EVALUATING THE TECHNIQUE OF USING NITROGEN RETENTION AS A RESPONSE CRITERION FOR AMINO ACID STUDIES IN THE HORSE

A Thesis

by

TERI JILL ANTILLEY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2006

Major Subject: Animal Science

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Approved by:

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	Pete Gibbs
Committee Members,	Brett Scott
	Larry Claborn
Head of Department	Gary Acuff

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ABSTRACT

Evaluating the Technique of Using Nitrogen Retention as a Response Criterion for Amino Acid Studies in the Horse. (May 2006)

Teri Jill Antilley, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Gary Potter
Dr. Pete Gibbs

Six Quarter Horse yearling fillies were used in a duplicated 3 x 3 Latin square designed experiment to evaluate the technique of nitrogen retention as a response criterion for amino acid studies in the horse. The yearlings were paired by age and randomly assigned to one of three concentrates fed with a medium quality Coastal Bermudagrass hay throughout the study. Diets were fed at approximately 1.9% of horse body weight per day, divided into twice daily feedings with a 60:40 concentrate: hay ratio. With the exception of lysine and threonine, proposed amino acid requirements for yearling horses were calculated using nutrient to calorie ratios of gilts weighing 80-120 kg and gaining 325 g/d.

Diet A was amino acid sufficient, as provided by a soybean meal-based concentrate. Diet B was amino acid deficient, with a cottonseed hull-based concentrate. Diet A and Diet B were isonitrogenous, containing approximately 12% crude protein. Diet C used the identical concentrate as Diet B, with synthetic essential amino acids and cysteine orally dosed to match the amino acid levels in Diet A. Nitrogen retention was not different between Diet A and Diet B. Diet C resulted in differences from Diets A and B in nitrogen retention; however, differences were a consequence of nitrogen intake.

Nitrogen retained as a percent of nitrogen absorbed was lower ($P < 0.05$) for Diet B than for Diet A, for data not accounting for endogenous fecal and urinary losses. There were no differences in nitrogen retained as a percent of nitrogen absorbed for horses fed Diet C, when compared to either Diet A or Diet B, for data not accounting for endogenous losses.

It was concluded that differences in nitrogen retained as a percent of nitrogen absorbed were observed between amino acid sufficient diets and amino acid deficient diets. However, horses fed amino acid deficient diets and orally dosed with synthetic amino acids, likely require some modified dosage level to achieve the same or higher values in nitrogen retained as a percent of nitrogen absorbed as those values for amino acid sufficient diets.

DEDICATION

To my parents, Mr. and Mrs. Jon Mark Antilley, for their love and support

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I would like to thank my committee co-chairs, Dr. Potter and Dr. Gibbs, and my committee members, Dr. Scott and Dr. Claborn, for their guidance and support throughout this research project. In addition, I would like to extend my gratitude for Dr. Knabe’s influence and help in the initial stages of the project design.

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CHAPTER I

INTRODUCTION

Numerous factors have played a role in the lack of research on amino acid requirements of horses. Research costs can quickly escalate with large numbers of horses on trial. Horses are very labor intensive, and require individual handling during total fecal and urine collections. However, one of the biggest factors is related to diets consumed by horses compared to other non-ruminants. Horses are fed diets made up of a wider variety of feedstuffs. While total tract absorption of protein is often similar across many feeds, there are tremendous differences in the site of absorption, depending on feedstuffs used. Consequently, recommendations for dietary protein are currently expressed on a crude rather than digestible basis. Past research has clearly identified lysine as the first limiting amino acid in horses (Breuer et al., 1970; Hintz et al., 1971a; Potter and Huchton, 1975). Numerous studies have compared growth in horses fed various sources of natural lysine and response to poor lysine sources supplemented with synthetic lysine (Ott et al., 1979; McCall et al., 1981; Wall et al., 1998). More recently, work has been published from studies investigating threonine requirements of horses (Graham et al., 1994). Beyond these amino acids, more work is needed to further identify amino acid requirements of horses, particularly the growing horse. There is merit to investigating potential procedures that might be used to measure response by horses to diets that differ in amino acid content.

This thesis follows the style of Journal of Animal Science.

The 1989 NRC recommends yearling horses of moderate growth receive 36 grams of lysine daily, or 1.9 grams of lysine per megacalorie (Mcal) of digestible energy (DE). Yearlings of rapid growth should receive 48 grams of lysine daily, or a nutrient to calorie ratio of 1.9 grams of lysine per Mcal of DE, according to Ott et al. (1981). A nutrient to calorie ratio of 1.3 grams of threonine per Mcal of DE was indicated in a study conducted by Graham et al. (1994). There are no published requirements for the other essential amino acids. Feed companies often formulate feeds based on crude protein concentrations in an attempt to meet or exceed the horse's anticipated requirements. Although many feedstuffs commonly used in horse feeding appear to meet the horse's protein requirements, it is possible for these feeds to be nitrogen sufficient but amino acid deficient, due to protein quality and site of absorption (Gibbs et al., 1988, 1996; Wall et al., 1998; Gibbs and Potter, 2002).

Several factors must be taken into account when balancing diets. Horses absorb amino acids primarily from the small intestine, and it appears that absorption is influenced by the rapid rate of ingesta flow through the upper tract (Nyberg et al., 1995), meal size, and feedstuff digestibility (Ball et al., 1991; Farley et al., 1995). Therefore, site of absorption, amount fed, and number of feedings per day are important considerations (NRC, 1989; Gibbs and Potter, 2002). Concentrate to hay ratios are also important, because nutrients in concentrates are typically digested and absorbed in the small intestine, while nutrients in hays are absorbed primarily from the large intestine (Hintz et al., 1971b; Gibbs et al., 1988, 1996). These ratios are also a consideration in routine attempts to meet a horse’s protein quality needs in a reasonable amount of daily feed.

Knowledge of amino acid requirements for horses would be of benefit for several reasons. Feed companies would be able to formulate a ration containing exact amounts of amino acids, while avoiding excess crude protein. This could reduce the unnecessary production of excess nitrogen emitted into the atmosphere as ammonia and nitrous oxide and the nitrate leached into groundwater, via the urine and feces, and therefore be more environmentally friendly (de Vries et al., 2001; Rotz, 2004). Furthermore, by fine-tuning the amino acid profile of the feed, higher quality diets could be utilized more efficiently without compromising growth and performance of the young horse.

Research on various techniques to determine which amino acids are limiting and to what extent has been done in some species. Limiting amino acids are those present in the least amount in a feed relative to the requirements. Loest et al. (2001) successfully determined limiting amino acids by using nitrogen retention as a response criterion in cattle. The experiments were relatively short term, consisting of 7-day periods, and used the method of amino acid deletion. Wang and Fuller (1989) were also successful in identifying limiting amino acids, the order in which they were limiting, and the optimum dietary amino acid pattern for growing pigs fed a casein diet. Nitrogen retention was also a response criterion used in this method of amino acid deletion. Although not previously attempted in the horse, this technique could prove to be beneficial in determining limiting amino acids and possibly the order in which they are limiting.

Objective. The purpose of this study was to evaluate the technique of using nitrogen retention as a response criterion for amino acid studies in the horse.

CHAPTER II

REVIEW OF LITERATURE

Importance of Diet Composition. Compared to other species, it is harder to determine the amino acid requirements of the horse, because of the wide variety of feedstuffs used in the horse industry. Many diets appear to be amino acid sufficient on paper, but when the site of absorption is taken into account, it has been found that these diets are often amino acid deficient. Therefore, it is a possibility that a horse could be nitrogen sufficient but amino acid deficient (Gibbs et al., 1988, 1996; Gibbs and Potter, 2002). For example, alfalfa hay is high in nitrogen and amino acids, but it is digested primarily in the hindgut, while amino acids are absorbed primarily in the foregut (NRC, 1989; Wall et al., 1998; Gibbs and Potter, 2002). One study showed that yearlings fed oats and alfalfa hay grew fatter, while yearlings fed a balanced concentrate and alfalfa hay grew taller. Horses fed higher nutrient to calorie ratios tended to have more skeletal growth. This indicates the importance of feeding balanced diets with adequate nutrient to calorie ratios (Gibbs et al., 1989). In addition, foals nursing mares fed supplemental soybean meal, a concentrate which is high in amino acids, had greater daily weight gain and total heart girth gain, when compared with foals nursing mares fed supplemental urea (Meadows et al., 1979).

Limiting Amino Acids. Of the ten essential amino acids, lysine has been the one most studied and has been found to be the first limiting amino acid. Multiple studies have shown that the addition of lysine to low lysine diets resulted in improved growth (Breuer et al., 1970; Hintz et al., 1971a; Potter and Huchton, 1975; Ott et al., 1979; McCall et al., 1981). Ott et al. (1981) found that when lysine intake is adequate, crude protein needs of the yearling horse are decreased. This study indicates that diets for yearlings of rapid growth should provide at least 48 grams of lysine per day or 1.9 grams of lysine per Mcal of DE. Lysine is the only amino acid requirement listed in the 1989 NRC for horses. In yearlings of moderate growth, the requirement is 36 grams of lysine per day, with a nutrient to calorie ratio of 1.9 grams of lysine per Mcal of DE.

Although it is still debated, threonine has been suggested to be the second limiting amino acid (Graham et al., 1994). A study with yearling horses fed a basal diet of corn, oats, and soybean meal plus 0.2% synthetic lysine and 0.1% synthetic threonine showed increased weight gain (due to muscle not fat), girth gain, and gain to feed ratios. It also showed a decrease in serum urea nitrogen, which has a negative correlation to protein quality. Adding threonine improved the amino acid balance of the diet and resulted in measured growth responses. This study indicated that 0.65 % lysine and 0.51% threonine are adequate for yearlings. This requirement could be met by feeding a concentrate with 12% crude protein plus 0.2% lysine and 0.1% threonine or by feeding a concentrate with 14% crude protein using corn, oats, and soybean meal. Using the ingredients from this study, a nutrient to calorie ratio of 1.3 grams of threonine per Mcal of DE was indicated. While some companies with proprietary data suggest methionine as second limiting for horses, such results have not been published.

Methods. Various methods have been used to determine amino acid requirements. In some studies, basal diets have been supplemented with synthetic amino acids (Ott et al., 1979; McCall et al., 1981; Graham et al., 1994). However, one study done by Loest et al. (2001) used the method of amino acid deletion. The experiment involved young, growing steers fitted with cannulas and limit-fed soybean hull-based diets. The diets were low in ruminally undegradable protein. Acetate was continuously infused into the rumen, and five treatments were continuously infused into the abomasum. The first treatment consisted of 115 grams per day of a mixture of ten essential amino acids designed to exceed the steers' requirements. The second, third, and fourth treatments contained the ten essential amino acids with one amino acid removed per treatment: leucine, isoleucine, and valine respectively. The final treatment had the ten essential amino acids with all three branched-chain amino acids removed. This experiment measured nitrogen retention as a response criterion. Nitrogen retention decreased when leucine was removed ($P < 0.05$), when valine was removed ($P < 0.05$), or when all three branched-chain amino acids were removed ($P < 0.05$). When leucine and all three branched-chain amino acids were removed, plasma leucine concentrations decreased ($P < 0.05$). When isoleucine and all three branched-chain amino acids were removed, plasma isoleucine concentrations decreased ($P < 0.05$). However, plasma isoleucine concentrations increased ($P < 0.05$) when leucine was removed. Plasma valine concentrations also decreased, when valine and all three branched-chain amino acids were removed but increased ($P < 0.05$) when leucine was removed. Conclusions drawn from this study indicate leucine and valine were among the limiting amino acids, due to a 10-13 % decrease in nitrogen retention, when they were removed. Isoleucine was not found to be limiting, because it did not elicit a response in nitrogen retention, when taken out of the diet by itself.

