

THE INTERACTION OF DIETARY PHOSPHORUS AND EXOGENOUS PHYTASE
CONCENTRATIONS ON NUTRIENT METABOLISM OF BEEF CATTLE

BY

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THESIS

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ABSTRACT

Phytate, the salt of phytic acid, is the main form of phosphorus (P) in cereal grains and by-product feeds and P must be cleaved from phytate to be absorbed. The P associated with phytate can make up to 60 to 80% of total P in cereal grains (Eeckhout and De Paepe, 1994; Viveros et al., 2000). Thus, beef cattle are fed dietary inclusions of P above their requirement to ensure adequate amounts are absorbed. As P intake increases above the requirement of the animal, endogenous fecal P and feed P excretion may also increase (Braithwaite et al., 1985; Scott et al., 1985; Ternouth, 1989), contributing to P pollution of surface water (U.S. EPA, 1996). Reducing P excreted in manure would help mitigate the impact beef cattle have on eutrophication of fresh waters. One way to reduce excreted P is by increasing the availability of P to the animal, thereby increasing absorption. A popular feeding strategy to increase P availability for nonruminants is the dietary addition of phytase, an enzyme that releases P bound to phytate, making it more available for absorption (Lei et al., 1993; Ravindran et al., 2000; Jongbloed et al., 2004). The microbial population in the rumen produces phytases; however, passage rate, Ca-complexes formed with phytate, and processing of dietary ingredients can reduce ruminal phytase activity (Konishi et al., 1999; Park et al., 1999; Kincaid et al., 2005). Limited data exists on the addition of supplemental, exogenous phytase to beef cattle diets to improve P availability to reduce the need for supplemental P.

Objectives were to determine the interactions of phytase inclusion and dietary P concentration on metabolism and digestibility of beef cattle fed starch-based diets. Six ruminally-fistulated steers (initial BW = 750 ± 61 kg) were fed in a 6 × 6 Latin square design with a 3 × 2 factorial arrangement of treatments: 1) 0 FTU phytase, 0.10% dietary P, 2) 500 FTU phytase, 0.10% dietary P, 3) 2,000 FTU phytase, 0.10% dietary P, 4) 0 FTU phytase, 0.30%

dietary P, 5) 500 FTU phytase, 0.30% dietary P, 6) 2,000 FTU phytase, 0.30% dietary P. Where one FTU is defined as the amount of phytase that liberates 1 μmol of inorganic phosphate per minute from 0.0051 M Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al., 1994).

There were no main effects of phytase inclusion on any parameter measured. However, dietary concentrations of P affected ($P < 0.01$) DMI, total fecal output, and apparent DM digestibility. Steers fed the 0.10% P diet, consumed less DM and excreted less feces, but had increased apparent DM digestibility compared with steers fed the 0.30% P diet. Diets deficient in P often reduce intake (Riddell et al., 1934; Call et al., 1986; Geisert et al., 2010), thus, the 0.10% P diet, likely did not supply adequate P, despite dietary phytase inclusions. In addition, the 0.10% P diet contained corn starch instead of corn to achieve the reduced P. Highly processed ingredients, like corn starch, are known to increase digestibility (Murphy et al., 1994). However, they may also decrease pH. In fact, steers fed the 0.10% P diet had ruminal pH below 5.5 by 6 hours post-feeding, and, thus, were more acidotic ($P = 0.01$) than steers fed 0.30% P. There were no differences ($P > 0.35$) in water intake and urine output between steers fed differing concentrations of dietary P. There was no difference ($P = 0.86$) in g/d intake of N nor was N retention affected ($P = 0.84$). Nitrogen retention was unaffected because steers fed the 0.10% P diet tended ($P = 0.10$) to absorb more N and excreted more ($P = 0.02$) N in the urine and less ($P < 0.01$) N in the feces compared to steers fed 0.30% P.

Concentration of P in the diets fed in this study, affected P digestibility. Steers fed 0.10% P consumed less ($P < 0.01$) P and excreted less P in feces ($P < 0.01$) and urine ($P < 0.01$) than steers fed 0.30% P. In steers consuming the 0.10% P diet, excretion of P was greater than intake of P. Therefore, both retention of P ($P < 0.01$) and absorption of P ($P < 0.01$) were negative. Steers fed the 0.10% P diet had an average plasma P concentration of 2.87 mg/dL, suggesting

they were, in fact, P-deficient. While steers on the 0.30% P diets, had plasma P concentrations in the P-adequate range, defined as greater than 4.50 mg/dL (NRC, 2000).

Water-soluble P concentration in the feces was greater ($P < 0.01$) in steers fed 0.30% P. However, the proportion of total fecal P that was excreted as water-soluble P was increased by 23.0% in steers fed 0.10% P compared to steers fed 0.30% P, regardless of phytase inclusion level. Not only did steers fed 0.30% P consume more ($P < 0.01$) total P but they consumed 100% more phytate-P as a proportion of total dietary P than cattle fed 0.10% P.

Although there was no interaction between phytase and P concentration in the current study, conclusions can be drawn from metabolism of P in steers fed either P-deficient or P-adequate diets. Steers that consumed a P-deficient diet, 0.10% P, had decreased DMI, which led to decreased fecal output, and these steers were considered to be in negative P balance due to negative absorption and retention values. Diets were formulated to provide similar N per day, and although steers tended to absorb more N during P deficiency, they excreted more N in their urine, thus N retention was not altered. Changes in blood P concentration, as a method to evaluate P status in ruminants, can be determined within 20 d consumption of a P-deficient diet.

DEDICATION

To my sister, Phoebe Long, and my parents, Rich and Roxann Long. For your love and support in this journey. Thank you for allowing me to come to you at any time for advice and guidance whether it be a shoulder to cry on or a smile celebrating a victory. You all have demonstrated patience, kindness, persistence, and endurance when the going gets tough and truly are my backbone. These characteristics I aim to emulate and with your demonstration, allowed for a successful completion of my master's degree.

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TABLE OF CONTENTS

CHAPTER 1: LITERATURE REVIEW	1
Introduction.....	1
Phosphorus Metabolism in Beef Cattle.....	3
Environmental Concerns with Phosphorus.....	8
Plant-bound Phosphorus and Phytate.....	12
Phytase	15
Summary	19
CHAPTER 2: EFFECTS OF THE INTERACTION OF DIETARY PHOSPHORUS AND EXOGENOUS PHYTASE CONCENTRATIONS ON PHOSPHORUS METABOLISM OF BEEF CATTLE	20
Abstract	20
Introduction.....	21
Materials and Methods.....	22
Results and Discussion	27
Tables	36
Figures.....	40
CHAPTER 3: IMPLICATIONS	44
LITERATURE CITED	45

CHAPTER I: LITERATURE REVIEW

Introduction

Consolidated animal agriculture is identified as one of the major contributors to phosphorus (**P**) pollution of surface water (EPA, 2001). Beef cattle contribute to this statistic because they are fed high dietary inclusions of P. One of the reasons cattle are fed elevated P in the diet is to ensure adequate amounts are absorbed. As P intake increases above the requirement of the animal, endogenous fecal P and urinary P excretion increase (Braithwaite, 1985; Scott et al., 1985; Ternouth, 1989), causing nutrient management and environmental concerns.

Phosphorus is not found in its free-form in nature because it is too reactive (McDowell, 1992). Therefore, it must always be attached to an organic molecule of some kind in nature, such as, phosphate (**PO₄³⁻**). Phytate, the salt of phytic acid (*myo*-inositol hexakisphosphate; inositol and six phosphate groups), is the molecule that binds P as **PO₄³⁻** in cereal grains and by-product feeds. Phytate has a molecular weight of 660 g/mol (Selle et al., 2011). The P associated with one phytate molecule is 282 g P per kg (Selle et al., 2011) which makes up 60 to 80% of total P in cereal grains (Eeckhout and De Paepe, 1994; Viveros et al., 2000). In addition, cereal grains are the main feed ingredient used in beef cattle finishing diets in the Midwest. The phytate molecule itself is indigestible and tightly binds P (Simons and Versteegh, 1990) thereby decreasing P availability (Clark et al., 1986; Simmons et al., 1990; Kincaid et al., 2005). For P to be detached from phytate, an enzyme, phytase, must be present (Simons and Versteegh, 1990). The enzyme phytase catalyzes the hydrolysis of the phytate molecule, liberating the attached **PO₄³⁻** groups from the inositol ring so that they may be absorbed by the animal. In cattle, the microbial population in the rumen produces phytase. The microbial population in the rumen is

responsible for the majority of ruminal phytase activity, and phytase is associated with the bacteria, not free in the fluid (Yanke et al., 1998). More specifically, the starch-fermenting bacteria, rather than protozoa or fungi, have the most phytase activity (Kincaid and Rodehutsord, 2005). Raun et al. (1956) discovered that when phytase from the rumen of a Shorthorn steer was added to an in vitro culture with organic phytate (as calcium phytate), the PO_4^{3-} availability was comparable to that of inorganic P. However, in vivo, passage from the rumen to the remaining GI tract may occur before ruminal phytases can cleave all of the phosphates from the phytate molecule (Kincaid et al., 2005). Ca-complexes formed with phytate during processing of dietary ingredients, like formaldehyde and heat treatment, can also reduce ruminal phytase activity (Konishi et al., 1999; Park et al., 1999).

Geisert et al. (2010) reported a direct, linear relationship between dietary P concentrations and fecal P concentrations in finishing crossbred steers. If P is not released from phytate in the GI tract, then the amount of P in the feces increases in the form of phytate-bound P and dissolved, water-soluble P that is associated with the liquid portion of feces. Phytate-bound P in the manure still presents an environmental concern because phytases in nature and in the aquatic environment, can cleave phytate bonds, releasing the PO_4^{3-} molecules (Cheng and Lim, 2006) attributing to eutrophication. These concerns are heightened when the P is applied to the land as liquid manure. The form of P in the liquid manure is known as dissolved-reactive P (**DRP**). Dissolved-reactive P is P in the form of PO_4^{3-} and is in aqueous solution. The DRP is increased when liquid manure is surface-applied to crop land (Yli-Halla et al., 1995; Schreiber, 1998; Pote et al., 1999). Liquid and solid state of manure have different concentrations of water-soluble P. Dry manure has 28% less water-soluble P concentrations than liquid manure (Kleinman et al., 2005). A strong negative correlation ($r = -0.72$) exists between DM and water-

soluble P in dairy manure, indicating increased dissolution of P with increasing water in manure (Kleinman et al., 2005). Thus, when liquid manure is applied to crop land, the proportion of P subject to runoff increases relative to the land application of solid manure (Dou et al., 2002; 2003). Therefore, water-soluble P, as PO_4^{3-} , in manure or mineral fertilizer worsen P contributions to runoff because it is the most readily available form of P for algae growth and eutrophication in fresh water (Walton and Lee, 1972; Sharpley et al., 1998; Kleinman, 2000). There has been little work on the effects of feeding exogenous phytase to beef cattle as a means to increase P availability to the animal and decrease P excreted in the manure.

Phosphorus Metabolism in Beef Cattle

Before environmental reductions of P are discussed, it is important to understand the necessity of P in beef cattle to understand why it is not just eliminated from the diet. Phosphorus makes up about 1% of the total body weight (Berner, 1997) and about 29% of the total body minerals (McDowell, 1992). Minerals cannot be synthesized in the body; therefore, P needs to be supplemented in order to meet animal requirements. Minerals, in general, are required to support many functions in animals including: structural building blocks, constituents of bodily fluids and tissues as electrolytes, and catalysts in enzyme and hormone function (Underwood, 1981). Phosphorus, along with calcium, magnesium, fluorine, and silicon, play a crucial role in teeth and bone stability, contributing to mechanical and structural support (McDowell, 1992). Phosphorus is also closely tied to Ca because together they make up the majority of bone. Phosphorus in bone is in the form crystalline PO_4^{3-} and gives bone its ridged structure (Berner, 1997). Together, Ca and P form the hydroxyapatite crystals of the bones, sometimes written as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (McDowell, 1992; Berner, 1997). Bone contains about 98 to 99% of the body's total Ca and 80 to 85% of its total P (Centrella and Canalis, 1985; Berner, 1997). For

comparison, soft tissues and muscles contain 14% of the body's total P whereas blood only contains 1% (Berner, 1997). In mammal bone ash, there is 36% Ca and 17% P (McDowell, 1992) making the ratio of Ca:P in bone roughly 2:1, which does not usually change (Hegsted, 1973). In fact, an increase in one of these minerals, Ca or P, will lead to improper utilization of the other (McDowell, 1992).

Phosphorus is involved in a variety of metabolic functions, such as energy transfer in the form of ATP, ADP, or AMP, is a component in RNA and DNA, and contributes to phospholipid formation (McDowell, 1992; NRC, 1996). While, extracellular P in plasma is the main method used in mammals to evaluate P status in mammals (Berner, 1997), bone P composition is the most definitive way to test for P deficiency (McDowell, 2003). Phosphorus is also involved in appetite regulation and plays a role in feed utilization (Underwood, 1981; Berner, 1997). Specific to ruminants, P is required by the microorganisms that inhabit the rumen for growth and cellular metabolism (NRC, 1996), especially cellulolytic bacteria (McDowell, 1985). In the rumen, P also helps regulate pH, acting as a buffer (McDowell, 1992). In fact, mineral salts, such as calcium phosphate, have been used in ruminant diets for their buffering effect (NRC, 1989).

The primary buffer in ruminants is saliva. Phosphorus plus bicarbonate in saliva contributes a buffer effect in the rumen during volatile fatty acid (**VFA**) production (McDowell, 1992; Care, 1994). Unique to ruminants, saliva serves as a reservoir for inorganic P and is the main donor of P to the gut (McDowell, 1992; Valk et al., 2002). Phosphorus in saliva supplies 80% of P to the digestive tract when swallowed (Care, 1994). Tomas (1974) discovered that salivary P entering the rumen is dependent on the physical form of the diet where ruminants consuming a forage diet will secrete more saliva in a given amount of time than would ruminants consuming a concentrate diet that is pelleted or ground. This is likely due to the large particle

size of forages stimulating saliva production. Salivary P is correlated to blood P (Tomas et al., 1974; Braithwaite, 1984). In sheep fitted with a reentrant cannula, salivary P was also correlated to the net amount of P absorbed in the small intestine (McDowell, 1992) suggesting the benefit of P conservation in the body in times of P deficiency (Care, 1994).

Multiple studies have reported the efficiency of total absorption of supplemental inorganic P in both ruminants and non-ruminants to be about 65 to 85% (Braithwaite, 1986; Challa et al., 1989). Phosphorus is absorbed, both passively and actively, in the duodenum of the small intestine and the forestomach (Breves et al., 1988) in the form of PO_4^{3-} (Wasserman, 1981; Braithwaite, 1984; NRC, 2005). Passive absorption of P takes place in the distal small intestine, in the jejunum and ileum, where increased dietary P correlates to P concentration in the lumen of those sections in the small intestine (NRC, 2005). Passive absorption of P in the small intestine also depends on solubility of P at the duodenum (Maynard et al., 1979). In the forestomach of ruminants, passive absorption is dependent on an electrochemical gradient (Breves et al., 1988). Although passive absorption of P occurs in multiple places in the ruminant gastrointestinal tract, methods of active absorption are also important in ruminants.

One active method of active absorption is hormonal regulation (Berner, 1997; NRC, 2005; McDowell, 1992). Previously, it was believed that main hormone responsible for the absorption of P is the active circulating form of vitamin D (**1,25-(OH)₂D₃**), synthesized and secreted by the kidney and regulated by parathyroid hormone (Berner, 1997). Parathyroid hormone increases salivary P concentration via 1,25-(OH)₂D₃ (Wright et al., 1984) by first increasing P concentration in the blood (Fischer et al., 1973) from stores in the kidney (Barlet and Care, 1972). Vitamin D regulates active absorption of P via pump mechanisms, such as Na-dependent active transport in the duodenum (McDowell, 1992; NRC, 2005). However, vitamin D

is not as involved as previously thought. Field et al. (1983) and Scott et al. (1985) questioned endocrine regulation of P homeostasis in ruminants. They postulated instead that absorptive efficiency of P in the small intestine was the main regulator and was more affected by a combination of factors such as salivary P, P intake, endogenous P losses, and fecal P.

In addition to hormones, Ca concentration can play a role in P absorption both directly and indirectly. Direct regulation with Ca occurs when insoluble complexes form between Ca and P and decrease absorptive capacity (Berner, 1997). Indirectly, when circulating concentrations of Ca are high, synthesis of $1,25-(\text{OH})_2\text{D}_3$ is reduced (Hughes et al., 1975), thereby decreasing P reabsorption. Intestinal pH, animal age, and dietary intake of other minerals such as Ca, Fe, Al, Mn, K, and Mg, that can form insoluble phosphates, are factors that affect P absorption (Irving, 1964). For example, Budde and Crenshaw (2003) found that there was an 80% decrease in PO_4^{3-} excreted in the urine of weanling pigs fed an acidic diet compared with those fed an alkaline diet. Further, pigs fed the acidic diet retained 147.62 mmol/d PO_4^{3-} whereas pigs fed the alkaline diet retained only 129.15 mmol/d PO_4^{3-} . This has to do with the fact that PO_4^{3-} is negatively charged and plays a role as a buffer in the osmotic balance at the site of absorption. When pH becomes more alkaline, it is harder for PO_4^{3-} to stay in solution, thereby inhibiting absorption (Care, 1994). In ruminants, the effect of dietary pH may be negligible because the rumen buffers dietary pH before the digesta enters the abomasum. However, there is a dearth of information on the effects of dietary pH on P digestion in ruminants.

