# Growth of Thoroughbreds fed Different Levels of Protein and Supplemented with Lysine and Threonine

by

William Burton Staniar

Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Animal and Poultry Science

APPROVED BY:

David S. Kronfeld, Chairmen

Joseph H. Herbein

Larry A. Lawrence

November, 1998

Blacksburg, Virginia

# Growth of Thoroughbreds fed Different Levels of Protein and Supplemented with Lysine and Threonine

by

William Burton Staniar

David S. Kronfeld, Chairman

Department of Animal and Poultry Science

## (ABSTRACT)

Currently accepted optimum protein levels for growth in the horse may be reduced with amino acid supplementation. This study investigated the effects on growth and protein status of Thoroughbred foals offered a supplement with a typical CP level to a supplement that had a lower CP level fortified with limiting amino acids. The control supplement (CS) contained 14% CP, 3.0 Mcal/kg DM, 10% corn oil, 22% soybean meal, 1.4% calcium, and three sources of fiber. The experimental supplement (LTS) contained 9% CP, 3.0 Mcal/kg DM, 10% corn oil, 3% soybean meal, 1.4% calcium, three sources of fiber, and was fortified with .6% lysine and .4% threonine. Lysine and threonine are the first two limiting amino acids in common diets of the horse. Mares and foals were fed twice daily (0700 and 1400) and kept on 30-acre pastures (mixed grass/white clover) until weaning (7 months). Weanlings continued on specified supplements and pastures for seven additional months. Physical measurements and blood samples were taken monthly. Measurements included weight, average daily gain (ADG), body condition (BC), wither height, hip height, length, girth, forearm length, front and hind cannon length, physis and fetlock circumference. Blood analysis included total protein (TP), albumin (ALB), creatinine (CREA), and plasma urea nitrogen (PUN). Effect of diet and time were evaluated by analysis of variance with repeated measures. No differences were found in physical measurements between the CS and LTS groups (ADG 0.8±0.4 kg/day, BC 4.9±0.05) for the observational period. Blood data also showed no difference for the period (ALB 2.9  $\pm$  0.03 g/dl, TP 5.7  $\pm$  0.10 g/dl, CREA 1.1  $\pm$  0.02 mg/dl). These results Abstract

suggest that the foals offered the LTS faired as well or better than foals on the CS. Lower levels of crude protein (CP) in the diet will result in less nitrogen pollution of pasture lands due to better utilization in the horse. By fortifying a low protein diet with the first two limiting amino acids, the protein is used more efficiently for growth and development, thereby benefiting both the horse and the land.

(Key Words: crude protein, amino acids, lysine, threonine, growth, horse)

## Acknowledgments

First, I would like to thank my mother and brother, Dale and Mac Staniar. For being the close family that we have always been, for trips to Middleburg, when I was too busy to leave, and finally for their support of my continued fascination with my own education.

Next, I would like to thank Dr. David Kronfeld. He has not only become a most valuable reference for any bit of information that I seek, but also a good friend and consult. He has urged the expansion of my views of science and the world and has been patient with my somewhat unfocused way of doing so.

I am very grateful to Dr. Larry Lawrence for his monthly excursions to Middleburg for the purpose of helping me to X-ray 24 unruly foals in tight quarters. His knowledge of proper bone development, and close ties to the present equine community have been very helpful.

I would like to thank Dr. Joe Herbein for his patience with two years of unscheduled visits to his office whenever I managed to get down from Middleburg. His realism made for an excellent contrast with ideas of Dr. Kronfeld and allowed my work to be well balanced. Classes that I had with Dr. Herbein were invaluable in the development of my knowledge of nutrition.

I would like to thank Dr. Webb for the use of his lab and amino acid analysis equipment. Thanks also to Kris Lee for running the amino acid analysis and providing me with every bit of help and information I could have asked for.

My thanks to Louisa Gay, our laboratory technician, for the time she spent teaching me to use the centrifichem, only to find out that she would have to run all the samples again on the Beckman. For all the times e-mails arrived from Middleburg asking her to run one errand or another, and she got them done even though work in Blacksburg had already filled her schedule.

Special thanks to Dr. Judy Wilson for her help in getting this project started. She took time then, and still does to help me work out the little glitches that appeared, and she was always a source for a good laugh.

#### Acknowledgements

Thanks go out to all at the MARE Center, without whose presence my work would have been impossible. To Dr. Wendell Cooper, who has further developed my interest in equine reproduction. To Alvin Harmon, who has been a good neighbor and a source of entertainment in Middleburg. To Barbara Moriarty, for keeping everything running smoothly. To Bill Helsel and Scotty Gerbich, whose humor made some of those days in the barn pass much more quickly.

A very special thanks goes to my fellow graduate students; Amy Ordakowski, Tiffany McCullough, Dr. Rhonda Hoffman, Patty Thiers, Belinda Hargreaves, Janice Holland and Carey Williams. Their inestimable help during study sessions, before seminar, and running experiments on the farm has made it possible for me to finish my masters. In the off times, they have also been good friends and confidants.

## **Table of Contents**

ABSTRACT	ii
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	vi
LIST OF FIGURES	viii
LIST OF APPENDIX A TABLES AND FIGURES	X
LIST OF APPENDIX B TABLES	xi
INTRODUCTION	1
REVIEW OF LITERATURE	3
Amino Acids	3
Proteins	4
Food Sources	5
Protein Digestion and Absorption	6
Protein Metabolism	8
Protein Status	9
Protein Requirements	10
Cattle	12
Swine	13
Horses	14
Equine Growth	16
OBJECTIVES	19
MATERIALS AND METHODS	20
Supplements	20
Care of animals	21
Blood collection	22
Table of Contents	vi

Physical Measurements	22
Statistical Analysis	23
RESULTS	24
Physical Measurements	24
Blood Analysis	25
DISCUSSION	27
IMPLICATIONS	32
LITERATURE CITED	33
FIGURES	38
APPENDIX A TABLES	56
APPENDIX B TABLES	64
VITA	98

# **List of Figures**

Figure	1. Body weight (mean ± SE) of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine
Figure	2. Daily gain (mean ± SE) of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine
Figure	3. Mean cumulative weight gain of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine. Values were calculated as a percent of initial weight
Figure	4. Relative change in average daily gain from month to month in foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine
Figure	5. Wither heights (mean ± SE) of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine
Figure	6. Hip heights (mean ± SE) of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine
Figure	7. Girth (mean ± SE) of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine
Figure	8. Body length (mean ± SE) of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine
Figure	9. Forearm lengths (mean ± SE) of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine
Figure	<ul> <li>10. Front cannon bone lengths (mean ± SE) of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine. Decreases from month to month due to observer error.</li> </ul>
Figure	<ul><li>11. Hind cannon bone lengths (mean ± SE) of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine. Decreases from month to month due to observer error</li></ul>
Figure	<ul> <li>12. Physis circumference (mean ± SE) of foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine. Decreases from month to month due to observer error.</li> </ul>

Page 1

Figure	<ul><li>13. Fetlock circumference (mean ± SE) of foals fed a 14% CP supplement or a</li><li>9% CP supplement, fortified with .6% lysine and .4% threonine. Decreases from month to month due to observer error.</li></ul>	. 50
Figure	<ul><li>14. Plasma levels of albumin (mean ± SE) in foals fed a 14% CP supplement or a</li><li>9% CP supplement, fortified with .6% lysine and .4% threonine</li></ul>	. 51
Figure	15. Plasma levels of total protein (mean ± SE) in foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine	. 52
Figure	16. Plasma levels of creatinine (mean ± SE) in foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine	. 53
Figure	17. Plasma levels of urea (mean ± SE) in foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine.	. 54
Figure	18. Urea:Creatinine (mean ± SE) in foals fed a 14% CP supplement or a 9% CP supplement, fortified with .6% lysine and .4% threonine	. 55

## List of Appendix A Tables and Figures

## Page

Appendix A table 1. Nutrient composition on a DM basis of the pasture, 14% crude protein (CS), and 9% crude protein, fortified with lysine and threonine (LT) supplements <sup>a</sup>	56
Appendix A table 2. Ingredient composition (%) of the 14% CP supplement (CS) and the 9% CP supplement, fortified with lysine and threonine (LTS)	57
Appendix A table 3. Amino acid composition (g/kg) of the pasture, 14% crude protein (CS), and 9% crude protein, fortified with lysine and threonine(LTS) supplements	58
Appendix A table 4. Amino acid composition (mg/g CP) of the pasture, 14% crude protein (CS), and 9% crude protein, fortified with lysine and threonine(LTS) supplements	59
Appendix A table 5. Amount of supplement fed to mares, foals, weanlings and yearlings through the study period	60
Appendix A table 6. Description of body measurements used to monitor growth of foals	61
Appendix A table 7. Power analysis of test to find a difference between treatments and estimation of the number of subjects per treatment group required to find a difference.	63

# List of Appendix B Tables

Appendix B table 1. Body weight (kg) of foals fed a 14% crude protein supplement	64
Appendix B table 2. Body weight (kg) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	65
Appendix B table 3. Average daily gain (kg/d) of foals fed a 14% crude protein supplement	66
Appendix B table 4. Average daily gain (kg/d) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	67
Appendix B table 5. Body condition of foals fed a 14% crude protein supplement	68
Appendix B table 6. Body condition of foals fed a 9% crude protein supplement, fortified with lysine and threonine	69
Appendix B table 7. Wither height (cm) of foals fed a 14% crude protein supplement	70
Appendix B table 8. Wither height (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	71
Appendix B table 9. Hip height (cm) of foals fed a 14% crude protein supplement	72
Appendix B table 10. Hip height (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	73
Appendix B table 11. Girth (cm) of foals fed a 14% crude protein supplement	74
Appendix B table 12. Girth (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	75
Appendix B table 13. Body length (cm) of foals fed a 14% crude protein supplement	76
Appendix B table 14. Body length (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	77
Appendix B table 15. Forearm length (cm) of foals fed a 14% crude protein supplement	nt <b>78</b>
Appendix B table 16. Forearm length (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	, <b>7</b> 9
Appendix B table 17. Front cannon length (cm) of foals fed a 14% crude protein supplement	80
Appendix B table 18. Front cannon length (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	81
List of Appendix B Tables	xi

# Page

Appendix B table 19. Hind cannon bone lengths (cm) of foals fed a 14% crude protein supplement	82
Appendix B table 20. Hind cannon bone lengths (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	83
Appendix B table 21. Physis circumference (cm) of foals fed a 14% crude protein supplement	84
Appendix B table 22. Physis circumference (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	85
Appendix B table 23. Fetlock circumference (cm) of foals fed a 14% crude protein supplement	86
Appendix B table 24. Fetlock circumference (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine	87
Appendix B table 25. Albumin level (g/dl) in plasma of foals fed a 14% crude protein supplement	88
Appendix B table 26. Albumin level (g/dl) in plasma of foals fed a 9% crude protein supplement, fortified with lysine and threonine	89
Appendix B table 27. Total protein (g/dl) levels in plasma of foals fed a 14% crude protein supplement	90
Appendix B table 28. Total protein (g/dl) levels in plasma of foals fed a 9% crude protein supplement, fortified with lysine and threonine	91
Appendix B table 29. Creatinine (mg/dl) levels in foals fed a 14% crude protein supplement	92
Appendix B table 30. Creatinine (mg/dl) levels in foals fed a 9% crude protein supplement, fortified with lysine and threonine	93
Appendix B table 31. Plasma urea nitrogen (mg/dl) levels in foals fed a 14% crude protein supplement	94
Appendix B table 32. Plasma urea nitrogen (mg/dl) levels in foals fed a 9% crude protein supplement, fortified with lysine and threonine	95
Appendix B table 33. Urea: Creatinine ratio of foals fed a 14% crude protein supplement	96
Appendix B table 34. Urea:Creatinine ratio of foals fed a 9% crude protein supplement, fortified with lysine and threonine	97

## Introduction

Dietary proteins perform a foremost role in the health and growth of animals. The variety of complex structures of proteins enables them to perform many tasks in the living organism. In order to form these structures the body is in a continuous state of protein turnover. Dietary protein is important because the body has no dispensable stores of protein. When synthesis of proteins is required, the amino acids utilized come from those absorbed from the diet, from synthesis in the body, or from the breakdown of functional proteins in the body.

Protein requirements and roles may change with growth, maintenance, pregnancy, and old age. Protein requirements for growth have been studied extensively due to the importance of proper development to the usefulness of the horse. Horses are used in such a manner that their bodies are often pushed to the limits of physical stress. Correct muscular, skeletal, pulmonary, and cardiovascular development are essential for horses whether their destiny be Churchill Downs, Badminton horse trials, rounding up cattle in Wyoming, or running through hunt fields the world over.

The protein requirement for growing horses is about 14% (Jordan and Myers, 1972; Ott et al., 1979; Schryver et al., 1987; Yoakam et al., 1978). Subsequent work has shown that the level of crude protein may be decreased through improvement in the quality of protein (Breuer and Golden, 1971; Graham et al., 1994; Ott et al., 1981; Potter and Huchton, 1975). Higher quality protein is used more efficiently because the amino acid profile supplied is similar to the profile demanded for whole body protein synthesis. Digestibility of dietary protein may also play a very important role in the choice of the protein source to be included in the diet. The majority of amino acids are absorbed as small peptides or free amino acids in the small intestine (Silk et al., 1985; Hara et al., 1984). Because the digestibility of protein in different food sources varies (Potter et al., 1992; Gibbs et al., 1996) choice of the source will affect the availability of amino acids for absorption.

Growth of Thoroughbred foals has been surveyed extensively, and typical patterns of growth and average daily gain (ADG) established (Hintz et al., 1979;

#### Introduction

Thompson 1995; Hoffman et al., 1996; Jelan et al., 1996; Saastamoinen 1996). These studies have revealed periods during the year when foals have sudden changes in growth patterns. Changes in growth rate, especially compensatory growth, have been associated with manifestations of developmental orthopedic disease in yearlings (Hintz et al., 1976).

The goal of this study was to examine the effects of diets with different amino acid compositions on the growth and protein status of foals and yearlings.

## **Review of Literature**

The word protein is derived from the Greek word *proteios* which means "of the first rank". Jöns J. Berzelius coined the word in 1838 to emphasize the importance of the nitrogenous component of the diet in growth and health (Stryer, 1995). Proteins are organic molecules consisting of chains of amino acids that have a carboxylic acid group at one end and an amino group at the other. Amino acids form proteins that function as contractile elements in muscle, enzymes that form the chemical engines in cells, antibodies and immunoglobulins, transporters in the blood, and certain hormones. Proteins make up over half the solid content of cells (Hunt and Groff, 1990), and approximately 18 percent of the body weight of animals (Swenson and Reece, 1993). They are important nutritionally because their constituent parts, amino acids, are essential in the manufacturing of new proteins and N containing molecules. Proteins are also important as sources of food energy.

## Amino Acids

The amino acid sequence of each protein specifies the function of that particular molecule. The almost infinite number of sequences allows for great variability in physical properties, and hence functions, of the protein molecule. Twenty amino acids are utilized to construct proteins. An amino acid is composed of an amino group, a carboxyl group, a hydrogen atom, and a distinctive side chain all connected to a single carbon atom. The different side chains impart certain characteristics to that particular amino acid (Stryer, 1995). Amino acids are classified by certain characteristics of their side chains. Examples of these side chain include aliphatic side chains, aromatic rings, sulfur atoms, hydroxylic groups, basic and acidic groups (Rodwell, 1993).

The same group of 20 amino acids are the building blocks for all proteins in all species. Some species, such as *Escherichia coli*, are able to synthesize all twenty amino acids; however, most mammals are unable to synthesize all the amino acids at rates fast

enough to supply the demands of protein production. This leaves a group of amino acids that are *essential*, and these must be harvested from the organism's environment.

## Proteins

Protein structure is characterized by four levels of organization. The primary structure is determined by the sequence of amino acids in accordance with a genetic code Amino acids are joined by peptide bonds into polypeptides. This covalent bonding occurs on the ribosomes within the cell. It is essential that while a protein is being synthesized all the amino acids necessary for its structure be present at once. If a limiting amino acid is missing, synthesis ceases.

The secondary structure of proteins is determined by non-covalent bonds between nearby amino acids in the polypeptide chain. Examples of these bonds include hydrogen, hydrophobic, and electrostatic bonds as well as Van der Walls forces. Secondary protein structure is characterized by a number of different conformations. An  $\alpha$ -helix is formed as a polypeptide chain coils upon itself with hydrogen bonds stabilizing the structure. The  $\alpha$ -helices provide rigidity to the portion of the molecule where they are present (Hunt and Groff, 1990). The  $\beta$ -conformation forms a sheet-like structure that is also held together by hydrogen bonds between strands of amino acids. Less ordered secondary structures include loops and coils in the polypeptide chain.

The tertiary protein structure involves the spatial relationship of secondary structural elements to each other, in other words the way the  $\alpha$ -helices,  $\beta$ -sheets, loops and coils fold onto each other. Whereas secondary structure usually involves the bonding of amino acids that are close to one another, tertiary structure often has amino acids far from each other on the polypeptide chain bonding to each other. Tertiary structure may begin to reveal some of the recognizable features of a protein, such as hydrophobic amino acids clustering towards the center of the structure, and disulfide bonds forming between cystine residues. These changes in structure are essential in determining the final function of the protein (Hunt and Groff, 1990).

The quaternary protein structure is the arrangement of separate polypeptide chains. This structure usually involves two (dimeric) or four (tetrameric) polypeptide subunits. The aggregate of these chains is called an oligomer. The spatial orientation and ability to shift this orientation allows these subunits to properly facilitate function of the protein. In certain cases, the individual subunits may perform different functions. These characteristics of proteins allow them to play important roles in intracellular regulation (Hunt and Groff, 1990, Rodwell, 1993).

## Food Sources

The function of proteins in the diet is to supply adequate amounts of needed amino acids. There are many sources of essential amino acids available and different species use different strategies to attain these. In ruminants, for example, a portion of the essential amino acids are available from dead ruminal bacteria passing into the small intestine (Richardson, 1978). Non-ruminants must rely more on the amino acid composition in feeds and forages. Amino acid composition is different from source to source. Non-ruminants must obtain the proper profile of amino acids to construct the proteins necessary for its survival. Limiting amino acids are those that are not present in sufficient quantity for production of proteins, hence they are "limiting" to the construction of those molecules (Friedman, 1996).

Proteins are available from either animal or vegetable ingredients. Some examples of these ingredients are cereals, legumes, other seeds, meat, seafood, insects, and animal by-products (Friedman, 1996). Diets containing combinations of protein sources may have different limiting amino acids due to various amino acid percentages. Soybean meal (SBM), linseed meal, cottonseed meal, brewer's dried grains, milk byproducts, and fishmeal are all examples protein sources that are commonly used for feeding livestock (Hintz et al., 1971; Ott et al., 1979; Godbee and Slade, 1981; O'Mara et al., 1997). These are used as protein sources because their composition of amino acids is closer to that required for whole body protein synthesis (Friedman, 1996; O'Mara et al., 1997). Corn may be a poor source of protein due to both the lower digestibility of the

protein composition (O'Mara et al., 1997) and its lower content of lysine in comparison with other sources.