Wang and Fuller (1989) used the method of amino acid deletion in non-ruminants. The purpose of their study was to determine the optimum dietary amino acid pattern for growing pigs. Four of their five experiments evaluated nitrogen balance, while the fifth experiment estimated the digestibility of the synthetic amino acids in the diet. In the first experiment, eighteen gilts were fed a low-casein diet (low-control) and low-casein diets in which 20% of the nitrogen was replaced with synthetic amino acids to see if nitrogen retention would respond in a similar manner to the high-casein diet (high-control). Nitrogen retention was similar among diets supplemented with amino acids and the high-casein diet, with a significant decrease in nitrogen retention ($P < 0.05$) for the low-casein diet. Twenty-four gilts in the second experiment were fed twelve different casein diets. High and low-casein diets served as the controls, while nine others added synthetic amino acids, including all the essential amino acids but one, to the low-casein diet. The nonessential amino acids aspartate, glutamate, and alanine were added to these nine diets, while they were omitted from the final diet. Using nitrogen retention as a response criterion, the quality of amino acids that could be removed was calculated and then used to develop an "ideal" pattern. Nitrogen retention decreased when certain essential amino acids were deleted, while it increased when tryptophan, lysine, and phenylalanine + tyrosine were removed. Methionine + cysteine showed the greatest reduction in nitrogen retention when removed, implying that they were first limiting in casein diets. Threonine was found second limiting. In the third experiment, 20% of each essential amino acid was deleted from this "ideal" pattern, and a new pattern was formulated, in which each essential amino acid would be equally limiting.

Optimum balance among essential amino acids, relative to lysine = 100 was methionine + cysteine 63, threonine 72, tryptophan 18, valine 75, isoleucine 60, leucine 110, and phenylalanine + tyrosine 120. The fourth experiment determined that maximum nitrogen retention was achieved when the ratio of essential to nonessential amino acids was at least 45:55.

These studies could serve as useful models for the horse. Being a non-ruminant, the horse could consume synthetic amino acids in the diet, like the pig, instead of having them continuously infused, as in the steer (Ott et al., 1979; McCall et al., 1981; Graham et al., 1994). Once the amino acid was found to be limiting, due to a drop in nitrogen retention, further studies could be done to determine exact requirements by titration.

CHAPTER III

EXPERIMENTAL PROCEDURES

Horses and Diets. Six yearling Quarter Horse fillies, averaging 15 months of age and ranging in weight from 320 kg to 347 kg, were used in a duplicated 3 x 3 Latin square designed experiment. All horses were housed in 13 x 14 meter dry lots (two per lot) at the Texas A&M Horse Center, with water and shelter available ad libitum. The yearlings were paired by age and randomly assigned to one of three concentrates with a medium quality Coastal Bermudagrass hay provided with all concentrates throughout the study. A concentrate to hay ratio of 60:40 was used. Diets were fed at approximately 1.9% body weight. Horses were placed in individual feeding stalls twice daily, fed at 12-hour intervals, and given approximately 4 hours per feeding to eat. Feed was weighed and recorded, as were all refusals. The fillies were dewormed and vaccinated prior to the experiments. All methods were approved by the Institutional Agricultural Animal Care and Use Committee (IAACUC).

With the exception of lysine and threonine, proposed amino acid requirements for the horses were used to formulate amino acid sufficient and deficient diets. Since the digestive tract of the pig is most like that of the horse, nutrient to calorie ratios were calculated using various ages of pigs. Gilts, ranging in body weight from 80 kg to 120 kg and gaining 325 grams per day (NRC, 1998), had nutrient to calorie ratios that most resembled the yearling’s nutrient to calorie ratios of 1.9 grams of lysine per Mcal of DE (NRC, 1989) and 1.3 grams of threonine per Mcal of DE (Graham et al., 1994). From there, nutrient to calorie ratios for the other essential amino acids were calculated and converted into projected requirements for the yearling fillies, as seen in Table 1.

Table 1. Proposed amino acid requirements for yearling horses using nutrient to calorie ratios of gilts ranging in weight from 80-120 kg and gaining 325 g/d

AA	AA required (g/d)		DE required (Mcal)		Nutrient:calorie ratio	
	Equine	Swine	Equine	Swine	Equine	Swine
Lysine	36.00	18.50	18.90	9.75	1.90	1.90
Threonine	24.60	12.60	18.90	9.75	1.30	1.29
Methionine	9.45	4.80	18.90	9.75	0.50	0.49
Cysteine	11.50	6.00	18.90	9.75	0.61	0.62
Arginine	10.96	5.66	18.90	9.75	0.58	0.58
Histidine	11.34	5.85	18.90	9.75	0.60	0.60
Isoleucine	18.90	9.75	18.90	9.75	1.00	1.00
Leucine	32.13	16.58	18.90	9.75	1.70	1.70
Phenylalanine	20.79	10.73	18.90	9.75	1.10	1.10
Tryptophan	6.62	3.41	18.90	9.75	0.35	0.35
Valine	24.00	12.38	18.90	9.75	1.27	1.27

Concentrates were top-dressed with 10% corn oil. The first concentrate (Diet A) was amino acid sufficient, consisting of 14% soybean meal, 13.5% corn, 13% rice mill run, and 8% oats, and is presented in Table 2. Diet B was composed of 19.75% cottonseed hulls, 19% corn, and 8% oats, was deficient in the essential amino acids, and is shown in Table 3. Diet B also contained 2% urea to maintain the same crude protein (CP) level as that of Diet A. Values in Table 2 and Table 3 were calculated using multiple NRC tables (NRC, 1982; NRC, 1989; NRC, 1996; NRC, 2001). Yearling fillies on Diet C were fed the concentrate in Diet B (Tables 3, 4), with synthetic amino acids orally dosed (Table 5), in order to obtain the total amount of essential amino acids and cysteine provided by Diet A (Table 4). Only synthetic L-Histidine, L-Threonine, L-Arginine, L-Valine, DL-Methionine, L-Phenylalanine, L-Isoleucine, L-Leucine, L-Lysine, L-

Tryptophan, and L-Cysteine were orally dosed to horses fed Diet C, so that Diet C was still deficient in the nonessential amino acids aspartate, glutamate, serine, glycine, alanine, and tyrosine. Diet C served as a second positive control and nitrogen balance studies were performed to determine whether or not the nitrogen retention was similar to that of Diet A, which used natural ingredients. Feed and hay samples were analyzed for amino acid concentration by the Texas A&M University Protein Chemistry Laboratory (Table 4). The average amount of amino acids provided per day to the yearlings fed Diets A, B, and C are in Table 5.

Table 2. Composition of Diet A (amino acid sufficient) fed to yearling horses

		Provided on an as-fed basis			
Ingredient	% of diet as-fed	DE (Mcal)	% CP	Ca %	P %
Coastal Bermudagrass	40.00	0.36	2.96	0.12	0.08
Soybean Meal	14.00	0.20	6.23	0.05	0.09
Corn	13.50	0.21	1.23	0.01	0.04
Rice Mill Run	13.00	0.08	0.82	0.02	0.06
Corn oil	10.00	0.41			
Oats	8.00	0.10	0.94	0.01	0.03
Limestone	0.50			0.17	0.00
Dical	0.50			0.11	0.10
Salt	0.25				
Vitamin/mineral premix	0.25				
Total		1.36	12.18	0.48	0.38

* Values in Table 2 were determined by calculation using multiple NRC tables.

Table 3. Composition of Diet B (amino acid deficient) fed to yearling horses

		Provided on an as-fed basis			
Ingredient	% of diet as-fed	DE (Mcal)	% CP	Ca %	P %
Coastal Bermudagrass	40.00	0.36	2.96	0.12	0.08
Cottonseed hulls	19.75	0.17	0.81	0.18	0.02
Corn	19.00	0.29	1.73	0.01	0.05
Corn oil	10.00	0.41			
Oats	8.00	0.10	0.94	0.01	0.03
Urea	2.00		5.62		
Dical	0.50			0.11	0.10
Limestone	0.25			0.09	
Salt	0.25				
Vitamin/mineral premix	0.25				
Total		1.33	12.06	0.51	0.27

* Values in Table 2 were determined by calculation using multiple NRC tables.

Table 4. Analyzed amino acid composition of the concentrates and hay fed to yearling horses

Amino acid	Concentrate in Diet A (g/kg)	Concentrate in Diet B (g/kg)	Coastal Bermudagrass hay (g/kg)
Histidine	2.93	1.38	2.25
Threonine	3.74	1.72	3.08
Arginine	8.06	5.10	4.90
Valine	4.79	2.60	4.00
Methionine	1.21	0.73	0.93
Phenylalanine	4.87	2.74	3.70
Isoleucine	4.32	2.09	3.20
Leucine	7.75	4.22	5.63
Lysine	7.40	3.43	4.73
Tryptophan*	—	—	—
Cysteine*	—	—	—
Aspartate	10.37	5.38	7.85
Glutamate	17.70	10.85	8.83
Serine	5.03	3.57	3.60
Glycine	3.93	2.76	3.38
Alanine	4.40	2.92	4.55
Tyrosine	2.20	1.26	1.48

* Tryptophan and cysteine concentrations were not provided since they are destroyed in the acid hydolysis.

Table 5. Average daily amino acids provided to the yearling horses

Amino acid	Diet A (g/d)	Diet B (g/d)	Diet C † (g/d)
Lysine	36.35	23.43	36.35
Threonine	20.16	13.60	20.16
Methionine	6.35	4.78	6.35
Valine	25.96	18.84	25.96
Arginine	38.92	29.35	38.92
Phenylalanine	25.45	18.52	25.45
Isoleucine	22.37	15.11	22.37
Leucine	39.82	28.34	39.82
Histidine	15.39	10.33	15.39
Tryptophan*	10.40	6.50	10.40
Cysteine*	13.65	7.15	13.65
Aspartate	54.10	37.88	37.88
Glutamate	80.47	58.21	58.21
Serine	25.72	20.96	20.96
Glycine	21.56	17.74	17.74
Alanine	26.13	21.32	21.32
Tyrosine	10.97	7.92	7.92

* Tryptophan and cysteine concentrations in the feed and hay were determined by tabular values since they are destroyed in the acid hydolysis.

† Difference between Diet A and Diet B in essential amino acids and cysteine was given to horses fed Diet C via oral dosing with synthetic amino acids

The synthetic amino acids L-Lysine, L-Threonine, DL-Methionine, and L-Tryptophan were of feed grade quality and were donated by ADM Alliance Nutrition. L-Histidine, L-Arginine, L-Valine, L-Phenylalanine, L-Isoleucine, L-Leucine, and L-Cysteine were crystalline amino acids purchased from SIGMA-ALDRICH, St. Louis, Missouri. Although cysteine is not an essential amino acid, it was supplemented because of its relationship with methionine. Yearlings fed Diet C were dosed via syringe twice daily with a mixture of these eleven amino acids, 10 cc of water, and 2 ½ cc of molasses immediately prior to eating.

Experimental Period. There were three 11-day periods, with a 7-day diet adaptation and a 4-day total collection of urine and feces. During the total collection, fillies were confined to metabolism stalls and fitted with a harness suitable for collecting urine. The yearlings were tied in individual stalls, with access to feed, hay and water for 23 hours per day. The flooring of the stalls was concrete, and was covered by a rubber mat to minimize fluid retention in the lower leg and to collect fecal material free of dirt contamination.

Excretion of feces was collected immediately upon defecation and deposited into a bucket labeled with the horse's identification. Feces were weighed and recorded for each horse every three hours. Ten percent of total fecal output was placed in a labeled freezer bag, which was then refrigerated prior to further processing for laboratory analyses.

Urine was collected immediately upon urination into the harness, which was emptied into a bucket labeled with the filly's identification. The urine was then poured into a graduated cylinder, and the total volume was recorded. A ten percent sample was saved in individually labeled, plastic bottles and refrigerated to await further analysis. Every four hours, urine amounts were totaled for each horse and recorded.

