This is to certify that the
thesis entitled

PHOSPHORUS DIGESTABILITY IN MATURE HORSES

presented by

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has been accepted towards fulfillment
of the requirements for the

Master of Science degree in Animal Science

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Major Professor's Signature

June 4, 2009
Date

PHOSPHORUS DIGESTABILITY IN MATURE HORSES

BY

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Animal Science

2009

ABSTRACT

PHOSPHORUS DIGESATBILITY IN MATURE HORSES

By

Tara Erin Lavin

Experiments were conducted to increase the knowledge of phosphorus digestibility in mature horses as much of the available research has focused on young and growing animals or utilized ponies as a model. Concern is growing over the environmental impact of phosphorus entering bodies of water due to the application of animal manure to the land, it is likely that a greater percentage of the manure management practices of horse owners in the United States will fall under governmental regulation. The first study fed 6 mature horses diets of alfalfa hay, grass hay, or grass hay and oats. No differences were found in phosphorus output in the urine or feces resulting in no differences in apparent digestibility regardless of intake level. All horses maintained a near zero phosphorus balance. In the second study mature horses were fed six diets with three levels of phosphorus intake, each diet with a corresponding phytase supplemented diet. There was no effect of phytase supplementation on phosphorus output in the urine or feces resulting in no differences in phosphorus apparent digestibility. Analysis of the feed and feces for phytate revealed a 93% average disappearance rate of phytate, suggesting that horses are already highly capable of degrading phytate and that phytase supplementation would not be cost effective if effects are to be seen. The results of these two studies show that mature horses are highly capable of maintaining a near zero phosphorus balance, and that increased intakes only result in increased potentially detrimental outputs to the environment.

ACKNOWLEDGMENTS

I would like to thank the members of my committee (Dr. Nielsen, Dr. Hill, Dr. Orth, and Dr. Herdt) for their guidance and support. And also for allowing me to pick their brains and bounce ideas off them; it was a great opportunity to work with such talented and thought provoking people.

I would like to thank the people who helped so much in getting the work done, including Adrienne Woodward, Justine Zingsheim, Jane Link and Jim Leismann. I am forever in debt to my good friend Cara O'Connor-Robison whose countless hours of assistance kept me going throughout the past two years, not to mention the incredible Piña-Coladas. I would also like to thank all the other Animal Science graduate students who lent their hilarious anecdotes and great advice to aid in the maintenance of my sanity.

I would like to thank Jason Shelton and Cargill Animal Nutrition for their support of this research. I would like to thank the University of Wisconsin-River Falls faculty who encouraged me to pursue graduate school. And the Trussell and Bedner families who have adopted me and given me access to a wide range of horses and equine-experiences.

I would like to thank my family (Mom, Grandma, Chris, and Kelley) who shaped who I am today and have always been there to support me. Mom, thank you for encouraging us to chase our dreams and making it possible to do so. And, of course, I'd like to thank my ever-faithful sheepdog, Pemberly, who not only guarded us and the research horses every night, but also continuously found great new ways to make me smile.

Thanks to all of you it was a great two years!

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CHAPTER 1

INTRODUCTION

Concern is growing over the environmental impact of phosphorus entering bodies of water due to application of animal manure to the land. Many livestock operations are under regulation as to the amount of phosphorus they can emit from their operations, including some of the large equine facilities in the United States. The manure management practices of smaller equine facilities may soon fall under governmental regulation. It is therefore necessary to be able to estimate the amount of phosphorus that the average horse excretes in its manure, and to find ways to reduce phosphorous output. To accomplish this goal more information is needed on the digestibility of phosphorus in the wide variety of feedstuffs commonly used in the equine industry. Information is also needed on the differences in digestibility of phosphorus seen in all age groups and exercise statuses. Much of the available research has centered on the young growing animal, or animals entering training. The purpose of the following studies was to examine phosphorous digestibility in some common feedstuffs fed to mature horses that were either lightly exercised or at maintenance. And also to determine the efficacy of phytase supplementation to diets of mature maintenance horses to reduce phosphorus concentrations in the manure, as this is a common practice in both the swine and poultry industries as a method to reduce fecal P.

CHAPTER 2

LITERATURE REVIEW

Phosphorus: Functions

Phosphorus has more known functions than any other mineral element (NRC, 2001) and is second only to Ca in abundance within the body (Gropper et al., 2005). Eighty percent of the P in a horse is found in the skeleton (bones and teeth), 16% in the muscle, and the rest is distributed between the organs, blood and skin (Grace et al., 1999). Phosphorus forms various calcium-phosphate complexes, such as hydroxyapatite, which are the major constituents of bone (Gropper et al., 2005). The ratio of Ca:P in normal bone of horses is 2:1, while the whole body ratio is approximately 1.7:1 (Frape, 2004), this is because P is also distributed among the soft tissues for a variety of essential functions. All energy-utilizing processes in the animal eventually involve high-energy phosphate bonds such as those in adenosine triphosphate (ATP) or creatine phosphate in the muscles. All cells utilize P for the formation of DNA, RNA, and cell membranes. As part of several basic compounds, P influences the release of oxygen into the muscle tissue (2,3-diphosphogcerate), glycogen synthesis (UDP-glucose) and degradation (glycogen phosphorylase), insulin/glucagon release (cAMP), and muscle contractions (IP_3). Phosphorus is the main intracellular buffer (Gropper et al., 2005), and phosphate is an important buffer in the gastrointestinal (GI) tract.

When there is not enough P available in the diet, horses will have symptoms similar to those seen in humans suffering from rickets which include lameness, delayed growth, weak bones, and skeletal deformations. When there is too much P in the diet, it can interfere with Ca absorption and cause nutritional secondary hyperparathyroidism

(NSH), also known as "big head" syndrome due to the fibrous tissue that invades the Ca deprived bones and enlarges them (Ramirez and Seahorn, 1997). This most often results from inverted Ca:P ratios, or the use of feedstuffs that decrease Ca absorption such as those high in oxalates (Ramirez and Seahorn, 1997); NSH has become rare in the United States as Ca supplementation has become common (Hintz, 1997). Feeding imbalanced Ca:P ratios and excess magnesium (Mg) has been linked to enterolith formation in horses (Bray, 1995), which can be lethal if they become large enough to block the intestine. The maximum tolerable concentration of P in equine diets is estimated at 1%, assuming adequate Ca:P ratio (NRC, 2005).

The Equine GI Tract

Horses are non-ruminant herbivores and hindgut fermentors. While the stomach and small intestine are similar to other monogastrics, such as the pig, the ceacum and large intestine of the hindgut are similar to the ruminant animal's rumen. The majority of the horse's digestive tract is maintained at or near neutrality, except for the stomach, which can range in pH from 1.6-6.0 (Alexander, 1962), this varies with the fed or fasted state, type of feedstuff, and region of the stomach (Ellis and Hill, 2005). The low pH in the stomach helps to initiate the digestive process; digesta usually remains in the stomach for 2 hours or less (Frape, 2004) and then it enters the small intestine (pH range of 6.7 to 6.9), followed by the small colon, where pH ranges from 6.5-7.5 (Alexander, 1962) and is maintained by a buffering system including bicarbonate and phosphate from salivary, pancreatic and biliary secretions. It is essential that this buffering takes place, as large amounts of volatile fatty acids (VFA), lactate and hydrogen ions are produced during the

process of fermentation. This process occurs to some degree in the small intestine, but mainly in the large intestine. Passage through the small intestine typically proceeds at a rate of 30 cm/min in the 20 to 25 m long tube (Frape, 2004). The sacculated structure of the ceacum and large intestine serve to slow down the rate of passage so that the microbial population can convert structural carbohydrates from feeds into usable VFA for the horse. Feed spends most of its time in the ceacum and large intestine (36 to 72 h) and then moves onto the small colon, which is primarily involved in absorbing water and forming fecal balls, which move through the rectum and to the anus for excretion.

The inorganic phosphate concentration of the stomach of horses is reportedly small (8 mEq/L), increases in the jejunum to 22 mEq/L, and decreases in the small intestine (Alexander, 1962). There is another, larger increase in inorganic phosphate concentrate in the ventral colon (31 mEq/L), which is doubled in the dorsal colon (63 mEq/L) and decreased in the small colon (59 mEq/L) (Alexander, 1962). While the author did not report how horses had been housed or fed prior to the experiment, the higher concentration of phosphate in the large intestine raised the question of where the primary absorption of P actually occurs in the horse. In the ruminant (Khorasani, 1997, Reinhardt et al., 1988; Pfeffer et al., 1970), swine (Moore and Tyler, 1955a,b), and human (Gropper et al., 2005) the main site is the small intestine. Schryver et al. (1972) determined that the main site of P absorption in the horse is the dorsal large colon and small colon. They used ceacal and oral doses of ^{32}P and an un-absorbable tracer (chromium oxide) to map the net flow of P in the digestive tract. There is an initial increase from dietary intake in the first half of the small intestine and a net removal from the second half. There is also a large net flow into the ceacum and net removal from the

dorsal colon and from the small colon. Their findings supported those of Alexander (1962), as phosphorous concentrations were significantly higher in the large intestine than in the small intestine, presumably due to the various secretions associated with digestion. This higher concentration may serve as the primary buffer in the dorsal colon for the relatively larger proportion of VFA produced in the hindgut than in the stomach and small intestine, while bicarbonate is the primary buffer system in the ceacum and ventral colon (Alexander, 1963). Additionally, Matusi et al. (1999) observed the same pattern of phosphorous secretion and absorption as Schryver et al. (1972). Horses are therefore similar to ruminants, swine, dogs, and humans in that there is net absorption of P in the small intestine; however, they differ in that the major site of P absorption in the horse is the large intestine.

The walls of the small intestine secrete a variety of digestive enzymes responsible for degradation of soluble carbohydrates, proteins, and fats. Calcium, Mg, and P are absorbed here. There are two mechanisms of P absorption: a saturable Na and Vitamin D-dependent active transporter, which is separate from the Ca-transporter (Horst, 1986), and a gradient-dependant facilitated diffusion process (Gropper et al., 2005). At high dietary concentrations, diffusion is the primary method of P absorption (Wasserman and Taylor, 1976). Parathyroid hormone (PTH) is secreted from the parathyroid gland when plasma Ca is low, which in turn increases production of calcitriol, the active from of Vitamin D, from the kidney. Calcitriol increases the amount of Ca reabsorbed from the kidney, and increases Ca and P uptake from the intestine. Parathyroid hormone also causes Ca and P to be released from bone. Once serum Ca is in the normal range, these processes are reversed (Gropper et al., 2005). Very low serum P concentrations can also cause calcitriol

to be released, and thus cause increased P absorption (Horst, 1986), although P serum concentrations are not as tightly regulated as Ca (Frape, 2004). In the normal healthy horse, serum P values range from 2.3-5.4 mg/dl, while serum Ca ranges from 10.4-13.4 mg/dl (Merck, 1998).

Various anaerobic organisms are found in the stomach and small intestine of the horse. In the small intestine, the number of anaerobic bacteria range from 10^6-10^9 cfu/ml small intestinal content, which includes various lactate users, entercocci, streptococci, and enterobacteria (Julliand, 2005). The majority of digestion that occurs in the large intestine is dependant upon the flora and fauna found there, as the walls of the large intestine secrete only mucus, no digestive enzymes (Frape, 2004). Therefore, the hindgut contains more yeast, fungi, protoza, and bacteria (Julliand, 2005). The microbes in the hindgut of the horse utilize the insoluble carbohydrates (cellulose, hemicellulose, pectin) and undigested fats, proteins, and soluble carbohydrates from the horse's diet to produce VFA, which serve as a major energy source for the horse and are readily absorbed from the large intestine.

Endogenous Losses

The flow of P into the digestive tract is much greater than the intake from the diet. Salivary, pancreatic, and biliary secretions contain P and are secreted into the upper gastro-intestinal tract.. Approximately 10 to 12 L of saliva are secreted daily in a normal horse (Frape, 2004), which has been estimated to contain 0.25 mEq HPO_4^{2-}/liter and 50-mE/liter bicarbonate (Frape, 2004). The flow of pancreatic secretions in horses is continuous, and increases when the horse consumes food. Approximately 10 to 12 L can

be secreted in 24 h (Frape, 2004). The constant biliary flow in the horse is estimated at 4-L per day (Alexander and Hickson, 1970). The portions of these secretions that are not reabsorbed contribute to the endogenous losses seen in the feces. Other sources of endogenous loss include microbial cell loss and intestinal cell sloughing. The endogenous losses of P in ponies were estimated to be 10 mg/kg BW/d (Schryver et al., 1971a), when four 2 to 2.5 yr old geldings where given ^{32}P IV or IM injections. In a review of 30 studies, where nutrient balance data was collected, Pagan (1998b) estimated endogenous losses of P to be 8.5 mg/kg BW/d. These values are based on the Lucas regression technique of plotting retention of P (g/d) against multiple intake concentrations of P (g/d). Actual endogenous losses result in a negative Y-intercept, which represents the amount of P digested at an intake of zero. The slope of the regression line is representative of the estimated true digestibility. These studies were done in horses weighing between 500 and 600 kg and the estimated true P absorption was 25% (Pagan, 1998a). However, the actual calculated requirement using these values was only slightly higher than those using the older numbers of 10 mg/g BW/d and 45% true absorption efficiency, and therefore the NRC (2007) chose to stay with the lower estimate to minimize any environmental impact of feeding excess P.

Determining Requirements

The amount of P consumed is either available for absorption or unavailable, regardless of time in the digestive tract. The unavailable P will be excreted in the feces. The P that is absorbed enters the bloodstream and is available to body tissues and organs. As previously noted, bone serves as a storage form of P that can be released into the

bloodstream to augment dietary supply as needed. To determine requirements we must know the efficiency of absorption of a mineral from the intestine which maybe determined by injecting the animal with labeled P, allowing the injected P to equilibrate with the P pool of the bloodstream, followed by a balance study where the amount of P the animal is fed and the amount of P in the urine and feces is determined. The unlabeled P in the feces is presumably dietary P that could not be utilized or did not become labeled, and the labeled P is from endogenous sources that became labeled after equilibration. The fecal excretion can be thought of in three fractions, first P of dietary origin that is not available for absorption, second P of endogenous origin that is secreted into the feces such as P of microbial or intestinal cell origin or from secretions that are not reabsorbed, and finally P of endogenous origin that is secreted to maintain blood levels (NRC, 2001).

Balance data can be corrected for endogenous losses of P, and estimates of P absorption can be made by dividing (intake - fecal P + estimated endogenous P losses) by intake, the NRC (2007) calls this the "estimated true digestibility". Estimates of endogenous losses and digestibility of feedstuffs often must be made using less intensive methods than using labeled P. In these cases, balance studies are performed using a wide range of P concentrations in the diets. The amount of mineral retained (intake-fecal output) is than plotted against the levels of intake. This is called a Lucas regression, in which the Y-intercept will represent the estimated endogenous losses, or losses that would occur when intake was zero, while the slope represents estimated true digestibility (Pagan, 1998a; NRC 2007). This method is less than optimal since P digestibility is likely increased as amount of P in the diet decreases to zero. This relationship is not a straight

line. However, when a better model is not available, this serves as a good estimator (Schryver et al. 1971a). When estimates of endogenous losses are inadequate, studies often report apparent digestibilities which are [(intake – fecal output)/intake]*100. These may underestimate the actual digestibility of the mineral, as they do not account for endogenous P utilization and losses (NRC, 2007). For example, in ponies 6 mo to 2.5 yr of age, apparent P digestibility was 18.5%, while estimated true digestibility was 45% (Schryver et al., 1971b). Often studies simply report the fecal and urinary losses and an apparent absorption or retention value. Absorption and retention are often used interchangeably when discussing P metabolism in the horse, as urinary contributions are so low that they rarely affect the value. Therefore, absorption and/or retention often represent intake - fecal output and sometimes intake - fecal and urinary output.

For balance studies to be accurate, many factors must be controlled including feed monitoring, complete collection of excreta, and use of identical proportions of all samples (i.e. saving a 10% fecal/urine sample each time) unless complete collections are utilized. Equally important is the quality of laboratory analysis. As water intake influences the DM content of the feces, and dilutes the amount of mineral present, determining DM for sample mixing is ideal. Water intake often varies between horses, and between days within a horse. Therefore, feces should be pooled based on DM. The amount of exercise all of the animals receive must also be standardized. If they are to receive a period of turnout time during the balance study, they should be together in the same pen so they all receive the same stimuli for activity. Also, adult horses must have a constant energy balance so that results are not confounded by weight gain or loss. Because of the tedious

nature of balance studies, and previous lack of concern for P impact of the environment, there is a lack of data on P metabolism in the horse.

Requirements of/Recommendations for the Horse

The NRC (1989) states that true P absorption for horses ranges from 30 to 55%, depending on the age and type of horse and the source and concentration of P fed. In determining requirements, the NRC (2007) assumed a 35% absorption efficiency for sedentary, gestating, and/or working horses with the assumption that diets for this group of horses are primarily composed of plant sources. For lactating and growing horses, whose diets are often supplemented with inorganic P, they used an absorption efficiency of 45%. To determine the amount of nutrient the horse needs to absorb each day, one must know biological need, endogenous losses and nutrient requirement for all processes including maintenance, growth, pregnancy, and lactation. As already discussed, the NRC (2007) has chosen to stay with the endogenous P loss estimate of 10 mg/kg BW/d (Schryver et al., 1971a) for all horses.

To determine requirements for growth, mineral content was measured in 4-mo, 1-yr, and 2-yr old mixed breed horses. Horses of this age range contain 8 g P/kg BW, therefore a growing foal would require 8 g P/kg BW gained per day (Schryver et al., 1974a). In growing Quarter Horses, the estimated endogenous loss was 18 mg/kg BW/d (Cymbaluk et al., 1989), suggesting that growing horses have a higher requirement than previously believed if breed and activity are not considered. This was supported by Furtado et al. (2000) who demonstrated that endogenous losses in 10 month old Brazilian horses was 10.3 mg P/kg BW/d. Growing horses likely have a greater need and thus may

have a higher true absorption efficiency. Grace et al. (1999b) estimated true absorption efficiency to be 50% in growing 200-kg Thoroughbred horses gaining 1 kg/d. Growing horses are adding muscle mass which requires P for normal tissue growth. There is no evidence that mature exercising horses have an increased P requirement (NRC 2007), and it was assumed that any increased requirement that growing horses may have due to exercise would be met by the increased requirements established for growth.

Influence of Dietary P on Fecal/Urinary/Plasma Levels

In four studies using mature horses or ponies, fecal P as a percentage of intake ranged from 82.2 to 87% (Hintz and Schryver, 1973; Patterson et al., 2002; van Doorn et al., 2004; Morris-Stoker et al., 2001). This is in contrast to two studies using yearling horses and growing ponies whose fecal P output as a percent of intake ranged from 53.5 to 67.2% (Hainze et al., 2004; Schryver et al. 1971b). These studies demonstrate that there are differences in P digestibility by age, dietary composition, physiological state, and frame size.

When horses of various ages were fed 133% or 275% of the NRC (1989) recommendations for Ca and P, fecal P excretion followed intake, so horses receiving more P excreted more in the feces (28.48 vs. 53.79 mg/kg BW/d). This is to be expected since the feces is the primary mode of P excretion. Urinary excretion ranged from 0.23 to 0.28 mg/kg BW/d (Buchholz-Bryant et al., 2001). Schryver et al. (1971a) reported that higher P diets increased urinary and fecal excretion of P and raised plasma P concentrations in 2-yr old ponies. Fecal, but not endogenous losses were related to dietary intake. They also reported that urinary excretion of ^{32}P tracer, given IV or IM, increased directly with intake. It was therefore concluded that renal excretion is the

primary mechanism of regulation of P serum concentrations of horses, but the levels of P are so low in the urine that the primary losses are fecal. Fecal excretion of P in the horse is dependant on intake, diet composition, P source, and age, and physiological state, which makes it hard to determine average output values for horses. More studies are needed with diets similar to those fed in the industry, and with a wide population of horses before useful estimates can be made.

Dietary Phosphorus Physical Forms

Inorganic

There are two main types of P in animal diets: inorganic and organic, with the most common organic source being phytate (myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate). Forms of inorganic P utilized in diets are monocalcium phosphate, dicalcium phosphate, monosodium phosphate, defluoronated rock phosphates and animal by-product ingredients (which can also be organic in nature). The digestibility of P from monosodium phosphate is estimated to be 52.7% (Hintz et al., 1973), while the availability of P in steamed bone meal, dicalcium phosphate and monosodium phosphate is estimated to be 58% (Hintz and Schryver, 1972a). The majority of P in forages is in the inorganic form.