## Protein Digestion and Absorption

Breakdown of dietary protein begins with the action of pepsin in the stomach. Pepsin and hydrochloric acid denature and break proteins down into large polypeptides. Pepsin's activity ceases once the stomach's contents enter the small intestine. Digestion of protein continues in the small intestine with a group of pancreatic proteolytic enzymes. These exopeptidases leave a mixture of amino acids and small peptides having a chain length of two to six amino acid residues. This mixture continues to move through the small intestine where further breakdown by brush border enzymes, luminal enzymes, and cytoplasmic enzymes occurs. Transporters on the brush border membrane then actively absorb a variety of amino acids, dipeptides, and tripeptides. These transporters appear to be specific for certain groups of free amino acids, and small peptides. There is competition for absorption within each of these groups. For example, each amino acid does not have a separate transporter, instead certain transporters are responsible for groups of amino acids. This results in competition for sites between amino acids in each group. Further breakdown of peptides may occur in the cell cytoplasm. Amino acids and peptides that remain are then transported across the lumen and into circulation (Silk et al., 1985).

Amino acids are digested in various sites through the equine gastrointestinal tract and absorbed in several different forms. Horses with ileal cannulas were fed hay and grain diets over a period of 14 - 21 days (Potter et al., 1992). Sampling of ileal and fecal samples showed that  $70 \pm 6.9\%$  of the digestible protein was digested in the small intestine. The CP in diets containing SBM or cottonseed had a true digestibility of near 100% in the small intestine. Gibbs et al. (1996) examined the N digestibility of different concentrates and hay in the small and large intestine of ponies fitted with ileal cannulas and offered three diets; corn and hay, oats and hay, sorghum and hay. Nitrogen digestibilities were then determined for the grain portion of the diet in the small and large

intestine. The apparent prececal N digestibility of the hay was 9.42% and 52.5% postileal. Prececal digestion of N was greater than postileal for the grain portion of the diet ( $62.0 \pm 5.86\%$  vs  $38.0 \pm 5.86\%$ ). In a second trial the N digestibility of SBM and cottonseed meal were determined by the same methods. Three-quarters of the N digested from the supplements occurred in the small intestine, with only a quarter being digested in the large intestine  $(74.09 \pm 2.93\%, 25.91 \pm 2.93\%)$ . There is a large difference in the digestion that occurs in the foregut and hindgut. The absorption of crude protein from the diet occurs throughout the digestive tract, but the majority is digested in the small intestine. Only 1-12% of absorbed amino acids come from absorption in the hindgut (Lewis 1995, Frape 1998).

Absorption of free amino acids and small peptides was compared in rats fed a diet of hydrolysate of egg white protein (70% small peptides) and a corresponding mixture of free amino acids (100% free amino acids) (Hara et al., 1984). Samples of portal blood were analyzed for amino acid composition to get a measure of amino acid absorptive intensity. Absorptive intensity was 70-80% higher for the mixture of small peptides than the free amino acids. These results suggested that absorption of small peptides played an important role in overall protein absorption.

In the ruminant, dietary protein is exposed to digestion by ruminal microflora prior to entering the small intestine, the major site of protein absorption. This results in a portion of the dietary protein being absorbed and utilized by the rumen microflora. The protein that is then available for digestion and absorption in the small intestine is a combination of protein escaping degradation in the rumen and microbial protein. This is called the metabolizable protein (Wilkerson et al., 1993). The amino acid composition of this mixture may be very different from that of the original dietary protein (Purser, 1970). The amino acid composition of several protein sources after a 12-h rumen incubation, and then passage through the small intestine of lactating Friesian cows revealed that the portion of amino acids that disappeared in the rumen was different for each source (fishmeal, 31%; SBM, 62.2%; corn distillers grain, 16.6%; SEM = 3.6%) (O'Mara et al., 1997). The percentage of total amino acids that disappeared from the small intestine was also different for each source (fishmeal, 97.2%; SBM, 95.5%; corn distillers grain, **Review of Literature** 7

83.9%; SEM = 1.8%). Messman and coworkers (1996) examined the disappearance of protein from legume forages, differing in tannin content, after passage through the rumen. Forages with a higher tannin content had reduced degradability of protein.

## Protein Metabolism

Protein metabolism includes protein turnover and N balance. Protein turnover is comprised of protein synthesis and degradation, and within this growth of the animal. Nitrogen balance includes protein and non-protein nitrogen (NPN) intake as well as output (Millward et al., 1994). The body maintains a pool of amino acids in the blood that is used to meet different requirements of protein metabolism. These amino acids are used as the building blocks of proteins, sources of NPN, and sources of energy through oxidation.

Protein synthesis constructs tissue and plasma proteins, protein hormones, and protein secretions. Smaller peptides, such as neuropeptides and oxytocin, are included in the synthesis process. Some NPN molecules that are constructed from amino acids are niacin, pyrimidines and creatine. Oxidation of amino acids for energy begins with the removal of the amino group. The remaining carbon skeleton is then used to generate ATP through its entrance into the mitochondrial electron transport chain. If the amino group is not used to synthesize other amino acids, it is excreted as urea (Hunt and Groff, 1990).

The liver plays a large role in the regulation of protein homeostasis. Amino acids that enter the liver are separated into the following categories; 20% for protein synthesis, 23% free amino acids released back to circulation, and 57% are catabolized (Hunt and Groff, 1990). The regulation of protein metabolism appears to involve complex interactions between insulin, growth hormone, glucocorticoids, and dietary protein. Evidence suggests insulin has some control over both protein synthesis and breakdown. The effect of heightened insulin levels in fasted animals is first a decrease in protein degradation followed by an increase in RNA activity (Lobley, 1992). Elevated levels of amino acids in the blood increased sensitivity of muscle protein synthesis to insulin.

Growth hormone acts synergistically with dietary protein to increase protein synthesis (Seve and Ponter, 1997). An examination of protein turnover in underfed steers demonstrated a reduction in protein synthesis and an increase in mobilization of proteins from muscle (Boisclair et al., 1993). Hormonal control of protein turnover is complex and tightly linked to dietary status of the animal.

#### **Protein Status**

The body has no dispensable protein stores. When protein is lost from the system, it is taken from essential structural or functional elements. It is therefore important to be able to examine the effects of diet on protein status. In order to evaluate protein status the products of protein metabolism are examined. Examples of those that are most often used are Plasma urea nitrogen (PUN), creatinine (CREA), total protein (TP), albumin (ALB), N balance, 3-methylhistidine and plasma amino acid levels (PAA) (Gibson, 1990).

Total protein has been used as an indicator of protein status in several studies (Godbee and Slade, 1981; Greppi et al., 1996; Saastamoinen, 1996). Levels of TP increase with age in horses. Levels changed from weanling to yearling, and from yearling to 2-year-old showed significant differences (5.72 vs 6.41 vs 6.70 g/100 ml, respectively, P < .05) (Godbee and Slade, 1981). Other studies have found contradictory results, and indicated that TP levels to remain relatively constant through growth (Saastamoinen, 1996). Levels of PAA and more specifically the ratio of nonessential (NEAA) to essential amino acids (EAA) are used to evaluate nutritional health; as an indicator of the amino acid profile being absorbed from the diet; and as an indicator of protein metabolism (Cabrera et al., 1992; Zicker et al., 1994). Horses that were fed six meals a day had constant levels of PAA, whereas those that were fed one large meal had higher levels of PAA five hours post-prandial (Russell et al., 1986). Plasma free amino acids may be used to evaluate availability and absorption, as well as the requirements of amino acids.

Plasma urea nitrogen is a by-product of amino acid catabolism. Urea levels decrease as the biological value of dietary protein increases (Eggum, 1970). A diet that has a composition of amino acids that is closer to that needed will leave fewer amino acids to be catabolized, hence lower urea levels. Other factors that may affect PUN concentrations are levels of energy intake and conditions that change protein metabolism (e.g. pregnancy, lactation, growth, and physical stress) (Gibson, 1990).

Analysis of PUN was used as a method to determine optimal levels of lysine and threonine in pigs fed a low protein diet or one supplemented with lysine and threonine (Malmlof and Askbrant, 1988). Supplementation brought about a 29% decrease in mean PUN (P < .05). Plasma urea nitrogen has also been examined as a test for lysine requirements thought to change through growth in pigs (Coma et al., 1995). Changes in PUN allowed determination of lysine requirements in pigs at different stages of growth. Plasma urea nitrogen levels increased 24% in weanling horses from seven to twelve months of age (Saastamoinen, 1996). These results indicate a decrease in protein requirement with the aging of the foals. Levels of PUN decreased in two-year-old horses fed SBM in comparison to those fed urea as a source of N (Godbee and Slade, 1981). Plasma urea levels act as an indicator of how the horse is utilizing the protein being presented in the diet. Higher plasma urea-nitrogen levels have been found in horses fed 20% CP compared to those fed 14% CP (Schryver et al. 1987).

#### Protein Requirements

Protein requirements define animals' necessity for a level that will maintain life. This can also be seen as the minimum level of the nutrient that needs to be supplied. Contemporary work on protein requirements has looked to define adequate or optimal levels with more attention paid to the individual systems in an attempt to get optimum as opposed to minimum performance (Kronfeld, 1982; Wang and Fuller, 1989; Hahn and Baker, 1995, Zello et al., 1995).

Before protein becomes limiting to growth energy requirements must be met. ADG is primarily influenced by the level of digestible energy intake (Ott and Asquith,

1986). Ott and Asquith investigated the effects of feeding Thoroughbred and Quarter horse yearlings diets with different levels of digestible energy (DE), and CP. Yearlings allowed *ad libitum* access to feed showed maximum growth on a 13.5% CP diet with a DE intake of 22.1 Mcal/d. When levels of CP were increased to 16.8% in these yearlings while restricting DE intake to 17.3 Mcal/d maximum growth was not attained. Hence, energy requirements must be met before protein requirements become limiting for growth.

Study of protein requirements in livestock other than horses strives to find levels of protein and amino acids for optimum development. Research in cattle and swine has gone so far as to define levels of lysine, threonine, tryptophan, methionine, and sulfur amino acids that provide for maximum growth (Fenderson and Bergen, 1975; Hahn and Baker, 1995). Because levels of the first limiting amino acids have been defined in these species, the concept of ideal protein is used to determine suitable levels of other amino acids. The ideal protein requirement produces a ratio of each amino acid to the first limiting amino acid. Wang and Fuller (1989) further define an ideal protein as, "one which includes the minimum quantity of essential amino acid compatible with maximum utilization of the protein as a whole."

The quality of the dietary protein is closely linked to the concept of ideal protein and utilization of the protein as a whole. Efficiency of the digestion, absorption, and utilization of the amino acids is representative of the protein's quality. A number of different measures of protein quality have been developed (Oser, 1959; Friedman, 1996).

- Amino acid score: (mg of amino acid in 1 g of a test protein)/(mg of amino acid in a reference protein)
- Plasma amino acid ratio: the relative changes in concentration of each of the free EAA in plasma after consumption of a protein food, expressed as a function of the specific amino acid requirements of an animal
- Protein efficiency ratio: (weight gain of a test group)/(total protein consumed)
- Essential amino acid index: the geometric mean of the ratios of the EAA in a protein relative to corresponding values in a reference protein

Reference proteins that are used are whole egg protein or casein. These proteins are used in protein synthesis with high efficiencies and further supplementation with any amino acid did not improve the biological value (Kronfeld, 1982). A low quality dietary protein can be adequate if sufficient quantities are consumed to meet amino acid needs; however, much of the amino acid content will be catabolized due to it inappropriate profile.

#### Cattle

In beef cattle, protein requirements are based around the ADG, and muscle accretion of the animal, as these are important to the final product. Protein turnover is the rate at which protein is continuously synthesized and degraded, and is a useful variable to examine when trying to improve weight and muscle gain (Lobley 1992, Wessels et al., 1997). To examine the effects of amino acid supplementation on protein turnover in Holstein steers, amino acids were labeled with an isotope and then infused into the abomasum with other amino acids (Wessels et al., 1997). Supplementation with amino acids increased protein turnover from  $168.6 \pm 11.4$  to  $183.2 \pm 11.4$  g N/d. Labeling of amino acids with isotopes allowed protein turnover to be calculated by monitoring the amount of N retained and excreted from cattle over time.

In dairy cattle, the protein requirements are centered on quality and quantity of milk production. Protein content in milk is important for production of cheeses and other dairy products. Rumen protected amino acids allow a producer to supplement their animals so that the amino acid profile reaching the small intestine for absorption may be more appropriate for the animals' needs. An increase in amino acid flow to the small intestine can result in several benefits. Increases in milk production, and content and yield of milk protein result from improvement of the profile of absorbed amino acids, as well as total amino acid supply (Schwab, 1996). The profile of amino acids in digestible ruminally undegraded protein (RUP) is generally not adequate to maximize use of metabolizable protein for protein synthesis. There are a number of approaches that have been used to increase the amino acid flow to the intestines (Chase, 1996). These include;

maximizing dry matter intake, increasing the amount of microbial protein synthesis, altering the degradability of protein sources by chemical or heat treatments, selecting protein sources to control ruminal protein degradation, selecting protein sources which enhance the conversion of crude protein to absorbable protein, feeding high RUP sources, and the use of ruminally protected amino acids.

#### Swine

Dietary protein is important in swine because of its affect on the muscle to fat ratio. Consumers desire leaner cuts of meat; therefore, the producer wishes to feed swine a diet that will facilitate this. Kropf and coworkers (1959) fed three diets with varying levels of CP and protein quality (16% CP high quality, 16% CP low quality, 12% CP high quality). Both high quality protein diets contained soybean oil meal, dried skim milk, and brewers' yeast. The low quality diet contained corn gluten meal and peanut meal. They found no difference between the high quality protein diets except that those swine slaughtered at earlier stages of growth on the 12% CP had less carcass muscling than those eating the 16% CP diet. These differences were no longer significant at later stages of growth. The group receiving the low quality protein had significantly lower ADGs (P < .01) than either of the high quality protein groups. This experiment points to an important role of protein quality in the growth of swine and raises questions as to the appropriate level of CP for growth.

Research in swine has progressed to the point that an optimum balance between all EAA has been proposed (Wang and Fuller, 1989). The levels are expressed as ratios of each EAA to lysine. Optimum ratios of threonine, tryptophan, and sulfur amino acids to lysine have been tested for appropriate levels at different stages of growth. Barrows and gilts in early (EF = 56 to 90 kg) and late (LF = 90 to 112 kg) finishing periods were fed diets with low and high ratios of essential amino acids (EAA) to lysine (Hahn and Baker, 1995). Swine that were fed diets with an increased EAA:Lys ratio had improved gain:feed ratio (P < .05), whole-body and carcass protein concentration (P < .10), and whole-body and carcass protein accretion (P < .08), as well as decreased urinary N excretion and increased N retention. Results of this study show that patterns of amino acids that may be appropriate at one stage of growth may change at other stages. With **Review of Literature** 13 continued research, exact requirements of amino acids at different stages of growth may be realized.

#### Horses

While the horse is not often perceived as a production animal, growth is still an important factor in the horse properly fulfilling its performance role. The horse is unable to produce approximately ten amino acids so these must be a part of the horse's daily intake (Frape, 1998). Amino acid composition of the diet is very important for the early stages of the foals' growth, and becomes less important as growth begins to slow (Godbee and Slade, 1981; Saastamoinen 1996).

In determining optimum protein levels in the horse, researchers have examined the growth of foals and yearlings on diets differing in crude protein (CP) content as well as varying amino acid profiles. Jordon and Myers (1972) fed pony foals 11, 13, and 15% CP diets over a period of 17 months. Growth of foals was monitored by comparing weights, heights, and ADG's of the groups. Foals fed the 15% protein diet had a 32% (P < .05) higher rate of gain than foals fed the 11% protein diet. When the foals were 8 months of age, no difference was seen in the ADG between these two groups. Schryver and coworkers (1987) examined growth and calcium metabolism in weanlings fed varying levels of protein. Horses that were fed a 9% CP diet had significantly lower ADG than horses on either 14, or 20% CP (.06 kg vs .63 and .69 kg respectively, P <.01). Because there was no significant difference in ADG between the 14 and 20% CP diets, 14% was adequate for the weanlings' growth. Pony weanlings fed 11, 14, or 17 % CP had ADGs of .22 kg, .44 kg, and .40 kg. No significant difference was seen between the 14 and 17% CP (Yoakam et al., 1978). Pulse and coworkers (1973) found a significant linear response between CP levels of 10, 13, 16, and 19% and the response in ADG, .35, .42, .56 and .57 kg. (P < .01) in immature horses.

The quality of protein in the diet is closely tied to the profile of amino acids present. While the quality of protein may be less important for older horses, it appears to be important for growth in younger animals. Horses at 12 months of age may have attained 65% of their adult weight and 90% of their adult height (Saastamoinen, 1996). Weanlings, yearlings and two-year-old horses were fed low protein diets supplemented **Review of Literature** 14 with SBM or urea. The weanlings supplemented with the SBM had greater ADG's than those supplemented with the urea (0.64 vs. 0.29kg); however much lesser differences were seen between the yearlings and two year olds (0.40 vs. 0.24kg, 0.40 vs. 0.28kg respectively) (Godbee and Slade, 1981). These results show that horses are more sensitive to the composition of amino acids in the diet during early growth.

There are a number of different sources of CP that can be added to the horses diet. Hintz and coworkers (1971) compared diets containing linseed meal (LSM) or milk byproducts (MP). While the level of CP remained the same between these two diets the level of lysine differed due to the amino acid profile of the LSM and MP. The LSM diet contained 0.3 to 0.4% lysine, while the 20% MP diets contained 0.65 to 0.7% lysine. ADG for the LSM fed horses was 0.60 kg/d, whereas the MP grew at a rate of 0.95 kg/d (P < .05). SBM was compared to Brewers' dried grains (BDG) as a protein source for growth. ADG for horses fed SBM was 0.53 kg/d, while those fed BDG had an ADG of 0.44 kg/d (P < .05) (Ott et al., 1979). Each of these is an example of level of protein being the same, while the source is different and the effects on growth are different. Difference in protein quality between these sources is the reason for differences in growth variables. In each of these experiments, secondary trials were done examining supplementation of lower quality protein with lysine. In each trial an increase in growth to the level of those of the high quality protein was seen (Hintz et al., 1971, Ott et al., 1979). The significant change in growth due to supplementation with lysine indicate this amino acid as playing an important role in the quality of protein fed to growing horses.

In a study investigating the role of lysine in protein quality, Breuer and coworkers (1971) fed six rations containing levels of lysine from 0.25 to 0.70% to twelve Quarter horse weanlings. ADGs and feed efficiency in the horses were found to be significantly effected by the level of lysine in the diet. ADG increased from 371g/d at the lowest level of lysine to 730 g/d at 0.6% lysine (P < .01). There was no improvement in ADG above 0.65% lysine. Ott and coworkers (1981) examined the effects of diets with similar CP levels, but different lysine levels and concluded that 0.65% lysine was appropriate for growth. In a follow up study they investigated the outcome of supplementing a 14% CP diet with 0.15% lysine (total lysine 0.64%) in comparison with a 16% CP (total lysine **Review of Literature** 

0.67%) diet (Ott et al., 1981). The results showed that ADG was 0.72 kg/d for both diets. Comparisons of diets containing 0.65% lysine and 0.49% lysine showed a slight difference in ADG (0.56 vs. 0.43kg/d respectively) (Potter and Huchton, 1975). Supplementation with small amounts of lysine allowed for improvement of the amino acid profile while decreasing the amount of protein needed for optimum growth.

