Improving estimations of phosphorus bioavailability for lactating dairy cows

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ABSTRACT

Phosphorus (P) is an instrumental nutrient in numerous physiological processes, but can have detrimental environmental impact if fed in excess. Increased P intake in dairy cows leads to increased fecal excretion of P and a reduction in efficiency of use. Variability in P concentration or availability in feedstuffs can exacerbate P excretion. To investigate variability in P between and within feedstuffs, 170 feed samples (forages, concentrates, and by-products), were collected from across the U.S., classified by region fed, and analyzed for total P, inorganic P, and phytate. Forages contained a greater proportion of P in the inorganic form and less total P and phytate as compared to concentrates and by-products. The majority of total P (71.2, 81.8, and 81.9% of total P in forages, concentrates, and by-products, respectively) was associated with inorganic P and phytate. The enzyme phytase has been used successfully in swine and poultry nutrition, as a feed additive, to increase available P and reduce the need for supplemental inorganic P. An experiment was conducted to investigate the effect of phytase use and forage particle length, using a 2 x 2 factorial, on P availability in lactating dairy cows. Total P intake of the four diets was similar ($P > 0.15$). Total tract digestibility of total P tended ($P < 0.10$) to be reduced and total P excretion was increased ($P < 0.05$) with phytase supplementation. Milk fat yield, protein yield, 3.5% FCM, and ECM were increased ($P < 0.05$) with addition of exogenous phytase to the diet. This indicates that phytate may contain some anti-nutritional factors that reduce availability in other nutrients used for milk production. Variation in P compounds between feeds, and variation in P digestion and production performance with exogenous phytase suggests opportunity for improvement in prediction of P availability from feeds for lactating cows.

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"Opportunity is missed by most people because it is dressed in overalls and looks like work." –Thomas Edison

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"We rejoice in our sufferings because we know that suffering produces perseverance; perseverance, character; and character, hope." Romans 5: 3-4

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Chapter I: Introduction

Concerns about nutrient losses from concentrated animal feeding operations (CAFO) are on the rise. Phosphorus (P) losses to the surrounding environment negatively impact lakes and streams. Eutrophication of these water bodies lead to fish kills, odor, and an overall decline in their recreational value.

A reduction in P imports to CAFO will help reduce P losses from farms, and an improved understanding of P digestion, metabolism, and utilization will support this goal. While initial gains in dietary P feeding practices can be made through removing mineral P supplements, additional progress requires addressing the issue of the high organic P content of byproduct feeds. Recent surveys indicate that use of these feeds is increasing, and that farmers and nutritionists lack confidence in the full availability of the P in these feedstuffs.

To better quantify availability of P in byproduct feeds, the form of P in these feedstuffs must be determined and measured. For instance, phytate-P (Pp) may not undergo complete hydrolysis in the rumen. Any undigested or unabsorbed P increases P excretion from the animal. Quantification of the extent of Pp hydrolysis in the rumen and lower digestive tract will support improved ration formulation in dairy cattle to reduce P excretion. Clear definition of P-containing compounds in feeds will support the improved estimates of bioavailability of P in by-product feeds necessary to advance “precision P feeding” techniques.

Chapter II: Review of Literature

Phosphorus

Accumulation of soil P in areas of intensive animal agriculture is due to land application of manures, and its buildup can be detrimental (Ebeling et al., 2002). Phosphorus runoff causes eutrophication in otherwise homeostatic nearby lakes and streams because the limiting nutrient for algae growth in fresh water is P. Upon death and decomposition of algae and weeds, dissolved oxygen in these water bodies is reduced, leading to fish kills, odor, and a decline in the recreational opportunities (Carpenter et al., 1998). In the Chesapeake Bay watershed, P loss from farms is under strict regulation. In these states, land application of manure is highly regulated to alleviate P buildup in the soil, with regulations often requiring farmers to apply manure based on crop uptake of P.

Scarcity of supplemental phosphate sources is a second factor driving the need for more precise and accurate evaluation of dietary P inclusion in food animal production systems. United States, China, and Morocco each produce approximately 25% of the world's phosphate rock but P production in the U.S. declined about 20% from 2003 to 2007 (USGS, 2007). Other leaders in production such as Christmas Island, Nauru, and Banaba have depleted their sources of phosphate, thus increasing the price of P-based feed additives.

Phytate phosphorus

Nearly 70% of the P in cereal grains is found in phytate (Nelson et al., 1968; Morse et al., 1992). Phytate can be degraded into varying inositol phosphate (IP) species. An IP is a six carbon ring containing phosphate groups on carbons in the ring. Inositol phosphate-6 (IP_6) contains a phosphate group on all six carbons. Upon hydrolysis, phosphates can be removed

individually from the ring leaving the ring intact and producing lower IP species which are partial degradates of Pp (IP_5, IP_4, IP_3, and IP_2).

Phytate P is not susceptible to mammalian enzymes so is generally unavailable for absorption by monogastrics. Inclusion of exogenous phytase and incorporation of feedstuffs containing endogenous phytase in poultry and swine diets have been extensively investigated. Cromwell et al. (1993) reported that growth performance and feed efficiency in growing and finishing pigs were equivalent in pigs fed diets supplemented with phytase and in pigs fed diets supplemented with inorganic P (Pi). Bioavailability of P was improved by approximately 45% with inclusion of phytase in the diet. Lei et al. (1993) observed that inclusion of phytase at 500 U/kg yielded similar growth performance in weanling pigs as did diets supplemented with Pi (to provide similar available P) and phytase yielded a 41% reduction in P excretion. Similarly, Harper et al. (1997) reported a 21.5% reduction in P excretion from weanling pigs fed phytase.

Although Pp is virtually unavailable to the nonruminant animal, ruminants have the capacity to utilize the P bound in the phytate molecule. Microorganisms in the rumen possess phytase (Yanke et al., 1998). There is evidence that extent of ruminal phytase activity varies and this activity may be altered by amount of grain in the ration (Yanke et al., 1998).

Many cereal grains contain endogenous phytase. Phytase activity of the grain depends upon relative location of Pp and phytase in the grain as well as temperature and pH during storage and processing (Bergman et al., 2000). Not all cereal grains have similar phytase activity. Godoy et al. (2005) reported that wheat and wheat by-products have the highest phytase activity (~1000 U/kg), 10 times higher than rice, 60 times higher than corn, and 25 times higher than oilseed products. Eeckhout and DePaepe (1994) found similar results in wheat and also reported barley and triticale to have a significant amount of intrinsic phytase activity. These researchers

suggested that it may be beneficial in ration formulation to factor in P liberated by endogenous phytase for grains that have activity greater than 100 U/kg. Despite endogenous phytase activity of barley, Kincaid et al. (2005) did not report improvement in P absorption or digestibility when comparing feeding barley to feeding corn in lactating cows.

Large amounts of P in grains and forages are present as Pp and, in theory, Pp in the rumen should be completely hydrolyzed because of phytase activity of ruminal bacteria. Extent of rumen development is one factor dictating degree of Pp digestion. In 6 week old calves, Duskova et al. (2001) reported 8.9% of total P in feces was in Pp form while 3% of ingested Pp was excreted in feces by 70 day old calves (Skrivanova et al. 2004) and only traces of Pp were detected in feces of calves at 56 days old (Nelson et al., 1976). Thus phytate digestion increases in the ruminant with increasing gastrointestinal maturity. However, in high producing dairy cows the amount of time feed spends in the rumen may be too short to allow the rumen microbes to completely hydrolyze Pp bonds. Despite ruminal phytase activity, passage rate, and physical properties of the feed may keep the animal from maximizing P availability (Kincaid et al., 2005). Therefore, Pp may exit the rumen and be excreted (Park et al., 2002). Phytate flow to the duodenum of sheep fed 133°C and 143°C heat treated rapeseed meal was 37% and 55% of total P, respectively (Park et al., 2000). Godoy and Meschy (2001) also observed that Pp hydrolysis by rumen bacteria is not complete. They speculated that some Pp digestion must occur in the abomasum or further on in the digestive tract.

Although it is assumed that Pp degradation occurs primarily in the rumen, Hu et al. (1996) determined that the small intestinal mucosa of pigs has some capacity to liberate phosphate molecules from Pp and their degradates *in vitro*. Activity was highest in the jejunal tissue and phytase activity was highly selective for lower IP species with greatest degradation of

IP_3. Humans and rats also have some ability to hydrolyze Pp in the small intestine; this capacity is much greater in the rat (Iqbal et al., 1994). The ability of the small intestine to hydrolyze Pp leads to more absorbable P as digesta moves through the digestive tract.

Success with phytase in swine diets has led to an increasing interest in phytase use in dairy cow rations. For lactating dairy cows, Kincaid et al. (2005) reported feeding 427 FTU phytase/ kg of DM (0.09%) in barley and corn-based diets with no Pi supplementation (all P from dietary ingredients) and observed a tendency for reduced fecal P when compared to non-phytase controls. Phosphorus absorbed tended to increase for cows being fed rations containing phytase, Pp hydrolysis increased, and Pp in feces decreased. No effect on milk production or milk composition was reported (Kincaid et al., 2005). Additional research by Knowlton et al. (2007) reported a 17% reduction in P excretion when feeding lactating cows a phytase-cellulase enzyme in rations containing no supplemental P.

Evaluation of NRC (2001) P requirements

Phosphorus is required for physiological function and is located in every cell in the body because it is a necessity in energy transactions. Phosphorus is a component of phospholipids, nucleic acids, phosphoroproteins, and in the acid-base buffer system in body fluids (NRC, 2001). The NRC (2001) reports P requirements on an absolute basis (g/d) and these requirements are a function of breed, body weight, maturity, stage of lactation, and DMI. While the dietary concentration needed to supply a specific quantity of P varies with DMI, it is convenient to express it as a percentage of DM. The NRC (2001) recommended P concentrations range from 0.42% in early lactation to approximately 0.32% after peak lactation. Accurate prediction of DMI is critical in interpreting these concentrations.

Current research regarding P requirements

Research regarding P requirements over the past ten years has focused on precise evaluation of the true P requirement in dairy cattle to avoid overfeeding. Multiple studies in the late 70's, 80's, and early 90's have indicated that low P diets do not affect reproductive performance. Wu et al. (2000) fed cows one of three concentrations of dietary P (0.31%, 0.40%, or 0.49%) for a 308 day lactation and determined that there were fewer days to first estrus for cows being fed 0.31% and 0.49% P ration. Cows fed the 0.31% P ration tended to be open fewer days ($P < 0.12$) and had lower services per conception ($P < 0.10$). While animal numbers in this study were too low to draw solid conclusions about reproduction (n= 8 – 9 per treatment), reducing dietary P did not hinder reproductive performance. Despite this research, many producers still incorporate more than 0.40% dietary P (on average).

Adoption of low P diets may also be limited by concerns that reducing dietary P may negatively affect milk production. Wu et al. (2000) reported no overall effect on milk production across one lactation with low P diets but did observe a treatment by time interaction. Beginning at week 25 of lactation, milk production decreased for cows fed a 0.31% P ration in comparison to cows fed 0.40% and 0.49% P rations. Kincaid et al. (1981) compared cows being fed 0.30% to 0.54% dietary P and observed lower milk yield for cows being fed lower dietary P; the low P diet in that study provided less P than is recommended by NRC (2001). Studies reporting no difference in milk yield for cows being fed varying concentrations of P in diets include Brintrup et al. (1993), feeding 0.33 and 0.39% dietary P; De Boer et al. (1981), feeding concentrations of 0.34, 0.51, and 0.69%; Dhiman et al. (1995), at concentrations of 0.39 and 0.65%; and Steevens et al. (1971), at 0.37 and 0.55% P. Wu and Satter (2000) reported that cows fed 0.48% P had similar milk production throughout their entire lactation as cows that consumed 0.38% P. Wu et

al. (2000) suggested feeding 0.30% P for cows producing 7,500 to 9,000 kg per lactation and increasing dietary P concentration to 0.38 - 0.40% P for cows producing greater than 10,000 kg per lactation. Feeding 0.31% dietary P throughout lactation may not sustain high milk production into late lactation (Wu et al., 2000).

Fecal excretion of P is affected by P intake. When Wu et al. (2000) fed lactating cows 0.31, 0.40, and 0.49% P, the respective fecal P concentrations averaged over all weeks of lactation (308 days) were 0.51, 0.73, and 0.90% on a DM basis. In that study, reduction in dietary P from 0.49% to 0.40% reduced fecal P excretion by 23%. Wu et al. (2003) evaluated fecal P excretion of diets with high or low forage (58% vs. 48% of DM) and high or low P (0.48% vs. 0.38%). They reported that fecal excretion of P was 25% less for cows fed low P diets than for cows fed high P diets. Phosphorus absorption was not altered when forage to concentrate ratio was changed (Knowlton et al., 2002; Wu et al., 2003). Other studies reporting strong positive relationships between dietary P and P excretion include Weiss and Wyatt (2004) for cows consuming 45 to 133 g of P/d; Valk et al. (2002) for cows consuming 67, 80, or 100% of their P requirement; and Morse et al. (1992) for cows consuming 0.30, 0.41, and 0.56% dietary P with the exception of cows in the first balance trial on the low P diet.

Phosphorus digestion and metabolism in ruminants

Phosphorus plays an integral role in digestive processes in the rumen. Ruminal microorganisms require P for cellulose digestion (Burroughs et al., 1951). Phosphorus is a required nutrient to synthesize microbial protein (Breves and Schroder, 1991; Bravo et al., 2000). Witt and Owen (1983) reported P concentration of rumen fluid to range from 264 to 434 mg/L on low and high P diets, respectively. Rumen microbes require 5 g of P/ kg of digested organic matter for optimal fiber digestibility (Durand and Komisarczuk, 1987). If we assume 9 kg of OM

digested in the rumen, then 45 g of P/ day is required to meet microbial P requirements. The model developed by Hill et al. (2008) indicates 52 g of P being recycled via saliva in a 'typical' diet. This would indicate the microbial requirement for P is met by salivary contributions.

Approximately 40% of the P entering the rumen is recycled Pi via salivary secretion (Grace et al., 1985). Salivary P resecretion is crucial in the maintenance of P homeostasis (Clark et al., 1973). Phosphorus partitioned from the bloodstream to the salivary glands is resecreted into the rumen (Durand and Kawashima, 1980), into milk, plays a crucial role in composition of red blood cells, muscle tissue synthesis, DNA and RNA, and regulates cellular and enzyme activity through phosphorylation (McDowell, 2003). Recycling of P will be maintained at the expense of bone P (Puggaard et al., 2011).

Phosphorus absorption from the small intestine

Main absorption sites of P are the duodenum (Wasserman, 1981) and the jejunum (Ben-Ghedalia et al., 1975). Sklan and Hurwitz (1985) determined that P disappearance from the gastrointestinal tract of sheep was 54% of P intake at the ileocecal junction. Two routes of absorption are present in the small intestine. The active transport system of P absorption is saturable and vitamin D-dependent. This system is utilized when low blood P stimulates the production of 1, 25-dihydroxy vitamin D, which initiates P absorption (Horst, 1986). Passive absorption predominates when P consumption is in the normal to high range and depends on an electrochemical gradient (Holler et al., 1988). This route of P absorption is dependent on concentration of P in blood plasma and presence of P in the lumen of the small intestine (Wasserman and Taylor, 1976). Efficiency of P absorption is reduced with high P concentrations in the digestive tract (Bravo et al., 2003b).

In nonruminants, secondary active transport of Pi is sodium dependent (e.g. Pfeffer et al., 1970; Grace et al., 1974); it is not clear whether this is true in ruminants. Schroder and Breves (1996) reported that 65% of Pi uptake in the brush border membrane vesicles from goat jejunal tissue is sodium dependent but suggests intensity of absorption may depend on Ca and P concentrations in the diet and ratio of the two. Foote et al. (2011) reported expression of NaPi-IIb (sodium-dependent phosphate transporter in intestinal cells) in the distal jejunum and the ileum indicating sodium dependent active transport in the small intestine. This is contradictory to reports of Shirazi-Beechey et al., (1996) that Pi uptake in sheep duodenal tissue is H^+ driven when the pH of the inside of the vesicle is greater than outside the vesicle and still independent of Na^+ when there is no pH differential. This indicates a structural modification of Pi binding sites exist causing a mechanistic difference in Pi uptake for ruminants versus nonruminants. Regardless of the differences in the mechanism for uptake in ruminants and nonruminants, active transport predominates in low intestinal P concentrations and passive when digesta P concentration is medium to high.

