The utilization of phytate-phosphorus by the transition dairy cow

by

Sarah Louise Lowry

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Program of Study Committee:
Wendy Powers, Major Professor
Howard Tyler
Jesse Goff
James Russell

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Graduate College
Iowa State University

This is to certify that the master's thesis of

Sarah Louise Lowry

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

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CHAPTER ONE: INTRODUCTION

Understanding mineral availability to dairy cows, in all stages of production, is crucial for maintaining whole farm nutrient management. Phosphorus (P) availability and utilization can be inconsistent between feedstuffs, cows and stages of production. If P is overfed on the dairy farm, environmental consequences result. Phosphorus has the potential to leach out of fields and contaminate surface and ground water. Phosphorus may be the limiting nutrient for algal growth. If this nutrient is no longer limiting and is supplied in excess of needs to surface water, eutrophication may occur. In this way over-application of manure to farm land can contribute to eutrophication, via P from field runoff.

Ruminants have great potential to utilize most dietary P. From two-thirds to three-quarters of phosphorus in concentrates is in the form of phytate (Common, 1940; Nelson et al., 1968b). Phytase hydrolyzes phytate to form orthophosphates, an absorbable form of P. The microbial populations within the rumen produce phytase, rendering phytate P available for the ruminant.

As a cow transitions from a dry gestating period to a period of lactation, many changes occur. Nutritional demands increase and hormone levels fluctuate as gestation progresses. The cow also experiences many environmental changes from changing pens and pen mates, to a change in diet. All of these have the possibility to lead the transition cow to health stress and rumen stress.

The current study was undertaken to determine the ability of the transition dairy cow to utilize phytate P. The transition period was defined as the period 14 days prepartum to 28 days postpartum. Four aspects of P utilization were investigated. An in vitro study was

conducted to determine if the stresses of the transition period affected the rumen's microbial population's ability to hydrolyze phytate P. Cracked corn, soybean meal, whole cottonseed, a total mixed ration and phytic acid were analyzed. An apparent digestibility study was conducted to determine if the digestibility of P was influenced by the effects of transition. A P balance study was conducted to determine the degree that the balance of P differed as the cow transitioned from late gestation to early lactation. Lastly, an in situ study was conducted to determine the rate at which phytate P was digested in the rumen.

CHAPTER TWO: REVIEW OF THE LITERATURE

Introduction

Phosphorus (P) is an environmental concern, both in our nation's soil and water. According to the 1998 National Water Quality Inventory, which reported on assessments for 32% of the United States' waters, 40% of assessed waters were impaired (U.S.E.P.A., 2001). In that report, agriculture was identified among the sources contributing to the impairment in 60% of the impaired rivers and streams, and 30% of the impaired lakes (U.S.E.P.A., 2001). One contributor to pollution in surface waters is runoff from fields with manure and/or fertilizer applied. The dairy industry has begun moving toward fewer but larger operations. As a result, manure production within some geographic areas has become greatly concentrated (U.S.E.P.A., 2001). In addition, many of the larger facilities specializing in dairy farming are in non-agricultural areas where there is insufficient land to accommodate the useful application of the animal manure produced (U.S.E.P.A., 2001). Many of these farmers choose to purchase feed rather than diversify their operation and grow feed on their land. The manure generated is not used to provide nutrients for crops for the dairy operation's use. Instead, manure must be shipped elsewhere.

Phosphorus Utilization in the Environment

To increase the amount of P in soil, manure that is rich in P can be applied to agricultural land and then used for crop growth. Total P in surface soils varies from 0.005% to 0.15% (Havlin et al., 1999). The quantity of total P in soils has little or no relationship to the availability of P to plants (Havlin et al., 1999). The availability of minerals in the soil and uptake by plants is dependent on factors such as soil pH, moisture, drainage (Fahey Jr.,

1994) and clay content (USDA, 1992). Over 80% of the fertilizer and manure P applied to land is held in the soil in a form that is not immediately available to crops (Withers, 1996).

Phosphorus in manure is present as both organic and inorganic forms. Approximately 73% of the P in excreted dairy manure is in the organic form; primarily from microbial tissue and plant residue (USDA, 1992). Over time, organic P becomes soluble or converts to fixed P within the soil (USDA, 1992, Figure1.1). Phosphorus may become loosely bound to aluminum, iron, or calcium and be in an inorganic labile form (USDA, 1992). Because of the particular chemistry of P, it reacts readily with positively charged aluminum, iron, and calcium ions to form relatively insoluble substances. Organic P within the soil may also become fixed to aluminum-phosphates or iron-phosphates and would therefore exist in a fixed inorganic state (USDA, 1992). Inorganic P consists of soluble and attached P (Figure 1.1). Soluble P is the form used by plants as orthophosphates (usually HPO_4^{-2} or $H_2PO_4^-$), and is the form that is subject to leaching (liquid percolating through soil removing soluble matter). Soluble P generally accounts for less than 15% of the total P in most soils (USDA, 1992). Attached P includes compounds that are formed when the anionic forms of dissolved P become attached to cations, such as iron, aluminum and calcium. Labile P remains in balance with soluble P. When the concentration of soluble P is reduced because of the removal by plants, some labile P is converted to soluble P, maintaining the balance (USDA, 1992). When the labile P converts to soluble P, it typically occurs at a slow rate (Havlin et al., 1999). It appears that this balance of attached to soluble P is crucial in keeping P both available to the plant and not readily leached.

In addition to P uptake by roots, soluble P can be adsorbed on mineral surfaces and precipitated as secondary P minerals. Soil microbes immobilize soluble P as microbial P,

eventually producing readily mineralizable P compounds (labile organic P) and organic P compounds more resistant to microbial degradation (Havlin et al., 1999). Havlin et al. (1999) states that maintenance of soluble P concentrations (intensity) for adequate P nutrition in the plant depends on the ability of labile P (quantity) to replace soil soluble P taken up by the plant. The ratio of quantity to intensity is called the buffer capacity, which expresses the relative ability of the soil to buffer changes in soil soluble P. The larger the buffer capacity, the greater the ability to buffer soluble P so there will be enough readily available for the plant to utilize.