Organic: Phytic Acid

Phytic acid is the primary storage form of P in the seeds of plants. It is liberated during germination to provide energy (ATP), and inositol phosphate intermediates for transport of materials into cells and to serve as secondary messengers such as inositol

triphosphate (IP3) (Vohra and Satyanarayana, 2003). Its structure consists of an inositol ring surrounded by six phosphate groups. Each group has two alcohol groups. Phytic acid is a strong acid with the six most easily displaceable protons of the molecule having a pKa of 1.84. It also has two protons in the weak acid range (pKa 5.7 to 6.3), and three to four with very weak acid range (pKa 9.7 to 12.0) (Costello et al., 1976). This gives phytic acid the potential to bind with cations in a wide pH range (Reddy et al., 1989). Salts of phytic acid are called "phytates" and the Ca/Mg salt is called "phytin" (Vohra and Satyanarayana, 2003). At neutral pH, each phytic acid molecule has one or two negatively charged oxygen atoms to which positive cations can bind strongly between two phosphate groups of the same molecule to from insoluble salts (Erdman, 1979). Phytic acid is a strong chelating agent and binds to Ca, Mg, Zn, Cu, Fe, Ni, Mn, K, and Mo (Reddy et al., 1989). Once insoluble salts are formed, the availability of the P and cation for absorption in the animal is drastically decreased (Maga, 1982; Sandberg et al., 1993; Davies and Nightingale, 1975). Phytate can be considered an antinutritional factor by decreasing availability of minerals. It reduces the availability of proteins (Dvorakova, 1998; Saio et al., 1967; Cosgrove, 1980), starches (Deshpande and Cherya, 1984; Knuckles and Betschart, 1987), and lipids. There also appears to be a negative effect on the activity of some enzymes such as amylase (Thompson and Yoon, 1984; Harland and Morris, 1995), pepsin (Deshpande and Cheryan, 1984), and trypsin (Singh and Krikorian, 1982; Caldwell, 1992). These deleterious effects are most likely due to binding between phytic acid and the molecules or binding of a phytic acid-cation complex to one of these molecules via the cation (Reddy et al., 1989).

Phytate is very abundant in cereals and certain legumes and can be found in the aleurone, or pericarp of the seed in cereals and the cotyledons of legumes (Reddy et al., 1989). Phytate content increases throughout ripening and peaks at seed maturity (Abernethy et al., 1973; Nahapetian and Bassiri, 1975; Welch and House, 1982; Welch et al., 1974). In corn, about 80% of the phytate is found in the germ, while in rice and wheat 80 to 87% is found in the pericarp or aleurone layer (Reddy et al., 1989). Phytate P accounts for more than 80% of the total P in cereals and cereal products, and 50 to 70% of the P in soybeans (Reddy et al., 1989). Table 1 lists the total P, phytate P and % phytate P of some common feedstuffs used in the equine industry.

Table 1: Total P, phytic acid-P, and phytase activity in some common feedstuffs. (Eekhout and de Paepe, 1994; NRC, 2007, 2001.)			
Feedstuff	Total P, % of DM	Phytic acid-P, % of Total P	Phytase*, unit/kg
Wheat Middlings	1.02	66	4,381
Wheat Bran	1.16	84	2,957
Wheat	0.33	67	1,193
Barley	0.37	60	582
Corn distillers	0.90	21	385
Rice Bran	1.78	64	122
Alfalfa (dehydrated pellets)	0.23	0	60
Soybeans (heated)	0.57	46	55
Oats	0.36	59	42
Soybean Meal, 44% CP	0.66	53	40
Sorghum	0.27	70	24
Corn grain	0.28	68	15
Beet pulp	0.10	0	3
Legume Hay	0.28-0.31	-	-
Grass Hay	0.26-0.34	-	-
Bermudagrass	0.27	-	-
Blood meal	0.30	-	-
Fish meal	2.69	-	-
Meat and Bone Meal	4.73	-	-
*: Phytase unit defined as the amount of inorganic P released, μmol/min, from a 0.0015 M Na-phytate solution at pH 5.5 and 37 °C -: Indicates value not determined for that feedstuff			

Phytase/Alkaline Phosphatases

Phytase enzymes (myo-inositol hexaphosphate phosphorohydrolases) catalyze the removal of inorganic orthophosphates from the phytic acid molecule (Nayni and Markalds, 1986). Various cations, proteins, and starches that were bound to phytic acid may also be released. The Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NCIUBMB) lists phytases in two categories: 3-

phytase (myo-inositol hexakis phosphate 3-phosphorohydrolase; EC 3.1.3.8) and 6-phytase (myo-inositol hexakis phosphate 6-phosphorohydrolase; EC 3.1.3.26). The number is based on the carbon position of the inositol ring from which the enzyme hydrolyzes a phosphate from first; so 3-phytase first removes phosphate form the 3-C position (Vohra and Satyanarayana, 2003).

Phytase activity has been identified in the intestine of the rat, chicken, calf, and man (Bitar and Reinhold, 1972; Patwardhan, 1937; Spitzer and Phillips, 1945). Since it is currently unknown exactly how horses are able to utilize phytate-P, it might be a good idea to include alkaline phosphatase in the scope of research. Alkaline phosphatase, which is capable of hydrolyzing phosphate esters in an alkaline medium at the brush border, has activity in the small intestine of the horse (163.9 to 244.5 U/min/mg tissue) (Bertone, 1990). There is approximately a 25-fold decrease in activity between the small and large intestines. The levels of alkaline phosphatase found in the hindgut of the horse are much higher than those of the cat, dog or man, indicating uniquely high digestive and absorptive action occurring in the hindgut of the horse (Frape, 2004). Alkaline phosphatase is found throughout the body and is responsible for the dephosphorylation of many compounds.

The microbes of ruminants have high phytase activity and are able to free phytate-bound P almost 100% (Raun et al., 1956; Vohra and Satyanarayana, 2003). Many microbes have extracellular phytase activities (Vohra and Satyanarayana, 2003). The fungi, *Aspergillus niger,* was found to be highly active in phytase production, and has been used for various supplements such as Natuphos (BASF, Inc.) and Allzyme (Alltech, Inc). Other microbes have also been used for supplements, such as the fungi *Trichoderma*

reesei used to produce Finase (Alko, Ltd.). Thermal stability is ideal in a supplemental phytase, as it must often endure the pelleting process where temperatures can reach 80° C or more. Ronozyme (Roche Novo Nordisk, Inc.) is advertised to be more resistant to heat. More information is needed on the inherent intestinal phytase activity and microbial phytase activity in the horse.

A final source of phytase is that which is part of the feedstuffs provided. Eeckhout and Depepe (1994) reported that rye (5,130 units/kg), triticale (1,688 units/kg), wheat (1,193 units/kg), and barley (582 units/kg) had high activity levels of phytase. Wheat middlings also contain high phytase activity (Underwood and Suttle, 1999). Maize, oats, sorghum, and oilseeds had little or no phytase activity. High temperatures during pelleting can also inactivate the innate phytase in the feedstuffs (Simons et al., 1990). Interestingly, soaking some supplemental phytase in water can enhance the activity in diets fed to swine (Liu et al., 1997).

Availability of Phytate-P to Ruminants and Other Monogastrics

Phytate is quickly hydrolyzed in the rumen (Reid et al., 1947) due to the high phytase activity of the microbes present (Raun et al., 1956). Therefore, ruminants are not dependant on the phytase activity of the feedstuff. Phytate-P is considered almost completely available to dairy cattle (Morse et al., 1992; NRC, 2001), while sheep are reported to absorb 78 to 81% of the phytate-P in cereal and vegetable protein sources (Field et al., 1984).

Under normal conditions, the P in phytate is relatively unavailable to monogastrics because they produce little phytase in the intestine (Vohra and

Satyanarayana 2003; Taylor, 1965; Nelson, 1967). In comparison to highly available inorganic P sources such as sodium and potassium phosphates, the P of cereals and vegetable proteins is only about 20 to 45% as absorbable in pigs and 25 to 50% in chickens (Soares, 1995). In pigs, apparent absorption of P from wheat and barley is higher than in corn (47 and 39 vs. 17%) even though they have similar proportions of phytate-P (70.7 and 63.6 vs. 65.6% of total P) (Jongbloed and Kemme, 1990). Digestibility is not only a function of the amount of phytate-P in the feedstuff, but also the inherent phytase activity of the feedstuff (Underwood and Suttle, 1999; Pointillart et al., 1987). In an effort to improve phytate digestibility in monogastrics and decrease output to the environment, phytase supplementation of the diet is widely utilized in the United States. Supplementation of pigs with phytase can eliminate the need for adding inorganic P to the diets of finishing pigs (Murry et al., 1997; Liu et al., 1997; O'Quinn et al., 1997). In broilers, phytase supplementation can improve P availability by 20 to 40%, depending on the type of diet and amount of phytase added (Simons et al., 1990; Kornegay et al., 1996; Yi et al., 1996). About 50% of the phytate-P in the diet was released when chicks were fed phytase (Natuphos) at 850 U (activity not reported)(Schoner et al., 1993). In pigs fed phytase (derived from *Aspergillus niger*) at 400 U (activity not reported) (Hoppe et al., 1993) and 246 U (microbial phytase EC 3.1.3.26, one U is the quantity of enzyme which liberates 1-μmol inorganic P/min from 5.1 mM-sodium phytate at pH 5.5 and 37°C) (Kornegay and Qian, 1996), phytase demonstrated similar results.

The availability of phytate-P is not simply related to the proportion of phytate, but also the Ca and P concentrations and ratio in the diet, the age and physiological state of

the animal (Underwood and Suttle, 1999), and as previously stated, the inherent phytase activity of the feedstuff (Pointillart et al., 1987). The availability of P from phytate-P can be improved when the Ca:P ratio is kept low, 0.8 to 1.4 vs. 2.0 in chicks (Edwards and Veltmann, 1983) and 1.5 to 1.7 vs. 2.9 to 3.0 or 1.2 vs. 2.0 in pigs (Lei et al., 1994; Qian et al., 1996). Phytase supplementation in swine diets (Kornegay and Qian, 1996; Liu et al., 1997; O'Quinn et al., 1997) and poultry diets (Simons et al., 1990) increases Ca digestibility. The utilization of phytate-P has also been improved by the addition of 5 or 10-μg/kg diet of Vitamin D (1,25-dihydroxycholecalciferol) in chickens (Edwards, 1993), but this may have only been a secondary effect to an increased absorption of Ca and P from the gut (Biehl and Baker, 1997). Diets can be formulated to contain appropriate amounts of Ca and P in the correct ratio to meet animals needs without the use of phytase, it's primary purpose is to reduce the cost of feeding excess P sources and to decrease output of P by the animals by increasing their utilization of phytate-P sources.

Availability of Phytate-P to Horses

The first equine studies investigating phytate-P availability to ponies fed pelleted diets of grass hay, beet pulp, corn and soybean meal with sodium phytate demonstrated no difference in estimated true P absorption (44%) from those fed the same diet but containing monosodium phosphate (45% estimated true P absorption) (Schryver et al., 1971a). When mature horses were fed hay and mixed grain diets with or without phytase (Allzyme, Alltech Inc.) added at 200 U/g (activity definition not reported), no effect on apparent P digestibility was observed. They reported apparent digestibility from 5.77 to 8.13 mg P/kg BW/d (Morris-Stoker et al., 2001). When mature horses were fed 300, 600,

or 900 U/kg (one U is the quantity of enzyme that liberates 1-μmol of inorganic P per min from 5.1 mM Na-phytate at pH 5.5 and 37°C) phytase (Natuphos 600, BASF, Wyandotte, WI) on top of a grass hay and grain diet of corn, oats, and soybean meal, no effect of enzyme supplementation was seen on P digestibility (Patterson et al., 2002). In yearling horses fed grass hay and various concentrates including whole oats, sweet feed, a pelleted concentrate, and alfalfa cubes, phytase (Allzyme, Alltech, Inc., Nocholasville, KY) at 1500 U/g (one U is the quantity of enzyme that releases 1-μmol of inorganic P per min from 0.15 mM Na-phytate at pH 5.5 and 37°C) supplementation was found to have no effect on P digestibility (Hainze et al., 2004). In all of these studies, the amount of phytate-P in the feedstuffs and feces was not determined. Therefore, it is unknown how much of the phytate-P may have been liberated either by supplemental or microbial enzyme activity.

When mature horses were fed oat, corn, wheat bran, and rice bran diets that contained 55% of the total P as phytate-P the apparent digestibility of phytate-P was 60% (van Doorn, 2003). This was increased to 71% ($P<0.02$) by the addition of 740 U/kg (one U is the quantity of enzyme that releases 1-μmol of inorganic P per min from 0.5 mM Na-phytate at pH 5.5 and 37 °C) phytase (Natuphos 5,000 Liquid, DSM Food Specialties, Delft, The Netherlands) (van Doorn, 2003). These researchers found no difference in the apparent P digestibility when a phytate-rich diet, containing large amounts of wheat and rice bran, was compared to a diet containing inorganic P from monocalcium phosphate (van Doorn et al., 2004). In contrast, Hintz et al. (1973) found that P from wheat bran (high in phytate-P) is approximately half as available as P from an inorganic source (NaH_2PO_4). Van Doorn et al. (2004a) found that the Ca digestibility on the phytate-P

diet was increased when the diet was supplemented with phytase (31.5 vs. 26.4%), but both phytate diets (supplemented and not) had lower Ca absorption than the control and inorganic P diets (47.7% and 42.35%). Additionally, Hintz et al. (1973) found that the apparent digestibility of Ca was lower when wheat bran was fed to ponies. This is likely because phytate binds Ca into insoluble complexes that are excreted via the feces, and phytase activity releases the Ca from binding. No differences in Mg retention or apparent digestibility were reported between any of the treatment diets (van Doorn et al., 2004a). This concurs with Hintz and Schryver (1972a) who reported that sodium phytate or wheat bran did not affect Mg metabolism in ponies.

To more closely examine the digestion of phytate-P in the horse, Matsui et al. (1999) fed 2 yr old horses either a low or high phytate diet. The low diet was primarily timothy hay, alfalfa hay, and inorganic P supplied by sodium dihydrogenphosphate providing 21.1 g P/d that was 26% phytate-P, and a Ca:P ratio of 1.1. The high phytate diet consisted of timothy hay, oats, corn, soybean meal, wheat bran, and a much smaller amount of inorganic P to make the total P concentrations of the two diets equal. The high phytate diet had a Ca:P ratio of 1.2, and horses consumed 59% of the 22 g P/d as phytate-P. All horses received Cr_2O_3 in the feed as an un-absorbable marker. Horses were fed their respective diets for 5 days, and 3 hours after the last feeding horses were sacrificed. The daily passage of P in an intestinal segment was then calculated as: daily Cr intake x P in digesta divided by Cr in digesta. There was an 11 and 28% reduction of phytate-P on the low and high phytate diets, respectively, between the amount of intake and the concentration of the digesta in the upper small intestine, with no differences in

phytate-P content in the upper and lower small intestine. However, there was a decrease in phytate-P in the lower large intestine (rectum) as compared to the upper large intestine (0.8 m caudal to the ileocaecal valve) when horses were fed the high phytate diet. Similarly, on the low phytate-diet, the amount of phytate-P in the lower large intestine, but not the upper large intestine, was significantly less than that in the lower small intestine. There was no difference between diets in the total amount of phytate-P present in the lower large intestine (2.6 g phytate-P). These results suggest that phytate-P is partially digested in the small intestine, but digested to a larger extent in the lower large intestine. Digestion in the large intestine would be most likely attributed to microbial phytase activity. On the high-phytate diet, 82% of the dietary phytate-P was degraded by the time it was sampled at the rectum. Similarly, 47% was digested when a low phytate diet was fed. These results indicate that 47 to 82% of phytate-P is available to the horse without phytase supplementation.

Strategies to Improve Phytate Digestibility

The phytate content of feedstuffs is influenced by a variety of factors including: environmental conditions, location, irrigation, soil, fertilizer application, and year of growth (Reddy et al., 1989). When incubated with water at 55° C for 24 h, phytate in wheat, rye, and barley was reduced by 46-77%, and was reduced by 84-99% when incubated with acetate buffer (pH 4.8) at the same temperature and time. In oats soaked and ground, phytate was reduced by 8-26%; in dehulled oats it was reduced by 72-77%. (Fredlund et al., 1997). Decreasing the phytate content of feeds would create less P waste once fed to the animal.

Other options to decrease P waste include feeding supplemental phytase, however it will increase cost of the diet. Besides reducing phytate in the feeds and supplementing diets with phytase, researchers have now developed transgenic pigs that secrete phytase in their saliva (Golovan et al., 2001). Thus diets could be formulated to include high phytate-P feeds and eliminate the use of inorganic P sources and decrease P losses to the environment if the production values of these animals could be maintained at a level that was acceptable to producers and consumers.

Other Factors that Effect P Absorption

Minerals and Vitamins

There are many factors that affect the absorption, requirement, and excretion of P that further complicate the determination of daily P output by horses. These include other minerals, phytate/oxalate content of diet, vitamin D, age of the horse, exercise, dietary composition including fiber content, and passage rate. Phosphorus is also lost through various excretions including sweat, hair loss and hoof growth.

There is some controversy as to the effects of dietary Ca on P metabolism. The majority of horse studies have found that high dietary concentrations of Ca do not affect P metabolism when the Ca:P ratios are 1.4 vs. 3.2 (Highfill et al., 2005), 1.2 vs. 4.1 (Whitlock et al., 1970), and 0.4, 1.9, or 4.3 (Schryver et al., 1970). However, van Doorn et al. (2004b) reported that when feeding ponies varying concentrations of Ca and the same concentration of P resulting in Ca:P ratios of 1.1, 2.6, and 4.3, there was a depression in apparent P digestibility from the low Ca diet compared to the intermediate and high (24.8 vs. 10.8 and 13.3%), and a similar pattern was seen in P retention from the

low to intermediate and high diets (25.8 vs. 11.5 and 16.1 mg P/kg BW/d). Apparent digestibility of Ca was also lower on the intermediate and high Ca diets versus the low (27.9 and 27.4 vs. 42.2%), while Ca retention was highest on the high Ca diet (65.6 vs. 13.5 and 27.0 mg Ca/kg BW/d). While the high Ca diet had a wider Ca:P ratio than those tested in the previous studies, there was no difference in P metabolism between the high and intermediate concentrations of Ca supplementation. Another interesting note is that all of these studies were done in relatively young horses and ponies. Van Doorn et al. (2004b) used the oldest ponies at 4 yr of age. The other studies used horses from 2 to 5 months of age (Whitlock et al., 1970) and ponies from 6 to 14 months (Schryver et al., 1970). Growing animals are accruing muscle and bone tissue, therefore these results may not be applicable to a maintenance animal.

A review by Pagan (1998a) summarizing 30 balance studies regressed P digestibility against Ca intake and Ca:P ratio and found no correlation between them. The reviewed studies had an average Ca:P ratio of 2.3 and used primarily Thoroughbreds, with an average weight of 500 to 600 kg. These results might therefore be more applicable to the average horse. The studies also incorporated a wide range of feedstuffs: alfalfa hay, sweet feed and fescue hay, and pelleted concentrates with timothy hay. Perhaps Ca is more likely to have an effect on P digestibility when phytate-P is present, since high dietary concentrations of Ca inhibit phytase activity (Taylor and Coleman, 1979; Nelson and Kirby, 1979). Additional studies need to be conducted with animals representing other age and physiological states fed constant Ca:P ratios while varying concentrations of Ca.

While high Ca may or may not alter P metabolism, high dietary P, without proportional increases in Ca, does decrease Ca metabolism in horses (Schryver et al., 1971b; Frape, 2004; Ramirez and Seahorn, 1997). When dietary P was increased from 0.2 to 1.2% of diet while Ca was maintained at 0.4% of diet in young ponies, resulting in Ca:P ratios of 2 and 0.3, Ca absorption was decreased by more than 50% (Schryver et al., 1971b). Pagan (1998a) noted that Ca digestibility was negatively correlated to P and NDF, so that as the percentage of P in the diet increased, true digestibility of Ca decreased. The slope of this line was -0.65 with R^2=0.35. The depressive effects of excess P on Ca absorption is one of the reasons that it is recommended horses should receive a Ca:P ratio in the diet of no less than 2:1. Ratios below this may put the horse at risk for inadequate Ca absorption, which has been correlated with NSH (Ramirez and Seahorn, 1997), and various malformations of bone.