Phosphorus absorption from the large intestine

Recent work suggests varying degrees of P absorption in the large intestine (Park et al., 2002; Bravo et al., 2003b). Absorption from this section of the ruminant digestive tract would allow P utilization to occur from phytate-P liberated beyond the rumen. Park et al. (2002) reported an increase in IP_3 (the partial degradate of Pp with an intact inositol ring with three phosphate groups remaining) in the lower large intestine of sheep when compared to the upper large intestines. Additionally, a reduction of IP_6, IP_5, and IP_4 in digesta occurred indicating some specificity of large intestine microbes to liberate P from high IP structures. Mass balance

calculations including Pp, its partial degradates, and orthophosphates indicate that some P absorption in the large intestine occurred.

Although some research has reported P absorption from the large intestine, the absolute capacity for absorption has not been clearly defined. In four- month- old sheep, Smith et al. (1955) reported similar concentrations of P in the lumen of the cecum and the rectum and increased concentrations of P in the colon suggesting absorption of P in the large intestine. Similarly, Sklan and Hurwitz (1985) observed 9% disappearance of P from the large intestine. However, in ten- month- old sheep Smith et al. (1955) observed an increase in P concentration in rectum as compared to cecal concentrations. This indicates no measurable P absorption in the large intestine in growing sheep, and perhaps net secretion.

Absorption of P from the large intestine may be diet dependent (Table 2.1). Holler et al. (1988) observed net uptake of Pi in the colon of sheep when luminal concentration of Pi was 2.5 – 6.5 mmol/L and net secretion of Pi into the colon with a Pi-free infusate. This indicates that Pi uptake in the large intestine of sheep is concentration dependent. More P was absorbed from the large intestine of sheep fed an all hay diet (Pfeffer et al., 1970) as compared to sheep fed a diet consisting of hay and barley. The hay diet was lowest in P; therefore, a possible explanation for this outcome is that reduced P intake may cause the mucosal cells in the large intestine to "scavenge" for P.

Table 2.1. Phosphorus intake and content along the gastrointestinal tract[1]

	900 g Hay		600 g Hay, 300 g Barley		300 g Hay, 600 g Barley	
	Sheep 1	Sheep 2	Sheep 1	Sheep 3	Sheep 1	Sheep 3
P intake, g/d	2.44	2.44	3.18	3.18	3.18	3.18

P at terminal ileum, g/d	3.11	3.09	3.19	3.50	3.00	3.60
P in feces, g/d	2.60	2.31	3.12	3.24	2.94	2.53
Terminal ileum – fecal P, g/d[2]	0.51	0.78	0.07	0.26	0.06	1.07
% absorbed in large intestine as % of intake[1]	20.9	33.0	2.2	8.2	1.9	33.6

[1] Adapted from Pfeffer et al. (1970)

[2] Calculated from given data

Location of fermentation within the gastrointestinal tract has an effect on the opportunity for P absorption. Because sheep are foregut fermenters, the degree of P absorption in the large intestine is different than hindgut fermenters such as horses and rats. In sheep, the large intestine is a secondary or tertiary site of P absorption. In horses, the large intestine is the primary site of P absorption. Schryver et al. (1971) reported that regardless of diet, P absorption in the horse was greatest in the dorsal and small colon. The alfalfa diet in this experiment resulted in the highest apparent cumulative absorption of P although it had the lowest P concentration. In the mole-rat, a hindgut fermenter, primary sites of active P transport are the cecum and proximal colon (Pitcher and Buffenstein, 1993). In both the horse (Schryver et al., 1971) and the mole-rat (Pitcher and Buffenstein, 1993), high fiber diets increased mineral absorption. The increase in mineral absorption was attributed to a symbiotic interaction of fiber-digesting microorganisms and regions of active mineral uptake.

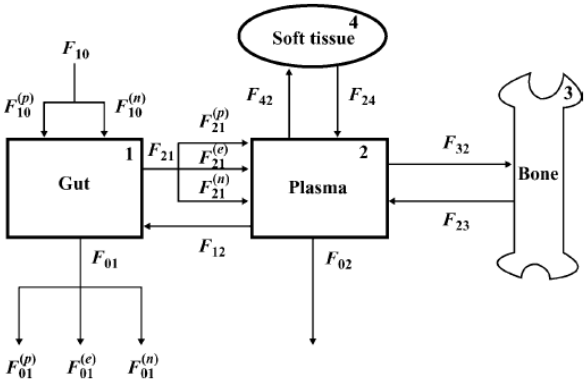
Presence of other minerals may also impact P availability in the large intestine. Total tract Pp hydrolysis in rats was approximately 20% on a high Ca diet (Wise and Gilburt, 1982). On a normal Ca diet, male and female rats achieved 43 and 70% total tract Pp hydrolysis,

respectively, indicating Ca chelation with Pp by intestinal bacteria causing reduction in digestibility. It can be concluded that P absorption in the large intestine does occur but it is more necessary in nonruminant hindgut fermenters as opposed to ruminants (foregut fermenters).

Model prediction of phosphorus digestion, absorption, and excretion

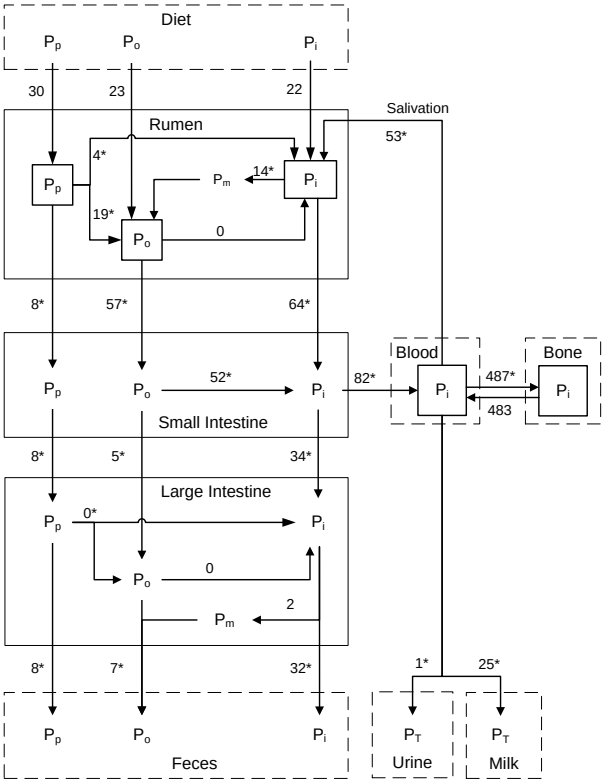
Most models of P metabolism in ruminants were derived from small ruminant studies using radioactive tracer kinetics to describe P digestion and absorption (Vitti et al., 2000; Bravo et al., 2003; Dias et al., 2006; Dias et al., 2011). These models aim to identify serum P, urinary P, and indicators of bone metabolism. Vitti et al. (2000) used growing goats fed high, medium, or low P diets to describe P fluxes in bone, plasma, soft tissue, and fecal excretion. Dias et al. (2006) expanded this model to incorporate forms of P (i.e. Pp and Po) into the intake and excretion fluxes of the model. Total tract digestibility of each fraction was a function of the model (Figure 2.1). A limitation to the Dias et al. (2006) model is its inability to predict what segment of the digestive tract and to what extent P degradation and absorption is occurring.

Figure 2.1. Model of P metabolism in growing goats (Dias et al., 2006; Vitti et al., 2000)



degradation,enters the small intestine. In the small intestine, P is absorbed and enters the bloodstream where it contributes to bone formation in concert with calcium. Phosphorus not entering the bloodstream passes into the large intestine where it will be excreted in feces (Durand and Kawashima, 1980). The model of Hill et al. (2008) allows prediction of excretion of P species to allow broader implications and conclusions about environmental pollution from dairy cows. However, the model is limited because of a lack of published data for flow of P containing compounds. For instance, the model assumes no degradation of Pp past the rumen because inadequate data exists to quantify that degradation. Evidence of Pp degradation beyond the rumen does exist (e.g. large intestine digestion of Pp in sheep, Park et al., 2002).

Figure 2.2. Model of P digestion and absorption in the lactating cow (Hill et al., 2008)



Phosphorus in feedstuffs

Phosphorus concentrations in traditional feedstuffs can vary with forage type, genetic strain, maturity, and soil test P. As forages mature, mineral content decreases because of a decrease in the leaf: stem ratio and an increase in forage fiber content (Fleming, 1973; Little, 1982). Neutral detergent fiber (NDF) is higher in more mature forage because the leaf: stem ratio decreases and mineral content is less in fiber rich portions. For example, immature hay contains

0.31% P as compared to mature hay containing 0.28% P (NRC, 2001). Inaccurate ration formulation based on forage maturity can exacerbate deficiencies or excesses.

Commonly fed grains also vary in P content. The NRC (2001) reported dried, ground corn grain contains 0.30% P, with a standard deviation of 0.05 (16.5% variation). Additionally, 48% SBM containing 0.70% P was associated with 11% variation (NRC, 2001).

Phytate chemistry

Phosphorus can exist as Pp bound to protein (Rham and Jost, 1979). These phytate-protein compounds contain solubility points for both nitrogen (N) and P, but N is not solubilized at the same pH as P. Degree of matrixed Pp and protein is associated with type of feed (Fontaine et al., 1946). These interactions have been reported in cottonseed, peanuts (Fontaine et al, 1946), and soybean products (Rham and Jost, 1979).

Feed type and extent of processing (Mjoun et al., 2008) are the two factors most affecting availability of P bound in Pp. Park et al. (2000) observed that untreated rapeseed meal is more subject to ruminal Pp degradation than rapeseed meal heated at 133°C and 143°C. Because of the contribution of processing to P availability, by-product feeds may vary in P availability. For instance, the Maillard reaction occurs with the combination of heat, moisture, and presence of sugar when processing feedstuffs. This not only binds protein, but it can also affect P forms and availability (Wahyuni et al., 1998). This illustrates that intrinsic feed properties dictate P degradation with processing, or lack thereof.

Phosphorus in by-product feeds

By-product feeds in dairy rations are becoming increasingly prevalent as ethanol production increases demand (and price) for corn and indirectly increases the price of traditional protein sources. By-product feeds are concentrated versions of their unprocessed counterparts

and they are generally higher in P than the original grain. For instance, corn grain contains 0.30% P, whereas dried distillers grains with solubles (DDGS) contain 0.83% P (NRC, 2001). It has been proposed that during fermentation in corn ethanol production, Pp in corn is hydrolyzed (Pedersen et al., 2007). Because of this, P in DDGS may be highly available (Mjoun et al, 2008). For instance, DDGS has a greater apparent total tract digestibility of P than corn when fed to pigs (Pedersen et al., 2007). Morse et al. (1992) reported that 12 hours *in vitro* digestion results in a minimum of 90% Pp hydrolysis in wheat middlings and DDG (dried distillers grains), compared to only 71.5% in corn.

Phytate chemistry in feed processing

Two processes used to make ethanol are wet milling and dry grinding (Rausch and Belyea, 2005). The purpose of wet milling is to recover the starch and in the process create a high protein by-product feed called corn gluten meal. The dry grind process is intended for maximal ethanol production through fermentation of the entire corn kernel (Rausch and Belyea, 2005). Variability of processing between and within processing types impacts nutrient content of the by-product feeds produced. Phytate degradation during this process is highly variable. Mahgoub and Elhag (1998) demonstrated that malting sorghum allowed for 68-87% of Pp to be degraded. Additionally, processes of soaking, extraction, and fermentation all led to Pp degradation but to varying degrees.

Processing method influences Pp degradation, so distillers grains and corn gluten feeds (the result of dry and wet milling, respectively), vary in Pp content. In wet milling, the steep water contains the majority of the Pp. Corn gluten feed is made by adding steep water back to bran. Liquid and solid fractions of whole stillage are low in total P (8.8 mg of P/g of sample) as compared to steep water (34.4 mg of P/ g of sample). Therefore, proportion of steep water to

bran influences total P concentration of the feed (Noureddini et al., 2009). In addition, proportion of steep water influences the proportion of that P that is Pp. Eighty percent of the P in steep water is in Pp form, whereas Pp is undetectable in whole stillage by-products (Noureddini et al., 2009). In the dry grind process resulting in distillers grains, solubles are equivalent to steep water. Condensed solubles contain 13.4 mg of P /g of sample while distillers grains without solubles contain 5.2 mg of P/g. When added back to distillers grains, the P concentration increases considerably.

Other forms of organic phosphorus in feedstuffs

Phospholipids and RNA and DNA associated-P may be the two other forms of P with the most contribution to total P and therefore biological importance. By calculation, it can be determined that P content of DNA and RNA is approximately 10%. In plants, DNA content is relatively low, ~2.8 mg/g of plant DM, in the terminal bud (Lee et al., 1966). Contribution of P from RNA is more substantial. At the terminal bud, RNA concentration ranges from 15 to 23 mg/g of plant DM (Lee et al., 1966; Sachdev and Debb, 1977), whereas three leaves away from the terminal bud, RNA content is much less (2.5 – 15 mg/g of plant DM), depending upon P concentration in the soil. Since RNA is 10% P, contribution of P from RNA origin can reach 2.3 mg of P/g of plant DM.

Phosphorus from phospholipids as a percent of total P in plants can be variable depending upon P content of the soil. For instance, in soybean roots, increasing P concentration of the soil from 0 to 18 ppm, increases total P concentration in the plant by 5 times (Lee et al., 1966). However, P from phospholipid only increased 2 fold. Phosphorus in phospholipid form, as a percentage of total P is greater (27%) in low soil P as compared to high soil P (10%). Plant

distribution of phospholipids is not uniform. The highest concentration of phospholipids is found in the seed of the plant and can be as great as 2% of DM (Robinson, 1980).

Laboratory techniques for determination of phosphorus-containing compounds

Sample extraction

The first step to all quantification methods is extraction of all P forms from the sample. Traditionally, 0.5 M HCl was used in a 1:20 ratio (sample to extractant) for 2 h at room temperature to extract phytate from feed and digesta samples prior to HPLC analysis (Graf and Dintzis, 1982; Kwanyuann and Burton, 2005). Few studies have investigated efficacy of feed P extraction techniques in feces, digesta, and manure samples. Yang (2006) reported that utilizing a 16 h, 2.4% HCl, extraction followed by nitric-perchloric acid wet digestion yields varying efficiencies of P extraction within and across sample type (

Table **2.2**). Alkaline extraction consisted of a 4 h extraction by 0.25M sodium hydroxide (NaOH) – 0.05M ethylenediaminetetraacetic acid (EDTA), followed by nitric-perchloric acid wet digestion. As with HCl extraction, percent P recovered was highly variable with NaOH-EDTA (

Table **2.2**). Similarly, Leytem et al. (2004) obtained 91% recovery of total P in swine manure with NaOH-EDTA extraction.

Table 2.2. Total phosphorus extraction of feed and digesta samples with HCl and NaOH-EDTA extraction (Yang, 2006)

	Extraction technique

Sample type	# of samples	HCl	NaOH-EDTA
Corn	4	70.9 – 90.6%	95.1 – 110.4%
Soybean meal	4	66.0 – 81.4%	92.7 – 100.0%
Dairy duodenal digesta	3	59.3 – 91.5%	85.0 – 112.7%
Dairy feces	3	54.2 – 72.6%	80.5 – 106.0%

Yang (2006) also compared the effect of the extraction technique (HCl versus NaOH-EDTA) on recovery of Pp measured with NMR analysis. Efficiency of extraction was dictated by sample type (Table 2.3). Extraction with HCl was more effective on feed samples whereas NaOH-EDTA extraction was more effective for digesta samples when measuring Pp.