Accumulation of P in soils, beyond the P buffer, can have a detrimental effect on the environment. Phosphorus not used by a season's crop will remain for the following season. Immobility of P in soil is caused by the presence of hydrous oxides of aluminum and iron oils that have high clay content, particularly kaolin. Soils high in volcanic ash or allophane, low or high soil pH, and high exchangeable aluminum also cause immobility in soils. These factors increase the immobility by causing an increased percentage of P to be attached. As long as the soil particles remain in place so will the P bound to the particles (USDA, 1992). Phosphorus applied in excess results in soil residual P (Fahey Jr., 1994). About 60% of the total P in manure can be available to crops the year of application if it is applied to soils that do not test "high" or "very high" in P (Killorn et al., 1997). When soils are high in P, the percentage of P that is in the "mobile" forms (soluble or labile) are then accessible to leaching which can occur with rain or irrigation. Following P entry into the surface water, the P pollution can result in eutrophication. Eutrophication is an increase in the fertility status of natural waters that causes accelerated growth of algae or aquatic plants, which is considered pollution (NRCS-Iowa, 2001). Phosphorus is often the limiting nutrient for algal

growth and when P enters the fresh water environment and the increase in growth of algae and aquatic weeds can accelerate the aging process in lakes (USDA, 1992). Once P supply is adequate or abundant eutrophication can occur.

In many states, regulations dictate that farmers test their soil and spread manure to meet the appropriate P needs of their crops (Lory and Scharf, viewed Sept. 2002). In other states, farmers still spread manure based on the nitrogen (N) needs of the crops. The majority of the time, when spreading to meet the N needs of crops, over-application of P occurs. For example, assume corn grain uses 1.2 lbs of N/ bushel (bu) and uses 0.1748 lbs of P/bu, approximately a 1:6.8 P:N ratio. Also, assume production is, on average, 150 bushels of corn/acre. Next, assume dairy manure (as excreted) contains 0.07 lbs of P/d/1000 animal lbs and 0.45 lbs of N/d/1000 animal lbs; that is a 1:6.4 P:N ratio. Therefore if manure was spread to meet the N demands of the crop, P would be over-applied. If manure was spread based on the P demands of the crop, N would be under-applied. Many dairies do not maintain and spread manure as excreted, but from a storage system of some sort, with parlor waste and bedding included, therefore it would be of interest also to look at the numbers relating to lagoon waste. Assume anaerobic supernatant lagoon waste contains 0.48 lbs of P per 1000 gallons and 1.67 lbs of N per 1000 gallons; a 1:3.5 P:N ratio. The extent of over-applying P or under-applying N is much greater, but the above explanation holds true for lagoon waste. When P is over-applied, all of the P applied in a given year may not be utilized by that season's crop. If the P stays in the soil in small amounts there is not usually a problem, especially if crops are rotated so that crops needing larger amounts of P are rotated with crops needing lesser amounts, creating a balance over the two or more crop cycles. The problem arises when too much P remains in the soil from one crop to the next, leaving a large

residual amount of P. A more conservative approach is to apply the manure to supply the P needs of the crop, either through reduced annual application rates, or less frequent applications. Correct application rate requires consideration of many variables, such as what crop is grown, time of year when spreading, manure source, current mineral load in the soil, manure texture, and manure moisture content (Killorn et al., 1997).

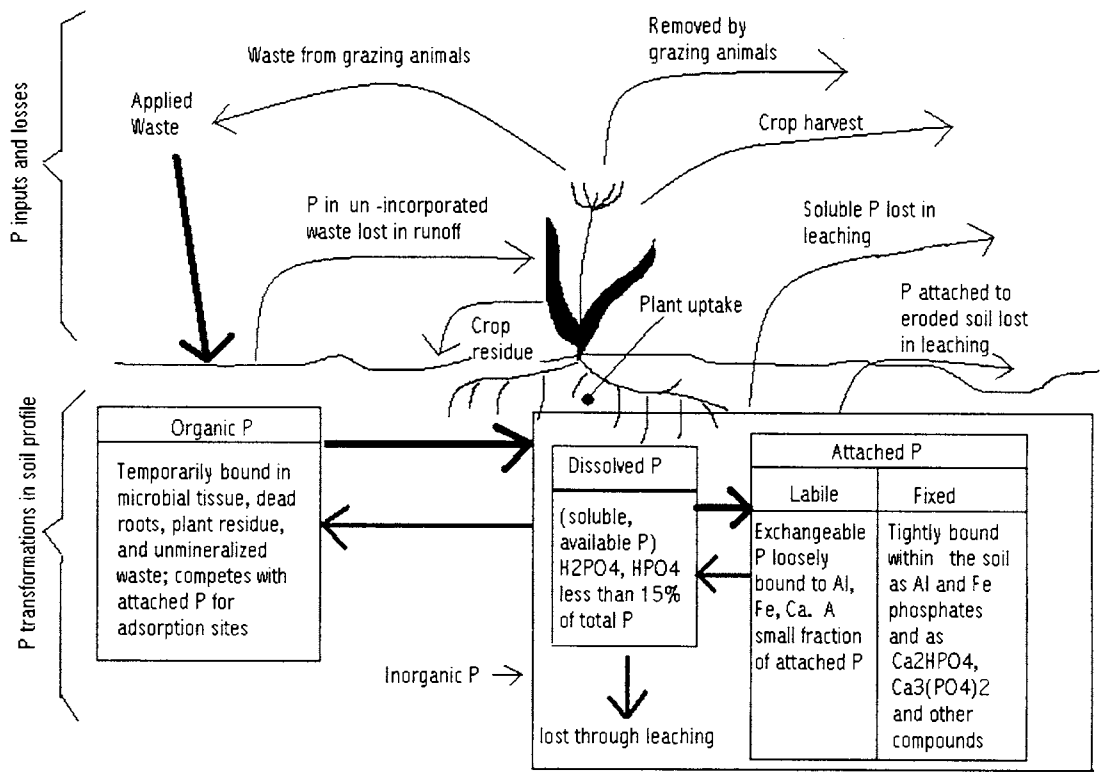

Figure 1.1 Phosphorus inputs and losses at a waste application site and phosphorus transformation within the soil profile (USDA, 1992).

Phosphorus Nutrition of the Dairy Cow

Phosphorus is essential to life function. This is true of both the dairy cow and the microorganisms that reside within the cow. Phosphorus is the second most plentiful mineral in the body of cattle (Ternouth, 1990). In blood plasma P concentration is normally 1.3 to

2.6 mmol/L (NRC, 2001). Whole blood contains six to eight times as much P as plasma. Approximately 5 to 8 g are present in the extracellular pool of a 600 kg animal. The intracellular concentration of P is approximately 25 mmol/L, and total intracellular P is about 155 g in a 600 kg cow (NRC, 2001). Phosphorus has more known biological functions than any other mineral element. Approximately 80% of P in the body is in bones and teeth. In bone, it is present with calcium (Ca) mainly as apatite salts and as calcium phosphate. Phosphorus is in every cell of the body, and almost all energy transactions involve formation or breaking of high-energy bonds that link oxides of phosphate to carbon or to carbon-nitrogen components such as ATP (adenosine tri-phosphate; NRC, 2001). The dairy cow also uses P in acid-base buffer systems of blood and other bodily fluids. Phosphorus is involved in cell differentiation. Phosphorus is a component of cell walls and cell contents as phospholipids, phosphoproteins, and nucleic acids. Ruminal microorganisms require P for digestion of cellulose (Burroughs et al., 1951) and synthesis of microbial protein (Breves and Schroder, 1991). These are just a few of the important functions of P in the bovine.

