Orally supplemented L-arginine impairs amino acid absorption depending on dose in horses


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ABSTRACT: The beneficial effect of L-arginine (L-Arg) supplementation on the physiology of several species has generated an interest in the use of L-Arg as a nutraceutical in horses, but dosage and absorption of orally supplemented L-Arg must be inferred from other species. The study objective was to determine the effect of 2 oral L-Arg doses on plasma arginine concentrations and the effect on absorption of other amino acids in mares. In Experiment 1, mares were blocked by age and breed and were fed L-Arg supplemented (supplemented with 0.025% BW L-Arg; n = 6) or control (no supplement; n = 6) concentrate on a single day with blood samples taken at 0, 0.5, 1, 2, 3, 4, and 5 h relative to feeding. In Experiment 2, mares (n = 6) were used in a 3 × 3 Latin square design with L-Arg (0.0125% of BW), urea (0.0087% of BW), and control fed mixed into a grain concentrate as single meal with blood samples taken at 0, 1, 2, 4, 6, 8, 10, and 12 h relative to feeding. In Experiment 1, L-Arg supplementation increased (P < 0.05) plasma L-Arg and ornithine concentrations and decreased (P < 0.05) lysine and methionine concentrations compared with the control group. At 1 h post feeding, L-Arg mares had lower (P < 0.05) plasma concentrations of histidine, glutamic acid, proline, isoleucine, threonine, phenylalanine, valine, alanine, and taurine. In Experiment 2, L-Arg supplementation increased (P < 0.05) arginine and ornithine concentrations compared with urea and control; there was no difference among other amino acids. These experiments indicate that L-Arg is absorbed and, dependent on the dose, alters the absorption of other amino acids in mares.

Key words: amino acid absorption, equine, L-arginine

INTRODUCTION

The beneficial effect of L-arginine (L-Arg) supplementation on the physiology of several species has generated an interest in the use of L-Arg as a nutraceutical in horses. In diabetic humans, L-Arg supplementation improved endothelial cell function, decreased gastric ulcers, enhanced wound healing, and improved insulin sensitivity (Gad, 2010). Additionally, L-Arg infused intravenously (183 mg/kg BW) increased plasma growth hormone concentrations in women 20-fold and in men 8.6-fold (Alba-Roth et al., 1988; Merimee et al., 1969). L-Arg supplementation improved vascular performance in humans (Gad, 2010) and reproductive performance in swine (Mateo et al., 2007) and increased blood flow to the reproductive tract (Mortensen et al., 2011) as well as improved the uterine environment post-foaling in mares (Kelley et al., 2013).

Current knowledge on dosage and absorption of orally supplemented L-Arg must be inferred from other species. Supplementation in swine ranges from 0.4 to 1.0% of the diet (DM basis) or approximately 0.007 to 0.014% of BW (Mateo et al., 2007; Wu et al., 2007; Li et al., 2010), and in human studies, doses up to 30 g are well tolerated (Gad, 2010). Arginine, lysine, cysteine, and histidine can all utilize the cationic amino acid transport system with varying affinities (Palacin et al., 1998). Thus, it is possible that increasing L-Arg in the diet may adversely affect the absorption of other amino acids, such as lysine, the limiting amino acid in horses (NRC, 2007). This altered absorption may result in amino acid deficiencies, although the diet may be formulated to meet the animal’s requirements.

Research from this lab has examined the effect of L-Arg supplementation on reproductive function in mares without data on the absorption of L-Arg or the impact it may have on other amino acids. The purpose
of this study was to observe the effect of L-Arg dose on plasma arginine concentrations and determine whether absorption of other amino acids is altered in horses. Our hypothesis for both experiments was that L-Arg administration would increase plasma L-Arg concentrations and not alter the absorption of other amino acids.