Table 2.3 Phytate-P recovery as a percentage of total P (

Table 2.2**) when analyzed by NMR (Yang, 2006)**

		Extraction Technique	
Sample Type	# of samples	HCl[1]	NaOH-EDTA[1]
Corn	4	45.3 – 83.5%	48.1 – 85.2%
Soybean meal	4	83.6 – 90.0%	73.0 – 87.1%
Dairy duodenal digesta	3	19.5 - 35.3%	44.5 – 50.2%
Dairy feces	3	ND – 26.2%	17.2 – 27.9%

[1] ND indicates non-detectable

Sequential extraction techniques using ddH_2O, HCl, NaOH, and $NaHCO_3$ have also been investigated (Turner and Leytem, 2004; He et al., 2010). He et al. (2010) extracted broiler litter

with ddH_2O, 0.5 M $NaHCO_3$, 0.1 M NaOH (+0.1mM EDTA), and 1.0 M HCl. The greatest P content was in the HCl extracted fraction followed by ddH_2O, NaOH, and $NaHCO_3$ with 44.6, 21.4, 20.7, and 12.9% of total P in each fraction, respectively. The residual P in the pellet contained approximately 0.4% of the total P. Turner and Leytem (2004) used the same sequential extraction in broiler litter, cattle manure, and swine manure with total P extraction efficiency of 94, 79, and 92%, respectively. These researchers also eliminated the ddH_2O extraction step and then used 1.0 M HCl or 0.5 NaOH + 50 mM EDTA and received similar extraction efficiency. McDowell et al. (2008a) determined that even though NaOCl would extract both inorganic and organic forms of P effectively, oxidation of Po was occurring and thus skewing Po and Pi concentrations in the sample. Additionally, the use of EDTA in extracts intended for the Mo-blue method (Murphey and Riley, 1958), for Pi determination, caused interference because the EDTA binds the Mo molecule (McDowell et al., 2008a). These reports indicate the need to choose an extractant based on sample type and P forms of interest.

Quantification of phytate in sample extracts

The original method of Pp determination was through precipitation of Pp with ferric ions (Heubner and Stadler, 1914). Sandberg and Ahderinne (1986) determined that HPLC values for Pp were lower than with the iron precipitation method. This is because the iron tends to precipitate other P forms in addition to Pp.

^{31}P nuclear magnetic resonance spectroscopy has been used to determine P compounds in extracts of feed, digesta, and feces (Kemme et al., 1999b; Turner, 2004; Toor et al., 2005). The major advantage to determination of P containing compounds using ^{31}P NMR is quantification of all P forms present through one process. This analytical technique is costly and the expense of ^{31}P NMR has encouraged researchers to find alternative methods.

Refinements in sample preparation, column type, mobile phase, and elution time may make HPLC the most promising technique to accurately quantify Pp in feed and lower inositol species in digesta samples. High pressure liquid chromatography and NMR methods were deemed comparable for speciating Pp and lower IP species (IP_5, IP_4, and IP_3) in ileal digesta and fecal samples from broilers (Leytem et al., 2008). Correlation between phytate measured by HPLC and phytate measured by ^{31}P NMR was 94 and 98% for the two sample types (Leytem et al., 2008). Sample preparation differs for these two methods of Pp quantification. Samples for ^{31}P NMR were extracted with NaOH-EDTA adapted from Turner (2004), whereas samples prepared for HPLC were extracted with HCl (adapted from Kwanyuen and Burton, 2005).

Sandberg and Ahderinne (1986) and Burbano et al. (1995) used similar methods for sample preparation using 0.5 M HCl extraction (Graf and Dintzis, 1982) for HPLC on different sample types, feed, digesta and legumes, respectively, and obtained similar results for efficiency in spike recovery of sodium phytate. These studies both indicate an optimal pH of 4.3 for the mobile phase regardless of type of acid used. Both methods also included isocratic elution and ion-pair C_{18} reverse phase HPLC to be ideal for Pp and lower IP quantification.

On farm implications of phosphorus feeding practices

Increased intake of P often increases feed costs. Currently, dicalcium phosphate costs $74.25 / 100 kg (Southern States, 2011). If dicalcium phosphate is used at the rate of 0.05% of the diet DM, a cow averaging 25 kg of DMI/day, consumes 0.125 kg of dicalcium phosphate at a cost of $0.09/ cow/ day. If a farm is milking 200 cows, feed costs are elevated by $18.50/day ($6,675/year). Therefore, inclusion of dicalcium phosphate in a TMR is a significant monetary expense and also has environmental implications because of increased P excretion (e.g. Morse et al., 1992; Wu et al., 2001; Knowlton and Herbein, 2002).

Dairy farms in the Chesapeake Bay watershed were feeding their cows 4.42 g of P/kg of DM on average (Dou et al., 2003). The NRC (2001) recommendations for those cows based on breed, production, stage of lactation, and body weight of cows in these herds was 3.3 g of P/kg of DM (25% overfeeding). Overfeeding P in dairy cattle is occurring in part because of high P by-products and increasing the importance of determining how to effectively manipulate the P present in the feed.

Manure is an economical source of N to maximize plant growth and production so when restrictions are placed on land application of P, manure P content needs to be minimized. Overfeeding P in dairy cows is common because of high forage P, too much P supplementation, improper/no analysis of feed, built in safety margins to the NRC (2001) P requirements, and inclusion of high P by-product feeds (Rausch and Belyea, 2005).

By-products tend to be inconsistent in their composition and undetected variation in their P content may increase overfeeding of P. Arosemena et al. (1994) reported 20% variation in NRC (1989) tabular values in all nutrients analyzed in by-product feeds, including P. High feed costs have encouraged farmers to replace corn and soybean meal in a typical ration with lower cost by-product feeds. By-product feeds are concentrated in some nutrients as compared to their unprocessed counterparts. Therefore, these feedstuffs tend to be high in P. These common feedstuff substitutions lead to increased P in the ration. This strategy of reducing ration costs with by-product feeds needs to be weighed against long-term environmental implications of increased P excretion from dairies implementing these feeding strategies.

Effects of phosphorus feeding practices on whole farm nutrient balance

Whole farm nutrient balance (WFNB) is the balance of nutrients (N, P, K) entering and leaving a farm. Nutrients enter in the form of animals, purchased feed, and fertilizers and leave

in animals or animal products, crops, and manure sold off farm. The balance between imports and exports of nutrients allows calculation of WFNB, an assessment of the risk of nutrient loss from a farm. Spears et al. (2003) reported that herd P utilization efficiency was a main factor affecting whole farm P balance. Reducing imported P (purchased feed and/or P supplements) would significantly improve herd P utilization efficiency.

With increased use of by-product feeds, P imports typically increase thus leading to negative impacts on WFNB (Koelsch and Lesong, 1999). Koelsch (2005) demonstrated a reduction in excess P by 43% through replacing a portion of corn gluten with corn or alfalfa. In beef operations, corn based diets average 3 g of P/ kg of DM whereas feeding programs using corn gluten and distillers grains contain 4-5 g of P / kg of DM (NRC, 1996).

Strategies like changes in crop rotation, manure handling, feeding practices, better forage selection based on P concentrations, not supplementing P in P adequate rations (Rotz et al., 2002), and mindful use of by-product feeds will allow maximization of P utilization to occur and minimization of negative environmental impacts. Implementing these strategies will improve whole farm P balance by improving efficiency of P utilization (Spears et al., 2003).

Objectives

The objectives of this dissertation work were to quantify P forms in a variety of feedstuffs and to investigate the effects of one method of improving P availability to cows, the use of exogenous phytase on digestive P flows and excretion. These data will be used to refine the model of Hill et al. (2008) to better predict P availability and utilization in lactating dairy cows.

Chapter III: Quantification of phosphorus forms in an array of feedstuffs

Abstract

Bioavailability of phosphorus (P) in feedstuffs has recently been reported to be unreliable. A recent survey of dairy nutritionists indicates a lack of confidence in the current coefficients for P availability which leads to overfeeding P on dairy farms (unpublished data). Quantification of total P and P-containing compounds in a variety of feedstuffs may help improve estimations of availability. One-hundred and seventy feed samples were gathered from two reputable commercial laboratories including forages, concentrates, and by-products. Each sample was classified by region fed. Samples were analyzed for total P, inorganic P (Pi), and phytate (Pp). The data was analyzed using the MIXED procedure of SAS to determine the effect of feed class and geographic region. Total P was greater ($P < 0.10$) in concentrates and by-products as compared to forages. Concentrates had the lowest ($P < 0.10$) concentration of Pi, followed by by-products and then forages. Two fractions of P analyzed (Pi and Pp) accounted for 71.2% of total P in forages and 81.8 and 81.9% in concentrates and by-products, respectively. Very few regional differences were present. Data characterizing P forms in feedstuffs in addition to total P may lead to better estimations of P bioavailability and more accurate feeding recommendations in dairy cows.

Introduction

Variability in nutrient content of forages, concentrates, and by-products may exacerbate inaccurate delivery of nutrients to dairy herds. Overfeeding due to undetected variation in nutrient content leads to unnecessary increase in feed costs and overfeeding nitrogen and phosphorus (P) increases their excretion (e.g. Wu et al., 2000; Knowlton and Herbein, 2002;

Groff and Wu, 2005) causing negative environmental implications. By-product feeds tend to be higher in P than their unprocessed counterparts so may contribute disproportionately to excessive P intake. Arosemena et al. (1994) collected samples of nine selected by-product feeds commonly fed on California dairies and reported 20% variability in nutrient composition of these feeds as compared to nutrient values reported in the NRC (2001). Kertz (1998) reported variability of the same magnitude in P concentrations of forages (~64,000 samples from one commercial laboratory) and concentrates (~37,000 samples from another laboratory).

Changes in P availability to ruminants have been reported between and within classes of feed with feed processing methods such as heat treatment and formaldehyde treatment (Koshini et al., 1999; Bravo et al., 2000; Park et al., 2000). However, the current NRC (2001) does not account for these differences. In feeds, P occurs in various forms including inorganic (Pi), non-phytate organic (Po), and phytate (Pp). These fractions vary in bioavailability because of intrinsic feed properties, physical form, and nutrient complexing (Fontaine et al., 1946). Characterization of these P forms may improve estimates of P availability in the same way that more complete characterization of fiber and protein fractions has improved prediction of availability of those nutrients.

The objective of this investigation was to describe the P in an array of forages, concentrates, and by-products by quantifying total P, Pi, and Pp in these feeds and assess regional differences in P fractions within feedstuffs.

Materials and methods

Feed samples (n=170) including forages, concentrates, and by-products were collected from two commercial laboratories and classified by region fed (Figure 3.1). All samples were

ground to 1.0 mm in a Wiley Mill (Arthur H. Thomas, Swedesboro, NJ) and analyzed for total P, using nitric-perchloric acid digestion (AOAC, 1984), Pi, and Pp.

For Pi and Pp analysis, samples were ground through a 0.2 mm screen in a Z-grinder (Retsch ZM 100, Haan, Germany). To quantify Pi, samples were extracted with 0.5 M HCl (1:20 sample: extractant ratio) for 4 h at ambient temperature on a horizontal mechanical shaker (Eberbach Reciprocal Shaker 6000, Ann Arbor, MI). After shaking, the samples were refrigerated (4°C) overnight and centrifuged at 30,000 x *g* for 10 minutes. If the supernatant was cloudy (common with samples high in starch and protein), it was further extracted 1:1 with 20% NaCl and filtered through a Whatman 55 mm glass microfiber filter. Inorganic P content of supernatants was determined with the molybdenum blue method as described by Murphy and Riley (1958). Extracts were further analyzed for total P by ICP (Inductively Coupled Plasma, Thermo electron IRIS Intrepid II XSP; Thermo Fisher Scientific Inc., Waltman, MA) to determine extraction efficiency of total P with 0.5 M HCl. Extraction efficiency was determined by dividing total P of HCl extracts by total P as measured by nitric-perchloric digestion. If extraction efficiency of total P for a feed type was greater than 85% (Table 3.1), Pp analysis was conducted on HCl extracts. If extraction efficiency was less than 85%, alkaline extraction was used (described below).

Samples for which HCl extraction was ineffective were extracted with 0.25 M NaOH + 50 mM EDTA (1:20 sample: extractant ratio) for 4 h and then centrifuged at 30,000 x *g* for 10 minutes. A 5 mL subsample of the supernatant was acidified with 500 µL of 6 M HCl + 1.2 M HF and refrigerated overnight (4°C). The following day, the acidified supernatant was re-centrifuged, the supernatant reserved, and the pellet discarded.

To prepare extracts (acid or alkaline) for Pp analysis, 5 mL was passed through a methanol-conditioned C_{18} cartridge (Sep-Pak Plus, Waters, MA) and a 0.2 µm IC membrane filter and reserved in a Dionex sample vial. Reserved extracts were analyzed by a colorimetric reaction with a post column reagent using a Tee connector and a 300 cm long reaction coil with a 0.5 mm I.D. and separation by HPIC (Dionex ICS 3000 with a Dionex 4 x 50 IonPac AG7 guard column and a 4 x 250 mm IonPac AS7 analytical column; Dionex, Sunnyvale, CA). Isocratic elution was used in a 10 minute run at 1 mL/min of 0.25 M HNO_3 mobile phase and Pp peak detection at 7.2 min. The post column reagent of 0.1% $Fe(NO_3)_3$ + 2% $HClO_4$ was pumped at 1 mL/min (Isco HPLC pump, model 2350, Isco Inc., Lincoln, NE) and absorbance was measured at 260 nm (Phillippy et al., 2003). Calibration standards for Pp (3, 15, and 30 µg P/mL) were prepared from sodium phytate.

Statistical analysis

All data were analyzed using the MIXED procedure of SAS (Cary, NC) with the model defined in Table 3.2. If data had a non-normal distribution, it was log transformed prior to statistical analysis. All data were reported as LS means and standard errors with the residual testing the main effects. Significance was declared at $P < 0.10$. When the effect of feed class, feed type, or region had a $P < 0.15$, Tukeys test was used to separate LS means.

Results and discussion

Effect of feed class

Total P concentration of forages was less than that of concentrates and by-products ($P < 0.10$; Table 3.3). Inorganic P content of concentrates was lower than Pi in forages ($P < 0.10$), but not different from by-products ($P > 0.10$). Phytate concentration was the lowest ($P > 0.10$) in forages, followed by by-products, and then concentrates. When comparing Pi as a percentage of

total P, forages have the greatest proportion in Pi followed by by-products and then concentrates (*P < 0.10*). Hydrolysis of Pp with processing has been reported to occur in sorghum and wet milling and dry grind in ethanol production (Mahgoub and Elhag, 1998; Noureddini et al., 2009). Interestingly, total P in corn and dry distillers grains with solubles (DDGS) is 0.30% and 0.83%, respectively. The data reported here between concentrates and by-products does not reflect the vast differences of total P that two of the most traditional unprocessed and processed feedstuff reveals. Phosphorus composition of concentrates and by-products do not differ (*P > 0.10*).

In forages, 71.2% of the total P was associated with Pi and Pp; these fractions accounted for greater than 80% of total P for concentrates and by-products (Table 3.3). The 20 - 30% of total P unaccounted for may be associated with phospholipids, DNA, RNA, or degradates of phytate hydrolysis (IP_5, IP_4, IP_3, and IP_2). The majority of phospholipids are found in the seed of the plant, and can be as much as 2% of plant DM (Robinson, 1980). Growing plants have a higher concentration of RNA per unit of DM (Lee et al., 1966). This indicates that forages should have a higher RNA content at harvest, because they are still growing, than concentrates. Younger growth (terminal bud) may contain 15 - 23 µg RNA/mg of DM with approximately 10% being P as compared to older growth (third leaf from terminal bud) containing 2.5 – 15 µg RNA/mg of DM. It is not unreasonable to assume, depending on maturity at harvest that up to 2.3 µg of P / mg of DM of the unexplained P in forages is from RNA origin (Lee et al., 1966). DNA content of plants is low. The contribution of P from DNA in young growth ranges from 0.20 – 0.28 µg of P/ mg of DM and 0.09 – 0.19 µg of P/ g of DM in older growth (Lee et al., 1966).

