA was derivatized on-line using o-phthaldehyde (Antonie et al., 1999). The elution of samples were performed at a flow rate of 2.0 mL/min by gradient elution, and the total run time was 30 min. Fluorescence detection was carried out at 340 (excitation) and 450 nm (emission).

9. Statistical analyses

A two way ANOVA (time and diet) was performed using the GLM procedure of SAS software (version 9.3). The Shapiro-Wilk test was used to verify the normality of the data set. Linear regression analyses as a function of dietary CP were performed to obtain protein and total amino acids intake data. Linear and quadratic regression analyses were also used to test the effect of dietary CP levels on serum free amino acids concentrations before and during exercise. Significance was declared when $P < 0.05$.

10. Results

10.1. Dietary amino acid

Total intake values of Lys, Thr, Leu, Ile and Val were affected by