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Original Research

Nitrogen Retention and Plasma Amino Acid Responses in Mature Geldings Fed Three Dietary Concentrations of Lysine

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ABSTRACT

Six mature Quarter Horse geldings (age, 11.5 ± 4.7 years; body weight [BW], 526 ± 9.2 kg) were used in a replicated 3 × 3 Latin square design to determine the effects of three dietary lysine (Lys) levels on nitrogen (N) retention and plasma amino acid (AA) concentrations. The geldings were fed a basal diet of concentrate and Bermuda grass hay supplemented with synthetic essential amino acids (EAA) to meet estimated requirements for EAA. Geldings were fed one of three dietary treatments: (1) a basal diet deficient in Lys (L−; 0.027 g of Lys•kg^{-1} BW•d^{-1}); (2) a basal diet supplemented with synthetic Lys to meet National Research Council (NRC; 2007) requirements (L+; 0.036 g of Lys•kg^{-1} BW•d^{-1}); or (3) a basal diet supplemented at twice the recommended Lys requirement (2 × L; 0.070 g Lys•kg^{-1} BW•d^{-1}). Horses fed the 2 × L diet had higher N intakes ($P = .0056$) than horses fed either the L− or L+ diet. However N retention ($P = .63$) was not different between treatments. Plasma Lys was greater ($P < .0001$) in 2 × L than L− and L+ diets. Plasma threonine (Thr; $P < .01$), methionine (Met; $P = .03$), and total plasma non-EAA ($P < .05$) concentrations decreased as dietary Lys increased. These results suggest N retention is not a good response criterion for evaluating the AA requirements of mature horses. However, plasma AA data indicated more efficient use of Thr and Met when horses were fed dietary Lys in concentrations greater than the current requirement recommended by the Nutrient Requirements of Horses (National Research Council, 2007).

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1. Introduction

In pigs, deletion of a limiting amino acid (AA) from the diet decreases nitrogen (N) retention (NR), but addition of a limiting AA improves NR [1,2]. Differences in NR due to dietary crude protein (CP) levels are documented in sedentary ponies [3] and mature working horses [4]. However, NR was shown to be an unsuitable response criterion with which to measure specific AA requirements in growing fillies [5]. Plasma AA concentrations have been successfully used in pigs [6] and chicks [7] as response criteria for determining AA requirements. There are limited data [8,9] describing the impact of diet supplementation with synthetic lysine (Lys) on plasma concentrations of other essential AA (EAA) in the horse. This study was conducted to characterize changes in NR and plasma AA concentrations in mature geldings fed three different concentrations of dietary Lys.

2. Materials and Methods

Six mature Quarter Horse geldings (age, 11.5 ± 4.7 years; body weight [BW], 526 ± 9.2 kg) were used in a replicated 3 × 3 Latin square design. All horses were individually housed at the New Mexico State University (NMSU) horse

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farm and provided ad libitum access to water. All management protocols and experimental procedures used in this study were approved by the NMSU Institutional Animal Care and Use Committee.

Geldings were fed a basal diet of concentrate and Bermuda grass hay in a 65:35 ratio. Concentrate was from Unifeed Hi-Pro, Inc. (Friona, TX). Bermuda grass hay was baled at the NMSU livestock farm. The basal diet (Table 1) was formulated to be deficient in CP and Lys based upon requirements for mature horses [1] when fed at 1.4% of BW in order to evaluate responses to synthetic Lys supplementation. Samples of hay and concentrate were analyzed for AA composition (Table 2) by the Texas A&M University Protein Chemistry Laboratory (College Station, TX).

Other than a requirement for Lys [1], insufficient data are available in the literature on which to base requirements for EAA in mature horses. Therefore, the National Research Council (NRC, 2007) [1] recommendation for dietary Lys requirement for mature sedentary horses (0.036 g Lys•kg^{-1} BW•d^{-1}) was used as a standard to estimate requirements for the other EAA. Bryden [10] and Graham-Thiers (Virginia Intermont College, Bristol, VA, personal communication, 2009) determined the EAA/Lys ratio in equine muscle (Table 3) and suggested this profile might be an appropriate means of estimating the proper balance of EAA in equine diets. These ratios were used as coefficients multiplied by 110% of the reported Lys requirement to provide ample estimates of dietary requirements for the other EAA. For example, the daily Lys requirement for a 500-kg horse at maintenance is 0.036 g Lys•kg^{-1} BW•d^{-1}. By multiplying 110% of this requirement by the appropriate coefficient for Met (0.28), we estimated the requirement to be 5.5 g/d for Met. Bryden [10] did not report a value for Trp, thus, the value from porcine muscle [11] was used where the ratio of muscle concentration of Trp/Lys was 0.10:1. Based on AA analysis of the feedstuffs, the basal diet met the 110% level of the predicted requirements for Leu, Phe, and Thr; however, the diet was deficient in Lys, Met, Ile, His, Val, and Arg. Although Trp was destroyed during AA analysis, Trp was supplemented to meet the full 110% level of the predicted requirement. The basal diet of each horse was supplemented with synthetic L-isomers of Met, Trp, Ile, His, Val, and Arg (Seltzer Ingredients, Carlsbad, CA) to meet the 110% level of the estimated requirements (Table 3) to ensure there were no deficiencies of these AA that could limit the horse's response to supplemental Lys. All supplemental AA were provided to each individual horse in an amount to meet the predicted requirement (Table 3) for its individual BW.