In by-product feeds, degradation of Pp is probable depending on a variety of factors including heat, moisture, pH, and degree of fermentation (Phillippy et al., 1986) associated with

processing method (and therefore, processing plant).

Concentrates may also contain lower IPs due to intrinsic phytase. Eeckhout and DePaepe (1994) report highest phytase activity in rye (5,130 phytase units /kg), triticale (1,688 phytase units /kg), barley (582 phytase units /kg), wheat (1,193 phytase units /kg), and fractions in wheat processing such as wheat fine bran, wheat middlings, wheat feed fiber and wheat bran with 4,601, 4,381, 3,350, and 2,957 phytase units /kg of feed, respectively. There is no correlation between total P content and phytase activity in the feed.

Variation by region

Very few regional differences were present within a feedstuff in this data set. For forages, there were trends for changes in total P content ($P < 0.15$; Table 3.4) and a significant difference in regions for Pi of alfalfa hay ($P < 0.10$; Table 3.5) with region 3 having the highest concentration at 2,659 μg /g of DM and region 4 having the lowest concentration of both TP and Pi at 1,079 μg /g of DM. These differences between regions may be due to differences in soil type, soil composition, or plant genetics. Availability of P in soil is dependent upon pH. In acidic soils, (pH < 6.0) the majority of P present as Pi is in the monovalent form, $H_2PO_4^-$. Ullrich-Eberius et al. (1984) observed that soil pH of 5.0 - 6.0 is most favorable for plant uptake of P.

Soybean meal from region 5 tended to have higher Pp than region 1 ($P < 0.15$; Table 3.6) at 5,520 and 4,367 μg /g of DM, respectively. Although the number of samples is small, regional differences were observed in concentration of Pi and Pp ($P < 0.10$) content of wheat middlings with Pi content of samples from region 1 and 3 being greater than from region 5 (Table 3.5). Phytate content of wheat middlings from region 3 and 5 was greater than from region 1 (Table 3.6). Wheat middlings are a blend of wheat bran, wheat shorts, wheat germ, wheat flour, and offal (tail of the mill; AAFCO). The incorporation rate of each fraction of wheat milling that

comprises wheat middlings, may influence the P content and P fractions that are present. Similarly, the Pi and Pp content of hominy tended to vary by region ($P < 0.15$). Inorganic P of hominy from region 6 was the highest, but not different ($P > 0.15$) from region 5. All other regions represented (1, 2, 3, 7, and 9) had similar ($P > 0.15$) Pi concentration to region 5 (Table 3.5). Phytate content of hominy was the lowest in samples from regions 3 (2,036 μg /g of DM) and 7 (4,099 μg /g of DM) and highest in region 2 (9,596 μg /g of DM; $P < 0.15$; Table 3.6). Hominy samples from regions 1, 5, 6, and 8 were similar in Pp concentration ($P > 0.15$) to both regions 3 and 7 as well as region 2 (Table 3.6). Hominy feed is produced from corn dry-milling and includes corn bran and corn germ cake (Rausch and Belyea, 2005). Incorporation rates of the germ and bran may impact P composition of the final product. Total P content of corn gluten feed, a by-product of corn wet-milling, varied by region ($P < 0.10$) with higher values from regions 1, 3, and 9 as compared to region 5.

Implications

Total P concentration and P form in feedstuffs can be highly variable and this variability cannot necessarily be attributed to region. With increasing concern of P loss from dairy farms because of detrimental environmental impacts, selection of feedstuffs on a farm based on P concentration of a feed may become a point of focus. This report provides insight on the importance of selecting one forage, concentrate, or by-product over another forage, concentrate, or by-product when managing P inputs to a farm to control P excretion on a farm. Future research may be able to better evaluate availability of these forms to the animal in order to further refine P feeding practices.

Table 3.1. Extraction efficiency of total phosphorus (P) in HCl extracts

	Total P, µg/ g DM					
	Nitric-perchloric	SE	HCl extract	SE	Extraction efficiency, %	SE
Feed type						
Alfalfa hay[4]	2,935	222	1,918	253	63.2	2.4
Alfalfa silage	3,114	256	2,747	292	87.5	2.7
Corn silage	2,138	330	2,279	377	106.4	3.5
Grass hay[4]	2,460	211	2,016	241	80.5	2.2
Barley[4]	4,403	405	3,476	462	78.7	4.3
Canola[4]	12,915	405	9,720	462	75.2	4.3
Corn grain	3,104	299	2,816	341	90.5	3.2
HMSC[1]	3,160	265	2,935	303	93.3	2.8
Soybean meal, 48%[4]	8,008	443	5,811	506	72.4	4.7
Almond hulls[4]	1,250	701	1,036	800	82.7	7.4
Corn gluten	10,235	375	9,468	428	100.2	4.0
Citrus pulp	1,440	443	1,306	506	92.5	4.7
Dried DGS[2]	9,729	275	9,289	1,132	94.4	2.9
Hominy	4,806	351	4,621	400	95.9	3.7
Soy hulls[4]	1,487	701	678	800	46.1	7.4
Wet brewers[4]	6,961	330	5,678	377	81.1	3.5
Wet DGS[2]	8,999	701	7,387	1,132	96.4	7.4

Wheat middlings	12,029	572	11,465	654	95.0	6.1
Whole cottonseed[4]	6,282	405	5,274	462	84.3	4.3
Feed class[3]						
Forage	2,560[a]	249	1,945[a]	285	76.5[a]	2.6
Concentrate	6,318[b]	165	4,952[b]	189	82.0[b]	1.8
By-product	7,340[c]	183	5,840[c]	209	86.2[c]	1.9

[1]High moisture shelled corn

[2]Distillers grains with solubles

[3]Means within columns with different superscripts are significantly different

[4]Samples in feed type were extracted with 0.25 M NaOH-50 mM EDTA for phytate analysis

Table 3.2. Statistical model used to analyze all variables

Source	df	Error df	Error term
Feed class	2	122	Residual
Feed type (feed class)	21	122	Residual
Region	8	122	Residual
Region x feed class	16	122	Residual
Residual	122		
Total	169		

Figure 3.1. Regional classifications of feeds[1]

[1]Region 1 = IN, IL, OH, MI, WI; Region 2 = AL, KY, MS, TN; Region 3 = DE, NJ, NY, PA; Region 4 = AZ, CO, ID, HI, MT, NM, NV, UT, WY; Region 5 = CT, MA, ME, NH, RI,VT; Region 6 = AK, CA, OR, WA; Region 7 = FL, GA, MD, NC, SC, VA, WV; Region 8 = IA, KS, MN, MO, NE, ND, SD; Region 9 = AR, LA, OK, TX

Table 3.3. Effect of feed class on total phosphorus (TP), inorganic phosphorus (Pi), and phytate phosphorus (Pp) content

Feed class[1]	TP		Pi		Pp		Pi		Pi + Pp	
	µg/g DM[2]						as a % of TP			
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Forage	2,652[a]	261	1,970[a]	376	BDL[a]	355	71.9[a]	4.7	71.2 [a]	4.2
Concentrate	6,380[b]	180	791[b]	260	4,625[b]	248	16.9[b]	3.2	81.8[b]	3.0
By-product	6,529[b]	193	1,543[ab]	278	4,192[c]	255	29.4[c]	3.5	81.9[b]	3.1

[1]Means within columns with different superscripts are significantly different

[2] Below detection limit

Table 3.4. Effect of region on total phosphorus content of feedstuffs

Feed[1]		Region[2], µg /g of DM									SEM	*P* <[3]
	n	1	2	3	4	5	6	7	8	9		
Alfalfa hay	18	3,324[ab]	2,989[ab]	3,727[a]	2,422[b]	-	2,597[ab]	3,099[ab]	2,482[ab]	3,389[ab]	460	0.15
Alfalfa silage	16	3,715	3,408	3,389	3,654	4,332	-	3,531	2,962	2,352	973	0.77
Corn silage	8	1,980	-	2,050	1,706	2,638	1,991	2,671	-	-	458	0.52
Grass hay	22	2,055	2,306	2,461	2,219	3,272	-	2,660	3,015	1,383	1,345	0.87
Corn grain	10	3,451	3,257	3,192	3,702	-	3,056	3,087	2,814	2,657	538	0.83
HMSC[4]	14	3,140	-	3,207	2,969	3,133	3,091	-	3,414	3,441	542	0.97
Soybean meal, 48%	5	7,898	-	-	-	8,173	-	-	-	-	160	0.27
Citrus pulp	4	2,010	-	1,280	-	-	1,339	-	-	-	264	0.40
Corn gluten	7	10,443[a]	-	11,438[a]	-	5,597[b]	-	-	-	10,910[a]	1,089	0.07
Dried DGS[5]	14	8,047	-	10,128	10,395	9,637	9,717	8,995	9,350	10,809	1,482	0.67
Hominy	8	4,773	6,630	2,888	-	3,916	6,219	3,829	-	6,363	636	0.31
Wet brewers	9	6,494	7,981	6,397	-	6,143	-	7,061	7,124	7,989	938	0.69

Wet DGS[5]	3	8,641	-	-	-	-	9,777	9,358	-	-	-	-
Wheat middlings	4	11,771	-	11,864	-	12,839	-	-	-	-	547	0.52
Whole cottonseed	6	-	-	6,807	6,516	-	5,528	5,174	-	8,144	1,044	0.55

[1]Means within rows with different superscripts are significantly different

[2]Region 1 = IN, IL, OH, MI, WI; Region 2 = AL, KY, MS, TN; Region 3 = DE, NJ, NY, PA; Region 4 = AZ, CO, ID, HI, MT, NM, NV, UT, WY; Region 5 = CT, MA, ME, NH, RI,VT; Region 6 = AK, CA, OR, WA; Region 7 = FL, GA, MD, NC, SC, VA, WV; Region 8 = IA, KS, MN, MO, NE, ND, SD; Region 9 = AR, LA, OK, TX

[3]Effect of region

[4]High moisture shelled corn

[5]Distillers grains with solubles

Table 3.5. Effect of region on inorganic phosphorus content of feedstuffs

Feed[1]		Region[2], µg /g of DM									SEM	*P* <[3]
	n	1	2	3	4	5	6	7	8	9		
Alfalfa hay	18	2,392[ab]	2,060[ab]	2,659[a]	1,079[b]	-	1,361[ab]	2,372[ab]	791[b]	1,939[ab]	560	0.08
Alfalfa silage	16	3,489	3,315	3,249	3,354	4,395	-	3,352	2,512	2,091	1,168	0.74
Corn silage	8	1,835	-	1,949	1,624	3,032	2,077	2,694	-	-	435	0.34
Grass hay	22	1,164	1,384	1,927	1,412	2,700	-	2,016	3,221	863	1,277	0.66
Corn grain	10	154	536	92	673	-	334	377	155	337	50	0.18
HMSC[4]	14	1,549	-	1,684	1,566	370	1,144	-	2,278	1,330	1,363	0.97
Soybean meal, 48%	5	538	-	-	-	606	-	-	-	-	57	0.43
Citrus pulp	4	719	-	368	-	-	662	-	-	-	209	0.60
Corn gluten	7	2,206	-	7,597	-	449	-	-	-	7,949	5,095	0.57
Dried DGS[5]	14	3,194	-	4,704	6,641	4,463	4,382	1,039	4,101	3,638	2,184	0.64
Hominy	8	168[a]	211[a]	254[a]	-	308[ab]	1,135[b]	179[a]	-	258[a]	63	0.14
Wet brewers	9	2,584	323	687	-	628	-	802	120	771	850	0.61

Wet DGS[5]	3	5,193	-	-	-	-	4,238	3,044	-	-	-	-
Wheat middlings	4	845[a]	-	871[a]	-	487[b]	-	-	-	-	17	0.05
Whole cottonseed	6	-	-	421	453	-	758	650	-	697	121	0.49

[1]Means within rows with different superscripts are significantly different

[2]Region 1 = IN, IL, OH, MI, WI; Region 2 = AL, KY, MS, TN; Region 3 = DE, NJ, NY, PA; Region 4 = AZ, CO, ID, HI, MT, NM, NV, UT, WY; Region 5 = CT, MA, ME, NH, RI,VT; Region 6 = AK, CA, OR, WA; Region 7 = FL, GA, MD, NC, SC, VA, WV; Region 8 = IA, KS, MN, MO, NE, ND, SD; Region 9 = AR, LA, OK, TX

[3]Effect of region

[4]High moisture shelled corn

[5]Distillers grains with solubles

Table 3.6. Effect of region on phytate content of feedstuffs

Feed[1]		Region[2], µg /g of DM									SEM	*P* <[3]
	n	1	2	3	4	5	6	7	8	9		
Alfalfa hay[6]	18	87	51	45	66	-	67	42	55	-	27	0.84
Alfalfa silage	16	ND	ND	ND	37	ND	-	ND	92	ND	-	-
Corn silage	8	ND	-	ND	ND	ND	ND	ND	-	-		-
Grass hay[6]	22	280	64	124	65	ND	-	ND	57	ND	41	0.22
Corn grain	10	1,952	1,768	2,496	2,796	-	2,639	2,656	2,541	1,954	529	0.71
HMSC[4]	14	1,410	-	1,567	864	2,351	1,275	-	676	941	579	0.45
Soybean meal, 48%[6]	5	4,369[a]	-	-	-	5,520[b]	-	-	-	-	362	0.15
Citrus pulp	4	ND	-	30	-	-	ND	-	-	-	-	-
Corn gluten	7	5,979	-	3,247	-	4,475	-	-	-	2,066	2,610	0.73
Dried DGS[5]	14	2,197	-	2,199	1,548	3,314	1,554	7,238	2,324	5,863	3,592	0.74
Hominy	8	4,340[ab]	9,596[a]	2,036[b]	-	3,757[ab]	4,703[ab]	4,099[b]	-	6,499[ab]	434	0.14
Wet brewers[6]	9	2,451	6,358	3,742	-	3,503	-	3,859	4,920	3,353	1,915	0.82

Wet DGS[5]	3	328	-	-	-	-	1,736	3,755	-	-	-	-
Wheat middlings	4	12,454[a]	-	15,332[b]	-	14,127[b]	-	-	-	-	124	0.06
Whole cottonseed[6]	6	-	-	6,602	5,511	-	3,866	5,870	-	7,821	1,400	0.54

[1]Means within rows with different superscripts are significantly different

[2]Region 1 = IN, IL, OH, MI, WI; Region 2 = AL, KY, MS, TN; Region 3 = DE, NJ, NY, PA; Region 4 = AZ, CO, ID, HI, MT, NM, NV, UT, WY; Region 5 = CT, MA, ME, NH, RI,VT; Region 6 = AK, CA, OR, WA; Region 7 = FL, GA, MD, NC, SC, VA, WV; Region 8 = IA, KS, MN, MO, NE, ND, SD; Region 9 = AR, LA, OK, TX

[3]Effect of region

[4]High moisture shelled corn

[5]Distillers grains with solubles

[6]Samples extracted with 0.25 M NaOH – 50 mM EDTA for analysis

References

Arosemena, A., E.J. DePeters, and J.G. Fadel. 1994. Extent and variability in nutrient composition within selected by-product feedstuffs. Anim. Feed Sci. Technol. 54: 103-120.

AOAC. 1998. Official Methods of Analysis. 16th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Bravo, D., F. Meschy, C. Bogaert, and D. Sauvant. 2000. Ruminal phosphorus availability from several feedstuffs measured by the nylon bag technique. Repro.Nutr. Dev. 40:149- 162.

Eeckhout, W. and M. De Paepe. 1993. Total phosphorus, phytate-phosphorus, and phytase activity in plant feedstuffs. Anim. Feed Sci. Technol. 47: 19-29.

Fontaine, T. D., W. A. Pons, Jr., and G. W. Irving, Jr. 1946. Protein-phytic acid relationship in peanuts and cottonseed. J. Biol. Chem. 164:487-507.