Exercise. On days prior to a total collection, fillies were exercised for fifteen minutes at a trot. The remainder of the time, they were allowed free exercise in dry lots. During total collections, horses were exercised for one hour per day, including a protocol of 10 minutes of walking, 10 minutes of trotting, 10 minutes of walking, 10 minutes of trotting, and 20 minutes of walking. Defecating and urinating of the horses was discouraged during exercise. Any defecation or urination that was excreted was measured and recorded. However, ten percent samples were not taken, due to contamination.

Laboratory Analysis. Dry matter (DM) content of feed, hay, and feces was determined after drying in a forced-air oven at 62^{o}C for 72 hours. Feed, hay, and fecal samples were ground in a Wiley mill with a three millimeter screen and later analyzed by Texas A&M University Soil, Water and Forage Testing Laboratory for nitrogen concentration determined by a LECO Nitrogen Analyzer. Urinary nitrogen was determined by Kjeldahl procedures.

Statistical Analysis. Analysis of variance (ANOVA) consistent with design was performed on all data using STATA (STATA Corp., College Station, TX). In addition, Fisher-Hayter pairwise comparisons were used for determining the location of statistically significant data.

CHAPTER IV

RESULTS

Nitrogen Balance Not Scaled to Body Weight. Means and standard errors of the nitrogen balance data not scaled to the body weight of the yearling fillies are presented in Table 6. Nitrogen intake was similar between Diet A and Diet B, as expected, because the diets were isonitrogenous. However, nitrogen intake was higher ($P < 0.01$) in Diet C, due to the additional nitrogen from the orally dosed synthetic amino acids. Fecal nitrogen was not different among diets. Urinary nitrogen was greater ($P < 0.01$) for Diets B and C than for Diet A. Some nitrogen balance studies for swine resulted in decreased urinary nitrogen, when low-protein diets were supplemented with synthetic amino acids to match the amino acid profile of a high-protein diet (Wang and Fuller, 1989; Kerr and Easter, 1995). More ($P < 0.05$) nitrogen was absorbed from Diet C than from Diet A or Diet B. Nitrogen absorbed as a percent of nitrogen intake was similar among diets. The amount of nitrogen retained was not significantly different among diets; however, there was a trend for horses fed Diet C to retain more ($P = 0.53$) nitrogen. This trend is a result of the increased nitrogen intake for Diet C, as nitrogen retention increases at higher levels of nitrogen intake. Nitrogen retained as a percent of nitrogen intake was similar among Diets A, B, and C. Nitrogen retained as a percent of nitrogen absorbed was lower ($P < 0.05$) for Diet B than for Diet A; however, Diet C was not different from Diet A or Diet B. Other research indicates nitrogen retained as a percent of nitrogen absorbed for low-protein diets with added synthetic amino acids to be similar to or higher than that of high-protein diets (Kerr and Easter, 1995).

Table 6. Nitrogen balance not scaled to the body weight of yearling fillies

	Diet A			Diet B			Diet C		
Item	Mean		SEM	Mean		SEM	Mean		SEM
Nitrogen intake (g/d)	253.38 [a]	±	7.25	259.13 [a]	±	5.24	287.74 [b]	±	7.31
Fecal nitrogen (g/d)	51.51	±	6.85	59.17	±	6.09	59.77	±	5.51
Urine nitrogen (g/d)	5.93 [a]	±	0.18	6.83 [b]	±	0.19	7.18 [b]	±	0.25
Nitrogen absorbed (g/d)	201.86 [c]	±	6.25	199.96 [c]	±	8.91	227.98 [d]	±	8.76
Nitrogen absorbed as a percent of intake	79.81	±	2.42	77.06	±	2.46	79.17	±	1.99
Nitrogen retained (g/d)	195.93	±	6.27	193.13	±	8.80	220.78	±	8.70
Nitrogen retained as a percent of intake	77.46	±	2.37	74.42	±	2.45	76.67	±	1.98
Nitrogen retained as a percent of absorbed	97.04 [c]	±	0.13	96.56 [d]	±	0.12	96.83 [c,d]	±	0.14

[a,b] Means in rows lacking common superscripts differ significantly ($P < 0.01$)

[c,d] Means in rows lacking common superscripts differ significantly ($P < 0.05$)

Nitrogen Balance Not Scaled to Body Weight, with Urea Nitrogen Subtracted from Nitrogen Intake in Diet C. Diet C was composed of the same ingredients as Diet B, including urea. As a result, nitrogen intake was increased in Diet C, when synthetic amino acids were given to the yearlings. The nitrogen from urea was subtracted from the nitrogen intake of each filly, and a new nitrogen balance table was constructed (Table 7). Nitrogen intake was again similar between Diets A and B, but it was lower ($P < 0.05$) for Diet C. Fecal nitrogen was similar among diets. Diet B and Diet C resulted in higher (P < 0.01) urinary nitrogen than did Diet A. Nitrogen absorbed from Diet C was less (P < 0.05) than nitrogen absorbed from Diet A or Diet B. No differences were seen among diets in nitrogen absorbed as a percent of nitrogen intake. Nitrogen retained was lower (P < 0.05) for Diet C than Diets A or B. Nitrogen retained as a percent of nitrogen intake was similar among diets; however, nitrogen retained as a percent of nitrogen absorbed was different among all three diets. Diet B was lower (P < 0.001) than Diet A, but higher (P < 0.001) than Diet C.

Table 7. Nitrogen balance not scaled to the body weight of yearling fillies, with urea nitrogen subtracted from nitrogen intake in Diet C

	Diet A			Diet B			Diet C		
Item	Mean		SEM	Mean		SEM	Mean		SEM
Nitrogen intake (g/d)	253.38 [f]	±	7.25	259.13 [f]	±	5.24	230.21 [g]	±	7.22
Fecal nitrogen (g/d)	51.51	±	6.85	59.17	±	6.09	59.77	±	5.51
Urine nitrogen (g/d)	5.93 [d]	±	0.18	6.83 [e]	±	0.19	7.18 [e]	±	0.25
Nitrogen absorbed (g/d)	201.86 [f]	±	6.25	199.96 [f]	±	8.91	170.44 [g]	±	8.66
Nitrogen absorbed as a percent of intake	79.81	±	2.42	77.06	±	2.46	73.93	±	2.54
Nitrogen retained (g/d)	195.93 [f]	±	6.27	193.13 [f]	±	8.80	163.25 [g]	±	8.60
Nitrogen retained as a percent of intake	77.46	±	2.37	74.42	±	2.45	70.80	±	2.53
Nitrogen retained as a percent of absorbed	97.04 [a]	±	0.13	96.56 [b]	±	0.12	95.73 [c]	±	0.23

[a,b,c] Means in rows lacking common superscripts differ significantly (P < 0.001)

[d,e] Means in rows lacking common superscripts differ significantly (P < 0.01)

[f,g] Means in rows lacking common superscripts differ significantly (P < 0.05)

Nitrogen Balance Accounting for Endogenous Losses. Nitrogen balance data not scaled to the yearlings' body weight were adjusted for endogenous fecal and urinary nitrogen losses using values of 0.05 grams of nitrogen per kilogram of metabolic body weight (g/kg BW $^{0.75}$) and 0.128 g/kg BW $^{0.75}$, respectively, which were calculated by Prior et al. (1974) for growing ponies (Table 8). Nitrogen intake was greater ($P < 0.01$) for Diet C than Diet A or Diet B, because of the supplemented amino acids. Fecal nitrogen was not different among diets, but Diets B and C resulted in more ($P < 0.05$) urinary nitrogen than Diet A. Values for urinary nitrogen were negative, which was most likely the result of overestimating endogenous losses. Nitrogen absorbed was higher ($P < 0.05$) for Diet C than Diets A or B, and nitrogen absorbed as a percent of nitrogen intake was similar among diets. As seen before, there was a trend for more ($P = 0.052$) nitrogen to be retained, when horses were fed Diet C, as a result of the higher nitrogen intake. No differences were seen among diets for nitrogen retained as a percent of nitrogen intake. Diet C was lower ($P < 0.01$) than Diet A for nitrogen retained as a percent of nitrogen absorbed; however, Diet B was not different from Diet A or Diet C.

Table 8. Nitrogen balance accounting for endogenous urinary[1] and fecal[2] losses in yearling horses

	Diet A			Diet B			Diet C		
Item	Mean		SEM	Mean		SEM	Mean		SEM
Nitrogen intake (g/d)	253.38 [a]	±	7.25	259.13 [a]	±	5.24	287.74 [b]	±	7.31
Fecal nitrogen (g/d)	47.59	±	6.85	55.25	±	6.07	55.85	±	5.50
Urine nitrogen (g/d)	-4.09 [c]	±	0.25	-3.20 [d]	±	0.23	-2.84 [d]	±	0.29
Nitrogen absorbed (g/d)	205.78 [c]	±	6.24	203.88 [c]	±	8.91	231.89 [d]	±	8.76
Nitrogen absorbed as a percent of intake	81.37	±	2.44	78.57	±	2.44	80.54	±	1.98
Nitrogen retained (g/d)	209.88	±	6.24	207.08	±	8.80	234.73	±	8.71
Nitrogen retained as a percent of intake	82.99	±	2.43	79.81	±	2.38	81.53	±	1.94
Nitrogen retained as a percent of absorbed	102.00 [a]	±	0.13	101.59 [a,b]	±	0.15	101.24 [b]	±	0.14

[a,b] Means in rows lacking common superscripts differ significantly ($P < 0.01$)

[c,d] Means in rows lacking common superscripts differ significantly ($P < 0.05$)

[1] Endogenous urinary N of 0.128 g N/kg $BW^{0.75}$

[2] Endogenous fecal N of 0.05 g N/kg $BW^{0.75}$

Nitrogen Balance Accounting for Endogenous Losses, with Urea Nitrogen Subtracted from Nitrogen Intake in Diet C. Urea nitrogen was subtracted from nitrogen intake in Diet C for the nitrogen balance data accounting for endogenous fecal and urinary nitrogen losses (Table 9). Nitrogen intake was again lower ($P < 0.05$) for Diet C than Diet A or Diet B. Fecal nitrogen was not different among diets. Diets B and C resulted in higher ($P < 0.05$) urinary nitrogen than Diet A. As a result of the lower nitrogen intake, horses fed Diet C absorbed less ($P < 0.05$) nitrogen than those fed Diets A or B. Nitrogen absorbed as a percent of nitrogen intake was similar among diets. Nitrogen retained was lower ($P < 0.05$) for Diet C, when compared to Diet A and Diet B. No differences among diets were seen for nitrogen retained as a percent of nitrogen intake or nitrogen retained as a percent of nitrogen absorbed.