Graham and coworkers (1994) investigated the second limiting amino acid for equine growth. Thirty-nine yearlings were split into three groups and fed one of three diets. The lysine and threonine contents of the 12% CP diets were: diet A contained 0.52% lysine and 0.43% threonine; diet B contained diet A plus 0.2% lysine; and diet C contained diet B plus 0.1% threonine. Average daily gains increased from diet A to diet C (0.57 ± 0.02, 0.64 ± 0.02, 0.67 ± 0.02 kg/d, respectively P < 0.02). There was also a significant increase in girth gains from diet A to C (9.7 ± 0.49, 10.1 ± 0.46, 11.3 ± 0.47 cm, respectively P < 0.05). This data showed threonine as the possible second limiting amino acid to growth in the horse.

## Equine Growth

Growth rates of Thoroughbreds have received close attention due to the importance of optimum growth and proper muscular and skeletal development. These factors play a large role in a horse's future performance ability. With a better understanding of normal growth, problems that occur with abnormal growth could be more quickly addressed.

In a review of several growth studies the greatest increases in growth occurred during the first month of life (Green, 1961). It was also pointed out that wither height may be a better indicator of growth than live-weight gain due to the variability in the latter. Growth rates of thoroughbreds in particular have been examined in a number of different surveys (Hintz et al., 1979, Jelan et al., 1996, Pagan et al., 1996). Jelan and coworkers (1996) examined growth data from 798 thoroughbred foals from Ireland. They split the growth of the foals into four phases; birth to 1 month, 1 to 12 months, 12 to 15 months, and 15 to 20 months. These were seen as characteristically different periods

of growth. During the first phase ADG was 1.6 kg/d, and then dropped to between 0.5 and 1.0 kg/d during the second period. Average daily gain increased during the third phase to 0.7 kg/d, and again decreased during the fourth phase to 0.2 kg/d. By the time foals were 12 months of age they had attained 76% of the weight and 94.5% of the wither height they would have at the start of training in month 20.

Over a period of three years, a group of 350 thoroughbred foals was surveyed in Kentucky to examine the growth patterns in this region (Pagan et al., 1996). Average daily gain of foals during their first month was 1.5-1.7 kg/d. Slowest growth was seen in these foals during the months of January, February, and March. In May an increase in ADG to 0.8 kg/d occurred, probably due to spring pasture growth.

In follow up to this pervious study, Pagan and coworkers (1996) compared the weight and height data from the foals that had been surveyed in Kentucky to data from foals raised in Canada. Foals in Kentucky were 102.0% the weight of foals in Canada at 14 days of age and 107.2% the weight of Canadian foals at 6 months. The sixth month corresponds to August. By February and March, the weights of the two groups were much closer. Some of the reason for the difference in August and the lack of difference in February and March may be due to the large contrast in seasons between Kentucky and Canada.

In two studies of growth in thoroughbreds Green (1969, 1976) found no differences in the growth rate of colts compared to fillies. In both studies, growth was measured by changes in height, girth, and circumference of the metacarpal bones just below the knee. Wither height in the initial study increased in colts and fillies 16.16 and 16.00 in respectively. Results from this study also showed no difference in the birth weights of foals born in February through May. Average wither heights, girths and bone circumference for foals in the second study at 14 months of age was 144, 160, and 18.5 cm respectively. Again, no differences were found between colts and fillies.

Further definition of skeletal growth rates was undertaken on 106 foals from 14 to 588 days of age (Thompson, 1995). Variables measured were body weight, wither height, hip height, body length, knee to pastern length, width of chest and depth of girth. Average weights of colts and fillies at 392 days of age were  $388.1 \pm 29.8$  kg and  $383.0 \pm$ **Review of Literature** 17 29.7 kg. Wither height and hip height had similar growth patterns; however, hip height tended to be 2 to 3 cm greater through the study. These results further establish a normal pattern of growth for Thoroughbred foals.

## Objectives

The general objective of this study was to examine growth and protein status in Thoroughbred foals, weanlings and yearlings offered pasture supplements with different protein levels and amino acid compositions. The primary goal of this study was to determine if a 9% crude protein supplement fortified with lysine and threonine would produce similar growth to an unfortified 14% crude protein supplement. The dietary effects were examined over an entire year to determine characteristic growth patterns. Finally, this study continues work towards development of a diet beneficial to both the horse and the environment.

## **Materials and Methods**

Protein and amino acid supplementation of pasture was studied on the growth of 22 foals from birth to 18 months of age at the Virginia Tech Middleburg Agricultural Research and Extension Center. Dams were paired by age, breeding date and sire, and then assigned randomly to two groups. Mares were maintained on the supplements from May of 1997 until time of weaning, at which point the foals were continued on the diets until July of 1998. The institution's animal care and research committee approved the experimental protocol.

### *Supplements*

This study compared a diet having a typical CP level with a diet that had a lower CP level fortified with limiting amino acids. The control supplement (CS) contained 14% CP, 3.0 Mcal/kg DM, 10% corn oil, 22% soybean meal, 1.4% calcium, and three sources of fiber. The experimental supplement (LTS) contained 9% CP, 3.0 Mcal/kg DM, 10 % corn oil, 3% soybean meal, 1.4% calcium, three sources of fiber, and was fortified with 0.6% L-lysine and 0.4% L-threonine. Supplements were designed to be isoenergetic, with mineral contents balanced to complement the pastures and meet or exceed current recommendations (NRC 1989; Kronfeld et al., 1996). Both groups were offered the supplements 7 to 10 days post partum and maintained on mixed grass/white clover pasture. Groups were rotated between pastures every 28 days to negate any pasture effect. Nutrient composition (Dairy One, Ithaca, NY) and make-up of the supplements and pasture are shown in Appendix A table 1 and 2. Pasture and supplements were analyzed for amino acid content (Bidlingmeyer et al., 1984) using a Pico•Tag Amino Acid Analysis System (Waters: Division of Millipore Corp., Milford, MA., Appendix A table 3). Horse muscle protein composition was used as a reference protein (Bryden, 1991).

Corn oil was used as a fat source because it is preferred by horses over other vegetable fat sources (Holland et al., 1998). Beet pulp, soybean hulls, and oat straw were

#### **Materials and Methods**

added to provide a range of different fiber sources. The intended role of these different fiber sources was to establish a diverse microbial population in the gastrointestinal tract with multiform fermentative capabilities. Mineral and vitamin supplementation of the diet was formulated to complement pastures in northern Virginia (Greiwe-Crandell et al., 1995; Kronfeld et al., 1996). The mineral premix was mixed by hand to insure the same mineral content for each ration. The vitamin premix was formulated in collaboration with Dr. Ted Frye and donated by Hoffman-LaRouche (Nutley, NJ).

The mares and foals were fed at 0700 and 1400 in feed tubs on the ground so that both had access to the supplement. Feed tubs were placed in a 30-meter circle with (n+1)buckets available, *n* equaling the number of horses in the pasture. This setup allowed each horse to eat its portion of the total amount of feed offered to the group. Careful observation indicated that some mares may have received more or less supplement than the desired amount, but the coefficient of variation in intake was approximately 10%, certainly less than variation associated with daily pasture intake (Kronfeld, 1997). The amount of supplement fed to the groups was such that a body condition of between 4.5 and 6 was maintained throughout the year. In order to maintain body condition the supplement:forage ratio during months when pasture growth was good was 1:2, while during some of the very dry or cold months it was raised to 1:1 (Appendix A table 5). For approximately a month in February and March of 1998, yearlings were also given free access to mixed grass/alfalfa hay (18.7% CP).

#### Care of animals

Twenty-four mares and foals were added to their respective groups five to seven days following foaling. They remained on pasture 24 hours a day, 7 days a week unless medical treatment was needed, in which case they would be brought into stalls. Mares and foals were on the anthelmintic, vaccination, and hoof trimming schedules routine at the MARE center (Ley et al., 1992). Two foals had to be taken off the study in the CS group leaving final number of 10 in the CS and 12 in the LTS group. Shelter was provided to each group by three-sided run-in sheds ( $18 \times 60$  ft) in each pasture. All

## **Materials and Methods**

horses in the pastures had *ad libitum* access to water. At six months, foals were weaned by removal of two mares every four days.

## Blood collection

Baseline samples were taken on mares and foals in May 1997 prior to being placed into their groups in the pasture. Blood samples were taken from mares and foals every 28 days post parturition between 0700 and 1200. The mares and foals were not fed on sampling mornings to reduce any effect feeding might have on samples being taken. Blood was collected by jugular venipuncture into 7 ml tubes (Lithium Heparin Vacutainer, Becton Dickenson, Rutherford, NJ). Samples were centrifuged within an hour of collection for 10 minutes at 3000 rpm at 20°C. Plasma was pipetted off and frozen, to be thawed later for analysis.

Blood plasma was analyzed for levels of total protein (TP), albumin (ALB), creatinine (CREA), and plasma urea nitrogen (PUN). Coloremetric analyses were conducted on a Beckman Synchron CX5 (Brea, CA). Total protein was measured by a timed endpoint biuret method (Synchron CX Clinical Systems, Kit # 442740, Brea, CA). Albumin was measured by a timed endpoint method (Synchron CX Clinical Systems, Kit # 442765, Brea, CA). Creatinine was measured by a modified rate Jaffe method (Synchron CX Clinical Systems, Kit # 442760, Brea, CA). Plasma urea nitrogen was measured by an enzymatic rate method (Synchron CX Clinical Systems, Kit # 442750).

#### Physical Measurements

Growth rates were evaluated every 28 d by a combination of body weight (BW), body condition score, wither and hip heights, length of body, forearm, and cannon bones, girth, and circumference of the physis and fetlocks. Body weights were measured using a portable electronic walk-on scale (Model TC-10S, Tyrel Corp.). Body condition was scored using the method of Henneke et al. (1983). Measurements were performed by the

#### **Materials and Methods**

same person each month so as to reduce variation in evaluation. Appendix A table 6 gives definitions of each measurement taken.

## Statistical Analysis

Dependent variables are summarized as least squares means and least squares standard errors (SE) of the mean. Dependent variables were analyzed for differences between the supplements over the entire sampling period by analysis of variance with repeated measures (SAS, 1989). The model statement included diet, horse within diet, time, and diet × time interaction. Tukey's multiple-comparison procedure was used to test differences between time periods. Means from months that appeared to show a difference in the variable measured were subjected to two sample t-tests to determine if there was a statistical difference between the diets during that month. Differences are considered significant at a P < .05.

## Results

#### Physical Measurements

Body weight (CS 261 ± 5.6 kg vs LTS 263 ± 5.1 kg) and average daily gain (ADG) (CS .80 ± .02 kg/d vs LTS .84 ± .02 kg/d) were not different (P = .75, P = .20, respectively) for the groups during the total observational period of 14 months (Figure 1 and 2). There was also no difference in mean BW or ADG between colts and fillies (P = .60). Cumulative percent increase in weight shows an increase over 14 months of 416% by the LTS group and 369% by the CS group (Figure 3). In March the ADG tended to be greater in yearlings fed LTS than in those fed the CS ( $1.02 \pm .064 \text{ vs} .77 \pm .071 \text{ kg/d}$ , respectively P = .065). The ADGs for March represent a 237% and 134% increase from the previous month in ADG for the LTS and CS groups, respectively (Figure 4). The ADG decreased in October and November, with the foals on the LTS tending to show less of a decrease in ADG than the foals on the Control supplement (Oct. 1.08 ± .064 kg vs .87 ± .071 kg/d, P = .080).

Body condition scores were not different (P = .86) between groups during the sampling period (Appendix B table 5 and 6). The mean condition scores for the LTS and CS groups were  $4.93 \pm .05$  and  $4.94 \pm .04$ , respectively. Condition scores were maintained with no changes between seasons.

Mean wither heights (CS  $138 \pm .97$  cm vs LTS  $138 \pm .88$  cm, P = .88) and hip heights (CS  $141 \pm 1.0$  cm vs LTS  $140 \pm .95$  cm, P = .56) showed no difference between supplements over the period of the study (Figure 5 and 6). The mean wither heights at 14 months for the LTS and CS groups were  $150.7 \pm .60$  cm and  $150.4 \pm .66$  cm, respectively. These tended to be 2 to 3 cm lower than mean hip heights at 14 months ( $152.0 \pm 0.62$  cm and  $152.7 \pm 0.68$  cm, respectively).

Girth measurements (CS 141 ± .97 cm vs LTS 142 ± .89 cm, P = .48) and length measurements (CS 136 ± 1.2 cm vs LTS 135 ± 1.1 cm, P = .49) were not different over the period of the study (Figure 7 and 8). Mean girth circumference and length at 14 months in the LTS and CS groups (166.3 ± .79 cm vs 163.8 ± .87 cm; 159.7 ± 1.00 cm vs **Results** 24
$160.7 \pm 1.10$  cm, respectively) were similar to known growth patterns (Hintz et al., 1979, Jelan et al., 1996, Pagan et al., 1996).

Mean forearm length (CS  $38 \pm .39$  cm vs LTS  $38 \pm .35$  cm, P = .94), front cannon length (CS  $31 \pm .27$  cm vs LTS  $31 \pm .24$  cm, P = .63), and hind cannon length (CS  $44 \pm .35$  cm vs LTS  $43 \pm .32$  cm, P = .21) all showed no difference in growth between groups (Figure 9, 10 and 11). Growth of these bones did not seem to show the same trends in ADG as other measurements. Front cannon bones increased  $2.0 \pm .40$  cm, hind cannon bones grew  $3.8 \pm .55$  cm, and the forearm increased  $13 \pm .42$  cm in length during the observational period.

Circumference of the physis and fetlock did not differ (P = .33, P = .28, respectively) between the groups (Figure 12 and 13). Mean physis circumferences for the LTS and CS groups were 29.1 ± .25 cm and 29.4 ± .20 cm. Mean fetlock circumferences for the LT and Control groups were 24.5 ± .17 cm and 24.8 ± .19 cm. Fetlock and physis circumference tended to be larger in the Control group in early spring than those of the LT group.

Power analysis of measurement data showed low power of the tests for all variables measured ( $\alpha = .05$ , n = 11, power = .050 to .238). The number of subjects in each group that would be needed to find a difference in each variable over the period of the study ranged from 41 for the hind cannon, to 20,574 for the forearm ( $\beta = .80$ ,  $\alpha = .10$ ) (Appendix A table 7).

## Blood Analysis

Mean plasma ALB levels were not different between the CS and LTS groups  $(2.89 \pm 0.03 \text{ and } 2.90 \pm 0.03 \text{ g/dl}$ , respectively, P = .78), and showed no change over the study period (Figure 14). Mean TP levels also did not vary over the study period and no difference was perceived between the CS and LTS groups  $(5.73 \pm .05 \text{ and } 5.78 \pm .04 \text{ g/dl}, \text{ respectively}, P = .46)$  (Figure 15).

#### Results

Mean plasma CREA levels for the CS and LTS groups  $(1.07 \pm .03 \text{ and } 1.06 \pm .03 \text{ mg/dl}$ , respectively, P = .77) were not different over the 14 months. Levels of CREA tended to increase after birth until July and August, then to decrease slightly and remain constant through 14 months (Figure 16). Plasma urea nitrogen levels for the CS and LTS groups  $(18.75 \pm 0.38 \text{ and } 18.80 \pm 0.35 \text{ mg/dl}$ , respectively, P = .92) were not different between treatments. Levels of urea in both groups showed a steady increase from May through November. This became less pronounced through the following months (Figure 17). The ratio of urea to creatinine was not different between the CS and LTS groups  $(17.87 \pm .68 \text{ and } 18.53 \pm .61$ , respectively, P = .48) (Figure 18). The pattern the ratio followed over the 14 months was similar to that of urea due to the fact that creatinine levels remained close to 1.0 mg/dl (Appendix B table 33 and 34). In November the urea:creatinine ratio was higher (P = .018) in the LTS group than the CS.

## Discussion

These results support the hypothesis that an improvement in the quality of protein fed will allow a decrease in the percentage of CP needed to maintain optimum growth. The evidence from this experiment includes: 1) no difference between treatment groups over the observational period for any of the indicators of protein status; 2) no difference between treatment groups over the observational period for any of the growth measurement taken; 3) similar growth in both groups to that seen in past growth surveys of Thoroughbreds.

Further examination of the results over restricted periods show differences between the two diets. This evidence includes: 1) a higher ADG in the LTS group in October and March; 2) a cumulative percent increase of weight in the LTS group of 416% compared to 369% in the CS group; 3) a 237% increase in ADG from February to March for the LTS group in comparison to 134% for the CS group.

The LTS group was provided a supplement with a higher quality protein. First, the supplement contained a greater percentage of lysine and threonine, the first and second limiting amino acids in the horse. Second, the percentage of crude protein in the LTS was lower than that in the CS. These two factors should have led to a more efficient utilization of dietary protein. Previous work has shown that yearlings offered a diet fortified with lysine and threonine had higher ADG's than yearlings fed as isonitrogenous diet with endogenous levels of these two amino acids (Graham et al., 1994).

Comparison of the two supplements on the basis of crude protein should take into account an average of 18% CP in the pasture (Appendix A table 1). The total dietary CP averaged 15.5% for the LTS group and 16.8% for the CS group (1:2 supplement to pasture ration). These levels of protein place both groups within the optimal range for CP% for growth. Blood measurements of protein status confirm the equivalence of protein intakes.

No differences in PUN concentration between the two groups were found due to both diets supplying more protein than was required for optimum growth. The PUN and urea:creatinine ratio reflected an increase in urea concentrations through the first seven

#### Discussion

months of the study, because creatinine remained unchanged this increase in the urea: creatinine ratio was correlated negatively with the decrease in ADG during the same months (P < .0001). These results suggest a reduction in protein requirement and heightened protein catabolism as the growth rate diminishes. A similar relationship appeared in a study of weanlings 7 to 10 months of age (Saastamoinen, 1996). Other indicators of protein status showed no difference between the two treatment groups and no trends over the observational period.

In general, PUN concentration decreases as the quality of dietary protein increases (Eggum, 1970). Low quality protein, as well as excesses of protein above that required will increase PUN concentrations. Higher PUN levels have been found in horses fed 20% CP compared to those fed 14% CP (Schryver et al. 1987). The levels of CP as well as lysine were above those recommended by the NRC from the time the foals were weaned until the end of the study for both diets (Appendix A figure 1).

Weight data from previous studies done at the MARE Center were interpreted to show a "spring slump" in the weights of yearlings in early spring (Hoffman, 1997). This "slump" in growth curve is formed due to a steadily decreasing ADG during the winter and then a sudden increase in ADG in the early spring. This period of growth may be more appropriately called a spring recovery. This trend is best seen in the ADG data.

