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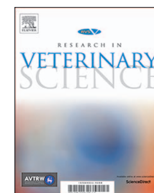

Effects of grain species, genotype and starch quantity on the postprandial plasma amino acid response in horses

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ABSTRACT

Postprandial alterations of plasma amino acid (PAA) levels partly reflect a temporal contribution of the feed. How cereal grains affect PAA levels is not known. We hypothesized that a meal of cereal grains causes a temporal increase of PAA, affected by grain species, grain genotype and meal size. Six mares were used in three consecutive trials, receiving four oats, barley and maize genotypes, respectively. Individual grain genotypes were provided as 3 meal sizes corresponding to 1.0, 1.5 or 2.0 g starch/kg body weight. Meadow hay (1.5 kg/100 kg body weight) was offered daily. At the test days, 1 kg hay was fed 60 min prior to the grain meal. Blood samples were taken before grain feeding (0 min) and 30, 60, 90, 120, 180, 240 and 300 min thereafter. Subsequently, the remaining hay was offered. The genotype × starch quantity (i.e., meal size) interaction had a major effect on postprandial PAA concentrations ($P < 0.05$). Availability of amino acids (AA), ingested from different grain genotypes, apparently differed at both the digestive and post-digestive level. Thus, AA supply from grain feeding can better be assessed on the genotype level. The concentrations of most PAA increased rapidly with a postprandial maximum at around 30 min. Hay feeding might have an underrated capability for AA provision because increases of PAA levels were initialized already by ingestion of a 1 kg hay. It remains unclear which portion of the PAA kinetics response originates from hay feeding and which one from the cereal grain meal.

1. Introduction

A large quantity of available amino acids (AA) has been reported to stimulate both the synthesis of muscle (Graham-Thiers and Kronfeld, 2005; Mastellar et al., 2016a, 2016b) and milk protein (Schubert et al., 1991). Therefore, an adequate supply of essential AA (EAA) is particularly important in lactating mares, growing horses, sport horses and older horses. Postprandial plasma AA (PAA) levels reflect the amount of dietary AA which is absorbed by the small intestine and not utilized by the gut wall, but only if the horses are not undersupplied with EAA (Krumbiegel et al., 2012). Furthermore, postprandial alterations of PAA levels can be impacted by AA utilization for, e.g., gluconeogenesis or by AA release from tissue proteolysis. In particular, exercise (Bergero et al., 2005; Hackl et al., 2009; Westermann et al., 2011), growth (Hackl et al., 2006; Krumbiegel et al., 2012; Tanner et al., 2014), lactation (Witten et al., 2011) and immunological restraints (Routledge et al., 1999) have been reported to affect postprandial PAA levels, as well as feeding sequence (Cabrera et al., 1996), feeding frequency and feed quantity (Russell et al., 1986). Thus, these factors need to be standardised or at least being kept constant in the experimental design to investigate the contribution of the feed on postprandial PAA concentrations.

In practical horse feeding, cereal grains are the most prominent concentrate components. However, there is a lack of information regarding their effect on postprandial PAA concentrations when different grain species and grain genotypes without supplementation of individual AA, AA concentrates or AA preparations are fed (Coenen et al., 2006; Harris et al., 2006). We hypothesized that meal feeding of cereal grains as part of a typical horse diet leads to a transient increase of PAA concentrations, which will be affected by the grain species, the grain genotype and the meal size. The aim of this study was to investigate the impact of feeding four different genotypes of crushed oats, barley grains

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and cracked maize grain, respectively, in quantities equal to 1.0, 1.5 and 2.0 g starch/kg body weight (BW) per meal on postprandial PAA changes.

2. Materials and methods

2.1. Ethical note

The experimental procedures used in this study were approved by the Animal Welfare Committee of Mecklenburg-Western Pomerania (LALLFM-V/TSD/7221.3.1.1-036/11).

2.2. Animals

Four Warmblood and two Trotter mares with a mean BW of 530 ± 56.0 kg, a mean body condition score of 5.9 ± 0.58 (on a scale from 1 to 9 according to Kienzle and Schramme, 2004) and 6 ± 2 years of age, were used in this study. The horses were housed in individual boxes fitted with rubber mats and a bedding of wood shavings. The horses had free access to a paddock each day, except for the days of blood sampling, but have not been exercised. All horses were under regular veterinary supervision and were regularly dewormed and vaccinated (EHV-1, EHV-4, Equine Influenza, Tetanus). Clinical diseases or abnormalities with respect to dental health were not detected.

2.3. Diets and experimental design

During the experimental period, the basal feed consisted of meadow hay with 1.5 kg/100 kg BW/d, i.e., 6.9–9.0 kg dry matter (DM)/d, provided in two equal meals per day (except for the test days). With the daily hay ration the mares received between 46.2 and 60.3 MJ of metabolizable energy (ME). Tap water was offered ad libitum. The hay was supplemented with one of four different genotypes of crushed grains from oat (Scorpion, Energie, Melody, Sandokan), barley (Yoole, ACK 2927, Lomerit, Campanile) and cracked maize grains (P9494, PR37Y12, Öl 3, Öl 4), respectively. All oat and barley genotypes examined were with husks with the exception of the oat genotype Sandokan. The meal sizes did correspond either to 1.0, 1.5 or 2.0 g starch/kg BW. This means the mares received 0.67–2.66 kg DM with the oats meals, 0.77–2.11 kg DM with the barley meals and 0.64–2.32 kg DM with the maize meals, which corresponded to a ME intake of 10.1–36.4 MJ, 10.1–27.6 MJ and 7.6–35.0 MJ, respectively. The feeding levels, expressed as a manifold of maintenance, were between 1.1 and 1.3 at 1.0 or 2.0 g starch/kg BW and grain meal, respectively (GfE, 2014). During adaptation periods, grain meals were offered at 1.5 g starch/kg BW. The analysed proximate nutrient composition and the calculated ME contents in the hay and the cereal grain genotypes are given in Table 1. Analysed contents of AA are shown in Table 2.