Table 9. Nitrogen balance accounting for endogenous urinary[1] and fecal[2] losses in yearling horses, with urea nitrogen subtracted from nitrogen intake in Diet C

	Diet A			Diet B			Diet C		
Item	Mean		SEM	Mean		SEM	Mean		SEM
Nitrogen intake (g/d)	253.38 [a]	±	7.25	259.13 [a]	±	5.24	230.21 [b]	±	7.22
Fecal nitrogen (g/d)	47.59	±	6.85	55.25	±	6.07	55.85	±	5.50
Urine nitrogen (g/d)	-4.09 [a]	±	0.25	-3.20 [b]	±	0.23	-2.84 [b]	±	0.29
Nitrogen absorbed (g/d)	205.78 [a]	±	6.24	203.88 [a]	±	8.91	174.36 [b]	±	8.67
Nitrogen absorbed as a percent of intake	81.37	±	2.44	78.57	±	2.44	75.64	±	2.52
Nitrogen retained (g/d)	209.88 [a]	±	6.24	207.08 [a]	±	8.80	177.20 [b]	±	8.61
Nitrogen retained as a percent of intake	82.99	±	2.43	79.81	±	2.38	76.88	±	2.47
Nitrogen retained as a percent of absorbed	102.00	±	0.13	101.59	±	0.15	101.66	±	0.19

[a,b] Means in rows lacking common superscripts differ significantly ($P < 0.05$)

[1] Endogenous urinary N of 0.128 g N/kg $BW^{0.75}$

[2] Endogenous fecal N of 0.05 g N/kg $BW^{0.75}$

Nitrogen Balance Scaled to Body Weight. The nitrogen balance data were scaled to the body weight of the yearling fillies to minimize variation (Table 10). Since Diet C was supplemented with synthetic amino acids, the nitrogen intake was higher ($P < 0.05$)

for Diet C, when compared to Diet A and Diet B. Fecal nitrogen was similar among diets, but urinary nitrogen was higher ($P < 0.05$) for Diets B and C. Nitrogen absorbed and nitrogen absorbed as a percent of nitrogen intake were not different among diets. Nitrogen retained and nitrogen retained as a percent of intake were also not different among diets. Nitrogen retained as a percent of nitrogen absorbed was higher ($P < 0.05$) for Diet A than for Diet B; however, Diet C was not different from Diet A or Diet B.

Table 10. Nitrogen balance scaled to the body weight of yearling fillies

	Diet A			Diet B			Diet C		
Item	Mean		SEM	Mean		SEM	Mean		SEM
Nitrogen intake ($mg•kg\ BW^{-1}•d^{-1}$)	756.33 [a]	±	26.43	772.54 [a]	±	13.22	858.19 [b]	±	21.84
Fecal nitrogen ($mg•kg\ BW^{-1}•d^{-1}$)	153.64	±	21.24	175.81	±	17.00	178.04	±	16.57
Urine nitrogen ($mg•kg\ BW^{-1}•d^{-1}$)	17.72 [a]	±	0.71	20.39 [b]	±	0.69	21.45 [b]	±	0.86
Nitrogen absorbed ($mg•kg\ BW^{-1}•d^{-1}$)	602.69	±	22.64	596.72	±	28.84	680.14	±	27.58
Nitrogen absorbed as a percent of intake	79.81	±	2.42	77.06	±	2.46	79.17	±	1.99
Nitrogen retained ($mg•kg\ BW^{-1}•d^{-1}$)	584.97	±	22.42	576.33	±	28.39	658.69	±	27.25
Nitrogen retained as a percent of intake	77.46	±	2.37	74.42	±	2.45	76.67	±	1.98
Nitrogen retained as a percent of absorbed	97.04 [a]	±	0.13	96.56 [b]	±	0.12	96.83 [b]	±	0.14

[a,b] Means in rows lacking common superscripts differ significantly ($P < 0.05$)

Nitrogen Balance Scaled to Body Weight, with Urea Nitrogen Subtracted from Nitrogen Intake in Diet C. Urea nitrogen was subtracted from nitrogen intake in Diet C, for the nitrogen balance data scaled to the fillies' body weight, and a new nitrogen balance table was constructed (Table 11). Nitrogen intake was lower ($P < 0.05$) for Diet C, when compared to Diet A and Diet B. Fecal nitrogen was similar among diets. Urinary nitrogen remained higher ($P < 0.05$) for Diets B and C than Diet A. Horses fed Diets A and B absorbed more ($P < 0.05$) nitrogen than those fed Diet C. Nitrogen absorbed as a percent of nitrogen intake was similar among diets. Fillies fed Diets A and B retained more ($P < 0.05$) nitrogen than fillies fed Diet C. Nitrogen retained as a percent of nitrogen intake was not different among diets; however, nitrogen retained as a percent of nitrogen absorbed was different among all three diets. Diet B was lower ($P < 0.001$) than Diet A, and Diet C was lower than both Diet A and Diet B.

Nitrogen Balance Accounting for Endogenous Losses and Scaled to Body Weight. Table 12 depicts data accounting for endogenous fecal and urinary nitrogen losses that were scaled to the body weight of the yearling fillies. Nitrogen intake was higher ($P < 0.05$) for Diet C than for Diet A or Diet B, because of the supplemented synthetic amino acids. Fecal nitrogen was not different among diets. Urinary nitrogen was higher ($P < 0.01$) for Diets B and C, when compared to Diet A. Nitrogen absorbed and nitrogen absorbed as a percent of nitrogen intake were similar among diets, as were nitrogen

Table 11. Nitrogen balance scaled to the body weight of yearling fillies, with urea nitrogen subtracted from nitrogen intake in Diet C

	Diet A		Diet B		Diet C	
Item	Mean	SEM	Mean	SEM	Mean	SEM
Nitrogen intake (mg•kg BW $^{-1}$•d $^{-1}$)	756.33 ^{d}	± 26.43	772.54 ^{d}	± 13.22	686.50 ^{e}	± 20.73
Fecal nitrogen (mg•kg BW $^{-1}$•d $^{-1}$)	153.64	± 21.24	175.81	± 17.00	178.04	± 16.57
Urine nitrogen (mg•kg BW $^{-1}$•d $^{-1}$)	17.72 ^{d}	± 0.71	20.39 ^{e}	± 0.69	21.45 ^{e}	± 0.86
Nitrogen absorbed (mg•kg BW $^{-1}$•d $^{-1}$)	602.69 ^{d}	± 22.64	596.72 ^{d}	± 28.84	508.46 ^{e}	± 26.54
Nitrogen absorbed as a percent of intake	79.81	± 2.42	77.06	± 2.46	73.93	± 2.54
Nitrogen retained (mg•kg BW $^{-1}$•d $^{-1}$)	584.97 ^{d}	± 22.42	576.33 ^{d}	± 28.39	487.00 ^{e}	± 26.23
Nitrogen retained as a percent of intake	77.46	± 2.37	74.42	± 2.45	70.80	± 2.53
Nitrogen retained as a percent of absorbed	97.04 ^{a}	± 0.13	96.56 ^{b}	± 0.12	95.73 ^{c}	± 0.23

^{a,b,c} Means in rows lacking common superscripts differ significantly ($P < 0.001$)

^{d,e} Means in rows lacking common superscripts differ significantly ($P < 0.05$)

Table 12. Nitrogen balance accounting for endogenous urinary[1] and fecal[2] losses and scaled to body weight of yearling fillies

	Diet A		Diet B		Diet C	
Item	Mean	SEM	Mean	SEM	Mean	SEM
Nitrogen intake (mg•kg BW^{-1}•d^{-1})	756.33 [c]	± 26.43	772.54 [c]	± 13.22	858.19 [d]	± 21.84
Fecal nitrogen (mg•kg BW^{-1}•d^{-1})	141.95	± 21.24	164.13	± 17.02	166.35	± 16.57
Urine nitrogen (mg•kg BW^{-1}•d^{-1})	-12.19 [a]	± 0.63	-9.52 [b]	± 0.64	-8.46 [b]	± 0.81
Nitrogen absorbed (mg•kg BW^{-1}•d^{-1})	614.37	± 22.67	608.40	± 28.86	691.83	± 27.59
Nitrogen absorbed as a percent of intake	81.37	± 2.44	78.57	± 2.44	80.54	± 1.98
Nitrogen retained (mg•kg BW^{-1}•d^{-1})	626.57	± 22.51	617.93	± 28.44	700.29	± 27.29
Nitrogen retained as a percent of intake	82.99	± 2.43	79.81	± 2.38	81.53	± 1.94
Nitrogen retained as a percent of absorbed	102.00 [a]	± 0.13	101.59 [a,b]	± 0.15	101.24 [b]	± 0.14

[a,b] Means in rows lacking common superscripts differ significantly ($P < 0.01$)

[c,d] Means in rows lacking common superscripts differ significantly ($P < 0.05$)

[1] Endogenous urinary N of 0.128 g N/kg $BW^{0.75}$

[2] Endogenous fecal N of 0.05 g N/kg $BW^{0.75}$

retained and nitrogen retained as a percent of nitrogen intake. Nitrogen retained as a percent of nitrogen absorbed was higher ($P < 0.01$) for Diet A than for Diet C, and Diet B was not different from either Diet A or Diet B.

Nitrogen Balance Accounting for Endogenous Losses and Scaled to Body Weight, with Urea Nitrogen Subtracted from Nitrogen Intake in Diet C. Nitrogen from urea was subtracted from nitrogen balance data accounting for endogenous fecal and urinary nitrogen losses and scaled to the body weight of the yearling fillies (Table 13). Nitrogen intake was lower ($P < 0.05$) for Diet C than for Diets A or B. There were no differences among diets for fecal nitrogen, but urinary nitrogen was higher ($P < 0.01$) for Diets B and C, when compared to Diet A. Fillies fed Diet C absorbed less ($P < 0.05$) nitrogen than fillies fed Diet A or Diet B. Nitrogen absorbed as a percent of nitrogen intake was similar among diets. Horses fed Diets A and B retained more ($P < 0.05$) nitrogen than horses fed Diet C. No differences among diets were seen for nitrogen retained as a percent of nitrogen intake or nitrogen retained as a percent of nitrogen absorbed.

Table 13. Nitrogen balance accounting for endogenous urinary[1] and fecal[2] losses and scaled to body weight of yearling fillies, with urea nitrogen subtracted from nitrogen intake in Diet C

	Diet A			Diet B			Diet C		
Item	Mean		SEM	Mean		SEM	Mean		SEM
Nitrogen intake (mg•kg BW $^{-1}$•d $^{-1}$)	756.33 ^{c}	±	26.43	772.54 ^{c}	±	13.22	686.50 ^{d}	±	20.73
Fecal nitrogen (mg•kg BW $^{-1}$•d $^{-1}$)	141.95	±	21.24	164.13	±	17.02	166.35	±	16.57
Urine nitrogen (mg•kg BW $^{-1}$•d $^{-1}$)	-12.19 ^{a}	±	0.63	-9.52 ^{b}	±	0.64	-8.46 ^{b}	±	0.81
Nitrogen absorbed (mg•kg BW $^{-1}$•d $^{-1}$)	614.37 ^{c}	±	22.67	608.40 ^{c}	±	28.86	520.14 ^{d}	±	26.55
Nitrogen absorbed as a percent of intake	81.37	±	2.44	78.57	±	2.44	75.64	±	2.52
Nitrogen retained (mg•kg BW $^{-1}$•d $^{-1}$)	626.57 ^{c}	±	22.51	617.93 ^{c}	±	28.44	528.60 ^{d}	±	26.26
Nitrogen retained as a percent of intake	82.99	±	2.43	79.81	±	2.38	76.88	±	2.47
Nitrogen retained as a percent of absorbed	102.00	±	0.13	101.59	±	0.15	101.66	±	0.19

[a,b] Means in rows lacking common superscripts differ significantly ($P < 0.01$)

[c,d] Means in rows lacking common superscripts differ significantly ($P < 0.05$)

[1] Endogenous urinary N of 0.128 g N/kg BW $^{0.75}$

[2] Endogenous fecal N of 0.05 g N/kg BW $^{0.75}$

CHAPTER V

DISCUSSION

Diet B was deficient in essential amino acids and most likely nonessential amino acids, and although urea was added to keep nitrogen intake the same as that of Diet A, it was not well utilized. As a result, there was an increase ($P<0.01$) in urinary nitrogen for data not scaled to body weight and data accounting for endogenous losses and scaled to body weight, as well as an increase ($P < 0.05$) in urinary nitrogen for data scaled to body weight and data accounting for endogenous losses but not scaled to body weight. Other research has also indicated little or no benefit to adding urea to the diet of horses (Godbee and Slade, 1981; Martin et al., 1996) and resulted in increased urinary nitrogen when urea was added (Glade and Biesik, 1986).