Phosphorus Digestion

The true digestibility of P in diets of ruminants has been reported to range from 50 to 94% (Braithwaite, 1986; Field et al., 1984; Kleiber et al., 1951; Lofgreen and Kleiber, 1954). Range of digestibility is due to differences in feeds, sheep versus cows and stage of lactation or pregnancy. Once digested, P enters a center pool of exchangeable P (blood inorganic P in part) via absorption from the intestine, absorption of digestive juice P (saliva) and by resorption from bone and soft tissues. It leaves the center pool by secretion of digestive juices, excretion in the urine, accretion into bone and soft tissues, secretion into milk and excretion in the feces (Braithwaite, 1983). Efficiency of absorption of dietary P in the

ruminant ranges between 63 and 79% depending on feedstuffs, feed intake, and individual animal demands (Braithwaite, 1986; Field et al., 1984). The 2001 NRC model estimates 64% absorption of P from forages, 70% absorption of P from concentrates and > 70% absorption of P from most of the inorganic P sources (NRC, 2001).

Horst (1986) reported that a cow producing 9,000 kg (19,800 lbs) of milk per lactation must consume an average of 60-70 g P/d to meet her lactation needs. An additional 15-20 g of P must be supplied for maintenance (Horst, 1986). The additional P maintenance demands during early lactation are primarily supplied from P mobilized from bone and soft tissues, rather than from P absorbed via saliva or feed intake (Braithwaite, 1983). Phosphorus in milk is typically 0.089% or approximately 0.9 g P/ kg milk (Spiekers et al., 1993).

Saliva is the chief path of P entry into the center pool of ruminants with estimates ranging from 50 to 77% (Care, 1994; Wadsworth and Cohen, 1977). Daily saliva flow for a cow ranges from 25 to 190 L/day (Bailey, 1961; Yarns et al., 1965). Bailey (1961), using cattle with ruminal cannulas, removed feed boluses from the reticulum/rumen as the boluses entered from the esophagus and from the cattle's mouth during rumination. Moisture of feed and bolus were utilized to determine saliva produced. Cattle were fed one of nine diets, thus giving a range of 98 to 109 L of saliva produced per day, depending on diet consumed (Bailey, 1961). Yarns et al. (1965), using beef steers with esophageal and ruminal cannulas, collected salivary secretions every 15 min for 24 consecutive hours. Animals were fed either a Bermuda grass/corn diet or an alfalfa diet. Cows maintained on the Bermuda grass/corn ration ranged in salivary production from 38 to 68 L/d and cows maintained on an alfalfa diet ranged in salivary production from 25 to 51 L/d (Yarns et al., 1965).

Poutiainen (1971) reported that 21-62 g of P enters the rumen every 12 h, depending on amount of long hay consumed. Forty-two percent (approximately 34 g) of the P entering the rumen is from saliva. Salivary P secretions constitute approximately 80% of the endogenous P (non-feed P) recycled to the gastrointestinal tract (Care, 1994). Cells and cell fragments sloughed from intestinal lining and phagocytes make up the remainder of the endogenous P excretions (Kleiber et al., 1951). Clark (1953) concluded that, of the species he examined (goats, sheep, cattle, horses, and dogs), only ruminants secrete P in their saliva. The large amount of salivary P is thought necessary to supply the P needs of rumen microorganisms and to buffer against the volatile fatty acids produced in the rumen. Salivary P mixes with the dietary P in the rumen before a portion of the total P is absorbed during its passage through the small intestine.

Factors that affect the net loss of endogenous phosphates secreted from the salivary glands also influence the absorption of dietary P (Horst, 1986). Salivary glands play an important role in P homeostasis by controlling the amount of P secreted into the gut (Tomas, 1974; Tomas and Somers, 1974). In more fibrous diets, saliva secretion will be increased and, although the salivary P concentration may be decreased due to dilution, total P output will still increase (Valk et al., 2000, Poutiainen, 1971).

Fecal P plays a large role in P excretion from the ruminant. Scott and McLean (1981) reported that 93.5 to 99.4% of P excreted by sheep is excreted in feces. There are two forms of fecal P, metabolic (exogenous or dietary) and endogenous. Endogenous P reaches the intestinal contents by 1) diffusion from blood or interstitial tissue fluid, 2) as part of secretions such as saliva, 3) as a component of cells or cell fragments sloughed off from the intestinal lining, or 4) P contained in phagocytes (Kleiber et al., 1951). Even though the

amount of endogenous fecal P loss is directly related to P intake (Dayrell and Ivan, 1989; Field et al., 1983; Scott et al., 1985) there is some unavoidable endogenous loss (Braithwaite, 1983; Braithwaite, 1984; Challa et al., 1989) due to continuous sloughing of cell fragments from the intestinal lining, saliva secretions, and other functions that contribute to endogenous losses. There is also an endogenous fraction that is excreted to maintain P homeostasis, representing P absorbed by the intestine in excess of the need to maintain normal blood P (NRC, 2001). Because ruminants absorb P in direct relation to intake and normally excrete very little in their urine, fecal secretion is the primary means of eliminating surplus and endogenous P and is therefore important in P homeostasis (Tomas, 1974). Metabolic fecal P is undigested P from food and it exceeds endogenous fecal P (Braithwaite, 1983; Kleiber et al., 1951; Lofgreen, 1960). Lofgreen and Kleiber (1954), Braithwaite (1983) and Kleiber et al. (1951) utilized the radioactive tracer P^{32} to determine endogenous P and metabolic P. Lofgreen and Kleiber (1954) reported metabolic P to be 92% of fecal P in sheep fed an alfalfa diet with highly available P, while Braithwaite (1983) reported metabolic P to be 60 to 68% of total fecal P, depending on stage of lactation. Kleiber et al. (1951) reported metabolic P to be 57% of fecal P in Jersey cows. Lofgreen and Braithwaite analyzed fecal loss in sheep. Differences observed in these studies may be due to the methods of administering the marker. Kleiber and Braithwaite injected P^{32} intravenously while Lofgreen administered it subcutaneously. Differences in methods of marker administration may lead to differences in rate of passage and the time it takes for a marker to be excreted (Miller and Byrne, 1970).