**MATERIALS AND METHODS**

These studies were approved by the University of Florida Institute of Food and Agricultural Sciences (IFAS) Animal Research Committee and were conducted at the IFAS Equine Science Center (Ocala, FL). Mares were fasted overnight before the experiment and then randomly assigned to an L-Arg group or an unsupplemented control group. The mean age and BW of the L-Arg group was 10.2 ± 4.2 yr and 537 ± 14 kg and control was 9.2 ± 6.8 yr and 544 ± 16 kg, respectively. A composite sample of the forage and grain concentrate mares received before study had the amino acid composition analyzed (Table 1; University of Missouri–Columbia, College of Agriculture, Food and Natural Resources, Agricultural Experimental Station, Chemical Laboratories, Columbia, MO). Mares received the same grain concentrate before study during the study. Amino acid absorption was evaluated in response to a single meal of a commercial grain concentrate (minimum guarantees: 16% CP, 3.5% crude fat, 0.9% Ca, 0.55% P; Ocala Breeder’s Feed and Supply, Ocala, FL) fed at 0.25% of BW. L-Arg mares had their grain top dressed and then mixed with L-Arg (Chemical Abstracts Service [CAS] number 74-79-3; Ajinomoto AminoScience LLC, Raleigh, NC) at 0.025% of BW, L-Arg group was 10.2 ± 4.2 yr and 537 ± 14 kg and control was 9.2 ± 6.8 yr and 544 ± 16 kg, respectively. A composite sample of the forage and grain concentrate mares received before study had the amino acid composition analyzed (Table 1; University of Missouri–Columbia, College of Agriculture, Food and Natural Resources, Agricultural Experimental Station, Chemical Laboratories, Columbia, MO). Mares received the same grain concentrate before study during the study. Amino acid absorption was evaluated in response to a single meal of a commercial grain concentrate (minimum guarantees: 16% CP, 3.5% crude fat, 0.9% Ca, 0.55% P; Ocala Breeder’s Feed and Supply, Ocala, FL) fed at 0.25% of BW. L-Arg mares had their grain top dressed and then mixed with L-Arg (Chemical Abstracts Service [CAS] number 74-79-3; Ajinomoto AminoScience LLC, Raleigh, NC) at 0.025% of BW (mean ± SEM L-Arg supplemented: 134.3 ± 3.6 g). Mares were fed grain only with no access to hay and housed individually in stalls for the duration of the study with free access to water. Indwelling catheters were aseptically placed into each mare’s left jugular vein by first clipping a 5-cm by 5-cm area and then scrubbing the area alternating 3 times between chlorhexidine (Zoetis, Florham Park, NJ) and Isopropyl alcohol. A 16-g by 15–cm Abbo cath (Hospira, Lake Forest, IL) was placed into the jugular vein attached to a sterile IV extension set (Hospira, Lake Forest, IL) and flushed with heparinized saline solution. The catheter was then sutured in place using 2-0 nylon suture (Butler Shein Animal Health, Dublin, OH). Blood samples (10 mL) were obtained before feeding (0 h) and at 0.5, 1, 2, 3, 4, and 5 h post feeding (relative to completion of the meal) and placed in heparinized Vacutainer tubes (BD Vacutainer, Mansfield, MA). Samples were then cen-trifuged at 1050 × g for 15 min and plasma was harvested and stored at −80°C until analyzed.

Experiment 2 was performed after analyzing the results from Experiment 1, using a lower L-Arg dose to determine if absorption of other amino acids was altered. An isonitrogenous group was added to determine if the difference between control and L-Arg supplementation was attributed to the difference in nitrogen content. Experiment 2 utilized Thoroughbred (n = 3) and Quarter Horse (n = 3) mares in a 3 × 3 replicated Latin square design. Mean (±SEM) BW of all mares was 559 ± 17 kg. On the first day of the experiment, mares were randomly assigned to either L-Arg (n = 2), urea (isonitrogenous; n = 2), or control (no supplement; n = 2) group. Similar to Experiment 1, mares were fasted overnight before the experiment and amino acid absorption was evaluated in response to a single meal of a commercial grain concentrate (minimum guarantees: 16% CP, 3.5% crude fat, 0.9% Ca, 0.55% P; Ocala Breeder’s Feed and Supply, Ocala, FL) fed at 0.25% of BW. Mares were given a 6-d washout period, rotated to another treatment, and the trial was repeated until each mare received each treatment. Depending on treatment assignment, mares had their grain supplemented with L-Arg (CAS number 74-7-3; Ajinomoto AminoScience LLC, Raleigh, NC) at 0.0125% of BW (mean ± SEM L-Arg supplemented per mare: 69.9 ± 2.2 g), urea at 0.0087% of BW (mean ± SEM urea supplemented per mare: 48.9 ± 1.5 g), or no supplement. Mares were fed grain only with