To give a better estimate of the true nitrogen digestibility of each diet, endogenous fecal and urinary losses were taken into account. Since there were no such data available for the yearlings used in this project, data from other research had to be used. Hintz and Schryver (1972) reported endogenous urinary losses for mature ponies to be 0.153 g/kg $BW^{0.75}$. Olsman et al. (2003) found metabolic fecal losses to be 0.035 g/kg $BW^{0.75}$ and endogenous urinary losses to be 0.131 g/ kg $BW^{0.75}$ for adult ponies; however, the values of 0.05 g/kg $BW^{0.75}$ for metabolic fecal nitrogen and 0.128 g/kg $BW^{0.75}$ for endogenous urinary nitrogen were used from research conducted by Prior et al. (1974), because these values were calculated from a study using yearling ponies. It is possible that the values in Prior's research overestimated the endogenous losses of the yearling fillies used in the more current research and resulted in negative values for urinary nitrogen in data accounting for endogenous losses.

Diet A and Diet B were isonitrogenous, and therefore, there were no differences seen between the two diets in nitrogen intake. However, Diet C was composed of the same ingredients as Diet B, which included two percent urea in the total diet. Horses fed Diet C were orally dosed with synthetic amino acids, and the additional nitrogen caused an increase ($P < 0.01$) in nitrogen intake for data not scaled to the body weight of the yearling fillies and an increase ($P < 0.05$) in nitrogen intake for data scaled to the body weight of the yearling fillies. The nitrogen from urea was subtracted from the total nitrogen intake for Diet C, which resulted in lower ($P < 0.05$) nitrogen intake for Diet C, when compared to Diet A and Diet B.

In addition to poor utilization of urea by the yearling fillies, further increases in urinary nitrogen for Diet C could possibly be explained by either decreased absorption of the synthetic amino acids or by nonessential amino acids becoming a limiting factor. Although differences among diets were seen in the amount of nitrogen absorbed, there were no significant differences among diets for nitrogen absorbed as a percent of intake. In addition, numerous studies have supplemented diets with synthetic amino acids with positive results in young growing horses (Ott et al., 1979; McCall et al., 1981; Graham et al., 1994) and in pigs (Tuitoek et al., 1997; Lenis et al., 1999; Otto et al., 2003).

It is possible, however, that an unbalanced ratio of essential amino acids to nonessential amino acids in Diet C resulted in increased urinary nitrogen. In order for protein synthesis to occur, essential and nonessential amino acids must be available to the tissues at the same time (Wang and Fuller, 1989). Horses on Diet C were orally dosed with ten essential amino acids and cysteine to match the essential amino acid profile in Diet A. Nonessential amino acids were most likely left very deficient. As a result, only a portion of the essential amino acids could be utilized, while the rest were excreted in the urine. The optimum ratio of essential amino acids to nonessential amino acids has been proposed in swine as being at most 50:50 (Lenis et al., 1999) and at least 45:55 (Wang and Fuller, 1989; Otto et al., 2003). This ratio leads to greater nitrogen utilization and less nitrogen excretion.

There was a trend for nitrogen retention to be higher for Diet C in data not excluding urea nitrogen from the nitrogen intake of horses fed Diet C. This trend was a result of the higher nitrogen intake for Diet C. Nitrogen retention was significantly lower for Diet C, when nitrogen intake was lower, due to the removal of urea nitrogen from the nitrogen intake. However, no differences in nitrogen retention were seen in Diets A and B. Therefore, nitrogen retention may not be the best value to study.

The biological value, or nitrogen retained as a percent of nitrogen absorbed, is a better indicator of the nitrogen the horse was actually able to utilize. Nitrogen retained as a percent of nitrogen absorbed was significantly decreased in Diet B when compared to Diet A, and Diet C had an intermediate value in data not accounting for endogenous losses. Decreased values in Diet B were expected, because the diet was deficient in essential amino acids. Diet C was significantly lower than Diet A in nitrogen retained as a percent of nitrogen absorbed, in data accounting for endogenous losses. The significant decreased biological value in Diet C proved that the amino acids supplemented diet was not as efficiently utilized as the naturally occurring amino acids in Diet A.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of this study was to evaluate the technique of using nitrogen retention as a response criterion for amino acid studies in the horse. No significant differences in nitrogen retention were seen between the amino acid sufficient diet (Diet A) and the amino acid deficient diet (Diet B). The amino acid deficient diet with orally dosed essential amino acids and cysteine (Diet C) resulted in significant differences in nitrogen retention; however, the differences were a consequence of the nitrogen intake. Consequently, at these levels of intake, nitrogen retention may not be the best indicator of amino acid status.

Nitrogen retained as a percent of nitrogen absorbed was a better indicator of protein quality, because it indicated the nitrogen that the horse was able to utilize. There was a significant decrease in nitrogen retained as a percent of nitrogen absorbed for fillies fed Diet B, when compared to fillies fed Diet A, before accounting for endogenous losses. Diet B was expected to cause a decrease in nitrogen retained as a percent of nitrogen absorbed, because the diet was of inferior protein quality, having lower levels of essential amino acids than Diet A. Diet B was not different from Diet A in data accounting for endogenous losses. There were no differences in nitrogen retained as a percent of nitrogen absorbed for horses fed Diet C, when compared to either Diet A or Diet B, for data not accounting for endogenous losses. In data accounting for endogenous losses, Diet C was significantly lower than Diet A, in nitrogen retained as a percent of nitrogen absorbed.

It was concluded that differences in nitrogen retained as a percent of nitrogen absorbed were observed between amino acid sufficient diets and amino acid deficient diets. However, horses fed amino acid deficient diets and orally dosed with synthetic amino acids likely require some modified dosage level to achieve the same or higher values in nitrogen retained as a percent of nitrogen absorbed as those values for amino acid sufficient diets. Diet C likely should have been formulated without urea, and it is possible that the supplemented amino acids should be given in excess of the horse's requirements to allow all diets to be isonitrogenous. In addition, improving the ratio of supplemented essential:nonessential amino acids should be considered.

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APPENDICES

APPENDIX 1. AMOUNT OF DIET FED PER DAY TO YEARLING FILLIES

Horse	Body weight (kg)	Concentrate (kg/d)	Oil (kg/d)	Hay (kg/d)	Diet as percent of BW
1A	322.73	3.25	0.58	2.60	1.99
2A	342.73	3.25	0.58	2.60	1.88
1B	320.91	3.25	0.58	2.60	2.01
2B	348.18	3.18	0.57	2.55	1.81
1C	337.27	3.23	0.58	2.55	1.88
2C	347.27	3.25	0.58	2.60	1.85

APPENDIX 2. NITROGEN BALANCE NOT SCALED TO BODY WEIGHT

Horse	Period	Diet	Total N Intake g/d	Fecal N g/d	Urine N g/d	N Absorbed g/d	N Abs. % intake	N Retained g/d	N Ret. % Intake	N Ret. % Abs.
1A	1	A	240.73	23.32	6.29	217.41	90.31	211.12	87.70	97.11
2A	1	A	234.55	45.46	6.33	189.09	80.62	182.76	77.92	96.65
1B	1	B	235.27	61.42	6.65	173.85	73.89	167.20	71.07	96.18
2B	1	B	272.70	55.23	7.17	217.48	79.75	210.30	77.12	96.70
1C	1	C	293.80	57.20	8.10	236.60	80.53	228.50	77.77	96.58
2C	1	C	319.85	68.35	7.02	251.50	78.63	244.48	76.44	97.21
1A	2	B	267.05	36.13	7.34	230.92	86.47	223.57	83.72	96.82
2A	2	B	260.88	74.78	7.18	186.09	71.33	178.92	68.58	96.14
1B	2	C	279.17	75.19	6.85	203.98	73.07	197.12	70.61	96.64
2B	2	C	267.20	65.93	7.30	201.28	75.33	193.97	72.59	96.37
1C	2	A	245.93	60.11	5.39	185.82	75.56	180.43	73.36	97.10
2C	2	A	248.40	56.61	6.07	191.78	77.21	185.71	74.76	96.83
1A	3	C	284.25	36.57	7.56	247.68	87.14	240.12	84.47	96.95
2A	3	C	282.19	55.39	6.29	226.81	80.37	220.52	78.15	97.23
1B	3	A	278.62	73.33	6.20	205.29	73.68	199.10	71.46	96.98
2B	3	A	272.06	50.24	5.32	221.82	81.53	216.50	79.58	97.60
1C	3	B	257.92	51.95	6.52	205.97	79.86	199.45	77.33	96.84
2C	3	B	261.01	75.54	6.12	185.48	71.06	179.35	68.71	96.70

APPENDIX 3. NITROGEN BALANCE ACCOUNTING FOR ENDOGENOUS LOSSES

Horse	Period	Diet	BW kg	MBW kg	End. Fecal Loss g/d	End. Urine Loss g/d	Exog. Fecal N g/d	Exog. Urine N g/d	True N Absorbed g/d	True N Abs. % Intake	True N Retained g/d	True N Ret. % intake	True N Ret. % Abs.
1A	1	A	322	76.01	3.80	9.73	19.52	-3.44	221.21	91.89	224.65	93.32	101.56
2A	1	A	342	79.53	3.98	10.18	41.49	-3.85	193.06	82.31	196.91	83.95	101.99
1B	1	B	320	75.66	3.78	9.68	57.64	-3.04	177.63	75.50	180.67	76.79	101.71
2B	1	B	347	80.40	4.02	10.29	51.21	-3.12	221.50	81.22	224.62	82.37	101.41
1C	1	C	336	78.48	3.92	10.05	53.28	-1.95	240.53	81.87	242.47	82.53	100.81
2C	1	C	346	80.22	4.01	10.27	64.34	-3.25	255.51	79.88	258.76	80.90	101.27
1A	2	B	322	76.01	3.80	9.73	32.33	-2.39	234.72	87.89	237.10	88.79	101.02
2A	2	B	342	79.53	3.98	10.18	70.81	-3.00	190.07	72.86	193.07	74.01	101.58
1B	2	C	320	75.66	3.78	9.68	71.41	-2.83	207.76	74.42	210.59	75.43	101.36
2B	2	C	347	80.40	4.02	10.29	61.91	-2.99	205.30	76.83	208.28	77.95	101.45
1C	2	A	336	78.48	3.92	10.05	56.19	-4.65	189.74	77.15	194.40	79.04	102.45
2C	2	A	346	80.22	4.01	10.27	52.60	-4.19	195.80	78.82	199.99	80.51	102.14
1A	3	C	322	76.01	3.80	9.73	32.77	-2.17	251.48	88.47	253.65	89.23	100.86
2A	3	C	342	79.53	3.98	10.18	51.41	-3.89	230.78	81.78	234.68	83.16	101.69
1B	3	A	320	75.66	3.78	9.68	69.55	-3.49	209.08	75.04	212.57	76.29	101.67
2B	3	A	347	80.40	4.02	10.29	46.22	-4.97	225.84	83.01	230.81	84.84	102.20
1C	3	B	336	78.48	3.92	10.05	48.02	-3.53	209.89	81.38	213.42	82.75	101.68
2C	3	B	346	80.22	4.01	10.27	71.53	-4.14	189.49	72.60	193.63	74.18	102.19