Urinary excretion of P in ruminants is normally very low, only 1% of fecal P excretion (Kleiber et al., 1951). Occasionally greater values are observed in some animals.

When forage diets are fed, ruminants usually excrete only negligible amounts of P in the urine. The relatively high maximum reabsorptive capacity for P in the renal tubules of the kidney is largely responsible for such small amounts of urinary P. The salivary glands largely replace the kidneys as the means of removing excess P from the circulation (Care, 1994). It has been suggested that excessive excretion of P in the urine occurs only when the salivary secretion mechanism becomes saturated (Field, 1981) or when there are factors that reduce the flow of saliva (fasting) and divert part of the endogenous P excretion from saliva to urine (Tomas and Somers, 1974). Tomas (1974) showed urinary P was increased with a modification of the physical nature of the diet (fine grinding), leading to an adaptation within the sheep in terms of pathways for P excretion. Scott and McLean (1981) addressed the advantage a ruminant may derive by maintaining the renal threshold for P excretion at such a high concentration compared to that observed in monogastric species. The authors explained this with an example of a poor quality roughage diet that contained little P, creating a wide range between the quantity of P required for saliva production and the quantity of P consumed. Fluctuating levels in the concentration of inorganic P in the plasma were observed. This occurred due to episodic feeding and/or a site of absorption (end of small intestine) far removed from the site of secretion (start of small intestine). If there was not a large renal threshold for P excretion at times when the concentration of P in the plasma increased, due to resorption, this excess would be excreted in the urine, but would not really be excess at all (Scott and McLean, 1981). This explains why there is approximately 5-6 g of P in saliva when only 200 mg of P are present in the circulation of sheep (Scott and McLean, 1981).

Completely eliminating or greatly reducing P from a dairy cow's diet is not an option when facing environmental issues. Accurately determining nutrient needs and feeding to those needs is a better option. Wu et al. (2000) observed that cows fed as low as 0.32% P (DM basis) of their diet would still have comparable milk production (10,790 vs. 11,226 and 11,134 kg/308d) to cows with greater percentages of P for two-thirds of their lactation. They also showed diets containing 0.41% P (DM basis) of the diet were adequate for top milk production (Wu et al., 2000).

Phosphorus Absorption and Regulation

Many factors come into play when determining the efficiency of P absorption, such as diet, intake, hormones, stage of lactation, pregnancy, and the fact that every cow partitions P differently (Tomas, 1974; Braithwaite, 1984). The efficiency of P absorption from mixed diets commonly fed to lactating dairy cows is approximately 50% (NRC, 1989). Horst (1986) identified factors affecting absorption. Specifically, the amount of P absorbed varies with the source of the P, the quantity of intake, Ca:P ratio, vitamin D status, intestinal pH, age of the cow, and dietary intake of other minerals and fat (Horst, 1986). Regulation of the P pool is under the influence of two calcitropic hormones: parathyroid hormone (PTH) and 1, 25-dihydroxyvitamin D (1, 25 $(OH)_2D$). Parathyroid hormone, secreted by the parathyroid gland, increases P concentration in saliva, thereby increasing P secretion into the rumen (Wadsworth and Cohen, 1977). 1, 25-dihydroxyvitamin D (1, 25 $(OH)_2D$) is the metabolite of vitamin D produced in the kidney (Horst, 1986). Vitamin D enters the ruminant in three ways. One way is from the photochemical conversion of 7-dehydrocholesterol to vitamin D_3 which is absorbed through the skin. Second, vitamin D enters as the result of intake of plants. Plants contain vitamin D as a result of photochemical conversion of ergosterol to

vitamin D_2. The third entry point is as vitamin D_2 or D_3 which is supplemented in the diet as commercially available crystalline forms (Horst, 1986). Vitamin D_2, D_3, or both are transported to the liver where they are converted to 25-hydroxyvitamin D (25-OHD; Horst, 1986). 25-OHD is then converted to 1, 25 $(OH)_2D$ in the kidney along with many other vitamin D metabolites (Horst, 1986). When plasma P is low, 1, 25 $(OH)_2D$ synthesis is stimulated by pituitary-mediated increase in kidney 1ά-hydroxylase (Horst, 1986). The resulting increase in 1, 25 $(OH)_2D$ stimulates the intestine to absorb P more efficiently (Horst, 1986).

Phosphorus intake increases the rate of absorption of dietary P, while the efficiency of absorption decreases. Phosphorus demand increases both the rate and efficiency of P absorbance (Braithwaite, 1984). In this case, rate is defined as the amount of P absorbed per unit of time and efficiency is defined as the amount of energy used to absorb a certain concentration of P. Endogenous loss of P in the feces is directly related to both P intake and P absorption but inversely related to P demands (Braithwaite, 1984). The rate of P secretion in the saliva is directly related to P absorption. Salivary secretion increases at exactly the same rate as does P absorption (Braithwaite, 1984). Rate of P absorption is related to P intake. Efficiency of P absorption increases in response to increased demand, suggesting that not all of the available P is normally absorbed. Two different processes may be involved in absorption: one, a passive process, related to P intake, and the other, an active process, related to P demand (Braithwaite, 1984). When the demand for P increases (e.g. during pregnancy or increased lactations) more P is absorbed due to improved efficiency, causing increases in intake and rate of absorption (Braithwaite, 1984). This increase in efficiency is determined by the saturable vitamin D-dependent active transport system (NRC, 2001). But,

if intake increases for reasons other than demand (e.g. better weather or improved feed quality) the increase in P absorption will be less efficient than it would be at a lower intake, yet the rate of absorption will increase. This increase in rate is due to the passive transport system. Horst (1986) showed low plasma P will stimulate an increase in 1, 25 $(OH)_2D$ synthesis, which stimulates an increase in P absorption efficiency from the intestine. Challa et al. (1989) attributes the increase in P absorption efficiency from the intestine to homeostatic control. The serum P concentration, which is directly related to the rate of P absorption, appears to determine the rates of P retention, salivary P secretion and urinary P excretion (Challa et al., 1989).

