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Controlled trial of whole body protein synthesis and plasma amino acid concentrations in yearling horses fed graded amounts of lysine

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ABSTRACT

Lysine has been reported as the first limiting amino acid in typical equine diets. Indicator amino acid oxidation (IAAO) has become the standard method for determining amino acid requirements in other species, but prior to this study, it has not been used to determine equine requirements. The aim of this study was to evaluate whole body protein synthesis and plasma and muscle amino acid concentrations in response to graded levels of lysine intake in yearling horses. Six Thoroughbred colts (358 ± 5 kg) were fed each of six treatment lysine intakes ranging from 76 to 136 mg/kg body weight/day. Blood samples were taken before and 90 min after the morning concentrate meal. Gluteal muscle biopsies were taken ~100 min after the morning concentrate meal. The next day, whole body phenylalanine kinetics were determined using a 2 h primed, constant infusion of [^{13}C] sodium bicarbonate followed by a 6 h primed, constant infusion of [1-^{13}C] phenylalanine. Plasma lysine concentrations increased linearly ($P < 0.05$) at both the 0 and 90 min time points with increasing lysine intakes. Free muscle asparagine, aspartate, arginine, glutamine, lysine, taurine and tryptophan concentrations responded quadratically to lysine intake ($P < 0.05$). Phenylalanine kinetics did not differ between treatment intakes ($P > 0.10$). A broken line analysis of lysine intake and phenylalanine oxidation failed to yield a breakpoint from which to determine a lysine requirement. These diets may have been limiting in an amino acid other than lysine, underscoring the lack of data concerning amino acid requirements and bioavailability data in the horse.

Introduction

Compared to human beings and pigs, relatively little work has been done to elucidate amino acid requirements for horses. Currently, the NRC (2007) lists only a crude protein requirement and a lysine requirement in the requirement tables and software. Identifying amino acid requirements for various physiological states (NRC, 2012) has helped the pig industry to minimize nitrogenous waste (Panetta et al., 2006) without compromising pig growth (Tuitoek et al., 1997). The environmental impacts of excess nitrogen output from equine operations have been reviewed by Bott et al. (2015). Other consequences of overfeeding protein to horses could be compromised respiratory health from increased ammonia output (Whittaker et al., 2009), disrupted acid–base balance (Graham-Thiers and Kronfeld, 2005b) and decreased bone mineralization from increased calcium excretion through urine (Glade et al., 1985). Determining amino acid requirements in horses will allow diet formulation to more closely match equine needs.

Lysine is a common limiting amino acid for many species (Metges et al., 2005; Tujioka et al., 2005; Tome and Bos, 2007). It has been found to be limiting in some diets for growing horses (Breuer and Golden, 1971; Potter and Huchton, 1975; Ott et al., 1981; Fisher et al., 1989; Graham et al., 1994). However, these studies in horses generally were not designed to measure specific lysine requirements and they did not use isonitrogenous treatments, but rather used treatments with different protein sources and/or did not include many levels of lysine intake. The NRC (2007) applied broken line analysis to seven studies that reported diet composition, intake and nitrogen retention to estimate a lysine requirement at 4.3% of the crude protein requirement (NRC, 2007). This lysine requirement as a percentage of crude protein is in line with other species (Ball et al., 2007), but has not been confirmed experimentally in horses. The current NRC (2007) recommendation for lysine for yearling horses is 113 mg/kg body weight (BW)/day.

Isotopic methods have been used in other species to determine amino acid requirements (Bertolo et al., 2005; Kurpad and Thomas, 2011). These methods are based on the concept that amino acids that cannot be used for protein synthesis are oxidized to CO_2. The production of CO_2 from amino acid metabolism can be determined from an infusion of a 1-^{13}C-labeled amino acid and measuring $^{13}CO_2$ expelled in the breath. Using an isotopic essential amino acid other than the amino acid being tested as an indicator of amino acid

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metabolism is known as the indicator amino acid oxidation (IAAO) method (Brunton et al., 1998; Elango et al., 2008). As the dietary intake of a test amino acid, such as lysine, increases, less of the indicator or 1-^{13}C-labeled amino acid is oxidized until a plateau is reached. The intersection of this decline and plateau of the rate of $^{13}CO_2$ release from the indicator amino acid defines the requirement of the test amino acid. The isotope infusion technique necessary to determine requirements via the IAAO method has been adapted to horses and validated (Urschel et al., 2012). In addition to calculating amino acid requirements, the isotopic data can also yield estimates of rates of whole body protein synthesis and breakdown (Waterlow et al., 1978).

This study investigated the responses of whole body protein synthesis in addition to plasma and tissue amino acid concentrations in yearling horses receiving graded levels of lysine. The lysine levels included levels both above and below the current NRC (2007) recommendations. We hypothesized that a breakpoint would be seen in our phenylalanine kinetics data denoting a lysine requirement for yearling 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 2008-0385). Six yearling Thoroughbred colts were obtained from the University of Kentucky Maine Chance Farm for use in this study in May 2011. The IAAO method has successfully been used to determine amino acid requirements in other species using five to six subjects (Bertolo et al., 2005; Elango et al., 2007; Levesque et al., 2011). A sample size calculation confirmed that six horses were appropriate (PS Power and Sample Size Calculations Program, version 2.1.3.1).

The adaptation diet consisted of hay cubes and a half and half mix of the concentrates used in the treatment diets. The 2 week adaptation period to diet and housing was followed by six 1 week treatment periods. Yearlings were housed in individual dry lot pens and all sampling procedures were conducted in individual sawdust bedded stalls (3.7 m × 3.7 m) except for muscle biopsies, which were collected from horses in stocks. A livestock scale (TI-500, Transcell Technology) was used to obtain daily body weights.