APPENDIX 4. NITROGEN BALANCE SCALED TO BODY WEIGHT

Horse	Period	Diet	BW kg	Total N Intake mg/kgBW/d	Fecal N mg/kgBW/d	Urine N mg/kgBW/d	N Absorbed mg/kgBW/d	N Abs. % Intake	N Retained mg/kgBW/d	N Ret. % Intake	N Ret. % Abs.
1A	1	A	322	747.61	72.43	19.52	675.17	90.31	655.65	87.70	97.11
2A	1	A	342	685.82	132.93	18.51	552.89	80.62	534.38	77.92	96.65
1B	1	B	320	735.22	191.95	20.78	543.27	73.89	522.50	71.07	96.18
2B	1	B	347	785.88	159.15	20.67	626.73	79.75	606.06	77.12	96.70
1C	1	C	336	874.40	170.24	24.11	704.17	80.53	680.06	77.77	96.58
2C	1	C	346	924.42	197.55	20.28	726.87	78.63	706.59	76.44	97.21
1A	2	B	322	829.35	112.21	22.81	717.14	86.47	694.33	83.72	96.82
2A	2	B	342	762.81	218.67	20.99	544.14	71.33	523.15	68.58	96.14
1B	2	C	320	872.41	234.98	21.42	637.43	73.07	616.01	70.61	96.64
2B	2	C	347	770.03	189.99	21.05	580.04	75.33	558.99	72.59	96.37
1C	2	A	336	731.93	178.91	16.05	553.02	75.56	536.98	73.36	97.10
2C	2	A	346	717.92	163.62	17.56	554.30	77.21	536.75	74.76	96.83
1A	3	C	322	882.76	113.56	23.49	769.20	87.14	745.71	84.47	96.95
2A	3	C	342	825.12	161.95	18.38	663.17	80.37	644.79	78.15	97.23
1B	3	A	320	870.69	229.16	19.36	641.53	73.68	622.17	71.46	96.98
2B	3	A	347	784.03	144.79	15.32	639.24	81.53	623.92	79.58	97.60
1C	3	B	336	767.62	154.61	19.40	613.01	79.86	593.61	77.33	96.84
2C	3	B	346	754.36	218.32	17.70	536.05	71.06	518.35	68.71	96.70

APPENDIX 5. NITROGEN BALANCE SCALED TO BODY WEIGHT AND ACCOUNTING FOR ENDOGENOUS LOSSES

Horse	Period	Diet	BW kg	MBW kg	End. Fecal Loss mg/kgBW/d	End. Urine Loss mg/kgBW/d	Adj. Fecal N mg/kgBW/d	Adj. Urine N mg/kgBW/d	Adj. N Absorbed mg/kgBW/d	True N Absorbed as % intake	Adj. N Retained mg/kgBW/d	N Retention as % intake	N Retention as % absorbed
1A	1	A	322	76.01	11.80	30.22	60.63	-10.69	686.98	91.89	697.67	93.32	101.56
2A	1	A	342	79.53	11.63	29.76	121.30	-11.26	564.51	82.31	575.77	83.95	101.99
1B	1	B	320	75.66	11.82	30.26	180.13	-9.49	555.09	75.50	564.58	76.79	101.71
2B	1	B	347	80.40	11.58	29.66	147.57	-8.99	638.32	81.22	647.31	82.37	101.41
1C	1	C	336	78.48	11.68	29.90	158.56	-5.79	715.85	81.87	721.64	82.53	100.81
2C	1	C	346	80.22	11.59	29.68	185.96	-9.40	738.46	79.88	747.86	80.90	101.27
1A	2	B	322	76.01	11.80	30.22	100.40	-7.41	728.94	87.89	736.35	88.79	101.02
2A	2	B	342	79.53	11.63	29.76	207.04	-8.78	555.77	72.86	564.54	74.01	101.58
1B	2	C	320	75.66	11.82	30.26	223.15	-8.85	649.25	74.42	658.10	75.43	101.36
2B	2	C	347	80.40	11.58	29.66	178.40	-8.61	591.63	76.83	600.23	77.95	101.45
1C	2	A	336	78.48	11.68	29.90	167.23	-13.85	564.70	77.15	578.55	79.04	102.45
2C	2	A	346	80.22	11.59	29.68	152.02	-12.12	565.90	78.82	578.02	80.51	102.14
1A	3	C	322	76.01	11.80	30.22	101.76	-6.73	781.01	88.47	787.73	89.23	100.86
2A	3	C	342	79.53	11.63	29.76	150.32	-11.38	674.80	81.78	686.18	83.16	101.69
1B	3	A	320	75.66	11.82	30.26	217.34	-10.90	653.35	75.04	664.26	76.29	101.67
2B	3	A	347	80.40	11.58	29.66	133.21	-14.33	650.83	83.01	665.16	84.84	102.20
1C	3	B	336	78.48	11.68	29.90	142.93	-10.50	624.69	81.38	635.19	82.75	101.68
2C	3	B	346	80.22	11.59	29.68	206.72	-11.98	547.64	72.60	559.62	74.18	102.19

APPENDIX 6. NITROGEN BALANCE NOT SCALED TO BODY WEIGHT, WITH UREA NITROGEN SUBTRACTED FROM NITROGEN INTAKE IN DIET C

Horse	Period	Diet	Total N Intake g/d	Fecal N g/d	Urine N g/d	N Absorbed g/d	N Abs. % intake	N Retained g/d	N Ret. % Intake	N Ret. % Abs.
1A	1	A	240.73	23.32	6.29	217.41	90.31	211.12	87.70	97.11
2A	1	A	234.55	45.46	6.33	189.09	80.62	182.76	77.92	96.65
1B	1	B	235.27	61.42	6.65	173.85	73.89	167.20	71.07	96.18
2B	1	B	272.70	55.23	7.17	217.48	79.75	210.30	77.12	96.70
1C	1	C	236.68	57.20	8.10	179.48	75.83	171.38	72.41	95.49
2C	1	C	261.99	68.35	7.02	193.64	73.91	186.62	71.23	96.38
1A	2	B	267.05	36.13	7.34	230.92	86.47	223.57	83.72	96.82
2A	2	B	260.88	74.78	7.18	186.09	71.33	178.92	68.58	96.14
1B	2	C	221.31	75.19	6.85	146.12	66.02	139.26	62.93	95.31
2B	2	C	210.57	65.93	7.30	144.65	68.69	137.34	65.22	94.95
1C	2	A	245.93	60.11	5.39	185.82	75.56	180.43	73.36	97.10
2C	2	A	248.40	56.61	6.07	191.78	77.21	185.71	74.76	96.83
1A	3	C	226.39	36.57	7.56	189.82	83.85	182.26	80.51	96.02
2A	3	C	224.33	55.39	6.29	168.95	75.31	162.66	72.51	96.28
1B	3	A	278.62	73.33	6.20	205.29	73.68	199.10	71.46	96.98
2B	3	A	272.06	50.24	5.32	221.82	81.53	216.50	79.58	97.60
1C	3	B	257.92	51.95	6.52	205.97	79.86	199.45	77.33	96.84
2C	3	B	261.01	75.54	6.12	185.48	71.06	179.35	68.71	96.70

APPENDIX 7. NITROGEN BALANCE NOT SCALED TO BODY WEIGHT AND ACCOUNTING FOR ENDOGENOUS LOSSES, WITH UREA NITROGEN SUBTRACTED FROM NITROGEN INTAKE IN DIET C

Horse	Period	Diet	BW kg	MBW kg	End. Fecal Loss g/d	End. Urine Loss g/d	Exog. Fecal N g/d	Exog. Urine N g/d	True N Absorbed g/d	True N Abs. % Intake	True N Retained g/d	True N Ret. % intake	True N Ret. % Abs.
1A	1	A	322	76.01	3.80	9.73	19.52	-3.44	221.21	91.89	224.65	93.32	101.56
2A	1	A	342	79.53	3.98	10.18	41.49	-3.85	193.06	82.31	196.91	83.95	101.99
1B	1	B	320	75.66	3.78	9.68	57.64	-3.04	177.63	75.50	180.67	76.79	101.71
2B	1	B	347	80.40	4.02	10.29	51.21	-3.12	221.50	81.22	224.62	82.37	101.41
1C	1	C	336	78.48	3.92	10.05	53.28	-1.95	183.41	77.49	185.35	78.31	101.06
2C	1	C	346	80.22	4.01	10.27	64.34	-3.25	197.65	75.44	200.90	76.68	101.65
1A	2	B	322	76.01	3.80	9.73	32.33	-2.39	234.72	87.89	237.10	88.79	101.02
2A	2	B	342	79.53	3.98	10.18	70.81	-3.00	190.07	72.86	193.07	74.01	101.58
1B	2	C	320	75.66	3.78	9.68	71.41	-2.83	149.90	67.73	152.73	69.01	101.89
2B	2	C	347	80.40	4.02	10.29	61.91	-2.99	148.67	70.60	151.65	72.02	102.01
1C	2	A	336	78.48	3.92	10.05	56.19	-4.65	189.74	77.15	194.40	79.04	102.45
2C	2	A	346	80.22	4.01	10.27	52.60	-4.19	195.80	78.82	199.99	80.51	102.14
1A	3	C	322	76.01	3.80	9.73	32.77	-2.17	193.62	85.53	195.79	86.48	101.12
2A	3	C	342	79.53	3.98	10.18	51.41	-3.89	172.92	77.08	176.82	78.82	102.25
1B	3	A	320	75.66	3.78	9.68	69.55	-3.49	209.08	75.04	212.57	76.29	101.67
2B	3	A	347	80.40	4.02	10.29	46.22	-4.97	225.84	83.01	230.81	84.84	102.20
1C	3	B	336	78.48	3.92	10.05	48.02	-3.53	209.89	81.38	213.42	82.75	101.68
2C	3	B	346	80.22	4.01	10.27	71.53	-4.14	189.49	72.60	193.63	74.18	102.19

APPENDIX 8. NITROGEN BALANCE SCALED TO BODY WEIGHT, WITH UREA NITROGEN SUBTRACTED FROM NITROGEN INTAKE IN DIET C

Horse	Period	Diet	BW kg	Total N Intake mg/kgBW/d	Fecal N mg/kgBW/d	Urine N mg/kgBW/d	N Absorbed mg/kgBW/d	N Abs. % Intake	N Retained mg/kgBW/d	N Ret. % Intake	N Ret. % Abs.
1A	1	A	322	747.61	72.43	19.52	675.17	90.31	655.65	87.70	97.11
2A	1	A	342	685.82	132.93	18.51	552.89	80.62	534.38	77.92	96.65
1B	1	B	320	735.22	191.95	20.78	543.27	73.89	522.50	71.07	96.18
2B	1	B	347	785.89	159.15	20.67	626.73	79.75	606.07	77.12	96.70
1C	1	C	336	704.41	170.24	24.11	534.17	75.83	510.07	72.41	95.49
2C	1	C	346	757.19	197.55	20.28	559.64	73.91	539.36	71.23	96.38
1A	2	B	322	829.34	112.21	22.81	717.14	86.47	694.33	83.72	96.82
2A	2	B	342	762.80	218.67	20.99	544.13	71.33	523.14	68.58	96.14
1B	2	C	320	691.58	234.98	21.42	456.61	66.02	435.19	62.93	95.31
2B	2	C	347	606.84	189.99	21.05	416.85	68.69	395.80	65.22	94.95
1C	2	A	336	731.94	178.91	16.05	553.03	75.56	536.99	73.36	97.10
2C	2	A	346	717.91	163.62	17.56	554.29	77.21	536.74	74.76	96.83
1A	3	C	322	703.08	113.56	23.49	589.51	83.85	566.02	80.51	96.02
2A	3	C	342	655.94	161.95	18.38	494.00	75.31	475.61	72.51	96.28
1B	3	A	320	870.70	229.16	19.36	641.54	73.68	622.18	71.46	96.98
2B	3	A	347	784.04	144.79	15.32	639.25	81.53	623.93	79.58	97.60
1C	3	B	336	767.62	154.61	19.40	613.01	79.86	593.61	77.33	96.84
2C	3	B	346	754.37	218.32	17.70	536.06	71.06	518.36	68.71	96.70

APPENDIX 9. NITROGEN BALANCE SCALED TO BODY WEIGHT AND ACCOUNTING FOR ENDOGENOUS LOSSES, WITH UREA NITROGEN SUBTRACTED FROM NITROGEN INTAKE IN DIET C