Toxicity studies of vitamin D in horses by Breidenback et al. (1998) have shown that horses have an odd reaction to pharmacological doses (40,000 IU Vitamin D_3/kg BW/d). Toxic doses led to 2 and 20 fold increases in Ca and P excretion respectively. Apparent digestibility of P and plasma P increased significantly, but serum Ca was unchanged, and Ca apparent digestibility tended to decline. Horses have a lower normal plasma concentration of calcitriol (20-40 pmol/L) than other mammals. In other mammals, high doses of Vitamin D primarily lead to increased apparent digestibility and renal excretion of calcium as well as Ca deposition in soft tissue (Boass and Toverud, 1996; Hodgkinson et al., 1979; Bordeau et al., 1986; Smother et al., 1986). More research is needed on Vitamin D metabolism in horses, especially for horses that are continuously stalled and receive little sunlight.

Varying concentrations of Mg and K have no effect on P metabolism (Hintz and Schryver, 1973; Hintz and Schryver, 1976). This maybe because the major site of absorption for Ca, Mg, and K is the small intestine (Hintz et al., 1978; Hintz and Schryver; 1972a, 1973, 1976), while P is likely absorbed to a large extent in the hindgut of the horse as previously discussed. Adequate absorption of the other minerals is of more concern in high P diets, particularly if those diets are high in phytate-P. Binding of phytate with Zn decreases availability of this mineral (Reddy et al., 1989). Phytic acid may also reduce the availability of Fe via a similar mechanism (Reddy et al., 1989).

In a study conducted using 2-yr old ponies that remained in positive P balance throughout the study, indicating that they were not being fed above their requirement, high P diets (1.19% P) increased the retention of P in comparison to low (0.2% P) diets, but as a percent of intake differences were not significant. The high P diet increased plasma P in comparison to the low P diet (Schryver et al., 1971a). Buchholz-Bryant et al. (2001) studied young, mature, and aged horses. The young and mature horses were fed 66.7 mg P/kg BW on the high P diet or 33.25 mg P/kg BW on the low P diet; while aged horses were fed 47.8 mg P/kg BW on the high P diet, and 25.3 mg P/kg BW on the low P diet. All three age groups maintained a near zero P balance on average throughout the 168-d trial, as the increases in P retention between the high and low P diets were not great enough to move them far from a zero balance. Therefore feeding P above the amount necessary to maintain a zero P balance will not enhance P absorption, but is likely to depress it. The addition of 5% sodium chloride versus only 1% sodium chloride to the diets of ponies increases P absorption, from 28% for diets with 1% sodium chloride to 40% with 5 % sodium chloride in the diets (Schryver et al., 1987).

Much like Ca, there have been varied results relative to Al impact on P metabolism. When mature ponies were fed high concentrations of Al (4,500 ppm) in a 0.3% P, 0.7% Ca diet, P absorption was decreased and the ponies went into a negative P balance, with decreased plasma P (Schryver et al., 1986). Researchers found that there was high bone turnover when ponies were fed the high Al diet. Al depresses P absorption (Schryver et al., 1986). However, a more recent study found that when horses were fed 931 ppm Al/kg feed, or 12 mg Al/kg BW/d, there was no effect on P metabolism (Roose et al. 2001). This study utilized 23-d diet adaptations and 5-d total collections, the diets contained Ca:P ratios of 3.1 on the non-supplemented diet and 2.6 on the Al-supplemented diet. The higher P content of the Al-supplemented diet may have masked some of the effects of Al on P metabolism, or it is possible that horses have a dose response to Al, and 931 ppm was not enough to elicit negative effects on P metabolism.

The dietary content of phytate and oxalate can also alter P absorption. The digestibility of P from phytate was previously discussed. Rats have shown an adaptive increase in microbial phytase activity after a period of P deprivation (Moore and Veum, 1983). This idea may be applicable to horses that are deprived of P by poor nutrition, or those fed a low P diet, or diet with mainly phytate-P, especially with too much Ca. Oxalate in the diet causes decreased Ca absorption and increased endogenous losses of P, thus decreasing P retention (McKenzie et al., 1981; Blaney et al., 1981). Horses consuming diets that contain oxalates and poor Ca sources, such as wheat bran or poor quality grass hay, particularly tropical grasses, are at the greatest risk for developing NSH (Ramirez and Seahorn, 1997).

Management Considerations

The age of the horse is an important consideration for mineral metabolism as the true digestibility of both Ca and P decline (71 to 42% and 52 to 6%) between the ages of 6 and 24 months (Cymbaluk et al., 1989). When the animal is actively laying down bone and remodeling bone to deal with new stresses, such as those induced by training, there is likely an increased demand for P and Ca or more efficient absorption. Exercise affects P requirements largely in relation to bone metabolism. Yearlings entering training have an initial decrease in P absorption and an increase in P output with the onset of bone demineralization that often accompanies typical training practices (Nielsen et al., 1998a; Stephens et al., 2004). After the initial bone loss that accompanies the early stages of training, an increase in bone mineral occurs as speed is introduced and this results in an increase in Ca and P demand. Ca and P needs must be met to provide for adequate bone formation. However, as long as the Ca:P ratio is maintained there is no benefit to feeding horses entering race training P above 0.24% of the diet (Nielsen et al. 1998b).

Horses who have been on stall rest and are being returned to activity will likely face the same challenges as the young horse entering training. During stall rest, little force is applied to their bones. Since bone responds to the forces put on it, horses on stall rest will likely have some demineralization of the bone. As animals return to work, it is important that diets be formulated to meet their needs for bone remodeling to deal with the stresses of work. The mature horse may have a different response to exercise. A review of 15 studies with P intakes ranging from 19 to 200 mg P/d resulted in an average P retention of 4.7 mg/kg BW/d; this was not different if the horses were sedentary or

exercised (Lawrence et al., 2003). This may indicate that exercising mature horses do not have an increased P requirement over their sedentary counterparts.

Passage Rate

The rate of passage through the equine digestive tract is faster than that of the ruminant (Frape, 2004). This is most likely due to their evolutionary development to consume large quantities of low quality forage. Thus, it would be essential to be able to quickly process the material and derive as many nutrients as possible from it so that more could be consumed. The passage rate of feeds in the horse is dependant upon physical form such that a pelleted diet would pass more quickly than a primarily forage diet (Frape, 2004). A horse with proper dentition usually chews forage to particles less than 1.6 mm (Frape, 2004), so chopping that does not reduce particle size below 1.6 mm may have no effect. The amount of water consumed would also affect passage rate.

Studies using markers to identify passage rates have varying results. Vander Noot et al. (1967) reported almost 100% recovery rates of Cr_2O_3 at 4 days which is similar to rates observed by Olsson et al. (1949). A study that utilized yellow Styrofoam particles and pelleted or non-pelleted hay and grain diets had complete marker recovery at 63 h (Hintz and Loy, 1966). Two studies, one using carbon granules and the other Cr_2O_3, reported total collection of the marker by 48 h (Alexander, 1946; Haenlein et al., 1966). These studies did not report differences in passage rates between different feed materials, however most studies fed relatively similar fiber types. Horses on pasture have a much higher passage rate than those fed other types of roughage. This is most likely due to the higher water content of pasture; but high fiber meals have a faster transit time than low

fiber diets of the same particle size (Frape, 2004). Transit time of meals is also much faster following a 12-h fast (Frape, 2004).

The ratio of soluble to insoluble fiber entering the stomach alters the viscosity of digesta, the water holding capacity of the feeds, and potentially the rate of passage (Bach-Knudsen, 2001). Increased dietary fiber reduces the apparent absorption of P in the large intestine of swine (Partridge et al., 1986). In rats, high fiber supplementation increases fecal excretion of Ca, Mg, Fe, Mn, Zn, and Cu (Gralack, 1996). The digestibility of P in horses is negatively correlated with NDF in the diet, so that as NDF was increased from 35 to 60%, true P digestibility decreased from 40% to 10 to 20% (Pagan, 1998a). In horses, larger particles seem to increase passage rates through the small intestine, and ceacum (Drogoul et al., 1996). Rapid flow of digesta could potentially cause decreased fermentation which may be a protective mechanism in the horse, as the horse is unable to handle large amounts of gas production (Ellis and Hill, 2005). Cattle, who are capable of eructation, have an average fecal particle size of 830 μm, while horses average 1,600-1,630 μm (Uden and Van Soest, 1982). Ruminants may have a higher digestibility of phytate-P as feed is processed to a smaller size, theoretically being exposed to phytase enzymes for longer periods of time than in the horse.

An interaction of passage rate and/or fiber type might have an influence on phytate-P digestibility as the increase in passage rate through the hindgut or interference with enzyme activity such as binding with fibers would decrease availability of P. Also, since a large part of phytate-P availability to horses is thought to lie with the phytase activity of the microbes of the hindgut, it is important to note that large starch meals or quick changes in the diet drastically affect the microbial population in the hindgut and

would hinder phytate-P utilization. Seven hours after a meal of hay, concentrate + minimal hay, or fasted, the total bacteria per (ml x 10^{-7}) in the ceacum and ventral colon of horses averaged: 500, 800, and 5, respectively (Frape, 2004). The availability of soluble carbohydrates will also drastically affect microbial populations, particularly if large amounts of them reach the hindgut and are fermented.

Other Losses

Besides fecal and urinary losses, the horse also expends P in sweat, hair, and hoof growth. These inputs of P are eventually lost and unavailable to tissues. However, they are considered to have a very minimal impact of overall P balance. Equine saliva has been reported to contain 0.19-2.15 mmol P/L (Eckersall et al., 1985; Eckersall, 1984). When sweat was collected for 20 minutes from polo horses, it contained 11-17 mg P (Schryver et al., 1978), suggesting losses of about 73-113 mg P/kg BW/h. Hoof structures are estimated to contain 181.5 ppm P. This concentration varies depending on age, diet, and which part of the hoof is sampled (Evans, 1992). Samples of mane hair contain 294 to 324 μg P/g hair (Asano et al., 2002; Asano et al., 2005). Horse body hair contains 0.35 to 0.39 mg P/g hair (Wysocki and Klett, 1971).

Environmental Impacts

Phosphorus posses an environmental hazard because, as it is introduced to bodies of water, it causes an increase in algae and weed growth that, at first, is unsightly and can decrease the recreational value of the body of water. Eventually, if algae and weed growth go unchecked, decomposition of the organic matter utilizes the dissolved oxygen

in the water. This is detrimental to fish and other aquatic life, and can result in growth and reproductive problems and ultimately death (Knowlton and Cobb, 2006). Agriculture has been identified as the primary non-point source of pollutants that decrease water quality of ponds, streams, rivers, lakes, and reservoirs (EPA, 2000). The main constituents of manure that can cause problems include P, nitrates, ammonia, salts, heavy metals, and organic solids (Waskom and Davis, 1999). Concern is growing about animal wastes relative to the environment. The United States Environmental Protection Agency has set new standards for large livestock operations, called concentrated animal feeding operations or CAFO's (EPA, 2003). These regulations are only a minimum standard and states can choose to legislate more stringent requirements. This is particularly relevant in Michigan, where a large portion of the economy revolves around the natural water resources of the state. Michigan law already prohibits any direct or indirect discharge of nutrients that are or may become injurious to the designated uses of the lakes (MDE, 2007). The cost of treating water to remove pollutants is usually far more expensive and ineffective than simply preventing contamination in the first place (EPA, 2000).

The EPA bases its regulations of animal operations on the number of animal units they house. The amount of regulation the operation is subject to is based on whether they are considered a small, medium, or large CAFO. Small operations generally have less than 250 animal units while large operations have 1,000 animal units. The EPA defines an animal unit as 454.5 kg live weight. Beef cattle, average weight 454.5 kg, are therefore 1 unit; while dairy cows, average weight 636.4 kg are considered 1.4 units. Horses on the other hand are considered 2 units, although few light horses reach 909 kg. Race tracks account for 96% of all the horse operations in the United States that fall under the large

CAFO designation, as they are one of the few places were 500 or more horses are housed at one time (Warren, 2003). Much smaller facilities housing 20 horses or less account for 96% of all equine operations and 73% of the equine population in the United States (NAHMS, 1998). While these small facilities may not currently be under strict regulations, it is very likely that the state and federal governments will begin to regulate even the smallest contributors as concern for environmental contamination rises.

The average horse produces 2.8 kg manure/100 kg BW a day, or 5,110 kg of manure a year for a 500-kg horse (Warren, 2003). The manure from even one horse can have an impact on the environment when it is compiled throughout the years. Soiled stall bedding that is disposed of with manure may also contribute. Bedding can add 3 to 7 kg of waste a day (Warren, 2003). The concern over additional waste due to bedding is one of the reasons that horses are considered 2 animal units. However, more research is needed to determine the actual contribution of the various bedding types. Research conducted on 6 common bedding types found a wide range of removal rates (10.5 to 21.1 kg bedding/day). The P concentration of all bedding types was 1.1 to 3.1 g P/kg bedding (Woodward et al., 2008). Therefore, the P contribution due to bedding is likely dependant upon removal rate, and could be modified through management considerations. Composts with large amounts of bedding reduce the water-soluble P content of the waste (Gagnon et al., 1999), while other studies have found no difference in P concentration after composting with bedding materials (Airaksinen et al., 2001).

Besides additional waste due to bedding, horses are often fed P, and many other nutrients, in great excess of their needs, resulting in higher P in manure. This also adds to the argument for defining horses as 2 animal units. The majority of feed companies do

not limit the amount of P included in their products, and there has been no maximum P concentration determined. In a review of nutrient balances performed in horses, Lawrence et al. (2003 a,b) found a positive correlation between intake and excretion of N, P, and K. If dietary concentrations of P can be decreased, fecal P will also be decreased if P is fed above the requirement. The third argument for horses contributing more P to the land is that horses do not produce a product that removes nutrients from the farm, such as milk, eggs, or meat.

Over 80% of horse facilities in the United States dispose of manure on site (NAHMS, 1998), which means that more P stays on the farm and only continues to stockpile instead of being exported from the farm. Over half of the farms that retain their manure report spreading it on pastures and fields as the primary method of disposal (NAHMS, 1998). The increasing amounts of feed that are imported rather than grown on site further exacerbates this imbalance of P inputs and exports. This may lead to P buildups in the soil. Also, since plants require a N:P ratio of 8:1, applying manure with a ratio of 2.1 to 5.6:1 N:P to meet nitrogen needs means applying large excesses of P that will not be used to support plant growth (Hainze et al., 2004). The National Research Council (1993) estimates that only 30% of the P imported onto farms (such as feeds and fertilizers) is exported (such as crops and animal products). The surplus 70% remains on the farm and potentially causes excessive soil P concentrations (Sturgul and Bundy, 2004). This is often seen in horse management systems where some portion of concentrate, forage, or both is imported. Larger operations are generally limited in the acres of land upon which they are able to apply manure on; which often results in excessive amounts of P being applied (Sturgul and Bundy, 2004). Limited distribution of

nutrients and export of nutrient-rich product from the farm both contribute to excessive P loads in soils of horse farms.

The EPA (2003) recognizes three primary ways that P from manure can enter a body of water. First, horses can be allowed direct access to a water source or there can be intentional dumping of manure into a water source. Second, surface runoff and erosion account for a large amount of the P that makes its way into bodies of water. Contamination via runoff is caused by water passing over soils, collecting nutrients, including P, and eventually dumping them into streams, lakes, and rivers. Drainage tiles used in fields can be a direct line for P to surface waters (MDE, 2007). Finally, P can enter bodies of water via leaching into soil and ground water. Leaching is when P moves, often without the aid of water, through the soil until it reaches groundwater; this process is especially prevalent in solids that are highly saturated with P, sandy soils, fields with high organic matter, or when water tables are very high (Sims et al., 1998). Once the P is in the groundwater, it can travel to surface water. In a P budget prepared for the state of Wisconsin, the major inputs of P to cropland were animal manure and commercial fertilizer, while the major P removals were crop uptake and losses in runoff and erosion (Bundy, 1998). The estimated losses of P due to runoff and erosion represented 1.6% of the P applied to croplands in WI each year (Bundy, 1998).

Two primary physical forms of P in manure affect water quality. The water-soluble fraction, which contains mainly inorganic P, and an insoluble or organic fraction, that would account for P bound to soils or phytate. Inorganic P is more readily available and utilized by plants during the growing season than organic P (Hainze et al., 2004). However, both create environmental concerns as potential contaminates of ground and

surface water (Sharpley et al., 1994). The water soluble-inorganic fraction is more prone to runoff in field management systems that do not incorporate manure or fertilizer. This would be seen in a pasture-based horse management system where P runoff is most prevalent during heavy rains (Sharpley et al., 1994). The insoluble or organic P is lost along with soil particles and other organic matter through erosion. This insoluble fraction can account for 60 to 90% of the P lost from tilled agricultural fields (Sharpley et al., 1994).

Horse manure is unique in that 60 to 70% of total P in manure is in the insoluble fraction and only 30 to 40% in the soluble fraction (Hainze et al., 2004). Other livestock species have a greater proportion of fecal P in the soluble fraction (Barnett, 1994b). In cattle, 62% of total P is inorganic or soluble while 44% in poultry manure is inorganic or soluble (Dou et al., 2000). Therefore, as horse manure contains a higher proportion of this insoluble or organic P, it might be a higher potential contaminant when used in a tilled farming system (Hainze et al., 2004) as the insoluble portion of P is the greatest contributor of P contamination during erosion or runoff from fields. However, horse manure in a traditional pasture setting would be less prone to runoff as it contains less inorganic or water soluble P (Hainze et al., 2004). When runoff reaches a body of water, the smaller soluble portion can have an immediate effect on the algae and weed growth (Sharpley et al.,1996). The insoluble portion generally settles to the bottom of the body of water, and overtime the P is released into the water (Sturgul and Bundy, 2004).

Variations in diet formulation can alter the amount of P a horse excretes. In a study conducted using three commercially available equine feeds, researchers found variability in the P concentrations and the apparent digestibility of P (Wilson et al.,

2003). Many commercial concentrates are available, and they could result in a wide range of fecal P concentrations. Increasing P concentrations in the diet by addition of varying concentrates to a grass hay diet increases fecal P outputs of horses (Hainze et al., 2004). Additionally, digestibility of forage can be increased by the addition of concentrates (Kienzle et al., 2002). Forages can vary in P content and digestibility as much as concentrate diets. A study using Arabian geldings found that alfalfa had a higher apparent digestibility than two grass hays (tall fescue and Caucasian bluestem), and that the alfalfa alone exceeded P requirements for the horses (Crozier et al., 1997). Phosphorus concentration and digestibility within the same general species of hay can vary depending on growth and harvesting conditions. Even diets with the same amount of total P can result in different amounts of fecal P due to variations in feedstuff digestibility. The P content of the hay and concentrate portions of equine diets vary widely, therefore more research is needed on complete diets to begin to quantify an average production of P in horse manure.

Conclusions

The horse derives no benefit from excess P concentrations in the diet, and high P concentrations can be detrimental to the metabolism of other minerals. When P is fed above the true requirement fecal P concentrations increase. Contamination by P originating from horse feces can be detrimental to bodies of water, decreasing their ability to sustain aquatic life and their usefulness for leisure activities. It is therefore important to formulate diets that meet, but do not exceed, the horses true requirement. Studies are needed to define this true requirement in a wide range of horses and to

determine the digestibility of P from the many feedstuffs used commonly in the industry. The investigation of the efficacy of supplemental phytase to decreasing P output in equine feces may prove useful if diets can be formulated to contain P at levels low enough so that any decrease in P output due to increased availability of P from phytate-P would be environmentally significant.

CHAPTER 3

PHOSPHOROUS BALANCE IN MATURE HORSES FED ALFALFA HAY, GRASS HAY, AND GRASS HAY PLUS OATS

Introduction

Phosphorus (P) is the second most abundant mineral in the animal body. As an essential component of equine diets, it is involved with, or can be found in, bone formation, high-energy phosphates, phospholipids, and genetic material. Due to the nature of common equine feedstuffs (Eeckhout and De Paepe, 1994; NRC, 2007) P is often fed in excess; when applied to the land, such as spreading of manure on fields, it is susceptible to runoff into ponds, streams, and lakes. Increasing levels of P leads to overproduction of algae, which can drastically decrease the oxygen supply of the water available to fish and other aquatic life (Knowlton and Cobb, 2006). The United States Environmental Protection Agency has recently set standards for large livestock operations, which primarily affect equine operations that house 500+ horses (EPA, 2003). While this might not seem like a concern to the average horse owner, the EPA has only set minimum standards, and states are capable of enacting legislation to impose higher regulations on a greater percentage of the equine industry. Therefore, many more horse owners' manure management systems may soon fall under state and federal regulation to protect water quality (Warren, 2003). In order to formulate equine diets to maintain P-balance while minimizing output to the environment, it is important to understand how the horse utilizes the P from specific feedstuffs incorporated into the diet. This requires balance studies. Unfortunately, due to their intensive nature, such information is limited.