Groff, E. B. and Z. Wu. 2005. Milk production and nitrogen excretion of dairy cows fed different amounts of protein and varying proportions of alfalfa and corn silage. J. Dairy Sci. 88:3619-3632.

Kertz, A. F. 1998. Variability in delivery of nutrients to lactating dairy cows. J. Dairy Sci. 81:3075-3084.

Knowlton, K. F. and J. H. Herbein. 2002. Phosphorus partitioning during early lactation in dairy cows fed diets varying in phosphorus content. J. Dairy Sci. 85:1227-1236.

Konishi, C., T. Matsui, W.-Y. Park, H. Yano, and F. Yano. 1999. Heat treatment of soybean meal and rapeseed meal suppresses rumen degradation of phytate phosphorus in sheep. Anim. Feed Sci. Technol. 80: 112-115.

Lee. K.W., A.C. Caldwell, and C.E. Clapp. 1966. RNA, DNA, and other phosphorus fractions in soybeans as affected by high phosphorus nutrition. Plant and Soil. 25: 406-413.

Mahgoub, S. and S. Elhag. 1998. Effect of milling, soaking, malting, heat-treatment and fermentation on phytate level of four Sudanese sorghum cultivars. Food Chem. 61:77-80.

Murphy, J. and J. P. Riley. 1958. A single-solution method for determination of soluble phosphate in natural waters. J. Marine Biol. Assoc. UK. 37:9-14.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, D.C.

Noureddini, H., M. Malik, J. Byun, and A.J. Ankey. 2009. Distribution of phosphorus compounds in corn processing. Bioresource Technol. 100: 731-736.

Park, W.-Y., T. Matsui, F. Yano, and H. Yano. 2000. Heat treatment of rapeseed meal increases phytate flow in the duodenum of sheep. Anim. Feed Sci. Technol. 88: 31-37.

Phillippy, B.Q., K.D. White, M.R. Johnston, S.H. Tao, and M.R.S. Fox. 1986. Preparation of inositol phosphates from sodium phytate by enzymatic and nonenzymatic hydrolysis. Anal. Biochem. 162: 115-121.

Rausch, K . and R. Belyea. 2006. The future of co-products from corn processing. Appl. Biochem. Biotechnol. 128: 47-86.

Robinson, T. 1980. The Organic Constituents of Higher Plants. 4th ed. Cordus Press. North Amherst, MA.

Ullrich-Eberius, C.I., A. Novacky, and A.J.E. van Bel. 1984. Phosphate uptake in *Lamna gibba* G1: Energetics and kinetics. Planta. 161: 46-52.

Wu, Z., L. D. Satter, and R. Sojo. 2000. Milk production, reproductive performance, and fecal excretion of phosphorus by dairy cows fed three amounts of phosphorus. J. Dairy Sci. 83:1028-1041.

Chapter IV: The effects of forage particle length and exogenous phytase inclusion on phosphorus digestion and absorption in lactating cows

Abstract

Feeding high concentrations of P in dairy rations is not uncommon because of the high proportions of concentrates and by-product feeds in modern dairy rations. High P intake leads to increased P excretion, which can have negative environmental implications. Dairy nutritionists, in a recent survey, report a lack of confidence in bioavailability of P in feedstuffs to ruminants and therefore may incorporate more P in the ration than recommended by the NRC (2001). Maximizing availability of intrinsic P is instrumental in reducing P excretion. Exogenous phytase supplementation in poultry and swine diets improves bioavailability of P and some research suggests that this strategy may have some application in dairy cattle rations. To evaluate site and extent of P digestion in lactating cows, an experiment was conducted using a 2 X 2 factorial arrangement with phytase and forage particle length as the two factors. Five ruminally and ileally cannulated cows (188 ± 35 DIM) were utilized in two incomplete Latin squares with four 21-d periods, reserving the last four days for total collection. Feed, orts, omasal, ileal, and fecal samples were collected and analyzed for total P, inorganic P (Pi), and phytate (Pp). Milk yield was recorded daily and samples were taken at each milking during total collection and analyzed for components. Total P intake was similar ($P > 0.15$) across the four dietary treatments. Inorganic P intake was increased ($P < 0.05$) and Pp intake was reduced ($P < 0.05$) with supplemental phytase. Omasal flow of Pi was reduced ($P < 0.05$) with phytase, but no effect of diet ($P > 0.15$) was observed in ileal flow for any P fraction. Fecal excretion of total P was greater ($P < 0.05$) and Pp excretion was reduced ($P < 0.05$) for cows supplemented with

phytase. There was no effect ($P > 0.15$) of dietary treatment on small intestine digestibility of P. Large intestine digestibility of total P was increased ($P < 0.05$) with short forage. Total tract digestibility of P tended to be reduced ($P < 0.10$) with phytase. Energy-corrected milk, 3.5% FCM, milk protein yield, and fat protein yield were increased ($P < 0.05$) with dietary phytase addition. Somatic cell count was reduced ($P < 0.05$) with phytase. This data indicates that in late lactation cows, phytase supplementation may not be beneficial for P utilization in P adequate diets; however, beneficial effects on milk production and components as well as reduced SCC may justify its application.

Introduction

Dietary manipulation to reduce phosphorus (P) excretion by dairy cattle is an important avenue to optimize whole farm nutrient balance and minimize environmental P pollution from farms (e.g. Wu et al., 2000; Knowlton and Herbein, 2002). Strategies to increase P digestibility may contribute to this goal. Exogenous phytase has been used successfully in swine nutrition to increase P digestibility, decrease supplemental P use, and reduce P excretion (e.g. Cromwell et al., 1993; Lei et al., 1993; Harper et al., 1997). Similarly, exogenous phytase inclusion has been reported to reduce fecal P in lactating dairy cows (Kincaid et al., 2005; Knowlton et al., 2007). Phytate is approximately 70% of the total P in grains (Nelson et al., 1968; Morse et al., 1992) and while ruminal microorganisms have the capacity to liberate phosphate molecules from the inositol ring of the phytate molecule (Park et al., 2002), the degradation of phytate in the rumen may not be complete (Godoy and Meschy, 2001). Ruminal phytate degradation is influenced by passage rate and physical properties of the ration and grain type (Kincaid et al., 2005). Feed processing methods have also been reported to alter phytate availability in sheep (Bravo et al.,

2000; Park et al., 2000). Variation in availability of phytate and other organic P forms is not accounted for in current ration formulation programs for dairy cows.

In situations where phytate escapes the rumen (e.g. where passage rate is high due to high DMI or finely chopped diets), inclusion of ruminally protected phytase may support increased phytate digestion in the proximal small intestine to allow phosphate absorption in the distal small intestine. Therefore, the objective of this study was to investigate site and extent of phytate degradation in diets varying in forage particle length with and without exogenous ruminally protected phytase.

Materials and methods

Animals and diets

Five crossbred [Swedish Red or Brown Swiss X (Holstein X Jersey)] ruminally- and ileally-cannulated first lactation cows (BW of 472 ± 36 kg and 188 ± 35 DIM) were utilized in two incomplete 4 x 4 Latin squares. The treatments, inclusion of phytase (1,500FTU/kg diet DM) and forage particle length, were administered in a 2 x 2 factorial arrangement. Diets were otherwise identical in composition (Table 4.1) and formulated to meet or exceed NRC (2001) requirements with dietary P at 0.43% of DM. Treatments were short forage (SF) no phytase, long forage (LF) no phytase, SF plus phytase, and LF plus phytase. Reduction in particle length for the SF diets was achieved by grinding grass hay through a 0.64 cm screen in a tub grinder (New Holland 390, Racine, WI) and by passing corn silage through a leaf mulcher (Flowtron Leaf Eater, Malden, MA) on the fine setting. Hay sufficient for the duration of the study was ground in advance and corn silage was processed daily prior to mixing the diets. Phytase was added to the vitamin –mineral mix (pre-made in large batches). Corn silage, grass hay, grain mix, and

vitamin-mineral mix were combined daily immediately prior to the 1200 h feeding. Samples were obtained at mixing.

The four periods each consisted of 21-d with the first 14 days of each period for dietary adaptation with cows group-housed and fed once daily via the Calan door system (American Calan, Northwood, NH). Cows were fed 10% excess of the previous day's intake at 1200 h, and feed refusals were measured daily. Body weight and milk yield were obtained twice daily at each milking, at 0300 h and 1400 h. All protocols and procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee.

Sample collection

On d 15 of each period, cows were moved to a metabolism barn for 3-d of barn adaptation followed by 4-d of total collection. Diets were mixed daily and sampled at mixing. Cows were fed 25% of their daily feed allowance four times daily at 0600, 1200, 1800, and 2400 h beginning at 2400 h on d 18 of each period. Immediately prior to each feeding, LiCo-EDTA solution (Uden et al., 1980) and Yb-labelled corn silage (Harvatine et al., 2002) were dosed ruminally to supply 0.11 g/d of Co and 0.11 g/d of Yb. Cows were milked twice daily at 0600 and 1800 h. Milk weights were recorded at each milking and milk samples were collected.

On d 17, 24-h before the onset of total collection, cows were fitted with harnesses that linked to a cup covering the vulva (Fellner et al., 1988) for total daily urine collection into a 12 L jug that was maintained on ice (Knowlton et al., 2010). Beginning at 1800 h on d 18, feces were collected from behind each cow four times daily and stored in 130 L tub. At 1800 h daily, feces, urine, and orts were weighed, thoroughly mixed, sub-sampled and samples were frozen (-20°C) until further analysis.

At 1400 h on d 17 (the evening before total collection commenced) a sampler was placed in the omasal orifice for sampling as described by Huhtanen et al. (1997). Omasal and ileal samples were taken every 8 h on d 18 to 21 advancing by 2 h on each consecutive day to represent every 2 h of a 24 h period.

Coccygeal blood samples were collected into serum separator tubes (Fisher Scientific, Pittsburgh, PA) on d 18 to 21 prior to the 1200 h feeding. Upon collection, samples were immediately centrifuged (2,200 x *g* for 20 min), and serum was harvested and stored at -20°C until further analysis.

On d 21, rumens were evacuated and every tenth handful reserved as a sample. Contents were weighed and returned to the rumen. Rumen microbes were isolated, by period, from a composite rumen sample immediately following sampling through differential centrifugation (Cecava et al., 1980). Briefly, equal parts of rumen contents (300 mL) from each cow were combined to create one composite sample per period, 500 mL of saline was added, and the mixture was blended in a Waring blender for 90 seconds. After blending, the sample was strained through four layers of cheesecloth, reserving the liquid and discarding the solids that remained. Liquid was centrifuged at 500 x *g* for 20 min to remove the remaining feed particles and then centrifuged at 8,000 x *g* for 20 min to isolate the microbes. Microbes were stored frozen at -80 °C and then lyophilized (Genesis 25EL, Stone Ridge, NY).

Sample analysis

Feed, orts, omasal, ileal, and fecal samples were pooled by wet weight by cow and period. Samples of feed and refusals were oven dried in a 55°C forced air oven (Thermo Scientific Precision 645, Danville, IN) to a constant weight. Rumen, omasal, ileal, and fecal samples were lyophilized (Genesis 25EL, Stone Ridge, NY). All samples, except omasal fluid,

were ground through a 1.0 mm screen in a Wiley Mill (Arthur H. Thomas). Omasal samples were separated to liquid and solid fractions and ground in a freezer mill (described below). All samples were analyzed in duplicate for N, P (AOAC, 1984), aNDF (Mertens, 2002), and ADF sequentially (Van Soest et al., 1991), and for K, Ca, Mg, and Cl using ICP (Inductively Coupled Plasma, Thermo electron IRIS Intrepid II XSP; Thermo Fisher Scientific Inc., Waltman, MA). Milk samples were analyzed for fat, protein, solids non-fat, lactose, milk urea N and somatic cells (DHIA, Blacksburg, VA).

Isolated microbes were subjected to purine analysis as detailed by Zinn and Owens (1986). Additionally, total P content of isolated microbes was measured (AOAC, 1984) and the P to purine ratio specific to this microbial population was calculated for each period. Omasal, ileal, and fecal samples were analyzed for purines (Zinn and Owens, 1986) and total P (AOAC, 1984). The P to purine ratio calculated from microbial isolates and purine content of digest and feces were used to calculate microbial P (Pm) flow.

Omasal samples from each time point were pooled on an equal fresh weight basis per cow per period, and the same was done for ileal samples. After pooling, omasal samples were centrifuged at 1,000 x *g* for 5 minutes to separate flow into two phases; fluid and particulate (the latter containing small and large particles; adapted from Reynal and Broderick, 2005). Both phases were frozen at -80 °C and then lyophilized (Genesis 25EL, Stone Ridge, NY). The fluid phase was ground using a freezer mill (6850 Freezer Mill, Santa Clara, CA) and the particulate phase was ground through 0.2 mm screen in a Z-grinder (Retsch ZM 100, Haan, Germany). Both phases were analyzed for Co and Yb by ICP (Inductively Coupled Plasma, Thermo electron IRIS Intrepid II XSP; Thermo Fisher Scientific Inc., Waltman, MA) and data were used to define relative flow of fluid and particulates at the omasum. Freeze-dried fluid and particulate phases

were recombined in proportions to their relative contribution to true omasal DM flow using the two marker system (France and Siddons, 1986). Omasal, ileal, and fecal samples were analyzed for Co and Yb to calculate nutrient flow and actual daily dose of each marker each day, respectively. Nutrient flow at the ileum was calculated as detailed in Equation 4. 1.

Equation 4. 1. Calculation of nutrient flow at the omasum and ileum

$$\left(\frac{Dose\ of\ the\ marker\ appearing\ in\ feces\ g/d}{Concentration\ of\ the\ marker\ in\ sample, mg/g}\right) * Concentration\ of\ nutrient\ in\ sample, mg/g$$

Urine samples were analyzed for P (AOAC, 1984). Serum inorganic P was determined using colorimetric methods (Inorganic Phosphorus Reagent Set, Pointe Scientific, Canton, MI).

Phosphorus fractionation

Feed, orts, digesta, and feces samples were ground through a 0.2 mm screen in a Z-grinder (Retsch ZM 100, Haan, Germany). For inorganic P analysis, 1.0 g sample was mixed with 20 mL of 0.5 M HCl and shaken ambient temperature on a horizontal mechanical shaker (Eberbach Reciprocal Shaker 6000, Ann Arbor, MI) for4 h. After shaking, the samples were refrigerated (4°C) overnight. Samples were then centrifuged at 30,000 x g for 10 minutes. The supernatant was reserved and the pellet discarded. Inorganic P content of supernatants was determined with the molybdenum blue method as described by Murphy and Riley (1958).

To extract samples for phytate and lower inositol species (IP_5, IP_4, and IP_3) analysis, 20 mL of 0.25 M NaOH + 50 mM EDTA was mixed with 1.0 g of sample in a centrifuge tube. This was shaken at ambient temperature on a horizontal mechanical shaker (Eberbach Reciprocal Shaker 6000, Ann Arbor, MI) for 4 h and then centrifuged at 30,000 x g for 10 minutes. The supernatant was reserved and the pellet discarded. A 5 mL subsample of the supernatant was acidified with 500 µL of 6 M HCl + 1.2 M HF and refrigerated overnight (4°C). The following

day, the acidified supernatant was re-centrifuged and again the supernatant reserved and the pellet discarded.