| Table 1. Amino acid analysis of forage and grain (wt/wt% is grams per 100 grams of sample) |
|---------------------------------------------|------------------|------------------|
| Amino Acid | Forage, wt/wt % | Grain, w/w % |
| Essential: | | |
| Arg | 0.32 | 0.85 |
| His | 0.12 | 0.36 |
| Ile | 0.33 | 0.54 |
| Leu | 0.63 | 1.04 |
| Lys | 0.42 | 0.83 |
| Met | 0.13 | 0.2 |
| Phe | 0.39 | 0.68 |
| Thr | 0.34 | 0.5 |
| Trp | < 0.04 | 0.17 |
| Val | 0.44 | 0.67 |
| Nonessential: | | |
| Ala | 0.51 | 0.7 |
| Asp | 0.96 | 1.56 |
| Cys | 0.1 | 0.27 |
| Glu | 0.81 | 2.37 |
| Gly | 0.4 | 0.68 |
| Orn | 0.01 | 0.01 |
| Pro | 0.52 | 0.81 |
| Ser | 0.31 | 0.59 |
| Tau | 0.03 | 0.04 |
| Tyr | 0.18 | 0.4 |
no access to hay and housed individually in stalls for the duration of the study (12h). Indwelling catheters were aseptically placed into each mare’s left jugular vein and blood samples were obtained before feeding (0 h) and at 1, 2, 4, 6, 8, 10, and 12 h post feeding (relative to completion of the meal) and placed into heparinized tubes. Samples were then centrifuged at 1050 × g for 15 min and plasma was harvested and stored at −80°C until analyzed.

Amino Acid Analysis

Plasma samples from both experiments were deproteinized using 35% (w/v) sulfosalicylic acid. The acid soluble fraction was separated by centrifugation (4°C, 11,000 × g for 20 min). The supernatant was filtered (0.2 mm, Fisher Scientific, Pittsburgh, PA) and then mixed 1:1 with 0.02 N HCl (Le Boucher et al., 1997). Plasma samples were then analyzed for amino acid composition using an Amino Acid Analyzer (L-8900, Hitachi-High Technologies, Pleasanton, CA) as previously described (Ma et al., 2010).

Statistical Analysis

For Experiment 1, data were analyzed using the SAS MIXED procedure with a random statement to account for variability of mares within treatment and a repeated measures statement to account for sequential measurements taken over time (SAS version 9.2: SAS Inst., Inc., Cary, NC). Dietary treatment, hour, and hour × dietary treatment were included in the model as fixed effects and a compound symmetry covariance structure was used. When an hour × treatment effect was significant, Tukey’s adjusted pairwise comparison was performed. Data are represented as least squared means (±SEM). A probability of \( P < 0.05 \) was considered significant and a probability between \( P \geq 0.05 \) and \( \leq 0.10 \) indicated a trend toward significance.

RESULTS

Experiment 1

Mares fed 0.025% of BW L-Arg had higher mean plasma arginine concentration compared with unsupplemented controls (278 ± 25 mmol/L and 103 ± 25 mmol/L, respectively; \( P < 0.05 \)). Plasma arginine was higher \( (P < 0.05) \) in L-Arg mares at 2 h post feeding and remained elevated \( (P < 0.05) \) through 5 h post feeding compared with control mares (Fig. 1).

There were no differences in plasma citrulline concentrations between treatments (Fig. 2A). Mean plasma citrulline concentrations in L-Arg–treated mares was
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Mean plasma ornithine concentration was higher \((P < 0.05)\) in L-Arg–treated mares \((112 \pm 10 \, \text{mmol/L})\) compared with control mares \((75 \pm 10 \, \text{mmol/L})\), with concentrations elevated \((P < 0.05)\) from 2 to 5 h post feeding in arginine-treated mares (Fig. 2B).

Mean plasma lysine concentration (Fig. 3A) was lower \((P < 0.05)\) in L-Arg mares \((91 \pm 9 \, \text{mmol/L})\) than control mares \((157 \pm 9 \, \text{mmol/L})\), with L-Arg mares having lower \((P < 0.05)\) plasma lysine concentrations at 0.5, 1, 2, 3, 4, and 5 h post feeding. Mean plasma methionine concentrations (Fig. 3B) were lower in L-Arg mares \((20.6 \pm 1.5 \, \text{mmol/L})\) than control mares \((25.9 \pm 1.5 \, \text{mmol/L}; P < 0.05)\). There was a treatment \(\times\) time effect for methionine concentration which was lower \((P < 0.05)\) at 1 h in L-Arg mares than control mares (Fig. 3B). Mean plasma valine concentration was lower in L-Arg mares \((133 \pm 5 \, \text{mmol/L})\) than control mares \((146 \pm 5 \, \text{mmol/L}; P < 0.05)\). There were no differences in the mean plasma concentrations of histidine, glutamic acid, proline, and isoleucine (Fig. 3B through F) between arginine- and control-treated mares. The concentrations of these amino acids peaked at 1 h post feeding in control mares and were lower \((P < 0.05)\) at 1 h post feeding in arginine supplemented mares.