Once digested, there are two routes of excretion for P, either in the feces or urine. Absorbed P that is not retained is excreted in the urine. Urinary P losses in cattle are far less than fecal losses (NRC, 1996) because ruminants have a higher renal threshold for P than nonruminants (McDowell, 1992). This ability to store P in the kidney for times of greater need

represents an evolutionary advantage for grazing ruminants. Poor-quality forages are not as readily digested by ruminants as good-quality forages, and they can also be deficient in nutrients, like P (McDowell, 1985). In situations when ruminants are required to graze low-quality forages, intake is the limiting factor at getting enough P to meet their requirement. Therefore, through their ability to store P in the kidney, they are able to use those stores when grazing poor-quality forages without removing P from the bone.

Phosphorus that is not absorbed is excreted in the feces. In addition to unabsorbed P, feces may contain “endogenous losses” of P. Endogenous fecal losses of P vary widely depending on intake and factors that affect salivary P (Challa et al., 1989; NRC, 1996). Other major contributions to endogenous losses of P include sloughed intestinal cells and microbial cells (Mason, 1984). Fecal endogenous losses of P can even occur during times of P deficiency (NRC, 2005). A few studies have evaluated P digestibility in feedlot steers. Hankins-Herr et al. (2009) fed feedlot steers (345 kg BW) a common finishing diet containing 0.30% P diet DM, that was corn and silage-based. Steers consumed 23.9 g/d P, excreted 6.0 g/d P in the feces, and absorbed 18.8 g/d P. Geisert et al., (2010) did a similar study in feedlot steers (386 kg BW) and fed another common finishing corn-based diet containing no silage with dietary DM P concentrations of 0.30% P. In this study, steers consumed 28.9 g/d P, excreted 12.1 g/d P, and absorbed 14.8 g/d P. The differences in P excreted and absorbed could be due to the addition of silage in the diet of the first study. Because silage is a fermented feedstuff with microbial activity, it contains bacterial phytases that make P in silage more available for digestion than P from unfermented feeds, like dry corn grain for example (Brask-Pedersen et al., 2013). Phytase activity will be discussed in further detail below. Regardless of how is it excreted, in the urine or

feces, excreted P can be applied to land, crops, or pasture, as fertilizer. However, excess land application of P can cause environmental concerns.

Environmental Concerns with Phosphorus

Phosphorus is an irreplaceable, essential, elemental nutrient required to sustain life and food production (Cordell and White, 2011). The Law of Mass Conservation applies to P, P cannot be manufactured nor destroyed. Phosphorus used in mineral supplements in both human and animal nutrition originates from rock phosphate found in the Earth's crust, but there is a limited supply on Earth. Exploitation of P has led to its scarcity (Cordell and White, 2011). The depleting world rock phosphate supply generated an 800% increase in the price of phosphate in 2008 (Dery and Anderson, 2009). However, in some circumstances, P could be considered “renewable” because there is a constant cycle between animals, soil, and plants (Cordell and White, 2011). Unfortunately, the depletion of rock phosphate in Earth's crust caused by P exploitation are still public concerns and the issues with P are multifaceted. As we continue to use rock phosphate from the Earth's crust, new sources of P need to be explored, like harvesting P from human feces (Mihelcic et al., 2011). In addition, due to the abundant use of P for decades, growing concerns about P pollution will need to be addressed.

Phosphorous pollution is a complex issue and there are a number of factors that affect runoff and subsequent P pollution potential (Ongley, 1996). First, rate of rainfall controls the amount of water as well as the soil detachment factor: the higher the rate of the rainfall, the higher the detachment factor, meaning increased lost soil particles to runoff. Second, in saturated conditions, permeability of the soil contributes to its ability to pass water. Mineral and organic composition, as well as porosity of the soil, all play a role in the permeability of the soil. Furthermore, one of the most common soil characteristics used to evaluate soil surface runoff is

infiltration rate of the soil measured in centimeters per hour. Infiltration rate is controlled by capillary forces correlated to soil compaction and will be very high at the beginning of a rainfall and then decrease over time as the soil becomes more saturated. Thus, soil infiltration varies among rainfall events and differs by soil type and geographic location.

Reviews on P pollution have been extensive (Sharpley et al., 1994; Correll, 1996; Sims et al., 1997). The following review will discuss specifically how beef cattle contribute to P pollution. In 1998, the EPA established a Unified Nutrient Strategy for animal feeding operations to implement nutrient management programs on the farm-enterprise level (EPA, 2015). The main goal of the program was to establish federal guidelines in the agriculture sector to manage water quality. Through the Unified Nutrient Strategy, concentrated animal feeding operations (**CAFO**) are required to develop a Comprehensive Nutrient Management Plan specific to their operation (EPA, 2015). In the state of Illinois, a beef cattle operation qualifies as a CAFO if 1,000 animal units or more are maintained in a confined space for 45 days during a 12-month period (EPA, 2015). Consolidated animal agriculture has been identified as one of the major contributors to P pollution of surface water (EPA, 2015).

Concentrated animal feeding operations that house cattle are called feedlots. Feedlots vary in animal inventory and management; but, in a feedlot, cattle are confined and have feed brought to them. As a result, manure, and the nutrients therein, like N and P, are consolidated. Feedlot cattle diets contain elevated concentrations of P due to the nature of the feedstuffs used. For example, corn and corn-byproducts contain 0.31% and up to 0.95% P on a DM basis, respectively (NRC, 1996). These feeds are the primary ingredients in feedlot cattle diets in the Midwestern region of the U.S. By comparison, grazed forages contain 0.20 to 0.30% P (DM basis; NRC, 1996). The elevated concentrations of P in the feedlot cattle diets lead to elevated

concentrations of P in the manure because there is a direct linear relationship between P concentrations in the diet and P concentration in the manure (Geisert et al., 2010). Continued over-application of P to the ground can lead to a P imbalance in the soil and increase the likelihood that P will move through the soil (Sharpley et al., 1998). Commonly planted corn hybrids in Illinois uptake a N:P ratio of about 5.8 (Bender et al., 2013). Uncomposted beef feedlot manure provides an N:P ratio of 2.6 (Eghball et al., 1997), which is 55% less than what the crop needs. Because of this, manure application to corn with beef feedlot manure to meet the requirements for N would increase soil levels of P beyond what is required. Or, that manure would have to be spread across greater distances to avoid over-applying. This is an added cost to the farmer because the manure would be hauled farther.

Phosphorus pollution from agricultural land, whether synthetic fertilizer or manure is used, occurs mainly via water runoff or soil erosion and typically happens after a rainfall event (Sharpley et al., 1994). The P can enter the water cycle after rainfall events via streams, rivers, and lakes (Ongley, 1996), and can accelerate eutrophication (Daniel et al., 1998).

Eutrophication occurs when the nutrient supply in a body of fresh water increases (Smith et al., 1999). Algae in these bodies of fresh water require N and P as the two main nutrients for growth (Schlesinger, 1991; Vitousek and Howarth, 1991). When N and P enter the water via runoff, they can exceed the N and P requirements of the algae, providing an oversupply of nutrients (Smith et al., 1999). When excess nutrients are available, algae, namely phytoplankton, continues to grow producing an algal “bloom” (Sharpley et al., 1994). As the phytoplankton settles to the bottom, they decay and remove oxygen from the water causing anoxia, or a lack of oxygen (Sharpley et al., 1994). Because of this anoxia, eutrophication is detrimental to aquatic life.

One of the most detrimental examples of eutrophication in the U.S. is the hypoxic zone in the Gulf of Mexico. Hypoxia is the depletion of oxygen to very low levels whereas anoxia, mentioned above, is the complete lack of oxygen. Thirty-one states in the United States are part of the Mississippi Watershed. Runoff from over half of the U.S. feeds into the Mississippi River and gets deposited in the Gulf of Mexico (The Nature Conservancy, 2012). The concentration of P in the waterways that flow into the Mississippi River, and subsequently into the Gulf of Mexico, creates a “dead zone” in the Gulf where oxygen supplies are depleted. In Illinois, for example, 48% of agriculture contributes an estimated 11% of P to the hypoxic zone in the Gulf of Mexico (David et al., 2013), making Illinois one of the top 4 states contributing to the hypoxic zone (EPA, 2014). Thus, Illinois agriculture accounts for 6% of the total P in the Gulf of Mexico (EPA, 2014).

Historically, beef cattle manure was considered a “sustainable” source of P that could be returned back to the soil as fertilizer, continuing the cycle from benefitting a living animal to then benefitting soil. However, manure application processes and P concentrations in manure may increase the risk for runoff and other associated issues discussed above. There are ways to manage manure runoff in an effort to reduce these negative effects. For example, Eghball and Gilley (1999) used a simulated model to show that manure from beef feedlots that was applied to winter wheat land and disked, increased runoff concentration of total P and led to dissolved P concentrations that were within a critical range when compared to manure applied in a no-till application method. The authors noted this is an indication that application methods that result in erosion, such as disking, cause an increase in P runoff (Eghball and Gilley, 1999). Therefore, increased efforts and research on ways to reduce P excretions in manure and subsequent runoff need to be evaluated and promoted. As several environmental papers have examined the runoff

issues (Shapley et al., 1994; Correll, 1998; Eghball and Gilley, 1999; Knowlton et al., 2004; Vasconcelos et al., 2007; Smith et al., 2015), there are several strategies to reduce P excreted in manure. Perhaps the most obvious strategy is to increase the availability of the P to the animal, thereby reducing fecal P. A popular feeding strategy to increase P availability for nonruminants is the dietary addition of phytase, an enzyme that releases P bound to phytate, making it more available for absorption. For example, phytase supplementation reduced P output by 26% and improved P digestibility by 20% in growing pigs (Sotak-Peper et al., 2016), reduced fecal P output by 10% and improved P digestibility by 38% in broilers (Ravindran et al., 2000), and improved P digestibility by 52% in lactating sows (Jongbloed et al., 2004). Phytate and phytase will be discussed in more detail below because for livestock producers, one major challenge to addressing the manure P values and subsequent runoff of P, occurs due to the plant bound, phytate-P in commonly used feedstuffs.

Plant-bound Phosphorus and Phytate

The plant bound P in common feedstuffs makes P reduction research challenging in livestock, in addition research in ruminants is challenging for a number of other reasons. First and foremost, P deficiencies are the most common mineral deficiency in grazing cattle around the world (McDowell, 1992). As early as 1785, there were observations and descriptions of P deficiencies in animals (McDowell, 1992). Phosphorus deficiencies are more commonly seen in animals on pasture compared to animals on concentrate diets (McDowell, 1992), due to the differences in P concentrations in grains and forages discussed previously. Briefly, the primary reason P deficiencies are more common in grazing animals is that P-deficient soils are widespread. Forages grown on P-deficient soil will also be P-deficient. Even in P-sufficient soils, drought and increasing forage maturity lead to P-deficient forages (NRC, 1996). In addition,

factors that affect DMI of a given forage can also impact mineral intake. For example, low protein composition of the diet (< 7%) and increased forage maturity (lignification) reduce total mineral intake (McDowell, 1992).

An animal is considered P-deficient when plasma P levels are below 4.5 mg/dL (McDowell, 1992). Beef cattle deficient in P have reduced growth and efficiency, appetite, and milk production, impaired reproduction, and weakened bones (Underwood, 1981; Shupe et al., 1988). In young animals, P deficiency will manifest itself as rickets, whereas in older animals it will manifest as osteomalacia (McDowell, 1992). Rickets is caused by improper calcification of bone during early stages of growth, whereas osteomalacia is the result of an excess of P taken from bone to meet the requirement of the animal (NRC, 1996). The clinical signs of rickets and osteomalacia are bone fractures, bone weakness, and stiffness (NRC, 1996). Deficiencies of P are often more concerning for young growing cattle as they have a greater requirement for P than mature cattle (Lofgreen et al., 1952; Wise et al., 1958). According to the NRC (1996), the P requirement for maintenance of a growing steer is 16 mg of P per kg of body weight. Consequently, daily endogenous losses are also 16 mg of P per kg of BW in cattle fed diets at or close to the P requirement (Tillman and Brethour, 1958; Tillman et al., 1959; Challa and Braithwaite, 1988a; Challa et al., 1989). The P requirement increases with increasing gain. In fact, the requirement for retained P, in excess of maintenance, is calculated to be 3.9 g of P for every 100 g of protein gain (NRC, 1996). If dietary P exceeds what is required by the calf, or if the diet is poorly digested, P is excreted in the feces (Lei et al., 1993; Knowlton et al., 2001; Dou et al., 2002; Dou et al., 2003; Bravo et al., 2003; Weiss and Wyatt, 2004). Particularly for grazing cattle, this means that their diet has too little P to supply their needs. Therefore, P is often supplemented in the diet of ruminants. Supplemental P sources ranked on availability are

monocalcium phosphate, dicalcium phosphate, defluorinated phosphate, and meat and bone meal (Peeler, 1972; Potter, 1988).

In ruminants fed concentrates, P deficiency is less of a concern because, in general, grains contain more P than forages (Kellems and Church, 1998). Within an individual plant, P is more concentrated in the seed and seed by-products, like bran and oil meal, than it is in the roughage portion of the plant (McDowell, 1992). However, up to 60 to 80% of total P in cereal grains, and certain cereal by-product feeds, is bound to phytate, the salt of phytic acid (*myo*-inositol hexakisphosphate; inositol and 6 phosphate groups; Eeckhout and De Paepe, 1994; Viveros et al., 2000). However, if fermentation is involved in the production of a particular by-product feed, phytate P as a percent of total P can be lower. For example, corn has 78% of total P bound to phytate and corn distillers grains has 21% of total P bound to phytate because of the fermentation process corn distillers undergoes during ethanol production resulting in partial phytate hydrolysis of that feedstuff (Eeckhout and De Paepe, 1994). The phytate molecule itself is indigestible and is known to have anti-nutritive properties because it is a polyanionic molecule (12 negative charges) allowing it to bind positively charged nutrients creating a chelate (Selle et al., 2011). Notably, phytate binds many divalent minerals like Ca^{2+} , Zn^{2+} , Fe^{2+} , and Cu^{2+} (Selle et al., 2011) inhibiting absorption of those minerals in the small intestine. Early on, it was suggested that phytate caused osteomalacia because phytate forms insoluble complexes with Ca in the small intestine reducing absorption of both Ca and P (Mellanby, 1949). Phytate has the same chelating effect on Zn, potentially causing parakeratosis in nonruminants (Oberleas et al., 1962).

Availability of phytate-bound P depends on the animal's ability to hydrolyze phytate to access the absorbable form of P, PO_4^{3-} (Simons and Versteegh, 1990; Greiner and Konietzny,

2011). The enzyme phytase allows that hydrolysis of PO_4^{3-} to take place. Better understanding of the chemistry behind phytase molecules may improve the understanding of their function in the animal.

Phytase

Commercial phytases were first introduced to the market in 1991 (Greiner and Konietzny, 2011). According to in vitro work by Konietzny and Greiner (2002), phytases dephosphorylate phytate in a stepwise fashion, generating PO_4^{3-} , the form of phosphate animals absorb, and partially phosphorylated myo-inositol phosphates. Those myo-inositol phosphates then become the substrate in the next phytase reaction (Greiner and Konietzny, 2011). To clarify, the starting molecule is a full phytate (myo-inositol 6 phosphate; inositol ring with 6 phosphate groups). One phytase molecule hydrolyzes phytate and the resulting compound is inositol (1,2,3,4,5) pentaphosphate and a free PO_4^{3-} that can be used by the microbes or the animal. Inositol (1,2,3,4,5) pentaphosphate is the substrate for the following phytase to hydrolyze it making it inositol (2,3,4,5) tetraphosphate where another PO_4^{3-} is freed. The same trend follows, inositol (2,3,4) triphosphate, then inositol (2,3) diphosphate, then finally inositol (2) monophosphate (Hayakawa et al., 1990; Greiner et al., 2000; Nakano et al., 2000). However, the PO_4^{3-} at the carbon-2 position is resistant to dephosphorylation as demonstrated by many researchers (Cosgrove, 1970; Nakano et al., 2000; Greiner and Carlsson, 2006). However, phytase may not dephosphorylate all of the five available orthophosphate groups in the stomach due to 1) decreased rate of dephosphorylation caused by product inhibition, 2) a lower hydrolysis rate of the lower, partially dephosphorylated, inositol phosphates, or 3) both (Greiner and Konietzny, 2011). The lack of total dephosphorylations is not necessarily a problem because as more of the PO_4^{3-} groups are removed, the chelating effect of phytate decreases, resulting in

reduced binding of the other essential elements. For example, inositol (2,3,4,5) tetrphosphate has 32% of the Ca-binding affinity of a full phytate molecule (inositol-1,2,3,4,5,6-hexakisphosphate) and the Ca-binding affinities of inositol tri-, di-, and monophosphate, are considered negligible (Luttrell, 1993). In fact, studies in swine have demonstrated an abundance of myo-inositol phosphates containing four or less phosphates has a higher chance of being dephosphorylated in the small intestine by mucosal phytases (Hu et al., 1996) rather than phytate, which is considered to be a poor substrate to mucosa phytases in the small intestine (Pointillart et al., 1984; 1985).