Horse	Period	Diet	MBW kg	End. Fecal Loss mg/kgBW/d	End. Urine Loss mg/kgBW/d	Adj. Fecal N mg/kgBW/d	Adj. Urine N mg/kgBW/d	Adj. N Absorbed mg/kgBW/d	True N Absorbed as % intake	Adj. N Retained mg/kgBW/d	N Retention as % intake	N Retention as % absorbed
1A	1	A	76.01	11.80	30.22	60.63	-10.69	686.98	91.89	697.67	93.32	101.56
2A	1	A	79.53	11.63	29.76	121.30	-11.26	564.51	82.31	575.77	83.95	101.99
1B	1	B	75.66	11.82	30.26	180.13	-9.49	555.09	75.50	564.58	76.79	101.71
2B	1	B	80.40	11.58	29.66	147.57	-8.99	638.32	81.22	647.31	82.37	101.41
1C	1	C	78.48	11.68	29.90	158.56	-5.79	545.85	77.49	551.64	78.31	101.06
2C	1	C	80.22	11.59	29.68	185.96	-9.40	571.23	75.44	580.63	76.68	101.65
1A	2	B	76.01	11.80	30.22	100.40	-7.41	728.94	87.89	736.35	88.79	101.02
2A	2	B	79.53	11.63	29.76	207.04	-8.78	555.76	72.86	564.54	74.01	101.58
1B	2	C	75.66	11.82	30.26	223.15	-8.85	468.43	67.73	477.28	69.01	101.89
2B	2	C	80.40	11.58	29.66	178.40	-8.61	428.44	70.60	437.04	72.02	102.01
1C	2	A	78.48	11.68	29.90	167.23	-13.85	564.71	77.15	578.56	79.04	102.45
2C	2	A	80.22	11.59	29.68	152.02	-12.12	565.88	78.82	578.01	80.51	102.14
1A	3	C	76.01	11.80	30.22	101.76	-6.73	601.32	85.53	608.04	86.48	101.12
2A	3	C	79.53	11.63	29.76	150.32	-11.38	505.62	77.08	517.00	78.82	102.25
1B	3	A	75.66	11.82	30.26	217.34	-10.90	653.36	75.04	664.27	76.29	101.67
2B	3	A	80.40	11.58	29.66	133.21	-14.33	650.84	83.01	665.17	84.84	102.20
1C	3	B	78.48	11.68	29.90	142.93	-10.50	624.69	81.38	635.19	82.75	101.68
2C	3	B	80.22	11.59	29.68	206.72	-11.98	547.65	72.60	559.63	74.18	102.19

APPENDIX 10. ANOVA FOR NITROGEN BALANCE NOT SCALED TO BODY WEIGHT

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Intake					
Total	17	8075.4395	475.0258		
Model	4	4446.2961	1111.5740	3.98	0.0253
Error	13	3629.1434	279.1648		
Diet	2	4064.2197	2032.1098	7.28	0.0076
Period	2	382.0763	191.0381	0.68	0.5217
Fecal Nitrogen					
Total	17	3692.1671	217.1863		
Model	4	533.8410	133.4602	0.55	0.7029
Error	13	3158.3261	242.9481		
Diet	2	254.6205	127.3102	0.52	0.6041
Period	2	279.2204	139.6102	0.57	0.5766
Urine Nitrogen					
Total	17	9.1122	0.5360		
Model	4	6.0675	1.5168	6.48	0.0043
Error	13	3.0446	0.2342		
Diet	2	5.0041	2.5020	10.68	0.0018
Period	2	1.0634	0.5317	2.27	0.1427

APPENDIX 10. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Absorbed					
Total	17	8804.6257	517.9191		
Model	4	3836.1219	959.0304	2.51	0.0930
Error	13	4968.5037	382.1925		
Diet	2	2939.4813	1469.7406	3.85	0.0488
Period	2	896.6406	448.3203	1.17	0.3401
Nitrogen Absorbed as a Percent of Intake					
Total	17	502.8374	29.5786		
Model	4	76.6814	19.1703	0.58	0.6793
Error	13	426.1559	32.7812		
Diet	2	25.0106	12.5053	0.38	0.6902
Period	2	51.6707	25.8353	0.79	0.4753
Nitrogen Retained					
Total	17	8561.5714	503.6218		
Model	4	3689.1338	922.2834	2.46	0.0974
Error	13	4872.4376	374.8028		
Diet	2	2780.0286	1390.0143	3.71	0.0532
Period	2	909.1052	454.5526	1.21	0.3289
Nitrogen Retained as a Percent of Intake					
Total	17	497.3890	29.2581		
Model	4	81.1679	20.2919	0.63	0.6473
Error	13	416.2210	32.0170		
Diet	2	29.8819	14.9409	0.47	0.6372
Period	2	51.2860	25.6430	0.80	0.4699
Nitrogen Retained as a Percent of Absorbed					
Total	17	2.3553	0.1385		
Model	4	1.2285	0.3071	3.54	0.0364
Error	13	1.1267	0.0866		
Diet	2	0.6986	0.3493	4.03	0.0435
Period	2	0.5298	0.2649	3.06	0.0817

APPENDIX 11. ANOVA FOR NITROGEN BALANCE NOT SCALED TO BODY WEIGHT, WITH UREA NITROGEN SUBTRACTED FROM NITROGEN INTAKE IN DIET C

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Intake					
Total	17	6784.5414	399.0906		
Model	4	3181.7133	795.4283	2.87	0.0662
Error	13	3602.8280	277.1406		
Diet	2	2813.4811	1406.7405	5.08	0.0235
Period	2	368.2321	184.1160	0.66	0.5312
Fecal Nitrogen					
Total	17	3692.1671	217.1863		
Model	4	533.8410	133.4602	0.55	0.7029
Error	13	3158.3261	242.9481		
Diet	2	254.6205	127.3102	0.52	0.6041
Period	2	279.2204	139.6102	0.57	0.5766
Urine Nitrogen					
Total	17	9.1122	0.5360		
Model	4	6.0675	1.5168	6.48	0.0043
Error	13	3.0446	0.2342		
Diet	2	5.0041	2.5020	10.68	0.0018
Period	2	1.0634	0.5317	2.27	0.1427
Nitrogen Absorbed					
Total	17	9539.0187	561.1187		
Model	4	4604.1277	1151.0319	3.03	0.0570
Error	13	4934.8909	379.6069		
Diet	2	3725.3647	1862.6823	4.91	0.0258
Period	2	878.7630	439.3815	1.16	0.3447
Nitrogen Absorbed as a Percent of Intake					
Total	17	565.6914	38.6289		
Model	4	177.5586	44.3896	1.20	0.3553
Error	13	479.1328	36.8563		
Diet	2	103.9752	51.9876	1.41	0.2790
Period	2	73.5834	36.7917	1.00	0.3951
Nitrogen Retained					
Total	17	9665.9768	568.5868		
Model	4	4828.2864	1207.0716	3.24	0.0472
Error	13	4837.6903	372.1300		
Diet	2	3937.5667	1968.7833	5.29	0.0208
Period	2	890.7197	445.3598	1.20	0.3334

APPENDIX 11. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Retained as a Percent of Intake					
Total	17	676.3701	39.7864		
Model	4	208.5576	52.1394	1.45	0.2735
Error	13	467.8124	35.9855		
Diet	2	133.4677	66.7338	1.85	0.1956
Period	2	75.0898	37.5449	1.04	0.3800
Nitrogen Retained as a Percent of Absorbed					
Total	17	7.9187	0.4658		
Model	4	6.1395	1.5348	11.21	0.0004
Error	13	1.7792	0.1368		
Diet	2	5.2400	2.6200	19.14	0.0001
Period	2	0.8994	0.4497	3.29	0.0700

APPENDIX 12. ANOVA FOR NITROGEN BALANCE NOT SCALED TO BODY WEIGHT AND ACCOUNTING FOR ENDOGENOUS LOSSES

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Intake					
Total	17	8075.4395	475.0258		
Model	4	4446.2961	1111.5740	3.98	0.0253
Error	13	3629.1434	279.1648		
Diet	2	4064.2197	2032.1098	7.28	0.0076
Period	2	382.0763	191.0381	0.68	0.5217
Fecal Nitrogen					
Total	17	3679.5344	216.4432		
Model	4	533.7208	133.4302	0.55	0.7015
Error	13	3145.8136	241.9856		
Diet	2	254.5143	127.2571	0.53	0.6031
Period	2	279.2064	139.6032	0.58	0.5754
Urine Nitrogen					
Total	17	11.1819	0.6577		
Model	4	6.0493	1.5123	3.83	0.0286
Error	13	5.1326	0.3948		
Diet	2	4.9898	2.4949	6.32	0.0121
Period	2	1.0595	0.5297	1.34	0.2953

APPENDIX 12. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Absorbed					
Total	17	8800.7565	517.6915		
Model	4	3835.7604	958.9400	2.51	0.0928
Error	13	4964.9962	381.9227		
Diet	2	2939.3192	1469.6596	3.85	0.0487
Period	2	896.4411	448.2205	1.17	0.3400
Nitrogen Absorbed as a Percent of Intake					
Total	17	500.6515	29.4500		
Model	4	75.9995	18.9998	0.58	0.6813
Error	13	424.6519	32.6655		
Diet	2	24.7318	12.3659	0.38	0.6922
Period	2	51.2676	25.6338	0.78	0.4767
Nitrogen Retained					
Total	17	8550.6698	502.9805		
Model	4	3689.4805	922.3701	2.47	0.0969
Error	13	4861.1892	373.9376		
Diet	2	2780.1755	1390.0877	3.72	0.0529
Period	2	909.3050	454.6525	1.22	0.3281
Nitrogen Retained as a Percent of Intake					
Total	17	491.7019	28.9236		
Model	4	79.6458	19.9114	0.63	0.6509
Error	13	412.0561	31.6966		
Diet	2	30.3412	15.1706	0.48	0.6301
Period	2	49.3046	24.6523	0.78	0.4797
Nitrogen Retained as a Percent of Absorbed					
Total	17	3.6378	0.2139		
Model	4	1.9656	0.4914	3.82	0.0289
Error	13	1.6721	0.1286		
Diet	2	1.7424	0.8712	6.77	0.0097
Period	2	0.2232	0.1116	0.87	0.4428

APPENDIX 13. ANOVA FOR NITROGEN BALANCE NOT SCALED TO BODY WEIGHT AND ACCOUNTING FOR ENDOGENOUS LOSSES, WITH UREA NITROGEN SUBTRACTED FROM NITROGEN INTAKE IN DIET C

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Intake					
Total	17	6784.5414	399.0906		
Model	4	3181.7133	795.4283	2.87	0.0662
Error	13	3602.8280	277.1406		
Diet	2	2813.4811	1406.7405	5.08	0.0235
Period	2	368.2321	184.1160	0.66	0.5312
Fecal Nitrogen					
Total	17	3679.5344	216.4432		
Model	4	533.7208	133.4302	0.55	0.7015
Error	13	3145.8136	241.9856		
Diet	2	254.5143	127.2571	0.53	0.6031
Period	2	279.2064	139.6032	0.58	0.5754
Urine Nitrogen					
Total	17	11.1819	0.6577		
Model	4	6.0493	1.5123	3.83	0.0286
Error	13	5.1326	0.3948		
Diet	2	4.9898	2.4949	6.32	0.0121
Period	2	1.0595	0.5297	1.34	0.2953
Nitrogen Absorbed					
Total	17	9535.8017	560.9295		
Model	4	4604.1531	1151.0382	3.03	0.0569
Error	13	4931.6485	379.3575		
Diet	2	3725.5876	1862.7938	4.91	0.0258
Period	2	878.5654	439.2827	1.16	0.3445
Nitrogen Absorbed as a Percent of Intake					
Total	17	647.7368	38.1021		
Model	4	170.4579	42.6144	1.16	0.3724
Error	13	477.2789	36.7137		
Diet	2	98.3450	49.1725	1.34	0.2959
Period	2	72.1128	36.0564	0.98	0.4007
Nitrogen Retained					
Total	17	9655.5931	567.9760		
Model	4	4828.2479	1207.0619	3.25	0.0469
Error	13	4827.3452	371.3342		
Diet	2	3937.3302	1968.6651	5.30	0.0207
Period	2	890.9177	445.4588	1.20	0.3326