As there are known interactions between P, Ca and Mg that could affect absorption Ca and Mg balance were also studied.

This study aims to determine the P-balance in mature horses when fed a variety of common equine diets. Our hypothesis was that P digestibility varies with total P, and that overall digestibility of P will decrease as dietary P increases.

Materials and Methods

Animal Management

The Michigan State University Institutional Animal Care and Use Committee approved all methods. Six mature Arabian geldings (16.0 ± 0.3 yr, 452 ± 11 kg) were used in a 6x6 Latin square design study, such that during each period each diet was fed and that by the end of the study each horse had received all diets. Prior to the start of the experiment, all animals were housed in large mixed grass pastures at the Michigan State University Horse Teaching and Research Center with free access to grass hay, water, and a mineral block. On the first day of the study, animals were moved into 3x3 m stalls bedded with wood shavings and the horses were housed in the stalls while on the study. The first 6 weeks were conducted October 8th, 2007 through November 18th, 2007. Horses were returned to pasture and fed regularly for a 2-mo washout period, then the second 6 weeks ran from January 21st, 2008 through March 3rd, 2008. Horses received light exercise 5 days a week as beginning equitation mounts. On non-collection weekends, horses were turned out in a dry lot for approximately 5 hours on Saturday and Sunday. The study consisted of six 14-d feeding periods, each with 11 days of diet adaptation and 3 days of total urine and fecal collection, similar collection protocols have

been previously used by other researchers (Patterson et al., 2002; Hainze et al., 2004; O'Connor et al., 2008). Horses were weighed twice before the start of each new period; the average of the two weights was used for feed calculations. The body condition score (BCS) was determined using the Henneke system (Henneke et al., 1983) before the start of each new period; observations from multiple researchers were averaged to obtain the most accurate score. Hay was fed at 1.6% (as fed) of each horse's adjusted body weight. To prevent overfeeding, the horses' BWs were adjusted by BCS using the following formula: Adjusted Weight=BW- [(BCS-5)*0.04*BW]. This was derived from the NRC (2007) statements that for an average 500-kg horse, the amount of weight to be gained or lost to move one BCS unit is 16 to 20 kg. Thus, 20 kg represents 4% of 500 kg so the BW was adjusted by 4% for each BCS unit greater than 5.

Diets

All hay bales were core-sampled prior to feeding and oats were sampled throughout the experiment. The diets included first cutting alfalfa hay (A1), second cutting alfalfa hay (A2), third cutting alfalfa hay (A3), timothy grass hay (GH), timothy grass hay and 1 kg oats/d (GH+1), and timothy grass hay and 2 kg oats/d (GH+2). All alfalfa hay was taken off the same field, and the timothy grass hay was all from the same cutting. Feed samples for each period were pooled and analyzed separately. Horses were fed at 700 and 1600; these hours best accommodated the horses' other duties as instructional mounts at the University farm. Orts were collected before the morning feeding and weighed; feed refusals were rare. Horses had continuous access to water.

Total Collections

At the start of each collection period, total collection devices (TCD; Equisan Marketing, Melbourne, Australia) were fitted to each animal. The TCDs were completely emptied of feces and urine every 8 hours into plastic containers. If the horse refused to use the TCD to urinate, the TCD was removed and a researcher collected the urine in a pitcher. Total volume of the urine was measured and recorded; a 10% sample of urine was kept in tightly capped Nalgene bottles. Feces were weighed, recorded, mixed and a 10% sample of feces was kept in a plastic bag. All samples were frozen at -20° C immediately after collection. After the completion of the collection period, all samples for a horse were pooled, thoroughly mixed, and a representative sample of urine and feces was retained for analysis. A fasting 10-ml blood sample was obtained from each horse via jugular venipuncture into a glass tube (BD Vacutainer, BD Frankline Labs, NJ) prior to the start of each collection. All blood samples were kept on ice for approximately 20 min. Samples were centrifuged (GS-6KR Centrifuge, Beckman, Fullerton, CA) for 15 min. Serum was pipetted off the top, placed into duplicate micro-centrifuge tubes, and then serum was frozen at -20° C. Frozen fecal samples were oven-dried (Thelco, Precision Scientific, Winchester, VA), then fecal and feed samples were ground through a 1-mm screen (Cyclotec 1093 Sample Mill, Foss, Eden Prairie, MN).

Mineral Analysis

A portion of urine was acidified at a rate of 25 µl per ml of urine with 12 M HCl (EMD Chemicals, Inc., Gibbstown, NJ) to insure all precipitate was in solution (O'Connor et al., 2006); acidified urine was used for Ca and Mg analysis, while non-acidified urine was used for P analysis

Fecal and feed samples were microwave digested as described by Shaw et al. (2002). Briefly, approximately 0.400 g of fecal or feed sample was measured into a Teflon-lined digestion vessel, 10-ml of 70% nitric acid (70% trace-metal grade; Fisher Scientific, Pittsburgh, PA) was added to each sample, and then samples were allowed to digest for 24 h lightly covered at room temperature. Vessels were placed in the microwave assembly and inserted into the microwave accelerated reaction system (MARS-5, CEM Corp., Matthews, NC). Samples were run at 1,200 W, 100% power, 200 PSI max, 190° C, with a ramp time of 30 min and a hold time of 10 min. Samples were allowed to cool in a hood for 5 min and then 2 ml of 30% hydrogen peroxide (J. T. Baker, Phillipsburg, NJ) was added. After 15 min, samples were brought to volume with ddH_2O, and samples were stored at room temperature. All glassware used in the mineral analyses was washed in 30% nitric acid and rinsed with double-deionized water.

Samples were vortexed thoroughly before being used for assays. Digested fecal and feed samples were all diluted 10x, while urine was run with no dilution. Serum samples were first precipitated with 12.5% Trichloroacetic acid (TCA) to remove interfering proteins for P assay, samples were then centrifuged and the supernatant was used for the assay; samples were run at a 4x dilution. Water from the spigot from which horses were watered was sampled and assayed. For all samples, 50 µl was pipetted, in duplicate, into well plates, then 250 µl of Molybdate-Sulfuric (MS) solution and 25 µl of p-Methylaminophenol (Elon) solution were added; samples were vortexed and incubated for 45 min. The liquid digests, urine and serum samples were analyzed for P using a spectrophotometer at 700 nm (Gomori, 1942).

Calcium and Mg concentrations in feeds, feces, urine, and serum were determined by atomic absorption spectroscopy (Unicam 989, Thermo Electron Corp., Franklin, MA). A bovine liver standard (1577b, National Institute of Standards and Technology, Gaitherburg, MD) was digested and analyzed with the samples against the same inorganic standard for each element (atomic absorption standards: CertiPUR, EMD Chemicals Inc., Gibbstown, NJ; P standard: LabChem Inc., Pittsburgh, PA).

Calculations

Mineral concentrations were used to calculate average mineral intake per day, as well as urinary and fecal mineral excreted per day. As urinary P was negligible (0.03 to 0.04 g/d), P balance was calculated as:

P Balance= (Intake of P)-(Output of P in Feces)

Ca and Mg balance were calculated as:

Ca (or Mg) Balance= (Intake of Mineral)-(Output of Mineral in Feces and Urine)

The percent AD of P was calculated as:

$$\text{Apparent Digestibility, \%} = \frac{\text{P Balance}}{\text{Total Intake of Mineral}} \times 100$$

The percent AD of Ca and Mg was calculated as:

$$\text{Apparent Digestibility, \%} = \frac{\text{Ca (or Mg) Balance}}{\text{Total Intake of Mineral}} \times 100$$

Statistical Analysis

Data were analyzed using the MIXED method procedure of SAS software (Version 9.1, SAS Inst., Inc., Cary, NC), with period and horse set as random variables.

Results are reported on a DM basis as LS mean ± SEM. The GLM procedure of SAS software and orthogonal contrasts were used to examine the effect of hay type.

Results

The GH had the lowest P content of 2.9 ± 0.3 g/kg DM. The P content of the three cuttings of alfalfa hay was: A1 = 5.0 ± 0.1 g/kg, A2 = 4.6 ± 0.1 g/kg, and A3= 4.3 ± 0.1 g/kg. The oats had an average P concentration of 5.3 ± 0.4 g P/kg. The percent composition of each diet of P, Ca, and Mg are presented in Table 2, along with the Ca:P ratios for each diet. Average feed intake, urine and fecal output, and total P concentration of the excreta are presented in Table 3. The different P concentrations of the feedstuffs resulted in differences in P intake between diets. The P intake, output, balance, and apparent digestibilities are presented in Table 4. Contrary to our hypothesis, there was no difference in the P-balance or AD between any of the diets. The water was found to contain 0.21 to 0.41 mg P/L. This value was so low that it was not considered in any of the balance calculations.

Table 2: Feed analysis by diet for P, Ca and Mg as percentage of total diet.

	Diet					
Percent of Total Diet	A1	A2	A3	GH	GH+1	GH+2
% P	0.5	0.5	0.4	0.3	0.4	0.4
% Ca	1.9	1.6	2.0	0.5	0.5	0.4
Ca:P	3.8	3.5	4.7	1.5	1.4	1.2
% Mg	0.4	0.3	0.3	0.2	0.2	0.2

Table 3: Intake of hay and oats on a DM basis, fecal and urine total output per day, and total P content of excreta per day.

	Diet						
Item	A1	A2	A3	GH	GH+1	GH+2	SEM
Hay Intake, kg DM/d	6.2	6.0	6.2	6.2	6.2	6.2	0.2
Oat Intake, kg DM/d					0.9^{b}	1.8^{a}	0.01
Fecal Output, kg dm/d	2.9^{c}	2.9^{c}	2.6^{c}	3.3^{b}	3.4^{b}	3.7^{a}	0.2
Urine Output, L/d	8.5^{a}	7.8^{a}	10.5^{a}	3.3^{b}	3.4^{b}	4.8^{b}	1.2
Total P Output, g P/d	29.5	27.0	26.3	24.2	25.0	30.5	1.9

a, b, c: Values within the same row with differing superscripts differ significantly ($P<0.05$)

Table 4: Phosphorus, Calcium and Magnesium intake, output, balance, apparent digestibility and body weights for each of the six diets; P-value for the contrast of all alfalfa hay diets versus all grass hay diets (A vs. G).

	Diet								A vs G
Item	A1	A2	A3	GH	GH+1	GH+2	SEM	P-value	P-value
Weight	452	456	458	459	460	464	12	0.05	-
P									
Intake, mg · kg BW^{-1} · d^{-1}	69^{a}	61^{b}	57^{b}	45^{c}	55^{b}	65^{ab}	4	0.001	0.02
Urine, mg · kg BW^{-1} · d^{-1}	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.54	0.16
Feces, mg · kg BW^{-1} · d^{-1}	65	59	58	53	54	66	4	0.12	0.34
Balance, mg · kg BW^{-1} · d^{-1}	4	2	-1	-8	1	1	6	0.40	0.35
Apparent Digestibility, %	5	2	-1	-23	0	-8	11	0.14	0.17
Ca									
Intake, mg · kg BW^{-1} · d^{-1}	265^{a}	211^{b}	270^{a}	69^{c}	71^{c}	71^{c}	7	0.001	0.001
Urine, mg · kg BW^{-1} · d^{-1}	19^{a}	16^{a}	18^{a}	4^{b}	4^{b}	8^{b}	3	0.006	0.001
Feces, mg · kg BW^{-1} · d^{-1}	100^{a}	100^{a}	130^{a}	39^{b}	35^{b}	38^{b}	14	0.001	0.001
Balance, mg · kg BW^{-1} · d^{-1}	145^{a}	93^{b}	112^{ab}	26^{c}	32^{c}	25^{c}	15	0.001	0.001
Apparent Digestibility, %	55^{a}	42^{ab}	42^{ab}	37^{b}	44^{ab}	35^{b}	8	0.05	0.21
Mg									
Intake, mg · kg BW^{-1} · d^{-1}	48^{a}	41^{b}	42^{b}	26^{d}	29^{cd}	31^{c}	1	0.001	0.001
Urine, mg · kg BW^{-1} · d^{-1}	8^{a}	9^{a}	10^{a}	3^{b}	3^{b}	5^{b}	1	0.001	0.001
Feces, mg · kg BW^{-1} · d^{-1}	26^{a}	23^{ab}	21^{bc}	17^{d}	18^{cd}	18^{cd}	2	0.004	0.001
Balance, mg · kg BW^{-1} · d^{-1}	14	9	11	6	8	9	2	0.20	0.09
Apparent Digestibility, %	28	21	25	24	27	28	5	0.80	0.74

a, b, c, d: Values within the same row with differing superscripts differ significantly ($P<0.05$)

Intake of Ca was higher on the alfalfa diets than on the grass hay diets (Table 4). This resulted in higher outputs of Ca in the urine and feces and higher Ca balances on the alfalfa diets. However, there were no differences in Ca AD between diets. Intake of Mg was also higher on the alfalfa diets than on the grass hay diets, which resulted in higher Mg output in the urine and feces (Table 4). However, there were no differences in Mg balance or AD between diets. There was a trend for the alfalfa diets to result in a higher Mg balance than the grass hay diets.

As expected there were no differences in serum P, Ca, or Mg concentrations between diets (Table 5). All horses maintained serum concentrations within reference range.

Table 5: Phosphorus, Calcium, and Magnesium serum values.

	Diet							
Item	A1	A2	A3	GH	GH+1	GH+2	SEM	P-value
Serum P, mg/dL	3.6	3.7	3.3	3.5	4.2	3.8	0.3	0.23
Serum Ca, mg/dL	12.2	10.1	11.3	11.8	11.9	11.6	0.8	0.49
Serum Mg, mg/dL	1.5	1.8	1.7	1.6	1.7	1.6	0.1	0.26

Discussion

The requirement for horses of the age, BW, and exercise status used in this study is approximately 16 g P/d (NRC, 2007). Using very commonly fed equine diets, P intake exceeded the horses' daily P requirements. However, horses in this study were still able to maintain a near zero P-balance, which one would expect for mature horses at maintenance. Feeding horses above their true requirement for P seems to have no apparent benefit in the mature maintenance horse and can result in large detrimental outputs of P to the environment. That being said, it must again be emphasized that these

were very common equine diets with little to no concentrate fed. It may be impractical for the industry to formulate diets that contain lower amounts of P as the contribution of P from forages fed at standard rates can exceed P requirements.

The near zero or negative apparent digestibilities of P in these diets may be explained, in part, by the fact that these were mature animals receiving only light exercise. These data suggest that horses of this class are very capable of maintaining P homeostasis. Crozier et al. (1997) reported a low AD of P from alfalfa of 8% and from Caucasian bluestem of -9% when fed to 2 to 6 year old Arabian horses. These are fairly close to our average P-AD for the alfalfa (2%) and grass hay (-10%) diets. Sturgeon et al. (2000) reported P-AD of 24, 6, and -1% for diets of alfalfa hay, matua grass hay, or Bermuda grass hay, respectively, fed to mature horses. The higher P-AD for alfalfa seen on that study may have been due to lower levels of intake (32 mg P · kg BW^{-1} · d^{-1}), as compared to the current study (57-69 mg P · kg BW^{-1}· d^{-1}), eliciting higher absorption. Buchholz-Bryant et al. (2001) also reported negative P-AD in mature stock type horses fed coastal Bermuda grass hay and a pelleted concentrate.

Our P-AD values are lower than those obtained utilizing ponies as a model with additional concentrate portions of the diet. The P-AD of mature ponies fed alfalfa hays and oats ranged from 22-24% (Hintz et al. 1984), the intake levels (34-37 mg P·kg BW^{-1}·d^{-1}) were lower than the current study (57-69 mg P·kg BW^{-1}·d^{-1}). Additionally, the diets in this study consisted primarily of long-stem forages. Other studies have used diets containing greater amounts of concentrate or utilized chopped or ground roughage. Such as Hintz and Schryver (1973) who reported P-AD of 15-18% in mature ponies fed pelleted diets of timothy hay, cellulose, corn, soybean meal, calcium carbonate, and

varying levels of magnesium oxide. And in 2 yr old ponies fed diets containing 38% ground grass hay and 59% of beet pulp, ground corn and soybean meal P-AD was 19%; while there were concentrates added to this diet the intake levels (34-49 mg P · kg BW^{-1} · d^{-1}) were similar to intakes of the current study on the grass hay diets (45-65 mg P · kg BW^{-1} · d^{-1}). Hypothetically, the coarseness of the current study's diet could have contributed to greater endogenous losses of P from the gastrointestinal tract.

Phosphorus balance, and therefore the AD, could also have been affected by the average weight loss of nearly 22 kg between periods 1 and 6 (464 ± 8 vs. 443 ± 11 kg) associated with calorie restriction to prevent horses from reaching BCS that were considered hazardous. Horses were already being fed above maintenance levels. Decreases in total body mass depress the maintenance requirement for P, and therefore likely results in less P being absorbed and excess P being expelled into the feces.

Horses maintained a positive Ca balance on all diets. This is similar to other studies utilizing mature horses. Higher intake levels associated with the alfalfa diets did elicit higher Ca balance. However there was only a trend (P = 0.054) for a treatment difference for AD with the A1 diet being greater than the GH and GH+2 diets. The Ca-AD of 46% for alfalfa hay diets is lower than the 72% Ca-AD reported when mature horses were fed alfalfa hay diets with intakes of 248 mg Ca · kg BW^{-1} · d^{-1}, which is similar to the 211 to 270 mg Ca · kg BW^{-1} · d^{-1} horses received on our alfalfa hay diets (Sturgeon et al. 2000). On the same study when horses were fed either matua or Bermuda grass hay with Ca intakes of 92 or 54 mg Ca · kg BW^{-1} · d^{-1}, Ca-AD were 57 and 40% respectively which is again higher than the 30% Ca-AD seen on our grass hay diets. In this case intake of Ca was higher on the two grass hay diets than on our grass hay diets,

which may have lead to the higher Ca-AD. The Ca-AD for alfalfa from the current study closely matches the 46% reported by Crozier et al. (1997) when mature horses were fed alfalfa, and the Ca-AD reported by Hintz et al. (1984) of 44-48% when mature ponies were fed alfalfa hays and oats.

Horses are different from ruminants in that they absorb a greater percentage of dietary Ca; this is regulated by large urinary outputs of Ca that are not seen in ruminants (Schryver et al. 1983). The fact that Ca and P are not absorbed in a 2:1 ratio is partially explained by this very high absorption and excretion of Ca seen in the horse. Therefore the large urinary Ca outputs and relatively higher Ca-AD than P-AD were expected.

Horses remained in a positive Mg balance on all diets. In mature horses fed alfalfa, matua grass hay, or Bermuda grass hay Mg-AD were 58, 48, and 49% respectively (Sturgeon et al. 2000). These are higher than the 25 and 26% Mg-AD from our alfalfa and grass hay diets. Intakes on the study by Sturgeon et al. (2000) were 72, 33, and 26 mg $\cdot$ kg $BW^{-1} \cdot d^{-1,}$ on the alfalfa, matua grass hay, or Bermuda grass hay diets respectively; compared to 41 to 48 mg $\cdot$ kg $BW^{-1} \cdot d^{-1}$ and 26 to 31 mg $\cdot$ kg $BW^{-1} \cdot d^{-1}$ of Mg intake on the current study on the alfalfa or grass hay diets. In mature horses fed alfalfa hay Mg-AD was 27%, and 29 or 32% when those horses were fed Caucasian bluestem or tall fescue (Crozier et al., 1997). Higher values were reported by Hintz et al. (1984) for Mg-AD of 50-53% when mature ponies where fed alfalfa hays and oats.

Also of note, horse owners have long suggested a correlation between feeding alfalfa and increased water consumption. As Table 3 demonstrates, horses on alfalfa hay had a 43% increase in urine output per day. While this did not correlate with an increase in total P output, it would cause increased bedding waste.

These data suggest that any P intake above the amount required for the mature lightly-exercised horse is either not absorbed or is excreted with no evident benefit to the horse and potential negative effects on the environment. There was no difference in apparent P digestibility between alfalfa or grass hay diets; however, alfalfa diets resulted in much higher urine outputs and would increase bedding waste. It is possible to exceed a mature horse's P requirement with forage alone when fed at levels near industry standards; formulating diets that do not exceed the horses true requirement may prove impractical. More studies are needed to more precisely define the minimum P requirement of horses at various life stages and to investigate dietary manipulations to decrease excess P output to the environment.