Bacterial phytases, like *E. coli* phytases, are a commonly used phytase in production animal feeding because they have an optimum pH close to the pH of the abomasum, a high affinity for sodium phytate, and a strong catalytic effect (Lei and Stahl, 2001). Some phytases are marketed as “6-phytases” which denotes that dephosphorylation starts at the orthophosphate bound to the L-6 (D-4) carbon position on the *myo*-inositol ring (Hayakawa et al., 1990; Greiner et al., 2000; Nakano et al., 2000).

Much of the research on the functionality of the phytase enzyme for use in animal feeds has been conducted in nonruminants (Ravindran et al., 2000; Jongbloed et al., 2004; Sotak-Peper et al., 2016) and centers around release of P from phytate with the addition of phytase to increase P digestibility and decrease P output. In nonruminants, phytase addition to the diet reduces the need for supplemental P (Greiner and Konietzny, 2011). For instance, P equivalency has been established for pigs fed a corn-soy diet with an *Aspergillus niger* phytase such that 500 FTU phytase is equal to 1 g of P as monocalcium phosphate that does not need to be supplemented (Hoppe and Schwarz, 1993).

There is speculation that phytases have other benefits, associated with reducing the antinutritive properties of phytate when fed to nonruminants, such as increasing protein solubility and proteolytic digestibility (Greiner and Konietzny, 2011). For example, Cowieson et al. (2006) found an improvement in amino acid availability of the diets fed to broiler chicks when supplemented with a bacterial phytase compared to those fed no additional phytase. Also, supplemental phytase was shown to improve the use of other minerals in pigs (Lei et al., 1993; Adeola et al., 1995) and fish (Debnath et al., 2005). The stomach is the main functional site of supplemental phytase activity in pigs and fish (Jongbloed et al., 1992; Yi and Kornegay, 1996; Yan et al., 2002) and the crop in poultry (Selle and Ravindran, 2007). Studies where phytase was fed to nonruminants show increased release of P from phytate when phytase is included in the diet and, in turn, increase P availability to the animal (Simons et al., 1990; Augspurger et al., 2003; Esteve-Garcia et al., 2005; Adeola et al., 2006). A study by Simons et al. (1990) was the first of its kind to demonstrate that 1,000 FTU per kg phytase reduced P excretion by 35% in pigs and by 47% in broilers. Other work has suggested feeding supplemental dietary phytase to nonruminant animals could reduce the excretion of phosphate up to 50% (Greiner and Konietzny, 2011). Thus, the addition of the phytase enzyme to nonruminant diets may subsequently reduce P pollution. However, nonruminant and ruminant animals differ markedly in digestive physiology.

Ruminants are unique because of the symbiotic relationship they have with the microorganisms that reside in the rumen, the first chamber of the gastrointestinal tract. The microbial population in the rumen manufactures phytases, thus phytate can be used as a source of P in ruminants (Guyton et al., 2003; Nakashima et al., 2007). However, due to factors such as passage rate (Kincaid et al., 2005), there is evidence that ruminal phytase may not release all of

the P from phytate. For example, Turner and Leytem (2004) suggested that the effects of endogenous phytases, those produced in the rumen, were lessened in cattle fed grain. These authors found that there was more phytate in manure from cattle fed grain compared to cattle fed forage. It has been suggested that these differences are due to the differences in phytate concentration in the two diet types, concentrate versus forage, and not caused by phytase addition alone (Ravindran et al., 1994). These studies in beef cattle were brief and the majority of research in cattle has been on the ability of phytases to reduce fecal P excretion from dairy cows (Kincaid et al., 2005).

Brask-Pedersen et al. (2013) fed 23, 2,023, 3,982, or 6,015 FTU/kg phytase to dairy cows and found that increasing phytase in the diet decreased total inositol phosphate and phytate bound P. However, ruminal phytase increased with increasing exogenous phytase inclusion in the diet, which tended to increase flow of P to the ileum and increased P excreted in the feces (Brask-Pederen et al., 2013). Authors concluded that the increase in flow of P to the lower digestive tract is a result of phytate hydrolysis in the rumen. Thus, despite the efforts to increase P availability and reduce fecal excretion, fecal excretion in this study was increased. In other work, however, dairy cows fed 427 FTU/kg phytase did have decreased total fecal P and fecal phytate P concentrations compared to cows fed no dietary phytase (Kincaid et al., 2005). Total P digestibility also tended to increase when 427 FTU/kg phytase was added to dairy cow diets. The authors speculated that this increase in digestibility caused increased hydrolysis of phytate P (Kincaid et al., 2005). These results agreed with a study by Knowlton et al. (2007) where dairy cows that consumed 1,485 FTU/kg phytase, had decreased fecal P. Results of another study indicate no difference in fecal P or P digestibility when crossbred feedlot steers were fed 600

FTU/kg of phytase compared to those not fed phytase (Hankins-Herr et al., 2009). Thus, the ability of dietary phytase inclusion to reduce fecal P excretion in cattle has been variable.

Phosphorus pollution caused by runoff is a challenge to the beef industry. Feeding strategies, like phytase, used to decrease P excretion have had variable successes. These variations may be attributed to differences in cattle type (dairy versus beef), production stage (growing versus finishing versus lactating), and phytase inclusion and type (bacterial versus fungal). More research to clarify the variation in fecal P excretion noted among studies and on other methods to reduce P pollution from land-applied manure are needed. Limited information on the effects of phytase in beef cattle diets exists (Hankins-Herr et al., 2009).

Summary

Topics in agriculture that are environmentally linked, like P, are complex and multifaceted. Phosphorus is an essential nutrient for plants and beef cattle, but it is overfed in some situations (grain-based diets) and is deficient in others (grazing). Overfeeding of P to beef cattle contributes to eutrophication and other environmental concerns that must be addressed. Because a large portion of P in commonly fed plant-based feedstuffs is bound to phytate, this adds another level of complexity to resolving the issue. Further, the price of P is increasing due to increasing demand coupled with decreasing of Earth's supply making it an expensive feed ingredient for cattle. Methods to mitigate P pollution in the environment include alternative land management practices that reduce erosion, and, in animal feeding, reductions of P excretion and P solubility in the manure. One way to attempt to address the latter, animal feeding issue, is by supplementing phytase. However, there have been limited data evaluating phytase supplementation to beef cattle and its effects on P digestibility and P metabolism.

CHAPTER II: EFFECTS OF THE INTERACTION OF DIETARY PHOSPHORUS AND EXOGENOUS PHYTASE CONCENTRATIONS ON PHOSPHORUS METABOLISM OF BEEF CATTLE

ABSTRACT

Objectives were to determine the interactions of phytase inclusion and dietary phosphorus concentration on metabolism of beef cattle fed a starch-based diet. Six ruminally-fistulated steers (initial BW = 750 ± 61 kg) were allotted to a 6×6 Latin square design with a 3×2 factorial arrangement of treatments. Factors included phytase inclusion, at 0, 500, or 2,000 phytase units (FTU)/kg of diet DM, and dietary P concentration, at 0.10% and 0.30% of total diet DM. Data were analyzed using the MIXED procedure of SAS with animal as the experimental unit. The CORR procedure was used to compare P concentrations between samples. There were no treatment interactions ($P \geq 0.30$) for any parameter measured. There were no main effects ($P \geq 0.45$) of phytase on DMI, total fecal output, apparent DM digestibility, water intake, or urinary output. Steers fed 0.10% P had decreased ($P < 0.01$) DMI and total fecal output, but increased ($P < 0.01$) apparent DM digestibility compared with steers fed 0.30% P. Although N intake and retention were not affected by treatment, steers fed the 0.10% P diet tended ($P = 0.10$) to absorb more N, and excrete more N in the urine ($P = 0.02$) and less N in the feces ($P < 0.01$) compared with steers fed 0.30% P. Steers fed 0.10% P also consumed 70.1% less ($P < 0.01$) total P each day, and excreted 51.9% less P in the feces ($P < 0.01$) and 94.6% less P in the urine ($P < 0.01$) compared with steers fed 0.30% P. Water-soluble P in the feces were greater ($P < 0.01$) on a g/d basis in steers fed 0.30% P when compared with steers fed 0.10% P. However, the proportion of total fecal P excreted as water-soluble P increased by 23.0% in cattle fed 0.10% P compared with steers fed 0.30% P, regardless of phytase inclusion level. There was no effect of dietary phytase concentration on blood or urinary ($P \geq 0.27$) P concentrations. Blood P concentration was

positively correlated ($r = 0.60$; $P < 0.01$) to urinary P concentration when steers were fed 0.10% P; however, when steers were fed 0.30% P, there was no correlation ($r = 0.36$; $P = 0.16$). Regardless of dietary P concentration, phytase supplementation did not increase P absorption nor retention.

Key words: beef cattle, phosphorus, phytase

INTRODUCTION

Phytate binds 60 to 80% of total P as inorganic phosphates (PO_4^{3-}) in cereal grains and by-product feeds, the main ingredients fed to livestock in the U.S (Eeckhout and De Paepe, 1994; Viveros et al., 2000). Phytate makes P indigestible (Simons and Versteegh, 1990), decreasing P available for absorption by animals (Simmons et al., 1990; Kincaid et al., 2005). Phytase liberates PO_4^{3-} from phytate to increase absorption in nonruminants (Lei et al., 1993). Nelson et al. (1971) suggested that providing high doses of phytase, 2,000 FTU or more, in swine and poultry diets may be more beneficial than including lesser doses. This technique is known as “superdosing”.

Dairy cattle have been fed phytase with mixed results (Bravo et al., 2002; Guyton et al., 2003; Knowlton et al., 2007). The greatest dose of phytase used in beef research to date has been 600 FTU per kg diet DM (Hankins-Herr et al., 2009). While rumen microbial populations produce phytase, it has been suggested that passage rate (Kincaid et al., 2005), grain type (Eeckhout and de Paepe, 1994; Ravindran et al., 1994), processing method (Park et al., 1999; Bravo et al., 2000), and dietary concentration of Ca (Sansinena, 1999) are factors that may affect the ability of ruminal phytases to cleave PO_4^{3-} from phytate. Feedlot cattle are often fed

processed, grain-based diets that increase rate of passage. There is minimal information on feeding phytase, and no information on superdosing phytase, to feedlot cattle to our knowledge.

We hypothesized that feeding increasing concentrations of phytase to beef cattle would reduce the total amount of P required in the diet, thereby decreasing P, total and water-soluble, excreted in the manure in steers fed a 0.10% P diet. Objectives were to determine the interactions of phytase inclusion and dietary phosphorus concentration on metabolism of beef cattle fed a starch-based diet.

MATERIALS AND METHODS

Animals and Management

Six Angus-cross, ruminally-fistulated steers (initial BW = 750 ± 61 kg) were used in a 6×6 Latin square design with a 3×2 factorial arrangement of treatments: 1) 0 FTU/kg phytase (5,000 FTU/g of Quantum Blue; ABVista, Marlborough, United Kingdom), 0.1% dietary P, 2) 500 FTU/kg phytase, 0.1% dietary P, 3) 2,000 FTU/kg phytase, 0.1% dietary P, 4) 0 FTU/kg phytase, 0.3% dietary P, 5) 500 FTU/kg phytase, 0.3% dietary P, or 6) 2,000 FTU/kg phytase, 0.3% dietary P. One FTU is defined as the amount of enzyme that liberates 1 μmol of inorganic phosphate per minute from 0.0051 M Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al., 1994). Diets were fed once daily for ad libitum intake. Steers were housed in metabolism stalls at the University of Illinois Beef Cattle and Sheep Field Research Laboratory in Urbana, IL. Stalls (2.3×1.3 m) were equipped with individual feed bunks. Water was provided ad libitum via nonsiphoning, automatic water bowls equipped with flow meters and data loggers (OM-CP-PULSE 101A) to record water intake (OMEGA Engineering Inc., Stamford CT). The

barn was equipped with a heating, ventilation, and air-conditioning system, providing a controlled environment set at 18.3°C.

Collection of Samples

To aid in diet transition and to account for any ruminal microbial differences, on d 0, all steers were weighed and partial ruminal contents (approximately 10 L) were removed from each steer, mixed among all steers, and mixed contents redistributed to all steers. Steers were then allotted to 1 of 6 dietary treatments according to Patterson and Lucas (1962).

During each period, steers were fed for a 14 d adaptation period and then samples were collected for 5 d after that. Thus, each period of the square was 19 d. Feed samples, 100 g (as-is), were collected on d 1 through 5 of the collection period. Individual feed ingredient samples were analyzed for DM (24 h at 105°C) at the initiation of each period to adjust for dietary inclusion. If there happened to be orts present on a day of collection, orts were weighed back and subsampled. Feed and orts samples were stored at -20°C and saved for later analysis.

On d 1 of the collection period, fecal bags were attached on each steer to determine fecal output for 120 h. Feces were collected in canvas bags secured by a leather harness attached around the heart girth and under the neck. Feces were weighed 3 times daily, 0600, 1200, and 1800 and a 5% subsample of the total weight was saved at each collection. Fecal subsamples were combined as collected and stored in a -20°C freezer until analysis.

Urine collecting funnels were attached to steers on d 2 of collection and used to collect urine for 72 h. During the 72 h of urine collection, steers were under continuous observation by trained personnel to ensure the apparatus remained in place and collections accurately represented total urine output. Funnels were connected to 18.9 L plastic collection tanks via

plastic hose (0.64 cm thick with a 1.9 cm opening). Urine was acidified using 200 mL of 6 N HCl in the urine collection containers. This amount of acid was determined by sampling urine during the first period and testing pH to ensure the correct volume of 6 N HCl needed to keep urinary pH under 3 at all times. Every 24 h, urine volume was measured using a 2000 mL graduated cylinder. A 1% subsample of acidified urine was taken and saved at 4°C for later analysis.

On d 5 of collection, fecal bags and urine funnels were removed and ruminal fluid was collected for 24 hr. Samples of whole rumen contents (up to 200 mL) were obtained through the rumen cannula. An additional subsample of contents was strained through 2 layers of cheesecloth to measure pH of ruminal fluid at 0, 3, 6, 9, 12, 15, and 21 hr post-feeding (Metler Toledo FE20; Metler Toledo Inc., Columbus, OH).

Blood was collected via jugular venipuncture on d 1 of the next period, prior to feeding the new diet. Blood samples were taken in BD Vacutainer® Trace Element K₂ EDTA 10.8 mg plastic tubes (Catalog Number 368381). The blood was stored at room temperature for 3 h. While in the chute, rumen contents from individual steers (approximately 4 L) were mixed and the redistributed to steers fed the corresponding diets in the following period to reduce potential carryover effects on rumen microorganisms and facilitate diet transition.

A 200mL sample of water from the water cups in the metabolism barn was taken one time during the experiment to analyze for P in the water and there were 3.201 µg P/mL of water.

Laboratory Analysis

After collection, feces samples were thawed, thoroughly mixed by hand and a 454 g subsample was brought back to the lab for analysis. Feed, fecal, and orts samples were

composited within period and lyophilized (FreeZone, Labconco, Kansas City, MO), then ground through a Wiley mill (1-mm screen, Arthur H. Thomas, Philadelphia, PA). Ground feed samples were analyzed for NDF and sequential ADF (using Ankom Technology method 5 and 6, respectively; Ankom²⁰⁰ Fiber Analyzer, Ankom Technology, Macedon, NY), N (Leco TruMac, LECO Corporation, St. Joseph, MI), fat (Method 2; Ankom Technology), total ash (500° C for 12 h, HotPack Muffle Oven Model: 770750, HotPack Corp., Philadelphia, PA), and total P (Miles et al., 2001). The resulting values were used to calculate nutrient composition of the diets. Dietary ingredient composites were subjected to perchloric acid digestion and inductively coupled plasma atomic emission spectroscopy analysis of complete minerals at a commercial lab (method 975.03: AOAC, 1988; Wooster, OH).