This study was carried out in context with another study which has focussed on the horses' plasma glucose and insulin responses to the feeding of the present variety of cereal grain genotypes and meal sizes. Thus, the experiment was designed as follows: Three consecutive trials were carried out, in which either maize, barley or oats was fed. Each of these trials started with a 4 weeks adaptation period, in which the 6 mares received one of the target grain genotypes at a meal size corresponding to 1.5 g starch/kg BW. Afterwards, the mares received a specific genotype for 3 days without changing the meal size. This genotype was subsequently offered for another 3 days while changing the grain meal size from day to day in a randomized cross-over design (using 3 × 2 animals) according to 1.0, 1.5 or 2.0 g starch/kg BW. These final 3 days (test days) were used for blood sampling. Then, the next grain genotype was offered, again using 3 days for adaptation plus 3 days for changing the meal size. At the test days, 1 kg of hay was provided 60 min before the feeding of cereal grains in order to support a stable hindgut fermentation (Zeyner et al., 2004) and for reasons of standardization (Bochnia et al., 2017; Glatter et al., 2017; Zeyner et al., 2017). The remaining hay was offered after the blood sampling period of each test day.

2.4. Sampling

Representative samples of the grain genotypes and the hay were taken. All feed samples were stored dry until subsequent analyses.

Blood samples were taken via an indwelling catheter (Braunüle® MT, 14 G, 80 mm, B. Braun Melsungen AG, Melsungen, Germany) immediately before (0 min) and 30, 60, 90, 120, 180, 240 and 300 min after the grain meal was given. The catheter was inserted into the *V. jugularis externa*, connected to a Heidelberger extension set (Mediware® REF H7 1403, Servoprax GmbH, Wesel, Germany) using a three-way stopcock (Cat. 230648, Jørgen Kruuse A/S, Langeskov, Denmark) and covered with an injection cap (Fresenius Kabi AG, Bad Homburg, Germany). The catheter system was sewed on the skin for 3 consecutive test days. It was flushed with a physiological saline solution after each blood collection and with a heparin-saline solution at the end of each test day to avoid impurities and venal thrombi. For blood collection, S-Monovette® tubes (2.7 mL FE, 1.2 mg EDTA/mL blood and 1 mg fluoride/mL blood, Sarstedt AG & Co., Nümbrecht, Germany) were used. The samples were cooled down to 4 °C, centrifuged at 3800 × *g* for 10 min and blood plasma was stored at −20 °C for subsequent analyses.

2.5. Sample preparation, analyses and calculations

The feed samples were ground to pass through a 1 mm sieve of a standard laboratory sample mill. For starch analysis, the cereal grains were pulverized using a ball mill. The dry matter (DM) content, ash, crude protein (CP), acid ether extract (AEE), crude fibre (CF), the Van Soest detergent fibres and sugar were determined according to the official German key book for feed analysis (VDLUFA, 2012; methods no. 3.1, 4.1.1, 5.1.1 B, 6.1.1, 6.5.1, 6.5.2, 6.5.3, 7.1.2 and 8.1). The amounts of organic matter (OM) and the nitrogen-free extract (NFE) were calculated (NFE = OM − CP − AEE − CF). Starch was enzymatically determined with the amyloglucosidase method (VDLUFA, 2012, method no. 7.2.5). The ME content of the feeds was calculated on the basis of crude nutrient analyses according to Kienzle and Zeyner (2010) and GfE (2014). Neutral detergent-soluble CP was determined basing on Licitra et al. (1996) according to VDLUFA (2016; method no. 4.13.1) and used to calculate the neutral detergent-soluble and precaecal digestible (pcd) contents of dietary CP and AA (Zeyner et al., 2010; GfE, 2014; Zeyner et al., 2015).

Feeds proteins were hydrolysed with hydrochloric acid and AA were analysed using ion exchange chromatography (Biochrom 30 with PEEK-Sodium Prewash Column, 100 × 4.6 mm, and PEEK-Oxidised Feedstuff Column, 200 × 4.6 mm, Biochrom Ltd., Cambridge, UK) according to the protocol of VDLUFA (2012, method no. 4.11.1), both with preceding oxidation for cysteine (Cys) and methionine (Met) detection but without oxidation for tyrosine (Tyr) and histidine (His) detection. Tryptophan (Trp) was hydrolysed with phosphoric acid and hydrochloric acid and analysed using high performance liquid chromatography (HPLC; Agilent 1100 Series with ZORBAX Eclipse XDB-C8, 150 × 4.6 mm, 5 µm, Agilent Technologies Inc., Santa Clara, CA, USA) according to Fontaine et al. (1998).

Plasma AA were determined as described by Hackl et al. (2010) (including Trp as described above) using a HPLC modular unit (Shimadzu, Kyoto, Japan) fitted with a cation column (LC K06, Alltech-Grom GmbH, Rottenburg-Hailfingen, Germany). Post-column AA UV-detection was carried out using ninhydrine (flow rate of 0.25 mL/min) and wavelengths of 570 nm for AA and 440 nm for proline (Pro).

2.6. Statistical analysis

Statistical analysis was performed using the SAS 9.4 software package (SAS Institute Inc., Cary, NC, USA).

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