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Dietary crude protein intake influences rates of whole-body protein synthesis in weanling horses

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ABSTRACT

The objective of this study was to measure whole-body protein kinetics in weanling horses receiving forage and one of two different concentrates: (1) commercial crude protein (CCP) concentrate, which with the forage provided 4.1 g CP/kg bodyweight (BW)/day (189 mg lysine (Lys)/kg BW/day), and (2) recommended crude protein (RCP) concentrate which, with the same forage, provided 3.1 g CP/kg BW/day (194 mg Lys/kg BW/day). Blood samples were taken to determine the response of plasma amino acid concentrations to half the daily concentrate allocation. The next day, a 2 h-primed, constant infusion of [^{13}C]sodium bicarbonate and a 4 h-primed, constant infusion of [1-^{13}C]phenylalanine were used with breath and blood sampling to measure breath $^{13}CO_2$ and blood [^{13}C]phenylalanine enrichment.

Horses on the CCP diet showed an increase from baseline in plasma isoleucine, leucine, lysine, threonine, valine, alanine, arginine, asparagine, glutamine, ornithine, proline, serine, and tyrosine at 120 min post-feeding. Baseline plasma amino acid concentrations were greater with the CCP diet for histidine, isoleucine, leucine, threonine, valine, asparagine, proline, and serine. Phenylalanine, lysine, and methionine were greater in the plasma of horses receiving the RCP treatment at 0 and 120 min. Phenylalanine intake was standardized between groups; however, horses receiving the RCP diet had greater rates of phenylalanine oxidation ($P = 0.02$) and lower rates of non-oxidative phenylalanine disposal ($P = 0.04$). Lower whole-body protein synthesis indicates a limiting amino acid in the RCP diet.

Introduction

Horses are believed to have a dietary requirement for each of the individual indispensable amino acids; however, unlike other species (WHO, 2007; NRC, 2012), equine amino acid requirements have not been well defined. Feeding recommendations are thus primarily focused on total protein intake. Several different systems have been used to express protein requirements, including crude protein (CP) (NRC, 2007), horse digestible CP (Martin-Rosset and Tisserand, 2004), apparent digestible CP (Ellis, 2004), and pre-cecally digested protein (Coenen et al., 2011). A limiting amino acid is a dietary indispensable amino acid that is provided the most below its requirement and therefore protein synthesis is limited to the rate at which that amino acid is available.

Several studies have identified lysine as a commonly limiting amino acid in equine diets (Breuer and Golden, 1971; Ott et al., 1981; Graham et al., 1994). These studies are the basis of lysine requirements listed in the National Research Council (NRC, 2007) and in French feeding recommendations (Martin-Rosset and Tisserand, 2004). In North America and Europe, CP values are readily available on feed labels and in ration formulation software, whereas the amino acid contents of equine feeds are not as readily available.

In the United States, horses are often fed CP well above the NRC recommendations (Harper et al., 2009), which for 6-month old weanlings is 3.1 g/kg bodyweight (BW)/day (NRC, 2007). Reflecting its status as a commonly limiting amino acid, the NRC recommends a lysine requirement of 32 g/day for a 6-month old weanling with an estimated mature weight of 550 kg (NRC, 2007). It has also been suggested that threonine (Graham et al., 1994) and methionine (Winsco et al., 2011; Graham-Thiers et al., 2012) are potentially limiting amino acids in yearling and weanling horse diets, respectively.

The NRC suggests that the other amino acid requirements for horses can be estimated from the tissue composition of the amino acids relative to lysine based on the work of Bryden (1991), but these

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rough estimates are not currently included in either the requirement tables or software of the NRC (2007). Although there has been some in vivo evaluation of these estimates (Graham-Thiers and Bowen, 2013), amino acid requirements have not been evaluated individually or over a variety of life stages.

High protein diets could have detrimental effects on the horse itself and could affect acid–base balance (Graham-Thiers et al., 2001), heat production (Kronfeld, 1996), water requirements (Graham-Thiers et al., 2001), and, potentially, respiratory health (Whittaker et al., 2009). A refined understanding of dietary limiting amino acids for growing horses could result, at least potentially, in improved diet formulation by enabling total CP intake to be reduced while meeting requirements of those amino acids most likely to be limiting by the use of crystalline amino acids.