Blood plasma samples were deproteinated following the procedures of Miles et al. (2001). Total P was analyzed using the colorimetric method of Miles et al. (2001). The amount of P was then determined using a spectrophotometer (Synergy HT, BioTek, Winooski, VT) where absorbance was measured at 660nm. Results for total P analysis were deemed acceptable at a coefficient of variation (CV) of $\leq 5\%$ within duplicates of feed, feed refusals, blood, urine, and total fecal P. The inter-assay CV for feed, feces, blood, and urine P determination was 5.4%. To ensure minimal P contamination, all glassware was soaked in 10% HNO₃ for 3 days and rinsed 3 times in deionized water prior to use.

Urine was analyzed for N, and total P. Apparent DM digestibility was calculated by subtracting the weight of feces (DM basis) from the weight of feed consumed (DM basis) and dividing the resulting value by weight of feed consumed (DM basis). This value was converted to a percent basis by multiplying by 100.

Digestibility of NDF was calculated by multiplying the weight of feed consumed (DM basis) by the percent NDF of the diet and the resulting value was considered NDF offered. Orts were analyzed for NDF as described above and the weight of Orts (DM basis) was multiplied by the NDF content of the Orts to determine NDF refused. Feces were analyzed for NDF and this NDF value was used to determine NDF output.

Dry matter intake of P was calculated by multiplying the weight of feed consumed (DM basis) by the percent P of the diet and the resulting value was considered P offered. Orts were analyzed for P as described above and the weight of Orts (DM basis) was multiplied by the P content of the Orts to determine P refused. Feces were analyzed for P and this P value was used to determine P output.

The water-soluble portion of total P in feces was analyzed using a method adapted from Kleinman et al. (2007). In quadruplicate, representative wet fecal samples containing 0.5 g solids were mixed with distilled water at a 100:1 solution to solids ratio for 60 min while on an orbital shaker (New Brunswick Scientific Classic C2 Platform Shaker, Edison, NJ). Samples were then centrifuged at a speed of $1500 \times g$ and temperature of 4°C for 10 minutes (Thermo Scientific Sorvall Legend XFR, Waltham, MA) and the supernatant was analyzed for P concentration using the same colorimetric procedure (Miles et al., 2001) as described above. Results for fecal water-soluble P analysis were deemed acceptable at a CV of $\leq 10\%$ within quadruplicates. The inter-assay CV was 4.11% for the water-soluble P results.

Phytic acid content in each of the feed ingredients used in this trial were analyzed by a commercial lab (Enzyme Services and Consultancy; Memphis, TN). In this assay, samples were extracted with HCl to solubilize the attached phosphate groups. They were then treated with a phytase that is specific to phytic acid and its lower myo-inositol phosphate forms. Subsequent

phosphatase treatment was done to ensure all phosphate groups were removed from the inositol ring. Then, the total phosphate released was measured colorimetrically given as g of phosphorus per 100 g of sample from which the phytic acid concentration was calculated.

Statistical Analysis

The experimental design was a 6×6 Latin square with a 3×2 factorial arrangement of treatments. Data were analyzed using the PROC MIXED procedure and correlations were analyzed using PROC CORR procedure of SAS (SAS Inst. Inc., Cary, NC). Repeated measures were used to analyze ruminal pH using the Toeplitz covariance structure. Individual animal was the experimental unit. Contrast statements were used to test the linear and quadratic effects of phytase inclusion. There were no linear or quadratic effects ($P \geq 0.11$) of phytase inclusion; therefore, only treatment main effects and interactions are reported. Significance was declared at $P \leq 0.05$. Trends were discussed at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

There were no interactions of phosphorus concentration and phytase inclusion ($P \geq 0.30$) on any parameters measured. We had hypothesized that feeding steers 0, 500, and 2,000 FTU phytase per kg diet DM when they consumed 0.10% P would linearly increase total P absorption and retention in cattle, whereas feeding steers phytase with a 0.30% P diet would have no effect on P absorption or retention. In other words, we expected cattle fed 0.10% P would have a greater response to released P due to their borderline deficiency. However, this was not the case, no interaction was detected in feedlot cattle fed for ad libitum intakes in this metabolism trial. Therefore, the main effects of phytase inclusion and dietary P concentration will be discussed.

There were no main effects ($P \geq 0.40$) of phytase inclusion on any parameter measured in the current study. One of the reasons we fed phytase to cattle was because phytase is known to have beneficial effects in pigs. There have been numerous studies in pigs documenting the efficacy of 250 to 1,500 FTU phytase per kg diet DM to increase ADG, G:F, bone strength, P digestibility, and P absorption (Adeola, 1995; Harper et al., 1997; Gentile et al., 2003; Braña et al., 2006; Kerr et al., 2010; Almeida and Stein, 2012). However, no such attributes were observed in this ruminant study.

The microbial population in the rumen synthesizes phytases (Guyton et al., 2003; Nakashima et al., 2007). Thus, Hankins-Herr et al. (2009) reported no differences in DMI, total fecal output, and DM digestibility when 600 FTU phytase per kg diet DM was added to a corn and corn silage finishing diet fed to beef steers compared to those not fed dietary phytase. In addition, Kincaid et al. (2005) found no differences in DM digestibility in dairy cows when 427 FTU phytase/kg of diet DM compared to cows fed no phytase in either corn- or barley-based diets. These authors attributed their results to adequate microbial phytase production. However, there is evidence that ruminal phytase may not release all of the P from phytate due to factors such as passage rate (Kincaid et al., 2005), grain type (Tronier et al., 1971; Eeckhout and de Paepe, 1994; Ravindran et al., 1994), processing method (Konishi et al., 1999; Park et al., 1999; Bravo et al., 2000), and dietary concentration of Ca (Sansinena, 1999). Nelson et al. (1971) suggested that providing atypically high doses of phytase (up to 7,600 FTU/kg of feed) to broiler chick diets can cleave 94.4% of phytate-bound P. This concept is known as “superdosing,” in swine and poultry diets. The added benefits observed were a 131% increase in weight gain and a 59% increase in bone ash in chicks that were superdosed with phytase compared to chicks fed no phytase.

In poultry diets, phytase is sometimes included at levels up to 2,500 FTU/kg diet DM (Simons et al., 1990; Zhang et al., 2003; Walk et al., 2013), with some studies reporting dietary inclusions of up to 7,600 and 12,000 FTU/kg diet DM (Nelson et al., 1971; Shirley and Edward, 2003). One FTU is defined as the amount of phytase that liberates 1 μ mol of inorganic phosphate per minute from 0.0051 M Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al., 1994). In pigs, dietary inclusions have been fed in the range of 100 through 2,000 FTU/kg diet DM (Jongbloed et al., 1995; Adeola, 1995; Santos et al., 2014), but also at levels as high as 15,000 FTU/kg diet DM (Harper et al., 1999; Kies et al., 2005). There is no information on superdosing phytase to beef cattle. Therefore, we aimed at exploring the effects of superdosing phytase, at 2,000 FTU/kg of diet DM, in beef cattle. The diets fed in this study were highly processed diets. Specifically, the 0.10% P diet contained largely pure corn starch and chopped hay, ultimately resulting in a small particle size. As small feed particles are fermented in the rumen, their specific gravity increases and they sink more quickly to the bottom where rumen contents are solubilized in liquid and are more likely to pass through the reticulo-ruminal orifice (Faichney, 1986, 1993; Sutherland, 1987). The small particle size and subsequent rapid passage rate may have precluded the ability of phytase to cleave P from phytate prior to passage (Kincaid et al., 2005). In addition, the Ca:P for the diets were 3.33 and 2.48 for the 0.10% P and the 0.30% P diet, respectively. Phytate can form a chelate with excess Ca molecules in the rumen and not allow phytase access to the phytate to hydrolyze P (Sansinena, 1999). In scenarios where cattle are fed heavily processed diets and large amounts of Ca, inclusions of phytase greater than the 2,000 FTU fed in the current trial may be warranted.

Despite the lack of effect of phytase, differences in dietary P did affect ($P < 0.01$) DMI, total fecal output, and apparent DM digestibility. Steers fed 0.10% P consumed 16% less ($P <$

0.01) compared to steers fed 0.30% P. Geisert et al. (2010) reported a quadratic effect ($P < 0.01$) on DMI when finishing steers were fed 0.10, 0.17, 0.24, 0.31, and 0.38% P, where steers fed the 0.10% P diet consumed 11% less compared to steers fed 0.31% dietary P (Geisert et al., 2010). P is involved in appetite regulation and plays a role in feed utilization (Underwood, 1981; Berner, 1997). Researchers have observed decreased DMI in cattle fed P-deficient diets from as early as the 1930's (Riddell et al., 1934; Kleiber et al., 1936). Authors have historically attributed this decrease in DMI to decreased "appetite." One author went as far as to calculate the appetite response as ad libitum intake per day divided by the metabolic BW in kg ($BW^{0.75}$; Kleiber et al., 1936). Using this equation for appetite with the data in the current trial there was a 17% decrease in appetite in steers fed 0.10% P compared to those fed 0.30% P.

Despite the reduction in intake, steers fed 0.10% P had increased ($P < 0.01$) total tract apparent DM digestibility compared with steers consuming 0.30% dietary P. This difference in the current study is likely attributed to the nature of the diets fed. The objective in formulating the diets for this study was to have a P-deficient and a P-adequate diet. In order to accomplish this objective and have both diets be "starch-based," the 0.10% P diet was formulated by adding back in corn grain components without P: purified cornstarch, corn bran, and corn oil. The diets were formulated to have constant NDF and fat, and to differ only in P concentration. Murphy et al. (1994) reported that increased processing of grains increased digestibility when limit-fed to cattle, this may have increased digestibility in cattle fed the 0.10% P diet.

Similar to NDF and fat, dietary CP concentrations were formulated to be isonitrogenous in both the 0.10 and 0.30% P diets; however, when analyzed, the 0.10% P diet contained 15% more CP than the 0.30% P diet. This difference in CP is attributed to mixing error at the feed mill of one of the ingredients in the supplement. But both CP concentrations were above the

level assumed to decrease intake in feedlot steers, 6 to 8% of the diet DM (NRC, 1987). Even though dietary N concentration differed, there was no difference ($P = 0.86$) in g/d intake of N (Table 3) nor was N retention affected ($P = 0.84$). Nitrogen retention was unaffected because steers fed the 0.10% P diet tended ($P = 0.10$) to absorb more N and excreted more ($P = 0.02$) N in the urine and less ($P < 0.01$) N in the feces compared with steers fed 0.30% P. Steers in this study weighed 815 kg, on average, requiring 208 g/d N according to the NRC (2016) and were consuming, on average, 199.42 g/d. Thus, steers fed 0.10% P and 0.30% in the current study were not N-deficient.

Steers fed the 0.10% P diet had a more rapid decrease ($P = 0.01$) in rumen pH (Figure 1) by 6 h post-feeding than steers fed 0.30% P likely due to the rapid fermentation of the corn starch in the rumen. This decrease in pH at 6 h post-feeding in steers on 0.10% P diet was below a pH of 5.5 and remained less than 5.5 until 15 h post-feeding. Whereas ruminal pH of steers fed 0.30% P diet throughout all time points measured was 5.6 or greater. A reduction in ruminal pH from 7 to 4.9 reduces protein degradation in grain-based diets (Cardozo et al., 2000; 2002). Despite the fact that ruminal pH in steers fed 0.10% P dropped to the point at which protein degradation is decreased, steers on that diet were able to absorb more N compared with steers on the 0.30% P diet.

Steers fed 0.10% P consumed less ($P < 0.01$) P and excreted less P in feces ($P < 0.01$) and urine ($P < 0.01$) than steers fed 0.30% P (Table 4). In steers consuming the 0.10% P diet, excretion of P was greater than intake of P. Therefore, both retention of P ($P < 0.01$) and absorption of P ($P < 0.01$) were negative. Steers fed 0.30% P excreted 51.9% more fecal P/d compared to steers fed 0.10% P. A portion of the fecal losses would be endogenous P losses regardless of dietary P concentration. Braithwaite (1984) demonstrated in lambs fed varying

levels of P that fecal endogenous losses of P are increased when dietary P increases regardless of whether or not the requirement is being met. Additionally, fecal endogenous losses from cattle fed a P-free diet were equal to 10 mg/kg BW per day as demonstrated by the Agricultural Research Council (1980). Although fecal endogenous losses of P were not measured in our study, steers fed 0.10% P in the current trial would have an estimated 8 g of fecal endogenous losses each day (815 kg BW with 10 mg P excretion per kg of BW) and measured total fecal P losses averaged 11.19 g/d. In addition, Pfeffer et al. (2005) estimated that about 66 to 75% of fecal P in ruminants is of endogenous origin. Thus, steers fed 0.10% P would have been close to the published estimations, with an estimated 71% of fecal P being as endogenous losses. However, steers fed 0.30% P using the same 8 g estimate for endogenous losses, would have had just 35% fecal P as endogenous losses. The discrepancy between the two may be due to P requirements. Geisert et al. (2010) cited that steers continued to gain and grow well when fed only 0.17% dietary P. These authors suggested that 0.17% P was adequate for steers and any P above that may be fed in excess. Excess nutrients that are not used by the animal are simply excreted. Thus, the increased P excretion in steers consuming 0.30% may be due to the decreased need for P. Typically, an increase in P intake leads to an increase in endogenous losses of P in both lambs (Preston and Pfander, 1964; Braithwaite, 1984; 1985) and steers (Challa et al., 1989; Coates and Ternouth, 1992; Bortolussi et al., 1996). The difference between P-deficient and adequate diets also corresponded to changes in blood P.

There was no effect of dietary phytase concentration on blood P ($P \geq 0.45$) or urinary P ($P \geq 0.27$), but, steers fed 0.30% P had 46.2% greater ($P < 0.01$; Figure 2) circulating concentration of plasma P and 94.6% greater ($P < 0.01$; Table 4) urinary excretion of P than steers fed 0.10% P. Blood P concentrations were correlated ($r = 0.60$; $P < 0.01$; data not shown)

to urinary P concentrations when dietary P was 0.10%. However, when dietary P was 0.30%, there was no correlation ($r = 0.36$; $P = 0.16$). Cattle conserve P by reducing relative urinary P excretion to maintain blood P (McDowell, 2000); however, when P is adequate, cattle will excrete excess P in their urine. Thus, while plasma concentrations are controlled through multiple homeostatic mechanisms, urinary P fluctuates more with changes in dietary P concentration. Furthermore, plasma P concentrations of less than or equal to 4.50 mg/dL in cattle are indicative of deficiency (NRC, 2000). Steers fed the 0.10% P diet had an average plasma P level of 2.87 mg/dL and, based on the urinary correlations and plasma P, were P-deficient. When steers consumed 0.30% P diets, blood P concentrations exceeded 5 mg/dL, indicating they were P-adequate. Bortolussi et al. (1996) reported that when steers were fed a diet containing 0.04% P, blood plasma P concentrations were 3.50 mg/dL. Most compelling, was that due to the Latin square design, steers used throughout the entirety of this study, were adapted to a new diet every 20 d, thus blood P concentration changed within 20 d of dietary P concentration changes, despite the mature size of the steers used in the current study. This observation indicates that blood can be an accurate early indicator of P deficiency. In fact, when sheep consumed either 1.94, 4.01, or 6.03 g/d P, there was a linear increase in plasma P concentrations as dietary P intake increased, observed after 9 d (Scott et al., 1985). Also, steers consuming as little as 5.80 g/d P had plasma P concentrations of 3.12 mg/dL compared with steers consuming 27.2 g/d P, which had plasma P concentrations of 7.57 mg/dL after 14 d (Bortolussi et al., 1996).

Based on the NRC (2016) prediction model, the maintenance requirement for P in a steer weighing 815 kg is 17.42 g/d. The steers on the 0.10% P diet consumed, on average, 9.90 g P/d whereas steers on the 0.30% P diet consumed 33.99 g P/d. Thus, steers on the 0.10% P diet were not getting enough dietary P to meet their maintenance requirement, ultimately resulting in

negative values for absorbed and retained P. Ruminants have a greater renal threshold for P than nonruminants (McDowell, 1992) because of their ability to store P in the kidney for times of greater need, which represents an evolutionary advantage for grazing ruminants. In situations when ruminants are required to graze poor-quality forages intake is the limiting factor at getting enough P to meet their requirement. Renal storage of P can supply blood P in times of need. Even though steers fed 0.10% P had negative P retention they excreted P in the urine, indicating they were mobilizing small amounts from body reserves of P, either from the bone or kidney. Conversely, steers on the 0.30% P diet most likely were not mobilizing from their body reserves to meet their requirement for P. They had both positive absorption and retention values, and retained on average about 60% of what was absorbed.

One justification for studying P absorption and retention in beef steers was an attempt to reduce P-causing pollution from beef steers, thus water-soluble P concentrations were measured in the feces. Water-soluble P concentration in the feces was greater ($P < 0.01$) in steers fed 0.30% P (Table 4). However, the proportion of total fecal P that was excreted as water-soluble P was increased by 23.0% in cattle fed 0.10% P compared to steers fed 0.30% P, regardless of phytase inclusion level. Not only did steers fed 0.30% P consume more ($P < 0.01$) total P but they consumed 100% more phytate-P as a proportion of total dietary P than cattle fed 0.10% P. Intake of P bound to phytate made up 32.7% and 67.7% of total P intake in the 0.10% and 0.30% P diets with no supplemental phytase, respectively (Figure 3), potentially explaining the differences in water-soluble P.