The greatest ADG was seen during the first four to six weeks of life. Large ADG's in the first month of growth have been reported in other studies of Thoroughbreds (Jelan et al., 1996; Pagan et al., 1996). This large initial growth may merit further examination, as there has been suggestion that colostrum may have some effect on growth (Seve and Ponter, 1997). During the next four months, ADG remained constant.

In October and November, the weight gains shown during the summer months began to decrease. This decline may be due to several different reasons. First, the change in season and subsequent cooler temperatures cause increased energy demands for thermoregulation and the development of a thicker winter coat. Second, foals were weaned in November. Third, pasture quality begins to decrease at this time of year, supplying the weanlings with less of the energy they need to grow. The stress of weaning as well as changes in feeding habits may have played a role in the decrease in growth Discussion 28 rates. During the months of December, January, and February ADG remained low when foals were faced with cold weather conditions and the poorest pasture conditions of the year. A comparison of Thoroughbreds' growth in different climactic regions (Pagan et al., 1996) indicated that growth is affected by severity of seasons and availability of pasture.

In March, a considerable increase in ADG was observed in both treatment groups (Figure 4). This change is due to milder temperatures, improving pasture conditions, and presumably some compensatory growth after depressed gains during the winter months. The large increase in growth during this month was revealed by examining the percentage change in ADG from February to March (Figure 3). The greatest changes in ADG prior to March were less than 50%. There was also a considerable difference in ADG between the two groups during March. Changes in growth rate, especially compensatory growth, have been associated with manifestations of developmental orthopedic disease in yearlings (Hintz et al., 1976). Any way to lessen the degree of change may help in decreasing the amount of physitis that is often seen with spring growth and after weaning.

Energy intake may be the main causative factor in the changes in growth seen over the observational period. Protein to energy ratios are important in interpreting protein's effect on growth. Work with Thoroughbred and Quarter horses has shown that energy plays a first limiting role to growth with protein being limiting only when energy is supplied in sufficient quantities (Ott and Asquith, 1986). When energy intake was restricted in the yearlings increased protein intake had no effects on growth. The effect of low energy intakes may have been seen during the winter months of this trial, when both groups had similarly low ADG. When energy intakes increase in the spring differences due to protein content of the diet may become apparent.

Growth of the foals in this study was similar to growth shown in previous studies. Hip height was generally 2-3 cm greater than wither height. This trend in growth has also been encountered in Thoroughbred foals raised in Kentucky (Thompson 1995). Wither and hip heights of the Kentucky yearlings at 390 d ( $147 \pm .45$  and  $149 \pm .50$  cm, respectively) were similar to measurements from this study ( $149 \pm 1.3$  and  $151 \pm 1.2$  cm, **Discussion** 29 respectively). Growth of the forearms was relatively greater than that of either the front or hind cannon bones. Measurements of forearm, front cannon, and hind cannon length, as well as physis and fetlock circumference appeared to decrease in the third month of the study; however, this was regarded as an error due to a change in observers during this month. Girth and length were similar to measurements from a previous study done on the station (Hoffman, 1997). Mean physis circumference at 12 months in the LTS and CS groups ( $31.2 \pm 0.20$  cm,  $30.9 \pm 0.22$  cm) showed an increase from initial circumference of 6.8 cm and 6 cm, respectively. These increases were slightly greater than those observed by Green (1969), although differences in measuring technique may account for this.

The profile of amino acids that were available for absorption may have been different for these two treatment groups. The CS group had protein supplied in the supplement mainly from SBM and the remainder from the pasture. The LTS group had a smaller percentage of SBM in the supplement, with assumed equal pasture intake to the CS group. The LTS group also had their supplement fortified with .6% lysine and .4% threonine. If the lysine and threonine were more available for absorption than amino acids bound up in feed, then animals on the LTS diet probably had a more readily available source for these essential amino acids.

Different sources of protein vary in their digestibility in the small intestine (Potter et al., 1992). Proteins are broken down in the small intestine into free amino acids as well as di- and tri-peptides (Silk et al., 1985). The degradability of proteins and therefore profile of amino acids available for absorption may vary with the composition of the diet. Digestibility of corn protein tended to be lower than that of SBM in the small intestine of ponies (Gibbs et al., 1996).

The weanlings and yearlings on the LTS had the free forms of the first two limiting amino acids available for absorption. The result of this may have been a profile of amino acids reaching tissues that was more appropriate for protein synthesis. While most months show no difference in growth variables between the two groups, there are differences in the rate of gain at certain points in the growth curve. These differences may be attributed to a difference in the availability of amino acids in the supplements. **Discussion**  The difference in the two supplements is best seen in October, and November, when foals are being weaned, and in March, with the arrival of spring. These periods relate to nutritional and environmental challenges to the foals. The diet change associated with weaning is second only to that at birth, hence proper nutrition during this period is critical. The LTS group was better able to maintain growth during this period. In March, pasture conditions are improving and daily temperatures begin to rise. One study showed ADG of foals to increase in one month from .5 to .6 kg/d in the spring (Pagan et al., 1996). This study showed a particularly large increase from February to March in ADG from .3 to 1.0 kg/d in the LTS group, with the CS group's ADG increasing by half of this.

Power analysis of the data indicates that, in order to find a difference between the two treatment groups due to diet, the number of horses in each group would need to be much greater than was available for the study (Appendix A table 7). The number of horses needed to find a difference ranged from 41 for the hind cannon to 20,574 for the forearm. The number for the hind cannon was particularly high because of the variation in the measurements taken for this variable. The power of the test  $(1-\beta)$  for detecting the research hypothesis is low for all dependent variables. This verifies that differences between the groups were far too small to detect with 10 and 12 horses in the groups.

Crude protein levels in the pasture were particularly high during this study, with a high of 22% in March and a low of 15% in June. Forage levels of CP may decrease to levels of 10% or lower, particularly in dry summer conditions (Minson, 1990). High CP levels may have overshadowed effects of the supplements on protein status in this study. A 9% CP supplement fortified with lysine and threonine may show more benefits in horses raised on lower quality pasture.

#### Discussion

# Implications

Fortification with the free amino acid forms of lysine and threonine may improve the availability of these amino acids for absorption and utilization by the horse. One benefit of an improved amino acid profile is that it provides for optimal growth without forcing the horse to squander energy removing waste of amino acid catabolism. Another advantage of higher protein quality is that it allows reduction in protein intake, hence it diminishes contamination of the environment with nitrogen and phosphorus. This work suggests that closer attention to the amino acid composition of the diet will sustain the performance of the horse while at the same time protect and enhance the environment.

# **Literature Cited**

- Bidlingmeyer, B. A., S. A. Cohen, and T. L. Tarvin. 1984. Rapid analysis of amino acids using pre-column derivatization. J. Chrom. 336:93-104.
- Bosclair, Y. R., A. W. Bell, F. R. Dunshea, M. Harkins, and D. E. Bauman. 1993. Evaluation of the arteriovenous difference technique to simultaneously estimate protein synthesis and degradation in the hindlimb of fed and chronically underfed steers. J. Nutr. 123:1076-1088.
- Breuer, L. H., and D. L. Golden. 1971. Lysine requirement of the immature equine. J. Anim. Sci. 33:227.
- Bryden, W. L. 1991. Amino acid requirements of horses estimated from tissue composition. Proc. Nutr. Soc. Aust. 16:53.
- Cabrera, L., V. Julliand, F. Faurie, and J. L. Tisserand. 1992. Influence of feeding roughage and concentrate (soy bean meal) simultaneously or consecutively on levels of plasma free amino acids and plasma urea in the equine. Pferdeheilkunde. Sonderheft:144-146.
- Chase, L. E. 1996. Practical application of amino acid concepts for dairy cattle. In: Proc. Cornell Nutr. Conf. Rochester, NY. pp.208-214.
- Eggum, B. 1970. Blood urea measurement as a technique for assessing protein quality. Br. J. Nutr. 24:983-988.
- Fenderson, C. L., and W. G. Bergen. 1975. An assessment of essential amino acid requirements of growing steers. J. Anim. Sci. 41:1759-1766.
- Friedman, M. 1996. Nutritional value of proteins from different food sources. A review. J. Agric. Food Chem. 44:6-29.
- Gibbs, P. G., G. D. Potter, G. T. Schelling, J. L. Kreider, and C. L. Boyd. 1996. The significance of small vs. large intestinal digestion of cereal grain and oilseed protein in the equine. J. Equine Vet. Sci. 16:60-65.
- Gibson, R. S. 1990. Assessment of protein status. In: Principles of Nutritional Assessment. p 307-342. Oxford University Press. New York, NY.
- Godbee, R. G., and L. M. Slade. 1981. The effect of urea or soybean meal on the growth and protein status of young horses. J. Anim. Sci. 53:670-676.
- Graham, P. M., E. A. Ott, J. H. Brendemuhl, and S. H. TenBroeck. 1994. The effect of supplemental lysine and threonine on growth and development of yearling horses. J. Anim. Sci. 72:380-386.
- Green, D. A. 1961. A review of studies on the growth rate of the horse. Brit. Vet. J. 117:181-191.

### Literature Cited

- Green, D. A. 1969. A study of growth rate in thoroughbred foals. Brit. Vet. J. 125:539-546.
- Green, D. A. 1976. Growth rate in thoroughbred yearlings and two year olds. Equine Vet. J. 8:133-134.
- Greppi, G. F., L. Casini, D. Gatta, M. Orlandi, and M. Pasquini. 1996. Daily fluctuations of haematology and blood biochemistry in horses fed varying levels of protein. Equine Vet. J. 28:350-353.
- Griewe-Crandell, K. M., D. S. Kronfeld et al. 1995. Seasonal vitamin A depletion in grazing horses is assessed better by the relative dose response test than by serum retinol. J Nutr 125:2711-2716.
- Hahn, J. D., and D. H. Baker. 1995. Optimum ratio of lysine to threonine, tryptophan, and sulfur amino acids for finishing swine. J. Anim. Sci. 73:482-489.
- Hara, H., R. Funabiki, M. Iwata, and K. Yamazaki. 1984. Portal absorption of small peptides in rats under unrestrained conditions. J. Nutr. 114:1122-1129.
- Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983 Relationship between condition score, physical measurement, and body fat percentage in mares. Equine Vet. J. 15:371-372.
- Hintz, H. F., H. F. Schryver, and J. E. Lowe. 1971. Comparison of a blend of milk products and linseed meal as protein supplements for young growing horses. J. Anim. Sci. 33:1274-1277.
- Hintz, H. F., H. F. Schryver, and J. E. Lowe. 1976. Delayed growth response and limb confirmation in young horses. In:Proc. Cornell Nutr. Conf. Ithaca, NY.
- Hintz, H. F., R. L. Hintz, and L. D. Vleck. 1979. Growth rate of thoroughbreds: Effect of age of dam, year and month of birth, and sex of foal. J. Anim. Sci. 48:480-487.
- Hoffman RM, Kronfeld DS, Lawrence LA, Cooper WL, Dascanio and Harris PA. 1996. Dietary starch and sugar versus fat and fiber: growth and development of foals. Pferdeheilkunde 12(3): 312–316.
- Hoffman, R. M. 1997. Carbohydrate and fat supplementation in grazing mares and foals. Ph.D. dissertation. Virginia Polytechnic Institute and State University, Blacksburg.
- Holland, J. L., D. S. Kronfeld, G. A. Rich, K. A. Kline, J. P. Fontenot, T. N. Meachem, and P. A. Harris. 1998. Acceptance of fat and lecithin containing diets by horses. Appl. Anim. Behav. Sci. 56:91-96.
- Hunt, S. M., and J. L. Groff. 1990. Proteins. In: Advanced Nutrition and Human Metabolism. p 129-167. West Publishing Company. New York, NY.
- Jelan, Z. A., L. B. Jeffcott, N. Lundeheim, and M. Osborne. 1996. Growth rates in thoroughbred foals. Pferdeheilkunde 12(3):291-295.

- Jordan, R. M., and V. Myers. 1972. Effects of protein levels on the growth of weanling and yearling ponies. J. Anim. Sci. 34:578-581.
- Kronfeld, D. S. 1982. Protein quality and amino acid profiles of commercial dog foods. J. Am. Anim. Hosp. Assoc. 18:679-683.
- Kronfeld, D. S., W. L. Cooper, K. M. Crandell, L. A. Gay, R. M. Hoffman, J. L. Holland, J. A. Wilson, D. Sklan, and P. A. Harris. 1996. Supplementation of pasture for growth. Pferdeheilkunde. 12(3):317-319.
- Kronfeld, D. S. 1997. Variations in energy requirements of horses and errors in estimation of pasture intake. In: Proc. 15<sup>th</sup> Equine Nutr. and Physiol. Symp. pg. 383.
- Kropf, D. H., R. W. Bray, P. H. Phillips, and R. H. Grummer. 1959. Effect of protein level and quality in swine rations upon growth and carcass development. J. Anim. Sci. 18:755-762.
- Ley, W. B., J. M. Bowen, and C. D. Thatcher. 1992. Equine Preventative Medicine. Virginia Maryland Regional College of Veterinary Medicine, Blacksburg, VA.
- Lobley, G. E. 1992. Control of the metabolic fate of amino acids in ruminants: a review. J. Anim. Sci. 70:3264-3275.
- Malmlof, K., and S. Askbrant. 1988. A note on the potential of systematic plasma urea measurements as a basis for determining optimal supplementation levels of lysine and threonine in pig diets. Swedish J. Agric. Res. 18:191-193.
- Messman, M. A., W. P. Weiss, and K. A. Albrecht. 1996. In situ disappearance of individual proteins and nitrogen from legume forages containing varying amounts of tannins. J. Dairy Sci. 79:1430-1435.
- Meyer, H. 1987. Nutrition of the equine athlete. Equine Exercise Physiology 3:644-673.
- Millward, D. J. 1997. Human amino acid requirements. J. Nutr. 127:1842-1846.
- Millward, D. J., J. L. Bowtell, P. Pacy, and M. J. Rennie. 1994. Physical activity, protein metabolism and protein requirements. Proc. Nutr. Soc. 53:223-240.
- Minson, D. J. 1990. Crude protein in forage. In: Forage in Ruminant Nutrition. p 180-188. Academic Press, Inc., Boston, MA.
- NRC. 1989. Nutrient Requirements of Horses (5<sup>th</sup> ed). National Academy Press, Washington, DC.
- O'Mara, F. P., J. J. Murphy, and M. Rath. 1997. The amino acid composition of protein feedstuffs before and after ruminal incubation and after subsequent passage through the intestines of dairy cows. J. Anim. Sci. 75:1941-1949.
- Oser, B. L. 1959. An integrated essential amino acid index for predicting the biological value of proteins. In: A. A. Albanese (Ed.) Protein and Amino Acid Nutrition. p 281-295.

### Literature Cited

- Ott, E. A., and R. L. Asquith. 1986. Influence of level of feeding and nutrient content of the concentrate on growth and development of yearling horses. J. Anim. Sci. 62:290-299.
- Ott, E. A., R. L. Asquith, and J. P. Feaster. 1981. Lysine supplementation of diets for yearling horses. J. Anim. Sci. 53:1496-1503.
- Ott, E. A., R. L. Asquith, J. P. Feaster, and F. G. Martin. 1979. Influence of protein level and quality on the growth and development of yearling foals. J. Anim. Sci. 49:620-628.
- Pagan, J. D., S. G. Jackson, and S. Caddel. 1996. A summary of growth rates of thoroughbreds in Kentucky. Pferdeheilkunde 12(3):285-289.
- Poso, A. R., B. Essen-Gustavsson, A. Lindholm, and S. G. B. Persson. 1991. Exerciseinduced changes in muscle and plasma amino acid levels in the standardbred horse. Equine Exercise Physiology 3:202-208.
- Potter, G. D., and J. D. Huchton. 1975. Growth of yearling horses fed different sources of protein with supplemental lysine. In:Proc. 4<sup>th</sup> Equine Nutr. and Phys. Symp. pp. 19-20. Pomona, CA.
- Potter, G. D., P. G. Gibbs, R. G. Haley, and C. Klendshoj. 1992. Digestion of protein in the small and large intestines of equines fed mixed diets. Pferdeheilkunde. Sonderheft:140-143.
- Pulse, R. E., J. P. Baker, G. D. Potter, and J. Willard. 1973. Dietary protein level and growth of immature horses. J. Anim. Sci. 37:289-290.
- Purser, D. B. 1970. Nitrogen metabolism in the rumen: microorganisms as a source of protein for the ruminant animal. J. Anim. Sci. 70:988-1001.
- Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. J. Anim. Sci. 46:740-745.
- Rodwell, V. W. 1993. Proteins: Structure and Function. In: Harper's Biochemistry (24<sup>th</sup> Ed.). p 43, 24-25. Appleton & Lange. Stamford, CT.
- Russell, M. A., A. V. Rodiek, and L. M. Lawrence. 1986. Effect of meal schedules and fasting on selected plasma free amino acids in horses. J. Anim. Sci. 63:1428-1431.
- Saastamoinen, M. T. 1996. Protein, amino acid and energy requirements of weanling foals and yearlings. Pferdeheilkunde 12(3):297-302.
- SAS. 1989. SAS/STAT<sup>®</sup> User's Guide (Release 6.12). SAS Inst. Inc., Cary, NC.
- Schryver, H. F., D. W. Meakim, J. E. Lowe, J. Williams, L. V. Soderholm, and H. F. Hintz. 1987. Growth and calcium metabolism in horses fed varying levels of protein. Equine Vet. J. 19:280-287.
- Schwab, C. G. 1996. Amino acid nutrition of the dairy cow: current status. In: Proc. Cornell Nutr. Conf. Rochester, NY. pp.184-198.

### Literature Cited

- Seve, B., and A. A. Ponter. 1997. Nutrient-hormone signals regulating muscle protein turnover in pigs. Proc. Nutr. Soc. 56:565-580.
- Silk, D. B. A., G. K. Grimble, and R. G. Rees. 1985. Protein digestion and amino acid and peptide absorption. Proc. Nutr. Soc. 44:63-72.
- Stryer, L. 1995. Protein Structure and Function. In: Biochemistry (4<sup>th</sup> Ed.). p 17. W. H. Freeman and Company, New York, NY.
- Swenson, M. J., and W. O. Reece. 1993. Protein and Amino Acid Metabolism. In: Duke's Physiology of Domestic Animals (11<sup>th</sup> Ed.). p 473. Cornell University Press. Ithaca, NY.
- Thompson, K. N. 1995. Skeletal growth rates of weanlings and yearling thoroughbred horses. J. Anim. Sci. 73:2513-2517.
- Trottier, N. L., C. F. Shipley, and R. A. Easter. 1997. Plasma amino acid uptake by the mammary gland of the lactating sow. J. Anim. Sci. 75:1266-1278.
- Tuitoek, J. K., L. G. Young, C. F. M. de Lange, and B. J. Kerr. 1997. Body composition and protein and fat accretion in various body components in growing gilts fed diets with different protein levels but estimated to contain similar levels of ideal protein. J. Anim. Sci. 75:1584-1590.
- Wang, T. C., and M. F. Fuller. 1989. The optimum dietary amino acid pattern for growing pigs. Br. J. Nutr. 62:77-89.
- Wessels, R. H., E. C. Titgemeyer, and G. St. Jean. 1997. Effect of amino acid supplementation on the whole-body protein turnover in Holstein steers. J. Anim. Sci. 75:3066-3073.
- Wilkerson, V. A., T. J. Klopfenstein, R. A. Britton, R. A. Stock, and P. S. Miller. 1993. Metabolizable protein and amino acid requirements of growing cattle. J. Anim. Sci. 71:2777-2784.
- Yoakam, S. C., W. W. Kirkham, and W. M. Beeson. 1978. Effect of protein level on growth in young ponies. J. Anim. Sci. 46:983-991.
- Zello, G. A., L. J. Wykes, R. O. Ball, and P. B. Pencharz. 1995. Recent advances in methods of assessing dietary amino acid requirements for adult humans. J. Nutr. 125:2907-2915.
- Zicker, S. C., and Q. R. Rogers. 1994. Concentrations of amino acids in plasma and whole blood in response to food deprivation and refeeding in healthy two-day-old foals. Am. J. Vet. Res. 55:1020-1027



Figures

Figure 1. Weights (mean ± SE) of foals fed a 14% CP supplement ( **5** CS) or a 9% CP supplement, fortified with lysine and threonine ( CP LTS).