Reducing the CP of a pasture supplement from 14% to 9% did not affect the growth of Thoroughbreds, when fortified with 0.6% lysine and 0.4% threonine (Staniar et al., 2001). Formulating diets in this manner has been shown to reduce nitrogenous waste excretion in other species including livestock and poultry (Jongbloed and Lenis, 1992; Applegate et al., 2008). Moreover, nitrogenous waste can have a negative effect on the environment (Fenn et al., 2003) so improved diet formulation in regard to protein would provide benefits beyond the equine industry.

Amino acid intakes above an animal's requirements will not be used for protein synthesis, and must be metabolized to urea and CO_2. The conversion of an amino acid to CO_2 can be measured by infusing a 1-^{13}C-labeled amino acid and measuring $^{13}CO_2$ expired in the breath. Once the amino acid oxidation is known, non-oxidative disposal, an estimate of whole-body protein synthesis, can be calculated (Waterlow et al., 1978). Such isotopic methods have been used extensively in other species (Coleman et al., 2003; Moehn et al., 2005; Brunton et al., 2007; Kurpad and Thomas, 2011) to determine amino acid requirement and to identify limiting amino acids. The concept and application of isotopic methods have been reviewed elsewhere (Elango et al., 2008) and compared thoroughly to other methods of determining amino acid requirements (WHO, 2007). Our recently validated amino acid isotope infusion technique for use in horses (Urschel et al., 2012) can now be used to improve our understanding of protein metabolism in horses.

The objective of the present study was to compare whole-body protein kinetics between horses receiving two different diets: (1) a commercial concentrate for growing horses fed in combination with forage, which provided CP above the current NRC recommendations, and (2) a diet that provided a lower protein concentrate in combination with the same forage and a level of CP similar to current NRC recommendations for growing horses. Both rations supplied the recommended intake of lysine. This study was the first time that stable isotope kinetics has been used to estimate whole-body protein synthesis in growing horses.

Materials and methods

Animals, housing, and feeding

All procedures used in this study were approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC 2010 – 0709, granted 1 September 2010). Six weanling colts of similar age and weight were obtained from University of Kentucky's Maine Chance Farm. At the time of sampling, the weanlings were on average across treatments 182 ± 8 days and 236 ± 20 kg.

During the 2-week adaptation period and the week between sampling periods, the weanlings were kept in individual dry lot pens and were brought into individual sawdust bedded stalls (3.7 m × 3.7 m) twice daily for concentrate feeding. Forage was fed in the dry lots. After catheterization and during all sample collection and isotope infusion procedures, the horses remained in the stalls. Bodyweights were taken weekly through the adaptation and study periods on a livestock scale (TI-500, Transcell Technology).

During the 2-week adaptation period, when the horses were adapting to the research environment, diets were designed to meet or exceed all the 2007 NRC recommendations (NRC, 2007). The concentrate to forage ratio of 58:42 we used was similar to that of other growing horse diets (Ott et al., 1981; Gibbs and Potter, 2002). Concentrate and alfalfa hay cubes (10 g cubes/kg BW/day; Table 1) were fed twice daily at 0700 and 1400 h, and the horses had free access to salt and water at all times. The concentrate consisted of a commercial pellet designed for growing horses (Growth Pellet, Buckeye Nutrition; 11.6 g/kg BW/day) in addition to a ration balancer pellet (Gro 'N Win Alfa, Buckeye Nutrition; 1.9 g/kg BW/day). Feed was collected throughout the study into plastic bags and stored at room temperature before being sent to Dairy One Cooperative for proximate analysis via wet chemistry. The concentrates and the forage were also analyzed for amino acid content as described below.

Study design and procedures

After the 2-week adaptation period, this study was conducted as a crossover design, where each colt was studied while receiving each of two dietary treatments, in a randomly determined order. The two treatments were the commercial crude protein diet (CCP), where horses continued to receive the commercial concentrate, as above, and the recommended crude protein diet (RCP), where horses received a concentrate containing ~50% of the CP contained in the commercial pellet (Tables 1 and 2).