The concentration of water-soluble P in cattle manure has implications in nutrient management if that manure is going to be used as fertilizer. Water-soluble P concentration in manure is positively correlated to dissolved-reactive P on the soil surface and is a readily

available source of solubilized P that is responsible for the eutrophication of fresh-water bodies (Pote et al., 1999). Besides concentration of water-soluble P, total P and N in feces used as fertilizer are also of concern in nutrient management. Commonly planted corn hybrids in Illinois uptake a N:P ratio of about 5.8 (Bender et al., 2013). Uncomposted beef feedlot manure provides an N:P ratio of 2.6 (Eghball et al., 1997), thus contains excess P compared with what the crop actually needs. Because of this, manure application to corn with beef feedlot manure to meet the requirements for N would increase soil levels of P beyond what is required. In the current trial, the N:P ratio of manure was 6.76 and 2.30 from steers fed 0.10% P and 0.30% P, respectively.

There were no interactions between phytase and P concentration in steers and there were no main effects of phytase. However, cattle consuming only 0.10% P were in negative P balance, which impacted P absorption and retention. Blood P values responded rapidly to the P deficient diets, confirming that blood can be an adequate indicator of P in cattle within 20 d of deficiency.

TABLES

Table 1. Composition of diets fed to steers

Phytase FTU/kg diet DM	0.10% P ¹			0.30% P		
	0 ²	500	2,000	0	500	2,000
Item, % DM basis						
Dry Rolled Corn	-	-	-	75.0	74.99	74.96
Grass hay ³	15.0	15.0	15.0	15.0	15.0	15.0
Corn starch	59.5	59.49	59.46	-	-	-
Corn bran	13.0	13.0	13.0	-	-	-
Corn oil	2.5	2.5	2.5	-	-	-
Phytase ⁴	0	0.01	0.04	0	0.01	0.04
Supplement						
Ground corn	-	-	-	4.672	4.672	4.672
Corn starch	1.700	1.700	1.700	-	-	-
Soybean meal	6.072	6.072	6.072	3.700	3.700	3.700
Urea	1.700	1.700	1.700	-	-	-
Limestone	0.400	0.400	0.400	1.500	1.500	1.500
Trace mineral ⁵	0.100	0.100	0.100	0.100	0.100	0.100
Monensin ⁶	0.017	0.017	0.017	0.017	0.017	0.017
Tylosin ⁷	0.110	0.110	0.110	0.110	0.110	0.110
Analyzed Composition						
DM	88.23	88.23	88.23	83.87	83.87	83.88
OM	97.68	97.68	97.68	95.72	95.72	95.73
Fat	3.02	3.01	3.01	2.58	2.58	2.58
NDF	19.66	19.66	19.65	19.00	19.00	18.99
ADF	8.97	8.97	8.97	8.38	8.38	8.37
CP	11.54	11.55	11.57	9.80	9.81	9.83
Ca	0.30	0.30	0.30	0.67	0.67	0.67
P	0.09	0.09	0.09	0.27	0.27	0.27
Phytic acid	0.10	0.10	0.10	0.64	0.64	0.64

¹Diets were formulated to contain 0.10 and 0.30% P based on book values (NRC, 2000).

²Denotes phytase enzyme addition to diet; 0 = 0 FTU phytase/kg diet DM; 500 = 500 FTU phytase/kg diet DM; 2,000 FTU phytase/kg diet DM.

³Grass hay fed to cattle was 87.01% DM and contained 8.97% CP, 67.99% NDF, and 41.23% ADF (DM basis).

⁴Phytase (5,000 FTU/g of Quantum Blue; ABVista, Marlborough, United Kingdom) was hand-mixed at the time of feeding.

⁵Included: 8.5% Ca (as CaCO₃), 5% Mg (as MgO and MgSO₄), 7.6% K (as KCl₂), 6.7% Cl (as KCl₂) 10% S (as S₈, prilled), 0.5% Cu (as CuSO₄ and Availa-4 (Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN)), 2% Fe (as FeSO₄), 3% Mn (as MnSO₄ and Availa-4), 3% Zn (as ZnSO₄ and Availa-4), 278 mg/kg Co (as Availa-4), 250 mg/kg I (as Ca(IO₃)₂), 150 mg/kg Se (Na₂SeO₃), 2,205 KIU/kg VitA (as retinyl acetate), 662.5 KIU/kg VitD (as cholecalciferol), 22,047.5 IU/kg VitE (as DL- α -tocopheryl acetate), and less than 1% CP, fat, crude fiber, salt.

⁶Rumensin 90 (198 g monensin/kg of Rumensin 90 DM; Elanco Animal Health; Greenfield, IN).

⁷Tylan 40 (88 g tylosin/kg of Tylan 40 DM; Elanco Animal Health).

Table 2. Effects of the interaction of phytase concentration (0, 500, 2,000 FTU phytase/kg diet DM) and dietary P (0.10% P or 0.30% P) on steer DM digestibility

Item	0.10%P ¹			0.30%P			SEM	P-value ³		
	0 ²	500	2,000	0	500	2,000		P	E	P×E
n	6	6	6	6	6	5	-	-	-	-
DMI, kg/d	10.64	11.57	9.84	12.34	12.68	13.22	0.82	<0.01	0.64	0.32
Fecal output, kg/d	2.80	3.08	2.70	4.14	4.08	4.37	0.27	<0.01	0.91	0.41
Apparent DM Digestibility ⁴ , %	73.90	73.51	72.66	66.77	68.07	66.85	1.11	<0.01	0.61	0.94
Water intake, L/d	52.29	55.83	62.36	77.38	60.42	52.17	13.52	0.53	0.80	0.38
Urine output, L/d	19.05	15.62	25.40	40.66	22.16	18.06	9.79	0.35	0.45	0.30

¹Diets were formulated to contain 0.10 and 0.30% P based on book values (NRC, 2000).

²Denotes phytase enzyme addition to diet; 0 = 0 FTU phytase/kg diet DM; 500 = 500 FTU phytase/kg diet DM; 2,000 FTU phytase/kg diet DM.

³P=the main effect of dietary phosphorus (0.10% or 0.30% diet DM); E = the main effect of phytase enzyme inclusion (0, 500, 2,000 FTU phytase/kg diet DM); P × E = the interaction of dietary P inclusion and concentration of enzyme addition

⁴Calculated as ((Feed DM – Fecal DM)/Feed DM) × 100

Table 3. Effects of the interaction of phytase concentration (0, 500, 2,000 FTU phytase/kg diet DM) and dietary P (0.10% P or 0.30% P) on N metabolism in steers fed starch-based diets

Item	0.10%P ¹			0.30%P			SEM	P-value ³		
	0 ²	500	2,000	0	500	2,000		P	E	P×E
Intake, g N/d	197.31	214.77	183.15	193.53	198.46	209.28	15.47	0.86	0.68	0.33
Fecal output, g N/d	67.27	71.25	62.33	79.61	79.79	85.30	6.59	<0.01	0.93	0.49
Urinary output, g N/d	104.12	107.37	97.27	86.39	88.72	92.18	7.22	0.02	0.86	0.54
Retention ⁴ , g N/d	25.92	36.15	23.56	27.53	29.95	31.80	7.83	0.84	0.63	0.61
Retention, % of intake	11.32	15.56	9.80	14.09	15.05	15.59	3.70	0.34	0.67	0.66
Absorbed ⁵ , g N/d	130.04	143.52	120.83	113.92	118.67	123.98	9.87	0.10	0.52	0.31
Retention, % of absorbed	16.99	22.99	14.89	23.79	24.68	25.53	5.63	0.14	0.73	0.69

¹Diets were formulated to contain 0.10 and 0.30% P based on book values (NRC, 2000).

²Denotes phytase enzyme addition to diet; 0 = 0 FTU phytase/kg diet DM; 500 = 500 FTU phytase/kg diet DM; 2,000 FTU phytase/kg diet DM.

³P=the main effect of dietary phosphorus (0.10% or 0.30% diet DM); E = the main effect of phytase enzyme inclusion (0, 500, 2,000 FTU phytase/kg diet DM); P × E = the interaction of dietary P inclusion and concentration of enzyme addition

⁴Calculated as Intake N (g/d) – Fecal N (g/d) – Urinary N (g/d)

⁵Calculated as Intake N (g/d) – Fecal N (g/d)

Table 4. Effects of the interaction of phytase concentration (0, 500, or 2,000 FTU phytase/kg diet DM) and dietary P (0.10% P or 0.30% P) on P metabolism in steers fed starch-based diets

Item	0.10%P ¹			0.30%P			SEM	P-value ³		
	0 ²	500	2,000	0	500	2,000		P	E	P×E
Intake ⁴ , g P/d	9.82	10.69	9.18	33.45	33.53	34.99	1.57	<0.01	0.93	0.60
Fecal output, g P/d	11.90	11.62	10.05	22.02	25.02	22.71	2.01	<0.01	0.57	0.64
Fecal water-soluble P										
g water-soluble P/d	8.80	8.66	7.64	12.74	13.35	13.13	1.88	<0.01	0.94	0.91
% of total fecal P	74.88	77.67	73.85	56.26	58.27	60.52	8.85	0.02	0.95	0.92
Urinary output, g P/d	0.32	0.20	0.29	5.85	3.32	6.22	1.48	<0.01	0.49	0.54
Retention ⁵ , g P/d	-2.41	-1.12	-1.15	5.58	7.17	5.97	1.14	<0.01	0.40	0.85
Retention, % of intake	-26.94	-12.32	-18.07	16.66	23.01	17.52	8.08	<0.01	0.98	0.82
Absorbed ⁶ , g P/d	-2.08	-0.93	-0.87	11.43	10.54	12.19	2.17	<0.01	0.87	0.87

¹Diets were formulated to contain 0.10 and 0.30% P based on book values (NRC, 2000).

²Denotes phytase enzyme addition to diet; 0 = 0 FTU phytase/kg diet DM; 500 = 500 FTU phytase/kg diet DM; 2,000 FTU phytase/kg diet DM.

³P=the main effect of dietary phosphorus (0.10% or 0.30% diet DM); E = the main effect of phytase enzyme inclusion (0, 500, 2,000 FTU phytase/kg diet DM); P × E = the interaction of dietary P inclusion and concentration of enzyme addition.

⁴Intake includes feed P and water P (3.03 µg/mg).

⁵Calculated as Intake P (g/d) – Fecal P (g/d) – Urinary P (g/d)

⁶Calculated as Intake P (g/d) – Fecal P (g/d)

FIGURES

Figure 1.

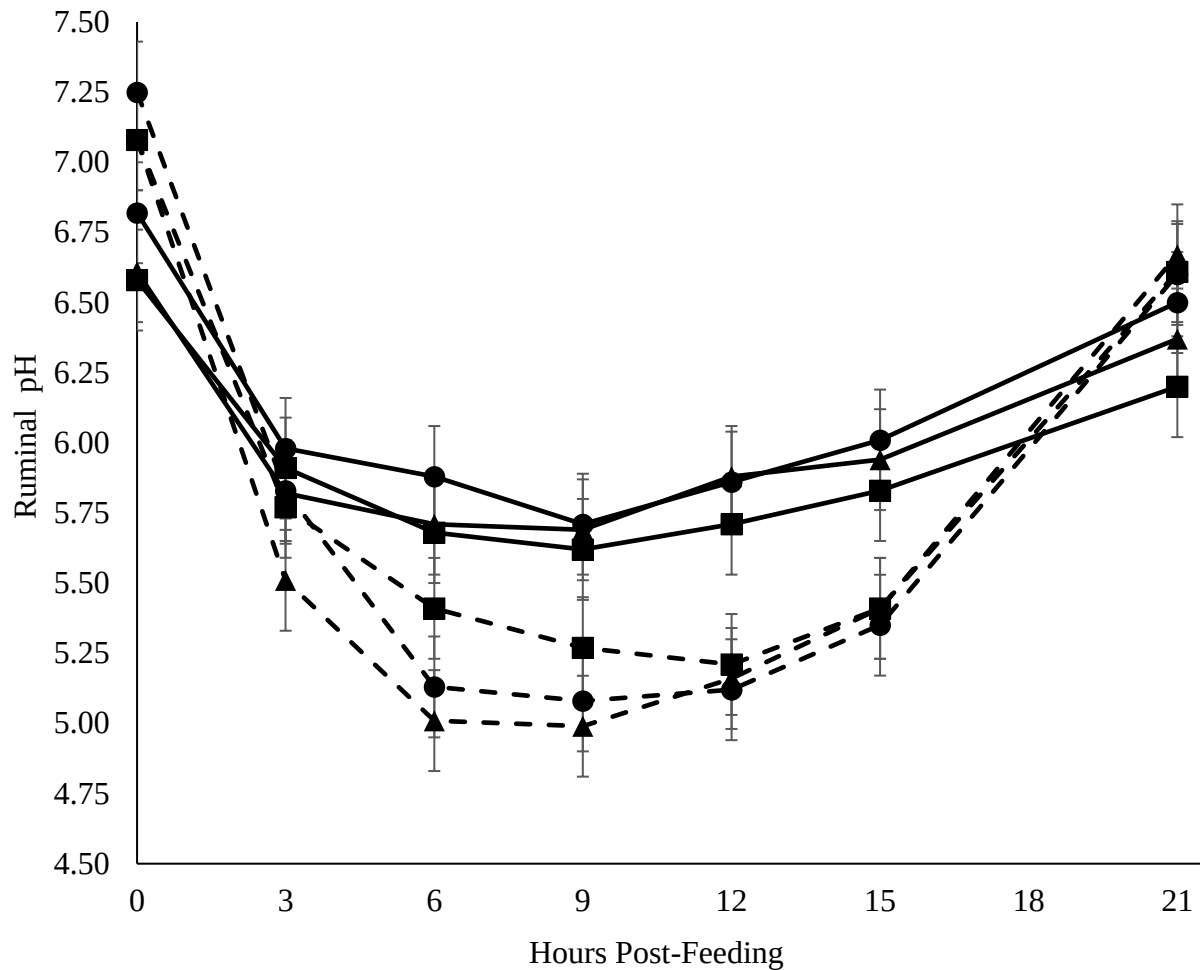


Figure 1. Effects of the interaction of phytase concentration (0, 500, or 2,000 FTU phytase/kg diet DM) and dietary P (0.10% P or 0.30% P) on ruminal pH in steers fed starch-based diets. Steers were fed P at 0.10% diet DM (---) or P at 0.30% diet DM (—) with varying concentrations of phytase: 0 FTU phytase/kg diet DM (●), 500 FTU phytase/kg diet DM (■), 2,000 FTU phytase/kg diet DM (▲). There was a $P \times \text{hr}$ interaction ($P < 0.01$) and a main effect of dietary P concentration ($P = 0.01$) for ruminal pH. There were no other treatment effects ($P \geq 0.48$) on ruminal pH. Standard error bars depict the variation associated with the interaction of $P \times E \times \text{hr}$ (SEM = 0.1798).

Figure 2.