Figure 2. Daily gain (mean ± SE) of foals fed a 14% CP supplement ( **so** CS) or a 9% CP supplement, fortified with lysine and threonine ( 🔄 LTS).

Figures







Figure 5. Wither heights (mean ± SE) of foals fed a 14% CP supplement ( **50** CS) or a 9% CP supplement, fortified with lysine and threonine ( C LTS).

Figures



Figure 6. Hip heights (mean ± SE) of foals fed a 14% CP supplement ( **50** CS) or a 9% CP supplement, fortified with lysine and threonine ( CP LTS).



Figure 7. Girth (mean ± SE) of foals fed a 14% CP supplement ( **\$50** CS) or a 9% CP supplement, fortified with lysine and threonine ( CP LTS).



Figure 8. Length (mean ± SE) of foals fed a 14% CP supplement ( **50** CS) or a 9% CP supplement, fortified with lysine and threonine ( C LTS).



Figure 9. Forearm lengths (mean ± SE) of foals fed a 14% CP supplement ( **\$50** CS) or a 9% CP supplement, fortified with lysine and threonine ( CP LTS).



Figure 10. Front cannon bone lengths (mean ± SE) of foals fed a 14% CP supplement ( **50** CS) or a 9% CP supplement, fortified with lysine and threonine ( 2 LTS). Decreases from month to month due to observer error.



Figure 11. Hind cannon bone lengths (mean ± SE) of foals fed a 14% CP supplement ( **50** CS) or a 9% CP supplement, fortified with lysine and threonine ( CLTS). Decreases from month to month due to observer error.



Figure 12. Physis circumference (mean ± SE) of foals fed a 14% CP supplement ( **50** CS) or a 9% CP supplement, fortified with lysine and threonine ( 2 LTS). Decreases from month to month due to observer error.



Figure 13. Fetlock circumference (mean ± SE) of foals fed a 14% CP supplement ( **\$57** CS) or a 9% CP supplement, fortified with lysine and threonine ( 2 LTS). Decreases from month to month due to observer error.



Figure 14. Plasma levels of albumin (mean ± SE) in foals fed a 14% CP supplement ( **50** CS) or a 9% CP supplement, fortified with lysine and threonine ( 2 LTS).



Figure 15. Plasma levels of total protein (means ± SE) in foals fed a 14% CP supplement ( **\$50** CS) or a 9% CP supplement, fortified with lysine and threonine (  $\swarrow$  LTS).



Figure 16. Plasma levels of creatinine (mean ± SE) in foals fed a 14% CP supplement ( **50** CS) or a 9% CP supplement, fortified with lysine and threonine ( 2 LTS).



Figure 17. Plasma levels of urea (means ± SE) in foals fed a 14% CP supplement ( **5** CS) or a 9% CP supplement, fortified with lysine and threonine ( CP LTS).



Figure 18. Urea to creatinine ratio (mean ± SE) in the blood of foals fed a 14% CP supplement ( **50** CS) or a 9% CP supplement, fortified with lysine and threonine ( 2 LTS).

# **Appendix A Tables**

Appendix A table	1. Nutrient co	mposition on a	DM basis of the	pasture, 14%	crude protein
(CS), and 9%	crude protein,	fortified with l	ysine and threor	nine (LT) supp	lements <sup>a</sup>

	Cont	rol	LT		Pastur	e
	Suppler	ment	Suppler	nent		
Item	Mean	SE	Mean	SE	Mean	SE
Crude protein, %	13.7 <sup>b</sup>	.21	8.5 <sup>b</sup>	.18	18.0 <sup>e</sup>	.43
Crude fat, %	14.5 <sup>b</sup>	.60	14.6 <sup>b</sup>	.70	$4.0^{\rm e}$	.12
Acid detergent fiber, %	31.2 <sup>b</sup>	.55	32.1 <sup>b</sup>	.73	33.0 <sup>e</sup>	.58
Neutral detergent fiber, %	43.0 <sup>c</sup>	.63	44.4 <sup>c</sup>	1.01	56.4 <sup>e</sup>	1.05
Non-structural carbohydrates, %	19.0 <sup>d</sup>	1.54	17.5 <sup>d</sup>	2.79	14.1 <sup>e</sup>	.74
Ca %	1.8 <sup>c</sup>	.07	1.8 <sup>c</sup>	.07	.5 <sup>e</sup>	.03
P %	$0.5^{\circ}$	.02	.6 <sup>c</sup>	.02	.3 <sup>e</sup>	.01
Mg %	$0.2^{c}$	.01	.2 <sup>c</sup>	.01	.2 <sup>e</sup>	.00
Κ%	$1.2^{\rm c}$	.04	.9 <sup>c</sup>	.02	.2 <sup>e</sup>	.07
Na %	$0.3^{\rm c}$	.02	.3 <sup>c</sup>	.03	$.0^{e}$	.00
Fe mg/kg	543 <sup>c</sup>	19.7	562 <sup>c</sup>	32.8	1078 <sup>e</sup>	118
Zn mg/kg	176 <sup>c</sup>	21.3	174 <sup>c</sup>	9.5	56 <sup>e</sup>	3.1
Cu mg/kg	38.6 <sup>c</sup>	2.32	40.8 <sup>c</sup>	1.87	6.2 <sup>e</sup>	3.19
Mn mg/kg	70.9 <sup>c</sup>	1.97	69.9 <sup>c</sup>	1.73	81.4 <sup>e</sup>	2.92
S %	$0.2^{c}$	.00	.2 <sup>c</sup>	.01	.2 <sup>e</sup>	.00
Cl ion %	$0.6^{\circ}$	.01	.8 <sup>c</sup>	.05	.6 <sup>e</sup>	.02

 $^{a}$  Analysis performed by Dairy One, Ithaca, NY  $^{b}$  n = 30,  $^{c}$  n = 10,  $^{d}$  n = 4,  $^{e}$  n = 46

Ingredient	CS	LTS
Oat straw	22.7	25.1
Medium cracked corn	3.9	20.1
Beet pulp	16.3	16.1
Soybean hulls	14.8	15.1
Corn oil	10.8	11.1
Molasses (cane)	4.9	5.0
Soybean meal	21.7	3.0
Limestone	2.2	1.8
Calcium phos dibasic	1.7	1.8
Lysine <sup>a</sup>	N/A	.6
Vitamin premix <sup>bc</sup>	.5	.5
Mineral premix <sup>d</sup>	.5	.5
Threonine <sup>a</sup>	N/A	.4

Appendix A table 2. Ingredient composition (%) of the 14% CP supplement (CS) and the 9% CP supplement, fortified with lysine and threonine (LTS)

<sup>a</sup> Courtesy of Dr. Dave Burnham (Heartland Lysine, Inc. Chicago IL)

<sup>b</sup> Courtesy of Dr. John Wilson (Hoffman-LaRoche, Nutley, NJ)

 $^{c}$  Provided the following amounts per kg of diet: vitamin A, 6,900 IU;  $\beta$ -carotene, 17.6; vitamin D<sub>3</sub>, 1,290 IU; vitamin E,

132 mg; vitamin C, 333 mg; Niacin, 15 mg; Thiamin, 7 mg; Riboflavin, 3.5 mg; Folic acid, .33 mg; Biotin, .21 mg.

<sup>d</sup> Provided the following amounts per kg of diet: Fe, 46.1mg; Zn, 105.8mg; Cu, 25.11mg; Mn, 18.02 mg; Se, .55 mg; I, .35 mg; NaCl used as carrier, 4160 mg.

	~ ~					
_	Control Su	ıpplement	LT Supple	ement	Pastu	re
Item	Mean <sup>b</sup>	SE	Mean <sup>b</sup>	SE	Mean <sup>c</sup>	SE
ASP	5.87	.067	3.80	.040	11.66	.068
GLU	10.60	.032	7.37	.027	14.37	.063
SER	3.17	.003	2.30	.000	4.46	.016
GLY	3.23	.007	2.63	.003	6.04	.022
HIS <sup>a</sup>	2.43	.007	1.97	.003	3.11	.012
ARG <sup>a</sup>	5.07	.012	3.50	.006	7.33	.030
THR <sup>a</sup>	3.20	.010	4.77	.003	6.10	.024
ALA	3.37	.003	3.10	.006	8.48	.036
PRO	4.20	.006	3.53	.007	7.44	.053
TYR	2.83	.007	2.10	.006	4.14	.016
VAL <sup>a</sup>	3.67	.007	2.83	.003	7.28	.031
MET <sup>a</sup>	.00	.000	.00	.000	1.28	.006
CYS	.00	.000	.00	.000	.00	.000
ILE <sup>a</sup>	3.40	.006	2.37	.003	5.87	.025
$LEU^{a}$	5.50	.006	4.40	.000	10.02	.039
$PHE^{a}$	3.30	.000	2.37	.003	6.39	.028
LYS <sup>a</sup>	4.57	.003	6.63	.023	6.41	.032

Appendix A table 3. Amino acid composition (g/kg) of the pasture, 14% crude protein (CS), and 9% crude protein, fortified with lysine and threonine(LTS) supplements

<sup>a</sup> Essential <sup>b</sup> Triplicate analysis <sup>c</sup> Composite of 16 samples

	FF I	Diet	LT Diet		Pasture	
Item	Mean <sup>b</sup>	SE	Mean <sup>b</sup>	SE	Mean <sup>c</sup>	SE
ASP	42.82	4.92	27.74	2.95	85.14	4.96
GLU	77.37	2.35	53.77	1.95	104.86	4.63
SER	23.11	0.24	16.79	0.00	32.56	1.15
GLY	23.60	0.49	19.22	0.24	44.07	1.58
HIS <sup>a</sup>	17.76	0.49	14.36	0.24	22.67	0.85
ARG <sup>a</sup>	36.98	0.88	25.55	0.42	53.52	2.20
THR <sup>a</sup>	23.36	0.73	34.79	0.24	44.51	1.73
ALA	24.57	0.24	22.63	0.42	61.93	2.66
PRO	30.66	0.42	25.79	0.49	54.30	3.88
TYR	20.68	0.49	15.33	0.42	30.21	1.15
VAL <sup>a</sup>	26.76	0.49	20.68	0.24	53.13	2.26
MET <sup>a</sup>	0.00	0.00	0.00	0.00	9.38	0.45
CYS	0.00	0.00	0.00	0.00	0.00	0.00
ILE <sup>a</sup>	24.82	0.42	17.27	0.24	42.84	1.82
LEU <sup>a</sup>	40.15	0.42	32.12	0.00	73.17	2.85
PHE <sup>a</sup>	24.09	0.00	17.27	0.24	46.67	2.03
LYS <sup>a</sup>	33.33	0.24	48.42	1.70	46.75	2.36

Appendix A table 4. Amino acid composition (mg/g CP) of the pasture, 14% crude protein (CS), and 9% crude protein, fortified with lysine and threonine(LTS) supplements

<sup>a</sup> Essential
<sup>b</sup> Triplicate analysis
<sup>c</sup> Composite of 16 samples

Appendix A table 5. Amount of supplement fed to mares, foals, weanlings and yearlings through the study period.

Horses	Dates fed	Amount per tub, kg/day
Mares, early lactation <sup>c</sup>	May - August	3.63 <sup>b</sup>
Mares and foals late lactation <sup>d</sup>	September - October	3.18 <sup>b</sup>
Weaned foals <sup>e</sup>	November - December	1.95 <sup>b</sup>
Yearlings, moderate growth <sup>f</sup>	January - March	$2.90^{a}$
Yearlings, moderate growth <sup>f</sup>	April - June	$2.27^{\rm b}$

<sup>a</sup> Supplement to pasture ratio of 1:1 <sup>b</sup> Supplement to pasture ratio of 1:2 NRC (1989) recommended daily DE<sup>c</sup> 35 Mcal/d, <sup>d</sup> 29 Mcal/d, <sup>e</sup> 18 Mcal/d, <sup>f</sup> 21 Mcal/d
Appendix A table 6. Description of body measurements used to monitor growth of foals

Variable	Measurement description
Wither height	distance from the ground to the highest point of the withers
Hip height	distance from the ground to the highest point of the croup
Body length	distance from the point of the shoulder to the point of the buttock
Girth	circumference of the girth behind the elbow and an inch behind the highest point of the withers
Forearm	distance from the point of the elbow to the accessory carpal bone
Front cannon	distance from the accessory carpal bone to the proximal sesamoids
Physis	circumference of the knee at the metaphysis of the distal radius, just above the accessory carpal bone
Fetlock	circumference of the fetlock at the metaphysis of the distal third metacarpal bone, just above the proximal sesamoids
Hind cannon	distance from the point of the hock (calcaneus) to the proximal sesamoids



Appendix A figure 1. NRC (1989) recommendations for CP and lysine (g/d) for weanlings and yearlings 6 and 12 months with mean weights of 245 and 375 (kg) and ADG's of .95 and .80 (kg/d) compared to estimated intakes of CP and lysine from pasture<sup>a</sup> and supplement<sup>b</sup>. <sup>a</sup> 6 kg/d intake, 18% CP, 6.41 g/kg lysine. <sup>b</sup> intake appendix table 4 kg/d, LTS 8.5% CP, 6.63 g/kg lysine, CS 13.7% CP, 4.57 g/kg lysine

## Appendix A

	Power $(1-\beta)$	n <sup>b</sup>
Variable	$n=11^{a}, \alpha=.05$	β=.80, α=.10
Length	0.097	152
Hip	0.084	209
Girth	0.099	146
Wither	0.052	2909
Forearm	0.050	20574
Hind cannon	0.238	41
Front cannon	0.077	265
Physis	0.157	70
Fetlock	0.187	55
BC	0.052	3380
ADG	0.229	43

Appendix A table 7. Power analysis of test to find a difference between treatments and estimation of the number of subjects per treatment group required to find a difference.

<sup>a</sup> Mean of 10 and 12

<sup>b</sup> Number of subjects required

## **Appendix B Tables**

							Contro	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	70.3	128.4	160.1	187.3	217.3	236.8	264.9	282.1	294.4	308.0	316.2	340.6	352.0	361.5
2	100.7	152.4	184.2	213.2	246.8	281.2	316.2	333.4	350.2	361.5	367.0	395.5	387.8	392.4
3	73.0	106.1	129.7	157.9	186.0	220.0	249.0	265.8	274.4	285.8	294.4	320.2	335.2	347.0
4	92.5	122.9	158.8	192.3	231.8	268.5	294.4	308.4	320.2	330.7	336.6	357.4	376.5	393.3
5	96.6	155.6	179.6	202.8	230.9	256.7	282.1	293.5	306.6	317.5	327.9	316.2	346.1	360.6
6	80.3	132.9	158.8	183.7	212.3	238.6	254.0	276.7	288.5	303.5	312.5	337.0	354.3	363.8
7	77.6	111.6	143.8	179.6	217.7	258.5	281.7	298.0	315.7	327.0	334.8	367.9	381.0	400.1
8	64.9	124.3	156.5	179.6	219.5	260.4	292.1	312.5	324.3	331.6	345.2	371.9	390.1	412.3
9	75.7	138.3	165.6	192.3	225.4	260.4	280.3	291.7	303.0	319.8	331.1	357.4	357.4	371.0
10	73.0	125.6	153.3	186.0	220.0	252.7	268.5	283.0	297.6	308.4	322.5	344.3	360.2	379.2
Means <sup>a</sup>	80.5	129.8	159.0	187.5	220.8	253.4	278.3	294.5	307.5	319.4	328.8	350.9	364.1	378.1

Appendix B table 1. Body weight (kg) of foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	110.2	134.7	152.4	188.2	226.8	265.8	299.8	318.9	333.4	344.7	356.1	383.3	401.4	423.2
2	76.7	140.6	173.7	200.0	232.2	266.3	297.1	315.2	316.6	332.9	332.5	367.4	379.2	386.9
3	89.8	141.1	169.6	196.4	224.5	252.2	274.9	289.4	301.2	311.2	323.9	356.5	372.9	382.8
4	67.6	117.9	151.5	188.2	230.0	260.8	293.5	318.4	331.1	339.7	349.3	377.4	395.5	407.3
5	61.2	114.8	137.4	156.5	181.9	207.3	233.1	247.7	256.3	259.5	272.2	290.3	301.2	301.2
6	57.6	106.1	136.1	168.3	200.9	236.8	265.4	285.3	301.6	317.5	331.1	356.1	371.0	385.1
7	74.8	121.1	150.6	184.6	222.3	257.6	291.2	312.5	327.9	343.4	352.0	381.9	400.5	411.4
8	82.1	140.6	167.8	203.7	235.4	276.7	314.8	338.8	352.4	366.5	374.2	392.4	386.0	416.4
9	78.5	135.2	157.9	187.3	217.3	249.5	280.8	307.1	318.0	327.9	339.3	377.4	396.9	401.9
10	84.8	136.1	164.7	193.2	220.0	249.5	276.2	281.2	288.9	300.3	302.1	339.3	352.9	366.0
11	51.3	97.1	131.5	164.2	198.2	226.8	260.4	284.9	302.1	312.5	325.7	357.9	379.2	395.1
12	70.3	133.4	156.9	186.9	219.5	252.2	286.7	306.2	319.3	332.9	334.8	362.9	385.6	388.7
Means <sup>a</sup>	75.4	126.6	154.2	184.8	217.4	250.1	281.2	300.5	312.4	324.1	332.7	361.9	376.9	388.8

Appendix B table 2. Body weight (kg) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