The CCP concentrate in combination with the forage cube provided protein well above requirements as is typical in the equine industry (Honore and Uhlinger, 1994; Harper et al., 2009), whereas the RCP treatment provided protein at a level consistent with the current NRC recommendations (NRC, 2007). The commercial and the RCP concentrates were formulated to contain similar amounts of lysine and micronutrients (Table 1).

Orts (remaining feed) were collected and weighed prior to each subsequent meal feeding, although after the adaptation period feed refusals were minimal. To standardize tyrosine and phenylalanine intakes between the two treatments the RCP concentrate was top dressed with tyrosine (15.3 mg/kg BW/day) and phenylalanine (51 mg/kg BW/day). Amino acid intakes from the treatment diets are provided in Table 3. Standardization of phenylalanine and tyrosine intake is common practice for studies evaluating different levels of protein and/or different feedstuffs using phenylalanine isotope kinetics (Moehn et al., 2005; Humayun et al., 2007). Phenylalanine is a common choice for an indicator amino acid and the criteria for an appropriate indicator amino acid are enumerated by Hsu et al. (2006a).

All horses received each treatment diet for 3 days before sampling. The short adaptation period required for this method (Moehn et al., 2004; Elango et al., 2009) makes it ideal for studying growing animals, as the animals can be monitored while receiving potentially deficient diets and the risk of detrimental effects associated with prolonged nutrient restriction is minimized. Any effect of the diet before receiving the treatment diet is likely to be minimal. Commentary on length of adaptation and the use of the indicator amino acid oxidation method can be found elsewhere (Elango et al., 2012).

On the afternoon of day 3 for each treatment, two jugular vein catheters were inserted, one for blood sampling and one for isotope infusion (Urschel et al., 2012). In order to determine the effect of concentrate CP on plasma amino acid concentrations, baseline blood samples (7 mL) were taken 15 min and immediately before feeding the 1400 h allocation of concentrate. Another sample was taken at 120 min post-feeding.

The next day, whole-body phenylalanine kinetics were determined using a 2 h-primed (7.1 μmol/kg BW), constant (6 μmol/kg BW/h) infusion of [^{13}C]sodium bicarbonate, to measure whole-body CO_2 production (Coggan et al., 1993), followed immediately by a 4 h-primed (8.4 μmol/kg BW), constant infusion of [1-^{13}C]phenylalanine (6 μmol/kg BW/h), to measure phenylalanine oxidation to CO_2 and phenylalanine flux. The isotope doses and lengths of infusion were previously validated in mature horses and resulted in a rapid rise to plateau isotopic enrichment in the plasma phenylalanine (Urschel et al., 2012). In order to infuse the isotope and allow the weanling horses to move freely in their stalls, cordless infusion pumps (J-1097 VetPro Infusion Pump, Jorgensen Laboratories) were attached to surcingles that were worn by the weanlings.

Starting at 90 min before the start of isotope infusion procedures, 1/24th of the morning meal, including both the hay cube and the concentrates, was given every 30 min during infusion in order to reduce fluctuations in phenylalanine oxidation (Mohn et al., 2003). Breath samples were collected every 30 min into gas impermeable bags using a modified equine Aeromask (Urschel et al., 2009), beginning 30 min prior to the start of the [^{13}C]sodium bicarbonate infusion until the end of the [1-^{13}C]phenylalanine infusion. Blood samples were collected every 30 min beginning at 30 min prior to the start of the [1-^{13}C]phenylalanine infusion until the end of the infusion procedures.

At the end of the isotope infusion procedures, catheters were removed and the weanlings had a 4-day washout period, during which they were fed and managed as described during the adaptation period, before being allocated to the other treatment. At the end of the study, the weanlings were returned to the research herd.

Blood sample processing

All blood samples were collected into heparinized Vacutainers (Becton-Dickinson) and promptly centrifuged at 1500 *g* for 10 min at 4 °C. Plasma was removed and frozen at –20 °C until the time of analysis.