Phytate analysis was performed in accordance with Ray et al. (2011). Briefly, to quantify phytate and IP_5, IP_4, and IP_3, 5 mL of NaOH-EDTA extract (for feed and digesta samples) was passed through a methanol conditioned C_{18} cartridge (Sep-Pak Plus, Waters, MA) and a 0.2 µm IC membrane filter and reserved in a Dionex sample vial. Filtered extracts were analyzed using HPIC (Dionex ICS 3000 with a Dionex 4 x 50 IonPac AG7 guard column and a 4 x 250 mm IonPac AS7 analytical column; Dionex, Sunnyvale, CA). A pH 4, 20.1 min gradient elution technique was utilized involving two mobile phases; 0.01 M 1-methylpiperazine and 0.5 M $NaNO_3$ + 0.01 M 1-methylpiperazine. Elution began with 100% 0.01 M 1-methylpiperazine and gradually decreased with increasing 0.5 M $NaNO_3$ + 0.01 M 1-methylpiperazine (75%) over the first 15 min. The remaining 5.1 min of elution utilized 100% 0.5 M $NaNO_3$ + 0.01 M 1-methylpiperazine as the mobile phase. Detection of Pp and lower inositols was achieved through colorimetric reaction with a post column reagent using a Tee connector and a 300 cm long reaction coil with a 0.5 mm I.D. The post column reagent used was Wade's reagent (0.015% $FeCl_3 \cdot 6H_2O$ (w/v) and 0.15% (w/v) sulfosalicylic acid), pumped at 1 mL/min using and Isco HPLC pump (model 2350, Isco Inc., Lincoln, NE), and absorbance measured at 500 nm (Rounds and Nelsen, 1993). Calibration standards for Pp (3, 15, and 30 µg P/mL) and lower inositols (1 and 2 h hydrolysates) were prepared from chemical hydrolysis of sodium phytate.

Statistical analysis

All data were analyzed using the mixed procedure of SAS (Cary, NC) for a replicated Latin square with the model defined in Table 4.2. Non-normal data was log transformed prior to statistical analysis. Data is reported as least squares means and the residual tested the main

effects and cow within square tested the effects of square. Significant differences were declared at $P < 0.05$ and trends at $P < 0.15$.

Results and discussion

Effect of grinding on particle size

To assess particle size reduction from grinding, particle separation was conducted on the TMR. There was a significant reduction ($P < 0.05$) in particles greater than 1.9 cm and particles 0.1 – 0.8cm (Table 4.3) with the SF diet. There was a tendency ($P < 0.10$) for the SF rations to have fewer fines, so particles less than 0.1cm. There was no difference ($P > 0.15$) in particles on the screen containing 1.9 – 0.8 cm. Thus, chopping the forage resulted in an overall reduction in forage particle length to achieve increased passage rate in the SF versus the LF rations.

Milk production

Effect of phytase

Milk production tended to increase ($P < 0.15$) with dietary phytase addition (Table 4.4). Phytase in the diet increased ($P < 0.05$) ECM, 3.5% FCM, protein, SNF, protein yield, and fat yield, and tended to increase SNF yield ($P < 0.10$) and lactose yield ($P < 0.15$). Kincaid et al. (2005) also reported numerical increases ($P < 0.13$) in milk protein concentration with supplemental phytase in mid-lactation dairy cows. Knowlton et al. (2005) observed no difference in milk yield or component production for lactating cows fed a phytase –cellulase enzyme blend.

One possibility for the improvements in production observed in the present experiment may be because phytase activity occurred in the feed (Table 4.6), prior to feeding, negative impacts of Pp on solubility of amino acids and perhaps an energy sparing effect. If so, the

resulting improved amino acid and gross energy availability would explain the increased milk, protein, and fat production.

Experiments in swine and poultry using phytase supplementation have yielded mixed effects on growth performance. Liao et al. (2005) reported no improvement in protein or energy digestibility in growing pigs fed phytase-supplemented diets with high or low Pp content. Low Pp diets did allow improvements in protein and energy utilization, suggesting an interference of Pp with protein and energy. On the contrary, Kemme et al. (1999a) observed increased apparent ileal digestibility of amino acids in growing and finishing pigs with phytase supplementation. These mixed results indicate that the composition of the basal diet may influence solubility of phytate-amino acid complexes and activity of phytase and therefore, alter amino acid availability. Bohlke et al. (2005) investigated amino acid digestibility in growing pigs fed low-phytate corn and normal corn diets and determined that although the amino acid profiles of the two corns were similar, apparent ileal digestibility of Arg, Ile, Lys, Phe, Thr, and Val were greater in low-phytate corn diets. Kemme et al. (1999a) saw similar results suggesting that removing the phytate-amino acid complexing with phytase can improve nutrient availability.

Exogenous phytase decreased ($P < 0.05$) SCC from 72,500 to 53,600 cells/ml (Table 4.4). Phytate is known to chelate minerals, thus reducing their availability. Low-phytate corn diets have been reported to improve apparent ileal and apparent total tract digestibility of Ca and P in pigs (Bohlke et al., 2005). vanDoorn et al. (2004) reported similar results in Ca digestibility with phytase supplementation in horses.

Evidence is growing suggesting Pp chelation with minerals such as Cu and Zn that are instrumental in immune function. In weanling pigs, Adeola et al. (1995) reported improvements in growth performance, plasma Zn concentration, and plasma P concentration as well as retained

Ca, P, and Cu with exogenous phytase. Kies et al. (2006) observed improved growth performance and digestibility of Ca, P, K, Mg, Na, and Cu in weanling pigs fed graded doses of phytase. Improved mineral status in dairy cows has beneficial effects on mastitis control and prevention. Copper supplementation improved liver Cu concentration in dairy cows on day -21 to day 42, relative to calving, and plasma Cu concentration on day 0 and 42 (Scaletti et al., 2003). A subset of these animals was subjected to intramammary challenge with *E. Coli*. Cows supplemented with Cu had reduced SCC and bacterial counts as compared to unsupplemented cows. Cope et al. (2009) observed a reduction in SCC and milk amyloid A concentration in lactating cows fed supplemental Zn. These reports implicate Cu and Zn as key minerals in mitigating mastitis incidence and improving health status. The results of reduced SCC with phytase supplementation in the present study may be explained by a reduction in mineral chelation with Pp allowing a more robust immune system because of increased mineral availability. This hypothesis requires additional exploration.

Effect of forage particle length

Milk protein was increased ($P < 0.05$) and SNF tended to increase ($P < 0.15$) with SF (Table 4.4). Long forage tended to increase ($P < 0.15$) lactose yield (1.02 vs. 0.98 kg/d). No effect of particle length was observed on milk yield, ECM, 3.5% FCM, fat yield, protein yield, or SCC with respect to forage particle size ($P > 0.15$).

Phosphorus intake, flow, excretion, and digestibility

Effect of phytase

There was no effect of treatment on DMI ($P > 0.15$) and therefore no effect ($P > 0.15$) on N, NDF, or ADF intake (Table 4.5). Phytase inclusion increased ($P < 0.05$) Pi intake (46.3 vs.

34.1 g/d) and consequently decreased ($P < 0.05$) Pp intake (18.4 vs. 40.7 g/d; Table 4.6). This shift in dietary P compounds with inclusion of exogenous phytase indicates phytase activity at the time feed was mixed, as feed was sampled at mixing. Kincaid et al. (2005) fed exogenous phytase to lactating dairy cows, but did not observe an alteration in P fractions in the TMR, indicating no phytase activity at the time of mixing. Flow of DM, total P, and Pp into the omasum was not affected ($P > 0.15$) by dietary treatment (Table 4.6). However, Pi flow at the omasum was reduced ($P < 0.05$) with phytase inclusion (148.3 vs. 178.0 g/d) indicating a possible increase in P absorption in the rumen or an alteration in P recycling in the saliva.

There was no effect ($P > 0.15$) of phytase inclusion on DM flow at the ileum. Phytate flow was greater ($P < 0.05$) at the ileum than at the omasum with exogenous phytase, with 95% confidence intervals of 1.84 – 1.36 and 1.39 – 0.52 (g of Pp/d), respectively. This biologically unlikely observation is likely due to limitations in sampling or analytical techniques. Omasal samples were very high in Pi and low in Pp. During HPIC analysis, the orthophosphate peaks were very large and eluted within the first two minutes of the run. This large peak may have interfered with precise quantification of Pp. The background during omasal sample analysis was 'noisy', which may have led to an underestimation of Pp in these samples.

As a percentage of P intake, apparent total tract digestibility of total P tended ($P < 0.10$) to be less with dietary phytase (30.0 vs. 41.6%; Table 4.6 and Table 4.7). There was no effect ($P > 0.15$) of phytase on apparent total tract digestibility of Pp or Pi. When total tract Pp digestibility was calculated for Pp concentration at feed mixing (i.e. ignoring phytase activity in the TMR after mixing and before feeding), Pp digestibility was increased ($P < 0.05$) with phytase supplementation. Kincaid et al. (2005) observed approximately 85% total tract Pp hydrolysis with phytase addition versus 80% without. Cows in the Kincaid et al. (2005)

experiment were consuming more P per day (~130 g/d), producing more milk, and had higher DMI than the cows in the present experiment. The much greater fecal excretion of Pp observed by Kincaid et al. (2005) (10 and 14 g/d with and without phytase addition, respectively, as compared to 0.98 and 1.33 g/ d of Pp with and without phytase supplementation, respectively in the present study), is not unexpected because of higher DMI and earlier DIM of the cows in the former study than the cows in the present study. Flow of Pm at the ileum was not affected ($P > 0.15$) by dietary treatment.

Apparent ruminal digestibility of Pp was reduced ($P < 0.05$) with phytase supplementation. This reduction in Pp digestion in the rumen is because Pp intake was reduced with phytase supplementation and a small amount of quantifiable Pp did escape form the rumen (Table 4.7). There was no effect ($P > 0.15$) of treatment on apparent large intestine digestibility of Pp. Phosphorus absorption and Pp degradation in the large intestine was present (Table 4.6 and Table 4.7). Park et al. (2002) observed similar trends for sheep for large intestine digestibility of Pp (~19%) and absorption of P in the large intestine. Fecal excretion of total P increased ($P < 0.05$) with phytase inclusion. Fecal Pp excretion decreased ($P < 0.05$) with dietary phytase (980 vs. 1,327 mg/d), but there was no effect of Pi excretion ($P > 0.15$).

Effect of forage particle length

Short forage tended to decrease ($P < 0.10$) DM digestibility (66.0 vs. 68.5%) similar to observations by Rode et al. (1985) for ground forage and Zebeli et al. (2007) with chopped hay, likely due to a more rapid passage rate (Table 4.5). Forage particle length did not affect ($P > 0.15$) intake or omasal flow of total P or Pi. Ruminal pool size (Table 4.6) and digestibility (Table 4.7) of TP, Pi, and Pp were not affected ($P > 0.15$) by forage particle length. Flow of total P and Pi at the ileum was increased ($P < 0.05$) with SF; however, flow of Pp and Pm were

unaffected ($P > 0.15$) by dietary treatment (Table 4.6). Fecal excretion of total P increased ($P < 0.05$) with SF (57.6 vs. 54.3 g/d; Table 4.6), which may be a result of increased passage rate attributed to decreased DM digestibility for SF. Microbial P in the feces was increased ($P < 0.05$) with LF, which may indicate increased microbial activity with increased retention time.

Apparent total tract digestibility of Pp tended to be reduced ($P < 0.15$) with SF. Apparent large intestine digestibility of total P as a percentage of total P from ileal flow, was increased ($P < 0.05$) with SF. Long forage tended ($P < 0.10$) to decrease apparent Pi digestibility in the large intestine (as a percentage of ileal flow; Table 4.7). No effect ($P > 0.15$) on small intestine digestibility of P was observed. Others have reported reduced forage particle length reduces nutrient utilization (Yang and Beauchemin, 2007) which is consistent with the present data set.

Serum, urine, and retained phosphorus

Serum P, urine output, and retained P were not affected ($P > 0.15$) by treatment (Table 4.8). No effect ($P > 0.15$) of phytase was observed on urinary P. Kincaid et al. (2005) reported an increase in serum P concentration in lactating cows fed exogenous phytase in barley and corn based diets. Valk et al. (2002) increased serum P and salivary P of lactating cows by increasing dietary P from 67 to 100% of P requirement. The lack of effect of phytase on serum P in the present study may be because dietary P exceeded the cow's requirements, in accordance with the cow's low P demand. Bravo et al. (2003c) attributed changes in urinary P excretion to alterations in plasma P through analysis of numerous previously published reports. Because there was no change in serum P, it is not surprising that no increase in urinary P occurred upon supplementation of phytase.

Biological significance of observed digestion and absorption of phosphorus compounds

Data in the present study may be beneficial to improve published models of P digestion, absorption, and metabolism. Tracer kinetics of P models that do exist are derived from small ruminant data (Vitti et al., 2000; Dias et al., 2006). The model of P metabolism most applicable to lactating dairy cows is by Hill et al. (2008; Figure 2.2) but this model in based on relatively limited data especially related to Pp digestion in the rumen and large intestines, Pm flow, and Pi absorption in the large intestine. In small ruminants, Park et al. (2002) reported large intestine absorption of P.

With phytase supplementation, 17 g of Pp were digested in the rumen and 40 g without phytase supplementation. Regardless of forage particle length, approximately 29 g of Pp was digested in the rumen. These values were different from zero ($P < 0.05$). In the small intestine, disappearnace of total P was 102 g/d and 129 g/d with and without phytase supplementation. Approximately 116 g/d of total P was disappeared from the small intestine for both forage particle lengths. Similarly, Pi disappearance from the small intestine was 110, 143, and 126 g/d for phytase supplementation, no phytase supplementation, and forage particle length, respectively. These values were different from zero ($P < 0.05$) and biologically significant because it gives an indication of P uptake in the small intestine. There was no degradation of Pp in the small intestine ($P > 0.15$). Phytate escape from the small intestine was approximately 1,700 mg/d for all dietary treatments. This value is different from zero ($P < 0.05$) and indicates that Pp escaping the upper gastrointestinal tract may contribute to fecal excretion of Pp.

Large intestine disappearance of total P both with and without phytase was about 9 g/d and this value tended to be different than zero ($P < 0.10$). For SF and LF, disappearance was 16 and 3 g/d, respectively, and were both different than zero ($P < 0.05$). Disappearance of Pi from

the large intestine was 0.7 and 0.2 g/d with and without phytase supplementation and not different than zero ($P > 0.15$). Phytate disappearance from the large intestine was 0.6 and 0.4 g/d with and without phytase and 0.6 and 0.4 g/d with SF and LF, respectively. Fecal excretion of Pp was approximately 1,155 mg/d for all dietary treatments. These values were significantly different than zero ($P < 0.05$). This small quantity digested in the large intestine may be due to high capacity of Pp degradation in the rumen and a small quantity entering the large intestine. Inorganic P in the large intestine stayed relatively constant perhaps due to Pp and Po degradation contributing to that pool. Thus, a disappearance of total P is observed, indicating a shift in P forms from degradation, and uptake of P in the large intestine.

These data contribute markedly to the Hill et al. (2008) model of P digestion and absorption in large ruminants. They provide additional data, though not the final word on capacity for P absorption in the small intestine and large intestine. Also, flow of Pm into and out of the large intestine can explain another portion of total P. Lastly, quantification of Pp digestion in the rumen and the large intestine will be a contribution to the large ruminant P model.

Limitations of the current data set include the cows were fairly late in lactation and consequently their demand for P was also reduced. In efforts to increase Pp content of the diet, total P content of the diet was in excess of the NRC (2001) requirement; thus reducing their drive to absorb P.

Conclusions

Phytase supplementation increased total P excretion, but reduced Pp excretion in lactating dairy cows. Total P digestibility was reduced with phytase supplementation, but no effect was observed on digestibility of Pi or Pp. Although no improvement was observed in P digestion, increased ECM, 3.5% FCM, milk protein yield, and milk fat yield was observed with exogenous

phytase addition to the diet. Somatic cell count was also reduced for cows supplemented with phytase. These improvements in yield of milk components and udder health with phytase suggest an improvement of nutrient utilization and immune function perhaps due to a reduction of anti-nutritional effects of Pp. The present experiment contributes biologically relevant data in understanding digestion and absorption of P compounds throughout the digestive tract of late lactation dairy cows.