CHAPTER 4

EFFECTS OF PHYTASE SUPPLEMENTATION IN MATURE HORSES FED ALFALFA HAY AND PELLETED CONCENTRATE DIETS

Introduction

Phytic acid (myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate), the primary storage form of P in the seeds of plants, is very abundant in cereals and some legumes. Phytase (myo-inositol hexaphosphate phosphorohydrolase) catalyzes the removal of inorganic orthophosphates from the phytic acid molecule (Nayni and Markalds, 1986). The P from phytic acid, or phytate, is highly available to ruminants (Morse et al., 1992; Field et al., 1984), but, under normal conditions, it is relatively unavailable to monogastrics who lack the microbial population housed in the rumen (Soares, 1995). Supplemental phytase from microbial sources has proven highly beneficial in increasing phytate-P availability in swine (Murry et al., 1997; Liu et al., 1997; O'Quinn et al., 1997) and poultry (Simons et al., 1990; Kornegay et al., 1996; Yi et al., 1996) and, therefore, decreasing P output into the environment. When P is applied to fields via manure, it is susceptible to runoff into ponds, rivers, and lakes. Increasing levels of P cause eutrophication of the waters, increased algae growth, decreased oxygen levels, and many detrimental effects to fish and other aquatic life (Knowlton and Cobb, 2006).

Previous studies in horses have shown no effect of phytase supplementation on P digestibility (Patterson et al., 2002; Hainze et al., 2004; van Doorn et al., 2004a), few have reported phytate content of the diet and feces. Determining the phytate balance may provide a better idea as to the efficacy of phytase supplementation to equine diets. The purpose of this study was to determine if phytase supplementation increased P

digestibility in diets where P amounts were low comparable to those used commonly in the industry, and to determine phytate digestibility in a fairly customary diet. Also, there are known interactions of P, Ca, and Mg that may affect absorption, and phytase has been shown to affect Ca and Mg availability, therefore Ca and Mg balance was studies. Our hypothesis was that feeding supplemental phytase would increase phytate and P digestibility, as well as decrease fecal P.

Materials and Methods

Animal Management

The Michigan State University Institutional Animal Care and Use Committee approved all methods. Six mature Arabian geldings (16.0 ± 0.3 yr, 478 ± 14 kg) were used in a 6x6 Latin square design study, such that during each period each diet was fed and that by the end of the study each horse had received all diets. Prior to the start of the experiment, all animals were housed in large mixed-grass pastures at the Michigan State University Horse Teaching and Research Center with free access to grass hay, water, and a mineral block. On the first day of the study, animals were moved into 3x3-m stalls bedded with wood shavings. Horses were housed in the stalls while on the study from June 9th through September 1st, 2008. Horses were turned out during the day between feeding times in a dry lot with free access to water. The study consisted of six 14-d feeding periods, each with 11 days of diet adaptation and 3 days of total urine and fecal collection; similar collection protocols have been previously used by other researchers (Patterson et al., 2002; Hainze et al., 2004; O'Connor et al., 2008). Horses were weighed twice before the start of each new period; the average of the two weights was used for

feed calculations. The body condition score (BCS) was determined using the Henneke system (Henneke et al., 1983) before the start of each new period; observations from multiple researchers were averaged to obtain the most accurate score.

Diets

Second-cutting alfalfa hay was included in all diets; hay bales were core-sampled prior to the start of the study, and mineral values were used to formulate the concentrate portion of the diets. The diets included: control diet (C), control diet plus phytase supplement (C+P), high P diet (H), high P diet plus phytase supplement (H+P), forage only (F), and forage plus phytase supplement (F+P). Phytase (Optiphos 4000 PF, JBS United, Sheridan, IN) was added to the pelleted concentrates before pelleting. The product used is considered very stable in normal pelleting temperatures, therefore significant loss of activity was not expected due to pelleting. All diets were initially fed at 1.5% of each horse's BW with intake being altered in three horses to prevent BCS changes greater than 1 unit and to comply with farm requirements. The first four diets consisted of a 40:60 concentrate to hay ratio on a weight basis. The F+P diet was fed forage at 1.498% BW and concentrate at 0.002% BW, while the F diet was completely forage. Concentrates were sampled throughout the study for analysis. Horses were fed at 700 and 1600. Orts were collected before the morning feeding and weighed; feed refusals were rare. Horses had continuous access to water.

Total Collections

At the start of each collection period, total collection devices (TCD; Equisan Marketing, Melbourne, Australia) were fitted to each animal. The TCDs were completely emptied of feces and urine every 8 hours into plastic containers. If the horse

refused to use the TCD to urinate, the TCD was removed and a researcher collected the urine in a pitcher. Total volume of the urine was measured and recorded; a 10% sample of urine was kept in tightly capped Nalgene bottles. Feces were weighed, recorded, mixed and a 10% sample of feces was kept in a plastic bag. Output results are summarized in Table 8. All samples were frozen at -20° C immediately after collection. After the completion of the collection period, all samples for a horse were pooled, thoroughly mixed, and a representative sample of urine and feces was retained for analysis. A fasting 10-ml blood sample was obtained from each horse via jugular venipuncture into a glass tube (BD Vacutainer, BD Frankline Labs, NJ) prior to the start of each collection. All blood samples were kept on ice for approximately 20 min. Samples were centrifuged (GS-6KR Centrifuge, Beckman, Fullerton, CA) for 15 min. Serum was pipetted off the top, placed into duplicate micro-centrifuge tubes, and then serum was frozen at -20° C. Frozen fecal samples were oven-dried (Thelco, Precision Scientific, Winchester, VA), then fecal and feed samples were ground through a 1-mm screen (Cyclotec 1093 sample mill, Foss, Eden Prairie, MN).

Mineral Analysis

A portion of urine was acidified at a rate of 25 µl per ml of urine with 12 M HCl (EMD Chemicals, Inc., Gibbstown, NJ) to insure all precipitate was in solution (O'Connor et al., 2006); acidified urine was used for Ca and Mg analysis, while non-acidified urine was used for P analysis.

Fecal and feed samples were microwave-digested as described by Shaw et al. (2002). Briefly, approximately 0.400 g of fecal or feed sample was measured into a Teflon-lined digestion vessel, 10-ml of 70% nitric acid (70% trace-metal grade; Fisher

Scientific, Pittsburgh, PA) was added to each sample, and then samples were allowed to digest for 24 h lightly covered at room temperature. Vessels were placed in the microwave assembly and inserted into the microwave accelerated reaction system (MARS-5, CEM Corp., Matthews, NC). Samples were run at 1,200 W, 100% power, 200 PSI max, 190° C, with a ramp-time of 30 min and a hold-time of 10 min. Samples were allowed to cool in a hood for 5 min and then 2 ml of 30% hydrogen peroxide (J. T. Baker, Phillipsburg, NJ) was added. After 15 min, samples were brought to volume with ddH_2O, and samples were stored at room temperature. All glassware used in the mineral analyses was washed in 30% nitric acid and rinsed with double-deionized water.

Samples were vortexed thoroughly before being used for assays. Digested fecal and feed samples were all diluted 10x, while urine was run with no dilution. Serum samples were first precipitated with 12.5% Trichloroacetic acid (TCA) to remove interfering proteins for P assay, samples were then centrifuged and the supernatant was used for the assay; samples were run at a 4x dilution. Water from the spigot used to water the horses was sampled and assayed. For all samples, 50 µl was pipetted, in duplicate, into well plates, then 250 µl of Molybdate-Sulfuric (MS) solution and 25 µl of p-Methylaminophenol (Elon) solution were added; samples were vortexed and incubated for 45 min. The liquid digests, urine and serum samples were analyzed for P using a spectrophotometer at 700 nm (Gomori, 1942).

Phytate concentration of feeds and feces was determined colormetrically as described by Latta and Eskin (1980). Briefly, 1 g of each sample was digested in duplicate with 20 ml of 2.4% HCl for one hour. Samples were centrifuged and the supernatant was used. For fecal samples, 6 ml of sample and 6 ml of ddH2O were mixed.

For feeds, 0.5 ml sample and 12 ml ddH2O were used; 10 ml of the diluted sample was then pipetted onto an anion column (BioRad Resin, Anion, 200-400 Mesh AG 1-X8, chloride form). The column was eluted with 15 ml of 0.1 M NaCl so that phytate alone remained on the column. The column was then eluted with 10 ml of 0.7 M NaCl, and the sample was collected. Wade's reagent was added to the sample and centrifuged. Samples were read on a spectrophotometer (DU 7400, Beckman-Coulter, Fullerton, CA) at 500 nm, with the Ca salt of phytic acid, Ca salt with 23.98% P (P9539, Sigma-Aldrich, St. Louis, MO), used to prepare the standard curve. The assay measures the number of phosphate ester bonds, and assumes that a phytate molecule contains 6 phosphorous molecules.

Calcium and Mg concentrations in feeds, feces, urine, and serum were determined by atomic absorption spectroscopy (Unicam 989, Thermo Electron Corp., Franklin, MA). A bovine liver standard (1577b, National Institute of Standards and Technology, Gaitherburg, MD) was digested and analyzed with the samples against the same inorganic standard for each element (atomic absorption standards: CertiPUR, EMD Chemicals Inc., Gibbstown, NJ; P standard: LabChem Inc., Pittsburgh, PA).

Phytase Activity Analysis

Analysis of phytase activity for the C+P, H+P, and F+P concentrate portions of their respective diets was conducted by Eurofins Scientific Inc. (Des Moines, IA) utilizing the AOAC Phytase Method QD176. Feed samples were taken 84 d into the study and frozen until analysis. The enzyme was incubated with a known concentration of the sodium salt of phytic acid. Incubation was terminated by the addition of molybdate/vanadate color stop reagent, forming a colored complex with the phytase-

liberated phosphate. The concentration of this colored complex was then determined spectrophotometrically at 415 nm. The concentration is directly proportional to the phytase activity of the sample.

Calculations

Mineral concentrations were used to calculate average mineral intake per day, as well as urinary and fecal mineral excreted per day. As urinary P was negligible (0.03 to 0.05 g/d), P balance was calculated as:

P Balance= (Intake of P)-(Output of P in Feces)

Ca and Mg balance were calculated as:

Ca (or Mg) Balance= (Intake of Mineral)-(Output of Mineral in Feces and Urine)

The percent AD of P was calculated as:

$$\text{Apparent Digestibility, \%} = \frac{\text{P Balance}}{\text{Total Intake of Mineral}} \times 100$$

The percent AD of Ca and Mg was calculated as:

$$\text{Apparent Digestibility, \%} = \frac{\text{Ca (or Mg) Balance}}{\text{Total Intake of Mineral}} \times 100$$

Phytate balance was calculated in the same manner as the corresponding P values, except phytate intake and output values were used. The difference in the amount of phytate from intake to fecal output was termed "phytate disappearance". The percent of phytate disappearance was calculated as:

$$\text{Phytate Disappearance, \%} = \frac{\text{Phytate Balance}}{\text{Total Intake of Mineral}} \times 100$$

Statistical Analysis

Data were analyzed using the MIXED method procedure of SAS software (Version 9.1, SAS Inst., Inc., Cary, NC), with period and horse set as random variables. Results are reported on a DM basis as least square mean ± SEM. The GLM procedure of SAS software and orthogonal contrasts were used to examine the effects of phytase supplementation and higher forage inclusion rate in the diet.

Results

Percent of total diet as-fed of dietary ingredients including concentrate and forage for each of the six experimental diets is summarized in Table 6. The diets contained 0.29 to 0.45% P and 0.04 to 0.08% phytate on a DM basis (Table 6). The contribution of each dietary component is summarized in Table 7. The targeted phytase activity of 800 FTU/kg was slightly higher than the actual activity in the C+P and H+P concentrates (Table 7). The actual activity of the F+P concentrate was also lower compared to targeted activity of 8000 FTU/kg. Although there was a greater intake of hay on the F and F+P diets than the other 4 diets, there were no differences between any of the diets in total output of feces (kg DM/d), urine (L/d), or total P output of the excreta (g P/d) (Table 8). As designed, intake of P differed between diets; it was highest on the H and H+P diets, and lower on the C, C+P, F and F+P diets (Table 9). This did not correlate with any significant differences in P output, balance, and apparent digestibilities (Table 9). The water was found to contain 0.21 to 0.41 mg P/L; this value was so low that it was not considered in any of the balance calculations.

Table 6: Percent of total diet as-fed of dietary ingredients including concentrate and forage for each of the six experimental diets, and total dietary percent of P, phytate-P, and Ca.						
	Diet					
	C	C+P	H	H+P	F	F+P
Concentrate, % as-fed of total diet						
Corn, fine ground	6.34	6.34	6.34	6.34	-	0.15
Wheat Midds By-Product 27 to 34% NDF	6.29	6.29	6.29	6.29	-	-
Soybean Hulls	8.00	8.00	8.00	8.00	-	-
Beet Pulp	5.72	5.72	5.72	5.72	-	-
Rice-Broken	6.00	6.00	6.00	6.00	-	-
Molasses-Cane	2.00	2.00	2.00	2.00	-	-
Salt	0.40	0.40	0.40	0.40	-	-
Calcium Carbonate	0.30	0.30	0.30	0.30	-	-
Fat-Corn Oil	0.50	0.50	0.50	0.50	-	-
Bentonite	0.80	0.78	0.60	0.58	-	-
Phosphate-Mono Dicalcium	-	-	0.40	0.40	-	-
Soybean Protein Concentrate	1.17	1.17	1.17	1.17	-	-
Corn Gluten Meal 60% Protein	2.00	2.00	2.00	2.00	-	-
Quest Anise 4X	0.02	0.02	0.02	0.02	-	-
776-Horse PX5098[1]	0.04	0.04	0.04	0.04	-	-
Maxi Bond	0.40	0.40	0.20	0.20	-	-
51102 Trace Mineral Premix[2]	0.02	0.02	0.02	0.02	-	-
Phytase[3]	-	0.02	-	0.02	-	0.001
Forage, % as-fed of total diet						
Long stem alfalfa hay	60	60	60	60	100	99.85
Percent of Total Diet, dry matter basis						
% P	0.32	0.29	0.45	0.39	0.30	0.30
% Phytate	0.08	0.08	0.07	0.08	0.05	0.04
% Ca	1.39	1.39	1.47	1.47	1.81	1.64
Ca:P	4.3	4.8	3.3	3.8	6.0	5.5
1:Provided the following per kilogram of diet: 13,216 IU/kg Vitamin A, 1,002 IU/kg Vitamin D, 99 IU/kg Vitamin E, 0.001 mg/kg Vitamin B12, 0.11 mg/kg Biotin, 9 mg/kg Folic Acid, 8 mg/kg Riboflavin, 11 mg/kg Thiamine						
2:Provided the following per kilogram of diet: 1 mg/kg Cobalt, 18 mg/kg Copper, 1 mg/kg Iodine, 12 mg/kg Iron, 84 mg/kg Manganese, 0.3 mg/kg Selenium, 84 mg/kg Zinc						
3: Optiphos 4000 PF, JBS United, Sheridan, IN						

Table 7: Analyzed dry matter, phytate, phosphorus, calcium, magnesium, and phytase activity of the concentrates (C, C+P, H, H+P, F+P) and forage used for the six diets.						
	Diet					
	C	C+P	H	H+P	F+P	Forage
Analyzed Composition, DM Basis						
DM, %	90.29	89.37	89.14	88.83	88.71	88.90
P, g/kg	3.75	2.26	6.77	5.29	2.55	3.01
Phytate P g/kg	1.25	1.24	1.14	1.34	0.26	0.45
Ca, g/kg	7.67	7.75	9.75	9.65	0.65	18.06
Mg, g/kg	2.48	2.51	2.60	2.61	0.94	3.28
Phytase Activity, FTU/kg[1]	-	440	-	530	3,400	-
1: One phytase unit (FTU) is defined as the amount of enzyme which liberates 1 μmol inorganic ortho-phosphate per min from 0.0051 mol/L Na-phytate at pH 5.5 and 37° C.						

Table 8: Intake of hay and oats on a DM basis, fecal and urine total output per day, and total P content of excreta per day.								
	Diet							
Item	C	C+P	H	H+P	F	F+P	SEM	P-Value
Hay Intake, kg DM/d	3.7	3.7	3.7	3.7	6.4	6.1	-	-
Concentrate Intake, kg DM/d	2.5	2.5	2.5	2.5	-	0.6	-	-
Fecal Output, kg DM/d	2.9	3.4	2.7	2.8	2.9	2.9	0.2	0.1
Urine Output, L/d	18.3	19.0	17.9	14.0	16.6	16.2	3.5	0.6
Total P Output, g P/d	21.7	22.2	26.6	26.4	26.5	24.3	3.2	0.6

Table 9: Phosphorus, Calcium, and Magnesium intake, output, balance, apparent digestibility and body weights for each of the six diets; P-values for the contrasts of all phytase diets versus all non-phytase diets (P vs. NP) and the two high forage diets versus the four lower forage diets (H vs. L).

	Diet								P vs. NP	H vs. L
Item	C	C+P	H	H+P	F	F+P	SEM	P-Value	P-Value	
Weight	472	473	471	471	474	474	14	0.91	-	-
P										
Intake, mg·kg $BW^{-1}·d^{-1}$	43^{c}	39^{d}	59^{a}	52^{b}	41^{cd}	43^{c}	1	0.01	0.01	0.01
Urine, mg·kg $BW^{-1}·d^{-1}$	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.22	0.36	0.63
Feces, mg·kg $BW^{-1}·d^{-1}$	46	47	56	56	55	52	6	0.59	0.83	0.75
Balance, mg·kg $BW^{-1}·d^{-1}$	-3	-8	3	-4	-14	-9	6	0.26	0.66	0.14
Apparent Digestibility, %	-8	-21	4	-8	-34	-19	14	0.26	0.75	0.15
Ca										
Intake, mg·kg $BW^{-1}·d^{-1}$	184^{b}	184^{b}	194^{b}	194^{b}	245^{a}	236^{a}	6	0.01	0.54	0.01
Urine, mg·kg $BW^{-1}·d^{-1}$	25	21	18	11	17	13	7	0.50	0.41	0.57
Feces, mg·kg $BW^{-1}·d^{-1}$	114	102	120	105	111	103	11	0.62	0.20	0.75
Balance, mg·kg $BW^{-1}·d^{-1}$	45^{c}	61^{bc}	56^{bc}	78^{b}	117^{a}	120^{a}	12	0.01	0.18	0.01
Apparent Digestibility, %	24^{c}	32^{bc}	29^{c}	40^{abc}	48^{ab}	51^{a}	6	0.02	0.15	0.01
Mg										
Intake, mg·kg $BW^{-1}·d^{-1}$	26^{b}	26^{b}	26^{b}	26^{b}	45^{a}	43^{a}	1	0.01	0.46	0.01
Urine, mg·kg $BW^{-1}·d^{-1}$	11	11	11	10	14	13	2	0.76	0.72	0.23
Feces, mg·kg $BW^{-1}·d^{-1}$	29	29	32	27	27	29	2	0.36	0.51	0.39
Balance, mg·kg $BW^{-1}·d^{-1}$	-1	-1	-4	2	4	3	4	0.38	0.48	0.20
Apparent Digestibility, %	-2	-3	-11	6	9	6	8	0.46	0.46	0.24

a, b, c, d: Values within the same row with differing superscripts differ significantly ($P<0.05$)

Intake of Ca was greatest on the F and F+P diets (Table 9), but no difference was seen between any of the diets in urine or fecal Ca output. The Ca balance was higher at higher levels of intake and horses on all diets maintained a positive Ca balance. There was no difference in Ca AD between the C, H, and F and their respective phytase added diets. Intake of Mg was also greatest on the F and F+P diets (Table 9), but there were no differences in urine Mg, fecal Mg, or Mg balance between all diets. Horses on all diets

maintained a near zero Mg balance, and there was no difference in AD between any of the diets.

Phytate intake ranged from 6.2 to 10.7 mg phytate/kg BW/d (Table 10). Differences in intake did not result in differences in phytate disappearance, which averaged 93%. Phytase supplementation improved the phytate balance on the H diet from 9.0 to 10.0 mg · kg BW^{-1} · d^{-1}, but there was no difference in the respective percentages of disappearance.

Table 10: Phytate intake, output, balance, and percent disappearance for each of the six diets; P-values for the contrasts of all phytase diets versus all non-phytase diets (P vs. NP) and the two high forage diets versus the four lower forage diets (H vs. L).