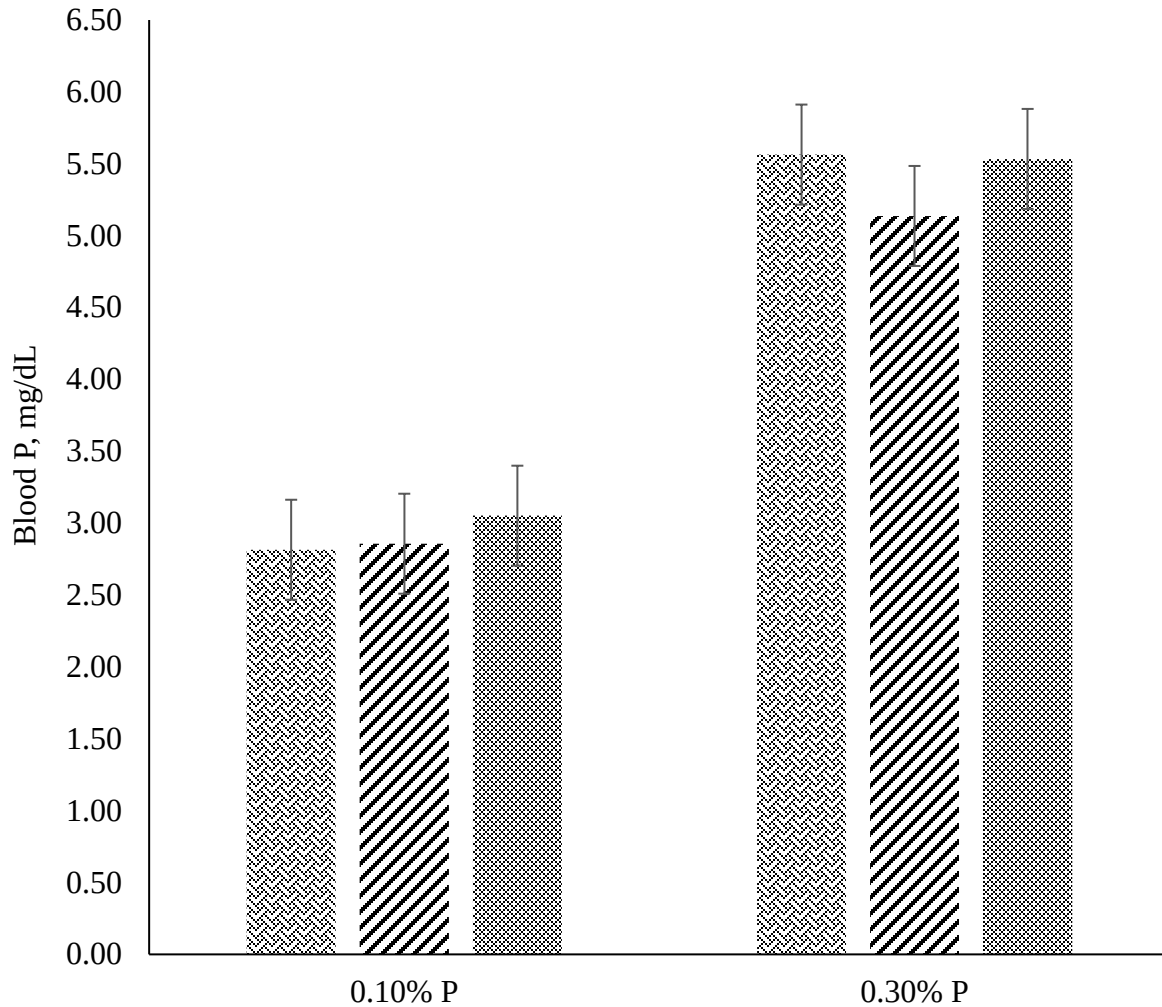


Figure 2. Effects of the interaction of phytase concentration (0, 500, or 2,000 FTU phytase/kg diet DM) and dietary P (0.10% P or 0.30% P) on blood P concentrations in steer fed starch-based diets. Steers were fed P at 0.10% diet DM or P at 0.30% diet DM with varying concentrations of phytase: 0 FTU phytase/kg diet DM (cross-hatched), 500 FTU phytase/kg diet DM (diagonal lines), 2,000 FTU phytase/kg diet DM (dotted) on a DM basis. There were no interactions of $P \times$ phytase inclusion ($P = 0.76$) nor main effects of phytase inclusion ($P \geq 0.45$) on blood P concentrations. Steers fed dietary concentrations of 0.30% P had greater ($P < 0.01$) blood P concentrations than steers fed dietary concentrations of 0.10% P. Standard error bars depict the variation associated with the interaction of $P \times E$ (SEM = 0.3478).

Figure 3.

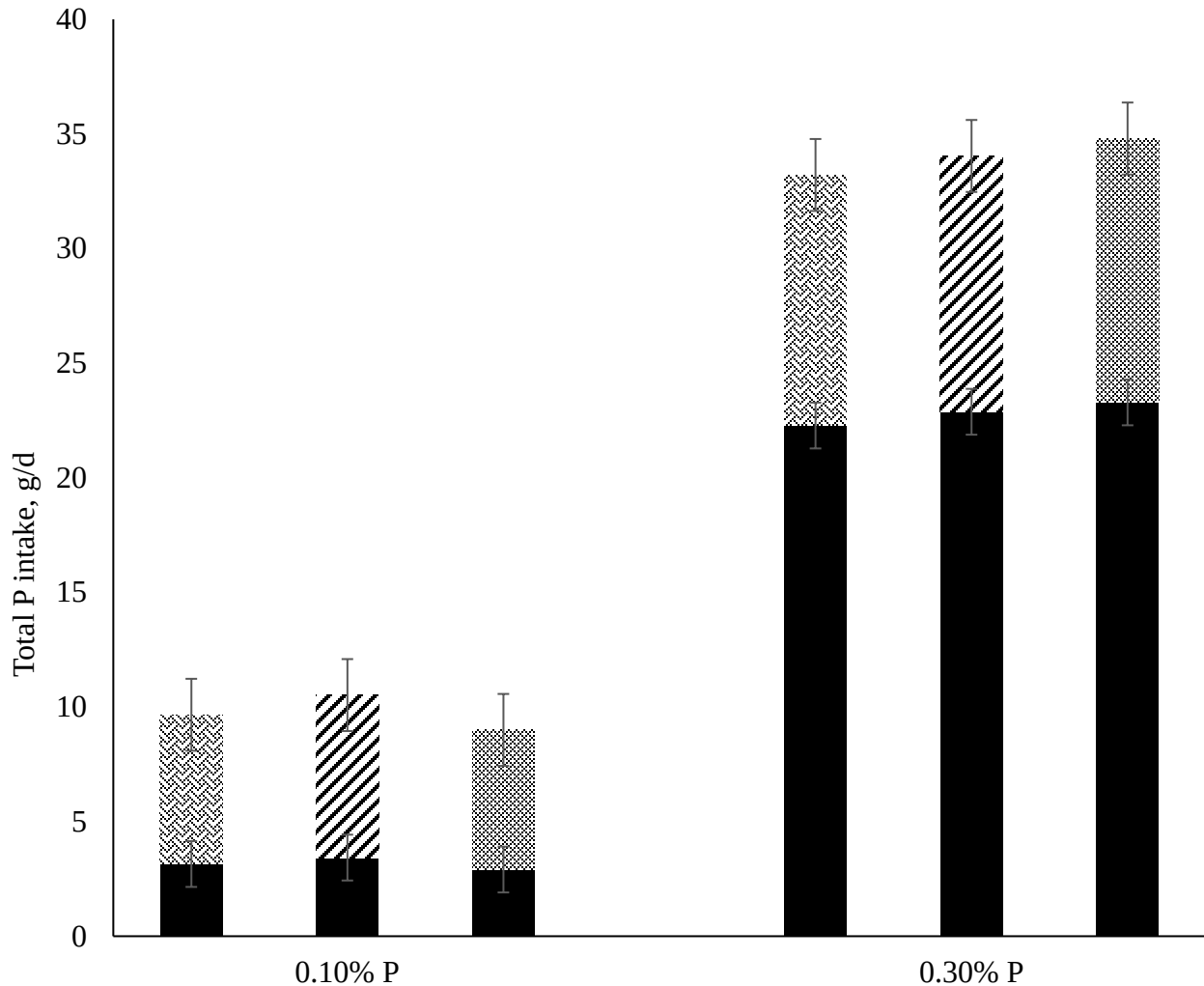


Figure 3. Effects of the interaction of phytase concentration (0, 500, or 2,000 FTU phytase/kg diet DM) and dietary P (0.10% P or 0.30% P) on the proportion of intake phytic acid-bound P in total P consumed by steers fed starch-based diets. Steers were fed P at 0.10% diet DM or P at 0.30% diet DM with varying concentrations of phytase: 0 FTU phytase/kg diet DM (white), 500 FTU phytase/kg diet DM (diagonal lines), 2,000 FTU phytase/kg diet DM (dots) on a DM basis with black denoting the proportion of phytic acid-bound P intake of total P intake within their respective treatments. There were no effects of phytase inclusion ($P \geq 0.57$) or the interaction of P $\times$ phytase inclusion ($P = 0.68$) on total P intake. Steers fed dietary concentrations of 0.30% P had greater ($P < 0.01$) total P intake compared to steers fed dietary concentrations of 0.10% P. There were also no effects of phytase inclusion ($P \geq 0.60$) or the interaction of P $\times$ phytase inclusion ($P = 0.79$) on phytic acid-bound P intake. Steers fed dietary concentrations of 0.30% P had greater ($P < 0.01$) phytic acid-bound P intake compared to steers fed dietary concentrations of 0.10% P. Standard error bars depict the variation associated with the interaction of P $\times$ E (SEM = 1.57 and 1.00, for total P intake and intake of phytic acid-bound P, respectively).

CHAPTER 3: IMPLICATIONS

Despite the fact that phytase had no effect on parameters measured in this study, insight can be drawn on the way P metabolism works when steers are fed P-deficient diets and P-adequate diets. Based on previous work, it has been demonstrated that there is a relationship between P intake, fecal endogenous losses of P, and plasma P concentration in ruminants (Preston and Pfander, 1964; Braithwaite et al., 1984; Challa and Braithwaite, 1988a; Challa et al., 1989; Bortolussi et al., 1996). Blood P fluctuated in the current study as P intake fluctuated, and this fluctuation occurred in a matter of about 20 d. Thus, these factors are in concert with each other in regulating P balance in the ruminant. Not only do processes in the live animal change with differing dietary P inclusions; but, total P and the proportion of fecal P most subject to run-off situations also change with dietary P inclusion. In the future, this should be taken into account if cattle producers intend to use manure for fertilizer. More research is warranted evaluating superdoses of phytase in ruminant diets and at different stages of production. The rumen holds a large volume of liquid and feed contents and perhaps requires more phytase to have a marked effect on P metabolism. Objectives of further research surrounding feeding strategies in cattle diets using phytase should be to make feces more environmentally-fitting in its nitrogen to phosphorus ratio for soil application.

LITERATURE CITED

- Adeola, O., B. V. Lawrence, A. L. Sutton, and T. R. Cline. 1995. Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. *J. Anim. Sci.* 73:3384-3391. doi:/1995.73113384x.
- Adeola, O. 1995. Digestive utilization of minerals by weanling pigs fed copper and phytase-supplemented diets. *Can. J. Anim. Sci.* 75:603-610. doi: 10.4141/cjas95-089.
- Adeola, O., O. A. Olukosi, J. A. Jendza, R. N. Dilger, and M. R. Bedford. 2006. Response of growing pigs to *Peniophora lycii*- and *Escherichia coli*-derived phytases or varying ratios of calcium to total phosphorus. *Animal Science*. 82:637-644. doi: 10.1079/ASC200676.
- Agricultural Research Council. 1980. The Nutrient Requirements of Ruminant Livestock. Slough: Commonwealth Agricultural Bureaux.
- Almeida, F. N. and H. H. Stein. 2012. Effects of graded levels of microbial phytase on the standardized total tract digestibility of phosphorus in corn and corn coproducts fed to pigs. *J. Anim. Sci.* 90:1262-1269. doi: 10.2527/jas.2011-4144.
- Augsburger, N. R., D. M. Webel, X. G. Lei, and D. H. Baker. 2003. Efficacy of an *E. coli* phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. *J. Anim. Sci.* 81:474-483. doi:/2003.812474x.
- Bailey, C. B. 1961. Saliva secretion and its relation to feeding cattle. 3. The rate of secretion of mixed saliva in the cow during eating, with an estimate of the magnitude of total daily secretion of mixed saliva. *Br. J. of Nut.* 15:443-451. doi: 10.1079/BJN19610053.
- Barlet, J. P., and A. D. Care. 1972. The influence of parathyroid hormone on urinary excretion of calcium, magnesium, and inorganic phosphorus in sheep. *Horm. Metab. Res.* 4:315-316. doi: 10.1055/s-0028-1097102.
- Bender, R. R., J. W. Haeghele, M. L. Ruffo, and F. E. Below. 2013. Modern corn hybrids' nutrient uptake patterns. *Better Crops*. 97:7-10.
- Berner, Y. N. 1997. Phosphorus. Pp. 63-92 in *Handbook of Nutritionally Essential Mineral Elements*, B. L. O'Dell and R. A. Sunde, eds. New York: Marcel Dekker.
- Bortolussi, G., J. H. Ternouth, and N. P. McMeniman. 1996. Dietary nitrogen and phosphorus depletion in cattle and their effects on liveweight gain, blood metabolite concentrations and phosphorus kinetics. *J. Agri. Sci, Cambridge*. 126:493-501. doi: 10.1017/S0021859600075584.
- Braithwaite, G. D. 1984. Some observations on phosphorus homeostasis and requirements of sheep. *J. Agric. Sci. Camb.* 102:295-306. doi: 10.1017/S0021859600042611.
- Braithwaite, G. D. 1985. Endogenous fecal loss of phosphorus in growing lambs and the calculation of phosphorus requirements. *J. Agric. Sci. Camb.* 105:67-72. doi: 10.1017/S0021859600055726.

- Braithwaite, G. D. 1986. Phosphorus requirement of ewes in pregnancy and lactation. *J. Agric. Sci. Camb.* 1016:271-278. doi: 10.1017/S0021859600063851.
- Braña, D. V., M. Ellis, E. O. Castañeda, J. S. Sands, and D. H. Baker. 2006. Effect of a novel phytase on growth performance, bone ash, and mineral digestibility in nursery and grower-finisher pigs. *J. Anim. Sci.* 84:1839-1849. doi:10.2527/jas.2005-565.
- Brask-Pedersen, D. N., L. V. Glitso, L. K. Skov, P. Lund, and J. Sehested. 2013. Effect of exogenous phytase on degradation of inositol phosphate in dairy cows. *J. Dairy Sci.* 96:1691-1700. doi: 10.3168/jds.2011-5278.
- Bravo, D., F. Meschy, C. Bogaert, and D. Sauvant. 2000. Ruminal phosphorus availability from several feedstuffs measured by the nylon bag technique. *Reprod. Nutr. Dev.* 40:149-162.
- Bravo, D., F. Meschy, C. Bogaert, and D. Sauvant. 2002. Effects of fungal phytase addition, formaldehyde treatment and dietary concentrate content on ruminal phytase availability. *An. Feed Sci and Tech.* 99:73-95.
- Bravo, D., D. Sauvant, C. Bogaert, F. Meschy. 2003. III. Quantitative aspects of phosphorus excretion in ruminants. *Reprod. Nutr. Dev.* 43:285-300. doi: 10.1051/rnd:2003021.
- Breves, G., H. Holler, P. Packheriser, G. Gabel, and H. Martens. 1988. Flux of inorganic phosphate across the sheep rumen wall in vivo and in vitro. *J. Exp. Physiol.* 73:343-351. doi: 10.1113/expphysiol.1988.sp003150.
- Budde, R. A., and T. D. Crenshaw. 2003. Chronic metabolic acid load induced changes in dietary electrolyte balance increased chloride retention but did not compromise bone in growing swine. *J. Anim. Sci.* 81:197-208. doi:/2003.811197x.
- Call, J. W., J. E. Butcher, J. L. Shupe, J. T. Blake, and A. E. Olson. 1986. Dietary phosphorus for beef cows. *Am. J. Vet. Res.* 47:475-481.
- Cassida, K. A., and M. R. Stokes. 1985. Eating and resting salivation in early lactation dairy cows. *J. Dairy Sci.* 69:1282-1292. doi: 10.3168/jds.S0022-0302(86)80534-3.
- Cardozo, P., S. Calsamiglia, and A. Ferrett. 2000. Effect of pH on microbial fermentation and nutrient flow in a dual flow continuous culture system. *J. Dairy Sci.* 83(Suppl. 1):265.
- Cardozo, P., S. Calsamiglia, and A. Ferrett. 2002. Effects of pH on nutrient digestion and microbial fermentation in a dual flow continuous culture system fed a high concentrate diet. *J. Dairy Sci.* 85(Suppl. 1):182.
- Care, A. D. 1994. The absorption of phosphate from the digestive tract of ruminant animals. *Br. Vet. J.* 150:197-205. doi:10.1016/S0007-1935(05)80227-1.
- Centrella, M. and E. Canalis. 1985. Local regulators of skeletal growth: a perspective. *Endocr. Rev.* 4:544-551. doi:10.1210/edrv-6-4-544.
- Challa, J., and G. D. Braithwaite. 1988a. Phosphorus and calcium metabolism in growing calves with special emphasis on phosphorus homeostasis: 1. Studies of the effect of changes in

- the dietary phosphorus intake on phosphorus and calcium metabolism. J. Agric. Sci. Camb. 110:573-581.
- Challa, J. and G. D. Braithwaite. 1988b. Phosphorus and calcium metabolism in growing calves with special emphasis on phosphorus homeostasis: 2. Studies of the effect of different levels of phosphorus, infused abomasally, on phosphorus metabolism. J. Agric. Sci. Camb. 110:583-589.
- Challa, J., G. D. Braithwaite, and M. S. Dhanoa. 1989. Phosphorus homeostasis in growing calves. J. Agric. Sci. Camb. 112:217-226.
- Cheng, C., and B. L. Lim. 2006. Beta-propeller phytases in the aquatic environment. Arch. Microbiol. 185:1-13. doi:10.1007/s00203-005-0080-6.
- Clark, R. 1953. A study of the water-soluble phosphate concentration of the ruminal contents in normal and phosphorus deficient animals. Onderstepoort J. Vet. Res. 26:137-140.
- Clark, Jr., W. D., J. E. Wohlt, R. L. Gilbreath, and P. K. Zajac. 1986. Phytate phosphorus intake and disappearance in the gastrointestinal tract of high producing dairy cows. J. Dairy Sci. 69:3151-3155. doi:10.3168/jds.S0022-0302(86)80780-9.
- Coates, D. B., and J. H. Ternouth. 1992. Phosphorus kinetics of cattle grazing tropical pastures and implications for the estimation of their phosphorus requirements. J. Agri. Sci., Cambridge. 119:401-409. doi:10.1017/S0021859600012247.
- Correll, D. L. 1996. The role of phosphorus in the eutrophication of receiving waters: A review. J. Environ. Qual. 27:261-266. doi:10.2134/jeq1998.00472425002700020004x.
- Cordell, D., and S. White. 2011. Peak phosphorus: Clarifying the key issues of a vigorous debate about long-term phosphorus security. Sustainability. 3:2027-2049. doi:10.3390/su3102027.
- Cosgrove, D. J. 1970. Inositol phosphate phosphatase of microbiological origin. Inositol pentaphosphate intermediates in the dephosphorylation of the hexaphosphates of myo-inositol, scyllo-inositol, and D-chiro-inositol, by a bacterial (*Pseudomonas* sp.) phytase. Australian J. of Biol. Sci. 23:1207-1220.
- Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006. Supplementation of corn-soy-based diets with an *Escherichia coli*-derived phytase: effects on broiler chick performance and the digestibility of amino acids and metabolizability of minerals and energy. Poultry Sci. 85:1389-1397.
- Daniel, T. C., A. N. Sharpley, and J. L. Lemunyon. 1998. Agricultural phosphorus and eutrophication: A symposium overview. J. Environ. Qual. 27:251-257. doi:10.2134/jeq1998.00472425002700020002x.
- David, M., G. McIsaac, G. Czapar, G. Schnitkey, and C. Mitchell. 2013. Science Assessment to Support an Illinois Nutrient Loss Reduction Strategy. UIUC. Available at <http://www.epa.state.il.us/water/nutrient/presentations/David.pdf>. June 19, 2015.