						Cont	rol Supple	ement					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98
1	2.03	1.11	0.95	1.05	.68	.98	.60	.43	.48	.29	.86	.40	.33
2	1.81	1.11	1.02	1.17	1.21	1.22	.60	.59	.40	.19	1.00	27	.16
3	1.16	.82	.98	.98	1.19	1.02	.59	.30	.40	.30	.90	.52	.41
4	1.06	1.25	1.17	1.38	1.28	.90	.49	.41	.36	.21	.73	.67	.59
5	2.06	.84	.81	.98	.90	.89	.40	.46	.38	.36	41	1.05	.51
6	1.84	0.90	.87	1.00	.92	.54	.79	.41	.52	.32	.86	.60	.33
7	1.19	1.13	1.25	1.33	1.43	.81	.57	.62	.40	.27	1.16	.46	.67
8	2.08	1.13	.81	1.40	1.43	1.11	.71	.41	.25	.48	.94	.63	.78
9	2.19	.95	.94	1.16	1.22	.70	.40	.40	.59	.40	.92	.00	.48
10	1.84	.97	1.14	1.19	1.14	.56	.51	.51	.38	.49	.76	.56	.67
Means <sup>a</sup>	1.73 <sup>b</sup>	$1.02^{cd}$	.99 <sup>cd</sup>	1.16 <sup>c</sup>	1.14 <sup>c</sup>	.87 <sup>cd</sup>	.57 <sup>e</sup>	.45 <sup>ef</sup>	.42 <sup>ef</sup>	.33 <sup>f</sup>	.77 <sup>d</sup>	.46 <sup>ef</sup>	.49 <sup>ef</sup>

Appendix B table 3. Average daily gain (kg/d) of foals fed a 14% crude protein supplement

<sup>a</sup> Least squares SE of means = 0.07 <sup>b, c, d, e, f,</sup> Values with different superscripts are different at P < .10

						L	Г Supplem	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98
1	.86	.62	1.25	1.35	1.36	1.19	.67	.51	.40	.40	.95	.63	.76
2	2.24	1.16	.92	1.13	1.19	1.08	.63	.05	.57	02	1.22	.41	.27
3	1.79	1.00	.94	.98	.97	.79	.51	.41	.35	.44	1.14	.57	.35
4	1.76	1.17	1.28	1.46	1.08	1.14	.87	.44	.30	.33	.98	.63	.41
5	1.87	.79	.67	.89	.89	.90	.51	.30	.11	.44	.63	.38	.00
6	1.70	1.05	1.13	1.14	1.25	1.00	.70	.57	.56	.48	.87	.52	.49
7	1.62	1.03	1.19	1.32	1.24	1.17	.75	.54	.54	.30	1.05	.65	.38
8	2.05	.95	1.25	1.11	1.44	1.33	.84	.48	.49	.27	.63	22	1.06
9	1.98	.79	1.03	1.05	1.13	1.09	.92	.38	.35	.40	1.33	.68	.17
10	1.79	1.00	1.00	.94	1.03	.94	.17	.27	.40	.06	1.30	.48	.46
11	1.60	1.21	1.14	1.19	1.00	1.17	.86	.60	.36	.46	1.13	.75	.56
12	2.20	.82	1.05	1.14	1.14	1.21	.68	.46	.48	.06	.98	.79	.11
Means <sup>a</sup>	1.79 <sup>b</sup>	.97 <sup>cd</sup>	1.07 <sup>cd</sup>	1.14 <sup>c</sup>	1.14 <sup>c</sup>	1.09 <sup>cd</sup>	.68 <sup>e</sup>	.42 <sup>ef</sup>	.41 <sup>ef</sup>	.30 <sup>f</sup>	1.02 <sup>d</sup>	.52 <sup>ef</sup>	.42 <sup>ef</sup>

Appendix B table 4. Average daily gain (kg/d) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

<sup>a</sup>Least squares SE of means = 0.06<sup>b, c, d, e, f,</sup> Values with different superscripts are different at P < .10

					Contr	ol Supple	ment					
Horse	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	4.5	4.5	4.5	4.5	4.5	4.5	4.0	4.0	4.0	4.5	5.0	5.0
2	5.0	4.5	5.0	5.0	5.5	5.0	5.0	5.0	5.5	5.0	4.5	4.5
3	5.5	5.0	4.5	5.0	5.0	4.5	4.5	4.5	4.5	4.5	5.0	4.5
4	5.0	5.0	5.0	5.0	5.0	4.5	5.0	5.0	5.0	4.5	5.0	5.5
5	5.0	4.5	5.0	5.5	5.0	5.0	5.0	4.5	5.0	4.5	4.5	5.0
6	5.0	5.0	5.0	5.0	5.0	5.0	4.5	4.5	4.5	5.0	5.0	5.0
7	5.0	5.0	5.0	5.0	5.0	5.0	5.5	5.0	4.5	5.0	5.0	5.0
8	5.5	5.0	5.0	5.5	5.5	5.0	5.0	5.0	5.0	5.5	5.5	5.0
9	5.0	5.0	5.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.5	5.0
10	5.0	5.0	5.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.5
Means <sup>b</sup>	5.1	4.9	5.0	5.1	5.1	4.9	4.9	4.8	4.8	4.9	5.0	5.0

Appendix B table 5. Body condition<sup>a</sup> of foals fed a 14% crude protein supplement

<sup>a</sup> Body condition measured on a scale of 1-10 (Henneke et al., 1983) <sup>b</sup> Least squares SE of means = 0.08

					LT	Supplem	ent					
Horse	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	4.5	5.0	5.0	5.5	5.0	5.0	5.5	5.0	5.0	5.0	5.5	5.5
2	4.5	4.5	5.0	5.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.5
3	5.0	5.0	5.0	5.0	5.0	4.5	5.0	4.5	4.5	5.0	5.0	5.0
4	5.0	5.0	5.0	5.0	5.0	4.5	5.0	5.0	5.0	5.0	5.5	5.5
5	5.0	5.0	5.0	5.5	5.0	5.0	5.0	4.5	4.5	5.0	5.5	5.5
6	5.0	4.5	4.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.5
7	4.5	4.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
8	4.5	4.5	5.0	5.0	5.5	5.0	5.0	5.0	5.0	5.0	4.5	5.0
9	5.0	5.0	4.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.5	5.5
10	5.0	4.0	5.0	5.0	5.0	4.0	4.5	5.0	5.0	4.5	5.0	5.0
11	5.0	4.5	4.5	5.0	5.0	4.5	5.0	4.5	4.5	4.5	5.0	5.0
12	5.0	4.5	5.0	5.0	5.0	4.5	5.0	5.0	5.0	4.5	5.0	5.0
Means <sup>b</sup>	4.8	4.6	4.9	5.1	5.0	4.8	5.0	4.9	4.9	4.9	5.1	5.3

Appendix B table 6. Body condition<sup>a</sup> of foals fed a 9% crude protein supplement, fortified with lysine and threonine

<sup>a</sup> Body condition measured on a scale of 1-10 (Henneke et al., 1983) <sup>b</sup> Least squares SE of means = 0.07

		Control Supplement												
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	106.7	119.4	121.9	132.1	134.6	137.2	139.7	142.2	144.8	147.3	147.3	147.3	149.9	149.9
2	116.8	127.0	129.5	134.6	139.7	142.2	142.2	147.3	149.9	147.3	149.9	152.4	154.9	152.4
3	106.7	116.8	127.0	129.5	134.6	139.7	139.7	142.2	144.8	147.3	147.3	147.3	149.9	149.9
4	114.3	119.4	124.5	132.1	137.2	139.7	142.2	144.8	147.3	147.3	149.9	149.9	149.9	152.4
5	116.8	127.0	129.5	132.1	137.2	139.7	142.2	142.2	144.8	144.8	147.3	149.9	147.3	149.9
6	109.2	121.9	121.9	129.5	137.2	139.7	139.7	144.8	147.3	144.8	147.3	149.9	149.9	149.9
7	106.7	114.3	124.5	129.5	137.2	142.2	139.7	144.8	144.8	147.3	152.4	149.9	152.4	152.4
8	106.7	111.8	124.5	127.0	137.2	139.7	142.2	142.2	147.3	144.8	144.8	149.9	149.9	147.3
9	109.2	119.4	124.5	129.5	137.2	137.2	139.7	144.8	142.2	144.8	147.3	147.3	147.3	149.9
10	106.7	116.8	121.9	129.5	137.2	139.7	139.7	142.2	144.8	144.8	147.3	147.3	147.3	149.9
Means <sup>a</sup>	110.0	119.4	125.0	130.6	136.9	139.7	140.7	143.8	145.8	146.1	148.1	149.1	149.9	150.4

Appendix B table 7. Wither height (cm) of foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	116.8	121.9	127.0	132.1	139.7	139.7	144.8	144.8	147.3	147.3	147.3	149.9	152.4	152.4
2	109.2	121.9	124.5	129.5	137.2	139.7	139.7	144.8	144.8	144.8	149.9	149.9	147.3	152.4
3	114.3	127.0	127.0	134.6	137.2	139.7	142.2	142.2	144.8	147.3	147.3	147.3	149.9	149.9
4	106.7	116.8	124.5	132.1	134.6	139.7	142.2	144.8	147.3	147.3	147.3	149.9	152.4	152.4
5	104.1	114.3	116.8	121.9	124.5	132.1	129.5	132.1	137.2	134.6	134.6	137.2	137.2	137.2
6	101.6	111.8	119.4	124.5	132.1	137.2	139.7	142.2	144.8	144.8	144.8	147.3	147.3	149.9
7	111.8	124.5	124.5	129.5	137.2	142.2	144.8	144.8	147.3	149.9	152.4	149.9	154.9	154.9
8	111.8	127.0	129.5	134.6	139.7	142.2	144.8	144.8	147.3	147.3	152.4	152.4	149.9	152.4
9	111.8	121.9	124.5	132.1	134.6	139.7	139.7	142.2	147.3	147.3	147.3	149.9	149.9	149.9
10	116.8	127.0	132.1	134.6	139.7	142.2	144.8	144.8	147.3	149.9	152.4	154.9	154.9	152.4
11	101.6	114.3	116.8	124.5	132.1	132.1	137.2	142.2	144.8	144.8	147.3	149.9	147.3	152.4
12	111.8	121.9	124.5	132.1	137.2	139.7	142.2	144.8	149.9	147.3	149.9	152.4	149.9	152.4
Means <sup>a</sup>	109.9	120.9	124.2	130.2	135.5	138.9	141.0	142.9	145.8	146.1	147.7	149.2	149.4	150.7

Appendix B table 8. Wither height (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

		Control Supplement												
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	109.2	124.5	127.0	134.6	139.7	142.2	142.2	147.3	147.3	149.9	152.4	149.9	152.4	152.4
2	119.4	129.5	129.5	137.2	142.2	147.3	147.3	149.9	152.4	149.9	152.4	154.9	154.9	154.9
3	109.2	119.4	127.0	129.5	137.2	142.2	142.2	144.8	147.3	147.3	149.9	147.3	152.4	152.4
4	111.8	127.0	127.0	134.6	139.7	142.2	147.3	147.3	147.3	149.9	152.4	152.4	152.4	154.9
5	119.4	132.1	134.6	137.2	139.7	142.2	144.8	147.3	149.9	149.9	149.9	152.4	149.9	152.4
6	111.8	124.5	129.5	132.1	137.2	142.2	142.2	147.3	147.3	149.9	149.9	152.4	152.4	152.4
7	109.2	116.8	124.5	132.1	139.7	144.8	144.8	147.3	147.3	149.9	152.4	152.4	152.4	154.9
8	109.2	119.4	127.0	129.5	137.2	142.2	144.8	147.3	149.9	149.9	149.9	149.9	149.9	149.9
9	109.2	124.5	127.0	132.1	139.7	142.2	142.2	147.3	144.8	149.9	152.4	152.4	149.9	149.9
10	111.8	121.9	127.0	134.6	139.7	142.2	142.2	144.8	144.8	147.3	149.9	149.9	149.9	152.4
Means <sup>a</sup>	112.0	124.0	128.0	133.4	139.2	143.0	144.0	147.1	147.8	149.4	151.1	151.4	151.6	152.7

Appendix B table 9. Hip height (cm) of foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	119.4	124.5	132.1	132.1	139.7	144.8	147.3	147.3	149.9	149.9	152.4	149.9	152.4	154.9
2	111.8	127.0	127.0	132.1	139.7	142.2	144.8	147.3	147.3	147.3	152.4	152.4	149.9	149.9
3	116.8	129.5	129.5	137.2	139.7	142.2	144.8	144.8	147.3	149.9	149.9	149.9	152.4	152.4
4	109.2	119.4	127.0	134.6	137.2	142.2	144.8	147.3	149.9	149.9	149.9	149.9	152.4	154.9
5	99.1	114.3	121.9	124.5	127.0	132.1	132.1	134.6	137.2	137.2	137.2	139.7	137.2	137.2
6	101.6	116.8	121.9	127.0	134.6	142.2	142.2	144.8	147.3	147.3	147.3	149.9	147.3	149.9
7	111.8	124.5	127.0	134.6	139.7	144.8	147.3	147.3	149.9	149.9	152.4	152.4	157.5	157.5
8	114.3	127.0	132.1	137.2	139.7	144.8	147.3	147.3	149.9	149.9	154.9	152.4	152.4	152.4
9	114.3	124.5	127.0	134.6	137.2	139.7	142.2	144.8	147.3	149.9	149.9	149.9	152.4	152.4
10	119.4	132.1	137.2	139.7	142.2	144.8	147.3	147.3	149.9	149.9	152.4	157.5	154.9	154.9
11	101.6	114.3	121.9	127.0	134.6	132.1	139.7	142.2	147.3	147.3	149.9	149.9	149.9	152.4
12	111.8	127.0	129.5	137.2	139.7	142.2	147.3	147.3	149.9	149.9	152.4	154.9	152.4	154.9
Means <sup>a</sup>	110.9	123.4	127.8	133.1	137.6	141.2	143.9	145.2	147.7	148.2	150.1	150.7	150.9	152.0

Appendix B table 10. Hip height (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	88.9	109.2	115.6	121.9	128.3	132.1	142.2	143.5	147.3	149.9	152.4	154.9	157.5	158.8
2	101.6	120.7	124.5	132.1	137.2	144.8	154.9	154.9	162.6	160.0	163.8	166.4	166.4	167.6
3	91.4	106.7	113.0	123.2	128.3	134.6	144.8	144.8	147.3	149.9	152.4	157.5	157.5	160.0
4	97.8	110.5	116.8	129.5	133.4	142.2	151.1	149.9	152.4	154.9	156.2	158.8	162.6	165.1
5	102.9	120.7	125.7	132.1	134.6	141.0	149.9	149.9	153.7	154.9	157.5	160.0	158.8	160.0
6	96.5	115.6	116.8	124.5	128.3	134.6	141.0	144.8	144.8	151.1	152.4	154.9	158.8	158.8
7	92.7	108.0	114.3	123.2	128.3	138.4	144.8	149.9	153.7	154.9	156.2	160.0	167.6	166.4
8	87.6	113.0	118.1	125.7	132.1	142.2	149.9	152.4	157.5	156.2	157.5	162.6	165.1	168.9
9	91.4	116.8	120.7	129.5	132.1	142.2	147.3	149.9	152.4	154.9	158.8	160.0	160.0	162.6
10	92.7	114.3	120.7	129.5	134.6	142.2	148.6	152.4	154.9	157.5	157.5	161.3	167.6	170.2
Means <sup>a</sup>	94.4	113.5	118.6	127.1	131.7	139.4	147.4	149.2	152.7	154.4	156.5	159.6	162.2	163.8

Appendix B table 11. Girth (cm) of foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	106.7	116.8	120.7	129.5	134.6	144.8	152.4	154.9	156.2	161.3	162.6	165.1	167.6	174.0
2	94.0	116.8	120.7	129.5	132.1	143.5	154.9	156.2	152.4	156.2	154.9	158.8	161.3	162.6
3	100.3	116.8	125.7	129.5	134.6	142.2	147.3	149.9	152.4	157.5	158.8	160.0	162.6	165.1
4	90.2	109.2	118.1	127.0	135.9	142.2	153.7	156.2	156.2	158.8	161.3	163.8	170.2	168.9
5	91.4	111.8	119.4	124.5	132.1	139.7	148.6	149.9	152.4	151.1	152.4	156.2	157.5	158.8
6	82.6	106.7	113.0	121.9	129.5	137.2	144.8	149.9	153.7	156.2	156.2	160.0	165.1	167.6
7	92.7	110.5	116.8	127.0	133.4	142.2	149.9	152.4	156.2	157.5	161.3	165.1	167.6	170.2
8	94.0	116.8	120.7	128.3	134.6	144.8	152.4	154.9	156.2	157.5	160.0	162.6	162.6	165.1
9	99.1	114.3	121.9	127.0	132.1	141.0	149.9	153.7	156.2	156.2	157.5	166.4	167.6	170.2
10	99.1	116.8	120.7	127.0	134.6	139.7	147.3	146.1	152.4	152.4	153.7	157.5	161.3	162.6
11	80.0	102.9	110.5	120.7	125.7	134.6	139.7	146.1	149.9	152.4	154.9	162.6	162.6	167.6
12	88.9	113.0	119.4	125.7	130.8	138.4	148.6	148.6	153.7	152.4	156.2	157.5	160.0	162.6
Means <sup>a</sup>	93.2	112.7	119.0	126.5	132.5	140.9	149.1	151.6	154.0	155.8	157.5	161.3	163.8	166.3

Appendix B table 12. Girth (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	86.4	106.7	118.1	124.5	130.8	138.4	138.4	146.1	146.1	149.9	156.2	157.5	162.6	161.3
2	97.8	119.4	121.9	134.6	134.6	143.5	146.1	144.8	152.4	154.9	158.8	163.8	161.3	162.6
3	82.6	99.1	106.7	119.4	121.9	130.8	134.6	138.4	143.5	142.2	143.5	153.7	152.4	154.9
4	88.9	109.2	121.9	123.2	135.9	141.0	138.4	139.7	148.6	148.6	152.4	160.0	161.3	161.3
5	95.3	115.6	130.8	128.3	133.4	139.7	139.7	139.7	146.1	144.8	152.4	154.9	156.2	160.0
6	87.6	110.5	118.1	121.9	125.7	130.8	133.4	141.0	142.2	148.6	147.3	154.9	158.8	160.0
7	82.6	106.7	116.8	124.5	127.0	133.4	138.4	142.2	147.3	152.4	152.4	154.9	160.0	166.4
8	80.0	104.1	120.7	128.3	124.5	134.6	139.7	142.2	147.3	152.4	152.4	152.4	156.2	161.3
9	83.8	111.8	115.6	127.0	129.5	138.4	142.2	141.0	147.3	147.3	149.9	156.2	156.2	160.0
10	83.8	106.7	113.0	123.2	124.5	134.6	134.6	139.7	144.8	143.5	147.3	152.4	153.7	158.8
Means <sup>a</sup>	86.9	109.0	118.4	125.5	128.8	136.5	138.6	141.5	146.6	148.5	151.3	156.1	157.9	160.7