Mean plasma concentrations of histidine, glutamic acid, proline, and isoleucine was not different between treatments. At 1 h, plasma concentrations of histidine, glutamic acid, proline, and isoleucine \((P < 0.05)\) were lower in arginine-treated mares, and the same pattern was seen with threonine, phenylalanine, leucine, valine, alalinine, and taurine (data not shown; \(P < 0.05)\). No differences were observed between L-Arg mares and control mares in either the mean or at any time point for plasma ammonia (mean: \(36 \pm 3 \, \text{mmol/L}\) and \(33 \pm 2 \, \text{mmol/L}, \text{respectively}\)) or plasma urea (mean: \(7872 \pm 394 \, \text{mmol/L}\) and \(7363 \pm 404 \, \text{mmol/L}, \text{respectively}\)).

**Experiment 2**

Mares fed 0.0125% of their BW L-Arg had an increased \((P < 0.05)\) mean plasma arginine concentration compared with urea or control \((167 \pm 19 \, \text{mmol/L}, 72 \pm 19 \, \text{mmol/L} \text{and} 69 \pm 19 \, \text{mmol/L}, \text{respectively})\). L-Arg mares had elevated plasma arginine concentration (Fig. 4) at 1, 2, 4, 6, and 8 h compared with urea or control mares. Plasma arginine concentration did not differ between urea and control mares.

Plasma citrulline concentration did not differ between treatments (Fig. 5A). Mean plasma ornithine concentration was higher \((P < 0.05)\) in L-Arg mares \((80 \pm 7 \, \text{mmol/L})\) than urea \((51 \pm 7 \, \text{mmol/L})\) and control \((50 \pm 7 \, \text{mmol/L})\) mares. Plasma ornithine concentrations (Fig. 5B) were elevated \((P < 0.05)\) in L-Arg mares at 1, 2, 4, 6, and 8 h compared with control and urea mares.
with urea and control mares. Plasma ornithine concentration did not differ between urea and control mares.

Mean plasma lysine (Fig. 6A), methionine (Fig. 6B), histidine (Fig. 6C), glutamic acid (Fig. 6D), proline (Fig. 6E), and isoleucine (Fig. 6F) concentrations were not different \((P \geq 0.05)\) between treatments, nor was any time point different. Plasma proline (Fig. 6E) concentration was greater \((P < 0.05)\) at 0 h in \(\ell\)-Arg mares than control mares but was not different \((P \geq 0.05)\) from the urea treatment.

No differences between treatments were observed in threonine, phenylalanine, leucine, valine, alanine, and taurine concentrations (data not shown). Mean plasma ammonia concentrations were not different between treatments; however, at 6 h post feeding ammonia concentration tended to be higher \((P = 0.093)\), with urea treatment \((89 \pm 11 \text{ mmol/L})\) compared with \(\ell\)-Arg \((62 \pm 11 \text{ mmol/L})\) and control treatment \((62 \pm 11 \text{ mmol/L})\). Mean plasma urea concentration was not different between treatments and there was no treatment \(\times\) time interaction (Fig. 7).

**DISCUSSION**

\(\ell\)-Arg has received much attention due to its effects on a variety of physiological systems. Arginine could benefit reproduction, insulin resistance, and gastric ulcers (Wu et al., 2007), each of which is of interest to the equine industry; however, knowledge on the proper dosage and absorption in horses is currently unknown. Our study demonstrates \(\ell\)-Arg supplemented at both 0.025 and 0.0125% of BW increased plasma arginine concentrations; however, at the higher dose, absorption of other amino acids was impacted.

Arginine supplementation has the potential to affect lysine, cysteine, and histidine absorption by competition for the same amino acid transporters (Palacin et al., 1998).