- Debnath, D., N. P. Sahu, A. K. Pal, K. K. Jain, S. Yengkokpam, and S. C. Mukherjee. 2005. Mineral status of *Pangasius pangasius* (Hamilton) fingerlings in relation to supplemental phytase; absorption, whole-body and bone mineral content. *Aquaculture Research*. 36:326-335. doi:10.1111/j.1365-2109.2004.01204.x.
- Dery, P., and B. Anderson. 2007. Peak phosphorus. *Energy Bull.* Available at <http://www.resilience.org/stories/2007-08-13/peak-phosphorus>
- Dias, R. S., S. Lopez, T. Silva, R. M. P. Pardo, J. C. Silva Filho, D. M. S. S. Vitti, E. Krebab, and J. France. 2009. Rumen phosphorus metabolism in sheep. *J. Agri. Sci.* 147:391-398.
- Dou, Z., K. F. Knowlton, R. A. Kohn, Z. Wu, L. D. Satter, G. Zhang, J. D. Toth, and J. D. Ferguson. 2002. Phosphorus characteristics of dairy feces affected by diets. *J. Environ. Qual.* 31:2058-2065. doi:10.2134/jeq2002.2058.
- Dou, Z., J. D. Ferguson, J. Fiorini, J. D. Toth, S. M. Alexander, L. E. Chase, C. M. Ryan, K. F. Knowlton, R. A. Kohn, A. B. Peterson, J. T. Sims, Z. Wu. 2003. Phosphorus feeding levels and critical control points. *J. Anim. Sci.* 86:3787-3795.
- Eeckhout, W., and M. De Paepe. 1994. Total phosphorus, phytate-phosphorus, and phytase activity in plant feedstuffs. *Anim. Feed Sci. and Tech.* 47:19-29. doi:10.1016/0377-8401(94)90156-2.
- Eghball, B., J. F. Power, J. E. Gilley, and J. W. Doran. 1997. Nutrient, carbon, and mass loss of beef cattle feedlot manure during compositing. *J. Environ. Qual.* 26:189-193.
- Eghball, B., and J. E. Gilley. 1999. Phosphorus and nitrogen in runoff following beef cattle manure or compost application. *J. Environ. Qual.* 28:1201-1210. doi:10.2134/jeq1999.00472425002800040022x.
- Engelen, A. J., F. C. Van Der Heeft, P. H. G. Randsdorp, and E. L. C. Smit. 1994. Simple and rapid determination of phytase activity. *J. AOAC Int.* 77:760-764.
- Esteve-Garcia, E., A. M. Perez-Vendrell, and J. Broz. 2005. Phosphorus equivalence of a consensus phytase produced by *Hansenula polymorpha* in diets for young turkeys. *Archives of Anim. Nutri.* 59:53-59.
- Faichney, G. J. 1986. The kinetics of particulate matter in the rumen. Pp. 173-195 in *Control of Digestion and Metabolism in Ruminants: Proceedings of the 6th International Symposium on Ruminant Physiology*, L. P. Milligan, W. L. Grovum, and A. Dobson, eds. Englewood Cliffs, NJ: Prentice Hall.
- Faichney, G. J. 1993. Digesta flow. Pp. 53-85 in *Quantitative Aspects of Ruminant Digestion and Metabolism*, J. M. Forbes and J. France, eds. Wallingford, Oxon, UK: CAB International.
- Field, A. C., J. Kamphues, and J. A. Woolliams. 1983. The effect of dietary intake of calcium and phosphorus on the absorption and excretion of phosphorus in chimaera-derived sheep. *J. Agric. Sci. Camb.* 101:597-602. doi: 10.1017/S0021859600038636.

- Fischer, J. A., U. Binswanger, and J. W. Blum. 1973. The acute parathyroid hormone response to changes in ionized calcium during phosphate infusions in the cow. *Europ. J. Clin. Invest.* 3:151-155. doi:10.1111/j.1365-2362.1973.tb00343.x.
- Geisert, B. G., G. E. Erickson, T. J. Klopfenstien, C. N. Macken, M. K. Lubbe, and J. C. MacDonald. 2010. Phosphorus requirement and excretion of finishing beef cattle fed different concentrations of phosphours. *J. Anim. Sci.* 88:2393-2402. doi:10.2527/jas.2008-1435.
- Gentile, J. M., K. R. Roneker, S. E. Crowe, W. G. Pond, and X. G. Lei. 2003. Effectiveness of an experimental consensus phytase in improving dietary phytate-phosphorus utilization by weanling pigs. *J. Anim. Sci.* 81:2751-2757. doi: /2003.81112751x.
- Golovan, S., G. Wang, J. Zhang, and C. W. Forsberg. 2000. Characterization and overproduction of the *Escherichia coli* appA encoded bifunctional enzyme that exhibits both phytase and acid phosphatase activities. *Canadian J. of Microbiol.* 46:59-71. doi:10.1139/cjm-46-1-59.
- González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. *J. Anim. Sci.* 93:4808-4818. doi:10.2527/jas2015-8992.
- Greiner, R., U. Konietzny, and K. D. Jany. 1993. Purification and characterization of two phytases from *Escherichia coli*. *Archives of Biochem. and Biophys.* 303:107-113. doi:10.1006/abbi.1993.1261.
- Greiner, R., N. G. Carlsson, and M. Larsson Alminger. 2000. Stereospecificity of myo-inositol hexakisphosphate dephosphorylation by a phytate-degrading enzyme of *Escherichia coli*. *J. of Biotech.* 84:53-62. doi:10.1016/S0168-1656(00)00331-X.
- Greiner, R., and N. G. Carlsson. 2006. Myo-inositol phosphate isomers generated by the action of a phytate-degrading enzyme from *Klebsiella terrigena* upon phytate. *Canadian J. of Microbiol.* 52:759-768.
- Greiner, R. and U. Konietzny. 2011. Phytases: biochemistry, enzymology and characteristics relevant to animal feed use In M. R. Bedford and G. C. Patridge (Eds.), *Enzymes in Farm Animal Nutrition*, 2nd Edition. (pp.96-128). Cambridge, MA:CABI.
- Guyton, A. D., J. M. McKinney, and K. F. Knowlton. 2003. The effect of steam-flaked or dry ground corn and supplemental phytic acid on phosphorus partitioning and ruminal phytase activity of lactating dairy cows. *J. Dairy Sci.* 86:3972-3982.
- Hankins-Herr, S. L., J. D. Arseneau, R. P. Lemenager, PAS, and A. L. Sutton, PAS. 2009. Effect of low-phytate corn and phytase on nutrient excretion and performance of feedlot cattle. *Prof. Anim. Sci.* 25:360-369.

- Harper, A. F., E. T. Kornegay, and T. C. Schell. 1997. Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. *J. Anim. Sci.* 75:3174-3186.
- Harper, A. F., J. H. Skaggs, H. P. Veit, and E. T. Kornegay. 1999. Efficacy and safety of Novo SP938 microbial phytase supplementation of a corn-soybean meal diet fed to growing pigs. *J. Anim. Sci.* 77:174-175. (Abstr.)
- Hayakawa, T., K. Suzuki, H. Miura, T. Ohno, and I. Igaue. 1990. Myo-inositol polyphosphate intermediates in the dephosphorylation of phytic acid by acid phosphatase with phytase activity in rice bran. *Agricul. and Biolo. Chem.* 54:279-286.
- Hegsted, D. M. 1973. *Modern Nutrition in Health and Disease*. 5th Ed. Lea and Febiger, Philadelphia, Pennsylvania.
- Hoppe, P. P. and G. Swartz. 1993. Experimental approaches to establish the phosphorus equivalency of *Aspergillus niger*-phytase in pigs. In *Proceedings of the 1st Symposium on Enzymes in Animal Nutrition*, Kartaue Ittingen, Switzerland, pp. 187-191.
- Hu, H. L., A. Wise, and C. Henderson. 1996. Hydrolysis of phytate and inositol tri-, tetra-, and pentaphosphates by the intestinal mucosa of the pig. *Nut. Res.* 16:781-787.
- Hughes, M. R., P. F. Brumbaugh, M. R. Hussler, J. E. Wergedal, D. J. Baylink. 1975. Regulation of serum of 1 α ,25-dihydroxyvitamin D3 by calcium and phosphate in the rat. *Science*. 190:578-580.
- Hristov, A. N., J. K. Ropp, K. L. Grandeem, S. Abedi, R. P. Etter, A. Melger, and A. E. Foley. 2004. Effect of carbohydrate source on ammonia utilization in lactating dairy cows. *J. Anim. Sci.* 83:408-421.
- Hristov, A. N. and J. P. Jouany. 2005. Factors affecting the efficiency of nitrogen utilization in the rumen In E. Peffer and A. Hristov (eds.), *Nitrogen and Phosphorus Nutrition of Cattle*. (pp. 117-166). Cambridge, MA: CABI.
- Irving, J. T. 1964. In *Mineral Metabolism*. C. L. Comer and F. Bronner, eds. Vol. 2, p. 249, Academic Press, New York.
- Jongbloed, A. W., Z. Mroz, and P. A. Kemme. 1992. The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. *J. Anim. Sci.* 70:1159-1168.
- Jongbloed, A. W., P. A. Kemme, Z. Mroz, and R. ten Bruggencate. 1995. Apparent total tract digestibility of organic matter, N, Ca, Mg and P in growing pigs as affected by levels of Ca, microbial phytase, and phytate. Pages 198-204 in *Proc. 2nd Eur. Symp. Feed Enzymes*. W. van Hartingsveldt, M. Hessing, J. P. van der Lugt, and W. A. C. Somers, ed. TNO, Zeist, The Netherlands.

- Jongbloed, A. W., J. Th. M. van Diepen, P. A. Kemme, and J. Broz. 2004. Efficacy of microbial phytase on mineral digestibility in diets for gestating and lactating sows. *Livestock Prod. Sci.* 91:143-155. doi:10.1016/j.livprodsci.2004.07.017.
- Karn, J. F. 2001. Phosphorus nutrition of grazing cattle: a review. *An. Feed Sci. and Tech.* 89:133-153. doi:10.1016/S0377-8401(00)00231-5.
- Kellems, R. O., and D. C. Church. 1998. *Livestock feeds and feeding*. 6th ed. Upper Saddle River, NJ.
- Kerr, B. J., T. E. Weber, P. S. Miller, and L. L. Southern. 2010. Effect of phytase on apparent total tract digestibility of phosphorus in corn-soybean meal diets fed to finishing pigs. *J. Anim. Sci.* 88:238-247. doi:10.2527/jas.2009-2146
- Kies, A. K., P. A. Kemme, L. B. J. Sebek, J. Th. M. van Diepen, and A. W. Jongbloed. 2005. Effect of graded doses and a high dose of microbial phytase on the digestibility of various minerals in weaner pigs. *J. Anim. Sci.* 84:1169-1175. doi:10.2527/2006.8451169x.
- Kincaid, R. L., D. K. Garikipati, T. D. Nennich, and J. H. Harrison. 2005. Effect of grain source and exogenous phytase on phosphorus digestibility in dairy cows. *J. Dairy Sci.* 88:2893-2902. doi:10.3168/jds.S0022-0302(05)72970-2.
- Kincaid, R. L., and M. Rodehutsord. 2005. Phosphorus metabolism in the rumen In E. Peffer and A. N. Hristov (eds.) *Nitrogen and Phosphorus Nutrition of Cattle*. Eds. Cambridge, MA:CABI.
- Kleinman, P. J. A. 2000. Source risk indicators of nutrient loss from agricultural lands. p. 237-252. In M. Salilus (ed.) *Managing nutrients and pathogens in animal agriculture*. Northeast Regional Agricultural Engineering Service, Ithica, NY.
- Kleinman, P. J. A., A. M. Wolf, A. N. Sharpley, D. B. Beegle, and L. S. Saporito. 2005. Survey of water-extractable phosphorus in livestock manures. *Soil Sci. Soc. Am. J.* 69:701-708. doi:10.2136/sssaj2004.0099.
- Kleinman, P. J. A., D. Sullivan, A. Wolf, R. Brandt, Z. Dou, H. Elliott, J. Kovar, A. Leytem, R. Maguire, P. Moore, L. Saporito, A. Sharpley, A. Shober, T. Sims, J. Toth, G. Toor, H. Zhang, T. Zhang. 2007. Selection of water-extractable phosphorus test for manures and biosolids as an indicator of runoff loss potential. *J. Environ. Qual.* 36:1357-1367. doi:10.2134/jeq2006.0450.
- Knowlton, K. F., J. H. Herbein, M. A. Meister-Weisbarth, and W. A. Wark. 2001. Nitrogen and phosphorus partitioning in lactating Holstein cows fed different sources of dietary protein and phosphorus. *J. Dairy Sci.* 84:1210-1217. doi:10.3168/jds.S0022-0302(01)74582-1.
- Knowlton, K. F., J. S. Radcliffe, C. L. Novak, and D. A. Emmerson. 2004. Animal management to reduce phosphorus losses to the environment. *J. Anim. Sci.* 82(E. Suppl.):E173-E195. doi:/2004.8213_supplE173x.

- Knowlton, K. F., M. S. Taylor, S. R. Hill, C. Cobb, and K. F. Wilson. 2007. Manure nutrient excretion by lactating cows fed exogenous phytase and cellulase. *J. Dairy Sci.* 90:4256-4360. doi:10.3168/jds.2006-879.
- Konietzny, U. and R. Greiner. 2002. Molecular and catalytic properties of phytate-degrading enzymes (phytases). *Int. J. of Food Sci. and Tech.* 37:791-812.
- Konishi, C., T. Matsui, W. Park, H. Yano, and F. Yano. 1999. Heat treatment of soybean meal and rapeseed meal suppresses rumen degradation of phytate in sheep. *Anim. Feed Sci. and Tech.* 13:115-122. doi:10.1016/S0377-8401(99)00044-9.
- Lei, X. G., P. K. Ku, E. R. Miller, and M. T. Yokoyama. 1993. Supplementing corn-soybean meal diets with microbial phytase linearly improves phytate phosphorus utilization by weanling pigs. *J. Anim. Sci.* 71:3359-3367. doi:10.2527/1993.71123359x.
- Lei, X. G. and C. H. Stahl. 2001. Biotechnological development of effective phytases for mineral nutrition and environmental protection. *Appl. Microbiol. Biotechnol.* 57: 474-781.
- Laboski, C. A. M. and J. B. Peters. 2007. Nutrient application guidelines for field, vegetable, and fruit crops in Wisconsin. <http://learningstore.uwex.edu/assets/pdfs/A2809.pdf>. Cited 7/14/2016.
- Lofgreen, G. P., M. Kleiber, and J. R. Luck. 1952. The metabolic fecal phosphorus excretion of the young calf. *J. Nutr.* 47:571-581.
- Luttrell, B. M. 1993. The biological relevance of the binding of calcium ions by inositol phosphates. *J. Biol. Chem.* 268:1521-1524.
- Maynard, L. A., J. K. Loosli, H. F. Hintz, and R. G. Warner. 1979. *Animal Nutrition*. 7th Ed. McGraw-Hill, New York.
- Mason, V. C. 1984. Metabolism of nitrogenous compounds in the large gut. *Proc. Of Nutri. Soc.* 43:45-53. doi:10.1079/PNS19840026.
- McDowell, L. R. 1985. *Nutrition of Grazing Ruminants in Warm Climates*. New York: Academic Press.
- McDowell, L.R. 1992. *Minerals in Animal and Human Nutrition*. New York: Academic Press.
- McDowell, L. R. 2003. *Minerals in Animal and Human Nutrition*, 2nd Ed. Amsterdam:Elsevier.
- Mellanby, E. 1949. The rickets-producing and anti-calcifying action of phytate. *J. Physiol.* 109:488-533. doi:10.1113/jphysiol.1949.sp004411.
- Mihelcic, J. R., L. M. Fry, and R. Shaw. 2011. Global potential of phosphorus recovery from human urine and feces. *Chemosphere.* 84:832-839. doi:10.1016/j.chemosphere.2011.02.046.
- Miles, P. H., N. S. Wilkinson, L. R. McDowell. 2001. *Analysis of minerals for animal nutrition research*. 3 ed.