Table 4.1. Ingredient composition of the diet

	% of DM
Ingredient	
Corn silage	41.7
Cottonseed meal	15.6
Grass hay	13.5
Corn, ground	12.3
Soybean meal,48%	7.9
Molasses, dehydrated	3.5
Beet pulp, dehydrated	2.9
Vitamin-mineral mix[1]	1.9
Sodium bicarbonate	0.6
Nutrient	
CP	17.7
NDF	32.6
ADF	18.8
Ca	0.66
P	0.43

[1] Vitamin-mineral mix contains Vit A 26,400 kIU/kg, Vit D 8,800kIU/kg, Vit E 44,000 IU/kg, 7.6% Ca, 5.9% Cl, 4.7% Na, 0.85% S, 0.45% Mg, 0.03% K, 3500 ppm Zn, 2000 ppm Fe, 2000 ppm Mn, 300 ppm Cu, 90 ppm Se, 70 ppm I, and 50 ppm Co.

Table 4.2. Statistical model used to analyze all variables

Source	df	Error df	Error term
Phytase	1	9	Residual
Particle length	1	9	Residual
Phytase x particle length	1	9	Residual
Square	1	3	Cow (square)
Cow(square)	3		
Period	3	9	Residual
Residual	9		
Total	19		

Table 4.3. Effect of particle size reduction on sieving the TMR with the Penn State Particle Separator

	No phytase		Phytase			Effect, *P* <		
Item, %	Short	Long	Short	Long	SEM	Phytase	FPL[1]	Phytase x FPL
> 1.9 cm	2.1	12.8	2.3	12.9	1.2	0.92	0.01	0.98
1.9 – 0.8 cm	32.3	33.1	28.7	31.2	2.1	0.31	0.33	0.87
0.1 – 0.8 cm	45.4	34.6	47.3	38.4	2.2	0.23	0.01	0.69
< 0.1 cm	21.2	19.4	21.7	17.4	1.4	0.60	0.06	0.40

[1]Forage particle length

Table 4.4. Effect of particle size and phytase on milk production and composition

Item	No phytase		Phytase		SEM	Effect, *P* <		
	Short	Long	Short	Long		Phytase	FPL[1]	Phytase x FPL
Milk, kg/d	19.5	20.3	20.2	20.9	0.59	0.15	0.12	0.87
3.5% FCM, kg/d	24.6	25.1	25.8	26.3	0.77	0.05	0.40	0.95
ECM, kg/d	24.5	25.1	25.8	26.2	0.73	0.04	0.42	0.90
Fat, kg/d	1.00	1.01	1.05	1.07	0.03	0.04	0.66	0.87
Protein, kg/d	0.76	0.79	0.82	0.82	0.02	0.03	0.52	0.43
SNF, kg/d	1.89	1.97	1.99	2.04	0.05	0.07	0.19	0.74
Lactose, kg/d	0.96	1.00	1.00	1.04	0.03	0.11	0.10	0.95
MUN, mg/dL	11.65	11.02	10.90	11.64	0.56	0.84	0.87	0.06
SCC (X 1,000)	88.9	56.1	58.4	48.8	24.9	0.02	0.43	0.60

[1]Forage particle length

Table 4.5. Effect of forage particle length and phytase on nutrient intake, excretion, and DM digestibility

Item	No phytase		Phytase		SEM	Effect, $P <$		
	Short	Long	Short	Long		Phytase	FPL[1]	Phytase x FPL
Intake								
DM, kg/d	16.1	17.1	17.0	17.2	0.57	0.35	0.23	0.43
N, g/d	430.0	447.4	447.7	465.2	28.3	0.55	0.55	0.99
NDF, kg/d	6.0	6.3	5.9	6.8	0.38	0.52	0.15	0.40
ADF, kg/d	3.0	3.1	3.2	3.4	0.21	0.26	0.40	0.70
Rumen DM pool, kg	8.4	9.1	8.9	9.4	0.77	0.63	0.48	0.93
DM digestibility, %	66.0	69.2	66.0	67.8	1.27	0.56	0.08	0.58
Fecal excretion								
DM, kg/d	5.4	5.3	5.7	5.6	0.25	0.27	0.65	0.82
N, g/d	193.4	175.8	188.3	203.6	9.88	0.27	0.92	0.13
NDF, kg/d	2.8	2.9	3.0	2.8	0.16	0.83	0.75	0.58

ADF, kg/d	1.5	1.6	1.6	1.5	0.08	0.94	0.61	0.22

[1]Forage particle length

Table 4.6. Effect of forage particle length and phytase on intake, rumen pool size, omasal flow, ileal flow, and fecal excretion of P, Pi, Pp, and Pm

Item	No phytase		Phytase		SEM	Effect, $P <$		
	Short	Long	Short	Long		Phytase	FPL[1]	Phytase x FPL
Intake								
Total P, g/d	74.5	76.7	77.8	74.1	4.08	0.93	0.86	0.49
Inorganic P, g/d	32.8	35.5	49.2	43.5	3.19	0.01	0.60	0.16
Phytate, g/d	44.0	37.3	13.8	23.1	3.88	0.01	0.74	0.07
Rumen pool								
Total P, g	65.4	71.9	63.9	67.5	5.8	0.58	0.35	0.78
Inorganic P, g	41.7	51.8	44.9	51.6	5.1	0.77	0.12	0.73
Phytate, mg[2]	54.3	24.0	93.9	92.7	37.0	0.17	0.70	0.68
Omasal flow								
Total P, g/d	195.9	181.9	173.7	157.8	17.6	0.12	0.28	0.94
Inorganic P, g/d	181.4	174.5	159.9	136.7	16.9	0.06	0.27	0.56

Phytate, g/d	0.87	0.94	0.99	1.10	0.55	0.73	0.83	0.97
Ileal flow								
Total P, g/d	72.6	53.6	74.0	61.4	5.4	0.24	0.01	0.41
Inorganic P, g/d	43.4	33.4	49.0	36.3	6.9	0.21	0.01	0.68
Phytate, g/d	2.03	1.58	1.67	1.54	0.28	0.42	0.25	0.51
Microbial P, g/d	27.4	28.3	32.5	27.8	2.89	0.42	0.49	0.32
Fecal excretion								
Total P, g/d	54.7	50.4	59.4	55.3	2.35	0.02	0.04	0.93
Inorganic P, g/d	41.4	34.2	41.3	42.0	3.21	0.21	0.29	0.20
Phytate, mg/d	1,493	1,161	974	986	239	0.01	0.18	0.15
Microbial P, g/d	18.0	22.7	20.9	19.0	3.48	0.84	0.62	0.42

[1]Forage particle length

[2] Phytate content was below detection limit in 65% of samples

Table 4.7. Effect of forage particle length and phytase on ruminal, small intestine, large intestine, and total tract digestibility of total P, Pi, and Pp

Item	No phytase		Phytase		SEM	Effect, *P* <		
	Short	Long	Short	Long		Phytase	FPL[1]	Phytase x FPL
Apparent ruminal digestibility, % of intake								
Phytate	98.4	97.5	86.2	95.9	3.8	0.07	0.24	0.16
Apparent small intestine digestibility, % of omasal flow								
Total P	60.0	67.2	57.4	61.5	6.3	0.60	0.16	0.52
Inorganic P	74.5	78.4	69.6	73.6	4.2	0.19	0.26	0.98
Phytate	-59.0	-111.4	-56.2	-102.1	79.9	0.92	0.40	0.95
Apparent large intestine digestibility, % of ileal flow								
Total P	24.9	3.5	14.4	8.5	6.6	0.60	0.03	0.16

Inorganic P	2.3	-1.7	7.5	-19.6	11.1	0.44	0.08	0.17
Phytate	25.4	25.0	40.1	34.6	9.4	0.18	0.74	0.77
Apparent total tract digestibility, %								
of intake								
Total P	26.1	33.8	22.7	24.5	3.3	0.09	0.18	0.40
Inorganic P	-33.4	5.7	8.2	-10.3	14.8	0.41	0.50	0.08
Phytate	95.4	96.4	82.6	96.1	4.6	0.16	0.13	0.18
Phytate[2]	96.5	96.9	97.8	97.4	0.6	0.01	0.97	0.05

[1]Forage particle length

[2]Calculated digestibility as if the phytase had not been active in the TMR

Table 4.8. Effect of forage particle length and phytase on urine output, urinary excretion of P, serum P, and retained P

Item	No phytase		Phytase		SEM	Effect, *P* <		
	Short	Long	Short	Long		Phytase	FPL[1]	Phytase x FPL
Urine, kg/d	11.5	10.8	10.3	11.6	1.25	0.80	0.71	0.23
Urinary P, g/d	0.77	2.62	0.99	1.70	1.08	0.68	0.15	0.50
Serum P, mg/dL	5.41	5.93	5.33	5.64	0.46	0.59	0.25	0.75
Retained P[2], g/d	-0.5	3.3	-2.8	-3.9	3.7	0.22	0.71	0.51

[1]Forage particle length

[2] Phosphorus intake – fecal phosphorus – milk phosphorus – urinary phosphorus

References

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.

AOAC. 1998. Official Methods of Analysis. 16th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Bohlke, R. A., R. C. Thaler, and H. H. Stein. 2005. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. J. Anim. Sci. 83:2396-2403.

Bravo, D., F. Meschy, C. Bogaert, and D. Sauvant. 2000. Ruminal phosphorus availability from several feedstuffs measured by the nylon bag technique. Repro.Nutr. Dev. 40:149-162.

Bravo, D., D. Sauvant, C. Bogaert, and F. Meschy. 2003c. III. Quantitative aspects of phosphorus excretion in ruminants. Reprod. Nutr. Dev. 43: 285-300.

Cecava, M.J., N.R. Merchen, L.C. Gay, and L.L. Berger. 1990. Composition of ruminal bacteria harvested from steers as influenced by dietary energy level, feeding frequency, and isolation techniques. J. Dairy Sci. 73: 2480-2488.

Cope, C. M., A. M. Mackenzie, D. Wilde, and L. A. Sinclair. 2009. Effects of level and form of dietary zinc on dairy cow performance and health. J. Dairy Sci. 92: 2128-2135.

Cromwell, G. L., T. S. Stahly, R. D. Coffey, H. J. Monegue, and J. H. Randolph. 1993. Efficacy of phytase in improving the bioavailability of phosphorus in soybean meal and corn-soybean meal diets for pigs. J. Anim Sci. 71:1831-1840.

Dias, R. S., E. Kebreab, D. M. S. S. Vitti, A. P. Roque, I. C. S. Bueno, and J. France. 2006. A revised model for studying phosphorus and calcium kinetics in growing sheep. J. Anim. Sci. 84:2787-2794.

France, J. and R. C. Siddons. 1986. Determination of digesta flow by continuous marker infusion. J. Theor. Biol. 121:105-119.

Godoy, S. and F. Meschy. 2001. Utilisation of phytate phosphorus by rumen bacteria in a semi-continuous culture system (Rusitec) in lactating goats fed on different forage to concentrate ratios. Reprod. Nutr. Dev. 41:259-265.

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.

Hill, S. R., K. F. Knowlton, E. Kebreab, J. France, and M. D. Hanigan. 2008. A model of phosphorus digestion and metabolism in the lactating dairy cow. J. Dairy Sci. 91:2021-2032.

Kemme, P. A., A. W. Jongbloed, Z. Mroz, J. Kogut, and A. C. Beynen. 1999a. Digestibility of nutrients in growing–finishing pigs is affected by *Aspergillus niger* phytase, phytate and lactic acid levels: 1. Apparent ileal digestibility of amino acids. Live. Prod. Sci. 58:107-117.

Kies, A. K., P. A. Kemme, L. B. J. Šebek, J. T. M. van Diepen, and A. W. Jongbloed. 2006. 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.

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.

Knowlton, K. F. and J. H. Herbein. 2002. Phosphorus partitioning during early lactation in dairy cows fed diets varying in phosphorus content. J. Dairy Sci. 85:1227.

Knowlton, K.F., C.M. Parson, C.W. Cobb, and K.F. Wilson. 2005. Exogenous phytase plus cellulase and phosphorus excretion in dairy cows. Prof. Anim. Sci. 21: 212-216.

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 cellulose. J. Dairy Sci. 90: 4356-4360.

Knowlton, K. F., M. L. McGilliard, Z. Zhao, K. G. Hall, W. Mims, and M. D. Hanigan. 2010. Effective nitrogen preservation during urine collection from Holstein heifers fed diets with high or low protein content. J. Dairy Sci. 93:323-329.

Lei, X. G., P. K. Ku, E. R. Miller, M. T. Yokoyama, and D. E. Ullrey. 1993. Supplementing corn-soybean meal diets with microbial phytase maximizes phytate phosphorus utilization by weanling pigs. J. Anim. Sci. 71:3368-3375.

Liao, S. F., A. K. Kies, W. C. Sauer, Y. C. Zhang, M. Cervantes, and J. M. He. 2005. Effect of phytase supplementation to a low- and a high-phytate diet for growing pigs on the digestibilities of crude protein, amino acids, and energy. J. Anim. Sci. 83: 2130-2136.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds using refluxing in beakers or crucibles: Collaborative study. J. AOAC. 85:1217-1240.

Morse, D., H. Head, C. J. Wilcox, H. H. Van Horn, C. D. Hissem, and B. Harris, Jr. 1992. Effects of concentration of dietary phosphorous on amount and route of excretion. J. Dairy Sci. 75:3039.

Murphy, J. and J. P. Riley. 1958. A single-solution method for determination of soluble phosphate in natural waters. J. Marine Biol. Assoc. UK. 37:9-14.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, D.C.

Nelson, T.S., L.W. Ferrara, and N.L. Storer. 1968. Phytate phosphorus content of feed ingredients derived from plants. Poult. Sci. 47: 1371- 1374.

Park, W.-Y., T. Matsui, and H. Yano. 2002. Post-ruminal phytate degradation in sheep. Anim. Feed Sci. Technol. 101: 55-60.

Park, W.-Y., T. Matsui, F. Yano, and H. Yano. 2000. Heat treatment of rapeseed meal increases phytate flow in the duodenum of sheep. Anim. Feed Sci. Technol. 88: 31-37.

Reynal and Broderick. 2005. Effect of dietary level of rumen-degradable protein on production and nitrogen metabolism in lactating dairy cows. J. Dairy Sci. 88: 4045-4064.

Rode, L.M., D.C. Weakley, and L.D. Satter. 1985. Effect of forage amount and particle size in diets of lactating dairy cows on site of digestion and microbial protein synthesis. Can. J. Anim. Sci. 65: 101-111.

Scaletti, R. W., D. S. Trammell, B. A. Smith, and R. J. Harmon. 2003. Role of Dietary Copper in Enhancing Resistance to Escherichia coli Mastitis[1]. J. Dairy Sci. 86: 1240-1249.

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

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

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.

Vitti, D. M., E. Kebreab, J. B. Lopes, A. L. Abdalla, F. F. De Carvalho, K. T. De Resende, L. A. Crompton, and J. France. 2000. A kinetic model of phosphorus metabolism in growing goats. J. Anim. Sci. 78:2706-2712.

Wu, Z., L. D. Satter, and R. Sojo. 2000. Milk production, reproductive performance, and fecal excretion of phosphorus by dairy cows fed three amounts of phosphorus. J. Dairy Sci. 83:1028-1041.

Yang, W.Z. and K.A. Beauchemin. 2007. Altering physically effective fiber intake through forage proportion and particle length: Digesition and milk production. J. Dairy Sci. 90: 3410-3421.

Zebeli, Q., M. Tafaj, I. Weber, J. Dijkstra, H. Steingass, and W. Drochner. 2007. Effect of varying dietary forage particle size in two concentrate levels on chewing activity, ruminal mat characteristics, and passage in dairy cows. J. Dairy Sci. 90: 1929-1942.

Zinn, R. A. and F. N. Owens. 1986. A rapid procedure for purine measurements and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66:157-166.

Chapter V: Conclusions

These two studies are steps forward in refining phosphorus (P) feeding requirements in dairy cattle. It has been long assumed that phytate (Pp) is completely available in ruminant animals because of the phytase capacity of ruminal microorganisms. In the past 15 years, research in small, and some large, ruminants have reported evidence contradictory to that conclusion. Quantification of P forms in feedstuffs is an instrumental step in elucidating the actual P availability of P-containing compounds. Characterization of P forms may lead to more insight as to why P availability may not be as complete as originally thought. These data should contribute to improvement of the model of Hill et al. (2008; Figure 2.2) in refining characterization of P compounds consumed by the cow.