In swine, feeding arginine \(>2.5\%\) of DM intake \((>1.0\%\) BW\) reduced growth and caused death due to amino acid imbalance (Edmonds et al., 1987) when fed for 16 d post weaning, but supplementation of up to 0.8% of the diet \((0.014\%\) BW\) had no effect on lysine or histidine absorption (Li et al., 2010) when fed from d 0 to 25 gestation. The explanation for the difference from these results and our study in horses is unclear. Woodward et al. (2012) reported no differences in L-lysine transporter’s \(V_{\text{max}}\) or \(K_M\) between porcine and equine jejunum and proximal colon, but that ponies had a greater diffusion of protein in brush border membrane vesicles from both sections than pigs. The question remains as to whether these transporters have varying affinity for \(\ell\)-Arg between species.

Our study fed a lower amount of \(\ell\)-Arg than has been shown to cause diarrhea in pigs, which were supplemented with 1.2% of the diet (1.6% BW) with \(\ell\)-Arg (Zhan et al., 2005), suggesting a gastrointestinal toxicity (Phillips, 1972). This may be caused directly by arginine or mediated by nitric oxide production from \(\ell\)-Arg, the substrate for nitric oxide production (Morris, 2006). Nitric oxide plays a role in water and electrolyte balance (Izzo et al., 1998), altering blood flow to the gastrointestinal tract (Stark and Szurszewski, 1992; Prins et al., 2005), intestinal motility (Kuiken et al., 2002; Prins et al., 2005; Kuiken et al., 2006), and the immune system.
L-arginine impairs amino acid absorption (Akisu et al., 2002). The role of nitric oxide in gastrointestinal function is complex, but depending on the concentration, may alter secretion and absorption (Grimble, 2007). Although this study does not address the intestinal effects of arginine supplementation, it raises the question of whether arginine or nitric oxide can inhibit amino acid absorption at high concentrations.

Arginine is synthesized in the hepatic urea cycle from arginosuccinate and also broken down into ornithine and urea (Wu and Morris, 1998). The use of urea for an isonitrogenous control in Experiment 2 appeared to function as an appropriate control based on comparing the plasma amino acid concentrations of the urea and nonsupplemented group. Surprisingly, l-Arg–treated mares from both Experiments 1 and 2 had a large increase in plasma ornithine concentrations but no difference in plasma urea. Arginine catabolism involves multiple organs, including the liver and intestinal epithelium (Wu and Morris, 1998). It has been reported that up to 70% of ingested l-Arg is converted to ornithine as it passes through the intestinal membrane (Wu and Meininger, 2000). Pregnant gilts supplemented at 1% of the diet (0.012% BW) with l-Arg–HCl beginning on d 30 gestation had increased plasma concentrations of proline, ornithine, and arginine and a lower plasma glutamine concentration on d 70 and 110 of gestation. Wu et al. (2007) found that in sheep and swine, arginine administration either i.v. or orally increased serum concentrations of ornithine, urea, and proline, whereas glutamine and ammonia decreased in a dose-dependent manner. Wu et al. (2007) did not note changes in any other amino acids.

Based on work performed in swine (Wu et al., 2007), we chose to sample blood for only 5 h post feeding in Experiment 1. Wu et al. (2007) found that in both pregnant and nonpregnant gilts supplemented once with l-Arg–HCl, arginine peaked around 1 h post feeding and after 5 h could find no difference in plasma l-Arg concentrations between supplemented and nonsupplemented gilts. Wu et al. (2007) fed l-Arg–HCl to gilts at

Figure 6. Least squares mean (± SEM) plasma lysine (A), plasma methionine (B), plasma histidine (C), plasma glutamic acid (D), plasma proline (E), and plasma isoleucine concentrations (F) in mares following a grain meal supplemented with 0.0125% of BW l-arginine, 0.0087% urea, or no supplementation. An “A” denotes a significant (P < 0.05) difference between l-arginine and control treatments.
a rate of 0.76% of diet DM (0.012% BW). This dose is between what we fed to horses in Experiments 1 and 2. Our data demonstrates that plasma arginine concentrations take longer to return to baseline than data has demonstrated in swine when fed comparable amounts orally.

In conclusion, our data demonstrates L-Arg is absorbed via the gastrointestinal tract and, depending on the amount fed, alters the absorption of other amino acids in horses. Although the exact mechanism by which L-Arg alters the absorption of other amino acids at the higher dose remains unclear, the amount of L-Arg supplemented to the diet needs to be considered to prevent the possibility of creating a deficiency in other amino acids.

**LITERATURE CITED**