Appendix B table 13. Length (cm) of foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	100.3	108.0	113.0	120.7	125.7	134.6	137.2	142.2	147.3	148.6	148.6	156.2	157.5	161.3
2	87.6	113.0	124.5	125.7	132.1	138.4	141.0	141.0	144.8	151.1	152.4	158.8	161.3	162.6
3	91.4	104.1	125.7	121.9	134.6	135.9	137.2	137.2	143.5	147.3	152.4	156.2	157.5	158.8
4	88.9	101.6	116.8	118.1	130.8	135.9	141.0	142.2	147.3	152.4	149.9	157.5	158.8	160.0
5	83.8	99.1	108.0	105.4	116.8	119.4	127.0	130.8	132.1	130.8	133.4	142.2	141.0	146.1
6	80.0	100.3	115.6	116.8	130.8	128.3	134.6	143.5	149.9	149.9	152.4	156.2	158.8	162.6
7	77.5	102.9	113.0	121.9	134.6	134.6	141.0	146.1	146.1	152.4	148.6	157.5	157.5	158.8
8	86.4	110.5	119.4	129.5	135.9	143.5	142.2	152.4	149.9	157.5	156.2	158.8	157.5	162.6
9	88.9	108.0	119.4	121.9	130.8	139.7	135.9	141.0	141.0	152.4	151.1	152.4	160.0	161.3
10	91.4	113.0	116.8	125.7	132.1	144.8	139.7	143.5	141.0	148.6	142.2	151.1	147.3	156.2
11	76.2	96.5	109.2	120.7	130.8	132.1	141.0	144.8	146.1	152.4	156.2	157.5	161.3	162.6
12	83.8	109.2	115.6	124.5	124.5	135.9	139.7	142.2	147.3	149.9	147.3	156.2	160.0	163.8
Means <sup>a</sup>	86.4	105.5	116.4	121.1	130.0	135.3	138.1	142.2	144.7	149.4	149.2	155.0	156.5	159.7

Appendix B table 14. Length (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	34.3	36.8	35.6	33.0	35.6	38.1	40.6	39.4	40.6	40.6	41.9	41.9	44.5	44.5
2	30.5	35.6	34.3	34.3	36.8	36.8	38.1	38.1	39.4	40.6	41.9	41.9	44.5	44.5
3	29.2	34.3	33.0	34.3	33.0	38.1	39.4	39.4	40.6	40.6	41.9	41.9	43.2	44.5
4	29.2	35.6	34.3	36.8	36.8	36.8	38.1	39.4	39.4	40.6	41.9	40.6	43.2	44.5
5	31.8	38.1	36.8	35.6	36.8	39.4	40.6	40.6	41.9	40.6	41.9	43.2	45.7	44.5
6	33.0	35.6	34.3	34.3	35.6	38.1	38.1	39.4	40.6	40.6	40.6	40.6	43.2	44.5
7	27.9	33.0	33.0	33.0	35.6	39.4	39.4	40.6	40.6	40.6	41.9	40.6	44.5	44.5
8	33.0	36.8	30.5	33.0	34.3	36.8	39.4	38.1	40.6	40.6	40.6	40.6	43.2	44.5
9	30.5	35.6	33.0	31.8	36.8	38.1	39.4	40.6	40.6	39.4	40.6	41.9	43.2	44.5
10	30.5	35.6	33.0	34.3	34.3	36.8	38.1	39.4	38.1	39.4	40.6	41.9	43.2	43.2
Means <sup>a</sup>	31.0	35.7	33.8	34.0	35.6	37.8	39.1	39.5	40.3	40.4	41.4	41.5	43.8	44.3

Appendix B table 15. Forearm length (cm) of foals fed a 14% crude protein supplement

							IТ	Suppleme	ont					
							LI	Suppleme	int					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	30.5	35.6	31.8	33.0	34.3	35.6	38.1	38.1	38.1	40.6	41.9	41.9	43.2	44.5
2	33.0	35.6	35.6	36.8	34.3	40.6	39.4	39.4	40.6	39.4	41.9	41.9	43.2	44.5
3	34.3	35.6	33.0	35.6	36.8	39.4	38.1	39.4	40.6	40.6	41.9	41.9	43.2	44.5
4	30.5	34.3	34.3	33.0	36.8	36.8	38.1	39.4	40.6	40.6	41.9	43.2	44.5	45.7
5	30.5	31.8	31.8	33.0	33.0	35.6	34.3	35.6	36.8	35.6	35.6	38.1	38.1	40.6
6	27.9	33.0	30.5	33.0	35.6	36.8	39.4	36.8	39.4	39.4	39.4	40.6	41.9	43.2
7	31.8	34.3	34.3	33.0	35.6	38.1	39.4	40.6	40.6	40.6	41.9	41.9	44.5	45.7
8	34.3	34.3	35.6	39.4	38.1	39.4	39.4	40.6	40.6	40.6	41.9	44.5	43.2	45.7
9	33.0	35.6	33.0	35.6	38.1	38.1	39.4	39.4	40.6	41.9	40.6	43.2	44.5	45.7
10	35.6	38.1	38.1	34.3	38.1	40.6	40.6	41.9	41.9	43.2	44.5	44.5	45.7	47.0
11	29.2	33.0	33.0	33.0	35.6	36.8	38.1	38.1	38.1	39.4	39.4	40.6	44.5	44.5
12	34.3	36.8	35.6	33.0	38.1	38.1	39.4	40.6	40.6	40.6	43.2	44.5	43.2	45.7
Means <sup>a</sup>	32.1	34.8	33.9	34.4	36.2	38.0	38.6	39.2	39.9	40.2	41.2	42.2	43.3	44.8

Appendix B table 16. Forearm length (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	31.8	30.5	30.5	30.5	31.8	30.5	30.5	31.8	33.0	30.5	31.8	31.8	31.8	31.8
2	30.5	33.0	31.8	31.8	31.8	31.8	31.8	31.8	33.0	31.8	31.8	31.8	31.8	33.0
3	29.2	30.5	29.2	30.5	29.2	31.8	31.8	30.5	31.8	31.8	31.8	30.5	31.8	33.0
4	27.9	31.8	30.5	30.5	30.5	30.5	31.8	30.5	30.5	33.0	31.8	31.8	33.0	33.0
5	33.0	31.8	30.5	30.5	30.5	30.5	31.8	30.5	31.8	31.8	30.5	30.5	33.0	31.8
6	31.8	30.5	30.5	30.5	29.2	31.8	30.5	30.5	31.8	33.0	31.8	31.8	33.0	33.0
7	29.2	30.5	30.5	31.8	31.8	30.5	31.8	31.8	33.0	31.8	31.8	31.8	33.0	33.0
8	31.8	31.8	30.5	30.5	31.8	31.8	33.0	31.8	33.0	31.8	31.8	33.0	33.0	33.0
9	31.8	31.8	30.5	30.5	31.8	31.8	31.8	30.5	31.8	31.8	31.8	30.5	31.8	31.8
10	29.2	31.8	27.9	29.2	30.5	29.2	29.2	30.5	30.5	30.5	30.5	30.5	31.8	30.5
Means <sup>a</sup>	30.6	31.4	30.2	30.6	30.9	31.0	31.4	31.0	32.0	31.8	31.5	31.4	32.4	32.4

Appendix B table 17. Front cannon length (cm) of foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	30.5	31.8	30.5	29.2	31.8	31.8	31.8	31.8	31.8	31.8	31.8	31.8	33.0	33.0
2	31.8	31.8	30.5	30.5	30.5	31.8	30.5	30.5	30.5	31.8	30.5	31.8	33.0	33.0
3	31.8	31.8	30.5	31.8	30.5	31.8	30.5	31.8	31.8	31.8	30.5	31.8	31.8	31.8
4	29.2	30.5	31.8	30.5	30.5	30.5	33.0	31.8	33.0	31.8	31.8	30.5	33.0	31.8
5	29.2	29.2	27.9	27.9	27.9	29.2	26.7	27.9	27.9	29.2	27.9	29.2	29.2	29.2
6	26.7	29.2	30.5	29.2	30.5	30.5	30.5	31.8	31.8	31.8	30.5	30.5	31.8	31.8
7	30.5	31.8	30.5	30.5	31.8	33.0	31.8	29.2	31.8	33.0	31.8	31.8	34.3	34.3
8	31.8	31.8	31.8	33.0	31.8	31.8	31.8	33.0	34.3	33.0	33.0	33.0	31.8	34.3
9	30.5	31.8	29.2	30.5	30.5	31.8	30.5	31.8	31.8	31.8	30.5	31.8	31.8	31.8
10	31.8	31.8	31.8	29.2	31.8	33.0	31.8	30.5	31.8	31.8	31.8	31.8	33.0	31.8
11	27.9	30.5	29.2	27.9	29.2	29.2	30.5	31.8	31.8	30.5	30.5	31.8	31.8	33.0
12	30.5	31.8	31.8	30.5	33.0	30.5	31.8	31.8	31.8	30.5	30.5	31.8	33.0	31.8
Means <sup>a</sup>	30.2	31.1	30.5	30.1	30.8	31.2	30.9	31.1	31.6	31.5	30.9	31.4	32.3	32.3

Appendix B table 18. Front cannon length (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	44.5	43.2	41.9	43.2	43.2	43.2	43.2	44.5	43.2	43.2	44.5	44.5	45.7	44.5
2	41.9	43.2	44.5	43.2	44.5	45.7	44.5	45.7	45.7	45.7	45.7	45.7	45.7	45.7
3	39.4	40.6	40.6	41.9	41.9	43.2	44.5	43.2	44.5	44.5	44.5	44.5	43.2	45.7
4	38.1	43.2	41.9	43.2	43.2	44.5	43.2	43.2	44.5	45.7	45.7	47.0	47.0	45.7
5	44.5	45.7	41.9	41.9	43.2	43.2	44.5	44.5	43.2	43.2	45.7	44.5	45.7	44.5
6	43.2	44.5	41.9	41.9	41.9	43.2	41.9	43.2	43.2	44.5	43.2	44.5	44.5	45.7
7	39.4	43.2	41.9	41.9	44.5	43.2	43.2	43.2	44.5	44.5	44.5	45.7	45.7	47.0
8	40.6	41.9	41.9	41.9	43.2	43.2	44.5	43.2	44.5	44.5	44.5	45.7	44.5	45.7
9	39.4	41.9	40.6	40.6	43.2	44.5	43.2	44.5	43.2	43.2	43.2	45.7	45.7	45.7
10	40.6	43.2	41.9	43.2	41.9	43.2	43.2	43.2	44.5	44.5	43.2	43.2	44.5	45.7
Means <sup>a</sup>	41.1	43.1	41.9	42.3	43.1	43.7	43.6	43.8	44.1	44.3	44.5	45.1	45.2	45.6

Appendix B table 19. Hind cannon bone lengths (cm) of foals fed a 14% crude protein supplement

							LT	Supplem	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	41.9	43.2	41.9	41.9	43.2	43.2	43.2	44.5	44.5	44.5	44.5	45.7	45.7	45.7
2	43.2	41.9	41.9	43.2	41.9	43.2	43.2	44.5	43.2	44.5	43.2	44.5	44.5	44.5
3	44.5	43.2	41.9	40.6	41.9	43.2	41.9	43.2	43.2	43.2	44.5	43.2	45.7	44.5
4	39.4	40.6	41.9	40.6	43.2	43.2	43.2	43.2	43.2	44.5	43.2	44.5	44.5	44.5
5	39.4	39.4	38.1	40.6	39.4	39.4	39.4	39.4	40.6	39.4	40.6	39.4	40.6	40.6
6	38.1	40.6	40.6	40.6	40.6	40.6	43.2	43.2	43.2	43.2	43.2	41.9	44.5	44.5
7	40.6	43.2	41.9	41.9	43.2	43.2	44.5	44.5	43.2	43.2	44.5	44.5	45.7	45.7
8	44.5	43.2	43.2	43.2	43.2	44.5	44.5	44.5	45.7	47.0	45.7	47.0	45.7	47.0
9	41.9	43.2	41.9	41.9	43.2	43.2	43.2	45.7	43.2	44.5	43.2	44.5	44.5	45.7
10	44.5	44.5	43.2	41.9	44.5	45.7	44.5	43.2	44.5	44.5	44.5	45.7	45.7	45.7
11	35.6	38.1	38.1	40.6	40.6	41.9	41.9	43.2	43.2	43.2	43.2	43.2	43.2	43.2
12	44.5	41.9	41.9	41.9	44.5	43.2	44.5	43.2	43.2	44.5	44.5	44.5	45.7	45.7
Means <sup>a</sup>	41.5	41.9	41.4	41.6	42.4	42.9	43.1	43.5	43.4	43.8	43.7	44.0	44.7	44.8

Appendix B table 20. Hind cannon bone lengths (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	24.1	27.9	27.9	27.9	27.9	30.5	30.5	30.5	30.5	30.5	30.5	31.8	31.8	33.0
2	26.7	30.5	27.9	30.5	29.2	30.5	30.5	30.5	30.5	31.8	31.8	31.8	33.0	33.0
3	24.1	26.7	26.7	27.9	26.7	27.9	30.5	30.5	29.2	30.5	30.5	30.5	30.5	30.5
4	25.4	27.9	27.9	27.9	29.2	29.2	29.2	29.2	29.2	30.5	29.2	30.5	30.5	31.8
5	25.4	27.9	27.9	27.9	29.2	29.2	27.9	27.9	29.2	30.5	30.5	30.5	30.5	30.5
6	24.1	27.9	27.9	27.9	27.9	27.9	29.2	29.2	30.5	30.5	31.8	30.5	31.8	30.5
7	25.4	27.9	27.9	29.2	30.5	30.5	30.5	30.5	30.5	31.8	33.0	31.8	33.0	33.0
8	25.4	27.9	27.9	26.7	27.9	30.5	30.5	29.2	30.5	30.5	31.8	30.5	31.8	31.8
9	24.1	27.9	26.7	27.9	27.9	29.2	30.5	29.2	30.5	30.5	30.5	30.5	31.8	30.5
10	24.1	26.7	26.7	27.9	26.7	29.2	30.5	29.2	30.5	30.5	30.5	30.5	30.5	30.5
Means <sup>a</sup>	24.9	27.9	27.6	28.2	28.3	29.5	30.0	29.6	30.1	30.7	31.0	30.9	31.5	31.5

Appendix B table 21. Physis circumference (cm) of foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	26.7	27.9	26.7	29.2	29.2	29.2	30.5	29.2	30.5	30.5	31.8	31.8	31.8	31.8
2	25.4	27.9	27.9	27.9	29.2	29.2	30.5	30.5	29.2	30.5	30.5	31.8	33.0	31.8
3	25.4	27.9	26.7	27.9	27.9	29.2	29.2	29.2	29.2	30.5	30.5	31.8	30.5	30.5
4	22.9	26.7	26.7	27.9	29.2	29.2	27.9	29.2	29.2	30.5	30.5	30.5	30.5	31.8
5	22.9	25.4	25.4	26.7	26.7	26.7	27.9	27.9	27.9	27.9	27.9	29.2	29.2	27.9
6	22.9	26.7	25.4	26.7	27.9	27.9	29.2	27.9	30.5	30.5	30.5	31.8	31.8	31.8
7	25.4	27.9	27.9	27.9	27.9	29.2	29.2	29.2	29.2	30.5	30.5	31.8	31.8	30.5
8	25.4	29.2	29.2	30.5	30.5	31.8	30.5	31.8	30.5	31.8	31.8	31.8	33.0	34.3
9	24.1	27.9	26.7	27.9	29.2	29.2	29.2	30.5	30.5	30.5	30.5	31.8	31.8	31.8
10	25.4	27.9	26.7	27.9	27.9	29.2	29.2	27.9	30.5	30.5	30.5	30.5	31.8	31.8
11	22.9	25.4	25.4	26.7	27.9	27.9	29.2	29.2	29.2	30.5	30.5	31.8	31.8	31.8
12	24.1	27.9	26.7	27.9	27.9	27.9	29.2	27.9	29.2	30.5	29.2	30.5	31.8	30.5
Means <sup>a</sup>	24.4	27.4	26.8	27.9	28.5	28.9	29.3	29.2	29.6	30.4	30.4	31.2	31.5	31.3

Appendix B table 22. Physis circumference (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	20.3	22.9	21.6	24.1	24.1	24.1	25.4	25.4	25.4	25.4	26.7	26.7	26.7	25.4
2	21.6	24.1	22.9	24.1	24.1	25.4	26.7	26.7	27.9	26.7	26.7	27.9	27.9	27.9
3	20.3	21.6	22.9	24.1	24.1	24.1	25.4	25.4	25.4	25.4	26.7	26.7	26.7	26.7
4	21.6	22.9	22.9	24.1	24.1	25.4	25.4	25.4	25.4	25.4	25.4	26.7	25.4	26.7
5	21.6	24.1	24.1	24.1	24.1	25.4	25.4	25.4	25.4	25.4	25.4	25.4	26.7	26.7
6	21.6	22.9	21.6	22.9	22.9	22.9	24.1	24.1	25.4	25.4	25.4	25.4	26.7	26.7
7	21.6	22.9	22.9	24.1	24.1	24.1	25.4	25.4	25.4	25.4	26.7	26.7	26.7	26.7
8	20.3	22.9	22.9	22.9	24.1	24.1	25.4	25.4	25.4	26.7	26.7	26.7	26.7	25.4
9	20.3	22.9	22.9	24.1	22.9	24.1	25.4	25.4	25.4	26.7	26.7	26.7	26.7	26.7
10	20.3	22.9	22.9	24.1	24.1	25.4	24.1	25.4	25.4	25.4	25.4	26.7	26.7	25.4
Means <sup>a</sup>	21.0	23.0	22.7	23.9	23.9	24.5	25.3	25.4	25.7	25.8	26.2	26.5	26.7	26.4

Appendix B table 23. Fetlock circumference (cm) of foals fed a 14% crude protein supplement

							I T	0 1	4					
							LI	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	22.9	24.1	22.9	22.9	25.4	25.4	25.4	25.4	25.4	25.4	26.7	26.7	26.7	26.7
2	21.6	22.9	22.9	22.9	24.1	25.4	25.4	25.4	25.4	26.7	25.4	25.4	26.7	26.7
3	20.3	22.9	22.9	22.9	24.1	24.1	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
4	20.3	22.9	22.9	24.1	24.1	24.1	25.4	25.4	25.4	25.4	25.4	26.7	25.4	25.4
5	19.1	21.6	20.3	22.9	22.9	22.9	22.9	24.1	24.1	24.1	24.1	24.1	24.1	24.1
6	19.1	21.6	22.9	22.9	24.1	24.1	25.4	25.4	25.4	25.4	25.4	26.7	26.7	26.7
7	20.3	22.9	22.9	22.9	24.1	25.4	24.1	25.4	25.4	25.4	25.4	26.7	26.7	26.7
8	21.6	24.1	24.1	25.4	25.4	25.4	26.7	26.7	26.7	26.7	27.9	27.9	27.9	27.9
9	20.3	22.9	22.9	22.9	24.1	24.1	24.1	25.4	25.4	25.4	25.4	25.4	26.7	26.7
10	20.3	22.9	22.9	22.9	24.1	25.4	24.1	25.4	25.4	25.4	25.4	25.4	25.4	26.7
11	17.8	21.6	21.6	22.9	22.9	24.1	24.1	24.1	25.4	25.4	25.4	25.4	25.4	26.7
12	20.3	22.9	22.9	24.1	24.1	25.4	25.4	24.1	25.4	25.4	25.4	25.4	26.7	26.7
Means <sup>a</sup>	20.3	22.8	22.6	23.3	24.1	24.7	24.9	25.2	25.4	25.5	25.6	25.9	26.1	26.4