- Murphy, T. A., F. L. Fluharty, and S. C. Loerch. 1994. The influence of intake level and corn processing on digestibility and ruminal metabolism in steers fed all-concentrate diets. *J. Anim. Sci.* 72:1608-1615. doi:10.2527/1994.7261608x.
- Nakano, T., T. Jon, K. Narita, and T. Hayakawa. 2000. The pathway of dephosphorylation of myo-inositol hexakisphosphate by phytases from wheat bran of *Triticum aestivum* L. cv. Nourin#61. *Bioscience, Biotechnology and Biochemistry.* 64:995-1003.
- Nakashima, B. A., T. A. McAllister, R. Sharma, and L. Brent Selinger. 2007. Diversity of phytases in the rumen. *Micro. Ecol.* 53:82-88. doi:10.1007/s00248-006-9147-4.
- Nelson, T. S., T. R. Shieh, R. J. Wodzinski, and J. H. Ware. 1968. The availability of phytate phosphorus in soybean meal before and after treatment with a mold phytase. *Poult. Sci.* 47:1842-1848. doi: 10.3382/ps.0471842.
- Nelson, T. S., T. R. Shieh, R. J. Wodzinski, and J. H. Ware. 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. *J. of Nut.* 101:1289-1294.
- NRC. 1987. Predicting Feed Intake of Food-Producing Animals. Washington, DC: National Academy Press.
- NRC. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl Acad of Sci. Washington D.C.
- NRC. 1996. Nutrient Requirements for Beef Cattle. 7th rev. ed. Natl. Acad. Press. Washington, D.C.
- NRC. 2005. Mineral Tolerance of Animals. 2nd Rev.. Ed. Natl. Acad. Press, Washington, D.C.
- NRC. 2016. Nutrient Requirements for Beef Cattle. 8th rev. ed. Natl. Acad. Press, Washington, D. C.
- Oberleas, D., M. E. Muhrer, and B. L. O'Dell. 1949. Effects of phytic acid on zinc availability and parakeratosis in swine. *J. Anim. Sci.* 21:57-61.
- Ongley, E. D. 1996. Control of water pollution from agriculture (No. 55). FAO.
- Owens, F. N., and A. L. Goetsch. 1986. Digesta passage and microbial protein synthesis. Pp. 196-223 in *Control of Digestion and Metabolism in Ruminants: Proceedings of the 6th International Symposium on Ruminant Physiology*, L. P. Milligan, W. L. Grovum, and A. Dobson, eds. Englewood Cliffs, NJ: Prentice Hall.
- Park, W. Y., T. Matsui, C. Konishi, S. W. Kim, F. Yano, and H. Yano. 1999. Formaldehyde treatment suppresses ruminal degradation of phytate in soya-bean meal and rapeseed meal. *Br. J. of Nut.* 81:467-471.
- Patterson, H. D., and H. L. Lucas. 1962. Change-over designs. North Carolina Ag. Exp. Station and USDA. Tech. Bul. No. 147 p 43.

- Peeler, H. T. 1972. Biological availability of nutrients in feeds: availability of major mineral ions. *J. Anim. Sci.* 35:695-712.
- Peffer, E., D. K. Beede, and H. Valk. 2005. Phosphorus metabolism in ruminants and requirements of cattle In E. Peffer and A. Hristov (eds.), *Nitrogen and Phosphorus Nutrition of Cattle*. Pp. 195-231. Cambridge, MA; CABI. doi: 10.1079/9780851990132.0000.
- Pointillart, A., N. Fontaine, and M. Thomasset. 1984. Phytate phosphorus utilization and intestinal phosphatases in pigs fed low phosphorus wheat or corn diets. *Nutri. Reports Int.* 22:473-483.
- Pointillart, A., N. Fontaine, M. Thomasset, and M. E. Jay. 1985. Phosphorus utilization, intestinal phosphatases and hormonal control of calcium metabolism in pigs fed phytic phosphorus: soyabean or rapeseed diets. *Nutri. Reports Int.* 32:155-167.
- Pote, D. H., T. C. Daniel, D. J. Nicholas, A. N. Sharpley, P. A. Moore, Jr., D. M. Miller, and D. R. Edwards. 1999. Relationship between phosphorus levels in three ultisols and phosphorus concentrations in runoff. *J. Environ. Qual.* 28:170-175. doi:10.2134/jeq1999.00472425002800010020x.
- Potter, L. M. 1988. Bioavailability of phosphorus from various phosphates based on body weight and toe ash measurements. *J. Poult. Sci.* 67:96-102. doi: 10.3382/ps.0670096.
- Preston, R. L., and W. H. Pfander. 1964. Phosphorus metabolism in lambs fed varying phosphorus intakes. *J. of Nut.* 83:369-378.
- Puggaard, L., N. B. Kristensen, and J. Sehested. 2010. Effect of decreasing dietary phosphorus supply on net recycling of inorganic phosphate in lactating dairy cows. *J. Dairy Sci.* 94:1420-1429. doi: 10.3168/jds.2010-3582.
- Raun, A., E. Cheng, and W. Burroughs. 1956. Phytate phosphorus hydrolysis and availability to rumen microorganisms. *Agric. and Food Chem.* 4:869-871.
- Ravindran, V., G. Ravindran, and S. Sivalogan. 1994. Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. *Food Chem.* 50:133-136. doi:10.1016/0308-8146(94)90109-0.
- Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response to broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolizable energy, nutrient digestibility and nutrient retention. *British J. Poult. Sci.* 41:193-200.
- Sansinena, M. J. 1999. Phytate phosphorus metabolism in holstein calves. M. S. Thesis. Louisiana State University, Baton Rouge.

- Santos, T. T., C. L. Walk, P. Wilcock, G. Cordero, J. Chewning. 2014. Performance and bone characteristics of growing pigs fed diets marginally deficient in available phosphorus and a novel microbial phytase. *Can. J. Anim. Sci.* 94: 493-497. doi: 10.4141/CJAS2013-190/
- Schlesinger, W. H. 1991. *Biogeochemistry: An analysis of global change*. Acad. Press, San Diego.
- Schnitkey, G. 2016. Revenue and costs for corn, soybeans, wheat, and double-crop soybeans, actual for 2009 through 2015, projected 2016. Farm Business Management- Department of Agricultural and Consumer Economics, University of Illinois. http://www.farmdoc.illinois.edu/manage/actual_projected_costs.pdf. Cited 7/14/2016.
- Schreiber, J. D. 1998. Estimating soluble phosphorus (PO₄-P) in agricultural runoff. *J. Miss. Acad. Sci.* 33:1-5.
- Scott, D., F. G. Whitelaw, W. Buchan, and L. A. Bruce. 1985. The effect of variation in phosphorus intake on salivary phosphorus secretion, net intestinal phosphorus absorption and fecal endogenous phosphorus excretion in sheep. *J. Agric. Sci. Camb.* 105:271-277. doi: 10.1017/S0021859600056331
- Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. *Anim. Feed Sci. and Tech.* 135:1-41. doi:10.1016/j.anifeedsci.2006.06.010.
- Selle, P. H., V. Ravindran, A. J. Cowieson, and M. R. Bedford. 2011. Phytate and Phytase In M. R. Bedford and G. C. Patridge (eds.), *Enzymes in Farm Animal Nutrition*, 2nd Edition. pp.160-206. Cambridge, MA; CABI.
- Sharpley, A. N., L. R. Ahuja, and R. G. Menzel. 1981. The release of soil phosphorus in relation to the kinetics of desorption. *J. Environ. Qual.* 10:386-391.
- Sharpley, A. N., S. C. Chapra, R. Wedepohl, J. T. 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. doi:10.2134/jeq1994.00472425002300030006x.
- Sharpley, A. N., J. J. Meisinger, A. Breeuwsma, T. Sims, T. C. Daniel, and J. S. Schepers. 1998. Impacts of animal manure management on ground and surface water quality In J. Hatfield (ed.), *Effective management of animal waste as a soil resource*. pp. 173-179. Lewis Publ., Boca Raton, Fl.
- Shirley, R. B., and H. M. Edwards, Jr. 2003. Graded levels of phytase past industry standards improves broiler performance. *Poult. Sci.* 82:671-680.
- Shupe, J. L., J. E. Butcher, J. W. Call, A. E. Olsen, and J. T. Blake. 1988. Clinical signs and bone changes associated with phosphorus deficiency in beef cattle. *J. Vet. Research.* 49:1629-1636.

- Simons, P. C., and H. A. J. Versteegh. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br. J. Nut.* 64:525-540. doi:10.1079/BJN19900052.
- Sims, J. T., R. R. Simard, and B. C. Joern. 1997. Phosphorus loss in agricultural drainage: historical perspective and current research. *J. Environ. Qual.* 27:277-293. doi:10.2134/jeq1998.00472425002700020006x.
- Smith, V. H., G. D. Tilman, J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Env. Poll.* 100:179-196. doi:10.1016/S0269-7491(99)00091-3.
- Smith, D. R., K. W. King, L. Johnson, W. Francesconi, P. Richards, D. Baker, and A. N. Sharpley. 2015. Surface runoff and tile drainage transport of phosphorus in the Midwestern United States. *J. Environ. Qual.* 44:495-502. doi:10.2134/jeq2014.04.0176.
- Sotak-Peper, K. M., J. C. González-Vega, and H. H. Stein. 2016. Effects of production area and microbial phytase on the apparent and standardized total tract digestibility of phosphorus in soybean meal fed to growing pigs. *J. Anim. Sci.* 94:2397-2402. doi:10.2527/jas.2016-0353.
- Sutherland, T. M. 1987. Particle separation in the forestomach of sheep In A. Dobson and M. J. Dobson, (eds) in *Aspects of Digestive Physiology in Ruminants*. Pp. 63-73. Ithaca, NY: Cornell University Press.
- Ternouth, J. H. 1989. Endogenous losses of phosphorus by sheep. *J. Agric. Sci. Camb.* 113:291-297. doi:10.1017/S0021859600069963.
- The Nature Conservancy. 2012. Mississippi River Basin. Available at <http://www.greatriverspartnership.org/en-us/northamerica/mississippi/pages/default.aspx>
- Tillman, A. D. and J. R. Brethour. 1958. Dicalcium phosphate and phosphoric acid as phosphorus sources for beef cattle. *J. Anim. Sci.* 17:100-103.
- Tillman, A. D., J. R. Brethour, and S. L. Hansard. 1959. Comparative procedures for measuring the phosphorus requirement of cattle. *Australian J. Anim. Sci.* 18:249-255.
- Tomas, F. M. 1974. Phosphorus homeostasis in sheep. II. Influence of diet on the pathway of excretion of phosphorus. *J. Agric. Res.* 25:485-493.
- Tronier, B., R. L. Ory, and K. W. Henningsen. 1971. Characterization of the fine structure and proteins from barley protein bodies. *Phytochemistry* 10:1207-1311.
- Turner, B. L. and A. B. Leytem. 2004. Phosphorus compounds in sequential extracts of animal manures: chemical speciation and a novel fractionation procedure. *Environ. Sci. Technol.* 38:6101-6108. doi:10.1021/es0493042.
- Underwood, E. J. 1981. *The Mineral Nutrition of Livestock*. Commonwealth Agricultural Bureaux, Longdon England.

- U. S. Environmental Protection Agency. 2001. Environmental and economic benefit analysis of proposed revisions to the national pollutant discharge elimination system regulation and the effluent guidelines for concentrated animal feeding operations. EPA-821-R-01-002.
- U. S. Environmental Protection Agency. 2014. Nutrient Pollution: EPA needs to work with states to develop strategies for monitoring the impact of state activities on the Gulf of Mexico hypoxic zone. Report No. 14-P-0348. Available at <http://www.epa.gov/oig/reports/2014/20140902-14-P-0348.pdf>. June 19, 2015.
- U. S. Environmental Protection Agency. 2015. The Sources and Solutions: Agriculture. Available at <http://www2.epa.gov/nutrientpollution/sources-and-solutions-agriculture>
- Valk, H. L., B. J. Sebek, and A. C. Beynen. 2002. Influence of phosphorus intake on excretion and blood plasma and saliva concentrations of phosphorus in dairy cows. *J. Dairy. Sci.* 85:2642-2649. doi:10.3168/jds.S0022-0302(02)74349-X.
- Vasconcelos, J. T., L. O. Tedeschi, D. G. Fox, M. L. Galyean, and L. W. Greene. 2007. Review: Feeding nitrogen and phosphorus in beef cattle feedlot production to mitigate environmental impacts. *Prof. Anim. Sci.* 23:8-17. doi:10.1532/S1080-7446(15)30942-6.
- Veum, T. L., D. W. Bollinger, C. E. Buff, and M. R. Bedford. 2006. A genetically engineered *Escherichia coli* phytase improves nutrient utilization, growth performance, and bone strength of young swine fed diets deficient in available phosphorus. *J. Anim. Sci.* 84:1147-1158. doi:/2006.8451147x.
- Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry.* 13:87-115. doi:10.1007/BF00002772.
- Viveros, A., C. Centeno, A. Brenes, R. Canales, and A. Lozano. 2000. Phytase and acid phosphatase activities in plant feedstuffs. *J. Agric. Food Chem.* 48:4009-4013. doi:10.1021/jf991126m.
- Wadsworth, J. C., and R. D. H. Cohen. 1976. Phosphorus utilization by ruminants. In: *Prospects for improving efficiency of phosphorus utilization. Reviews in Rural Science, III.* Pp 143–153. Univ. of New England Press, Australia.
- Walk, C. L., M. R. Bedford, T. S. Santos, D. Paiva, J. R. Bradley, H. Wlodecki, C. Honaker, and A. P. McElroy. Extra-phosphoric effects of superdoses of a novel microbial phytase. 92:719-725. doi: 10.3382/ps.2012-02727
- Walton, C. P., and G. F. Lee. 1972. A biological evaluation of the molybdenum blue method for orthophosphate analysis. *Tech. Int. Ver. Limnol.* 12:676-684.
- Wasserman, R. H. 1981. Intestinal absorption of calcium and phosphorus. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 40:68-72.

- Weiss, W. P., and D. J. Wyatt. 2004. Macromineral digestion by lactating dairy cows: Estimating phosphorus excretion via manure. *J. Dairy Sci.* 87:2158-2166. doi:10.3168/jds.S0022-0302(04)70035-1.
- Wise, M. B., S. E. Smith, and L. L. Barnes. 1958. The phosphorus requirement of calves. *J. Anim. Sci.* 17:89-99.
- Wright, R. D., J. R. Blair-West, J. F. Nelson, and G. W. Tregear. 1984. Handling of phosphate by a parotid gland (ovine). *American J. Physio.* 246:F916-F926.
- Yan, W., R. C. Reigh, and Z. Xu. 2002. Effects of fungal phytase on utilization of dietary protein and minerals, and dephosphorylation of phytic acid in the alimentary tract of channel catfish *Ictalurus punctatus* fed an all-plant protein diet. *J. of the World Aquacultural Society.* 33:10-22.
- Yanke, L. J., H. D. Bae, L. B. Senlinger, and K. J. Cheng. 1998. Phytase activity of anaerobic ruminal bacteria. *Microbiology.* 144:1565-1573. doi:10.1099/00221287-144-6-1565.
- Yi, Z., and E. T. Kornegay. 1996. Site of phytase activity in the gastrointestinal tract of young pigs. *Anim. Feed Sci. and Tech.* 61:361-368. doi:10.1016/0377-8401(96)00959-5.
- Yli-Halla, M., H. Hartikainen, P. Ekholm, E. Tortola, M. Puustinen, and K. Kallio. 1995. Assessment of soluble phosphorus load in surface runoff by soil analyses. *Agric. Ecosyst. Environ.* 56:53-62. doi:10.1016/0167-8809(95)00633-8.
- Zhang, Z. B., E. T. Kornegay, J. S. Radcliffe, D. M. Denbow, H. P. Veit, and C. T. Larsen. 2000. Comparison of genetically engineered microbial and plant phytase for young broilers. *Poult. Sci.* 79:709-717. doi: 10.1093/ps/79.5.709.