Phytate and Phytase

Phytate (phytic acid or myo-inositol hexaphosphoric acid) (Figure 1.2) is a compound that has been closely examined by environmentalists because of its prevalence as one of the forms of P leading to polluted soils (Havlin et al., 1999) and waterways. Microbiologists have tried to identify strains of bacteria, protozoa and other microbes which can hydrolyze phytate into usable phosphates (Suzuki and Ushida, 2000; Yanke et al., 1998). Animal scientists are also working in this area because many animal feed sources of plant origin contain from one-half to three-fourths of their total P as phytate (Common, 1940). Phytate concentration in feeds does depend on the feed ingredient itself; forages are relatively low in phytate-P (Clark et al., 1986), whereas wheat, oats, barley, corn (Common, 1940) and soybean meal can have two-thirds to three-quarters of their total P as phytate-P (Holden et al., 1996; Nelson et al., 1968a). For phytate-P to be available for absorption and utilization by animals, an inorganic form of P (PO_4) must be hydrolyzed from the inositol ring of

phytate by the enzyme phytase (myo-inositol hexakisphosphate 3-phosphohydrolase) (McCance and Widdowson, 1944; Morse et al., 1992; Nelson et al., 1968a; Pointillart, 1991; Pointillart et al., 1987). This enzyme is produced by protozoa and bacteria in the gut of livestock (Suzuki and Ushida, 2000; Yanke et al., 1998), among other places. One unit of phytase activity is defined as the quantity of enzyme that liberates one micro-mole of inorganic P per minute from 5.1 millimole sodium phytate solution at a pH of 5.5 and temperature of 37° C.

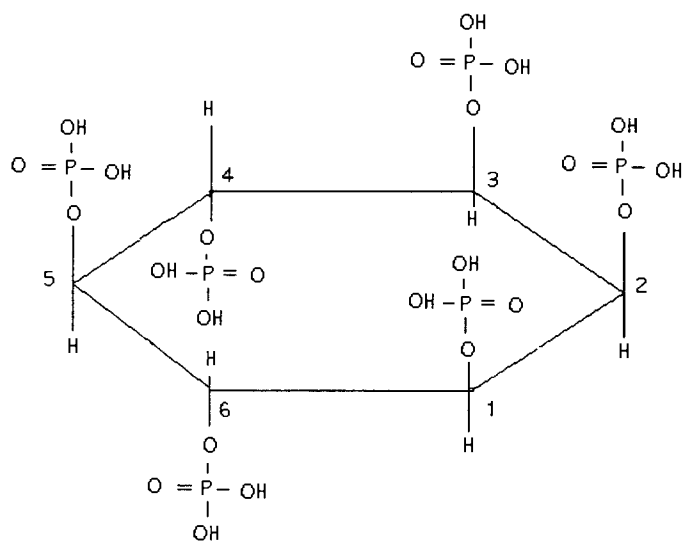

Figure 1.2. Phytic acid, *myo*-inositol hexakisphaosphate (Gibson and Ullah, 1990).

A large amount of phytate research has been conducted using non-ruminant animals. Non-ruminant animals cannot efficiently use phytate-P because the P is bound in an inositol ring (Bosch et al., 1998) and non-ruminants do not possess a sufficient microbial population in the upper intestine to produce an adequate quantity of the phytase enzyme to hydrolyze all of the feed phytate. Undigested phytate-P is excreted as fecal P. One way to improve dietary utilization, and therefore reduce fecal P, is to supplement non-ruminant feeds with the

phytase enzyme. Bosch et al. (1998) summarized many of the studies of phytase use in swine diets. The studies reviewed by Bosch et al. (1998), reported reductions in P content of manure varying from 5.5 to 62.4% following inclusion of 76 to 726 units phytase/lb feed. The use of phytase enables the non-ruminant to more efficiently utilize dietary P, reducing the need for supplemental P. The land area required for manure disposal is reduced, saving money in hauling the manure, and decreasing the land-base needed to spread manure.

Ruminants can hydrolyze phytate more efficiently because of the occurrence of bacterial-produced phytase in the rumen (Clark et al., 1986; Field et al., 1984; Morse et al., 1992; Raun et al., 1956; Valk et al., 2000). Morse et al. (1992) showed that greater than 90% of phytate-P disappeared from solids between 6 and 24 h of in vitro incubation and Clark et al. (1986) reported that 98% of phytate-P was digested in early lactation Holstein cows. Raun et al. (1956) reported that the availability of phytate-P to rumen microorganisms appeared to be close to 100%. Reid et al. (1947) showed that phytate-P is completely and rapidly hydrolyzed in the alimentary tract of sheep.

The exact location in the gastrointestinal tract of phytate hydrolysis becomes a matter of interest when discussing in vitro studies. If the hydrolysis occurs in the rumen, an in vitro study is an effective research tool for estimating phytate breakdown. If hydrolysis occurs in the intestine, in vitro studies are less reliable. An early paper by Mathur (1953) suggests the enzymatic cleavage of phytic acid may take place in the intestine. However other work disputes this finding (Morse et al., 1992; Reid et al., 1947). Morse et al. (1992) used an in vitro procedure and results suggested that the phytate hydrolysis occurs in the rumen fluid. Reid et al. (1947) showed that all phytate-P was hydrolyzed in the rumen of sheep, with the

exception of very small particles that passed quickly. Phytate in these small particles were hydrolyzed in the small intestine.

Given that ruminants have the ability to break down phytate in their rumen with no supplemented phytase, phytase sources in the rumen are of interest. Industrial production of phytase used in non-ruminant diets is currently utilizing the soil fungus *Aspergillus* (Yanke et al., 1998). Roche Vitamin Inc. utilizes the microbe *Aspergillus oryzae* with a gene from *Peniophor lycii* which codes for phytase expression (personal communication from Janet Snow, Ph. D., Technical Service Coordinator, Roche Vitamin Inc, Ames IA). Yanke et al. (1998) looked at 334 strains of bacteria, and found a large number of the strains that were positive for phytase activity including *Selenomonas ruminantium*, with a substantial percentage of the strains being *Megasphaera elsdenii* isolates that were also positive but with lower phytase activities (Yanke et al., 1998). Yanke states that rumen phytase activity appeared to be largely of bacterial origin with relatively little activity apparent in the particulate (protozoal/feed particle/fungal) fraction of the ruminal fluid. Suzuki and Ushida (2000) observed phosphohydrolyzing activity by both bacterial and protozoal populations with protozoal populations showing an approximately seven times greater initial velocity in releasing inorganic P than that of the bacterial population. Of the populations of bacteria that were present Suzuki and Ushida (2000) isolated two facultative anaerobic bacteria, *Klebsiella* sp. and *Corynebacterium* sp., which were phytase-producing organisms in the rumen of an adult Suffolk wether (Suzuki and Ushida, 2000). It appears that the key differences in these studies are the bacterial sources and the feed sources. In one study, ovines were fed a diet of timothy hay and commercial concentrate leading to protozoal phytase (Suzuki and Ushida, 2000), while in a second study, bovines were fed diets consisting of barley grain and a

barley/alfalfa hay mixture leading to bacterial phytase (Yanke et al., 1998). Yanke et al. (1998) observed phytase activity increased with higher grain diets, perhaps in response to the higher concentrations of phytate in cereal grains as compared to forages.