Table1
Composition of basal diet (100% DM basis)

Feedstuff	% of Diet	% DM	DE (Mcal/kg)[a]	% CP	% Ca	% P
Concentrate[b]	65	89.0	2.69	7.50	0.85	0.25
Bermuda grass hay	35	93.0	2.13	8.80	0.44	0.28
Total	100	90.4	2.49	7.96	0.71	0.26

[a]Calculated as: Digestible energy (kcal/kg DM) = 2,118 + 12.18 × (% CP) − 9.37 × (% ADF) − 3.83 × (% hemicellulose) + 47.18 × (% fat) + 20.35 × (% nonstructural carbohydrate) − 26.3 (% ash) (NRC, 2007) [1].

[b]Concentrated mixture of 25% beet pulp, 21% rice hulls, 20% cottonseed hulls, 10% cane molasses, 1% vegetable oil, 0.3% dicalcium phosphate, 0.75% salt, 0.125% trace mineral supplement, 0.1% mold inhibitor, and 0.075 % equine vitamin supplements.

Table 2
Amino acid composition of the concentrate and Bermuda grass hay in the basal diet

Amino Acid	% AA (100% DM Basis)	
	Concentrate	Bermuda Grass Hay
Lysine	0.12	0.33
Methionine	0.04	0.09
Threonine	0.13	0.27
Isoleucine	0.10	0.20
Leucine	0.24	0.48
Histidine	0.05	0.06
Phenylalanine	0.14	0.31
Valine	0.13	0.24
Arginine	0.14	0.29

Because Lys was the only source of variation for N intake in the diet, diets were not adjusted to be isonitrogenous as total N intake for an individual varied by less than 0.01 g N•kg^{-1} BW•d^{-1}.

The three dietary treatments were (1) a basal diet deficient in Lys (L−), (2) a basal diet supplemented with synthetic L-lysine (Ajinomoto Heartland, Inc., Eddyville, IA) to meet the Lys requirement (L+; NRC, 2007), and (3) a basal diet supplemented with synthetic L-lysine at twice the recommended requirement (2 × L). All synthetic AA were equally split between feedings and top-dressed onto the concentrate immediately prior to feeding and mixed with 60 mL of distilled water to insure adherence to the feed mixture.

2.1. Experimental Procedures

On the first day of each 11-day period, horses were fed only the basal diet without any AA supplementation. For the next 6 days, the horses were fed their appropriate treatment diet at 7:00 AM and 7:00 PM. At 6:45 PM on day 8 of the 11-day period, horses were fitted with collection harnesses (Equisan Marketing PTY Ltd., South Melbourne, Australia) for total collection of urine and feces for the next 4 days. On days 1 to 7 of each period, horses were kept in 3-m × 7-m pens and exercised daily for 10 min at a trot on a mechanical walker. During days 8 to 11, horses were confined to their 3-m × 3-m stalls and not exercised.

Table 3
Estimated essential amino acid (EAA) requirements of mature horses based upon muscle EAA/Lysine ratio

Essential Amino Acid	EAA/Lysine Ratio	Requirement (g AA•kg^{-1} BW•d^{-1})[a]
Lysine	1.00	0.036[b]
Methionine	0.28	0.011
Threonine	0.63	0.024
Tryptophan	0.10[c]	0.004
Isoleucine	0.51	0.022
Leucine	1.09	0.042
Histidine	0.60	0.024
Phenylalanine	0.52	0.024
Valine	0.66	0.025
Arginine	0.70	0.029

[a]Calculated as: EAA/Lysine ratio × 110% of Lys requirement (0.036 g AA•kg^{-1} BW• d^{-1}).

[b]Value from the NRC [1].

[c]Value from porcine tissue composition [11].