	Diet								P vs. NP	H vs. L
Item	C	C+P	H	H+P	F	F+P	SEM	P-Value	P-Value	
Phytate										
Intake, mg·kg BW^{-1} ·d^{-1}	10.3^{a}	10.2^{ab}	9.6^{b}	10.7^{a}	6.2^{c}	6.3^{c}	0.3	0.001	0.14	0.01
Feces, mg·kg BW^{-1} ·d^{-1}	0.4	0.7	0.6	0.7	0.5	0.6	0.1	0.55	0.13	0.58
Balance, mg·kg BW^{-1} ·d^{-1}	9.9^{a}	9.4^{ab}	9.0^{b}	10.0^{a}	5.7^{c}	5.6^{c}	0.3	0.001	0.47	0.01
Disappearance, %	96	93	94	93	92	91	2	0.37	0.27	0.04

a, b, c: Values within the same row with differing superscripts differ significantly ($P<0.05$)

As expected, there were no differences between diets in serum concentrations of P, Ca, or Mg; all horses maintained serum concentrations within reference ranges throughout the study (Table 11).

Table 11: Phosphorus, Calcium, and Magnesium serum values.								
	Diet							
Item	C	C+P	H	H+P	F	F+P	SEM	P-value
Serum P, mg/dL	3.1	2.9	3.4	3.2	2.9	3.0	0.2	0.37
Serum Ca, mg/dL	12.1	12.2	12.5	12.7	13.1	12.5	0.5	0.11
Serum Mg, mg/dL	1.9	1.9	2.0	1.9	1.9	2.0	0.1	0.82

Discussion

Horses maintained a near zero P balance. While 5 of the 6 diets resulted in negative balances, those values are not significantly different from the diet that was in positive balance. These were mature maintenance animals, so a near zero balance is expected. Negative balances are obtainable when animals are fed above their requirement for a nutrient, and also have an endogenous loss of the same nutrient. Horses were being fed on average well above their suggested requirement of approximately 13 g P/d (NRC, 2007) and this may have contributed to the large fecal P losses. The negative P balances and P-AD most likely indicate that the horses had a very low P requirement and where therefore excreting a large portion of dietary P and their normal level of endogenous P loss. Other researchers have reported low P-balances in mature horses. When horses received 18 to 44 g P/d, P-balance ranged from 0.4 to 5.6 g/d (van Doorn et al., 2004a). Which is similar to horses fed 28 to 33 g P/d (Patterson et al., 2002) or 20 g P/d (Morris-Stoker et al., 2001) with reported P-balances of 3 to 6 g P/d or 3 to 4.2 g P/d.

The digestibility of P was not affected by phytase supplementation. This may be due to the diets containing relatively low amounts of phytate. Any additional P liberated from phytate may not have had a large enough effect on overall P balance. The increase in phytate balance from the H to the H+P diet may have been due to the higher phytate intake on the H+P diet. The lack of effects on P balance could also be due to the level of

phytase activity in the diet, as perhaps higher levels of activity would result in differences in P balance. However, Van Doorn et al. (2004a) reported no differences in P balance when mature horses were fed hay and a textured ration consisting of oat, corn, wheat bran, and rice bran with and without supplementation of 740 U/kg phytase (one U is the quantity of enzyme that releases 1-μmol of inorganic P per min from 0.5 mM Na-phytate at pH 5.5 and 37 °C; Natuphos 5,000 Liquid, DSM Food Specialties, Delft, The Netherlands). When mature horses were fed 300, 600, or 900 U/kg phytase (one U is the quantity of enzyme that liberates 1-μmol of inorganic P per min from 5.1 mM Na-phytate at pH 5.5 and 37°C; Natuphos 600, BASF, Wyandotte, WI) on top of a grass hay and grain diet of corn, oats, and soybean meal, no effect of enzyme supplementation was seen on P digestibility (Patterson et al., 2002). And in yearling horses fed grass hay and various concentrates including whole oats, sweet feed, a pelleted concentrate, and alfalfa cubes, phytase at 1500 U/g (one U is the quantity of enzyme that releases 1-μmol of inorganic P per min from 0.15 mM Na-phytate at pH 5.5 and 37°C; Allzyme, Alltech, Inc., Nocholasville, KY) supplementation was found to have no effect on P digestibility (Hainze et al., 2004).

While these factors could play a role in whether phytase is effective in increasing P availability in equine diets, a more probable explanation exists as to why most researchers fail to detect benefits from supplementing phytase to horses. The high apparent digestibility of phytate in this study suggests that horses are capable of degrading phytate, probably due to the microbial population in the hindgut. The hindgut of the horse serves many similar functions to the rumen of the ruminant animal. Phytate is quickly hydrolyzed in the rumen (Reid et al., 1947) due to the high phytase activity of

the microbes present (Raun et al., 1956). Phytate-P is considered almost completely available to dairy cattle (Morse et al., 1992; NRC, 2001), while sheep are reported to absorb 78 to 81% of the phytate-P in cereal and vegetable protein sources (Field et al., 1984).

If P were liberated from phytate by the microbes in the hindgut, P absorption would be improved – but only if P were absorbed in the hindgut of the horse. In the ruminant (Khorasani, 1997, Reinhardt et al., 1988; Pfeffer et al., 1970), swine (Moore and Tyler, 1955a,b), and human (Gropper et al., 2005), the main site of P absorption is the small intestine. Schryver et al. (1972) determined that the main site of P absorption in the horse is the dorsal large colon and small colon. Their findings supported those of Alexander (1962), as phosphorous concentrations were significantly higher in the large intestine than in the small intestine, presumably due to the various secretions associated with digestion. This higher concentration may serve as the primary buffer in the dorsal colon for the relatively larger proportion of VFA produced in the hindgut than in the stomach and small intestine, while bicarbonate is the primary buffer system in the ceacum and ventral colon (Alexander, 1963). It would therefore make sense that a mechanism would exist to re-absorb P from these secretions before they were expelled in the feces. Additionally, Matusi et al. (1999) observed the same pattern of P secretion and absorption as Schryver et al. (1972). Horses are therefore likely similar to ruminants, swine, dogs, and humans in that there is net absorption of P in the small intestine; however, they likely differ in that the major site of P absorption in the horse is the large intestine. This is likely why there was no difference in P balance observed between phytase supplemented and non-supplemented diets. Horses are already capable of

releasing P from phytate via the microbes in the hindgut, and this P is then available for absorption in the hindgut.

Horses remained in positive Ca balance, and there was no effect of phytase supplementation on Ca-AD. This is in contrast to van Doorn et al. (2004a) in which horses fed alfalfa meal, wheat bran, and rice bran had improved Ca-AD with phytase supplementation. Phytate does form insoluble salts with Ca that reduce the availability of both Ca and P, it is unclear why no effect of phytase supplementation was seen on Ca-AD in the current study. It maybe due in part to the higher Ca intakes of 87 to 116 g/d on the current study versus 69 g Ca/d fed by van Doorn et al. (2004a). Higher intake levels could mask any effects of phytase releasing Ca from phytate. Horses are different from ruminants in that they absorb a greater percentage of dietary Ca; this is regulated by large urinary outputs of Ca that are not seen in ruminants (Schryver et al. 1983). The fact that Ca and P are not absorbed in a 2:1 ratio is partially explained by this very high absorption and excretion of Ca seen in the horse. Therefore the large urinary Ca outputs and relatively higher Ca-AD than P-AD were expected.

The horses maintained a near zero Mg balance on all diets, a near zero balance was expected as these were mature maintenance animals. Horses were receiving 15 to 24 g Mg/d, which exceeds their requirement of 7 g/d (NRC, 2007). The low balances resulted in an average Mg-AD of 0.8% which is much lower than that reported by Hintz et al. (1984), of 50-53%, when mature ponies were fed alfalfa hay and oats. It is also lower than that reported by Sturgeon et al. (2000) when mature horses were fed alfalfa hay resulting in a Mg-AD of 58% at higher intake levels of 72 mg $\cdot$ kg $BW^{-1} \cdot d^{-1}$ compared to the 43 to 45 mg $\cdot$ kg $BW^{-1} \cdot d^{-1}$ horses received on the current study. It has

been shown that high P diets decrease Mg-AD in ponies from 50 to 36% (Hintz and Schryver, 1972b). It is unclear why there were such low Mg-AD on the current study, it most likely relates back to the horses being mature maintenance animals. There was no effect of phytase supplementation on Mg digestibility, which agrees with previous studies (van Doorn et al., 2004a).

This study further demonstrates that feeding mature maintenance horses above their requirement for P results in higher P outputs with no apparent benefit to the horse. There was no effect on P, phytate, Ca, or Mg output or their digestibilities due to phytase supplementation. Theoretically, if phytase is to be a functional feed additive for horses, diets must contain low amounts of P and high amounts of phytate. Research is needed to determine if P balance in horses receiving high cereal grain concentrate (greater than the 40% used in this study) and low forage diets would be affected by phytase supplementation, as more phytate would be present in the diet. However, these data suggest that horses are already capable of degrading phytate, and any additional benefit to be derived from phytase supplementation may not be cost effective. The most effective way to decrease P output to the environment would therefore be to design low P diets, but more research is needed to make these practical for the horse industry.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Our research has shown that mature maintenance horses and horses receiving light exercise maintain a near zero P balance, even when fed in excess of current recommendations (NRC, 2007) for P intake. The mature horse derives no benefit from excess P concentrations in the diet, and high P concentrations can be detrimental to the metabolism of other minerals. The first study demonstrated that levels far above current recommendations were easily obtained on forage-only diets. It might be impractical for the industry to formulate diets that contain P that meets, but does not exceed, P requirements as the contribution from forages fed at standard rates can exceed P requirements of mature maintenance animals.

The P requirements of all processes including maintenance, growth, pregnancy, and lactation need to be better defined, as well as the endogenous losses from horses in all stages of life and activity levels. Once the true requirement has been defined, research is needed to design diets that contain the lowest amounts of P possible so as to meet, but not exceed, the true requirement, thereby reducing unnecessary output to the environment. The most likely target of diet manipulation would be the concentrate portion, as reductions in organic P supplementation, or reduction of high P feedstuff content would decrease fecal output when P is already supplied at the required levels. This may prove difficult as horses that are being fed large amounts of concentrate often have a high energy requirement. Feedstuffs would need to be identified that can meet these energy requirements at normal dry matter intakes while still addressing the problem of P concentration. This will also require identification of the true digestibility of the

wide range of feedstuffs utilized by the equine industry. Research will have to include diets that address the various ratios of forage to concentrate that are fed, as differences in ratio will affect digestibility.

As the second study demonstrates, phytase is not likely to be a useful feed additive for the equine industry. Horses have a large microbial population in the hindgut that functions much like the population housed in the rumen of ruminants. Ruminants are capable of degrading phytate to liberate P due to their microbial population. Horses most likely have similar microorganism that are responsible for the high apparent digestibility of phytate seen in our research. As the horse relies heavily on the hindgut for digestion of its largely forage diet, it makes inherent sense for a mechanism to exist for P absorption near or after the location of feed digestion. As previously discussed, research has supported the conclusion that P is absorbed to a significant extent in both the small and large intestine of the horse. As phytase is an enzyme, constructed of protein, it is likely degraded in the stomach and small intestine. Any benefit phytase may have would need to be derived early on in digestion. Phytase enhances Ca absorption in other species by liberating Ca from phytate. Our research did not find similar results; however, this may have been due to the low amount of phytate present in the diet. Higher phytate content would increase the likelihood of binding to Ca, and phytase might therefore have an appreciable effect.

Research is now needed to determine to what extent the P liberated from phytate in the hindgut is absorbed by the horse. This level of absorption efficiency could then be used in diet formulation to reduce the amount of inorganic P supplemented to the diet, and ultimately the amount of P introduced to the environment. More research is also

needed to determine how high phytate diets affect the availability or function of other minerals, such as Ca and Mg, starches, lipids, and proteins, in particular enzymes such as trypsin, pepsin, and amylase in horses.

The activities of the microbes in the hindgut of the horse are not well understood. Research is needed to define the population and its various functions and capabilities so that they can be maximized upon by producers. The process of canulating horse's intestinal tracts needs to be refined so that studies can be conducted over longer periods of time utilizing larger sample sizes. This must also be accompanied by research into the absorptive capacity of the hindgut of the horse. As a large portion of digestion occurs in the hindgut it would seem evolutionarily beneficial for the large intestine to have mechanisms for absorption of many of the nutrients derived from microbial processing.

There is only so much that animal science will be able to accomplish in reducing P emissions from livestock. Ultimately we will need to turn to crops and soil science to develop manure and field management strategies that minimize the possibilities of erosion and runoff into waterways, and also to develop feeds with lower P concentrations.

APPENDIX A

COMPARISON OF DATA OBTAINED FROM OPEN CELL DIGEST USING ICP-MS AND DATA OBTAINED FROM MICROWAVE DIGEST USING ICP-MS OR SPECTROPHOTOMETOR

Introduction

The study presented in Chapter 3 originally called for use of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for mineral analysis of all samples. Phosphorus data obtained from this analysis was then compared to data obtained via the colorimetric method of Gomori (1942) utilizing microwave digested samples. To determine the effect of digest method, the microwave digests were then analyzed using the ICP-MS and results were compared.

Materials and Methods

Mineral Analysis-ICP-MS

Fecal samples were freeze dried and ground, feed samples were also ground. Portions of all fecal and feed samples were retained for DM analysis. Ground samples were kept at room temperature until needed for further analysis. Approximately 0.25 g of fecal and feed samples were measured into digestion vials, 2.5-ml of 70% Nitric Acid was added to each sample, they were then allowed to digest for 24 h at 86°C in a heating oven. Double-deionized water was then used to bring the feed and fecal samples up to approximately 12.5 g. Feed and fecal samples were centrifuged before 200 µl of each sample was pipetted into an ICP-MS tube and then diluted with 5ml of ICP-MS Diluent Internal Standard (2% Butanol, 0.05% Triton X-100, 0.05% EDTA, 1% Ammonium Hydroxide, 10 ppb Scandium, 15 ppb Indium, 15 ppb Rhodium, 15 ppb Germanium, 15

ppb Bismuth). A portion of urine was acidified at a rate of 12 µl per ml of urine with 12M HCl to insure all precipitate was in solution (O'Connor, 2006). For mineral analysis, 200 µl of serum and acidified urine were pipetted into test tubes. All serum and urine samples had 5 ml of ICP-MS Diluent Internal Standard added to them. All samples were run in duplicate and vortexed shortly before being run through the ICP-MS (Agilent 7500 CE), located at the Michigan State University Diagnostic Center for Population and Animal Health.

Mineral Analysis-Microwave Digests and Colorimetric Method

Fecal and feed samples were microwave digested as described by Shaw et al. (2002). Briefly, approximately 0.400 g of fecal or feed sample was measured into a Teflon-lined digestion vessel, 10-ml of 70% nitric acid (70% trace-metal grade; Fisher Scientific, Pittsburgh, PA) was added to each sample, and then samples were allowed to digest for 24 h lightly covered at room temperature. Vessels were placed in the microwave assembly and inserted into the microwave accelerated reaction system (MARS-5, CEM Corp., Matthews, NC). Samples were run at 1,200 W, 100% power, 200 PSI max, 190° C, with a ramp time of 30 min and a hold time of 10 min. Samples were allowed to cool in a hood for 5 min and then 2 ml of 30% hydrogen peroxide (J. T. Baker, Phillipsburg, NJ) was added. After 15 min, samples were brought to volume with ddH_2O, and samples were stored at room temperature. All glassware used in the mineral analyses was washed in 30% nitric acid and rinsed with double-deionized water.

Samples were vortexed thoroughly before being used for assays. Digested fecal and feed samples were all diluted 10x, while non-acidified urine was run with no dilution.

Serum samples were first precipitated with 12.5% Trichloroacetic acid (TCA) to remove interfering proteins for P assay, samples were then centrifuged and the supernatant was used for the assay; samples were run at a 4x dilution. For all samples, 50 µl was pipetted, in duplicate, into well plates, then 250 µl of Molybdate-Sulfuric (MS) solution and 25 µl of p-Methylaminophenol (Elon) solution were added; samples were vortexed and incubated for 45 min. The liquid digests, urine and serum samples were analyzed for P using a spectrophotometer at 700 nm (Gomori, 1942).

Mineral Analysis-ICP-MS Utilizing Microwave Digest Samples

Microwave digest samples were run on the ICP-MS utilizing the same method previously described. A P standard and corn bran standard (NIST corn Bran 8433) were run at the same time for comparison.

Results and Discussion

Results obtained from the ICP-MS utilizing an open-cell digest did not closely match those obtained utilizing the colorimetric method and microwave digests, or utilizing the ICP-MS and microwave digests (Table 1A).

Table 1A: Results of phosphorus analysis from ICP-MS utilizing open-cell digests (A), ICP-MS utilizing microwave digests (B), and colorimetric method utilizing microwave digests (C). Coefficients of variation between A and B, A and C, and B and C.								
			A	B	C			
Sample	Period	Sample Type	ICP-MS Open Cell Digest	ICP-MS Microwave Digest	Colorimetric Method Microwave Digest	CV: A vs. B	CV: A vs. C	CV: B vs. C
			mg P/ g sample DM			%		
Hi	1	Fecal	3.9	3.4	3.0	10	19	9
Sand	1	Fecal	4.1	3.7	3.2	9	17	9
Masq	2	Fecal	5.6	4.7	4.5	13	16	4
Geo	2	Fecal	8.1	6.7	5.7	13	25	12
Bo	3	Fecal	5.7	5.1	5.4	7	4	3
Cav	3	Fecal	6.8	5.8	4.9	11	23	12
Hi	3	Fecal	8.7	6.4	5.4	22	33	12
Bo	4	Fecal	7.8	5.8	6.5	21	13	8
Masq	5	Fecal	10.9	8.9	8.8	14	15	1
Geo	5	Fecal	10.2	7.4	6.5	22	31	9
Bo	6	Fecal	10.6	7.2	8.0	27	20	7
GH	1	Hay	1.9	1.9	1.5	1	16	16
GH	2	Hay	1.2	2.3	2.4	44	44	1
GH	3	Hay	1.4	2.4	2.4	35	35	1
GH	6	Hay	4.2	3.4	2.8	14	27	13
A1	2	Hay	4.1	3.9	3.3	4	15	11
A1	5	Hay	4.7	4.5	4.0	3	11	8
A1	6	Hay	6.1	4.0	3.8	30	34	4
A2	1	Hay	4.0	3.7	3.0	5	20	15
A2	4	Hay	2.8	4.0	3.3	25	11	14
A2	6	Hay	3.7	3.8	3.2	2	9	11
A3	2	Hay	1.9	3.4	3.1	40	35	6
A3	4	Hay	3.8	3.6	3.1	4	16	12
A3	6	Hay	2.5	3.4	3.5	21	23	2
Oats	4	Grain	5.6	4.5	4.3	15	18	4
Oats	6	Grain	3.0	5.4	4.9	41	34	8
					Average:	17	22	8
					Fecal	15	20	8
					Hay	18	23	9
					Grain	28	26	6

The standards run on the ICP-MS at the same time as the microwave digests returned values that were within the expected range. It is unclear as to whether the discrepancy in

values is due to the digest or to some type of interference during sampling on the machine. As the variation between the results obtained via the colorimetric method utilizing microwave digests and the ICP-MS utilizing microwave digests was lower than the variations between the colorimetric method and the ICP-MS utilizing open cell digests, it is likely that the problem exists in the digest method. It appears that the open cell digest might be better for fecal samples than feed samples. As the colorimetric method is more standard in the industry, it was utilized for all phosphorus analysis in the two projects.

The average coefficient of variation between ICP-MS and colorimetric P analysis values for serum was 10%, with a range of 0 to 28% (Table 2A). This might be because the colorimetric method called for precipitation with TCA prior to the assay to eliminate interfering proteins. The level of P in urine was below the detection level of the ICP-MS, and therefore that data is not presented here.

Table 2A: Comparison of serum phosphorus analysis between ICP-MS and the colorimetric method.				
		ICP-MS	Colorimetric	CV
Horse	Period	mg P/dl Serum		%
Bo	1	4.1	4.7	10
Masq	1	3.0	3.4	8
Hi	1	3.4	2.9	10
Geo	1	3.8	3.4	8
Cav	1	3.6	3.9	5
Sand	1	2.8	4.0	26
Bo	2	3.4	3.7	5
Masq	2	3.5	3.3	3
Hi	2	4.0	3.2	16
Geo	2	3.5	3.7	4
Cav	2	3.4	5.0	27
Sand	2	3.8	4.0	3
Bo	3	3.8	4.0	3
Masq	3	3.4	4.1	13
Hi	3	3.6	3.1	9
Geo	3	3.6	3.6	1
Cav	3	3.2	4.8	28
Sand	3	3.6	3.6	0

APPENDIX B

ESTIMATED ENDOGENOUS LOSSES AND TRUE DIGESTABILITIES FROM BOTH REPORTED STUDIES

Introduction

A regression technique was utilized to estimate the endogenous losses of P, Ca, and Mg from the two reported studies. The studies were not designed to make this estimate reliable, and therefore it was not presented with the study results. The values may serve some purpose for comparisons with other work utilizing this technique and are therefore reported here.