The Transition Period

The transition period includes the last few weeks of gestation, parturition, the onset of lactation and the first few weeks of lactation. The transition period is associated with the most dramatic changes in endocrine status, metabolism, and blood metabolite profiles. The transition period precedes the time of most severe negative nutrient balance that a cow faces (Grummer, 1995). The extent of these changes may play an important role in determining the level of stress a cow experiences during the transition period, as well as her health and productivity during the early postpartum period (Grummer, 1995). Most infectious diseases and metabolic disorders occur during this time. Milk fever, ketosis, retained fetal membranes, metritis, and displaced abomasums primarily impact the transition cow (Drackley, 1999). Even though these diseases do not directly relate to P utilization, they do affect many functions of a cow's system, including rumen health. Rumen health does have a direct impact on P utilization.

Metabolic Effects

Plasma insulin decreases and growth hormone increases as the cow progresses from late gestation to early lactation, with acute surges in plasma concentration of both hormones at parturition (Kunz et al., 1985). Non-esterified fatty acids (NEFA) increase approximately two-fold between 17 d prepartum and 2 d prepartum, at which time the concentration of NEFA increases dramatically until completion of parturition (Grummer, 1995). It is not clear

if this rise in NEFA is due to the stress of parturition, hormones, changing of endocrine status or energy restriction from decreased intake (Grummer, 1995). Plasma glucose concentrations remain stable or increase slightly during the prepartum transition period, increase dramatically at calving, and then decrease immediately postpartum (Kunz et al., 1985).

The amount of energy required for maintenance and milk production exceeds the amount of energy the cow can obtain from the diet during early lactation. To meet these energy needs, the cow must utilize body fat as a source of energy (Goff and Horst, 1997). The liver is responsible for oxidizing these fatty acids from body fat through the TCA cycle. The liver can only oxidize a limited amount and can only produce a small amount of the low density lipoproteins used to move fat out of the liver. This can lead to ketosis which usually becomes clinically evident from 10 d to 3 wk after calving (Goff and Horst, 1997). Once ketotic, gluconeogenesis becomes impaired, resulting in hypoglycemia. The cow becomes more lethargic, reducing feed intake (Goff and Horst, 1997) and available energy.

Hormone Effects

The blood concentration of many hormones increase or decrease dramatically at parturition and may affect dry matter intake (DMI) (NRC, 2001). Progesterone is the prominent hormone of pregnancy. It's concentration during the dry period is elevated while maintaining pregnancy but declines rapidly approximately 2 d before parturition (Chew et al., 1979; Hunter et al., 1970). Goff and Horst (1997) reported that plasma progesterone concentrations increased steadily until approximately d 250 of gestation when progesterone peaked at approximately 8 ng/ml. From d 240 of gestation until the day before calving, plasma progesterone concentrations decrease to 3 to 4 ng/ml (Goff and Horst, 1997). At

parturition, progesterone concentrations fall quickly to concentrations near zero (Hunter et al., 1970; Senger, 1999). During the last 3 and 4 wk of gestation, progestin concentration averaged 11.2 ng/ml, followed by a reduction to 8.0 ng/ml by the last 2 wk prepartum and further decreased to 0.9 ng/ml at parturition (Edgerton and Hafs, 1973). In the weeks following parturition progestin slowly increased from 0.3 ± 0.1 ng/ml at 1 wk to 6.1 ± 1.2 ng/ml at 8 wk postpartum (Edgerton and Hafs, 1973). Estrogen increases in plasma during late gestation but decreases immediately at calving (Chew et al., 1979). Plasma estrogen concentrations remain approximately 20 pg/ml during early pregnancy, and then start to increase in mid-gestation, peaking immediately before calving at 400-6000 pg/ml (Goff and Horst, 1997). During estrus, estrogen increases with a concurrent decline in feed intake (Tarttelin, 1968). Intake might also decline during late gestation when estrogen increases (Forbes, 1971).

In a study by Edgerton and Hafs (1973), serum prolactin averaged 50 ± 4 ng/ml in the last 2 to 4 wk of gestation in Holstein cows, and then increased to approximately 106 ± 14 ng/ml 1 wk prior to parturition. By 1 wk postpartum, prolactin had fallen to about 30% of the concentration at parturition and remained relatively stable for about 6 wk (Edgerton and Hafs, 1973). Edgerton and Hafs (1973) also showed that serum luteinizing hormone (LH) remained at approximately 0.5 ng/ml until postparturition when it increased over the following 6 wk to 1.5 ng/ml (Edgerton and Hafs, 1973). Glucocorticoid concentrations averaged 3.4 ng/ml in Edgerton's study during the 4 wk prior to parturition then increased at parturition to 9.3 ng/ml and returned to prepartum concentrations by the first week after parturition. Edgerton and Hafs (1973) suggest this three-fold increase may be related to stress that may play an important role in parturition. Changes in endocrine status during late

gestation influences nutrient metabolism (Grummer, 1995). Current research is not clear on all of the effects of changing levels of hormones around parturition. These changes could affect not only nutrient metabolism, but also feed intake around calving and the level of stress the cow is enduring.

Diet Change and Rumen Effects

Feed intake is usually decreased 30 to 35% during the final 3 wk prepartum (Goff and Horst, 1997; Kunz et al., 1985), but negative energy and protein balances are not as severe as during the week following parturition. A reduction in feed intake is initiated during the prepartum period, yet at this same time nutrient demands for support of conceptus growth and initiation of milk synthesis are increasing (Grummer, 1995). Stanley et al. (1993) reported that DMI did not decrease in beef cattle right before calving, but others have shown that DMI did decrease in both sheep (Faichney and White, 1988; Forbes, 1968; Forbes, 1971; Forbes, 1970) and cattle (Aitken and Preston, 1964; Owen and Miller, 1968).

Reduced rumen size, because of the increasing size of the fetus through the transition period, decreases rumen capacity and can affect feed intake because of a simple lack of space for more feed (Forbes, 1971). Stanley et al. (1993) reported that rumen capacity in beef cows decreased from 127 L 61 d prepartum to 102 L 6 d prepartum, then increased to 142 L by 8 d postpartum. Faichney and White (1988a) showed no decrease in rumen volume during late gestation of Corriedale ewes. Forbes (1968) demonstrated that late pregnant ewes had significantly less rumen volume than either non-pregnant ewes or ewes in the early stages of pregnancy. Forbes (1968) and Faichney (1988a) slaughtered ewes at different stages of gestation to determine rumen volume, while Stanley et al. (1993) injected water into the reticulo-rumen through a canula to determine rumen volume. Stanley fed a chopped hay diet,

Faichney fed a pelleted mixture of hay and grain and Forbes fed a hay diet. Differences in technique may explain differences in the results of these studies, but the difference in results were not consistent. Diet processing may be a determining factor in rumen size. Faichney and White (1988a) reported no decrease in rumen volume in ewes fed a mixture of grain and hay as pellets, while Stanley et al. (1993) and Forbes (1968) did observe a decrease in rumen volume when a chopped hay diet was fed. The effect of the uterus increasing in size and causing the rumen to have less space may not be affected by the chemical makeup of the diet, but instead by the physical form of the diet.

