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Research

The effect of training sessions and feeding regimes on neuromodulator role of serotonin, tryptophan, and β-endorphin of horses

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ABSTRACT

We tested the hypothesis that diet affects equine feeding behavior and that diet composition affects a shift in energy metabolism characterized by a wide range of neuroendocrine changes. We investigated the effects of training sessions on circulating serotonin (5-HT), tryptophan, and β-endorphin (β-EN) concentrations in horses to ascertain whether two different isoenergetic diets would affect this response. Thirty-six Dutch Warmblood horses were randomly distinguished in 18 horses fed with a low-fiber diet (LF) and 18 horses fed with a high-fiber diet (HF). The training session was represented by a medium-heavy exercise and consisted of 21 minutes/day of walk, 36 minutes/day of trot, 15 minutes/day of canter, for a total of 72 minutes/day. At the end of this session, a set exercise test was performed. There was a significant increase in plasma 5-HT ($P < 0.0001$) and β-EN ($P < 0.0001$) concentrations following exercise compared to baseline values, in both HF and LF groups. No significant changes were observed for plasma tryptophan concentrations after exercise. A two-way analysis of variance showed significant effects of medium-heavy workload exercise treatments and time points of sampling during exercise on 5-HT and β-EN changes. Plasma 5-HT and β-EN patterns are presumably linked to the workload exercise effect, as shown by their increasing trend in both HF and LF groups.

Introduction

Equines are hindgut fermenters that physiologically evolved to consume small and frequent forage-based meals during the day and to digest and utilize high-fiber diets (HFs). Serotonin (5-HT) is a mediator between brain and intestine, the so-called brain-gut axis (Kim et al., 2000) and has a pivotal role in regulating appetite, satiety, and food intake (Gruninger et al., 2007). In a natural setting, horses spend about 14 hours grazing each day (Fleurance et al., 2001; Ellis, 2010). Sport horses are generally stalled, fed a large amount of concentrated grain meals, and have feedings limited to two or three times daily (Henderson, 2007). Loss of natural feed intake time was considered as an important trigger for predisposing animals to develop stereotypic behavior and health problems (Ellis, 2010; Wickens et al., 2010; Sarrafchi and Blokhuis, 2013). Horses those fed low-fiber diets (LFs) are more likely to develop stereotypic behavior than those fed HFs (Gillham et al., 1994; Ellis and Visser, 2003). Consistent lower 5-HT concentrations are found in cribbing horses than in control animals (Lebelt et al., 1998). Fat- and fiber-based diets have been reported to result in calmer patterns of behavior (Hothersall and Nicol, 2009). There is little evidence that herbal- or tryptophan-containing supplements influence equine behavior in any measurable way, although horses have high postprandial changes in plasma tryptophan concentrations (Hackl et al., 2006). Tryptophan infusion decreases endurance in horses (Farris et al., 1998) and oral tryptophan supplementation before exercise has no effect (Vervuert et al., 2005).

The complex interplay between 5-HT, its receptors, and the modulator role on feeding behavior has become of great interest in the scientific community (Keszthelyi et al., 2009; Magalhães et al., 2010). There are few published studies on the role of circulating

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5-HT, its precursor tryptophan and β-endorphin (β-EN), considered a marker of welfare, in response to potentially stressful stimuli, such as different management or feeding-housing regimes (Fazio et al., 2009; Ferlazzo et al., 2012a) and exercise training on fatigue (Medica et al., 2011; Ferlazzo et al., 2012b; Cravana et al., 2017).

Accordingly, the present study sought to characterize the changes in circulating 5-HT, tryptophan, and β-EN concentrations during medium-heavy exercise levels of trained Warmblood horses, with respect to the effect of two isoenergetic experimental diets that varied only in fiber content. Changes in neurohormonal parameters are expected after medium-heavy exercise, but diets could modulate such variation.

Material and methods

Horses

This research is the final part of a large project on the effects of exercised levels and feeding on welfare and performance of trained horses. This project had three consecutive phases that lasted 4 weeks, each represents an exercise level (light, medium, and medium-heavy). All exercises were performed on treadmill.

In this study, phase 3, only, was considered. Phase 3 (medium-heavy exercise level) consisted of 21 minutes/day of walk, 36 minutes/day of trot, 15 minutes/day of canter, for a total of 72 minutes/day. At the end of phase 3, a set exercise test (SET) was performed. Each horse was submitted to SET on the treadmill, for a total of time spent paired to 15 minutes. Speed in m/s and duration of set exercise ranged between ±1.8 m/s and 6 minutes (walk) to ±4.0 m/s and 9 minutes (trot), respectively.

Thirty-six healthy Dutch Warmblood horses (18 mares and 18 geldings), aged 3 years, weighting 554 ± 42 kg, housed in wood shavings bedding were used. All horses were reared under similar conditions and had been accustomed to individual housing and treadmill work over the previous 4 months. All horses had reached a light fitness level (20-minute walk and 8-minute trot on the treadmill).