Materials and Methods

Calculations

Mineral concentrations were used to calculate average mineral intake per day, as well as urinary and fecal mineral excreted per day. As urinary P was negligible (0.03 to 0.04 g/d), P balance was calculated as:

P Balance= (Intake of P)-(Output of P in Feces)

Ca and Mg balance were calculated as:

Ca (or Mg) Balance= (Intake of Mineral)-(Output of Mineral in Feces and Urine)

Estimated endogenous losses were calculated by plotting the mineral balance (Y-axis) against the average mineral intake (X-axis), with the Y-intercept being used as the amount of endogenous losses or the point where intake would be zero. Estimated true mineral digestibility was then calculated as:

$$\text{Est. True Dig, \%} = \frac{\text{(Mineral Balance + Endogenous Fecal Losses)}}{\text{Total intake of Mineral}} \times 100$$

Statistical Analysis

Data were analyzed using the REG procedure of SAS software (Version 9.1, SAS Inst., Inc., Cary, NC).

Results

The R^2 values were less than desirable, with the possible exception of the Ca estimates (Table 1B). The values of the estimated true digestibility and estimated endogenous losses (Table 2B) did not closely match those suggested by the NRC (2007) as shown in Table 3B.

Table 1B: Estimated true digestibilities for the six diets from both reported studies.

	Estimated true digestibilities, %						Estimate		Regression Line	
	Diet						SEM	P-Value	R^2	P-Value
Mineral	A1	A2	A3	GH	GH+1	GH+2				
P, %	39	41	40	32	44	29	8	0.60	0.3	0.002
Ca, %	60	48	47	54	60	52	8	0.22	0.7	0.001
Mg, %	37	31	35	40	42	41	5	0.48	0.3	0.001
	C	C+P	H	H+P	F	F+P				
P, %	74	69	63	60	51	62	15	0.81	0.1	0.10
Ca, %	75	83	77	88	85	90	6	0.33	0.5	0.001
Mg, %	86	85	77	96	86	84	10	0.70	0.2	0.013

Table 2B: Estimated endogenous losses for the six diets from both reported studies.		
	Estimated Endogenous Losses	
Mineral	Study 1	Study 2
P, g/d	16.7	9.4
P, mg/kg BW/d	37.0	20
Ca, g/d	2.4	37
Ca, mg/kg BW/d	5.2	78
Mg, g/d	0.8	14
Mg, mg/kg BW/d	2	31

Table 3B: Estimated true digestibilities and endogenous losses suggested by the NRC (2007).		
Mineral	Estimated True Digestibility (%)	Estimated Endogenous Losses (mg/kg BW/d)
P	30 to 55	10
Ca	50 to 75	20 to 32
Mg	40 to 60	2 to 6

Discussion

The high estimated endogenous losses of Ca, P, and Mg may illustrate the inherent problems with the regression technique utilized, particularly when dietary intakes are far from zero. The technique functions on the principle that the y-intercept represents losses when intake is zero. As intake approaches zero, absorption would be increased and excretion would be decreased to continue to meet demand and maintain blood levels, this could no longer be represented by a linear relationship. When intake levels are far from zero, the discrepancy is likely magnified, resulting in inflated estimations of endogenous loss and estimated true digestibility. To illustrate the changes in the estimation that wider intake ranges can have, values from both studies were combined. The regression line of the combined data of intake versus apparent absorption resulted in estimated endogenous losses of 7.92 g Ca/d (R^2=0.55), which would represent

16.7 mg Ca · kg BW^{-1} · d^{-1} for a 473-kg horse. These values are actually lower than those considered by the NRC (2007), which makes inherent sense, as these animals were mature maintenance horses. When the same thing was done for P intake and balance of the two studies, there was no real improvement in the estimate of endogenous P loss, which was 15.7 g/d (R^2=0.20), representing 33.1 mg · kg BW^{-1} · d^{-1} for a 473-kg horse, which is about 3 times the amount suggested by the NRC (2007). The lack of difference is likely because P intake was on average very similar between the two studies; intake on the first study was 12 to 36 g/d, while the second study ranged from 15 to 32 g/d of P intake. Data was also combined for Mg intake and balance. Intake of Mg on the first study ranged from 11 to 23 g/d, the estimated endogenous losses were 1.02 g Mg/d (R^2=0.03) or 2.2 mg · kg BW^{-1} · d^{-1}. The relatively small increase in range of Mg intake improved estimation of Mg endogenous losses but a similar increase in P intake range did not improve the estimation of P endogenous losses. This is likely because the range of P-balance did not change between the first and second studies (-22 to 7 g/d vs. -15 to 11 g/d), while the range of Mg-balance was different between the two studies (-13 to 18 g/d vs. 0.2 to 13 g/d).

LITERATURE CITED

Abernethy, R. H., G. M. Paulsen, and R. Ellis Jr. 1973. Relationship among phytic acid, phosphorus and zinc during maturation of winter wheat. J. Agric. Food Chem. 21:282-284.

Airaksinen, S., H. Heinonen-Tanski, and M. L. Heiskanen. 2001. Quality of different bedding materials and their influence on the compostability of horse manure. J. Eq. Vet. Sci. 21: 125-130.

Alexander, F., and J.C.D. Hickson. 1970. The salivary and pancreatic secretions of the horse. In: Physiology of Digestion and Metabolism of the Ruminant, A. T. Phillipson, E. F. Annison, D. G. Armstrong, C.C. Balch, R. S. Comline, R. N. Hardy, P. N. Hobson, R.D. Keynes-F.R.S., eds. Oriel Press Limited, Newcastle, England. 375-389.

Alexander, F. 1946. The rate of passage of food residues through the digestive tract of horses. J. Comp. Path. 56:266.

Alexander, F. 1962. The concentration of certain electrolytes in the digestive tract of the horse and pig. Res. Vet. Sci. 3:78-84.

Alexander, F. 1963. Digestion in the horse. In: Progress in Nutrition and Allied Sciences. D. P. Cuthbertson ed. Oliver and Boyd, London. 259-268.

Asano, K., K. Suzuki, M. Chiba, K. Sera, R. Asano, and T. Sakai. 2005. Twenty-eight element concentrations in mane hair samples of adult riding horses determined by particle-induced X-ray emission. Biol. Trace Element Res. 107: 135-140.

Asano, R., K. Suzuki, T. Otsuka, M. Otsuka, and H. Sakurai. 2002. Concentrations of toxic metals and essential minerals in the mane hair of healthy racing horses and their relation to age. J. Vet. Med. Sci. 64: 607-610.

Bach-Knudsen, K. E. 2001. The nutritional significance of "dietary fibre" analysis. An. Feed Sci. Tech. 90: 3-20.

Barnett, G. M. 1994. Manure phosphorus fractionation. Bioresource Tech. 49: 149-155.

Bertone, A. L., F. Toofanian, and T. S. Stashak. 1990. Alteration of intestinal enzyme activities associated with extensive large-colon resection in horses. Am. J. Vet. Res. 51: 1329-1334.

Biehl, R. R., and D. H. Baker. 1997. 1α-Hydroxycholecalciferol does not increase the specific activity of intestinal phytase but does improve phosphorus utilization in both cecectomized and sham-operated chicks fed cholecalciferol-adequate diets. J. Nutr. 127:2054-2059.

Bitar, K., and J. G. Reinhold. 1972. Phytase and alkaline phosphatase activities in the intestinal mucosa of rat, chicken, calf and man. Biochem. Biophys. Acta. 268: 442-452.

Blaney, B. J., R. J. W. Gartner, and R. A. McKenzie. 1981. The effects of oxalate in some tropical grasses on the availability to horses of calcium, phosphorus and magnesium. J. Agric. Sci., Camb. 97: 507-514.

Boass, A., and S. U. Toverud. 1996. Duodenal active calcium transport in female rats increases with serum calcitriol concentrations, but reaches a plateau far below maximal calcitriol levels. J. Bone Min. Res. 11: 1640-1645.

Bourdeau, J. E., D. A. Schwer Dymerski, P. H. Stern, and C. B. Langman. 1986. Calcium and phosphorus metabolism in chronically vitamin D-deficient laboratory rabbits. Miner. Electrolyte Metab. 12: 176-185.

Bray, R. E. 1995. Enteroliths: feeding and management recommendations. J. Eq. Vet. Sci. 15: 474-478.

Breidenbach, A., B. Révész, and J. Harmeyer. 1998. Effects of high doses of vitamin D on calcium and phosphate homeostasis in horses: a pilot study. J. Anim. Physiol. a. Anim. Nutr. 80: 101-107.

Buchholz-Bryant, M. A., L. A. Baker, J. L. Pipkin, B. J. Mansell, J. C. Haliburton, and R. C. Bachman. 2001. The effect of calcium and phosphorous supplementation, inactivity, and subsequent aerobic training on the mineral balance in young, mature, and aged horses. J. Eq. Vet. Sci. 21: 71-77.

Bundy, L. G. 1998. A Phosphorus Budget For Wisconsin Cropland. The Wisconsin Department of Natural Resources and The Wisconsin Department of Agriculture, Trade and Consumer Protection.

Caldwell, R. A. 1992. Effect of calcium and phytic acid on the activation of trypsinogen and the stability of trypsin. J. Agric. Food Chem. 40:43-46.

Capen, C. C. 1983. Nutritional Secondary Hyperparathyroidism. In: Current Therapy in Equine Medicine. N.E. Robinson, ed. W.B. Saunders, Philidelphia, PA. 1983.160-163.

Chew, D. J. 1992. Disorders of calcium: hypercalcemia and hypocalcemia. In: Fluid Therapy in Small Animal Practice. S.P. DiBartola, ed. W.B. Saunders, Philadelphia, PA. 116-130.

Cosgrove, D. J. 1980. Inositol Phosphates: Their Chemistry, Biochemistry, and Physiology. Elsevier Scientific Publishing Co., New York.

Costello, A. J. R., T. Glonek, and T. C. Meyers. 1976. Phosphorus-31 nuclear magnetic resonance-pH titrations of myoinositol hexaphosphate. Carbohydr. Res. 46:159

Crozier, J. A., V. G. Allen, N. E. Jack, J. P. Fontenot, and M. A. Cochran. 1997. Digestibility, apparent mineral absorption, and voluntary intake by horses fed alfalfa, tall fescue, and caucasian bluestem. J. Anim. Sci. 75: 1651-1658.

Cymbaluk, N. F., G. Christison, and D. H. Leach. 1989. Nutrient utilization by limit- and ad libitum fed horses. J. Anim Sci. 67: 414-425.

Davies, M. I., and I. Motzok. 1972. Intestinal alkaline phosphatase and phytase of chicks: separation of isoenzymes, zinc contents and in vitro effects of zinc. Comp. Biochem. Physiol. B. 42:345-356.

Davies, M. I., G. M. Ritcey, and I. Motzok. 1970. Intestinal phytase and alkaline phosphatase of chicks: influence of dietary calcium, inorganic and phytate phosphorus and vitamin D_3. Poult. Sci. 49:1280-1286.

Davies, N. T., and A. A. Flett. 1978. The similarity between alkaline phosphatase (EC 3.1.3.1) and phytase (EC 3.1.3.8) activities in rat intestine and their importance in phytate-induced zinc deficiency. Br. J. Nutr. 39: 307-316.

Davies, N. T., and R. Nightingale. 1975. The effects of phytate on intestinal absorption and secretion of zinc and whole body retention of zinc, copper, iron, and manganese in rats. Br. J. Nutr. 34:243-258.

Deshpande, S. S., and M. Cheryan. 1984. Effects of phytic acid, divalent cations and their interactions on alpha-amylase activity. J. Food Sci. 49:516-519.

Dou, Z., J. D. Toth, D. T. Galligan, K.C.F. Ramberg, and J. D. Ferguson. 2000. Laboratory procedures for characterizing manure phosphorus. J. Environ. Qual. 29:508-514.

Drepper , K., J. O. Gutte, H. Meyer, and F. J. Schwarz. 1982. Energie-und Nahrstoffbedarf landwirtschaftlicher Nutziere. Nr.2 Empfehlungen zur Energie-und Nahrstoffversorgung der Pferde. Frankfurt am Main, Germany: DLG Verlag.

Drogoul, C., F. Faurie, and J. L. Tisserand. 1996. Effects of forage granulation on the cacum and the colon metabolisms in ponies. Proc. 47th European Assoc. Anim. Prod., Lillehammer, Norway.

Dvorakova, J. 1998. Phytase: Sources, preparation, and exploitation. Folia Microbiol. 43 (4): 323-338.

Eckersall, P. D. 1984. Equine whole saliva: a sample collection system and biochemical analysis. Vet. Record. 115: 437-438.

Eckersall, P. D., T. Aitchison, K. M. Colquhoun. 1985. Equine whole saliva: variability of some major constituents. Eq. Vet. J. 17: 391-393.

Edwards, H. M., Jr. 1993. Dietary 1,25-dihydroxycholecalciferol supplementation increases natural phytate phosphorus utilization in chickens. J. Nutr. 123:567-577.

Edwards, H. M., Jr., and J. R. Veltmann Jr. 1983. The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chicks. J. Nutr. 113:1568-1575.

Eeckhout, W., and M. De Paepe. 1994. Total phosphorus, phytate-phosphorus and phytase in plant feedstuffs. Anim. Feed Sci. Technol. 47: 19-29.

Ellis, A. D., and J. Hill. 2005. Nutritional Physiology of the Horse. Nottingham University Press. Nottingham, UK.

EPA. 2000. National Water Quality Inventory: 2000 Report. United States Environmental Protection Agency, EPA-841-R-02-001. http://www.epa.gove/305b/2000report/

EPA. 2003. National Pollutant Discharge Elimination System Permit Regulation and Effluent Limitation Guidelines and Standards for Concentrated Animal Feeding Operations (CAFOs): Final Rule. Federal Register. 68: 7175-7274.

Erdman, J. W. Jr. 1979. Oilseed phytates: nutritional implications. J. Amer. Oil Chem. Soc. 56: 736-741.

Evans, J. W. 1992. Horse Breeding and Management. Elsevier Health Sciences. Amsterdam, Netherlands.

Field, A. C., J. A. Woolliams, R. A. Dingwall, and C. S. Munro. 1984. Animal and dietary variation in the absorption and metabolism of phosphorus by sheep. J Agric. Sci, Camb. 103: 283-291.

Frape, D. Equine Nutrition and Feeding, 3rd Ed. 2004. Blackwell Publishing Ltd. Oxford, UK.

Fredlund, K., N. G. Asp, M. Larsson, I. Marklinder, and A. S. Sandberg. 1997. Phytate reduction in whole grains of wheat, rye, barley and oats after hydrothermal treatment. J. Cereal Sci. 25: 83-91.

Furtado, C. E., H. Tosi, and D. M. S. S. Vitti. 2000. Endogenous loss and true absorption of phosphorus in the diet for growing horses. Pesq. Agropec. Bras. 35: 1023-1028.

Gagnon, G., R. Robitallie, and R. R. Simand. 1999. Characterization of several on-farm and industrial composted materials. Can. J. Soil Sci. 79: 201-210.

Golovan, S. , R. Meidinger, A. Ajakaiye, M. Cottrill, C. Planta, M. Fan, A. Hayes, R. Hacker, J. Phillips, and C. Forsberg. 2001. Enhanced phosphorus digestion and reduced pollution potential by transgenic pigs with salivary phytase. J. Anim. Sci. 79 (Suppl. 1) 753 (Abstr.) 182.

Gomori, G. 1942. A modification of the colorimetric phosphorous determination for use with photoelectric colorimeter. J. Clin. Lab. Med. 27:955-960.

Grace, N. D., S. G. Pearce, E. C. Firth, and P. F. Fennessy. 1999a. Content and distribution of macro- and micro-elements in the body of pasture-fed young horses. Aust. Vet. J. 77: 172-176.

Grace, N. D., S. G. Pearce, E. C. Firth, and P. F. Fennessy. 1999b. Content and distribution of macro- and micro-elements in the milk of pasture fed Thoroughbred mares. Aust. Vet. J. 77: 177-180.

Gralak, M. A., M. Morawiec, E. Bartnikowska, and G. W. Kulasek. 1996. Comparison of the influence of dietary fiber sources with different proportions of soluble and insoluble fiber on Ca, Mg, Fe, Zn, Mn, and Cu apparent absorption in rats. Arch. Tierernahr. 49: 293-299.

Gropper, S., J. L. Smith, J. L. Groff. 2005. Advanced Nutrition and Human Metabolism, 5th Ed. Wadsworth, Cengage Learning. Belmont, CA.

Haenlein, G. F. W., R. C. Smith, and Y. M. Yoon. 1966. Determination of the fecal excretion rate of horses with chromic oxide. J. Anim. Sci. 25: 1091-1095.

Hainze, M. T. M., R. B. Muntifering, C. W. Wood, C. A. McCall, and B. H. Wood. 2004. Faecal phosphorus excretion from horses fed typical diets with and without added phytase. Anim. Feed Sci. Tech. 117: 265-279.

Harland, B. F., and E. R. Morris. 1995. Phytate: a good or bad food component. Nutr. Res. 15: 733-754.

Harmeyer, J., and C. Schlumbohm. 2004. Effects of pharmacological doses of vitamin D3 on mineral balance and profiles of plasma vitamin D3 metabolites in horses. J. Steroid Biochem. Mol. Biol. 89-90: 595-600.

Henneke, D., G. Potter, J. Kreider, and B. Yates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15:371-372.

Highfill, J. L., G. D. Potter, E. M. Eller, P. G. Gibbs, B. D. Scott, and D. M. Hood. 2005. Comparative absorption of calcium fed in varying chemical forms and effects on

absorption of phosphorus and magnesium. Proc. 19th Eq. Sci. Soc. Tucson, AZ. 37-42.

Hintz, H. F., and R. G. Loy. 1966. Effects of pelleting on the nutritive value of horse rations. J. Anim. Sci. 25: 1059-1062.

Hintz, H. F., and H. F. Schryver. 1972a. Availability of calcium and phosphorus in various supplements when fed to ponies. J. Anim. Sci. 34: 979-980.

Hintz, H. F., and H. F. Schryver. 1972b. Magnesium metabolism in the horse. J. Anim. Sci. 35: 755-759.

Hintz, H. F., and H. F. Schryver. 1973. Magnesium, calcium and phosphorus metabolism in ponies fed varying levels of magnesium. J. Anim. Sci. 37: 927-930.

Hintz, H. F., and H. F. Schryver. 1976. Potassium metabolism in ponies. J. Anim. Sci. 42: 637-646.

Hintz, H. F., and H. F. Schryver. 1984. Oxalic acid content of alfalfa hays and its influence on the availability of calcium, phosphorus and magnesium to ponies. J. Anim. Sci. 58: 939-942.

Hintz, H. F., A. J. Williams, J. Rogoff, and H. F. Schryver. 1973. Availability of phosphorus in wheat bran when fed to ponies. J. Anim. Sci. 36: 522-525.

Hintz, H. F., H. F. Schryver, and C. E. Stevens. 1978. Digestion and absorption in the hindgut of nonruminant herbivores. J. Anim. Sci. 46: 1803-1807.

Hintz, H. F. 1997. Straight from the horse's mouth: nutritional secondary hyperparathyroidism still happens. Eq. Pract. 19: 5-6.

Hodgkinson, A., D. H. Marshall, and B. E. Nordin. 1979. Vitamin D and magnesium absorption in man. Clin. Sci. 57: 121-123.

Hoppe, P. P., F. J. Schoner, H. Wiesche, G. Schwarz, and S. Safer. 1993. Phosphorus equivalency of *Aspergillus niger*-phytate for piglets fed a grain-soya bean meal diet. J. An. Physiol. An. Nutr. 69: 1-9.

Horst, R. L. 1986. Regulation of calcium and phosphorus homeostasis in the dairy cow. J. Dairy Sci. 69: 604-616.

Jongbloed, A. W., and P. A. Kemme. 1990. Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. 1. Digestible phosphorus in feedstuffs from plant and animal origin. Neth. J. Agr. Sci. 38: 567-575.

Julliand, V. 2005. Impact of nutrition on the microflora of the gastro-intestinal tract in horses. In: Applied Equine Nutrition, Equine Nutrition Conference. A. Lindmer, ed. Wageningen Academic Publishers, The Netherlands. 85-103.