A decrease in rumen volume may also be attributed to an increase in abdominal fat. As the uterus enlarges during pregnancy, fat may have an even greater effect in the lack of abdominal space (Forbes, 1968). Forbes (1968) noted that in a study with Speckledface Welsh ewes, the fattest ewe on trial had a very low volume of rumen contents when compared with other ewes at the same stage of pregnancy. The thinnest ewe on trial had a larger volume of rumen contents when compared with other ewes at the same stage of prgnancy. Stanley et al. (1993) showed that even though rumen capacity measured at 22 d postpartum was approximately 5% greater than capacity measured 61 d prepartum, DMI was approximately 69% greater 22 d postpartum than 61 d prepartum. This suggests that although rumen capacity does play a role in DMI, it is not the only factor.

Graham and Williams (1962) suggests that pressure on the uterus of a fully distended rumen may reduce rumen capacity and increase the rate of excretion of food residues. Stanley et al. (1993) reported that, in cows, ruminal indigestible acid detergent fiber (IADF) passage rate increased across the prepartum period and peaked just before calving. In studies with ewes, rate of passage was markedly faster in late pregnancy than in early pregnancy

(Faichney and White, 1988; Graham and Williams, 1962). If DMI did remain constant in later pregnancy the decrease in rumen size might play a role in the increased rate of passage (Stanley et al., 1993). Egan et al. (1986) suggests that rate of passage from the reticulo-rumen is a major factor determining the level of intake possible, explaining that the faster space is made available by digestion or passage of space-occupying solids, the greater will be the intake. In a study conducted by Weston et al. (1983), feed intake was held constant throughout the transition period by intraruminal additions of feed. An increase in rate of passage was observed, yet it was no different from the increased rate of passage that was observed in the heifers who experienced a decrease in DMI prepartum (Weston et al., 1983). Graham (1962) fed four levels of intake and observed an increasing rate of passage from the lowest intake to the highest intake, even when less feed was consumed than capacity would allow and when the fetal lamb was still at an insignificant size. The digestive tract and abdominal cavity contained much less than capacity and still an increase in rate of passage was observed. The researchers suggest that pressure exerted on a particular section of the digestive tract may affect the overall rate of passage (Graham and Williams, 1962). Both Weston and Graham's research suggest that intake is not the main cause of a change in rate of passage and Graham's study suggests that neither is size of the uterus. One other suggestion is that some characteristics of plants can affect rates of digestion, retention times, and intakes (Egan et al., 1986). Also, the factors that increase passage rate involve both chemical and physical attributes of the feed and the physiological responsiveness of the animal (Egan et al., 1986).

Prior to parturition a decrease in space in the body cavity, an increase in rate of passage, and a decrease in DMI are observed (Forbes, 1971; Graham, 1962; Stanley et al.,

1993; Weston et al., 1983). All of these influence the others. The fact that it is uncertain which effect is controlling which does not take away from the fact that they may all be affecting P utilization. With increased rate of passage, the P present in feed may not have a chance to come fully in contact with phytase and, therefore, remain in an unusable form that passes through the GI tract unabsorbed to be excreted in feces. With decreased DMI, the rumen microflora are most likely not working at peak performance and may not be producing phytase as readily. With decreased rumen capacity, both of these previous effects are exaggerated.

As cows initiate and terminate the dry period, changes in rumen dynamics occur (NRC, 2001). These alterations are nutritionally induced rather than physiologically induced (NRC, 2001). Goff and Horst (1997) explain that during the transition from a lactation diet to a dry cow diet, the bacterial population shifts away from the lactate producers as a result of the decrease in readily fermentable starches in the diet. With a decrease in lactate, the concentration of bacteria that convert lactate to volatile fatty acids (VFA) also decrease (Goff and Horst, 1997). These lactate converters, *Selenomonas ruminantium* and *Megasphaera elsdenii,* are also the bacteria that make phytase (Yanke et al., 1998). Thus, if these bacteria are declining due to the change in diet, it is reasonable to project that the amount of phytase available in the rumen is declining also. As this bacterial shift takes place, lactate builds up and the pH of the rumen decreases because the acidity of lactate (pK_a= 3.86) is stronger than that of VFA (pK_a = 3.83-4.82; Goff and Horst, 1997). Rumen epithelia are compromised because of the change in diet. The rumen may then lack the ability to absorb the VFA quickly enough to prevent an accumulation of organic acids within the rumen. This causes the rumen pH to fall to the point where the protozoa and many of the bacteria within the

rumen are killed or are inactive (Goff and Horst, 1997). Raun et al. (1956) reported that phytase activity of rumen microorganisms is optimum at pH near 5.5. Therefore, not only is there a possibility of sub-optimal numbers of phytase-producing bacteria, but the ones present may either be not functioning well in the poor environment or are dying.

Phosphorus Utilization During the Transition Period

Phosphorus utilization is affected by pregnancy and lactogenesis during the transition phase. Braithwaite (1983, 1986) showed that ewes supplied sufficient dietary Ca exhibited an increased rate of P absorption in early lactation as the dietary P intake was increased. The efficiency of absorption, however, remained low. In mid- to late lactation, despite the falling demands for milk P, the quantity of P absorbed was increased even further than in early lactation. This increase was largely achieved by an increase in absorption efficiency because P intake remained fairly constant (Braithwaite, 1983).