To more completely describe flow of P compounds through the gastrointestinal tract, the data from the phytase and forage particle length feeding study will be beneficial. Omasal, ileal, and fecal flows of total P, inorganic P, Pp, and microbial P will also contribute to the description of digestion, absorption, and end fate of P compounds. Upon implementation of this data into Hill et al. (2008; Figure 2.2), more complete and refined estimates of P availably and excretion can be elucidated.

References

Ben-Ghedalia, D., H. Tagari, S. Zamwel, and A. Bondi. 1975. Solubility and net exchange of calcium and phosphorus in digesta flowing along the gut of sheep. Br. J. Nutr. 33: 87-94.

Bergman, E. L., K. Autio, and A. S. Sandberg. 2000. Optimal conditions for phytate degradation, estimation of phytase activity, and localization of phytate in barley. J. Agric. Food Chem. 48:4647-4655.

Braithwaite, G. D.1976. Reviews of the progress of dairy science Calcium and phosphorus metabolism in ruminants with special reference to parturient paresis. J. Dairy Res. 43: 501-520.

Bravo, D., D. Sauvant, C. Bogaert, and F. Meschy. 2003a. I. A bibliographic database for quantitative analysis of phosphorus flow in ruminants. Reprod. Nutr. Dev. 43: 251-269.

Bravo, D., D. Sauvant, C. Bogaert, and F. Meschy. 2003b. II. Quantitative aspects of phosphorus absorption in ruminants. Reprod. Nutr. Dev. 43: 271-284.

Breves, G. and B. Schroder. 1991. Comparative aspects of gastrointestinal phosphorus metabolism. Nutr. Res. Rev. 4:125-140.

Brintrup, R., T. Mooren, U. Meyer, H. Spiekers, and E. Pfeffer. 1993. Effects of two levels of phosphorus intake on performance and faecal phosphorus excretion of dairy cows. J. Anim. Physiol. Anim. Nutr. 69:29-36.

Burbano, C., M. Muzquiz, A. Osagie, G. Ayet, and C. Cuadrado. 1995. Determination of phytate and lower inositol phosphates in Spanish legumes by HPLC methodology. Food Chem. 52: 321-325.

Burroughs, W., A. Latona, P. DePaul, P. Gerlaugh, and R. M. Bethke. 1951. Mineral influences upon urea utilization and cellulose digestion by rumen microorganisms using the artificial rumen technique. J. Anim Sci. 10:693-705.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Eco. Appl. 8: 559-568.

Cerosaletti, P. E., D.G. Fox, and L.E. Chase. 2004. Phosphorus reduction through precision feeding of dairy cattle. J. Dairy Sci. 87: 2314-2323.

Clark, R.C., O.E. Budtz-Olsen, R.B. Cross, P. Finnamore and P.A. Bauert. 1973. The importance of the salivary glands in the maintenance of phosphorus homeostasis in sheep. Aust. J. Ag. Res. 24: 913-919.

De Boer, G., J. G. Buchanan-Smith, G. K. Macleod, and J. S. Walton. 1981. Responses of dairy cows fed alfalfa silage supplemented with phosphorus, copper, zinc, and manganese. J. Dairy Sci. 64:2370-2377.

Dhiman, T. R., J. Kleinmans, N. J. Tessmann, H. D. Radloff, and L. D. Satter. 1995. Digestion and energy balance in lactating dairy cows fed varying ratios of alfalfa silage and grain. J. Dairy Sci. 78:330-341.

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, and Z. Wu. 2003. Phosphorus feeding levels and critical control points on dairy farms. J. Dairy Sci. 86:3787-3795.

Durand, M. and R. Kawashima. 1980. Influences of minerals in rumen microbial digestion. Pages 375-408 in Digestive Physiology and Metabolism in Ruminants. Y. Ruckebusch and P. Thivend, ed. AVI Publishing Company Inc., Westport, CT.

Durand, M. and S. Komisarczuk. 1987. Influence of major minerals on rumen microbiota. J. Nutr. 118: 249-260.

Duskova, D., R. Dvorak, V. Rada, J. Doubek, and M. Morounek. 2001. Concentration of phytic acid in feces of calves fed started diets. Acta Vet. Brno. 70: 381-385.

Ebeling, A.M., L.G. Bundy, T.W. Andraski, and J.M. Powell. 2002. Dairy diet phosphorus effects on phosphorus losses in runoff from land-applied manure. Soil Sci. Am. J. 66: 284-291.

Fleming, G.A. 1973. Mineral composition of herbage. Pages 529-566 in Chemistry and Biochemistry of Herbage. G.W. Butler and R.W. Bailey, eds, Academic Press, New York, N.Y.

Foote, A. P., B. D. Lambert, J. A. Brady, and J. P. Muir. Short communication: Phosphate transporter expression in Holstein cows. J. Dairy Sci. 94:1913-1916.

Frank, B. and C. Swensson. 2002. Relationship between content of crude protein in rations for dairy cows and milk yield, concentration of urea in milk and ammonia emissions. J. Dairy Sci. 85:1829-1838.

Godoy, S. C. Chicco, F. Meschy, and F. Requena. 2005. Phytic phosphorus and phytate activity of animal feed ingredients. Interciencia. 30: 24-28.

Graf, E. and F.R. Dintzis. 1982. High-performance liquid chromatographic method for the determination of phytate. Anal. Biochem. 119: 413-417.

Hill, S. R., K. F. Knowlton, E. Kebreab, J. France, and M. D. Hanigan. 2008. A model of phosphorus digestion and metabolism in the lactating dairy cow. J. Dairy Sci. 91:2021-2032.

Holler, H. A. Figge, J. Richter, and G. Breves. 1988. Calcium and phosphate net absorption from the sheep colon and rectum perfused *in vivo*. J. Anim. Phys. Anim. Nutr. 59: 9-15.

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

Hu, H.L., A. Wise, and C. Henderson. 1996. Hydrolysis of phytate and inositol tri-, tetra-, and penta- phosphates by the intestinal mucosa of the pig. Nutr. Research. 16: 781-787.

Iqbal, T., K.O. Lewis, and B.T. Cooper. 1994. Phytase activity in the human and rat small intestines. Gut. 35: 1233-1236.

Kemme, P. A., A. Lommen, L.H. Jonge, J. D. Van der Klis, A.W. Jongbloed, Z. Mroz, and A.C. Beynen. 1999b. Quantification of inositol phosphates using ^{31}P nuclear magnetic resonance spectroscopy in animal nutrition. J. Agric. Food Chem. 47: 5116-512.

Kincaid, R. L., J. K. Hillers, and J. D. Cronrath. 1981. Calcium and phosphorus supplementation of rations for lactating cows. J. Dairy Sci. 64:754-758.

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.

Knowlton, K.F., J.M. McKinney, and C. Cobb. 2002. Effect of a direct-fed fibrolytic enzyme formulation on nutrient intake, partitioning, and excretion in early and late lactation Holstein cows. J. Dairy Sci. 85: 3328-3335.

Knowlton, K.F., C.M. Parson, C.W. Cobb, and K.F. Wilson. 2005. Exogenous phytase plus cellulase and phosphorus excretion in dairy cows. Prof. Anim. Sci. 21: 212-216.

Koelsch, R. 2005. Evaluating livestock system environmental performance with whole-farm nutrient balance. J. Environ. Qual. 34: 149-155.

Kwanyuen, P. and J.W. Burton. 2005. A simple and rapid procedure for phytate determination in soybean and soy products. J. Am. Oil Chem. Soc. 82:81-85.

Leytem, A.B., B.L. Turner, and P.A. Thacker. 2004. Phosphorus composition of manure from swine fed low-phytate grains: Evidence for hydrolysis in the animal. J. of Environ. Qual. 33: 2380-2383.

Leytem, A. B., P. W. Kwanyuen, R. O. Maguire, and J. Brake. 2008. Evaluation of phosphorus characterization in broiler ileal digesta, manure, and litter samples; ^{31}P-NMR vs. HPLC. J. Environ. Qual. 37: 494-500.

Little, D.A. 1982. Utilization of minerals. Pages 259-283 in Nutritional Limits to Animal Production from Pastures. J.B. Hacker, ed. Commonweatlth Agricultural Bureaux, Farnam Royal, UK.

McDowell, R.W. 2003. Minerals in Animal and Human Nutrition. 2nd Edition. Elsevier Sci. B.V.,Amsterdam, The Netherlands.

McDowell, R.W., L.M. Condron, and I. Stewart. 2008a. An examination of potential extraction methods to assess plant available organic phosphorus in soil. Biol. Fertil. Soils. 44: 707-715.

McDowell, R. W., Z. Dou, J. D. Toth, B. J. Cade-Menun, P. J. A. Kleinman, K. Soder, and L. Saporito. 2008b. A comparison of phosphorus speciation and potential bioavailability in feed and feces of different dairy herds using ^{31}P nuclear magnetic resonance spectroscopy. J. Environ. Qual. 37: 741-752.

Mjoun, K., K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe. 2008. Ruminal phosphorus disappearance from corn and soybean feedstuffs. J. Dairy Sci. 91:3938-3946.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, D.C.

National Research Council. 1996. Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, D.C.

Nelson, T.S., L.B. Daniels, J.R. Hall, and L.G. Shields. 1976. Hydrolysis of natural phytate phosphorus in the digestive tract of calves. J. Anim. Sci. 42: 1509-1512.

Pedersen, C., M. G. Boersma, and H. H. Stein. 2007. Digestibility of energy and phosphorus in ten samples of distillers dried grains with solubles fed to growing pigs. J. Anim. Sci. 85:1168-1176.

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

Pitcher, T. and Buffenstein, R. 1993. Passive uptake in the small intestine and active uptake in the hindgut contribute to the highly efficient mineral metabolism of the common mole-rat, *Cryptomys hottentotus*. Br. J. Nutr. 71: 573-582.

Puggaard, L., N. B. Kristensen, and J. Sehested. 2011. Effect of decreasing dietary phosphorus supply on net recycling of inorganic phosphate in lactating dairy cows. J. Dairy Sci. 94:1420-1429.

Rham, O. and T. Jost. 1979. Phytate-protein interactions in soybean extracts and low-phytate soy protein products. J. Food Sci. 44: 596-600.

Rotz, C. A., A.N. Sharpley, L.D. Satter, W.J. Gburek, and M. A. Sanderson. 2002. Production and feeding strategies for phosphorus management on dairy farms. J. Dairy Sci. 85: 3142-3153.

Sachdev, P. and D. Debb. 1977. Effect of zinc on protein and RNA content in wheat plants. J. Sci. Fd Agric. 28: 959-962.

Sanberg, A.S. and R. Ahderinne. 1986. HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. J. Food Sci. 51: 547-550.

Schachtman, D.P., R.J. Reid, and S.M. Ayling. 1998. Phosphorus uptake by plants: From soil to cell. Plant Physiol. 116: 447-453.

Schroder, B. and G. Breves. 1996. Mechanisms of phosphate uptake into brush border membrane vesicles from goat jejunum. J. Comp. Physiol. 166:230-240.

Schryver, H. F., H. F. Hintz, P. H. Craig, D. E. Hogue, and J. E. Lowe. 1972. Site of phosphorus absorption from the intestine of the horse. J. Nutr. 102:143-147.

Shirazi-Beechey, S. P., J. I. Penny, J. Dyer, I. S. Wood, P. S. Tarpey, D. Scott, and W. Buchan. 1996. Epithelial phosphate transport in ruminants, mechanisms and regulation. Kidney Int. 49:992-996.

Sklan, D. and S. Hurwitz. 1985. Movement and absorption of major minerals and water in ovine gastrointestinal tract. J. Dairy Sci. 68:1659-1666.

Skrivanova, V., M. Marounek, and R. Drorak. 2004. Digestibility of total and phytate phosphorus in young calves. Vet. Med.-Czech. 49: 191-196.

Smith, A. H., M. Kleiber, A. L. Black, and C. F. Baxter. 1955. Transfer of phosphate in the digestive tract: II. Sheep. J. Nutr. 57:507-527.

Spears, R. A., A. J. Young, and R. A. Kohn. 2003. Whole-farm phosphorus balance on western dairy farms. J. Dairy Sci. 86:688-695.

Steevens, B. J., L. J. Bush, J. D. Stout, and E. I. Williams. 1971. Effects of varying amounts of calcium and phosphorus in rations for dairy cows. J. Dairy Sci. 54:655-661.

Toor, G.S., B.J. Cade-Menum and J.T. Sims. 2005. Establishing a linkage between phosphorus forms in dairy diets, feces, and manures. J. Environ. Qual. 34:1380-1391.

Turner, B.L. 2004. Optimizing phosphorus characterization in animal manures by solution phosphorus-31 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 33: 757-766.

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.

United States Geological Survey. 2007. Minerals Yearbook. http://minerals.usgs.gov/minerals/pubs/commodity/phosphate_rock/. Accessed March 5th, 2009.

Wahyuni, M., S. Ishizaki, and M. Tanaka. 1998. Improvement of thermal stability of fish water soluble proteins with glucose-6-phosphate through the Maillard reaction. Fish Sci. 64:973-978.

Wasserman, R. H. 1981. Intestinal absorption of calcium and phosphorus. Fed. Proc. 40: 68-72.

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

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.

Wise, A., and D.J. Gilburt. 1981. Phytate hydrolysis by germfree and conventional rats. Appl. Enviro. Microbiol. 43: 753-756.

Witt, K.E. and F.V. Owens. 1983. Phosphorus: Ruminal availability and effects on digestion. J. Anim. Sci. 56: 930-937.

Wu, Z. and L. D. Satter. 2000. Milk production and reproductive performance of dairy cows fed two concentrations of phosphorus for two years. J. Dairy Sci. 83:1052-1063.

Wu, Z., L. D. Satter, A. J. Blohowiak, R. H. Stauffacher, and J. H. Wilson. 2001. Milk production, estimated phosphorus excretion, and bone characteristics of dairy cows fed different amounts of phosphorus for two or three years. J. Dairy Sci. 84:1738-1748.

Wu, Z., S. K. Tallam, V. A. Ishler, and D. D. Archibald. 2003. Utilization of phosphorus in lactating cows fed varying amounts of phosphorus and forage. J. Dairy Sci. 86:3300-3308.

Yang, T.H. 2006. Effects of diet on phosphorus digestion in dairy cattle. MS thesis. Virginia Polytechnic Institute and State University, Blacksburg.

Yanke, L.J., H.D. Bae, L.B. Selinger, and K.J. Cheng. 1998. Phytase activity of anaerobic ruminal bacteria. Microbiology. 144: 1565-1573.

Appendix A

Elaboration on reconstituting omasal samples (France and Siddons, 1986) based on marker flow

Abbreviations

R: reconstitution factor. This value makes adjustments to digesta flow by adjusting the relative association of the markers to one another.

f: particle

F = proportion of particles in the reconstituted sample (wet weight)

t: supernatant (or fluid)

T = proportion of supernatant in reconstituted sample (wet weight)

x: unrepresentative sample

Co: cobalt (mg/g of fresh weight)

Yb: ytterbium (mg/g of fresh weight)

Dose: actual dose of the specified marker. This should be determined from total excretion of feces and the marker analysis of feces. (mg/day)

Calculations

Using these calculations in this order in accordance with the referenced calculations from France and Siddons (1986) will allow ease of reconstitution recalculation.

- $Rf = (Co_x / \text{Dose Co}) – (Yb_x / \text{Dose Yb}) / (Yb_f / \text{Dose Yb}) – (Co_f / \text{Dose Co})$

- F = (Proportion of f in original sample + Rf) / (1+ Rf)
  T = 1- F

- Use equations #30a and #30b from France and Siddons (1986)

- Then use equation #31 for both markers

- Value obtained from equation #31 for Co * T = total flow from fluid phase
  Value obtained from equation #31 for Yb * F = total flow from particle phase