Khorasani, G. R., R. A. Janzen, W. B. McGill, and J. J. Kennelly. 1997. Site and extent of mineral absorption in lactating cows fed whole-crop cereal grain silage or alfalfa silage. J. Anim. Sci. 75: 239-248.

Kienzle, E., S. Fehrle, and B. Opitz. 2002. Interactions between the apparent energy and nutrient digestibilities of a concentrate mixture and roughages in horses. J. Nutr. 132 (Suppl.): 1788S-1780S.

Knowlton, K. F., and T. D. Cobb. 2006. ASDA Foundation Scholar Award: Implementing waste solutions for dairy and livestock farms. J. Dairy. Sci. 89: 1372-1383.

Knuckles, B. E., and A. A. Betschart. 1987. Effect of phytase and other myo-inositol phosphate esters on alpha-amylase digestion of starch. J. Food Sci. 2: 719-721.

Kornegay, E. T., and H. Qian. 1996. Replacement of inorganic phosphorus by microbial phytase for young pigs fed a maize-soyabean meal diet. Brit. J. Nutr. 76: 563-578.

Kornegay, E. T., D. M. Denbow, Z. Yi, and V. Ravindran. 1996. Response of broilers to graded levels of Natuphos phytase added to corn-soybean meal based diets containing three levels of nonphytate phosphorus. Br. J. Nutr. 75: 839-852.

Latta, M., and M. Eskin. 1980. A simple and rapid colorimetric method for phytate determination, J. Agric. Food Chem. 28:1313-1315.

Lantzsch, H. J., S. Wjst, and W. Drochner. 1995. The effect of dietary calcium on the efficacy of microbial phytase in rations for growing pigs. J. Anim. Physiol. a. Anim. Nutr. 73: 19-26.

Lawrence, L. M., J. Bicudo, J. Davis, and E. Wheeler. 2003a. Relationships between intake and excretion for nitrogen and phosphorus in horses. Proc. 18th Eq. Nutr. Physiol. Symp. Lansing, MI

Lawrence, L., J. R. Bicudo, and E. Wheeler. 2003b. Horse manure characteristics literature and data base review. 9th International Symposium on Animal, Agricultural and Food Processing Wastes. Raleigh, NC.

Lei, X. G., P. K. Ku, E. R. Miller, M. T. Yokyama, and D. E. Ullrey. 1994. Calcium level effects the efficacy of supplemental microbial phytase in corn-soybean meal diets for pigs. J. Anim. Sci. 72: 139-143.

Liu, J., D. W. Bollinger, D. R. Ledoux, M. R. Ellersieck, and T. L. Veum. 1997. Soaking increases the efficacy of supplemental microbial phytase in a low-phosphorus corn-soybean meal diet for growing pigs. J. Anim. Sci. 75: 1292-1298.

Maga, J. A. 1982. Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30: 1-9.

Matsui, T., Y. Murakami, H. Yang, H. Fujikawa, T. Osawa, and Y. Asai. 1999. Phytate and phosphorous movements in the digestive tract of horses. Eq. Vet. J. Suppl. 30: 505-507.

McKenzie, R. A., B. J. Blaney, and R. J. W. Gartner. 1981. The effect of oxalate on calcium, phosphorus and magnesium balances in horses. J. Agric. Sci. Cambr. 97: 69-74.

Merck Veterinary Manual. 8th Ed. 1998. Serum biochemical reference ranges. Merck and Company, Inc. Whitehouse Station, NJ.

Moore, J. H., and C. Tyler. 1955a. Studies on the intestinal absorption and excretion of calcium and phosphorous in the pig. 1. A critical study of the Bergeim technique for investigating the intestinal absorption and excretion of calcium and phosphorous. Brit. J. Nutr. 9: 63.

Moore, J. H., and C. Tyler. 1955b. Studies on the intestinal absorption and excretion of calcium and phosphorous in the pig. 2. The intestinal absorption and excretion of radioactive calcium and phosphorus. Brit. J. Nutr. 9: 81.

Moore, R. J., and T. L. Veum. 1983. Adaptive increase in phytate digestibility by phosphorus-deprived rats and the relationship of intestinal phytase (EC 3.1.3.8) and alkaline phosphatase (EC 3.1.3.1) to phytate utilization. Br. J. Nutr. 49: 145-152.

Morris-Stoker, L. B., L. A. Baker, J. L. Pipkin, R. C. Bachman, and J. C. Haliburton. 2001. The effect of supplemental phytase on nutrient digestibility in mature horses. Proc. 17th Eq. Nutr. Physiol. Soc Symp. Lexington, KY. 48-52.

Morse, D., H. H. Head, and C. J. Wilcox. 1992. Disappearance of phosphorous in phytate from concentrates in vitro from rations fed to lactating dairy cows. J. Dairy Sci. 75: 1979-1986.

Murry, A. C., R. D. Lewis, and H. E. Amos. 1997. The effect of microbial phytase in a pearl millet-soybean meal diet on apparent digestibility and retention of nutrients, serum mineral concentration and bone mineral density of nursery pigs. J. Anim. Sci. 75: 1284-1291.

Nahapetian, A., and A. Bassiri. 1975. Changes in concentrations and interrelationships of phytate, phosphorus, magnesium, calcium, and zinc in wheat during maturation. J. Agric. Food Chem. 23: 1179.

NAHMS. 1998. Equine 1998 Study. United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Animal Health Monitoring System. Centers for Epidemiology and Animal Health, Fort Collins, CO. http://www.aphis.usda.gov/vs./ceah/cahm/Equine/equin.htm

Nayni, N. R., and P. Markakis. 1986. Phytases. In: Phytic Acid: Chemistry and Applications. E. Graf, ed. Pilatus Press. Minneapolis, MN.

Nelson, T. S., and L. K. Kirby. 1979. Effect of age and diet composition on the hydrolysis of phytate phosphorus by rats. Nutr. Rep. Intern. 20: 729.

Nelson, T. S. 1967. The utilization of phytate phosphorus by poultry: a review. Poult. Sci. 46: 862-871.

Nielsen, B. D., G. D. Potter, L. W. Greene, E. L. Morris, M. Murray-Gerzik, W. B. Smith, M. T. Martin. 1998a. Characterization of changes related to mineral balance and bone metabolism in the young racing quarter horse. J. Eq. Vet. Sci. 18: 190-200.

Nielsen, B. D., G. D. Potter, L. W. Greene, E. L. Morris, M. Murray-Gerzik, W. B. Smith, M. T. Martin. 1998b. Response of young horses in training to varying concentrations of dietary calcium and phosphorus. J. Eq. Vet. Sci. 18: 397-404.

NRC. 1989. Nutrient Requirements of Horses. 5th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 1993. Soil and water quality: an agenda for agriculture. Natl. Acad. Press, Washington, DC.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2005. Mineral Tolerance of Animals, 2nd rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2007. Nutrient requirements of Horses. 6th rev. ed. Natl. Acad. Press, Washington, DC.

O'Connor, C. I., B. D. Nielsen, A. D. Woodward, H. S. Spooner, B. A. Ventura, and K. K. Turner. 2008. Mineral balance in horses fed two supplemental silicon sources. J. Anim. Phys. Anim. Nutr. 92:173-181.

O'Quinn, P. R., D. A. Knabe, and E. J. Gregg. 1997. Efficacy of Natuphos® in sorghum based diets for finishing swine. J. Anim. Sci. 75: 1299-1307.

Olsson, N., G. Kohlen, and W. Cagell. 1949. Smaltberhaetsfork med haster. Lantbrukshog-skolan Husdjursforsoksanstalen. Medd. Nr. 36 (English Summary)

Pagan, J. D. 1994. Nutrient digestibility in horses. Feeding the performance horse. In Proc. KER Short Course for Feed Manufacturers. Lexington, KY. 127-136.

Pagan, J. D. 1998a. Factors affecting mineral digestibility in horses. In: Advances in Equine Nutrition, Proc. Equine Nutrition Conference for Feed Manufacturers. Lexington, KY. 89-104.

Pagan, J. D. 1998b. Nutrient digestibility in horses. In: Advances in Equine Nutrition. J. D. Pagan, ed. Nottingham University Press, Nottingham, UK.

Pagan, J. D. 2005. Nutritional challenges of feeding the two-year-old. In: Advances in Equine Nutrition. J. D. Pagan, ed. Nottingham University Press, Nottingham, UK. 359-364.

Partridge, I. G., O. Simon, H. Bergner. 1986. The effects of treated straw meal on ileal and faecal digestibility of nutrients in pigs. Arch. Tierernahr. 36: 351-359.

Patterson, D. P., S. R. Cooper, D. W. Freeman, and R. G. Teeter. 2002. Effects of varying levels of phytase supplementation on dry matter and phosphorus digestibility in horses fed a common textured ration. J. Eq. Vet. Sci. 22: 456-459.

Patwardhan, V. N. 1937. The occurrence of a phytin-splitting enzyme in the intestines of albino rats. Biochem. J. 31: 560-564.

Pfeffer, E., A. Thompson, and D. G. Armstrong. 1970. Studies on intestinal digestion in the sheep. 3. Net movement of certain inorganic elements in the digestive tract on rations containing different proportions of hay and rolled barley. Br. J. Nutr. 24: 197-204.

Pileggi, V. J., 1959. Distribution of phytase in rat. Arch. Biochem. Biophys., 80: 1-8.

Pointillart, A. L., A. Fourdin, and N. Fontaine. 1987. Importance of cereal phytase activity for phytate phosphorus utilization by growing pigs fed triticale or corn. J. Nutr. 117: 907-913.

Qian, H., E. T. Kornegay, and D. E. Conner Jr. 1996. Averse effects of wide calcium:phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. J. Anim. Sci. 74: 1288-1297.

Ramirez, S., and T. L. Seahorn. 1997. How to manage nutritional secondary hyperparathyroidism in horses. Vet. Med. 92: 978-985.

Raun, A., E. Cheng, and W. Burroughs. 1956. Phytate phosphorus hydrolysis and availability to rumen microorganisms. J. Agric. Food Chem. 4: 869-871.

Ravindran, V., E. T. Kornegay, D. M. Denbow, Z. Yi, and R. M. Hulet. 1995. Response of turkey poults to tiered levels of Natuphos phytase added to soybean meal-based semi-purified diets containing three levels of non-phytate phosphorus. Poult. Sci. 74: 1843-1854.

Reddy, N. R., M. D. Pierson, S. K. Sathe, and D. K. Salunkhe. 1989. Phytates in Cereals and Legumes. CRC Press, Inc. Boca Raton, FL.

Reid, R. L., M. C. Franklin, and E. G. Hallsworth. 1947. The utilization of phytate phosphorus by sheep. Australian Vet. J. 23:136-140.

Reinhardt, T. A., R. L. Horst, and J. P. Goff. 1988. Calcium, phosphorus, and magnesium homeostasis in ruminants. In: Metabolic Diseases of Ruminant Livestock. Veterinary Clinics of North America: Food Animal Practice. 4: 331-350.

Roose, K. A., K. E. Hoekstra, J. D. Pagan, and R. J. Geor. 2001. Effect of aluminum supplement on nutrient digestibility and mineral metabolism in thoroughbred horses. Proc. 17th Eq. Nutr. Physiol. Symp. Lexington, KY. 364-369.

Rose, B. D. 1989. Hormonal regulation of calcium and phosphate balance. Clinical Physiology of Acid-Base and Electrolyte Disorders. McGraw-Hill. New York, NY. 150-167.

Saio, K., E. Koyama, and T. Watanabe. 1967. Protein-calcium-phytic acid relationship in soybean. 1. Effects of calcium and phosphorus on solubility characteristics of soybean meal protein. Agric. Biol. Chem. 31: 110-115.

Sandberg, A. S., T. Larsen, B. Sandstorm. 1993. High dietary calcium level decreases phytate degradation in pigs fed a rapeseed diet. J. Nutr. 123: 559-566.

SAS. Institute Inc. SAS/STAT User's Guide, Version 9.1, Cary, NC. (2003).

Schoner, F. J., P. P. Hoppe, G. Schwarz, and H. Wiesche. 1993. Comparison of microbial phytase and inorganic phosphate in male chickens: influence on performance data, mineral retention and dietary calcium. J. Anim Physiol. Anim. Nutr. 69: 235-244.

Schryver, H. F., P. H. Craig, and H. F. Hintz. 1970. Calcium metabolism in ponies fed varying levels of calcium. J. Nutr. 101: 955-964.

Schryver, H. F., T. J. Foose, J. Williams, and H. F. Hintz. 1983. Calcium excretion in feces of ungulates. Comp. Biochem. Physiol. 74A: 375-379.

Schryver, H. F., H. F. Hintz, and P. H. Craig. 1971a. Phosphorous metabolism in ponies fed varying levels of phosphorous. J Nutr. 101: 1257-1264.

Schryver, H. F., H. F. Hintz, and P. H. Craig. 1971b. Calcium metabolism in ponies fed a high phosphorous diet. J Nutr. 101: 259-264.

Schryver, H. F., H. F. Hintz, P. H. Craig, D. E. Hogue, and J. E. Lowe. 1972. Site of phosphorus absorption from the intestine of the horse. J. Nutr. 102: 143-148.

Schryver, H. F., H. F. Hintz, J. E. Lowe, R. L. Hintz, R. B. Harper, and J. T. Reid. 1974a. Mineral composition of the whole body, liver, and bone of young horses. J. Nutr. 104: 126-132.

Schryver, H. F., H. F. Hintz, and J. E. Lowe. 1974b. Calcium and phosphorus in the nutrition of the horse. Cornell Vet. 64: 493-515.

Schryver, H. F., H. F. Hintz, and J. E. Lowe. 1978. Calcium metabolism, body composition, and sweat losses of exercised horses. Am. J. Vet. Res. 39: 245-248.

Schryver, H. F., D. L. Millis, L. V. Soderholm, J. Williams, and H. F. Hintz. 1986. Metabolism of some essential minerals in ponies fed high levels of aluminum. Cornell Vet. 76: 354-360.

Schryver, H. F., M. T. Parker, P. D. Daniluk, K. I. Pagan, J. Williams, L. V. Soderholm, and H. F. Hintz. 1987. Salt consumption and the effect of salt on mineral metabolism in horses. Cornell Vet. 77:122-131.

Sharpley, A. N., S. C. Chapra, R. Wedepohl, J. G. Sims, T. C. Daniel, and K. R. Reddy. 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. J. Environ. Qual. 23: 437-451.

Sharpley, A.N., T.C. Daniel, J.T. Sims, and D.H. Pote. 1996. Determining environmentally sound phosphorus levels. J. Soil Water Conser. 51: 160-166.

Shaw, D. T., D. W. Rozeboom, G. M. Hill, and J. E. Link. 2002. Impact of vitamin and mineral supplement withdrawal and wheat middling inclusion on finishing pig growth performance, fecal mineral concentration, carcass characteristics, and the nutrient content and oxidative stability of pork. J Anim. Sci. 80:2920–2930.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Walters, R. F. Beudeker, and G. J. Verschoar. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Brit. J. Nutr. 64: 525-540.

Sims, J. T., R. R. Simard, and B. C. Joern. 1998. Phosphorus loss in agricultural drainage: Historical perspective and current research. J. Environ. Qual. 27: 277-293.

Singh, M., and A. D. Krikorian. 1982. Inhibition of trypsin activity in vitro by phytate. J. Agric. Food Chem. 30: 799-800.

Smothers, R. L., B. S. Levine, F. R. Singer, G. F. Bryce, J. P. Mallon, O. N. Miller, J. W. Coburn. 1986. Relationship between urinary calcium and calcium intake during calcitriol administration. Kidney Int. 29: 578-583.

Soares, J. H. 1995. Phosphorus bioavailability. In: Bioavailability of Nutrients for Animals. C. B. Ammerman, D. H. Baker, and A. J. Lewis, eds. Academic Press, New York, NY. 257-294.

Spitzer, R. S., and P. H. Phillips. 1945. Enzymatic relationships in the utilization of soybean oil meal phosphorus by the rat. J. Nutr. 30:183.

Stephens, T. L., G. D. Potter, P. G. Gibbs, and D. M. Hood. 2004. Mineral balance in juvenile racing horses in race training. J. Eq. Vet. Sci. 24: 438-450.

Sturgul, S. J., and L. G. Bundy. 2004. Understanding Soil Phosphorus: An overview of phosphorus, water quality, and agricultural management practices. University of Wisconsin-Extension, United States Department of Agriculture. A3771.

Sturgeon, L. S., L. A. Baker, J. L. Pipkin, J. C. Haliburton, and N. K. Chirase. 2000. The digestability and mineral availability of matua, Bermuda grass, and alfalfa hay in mature horses. J. Eq. Vet. Sci. 20: 45-48.

Taylor, T. G. 1965. The availability of the calcium and phosphorus of plant materials by animals. Proc. Nutr. Soc. 24: 105-110.

Taylor, T. G., and J. W. Coleman. 1979. A comparative study of the absorption of calcium and the availability of phytate phosphorus in the golden hamster (Mesocricetus auratus) and the laboratory rat. Br. J. Nutr. 42: 113.

Thompson, L. U., and J. H. Yoon. 1984. Starch digestibility as affected by polyphenols and phytic acid. J. Food. Sci. 49: 1228-1229.

Uden, P., and P. J. Van Soest. 1982. The determination of digesta particle size in some herbivores. Anim. Feed Sci. Tech. 7: 35-44.

Underwood, E. J., and N. F. Suttle. 1999. The Mineral Nutrition of Livestock. (3rd Ed.). CABI Publishing. New York, NY. 105-148.

Van Doorn, D. 2003. Equine phosphorus absorption and excretion. PhD Thesis, Utrecht University, The Netherlands.

Van Doorn, D.A., H. Everts, H. Wouterse, and A.C. Beynen. 2004a. The apparent digestibility of phytate phosphorus and the influence of supplemental phytase in horses. J. Anim. Sci. 82: 1756-1763.

Van Doorn, D. A., M. E. Van Der Spek, H. Everts, H. Wouterse, and A. C. Beynen. 2004b. The influence of calcium intake on phosphorus digestibility in mature ponies. J. Anim. Physiol. Anim. Nutr. 88: 412-418.

Vander Noot, G. W., L. D. Symons, R. K. Lydman, and P. V. Fonnesbeck. 1967. Rate of passage of various feedstuffs through the digestive tract of horses. J. Anim. Sci. 26: 1309-1311.

Vohra, A., and T. Satyanarayana. 2003. Phytases: Microbial Sources, Production, Purification, and Potential Biotechnological Applications. Crit. Rev. Biotechnol. 23: 29-60.

Warren, L. K. 2003. Conquering mount manure: the need for education, outreach, and research in the horse industry. Proc. 18th Eq. Nutr. Physiol. Soc. Symp. East Lansing, MI. 220-229.

Waskom, R. M., and J. G. Davies. 1999. Best Management Practices for Manure Utilization. Colorado State University Cooperative Extension, Bulletin 568A.

Wasserman, R. H., and A. N. Taylor. 1976. Gastrointestinal absorption of calcium and phosphorus. In: Handbook of Physiology. Sect. 7: Endocrinology. Vol. VII., Parathyroid Gland. G. D. Aubach, ed. Washington, D.C. Am. Physiol. Soc. 137-155.

Welch, R. M., and W. A. House. 1982. Availability to rats of zinc from soybean seeds as affected by maturity of seed, source of dietary protein, and soluble phytate. J. Nutr. 112: 879.

Welch, R. M., W. A. House, and W. H. Allaway. 1974. Availability of zinc from pea seeds to rats. J. Nutr. 104: 733-740.

Whitlock, R. H., H. F. Schryver, L. Krook, H. F. Hintz, and P. H. Craig. 1970. The effects of high dietary calcium in horses. Proc. 16th Am. Assoc. Eq. Pract. 127-130.

Wilson, J. A., C. W. Babb, and R. H. Prince. 2003. Digestibility of three commercially available equine pelleted feeds. Proc. 18th Eq. Nutr. Phys. Soc. Symp. East Lansing, MI. 95.

Woodward, A. D., B. D. Nielsen, C. I. Robison-O'Connor, and J. M. Witherspoon. 2008. Phosphorus contribution of six equine bedding types. J. Anim. Sci. 86 (E-Suppl.2): Abstr. 551. 517.

Wysocki, A. A,. and R. H. Klett. 1971. Hair as an indicator of the calcium and phosphorus status of ponies. J Anim Sci. 32: 74-78.

Yi, Z., E. T. Kornegay, V. Ravindran, and D. M. Denbow. 1996. Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphors equivalency values for phytase. Poult. Sci. 75: 240-249.