Diets were designed to meet or exceed the 2007 Nutrient Requirements of Horses recommendation, with the exception of lysine (NRC, 2007). Two isonitrogenous and isocaloric versions of the same concentrate pellets were formulated for this study (Tables 1 and 2). The only differences between the two concentrates were that one contained free lysine, and the other an isonitrogenous amount of free glycine and a small amount of rolled oats to account for the difference in weight of the added glycine versus lysine. By mixing the two concentrates at different ratios and feeding timothy hay cubes at the same rate for all treatments, six treatment diets were created with lysine intakes of 76, 90, 104, 118, 127 and 136 mg/kg BW/day. Three of the lysine intakes were above the estimated lysine requirement of 113 mg/kg BW/day and three of the intakes were below. Glycine was selected because it is a dispensable amino acid and not metabolically related to lysine. The concentrate was provided at 1.11% of BW/day with timothy hay cubes at 1.37% of BW/day (Table 1). Timothy was used as the forage component of the diets because of its relatively low lysine content (Woodward et al., 2011). Concentrate and hay cubes were fed twice daily (0700 and 1500 h) with water and salt available at all times. Feed was collected throughout the study and four samples of each feedstuff were sent to Dairy One Cooperative for proximate analysis at the conclusion of the study. Weekly feed samples were analyzed for amino acid content as described below.

Study design and procedures

Horses were studied in a group of two and a group of four with similar birth dates within each group to facilitate sample collection. Splitting the horses into groups allowed all the horses to be studied at similar ages and further minimize variation. Diets were fed in a random order within the groups, with the stipulation that no two horses were on the same treatment diet at the same time. Treatment assignments were made after horses were selected for the study using a random number generator, such that each treatment was represented in each period and each horse

Table 1
As-fed nutrient composition of feeds (mean ± standard error) used in creating treatments with six levels of lysine intake for a controlled trial of yearling horses.

	Timothy hay cubes[a]	Low lysine concentrate[b]	High lysine concentrate[c]
Overall nutrient composition			
Dry matter (%)	91.2 ± 0.2	89.3 ± 0.1	89.6 ± 0.5
DE (Mcal/kg) [c]	1.69 ± 0.11	2.97 ± 0.02	3.06 ± 0.06
Crude protein (%)	7.1 ± 0.1	13.9 ± 0.1	13.7 ± 0.3
Lignin (%)	4.9 ± 0.1	0.8 ± 0.3	0.9 ± 0.1
ADF (%)	39.4 ± 0.6	10.1 ± 0.5	9.3 ± 1.1
NDF (%)	60.4 ± 0.5	19.9 ± 0.7	17.5 ± 2.0
Water soluble carbohydrates (%)	10.9 ± 0.3	3.9 ± 0.1	4.1 ± 0.1
Ethanol soluble carbohydrates (%)	5.6 ± 0.2	2.3 ± 0.8	2.8 ± 0.3
Starch (%)	1.0 ± 0.1	32.3 ± 0.4	33.4 ± 1.9
Crude fat (%)	1.6 ± 0.1	6.7 ± 0.1	7.1 ± 0.1
Calcium (%)	0.37 ± 0.01	1.86 ± 0.10	1.94 ± 0.17
Phosphorus (%)	0.20 ± 0.01	0.30 ± 0.01	0.32 ± 0.02
Iron (mg/kg)	149 ± 6	175 ± 9	215 ± 61
Zinc (mg/kg)	101 ± 16	114 ± 6	126 ± 34
Amino acid composition (g/100 g feed)			
Alanine (%)	0.34 ± 0.03	0.62 ± 0.02	0.61 ± 0.01
Arginine (%)	0.35 ± 0.04	0.61 ± 0.03	0.60 ± 0.01
Aspartate + asparagine (%)	0.51 ± 0.04	0.61 ± 0.06	0.53 ± 0.05
Glutamate + glutamine (%)	0.64 ± 0.07	2.50 ± 0.09	2.57 ± 0.08
Glycine (%)	0.25 ± 0.02	1.06 ± 0.02	0.40 ± 0.01
Histidine (%)	0.05 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
Isoleucine (%)	0.28 ± 0.03	0.51 ± 0.02	0.47 ± 0.02
Leucine (%)	0.47 ± 0.04	1.19 ± 0.02	1.19 ± 0.02
Lysine (%)	0.25 ± 0.02	0.37 ± 0.02	0.92 ± 0.05
Methionine (%)	0.02 ± 0.01	0.59 ± 0.06	0.70 ± 0.05
Phenylalanine (%)	0.32 ± 0.02	0.60 ± 0.01	0.60 ± 0.02
Proline (%)	0.36 ± 0.03	0.98 ± 0.03	0.98 ± 0.01
Serine (%)	0.19 ± 0.01	0.32 ± 0.04	0.45 ± 0.03
Threonine (%)	0.18 ± 0.01	0.38 ± 0.01	0.38 ± 0.01
Tyrosine (%)	0.14 ± 0.02	0.39 ± 0.03	0.39 ± 0.02
Valine (%)	0.35 ± 0.02	0.56 ± 0.02	0.53 ± 0.01

[a] Timothy Balance Cube (Ontario Dehy).
[b] Mixed for this experiment by Buckeye Nutrition; for ingredient composition see Table 2.
[c] Diets were top-dressed with 0.43 mL/kg body weight/day canola oil to increase the DE of the diets.