APPENDIX 13. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Retained as a Percent of Intake					
Total	17	643.4300	37.8488		
Model	4	181.3416	45.3354	1.28	0.3293
Error	13	462.0884	35.5452		
Diet	2	111.8747	55.9373	1.57	0.2443
Period	2	69.4669	34.7334	0.98	0.4024
Nitrogen Retained as a Percent of Absorbed					
Total	17	3.0420	0.1789		
Model	4	0.8914	0.2228	1.35	0.3049
Error	13	2.1505	0.1654		
Diet	2	0.5627	0.2813	1.70	0.2207
Period	2	0.3287	0.1643	0.99	0.3967

APPENDIX 14. ANOVA FOR NITROGEN BALANCE SCALED TO BODY WEIGHT

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Intake					
Total	17	76478.9927	4498.7642		
Model	4	39392.3111	9848.0777	3.45	0.0394
Error	13	37086.6816	2852.8216		
Diet	2	35946.7233	17973.3616	6.30	0.0122
Period	2	3445.5878	1722.7939	0.60	0.5613
Fecal Nitrogen					
Total	17	32648.1567	1920.4798		
Model	4	4725.2814	1181.3203	0.55	0.7025
Error	13	27922.8752	2147.9134		
Diet	2	2184.8814	1092.4407	0.51	0.6128
Period	2	2540.3999	1270.1999	0.59	0.5678
Urine Nitrogen					
Total	17	96.4204	5.6717		
Model	4	53.2808	13.3202	4.01	0.0246
Error	13	43.1396	3.3184		
Diet	2	44.4374	22.2187	6.70	0.0100
Period	2	8.8434	4.4217	1.33	0.2976

APPENDIX 14. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Absorbed					
Total	17	89169.5362	5245.2668		
Model	4	33566.8567	8391.7141	1.96	0.1600
Error	13	55602.6795	4277.1292		
Diet	2	25988.6924	12994.3462	3.04	0.0827
Period	2	7578.1643	3789.0822	0.89	0.4358
Nitrogen Absorbed as a Percent of Intake					
Total	17	502.8374	29.5786		
Model	4	76.6814	19.1703	0.58	0.6793
Error	13	426.1559	32.7812		
Diet	2	25.0106	12.5053	0.38	0.6902
Period	2	51.6707	25.8353	0.79	0.4753
Nitrogen Retained					
Total	17	86140.0023	5067.0589		
Model	4	32290.4957	8072.6239	1.95	0.1622
Error	13	53849.5066	4142.2697		
Diet	2	24583.4440	12291.7222	2.97	0.0868
Period	2	7707.0512	3853.5256	0.93	0.4192
Nitrogen Retained as a Percent of Intake					
Total	17	497.3890	29.2581		
Model	4	81.1679	20.2919	0.63	0.6473
Error	13	416.2210	32.0170		
Diet	2	29.8819	14.9409	0.47	0.6372
Period	2	51.2860	25.6430	0.80	0.4699
Nitrogen Retained as a Percent of Absorbed					
Total	17	2.3553	0.1385		
Model	4	1.2285	0.3071	3.54	0.0364
Error	13	1.1267	0.0866		
Diet	2	0.6986	0.3493	4.03	0.0435
Period	2	0.5298	0.2649	3.06	0.0817

APPENDIX 15. ANOVA FOR NITROGEN BALANCE SCALED TO BODY WEIGHT, WITH UREA NITROGEN SUBTRACTED FROM NITROGEN INTAKE IN DIET C

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Intake					
Total	17	64192.6725	3776.0395		
Model	4	28314.3102	7078.5775	2.56	0.0881
Error	13	35878.3623	2759.8740		
Diet	2	25081.0165	12540.5083	4.54	0.0319
Period	2	3233.2937	1616.6468	0.59	0.5707
Fecal Nitrogen					
Total	17	32648.1567	1920.4798		
Model	4	4725.2814	1181.3203	0.55	0.7025
Error	13	27922.8752	2147.9134		
Diet	2	2184.8814	1092.4407	0.51	0.6128
Period	2	2540.3999	1270.1999	0.59	0.5678
Urine Nitrogen					
Total	17	96.4204	5.6717		
Model	4	53.2808	13.3202	4.01	0.0246
Error	13	43.1396	3.3184		
Diet	2	44.4374	22.2187	6.70	0.0100
Period	2	8.8434	4.4217	1.33	0.2976
Nitrogen Absorbed					
Total	17	94906.8017	5582.7530		
Model	4	40992.7913	10248.1978	2.47	0.0964
Error	13	53914.0103	4147.2316		
Diet	2	33410.1913	16705.0956	4.03	0.0435
Period	2	7582.6000	3791.3000	0.91	0.4251
Nitrogen Absorbed as a Percent of Intake					
Total	17	656.6914	38.6289		
Model	4	177.5586	44.3896	1.20	0.3553
Error	13	479.1328	36.8563		
Diet	2	103.9752	51.9876	1.41	0.2790
Period	2	73.5834	36.7917	1.00	0.3951
Nitrogen Retained					
Total	17	95233.7002	5601.9823		
Model	4	42996.2905	10749.0726	2.68	0.0794
Error	13	52237.4097	4018.2623		
Diet	2	35304.1647	17652.0823	4.39	0.0349
Period	2	7692.1257	3846.0629	0.96	0.4095

APPENDIX 15. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Retained as a Percent of Intake					
Total	17	676.3701	39.7864		
Model	4	208.5576	52.1394	1.45	0.2735
Error	13	467.8124	35.9855		
Diet	2	133.4677	66.7338	1.85	0.1956
Period	2	75.0898	37.5449	1.04	0.3800
Nitrogen Retained as a Percent of Absorbed					
Total	17	7.9187	0.4658		
Model	4	6.1395	1.5348	11.21	0.0004
Error	13	1.7792	0.1368		
Diet	2	5.2400	2.6200	19.14	0.0001
Period	2	0.8994	0.4497	3.29	0.0700

APPENDIX 16. ANOVA FOR NITROGEN BALANCE SCALED TO BODY WEIGHT AND ACCOUNTING FOR ENDOGENOUS LOSSES

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Intake					
Total	17	76478.9927	4498.7642		
Model	4	39392.3111	9848.0777	3.45	0.0394
Error	13	37086.6816	2852.8216		
Diet	2	35946.7233	17973.3616	6.30	0.0122
Period	2	3445.5878	1722.7939	0.60	0.5613
Fecal Nitrogen					
Total	17	32666.3255	1921.5485		
Model	4	4723.8329	1180.9582	0.55	0.7029
Error	13	27942.4925	2149.4225		
Diet	2	2184.5703	1092.2851	0.51	0.6131
Period	2	2539.2626	1269.6313	0.59	0.5682
Urine Nitrogen					
Total	17	88.4601	5.2035		
Model	4	53.4157	13.2864	4.89	0.0125
Error	13	35.3143	2.7164		
Diet	2	44.3413	22.1706	8.16	0.0051
Period	2	8.8044	4.4022	1.62	0.2353

APPENDIX 16. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Absorbed					
Total	17	89254.3525	5250.2560		
Model	4	33567.2205	8391.8051	1.96	0.1605
Error	13	55687.1320	4283.6255		
Diet	2	25989.2918	12994.6459	3.03	0.0829
Period	2	7577.9287	3788.9644	0.88	0.4364
Nitrogen Absorbed as a Percent of Intake					
Total	17	500.6515	29.4500		
Model	4	75.9995	18.9998	0.58	0.6813
Error	13	424.6519	32.6655		
Diet	2	24.7318	12.3659	0.38	0.6922
Period	2	51.2676	25.6338	0.78	0.4767
Nitrogen Retained					
Total	17	86429.1894	5084.0699		
Model	4	32291.5065	8072.8766	1.94	0.1639
Error	13	54137.6829	4164.4371		
Diet	2	24583.8697	12291.9349	2.95	0.0877
Period	2	7707.6369	3853.8184	0.93	0.4210
Nitrogen Retained as a Percent of Intake					
Total	17	491.7019	28.9236		
Model	4	79.6458	19.9114	0.63	0.6509
Error	13	412.0561	31.6966		
Diet	2	30.3412	15.1706	0.48	0.6301
Period	2	49.3046	24.6523	0.78	0.4797
Nitrogen Retained as a Percent of Absorbed					
Total	17	3.6378	0.2139		
Model	4	1.9656	0.4914	3.82	0.0289
Error	13	1.6721	0.1286		
Diet	2	1.7424	0.8712	6.77	0.0097
Period	2	0.2232	0.1116	0.87	0.4428

APPENDIX 17. ANOVA FOR NITROGEN BALANCE SCALED TO BODY WEIGHT AND ACCOUNTING FOR ENDOGENOUS LOSSES, WITH UREA NITROGEN SUBTRACTED FROM NITROGEN INTAKE IN DIET C

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Intake					
Total	17	64192.6725	3776.0395		
Model	4	28314.3102	7078.5775	2.56	0.0881
Error	13	35878.3623	2759.8740		
Diet	2	25081.0165	12540.5083	4.54	0.0319
Period	2	3233.2937	1616.6468	0.59	0.5707
Fecal Nitrogen					
Total	17	32666.3255	1921.5485		
Model	4	4723.8329	1180.9582	0.55	0.7029
Error	13	27942.4925	2149.4225		
Diet	2	2184.5703	1092.2851	0.51	0.6131
Period	2	2539.2626	1269.6313	0.59	0.5682
Urine Nitrogen					
Total	17	88.4601	5.2035		
Model	4	53.4157	13.2864	4.89	0.0125
Error	13	35.3143	2.7164		
Diet	2	44.3413	22.1706	8.16	0.0051
Period	2	8.8044	4.4022	1.62	0.2353
Nitrogen Absorbed					
Total	17	94985.0758	5587.3574		
Model	4	40992.8027	10248.2007	2.47	0.0968
Error	13	53992.2731	4153.2517		
Diet	2	33410.2024	16705.1012	4.02	0.0437
Period	2	7582.6002	3791.3001	0.91	0.4256
Nitrogen Absorbed as a Percent of Intake					
Total	17	647.7368	38.1021		
Model	4	170.4579	42.6144	1.16	0.3724
Error	13	477.2789	36.7137		
Diet	2	98.3450	49.1725	1.34	0.2959
Period	2	72.1128	36.0564	0.98	0.4007
Nitrogen Retained					
Total	17	95495.0236	5617.3543		
Model	4	42996.3528	10749.0882	2.66	0.0804
Error	13	52498.0236	4038.3592		
Diet	2	35304.6998	17652.3499	4.37	0.0353
Period	2	7691.6530	3845.8265	0.95	0.4112

APPENDIX 17. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Nitrogen Retained as a Percent of Intake					
Total	17	643.4300	37.8488		
Model	4	181.3416	45.3354	1.28	0.3293
Error	13	462.0884	35.5452		
Diet	2	111.8747	55.9373	1.57	0.2443
Period	2	69.4669	34.7334	0.98	0.4024
Nitrogen Retained as a Percent of Absorbed					
Total	17	3.0420	0.1789		
Model	4	0.8914	0.2228	1.35	0.3049
Error	13	2.1505	0.1654		
Diet	2	0.5627	0.2813	1.70	0.2207
Period	2	0.3287	0.1643	0.99	0.3967

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