To determine whether there was an effect of amount of dietary fiber on performance, horses were fed two isoenergetic diets for 4 weeks. Horses were randomly assigned to one of two groups of 18 animals, with each group containing nine mares and nine geldings. One group of horses was fed on a HF (HF group: DM ratio—concentrate:silage = 1:4), and one group was fed on a LF (LF group: DM ratio—concentrate:silage = 4:1). The chemical composition of the feed was as for Ellis and Visser (2003). Hay was given twice daily (8:00 and 17:00), whereas concentrate feed was divided into three equal meals per day. All horses were fed a minimum of 1.4 times digestible crude protein requirements as reported by Ellis and Visser (2003). The HF group was fed an additional mineral supplement in order to adjust mineral intakes as close as possible to those levels fed in the LF group. All diets fulfilled recommended requirements.

At the end of phase 3, all horses were kept in two groups by the sex (each consisting of 50% former HF group and 50% former LF group) and placed on grassland for 3 months. The feeding regime for the "control" diet was therefore grass and silage. Exact intake per horse could not be determined, but an average of 11 kg wet matter was fed per day per horse. Control blood samples were taken at the end of this period.

All horses were subjected to a standardized management, handling regimen, and blood sampling techniques. An equal number of each group (HF/LF) was tested either in the morning and afternoon. All methods and procedures used in this study were approved by the Animal Care and Use Committee of the Animal Sciences Group of Wageningen University and Research Centre in Lelystad, (the Netherlands), in accordance with EU Directive 2010/63/EU for animal experiments.

Sample collection

The first blood samples were collected at 11 AM and at 2 PM from jugular vein at rest in baseline conditions (T0). The second blood sample was taken 60 minutes after exercise, at 60 minutes after training on the treadmill and after the final bout of trotting (T1), and at the end of the 72 minutes exercise test, immediately following last trot (T2). A final blood sample was taken after 3 months of grass and silage contents (T3), for the "control" diet effect.

Horses had previously been accustomed to clinical routines and blood collections.

All samples were collected by the same operator into evacuated tubes (Venoject, Terumo) and into evacuated tubes (Venoject EDTA K_3; Terumo Europe, Rome, Italy) and were immediately refrigerated at 4°C.

5-HT and tryptophan analysis

Blood samples were centrifuged at 4°C at 4500 × *g* for 10 minutes, to obtain a platelet poor plasma (PPP) fraction which is devoid of 98% of platelets (Bruschetta et al., 2014). Equal volumes (100 μL) of internal standard of N-methylserotonin (Chromsystems, München, Germany) and protein precipitation reagent (Chromsystems) were added to 100 μL of PPP. The solutions were vortex mixed for 30 seconds, incubated at 4°C for 10 minutes, and then centrifuged at 4500 × *g* for 10 minutes. The resulting supernatants were stored at −20°C for the following high performance liquid chromatography (HPLC) analysis. Separation of PPP 5-HT and tryptophan were carried out by an isocratic reverse phase HPLC method. Qualitative and quantitative analyses of 5-HT and tryptophan were performed as detailed elsewhere (Bruschetta et al., 2013). The assay sensitivity of HPLC detector was 0.5 ng/mL for 5-HT and 3 ng/mL for tryptophan. The intraassay and inter assay coefficients of variation (CVs) for 5-HT were 4.2% and 5.3%, respectively. The intraassay and interassay CVs for tryptophan were 4.3% and 6.0%, respectively.

β-EN analyses

The blood was placed on ice until centrifugation and centrifuged within 1 hour after collection. In order to analyze β-EN concentrations, after collection an aliquot of the blood samples (2.5 mL) was transferred into polypropylene tubes containing ethylenediaminetetraacetic acid (EDTA) (1 mg/mL of blood) and aprotinin, protease inhibitor (500 KIU mL blood; ICN Biomedicals Inc., Aurora, OH, USA) and kept at 4°C. Plasma samples were harvested after centrifugation at 3000 × *g* for 15 minutes at 4°C and stored at −20°C until the day of analysis. Peptides were extracted from plasma samples with 1% trifluoroacetic acid (TFA, HPLC grade) and elution with 60% acetonitrile (HPLC grade) in 1% TFA.

Plasma β-EN concentrations were measured in duplicate utilizing a commercial RIA kit (Peninsula Lab. Inc., Belmont, CA, USA) for human β-EN, which has a 100% cross-reactivity with equine β-EN (Mehl et al., 1999; Mehl et al., 2000). The sensitivity of the assay β-EN was 5 pmol/L. The intraassay and interassay CVs were 7% and 15%, respectively.

Statistical analyses

Physiological data were tested for normality. An analysis of variance was applied for differences between treatments, with adjustment for the following factors: sex, diets, training phases, and blood sample time.

In order to analyze differences for treatments (increasing exercise levels, HF and LF diets, and sex), time, and the interaction