Appendix B table 24. Fetlock circumference (cm) of foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	2.41	2.56	2.96	3.08	3.02	2.88	2.94	2.74	2.75	2.85	2.93	2.85	2.99	2.80
2	2.78	2.82	2.92	3.29	3.15	3.11	3.15	3.26	3.01	2.76	2.93	2.83	3.33	2.90
3	2.62	2.72	2.69	3.12	3.02	3.05	3.10	3.04	2.96	2.78	2.89	2.99	3.15	3.05
4	2.65	2.59	2.57	2.86	2.99	2.91	2.96	2.95	3.06	2.83	2.85	2.73	3.05	2.95
5	2.79	2.83	2.90	3.14	3.02	3.20	3.02	2.82	2.75	2.91	2.85	3.05	2.98	2.90
6	2.50	2.83	2.65	2.99	3.13	2.93	2.96	2.64	2.31	2.96	2.90	2.88	3.00	3.10
7	2.50	2.78	2.39	3.10	3.09	2.92	3.03	2.82	2.42	2.53	2.81	2.88	2.88	2.85
8	2.57	2.77	2.67	2.93	2.74	3.08	3.06	2.94	2.67	2.96	2.70	2.88	3.03	3.00
9	2.46	2.66	2.45	3.08	2.49	3.04	3.22	3.07	2.30	2.93	2.88	2.94	3.18	3.10
10	2.52	2.91	2.82	3.04	2.59	2.90	2.74	2.87	2.64	2.63	2.82	3.00	3.13	3.00
Means <sup>a</sup>	2.58	2.74	2.70	3.06	2.92	3.00	3.02	2.91	2.69	2.81	2.85	2.90	3.07	2.97

Appendix B table 25. Albumin level (g/dl) in plasma of foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	2.62	2.54	3.13	2.99	2.36	3.12	2.91	2.86	2.81	2.87	2.83	2.77	2.90	2.85
2	2.41	2.72	2.85	2.93	2.22	2.91	2.68	2.88	2.71	2.82	2.85	2.90	2.85	3.15
3	2.72	2.90	2.60	2.94	2.10	3.18	3.06	3.01	2.78	3.00	3.07	2.78	3.24	3.10
4	2.58	2.25	2.49	2.66	2.18	2.85	2.77	2.81	2.88	2.98	2.89	2.68	3.06	3.05
5	2.91	3.25	3.17	3.03	2.43	3.31	2.94	3.41	3.24	3.27	3.39	3.30	3.48	3.35
6	2.40	1.73	2.44	2.71	2.67	3.02	2.65	2.99	2.83	3.00	2.99	2.94	3.14	3.20
7	2.82	2.76	2.54	2.82	2.85	2.83	2.62	2.71	2.26	2.99	2.92	2.67	3.00	2.90
8	2.51	2.90	2.83	2.99	2.82	3.05	2.91	2.85	2.63	2.86	2.97	2.77	3.06	3.10
9	2.87	2.73	2.86	2.95	2.85	2.78	2.66	2.53	2.45	2.86	2.79	2.79	3.29	3.30
10	2.77	2.94	2.82	2.96	2.75	3.17	2.92	3.28	2.51	3.00	3.09	2.70	3.21	3.30
11	2.47	2.73	2.94	2.95	2.95	3.11	3.09	2.85	2.38	3.02	3.06	2.92	3.32	3.25
12	2.70	2.84	3.18	2.93	3.03	3.01	2.82	3.06	2.74	2.99	3.15	2.85	3.01	3.10
Means <sup>a</sup>	2.65	2.69	2.82	2.90	2.60	3.03	2.83	2.93	2.68	2.97	3.00	2.84	3.13	3.14

Appendix B table 26. Albumin level (g/dl) in plasma of foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	5.27	5.61	6.16	6.27	5.65	6.01	5.97	5.48	5.41	5.41	5.51	5.53	6.08	5.60
2	5.62	5.88	5.78	6.28	5.86	5.64	5.83	5.77	5.47	5.07	5.61	5.32	6.64	6.20
3	5.74	5.78	5.56	6.21	5.82	5.71	5.73	5.50	5.46	5.24	5.57	5.47	6.17	6.55
4	6.64	6.51	5.68	5.95	5.40	5.40	5.63	5.36	5.56	5.06	5.57	5.29	5.98	6.30
5	5.08	5.42	5.38	5.98	5.29	5.81	5.65	5.33	5.12	5.50	5.24	6.53	6.52	6.40
6	5.60	5.87	5.26	5.86	5.67	5.50	5.55	5.15	4.46	5.51	5.47	5.74	6.23	6.60
7	6.38	5.97	5.00	6.46	5.49	5.53	5.81	5.21	4.55	4.83	5.37	5.66	6.31	6.50
8	5.58	5.76	5.52	6.42	5.39	6.00	5.97	5.70	5.39	5.55	5.81	5.67	6.50	6.45
9	4.85	5.20	4.90	6.09	4.64	5.72	6.03	5.66	4.26	5.33	5.25	5.49	6.51	6.45
10	5.13	6.62	5.74	6.28	5.13	5.68	5.35	5.64	5.12	4.92	5.12	6.00	6.45	6.55
Means <sup>a</sup>	5.59	5.86	5.50	6.18	5.43	5.70	5.75	5.48	5.08	5.24	5.45	5.67	6.34	6.36

Appendix B table 27. Total protein (g/dl) levels in plasma of foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	5.29	5.77	7.48	6.43	4.61	5.85	5.05	5.10	5.38	5.53	5.55	5.70	6.35	5.95
2	5.20	5.16	5.33	5.65	4.31	5.63	5.08	5.41	5.14	5.54	6.19	5.87	6.40	6.55
3	5.06	5.76	5.03	5.89	4.18	5.93	5.70	5.48	5.29	5.96	6.17	5.97	6.89	6.90
4	4.80	4.17	4.95	5.28	4.08	5.21	4.88	5.00	5.48	5.91	6.04	5.48	6.30	6.35
5	6.02	6.36	6.02	5.93	4.59	5.99	5.46	6.21	5.92	6.31	6.32	6.25	6.38	6.50
6	4.97	3.62	5.22	5.88	5.34	6.01	5.03	5.68	5.41	6.00	6.14	6.32	6.73	6.80
7	5.81	5.42	5.44	5.91	5.77	5.97	5.47	5.98	5.00	6.53	6.77	6.20	6.75	6.30
8	5.13	5.97	5.66	5.74	5.13	5.85	5.28	5.47	5.16	5.54	5.54	5.80	6.41	6.90
9	5.92	5.47	5.46	5.75	5.36	5.74	5.81	5.26	5.09	5.73	5.90	5.96	6.73	6.75
10	4.62	5.68	5.29	5.65	5.19	5.94	5.04	5.92	5.02	5.50	6.05	5.85	6.71	6.85
11	4.98	5.47	5.72	5.96	5.20	5.41	5.77	5.58	4.78	5.80	5.90	5.88	6.51	6.45
12	5.69	5.41	6.06	5.57	5.51	5.35	4.81	5.65	5.43	5.70	6.09	5.79	6.18	7.05
Means <sup>a</sup>	5.29	5.35	5.64	5.80	4.94	5.74	5.28	5.56	5.26	5.83	6.05	5.92	6.53	6.61

Appendix B table 28. Total protein (g/dl) levels in plasma of foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Suppler	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	0.91	1.03	1.07	1.10	0.91	0.88	0.82	0.92	1.10	0.94	0.86	0.85	1.05	0.90
2	1.00	1.14	1.21	1.34	1.20	1.10	1.07	1.02	1.21	1.13	1.02	0.94	1.25	1.05
3	0.99	0.98	1.00	1.18	1.06	0.93	0.96	1.03	1.10	0.94	0.88	0.82	0.98	0.95
4	1.17	1.16	1.31	1.51	1.16	1.05	1.11	0.99	1.42	1.21	0.96	1.00	1.14	1.10
5	0.93	1.21	1.29	1.38	1.17	1.17	1.05	1.06	1.19	1.18	1.09	1.16	1.20	1.15
6	0.96	1.21	1.13	1.29	1.11	0.97	0.99	1.05	1.14	1.04	0.95	0.84	1.07	1.05
7	0.87	0.89	1.00	1.23	1.01	0.96	1.06	1.07	1.10	1.04	0.93	0.88	1.06	1.05
8	0.96	1.07	1.20	1.26	1.01	1.04	1.05	1.09	1.24	1.19	0.92	0.85	1.16	1.15
9	0.85	1.16	1.14	1.25	0.98	1.03	1.07	1.10	1.02	1.07	0.91	0.90	1.17	1.05
10	1.05	1.14	1.26	1.34	1.09	1.11	1.13	1.13	1.18	1.13	0.93	0.99	1.06	1.15
Means <sup>a</sup>	0.97	1.09	1.16	1.29	1.07	1.02	1.03	1.04	1.17	1.08	0.94	0.92	1.11	1.06

Appendix B table 29. Creatinine (mg/dl) levels in foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	1.10	1.16	1.51	1.15	1.06	0.97	0.90	1.20	1.26	1.18	1.08	0.98	1.05	1.30
2	0.83	1.03	1.15	1.09	0.94	0.97	0.86	1.00	1.01	0.98	0.94	0.84	1.25	1.20
3	0.94	1.06	1.13	1.14	0.86	0.93	0.90	0.95	1.03	0.93	0.91	0.83	1.06	1.10
4	1.31	1.11	1.39	1.37	1.09	1.17	1.06	1.12	1.17	1.14	1.01	0.87	1.19	1.20
5	0.88	0.91	1.12	0.99	0.84	0.77	0.72	0.86	0.90	0.89	0.77	0.77	0.99	1.00
6	1.06	0.87	1.46	1.31	1.31	1.21	1.06	1.13	1.26	1.16	1.02	1.01	1.22	1.35
7	1.15	1.10	1.36	1.20	1.16	1.04	0.99	1.12	0.99	1.10	0.95	0.92	1.02	1.40
8	1.20	1.57	1.69	1.57	1.25	1.18	1.13	1.23	1.29	1.26	1.18	1.09	1.27	1.10
9	0.74	0.92	1.07	0.96	0.96	0.83	0.83	0.90	1.00	0.98	0.91	0.78	1.10	1.15
10	0.87	0.99	1.21	1.14	1.11	0.97	0.83	1.02	0.96	0.95	0.97	0.89	0.99	1.10
11	1.12	1.08	1.45	1.24	1.12	1.07	0.82	1.08	1.04	1.06	0.97	0.86	1.08	1.10
12	0.98	1.06	1.15	1.06	1.00	0.82	0.80	0.96	0.91	0.90	0.79	0.81	0.95	1.00
Means <sup>a</sup>	1.01	1.07	1.30	1.18	1.06	0.99	0.91	1.04	1.07	1.04	0.96	0.89	1.09	1.17

Appendix B table 30. Creatinine (mg/dl) levels in foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Supple	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	8.96	9.64	14.25	16.65	16.75	17.26	23.46	21.23	25.20	21.99	21.88	25.74	22.00	21.50
2	7.96	9.21	14.57	18.79	19.19	18.96	23.43	21.80	25.31	25.55	23.38	28.05	22.37	24.00
3	7.68	8.47	10.20	13.94	14.72	15.42	16.75	19.57	23.10	22.69	20.92	26.97	21.63	24.50
4	9.00	10.21	11.92	15.49	17.75	15.09	24.59	19.86	24.75	23.01	18.19	26.86	23.10	24.00
5	7.94	10.10	11.72	13.62	14.93	15.11	19.32	19.88	20.19	20.63	18.98	12.88	19.73	21.50
6	9.28	10.98	16.17	17.72	20.34	20.05	23.81	23.91	24.25	27.58	24.71	29.72	24.19	26.00
7	7.75	6.27	8.75	14.05	15.74	15.22	23.35	22.35	22.45	23.10	19.63	22.98	19.41	24.00
8	12.93	11.12	11.34	11.66	12.41	14.96	22.92	21.74	22.92	24.16	17.59	25.12	22.35	24.00
9	5.33	8.81	12.22	16.36	16.41	18.33	24.63	24.07	22.80	23.84	21.69	28.33	20.24	25.50
10	8.62	9.98	12.98	15.62	15.59	17.76	22.94	23.61	24.65	26.06	20.54	26.64	21.74	25.00
Means <sup>a</sup>	8.54	9.48	12.41	15.39	16.38	16.81	22.52	21.80	23.56	23.86	20.75	25.33	21.67	24.00

Appendix B table 31. Plasma urea nitrogen (mg/dl) levels in foals fed a 14% crude protein supplement

							LT	Suppleme	ent					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	8.08	8.64	15.00	14.32	21.04	19.66	22.01	19.46	22.79	24.22	18.89	27.21	21.00	22.00
2	8.16	11.41	12.54	15.20	16.61	17.96	24.36	19.40	20.13	21.73	19.12	25.67	21.00	16.00
3	10.95	8.42	11.05	12.17	14.59	18.67	21.19	19.79	20.13	21.57	20.48	26.12	21.96	18.00
4	7.67	6.84	8.77	12.28	15.46	18.38	17.82	18.56	24.15	23.98	22.14	27.86	22.08	20.00
5	13.74	10.70	15.87	15.72	19.86	17.78	24.77	20.46	23.89	22.19	21.50	26.06	22.30	14.00
6	11.17	8.90	12.82	12.05	15.19	18.22	27.03	20.70	23.30	23.54	21.33	29.74	23.94	21.50
7	10.75	8.30	14.62	17.60	22.29	21.38	25.26	19.88	24.51	24.32	21.01	25.70	21.42	21.00
8	7.44	9.62	13.71	15.12	20.07	18.44	22.72	19.16	22.72	23.81	20.13	23.63	19.68	16.00
9	11.38	8.99	12.18	12.93	18.21	15.86	24.23	20.17	22.58	22.29	20.84	27.67	22.72	16.00
10	7.73	10.15	14.22	15.98	18.57	19.67	26.93	20.39	23.03	24.78	22.07	25.50	24.22	20.00
11	11.90	9.02	13.51	15.75	22.77	22.96	24.55	21.75	22.97	23.41	23.05	28.32	23.07	21.50
12	8.00	10.39	15.45	15.15	21.01	21.59	24.25	18.38	24.58	25.30	19.25	24.92	21.94	21.50
Means <sup>a</sup>	9.75	9.28	13.31	14.52	18.80	19.21	23.76	19.84	22.90	23.43	20.82	26.53	22.11	18.96

Appendix B table 32. Plasma urea nitrogen (mg/dl) levels in foals fed a 9% crude protein supplement, fortified with lysine and threonine

							Contr	ol Suppler	ment					
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	9.84	9.40	13.38	15.14	18.51	19.61	28.78	23.07	23.01	23.52	25.44	30.28	21.05	23.89
2	7.96	8.11	12.09	14.07	15.99	17.24	21.89	21.37	20.91	22.71	23.03	29.84	17.90	22.86
3	7.76	8.69	10.20	11.86	13.95	16.58	17.54	19.00	21.09	24.14	23.77	32.88	22.07	25.79
4	7.73	8.84	9.13	10.25	15.36	14.44	22.15	20.16	17.43	19.09	18.95	26.99	20.26	21.82
5	8.54	8.38	9.12	9.87	12.76	12.97	18.40	18.84	16.96	17.48	17.49	11.10	16.51	18.70
6	9.72	9.07	14.31	13.79	18.32	20.77	24.05	22.88	21.36	26.52	26.15	35.59	22.71	24.76
7	8.91	7.08	8.75	11.42	15.66	15.85	22.13	20.99	20.40	22.31	21.11	26.11	18.31	22.86
8	13.54	10.44	9.49	9.25	12.28	14.38	21.83	20.04	18.48	20.39	19.11	29.72	19.35	20.87
9	6.26	7.62	10.72	13.08	16.83	17.88	23.01	21.98	22.35	22.12	23.84	31.47	17.30	24.29
10	8.20	8.79	10.34	11.65	14.30	16.00	20.39	20.98	20.97	23.06	22.21	27.05	20.51	21.74
Means <sup>a</sup>	8.85	8.64	10.75	12.04	15.40	16.57	22.02	20.93	20.30	22.13	22.11	28.10	19.60	22.76

Appendix B table 33. Urea:Creatinine ratio of foals fed a 14% crude protein supplement
		LT Supplement												
Horse	May-97	Jun-97	Jul-97	Aug-97	Sep-97	Oct-97	Nov-97	Dec-97	Jan-98	Feb-98	Mar-98	Apr-98	May-98	Jun-98
1	7.34	7.48	9.93	12.45	19.84	20.37	24.59	16.28	18.08	20.53	17.57	27.77	20.00	16.92
2	9.83	11.13	10.90	14.01	17.67	18.61	28.33	19.40	19.93	22.17	20.45	30.74	16.80	13.33
3	11.71	7.98	9.78	10.72	17.06	20.07	23.54	20.94	19.54	23.32	22.51	31.46	20.82	16.36
4	5.88	6.16	6.33	8.96	14.24	15.71	16.81	16.57	20.73	21.12	21.92	32.02	18.63	16.67
5	15.70	11.82	14.17	15.88	23.78	23.09	34.40	23.92	26.69	25.07	28.10	34.07	22.63	14.00
6	10.53	10.22	8.81	9.19	11.64	15.12	25.50	18.32	18.57	20.38	21.01	29.44	19.70	15.93
7	9.38	7.58	10.79	14.72	19.29	20.55	25.64	17.83	24.88	22.21	22.12	28.08	21.10	15.00
8	6.23	6.15	8.11	9.63	16.05	15.63	20.20	15.57	17.68	18.89	17.06	21.67	15.49	14.55
9	15.48	9.83	11.44	13.54	18.96	19.22	29.36	22.54	22.69	22.74	22.90	35.47	20.65	13.91
10	8.88	10.30	11.80	14.08	16.73	20.28	32.64	20.09	24.11	26.08	22.87	30.42	24.58	18.18
11	10.67	8.35	9.35	12.75	20.33	21.55	29.94	20.14	22.08	22.08	23.89	33.12	21.46	19.55
12	8.20	9.84	13.49	14.29	21.01	26.32	30.31	19.25	27.01	28.26	24.52	30.76	23.22	21.50
Means <sup>a</sup>	9.99	8.90	10.41	12.52	18.05	19.71	26.77	19.24	21.83	22.74	22.08	30.42	20.42	16.32

Appendix B table 34. Urea: Creatinine ratio of foals fed a 9% crude protein supplement, fortified with lysine and threonine

<sup>a</sup> Least squares SE of means = 0.60

## Vita

William Burton Staniar, son of Mrs. Dale Staniar and Mr. William Staniar, was born on August 26, 1974 in Princeton, New Jersey. He graduated from Pomfret School in Pomfret, Connecticut in 1992. He then attended the University of Richmond, where he majored in Biology. William received his Bachelor of Arts degree in May 1996. He then began his work towards a Master of Science degree in Equine Nutrition in the Department of Animal and Poultry Sciences at Virginia Polytechnic Institute and State University. William has done the majority of his research at the Middleburg Agricultural Research and Extension Center. He has received a John L. Pratt Fellowship to pursue a doctoral degree in Equine Nutrition at Virginia Polytechnic Institute and State University.