The endogenous loss of P in the feces increases at the onset of lactation but then decreases slightly in mid- to late lactation (Braithwaite, 1983). In spite of the plentiful supply of dietary P, P was mobilized from bone and soft tissues in late pregnancy and early lactation when the rate of resorption increased to a high value, relative to accretion (Braithwaite, 1983). Approximately 65 g of P were lost during pregnancy and early lactation in the ewes with plentiful Ca and P intake. Phosphorus was more than adequately replaced by 5 wk post-lactation. Although the rate of P absorption by the ewes was increased to a greater extent between late pregnancy and early lactation than were P demands, the extra P absorbed was not used to meet these demands but was instead excreted in the urine and feces. Although absorption of P increased between late pregnancy and early lactation from 77.8 to 199.3 mg/d per kg body weight, demands for P increased from 10.7 to 40.4 mg/d per kg body

weight, a difference of 91.8 mg/d per kg of body weight. The additional P demands for milk production were supplied from P mobilized from bone and soft tissues instead of via absorption (Braithwaite, 1983). Braithwaite (1983) suggested that P probably was mobilized from bone and soft tissues not because of an increased demand for P, but instead, because of a lack of Ca. Since bone mineral is a complex Ca-P salt, mobilization of bone Ca also results in the release of bone P (Braithwaite, 1983). Demands for P associated with fetal development and milk production increased rapidly in late pregnancy to peak in early lactation, and then decreased (Braithwaite, 1986). House and Bell (1993) showed that a multiparous Holstein would require approximately 5.4 g of P to meet the maximum requirements for conceptus growth. The efficiency of absorption of dietary P remained high and fairly constant at about 63% throughout the whole experimental period (28 days prepartum to 35 days postpartum) but the rate of absorption varied in direct relation to the P intake. Absorption tended to be high at peak lactation, when the sheep had a greater intake, but low in the dry period, when intake was decreased (Braithwaite, 1986). Braithwaite (1986) concluded that rather than feed pregnant and lactating ewes according to their immediate day-to-day needs for P, it would be better to provide less minerals than is indicated from calculated requirements during late pregnancy, when, because of limitation on Ca absorption, some mobilization of skeletal reserves is inevitable, and to give more minerals in mid- to late lactation when animals need to replace skeletal mineral reserves (Braithwaite, 1986).

Conclusion

The efficiency at which a dairy cow can utilize phytate-P can have a large impact on the environmental stability of our nation's water resources. If the many stresses of the transition period have a negative effect on the cow's ability to synthesize phytase and degrade phytate-P, less P will be absorbed in the small intestine and more will be excreted via feces into the environment. Therefore, it becomes necessary to determine if the stresses of transition do reduce the cow's ability to use phytate-P.

CHAPTER 3:
IN VITRO DIGESTION OF PHYTATE PHOSPHORUS IN THE TRANSITION DAIRY COW

Introduction

Understanding the availability and degradability of P in commonly fed feedstuffs can help reduce the concentration of P in manure. A reduction in fecal P can lead to a reduction in P runoff from fields and pollution. Phytate-P is the form of P present in the largest concentration in animal feed sources of plant origin. Phytate concentration in feeds depends on the feed ingredient itself; forages are relatively low in phytate-P (Clark et al., 1986), whereas wheat, oats, barley (Common, 1940), corn and soybean meal can have two-thirds to three-quarters of their total P as phytate-P (Holden et al., 1996; Nelson et al., 1968a). For phytate-P to be available for absorption and utilization by animals it must come into contact with the enzyme phytase. Phytase is produced by protozoa and bacteria in the gut of ruminants (Suzuki and Ushida, 2000; Yanke et al., 1998). Researchers have shown that 90 to 100% of phytate-P disappeared from solids between 6 and 24 h of in vitro incubation with rumen fluid (Clark et al., 1986; Morse et al., 1992b; Raun et al., 1956) suggesting that phytate is readily hydrolyzed.

The transition period includes the last few weeks prepartum, parturition, the onset of lactation and the first few weeks postpartum. The transition period also includes the time of most severe negative nutrient balance that a cow faces (Grummer, 1995). The cow is most prone to metabolic diseases during the transition period. The objective of the in vitro study was to determine if a change in the cow's ability to utilize phytate P existed as the cow

transitioned from late gestation to early lactation. The phytase activity of the rumen fluid was determined indirectly as the concentration of phytate loss during in vitro incubation.

Materials and Methods

Two pregnant Holstein heifers from the Iowa State University dairy herd were selected for this study based on projected calving date. Heifer number 1847 had a projected calving date of May 14, 2001 and heifer number 1854 was expected to calve on June 1st. Days prepartum will be denoted as negative days and day of calving will be denoted as d 0, throughout. On d -46 and -63 respectively the heifers underwent surgery at the Iowa State University School of Veterinary Medicine to implant ruminal canulas. Cows were restricted from feed for 48 h and water for 24 h prior to surgery. Cows were then transported to the veterinary school where the surgery was performed under local lidocaine anesthesia (Kikela block). Following surgery, each cow was administered 50 cc penicillin intra-muscularly. Five boluses of aspirin were administered orally (daily 0200 h and 1500 h) for 5 d. Following surgery, the cows were moved back to the Iowa State University dairy and placed in individual pens within the maternity barn for careful observation. For 7 d following surgery, each cows' temperature was recorded daily. On d 5 following surgery, stitches were removed. The area around each canula was washed daily for the first week then weekly for the remainder of the first month with antibacterial soap.

On day -18 for 1847 and -36 for 1854, heifers were moved to individual tie stalls in the east wing of the Iowa State University dairy. The heifers were allowed 4 d to adjust to the tie stalls before the trial began. Feed intake data were collected throughout the remainder of the trial. The cows were fed a total mixed ration (Table 3.1) once in the morning, between

0700 and 0800 h, and once in the evening, between 1800 and 1900 h. Prior to the morning feeding, orts were collected and weighed. A washable bench scale (Ohaus Corporation, Florham Park, NJ) was used to weigh feed and orts. The total mixed ration (TMR) offered to the cows was sub-sampled every 3 d during the course of the transition period, at the morning feeding. A 177 ml Whirl-pak bag of the TMR was frozen for future analysis.

Three days prepartum, cow 1847 was moved to an individual pen in the maternity barn to calve. Intake was measured throughout the time in maternity barn. Cow 1847 calved on May 14, 2001. Five days prepartum, 1854 was moved to the maternity barn and she calved on May 31, 2001. Cows were moved back into tie stalls in the east wing 1 d postpartum.

Feed Sample Preparations

Samples of individual ingredients (whole cotton seed (WCS), soybean meal (SBM), and cracked corn) and the composite TMR were collected, dried at 55° C for 12 h, and ground to 0.5 mm in a Wiley laboratory mill (model 4, Arthur H. Thomas Co., Philadelphia, PA; Marten and Barnes, 1980). Samples were collected once and used throughout the in vitro study. While not included in the TMR, phytic acid (Sigma, St. Louis, MO) was also included in the study to provide a known phytate concentration. Each feed and the phytic acid was weighed in triplicate (0.2500 to 0.2590 g; Marten and Barnes, 1980) into a pre-weighed 50 ml Nalgene incubation tube.

Table 3.1. Nutrient value of components of the total mixed ration. Values for alfalfa haylage, corn silage, and alfalfa hay are analyzed values. Values for corn grain ground, corn gluten feed, cottonseed, and soybean meal are reported values from the Michigan State University Spartan Feed Program.

Composition	lbs DM	% DM[1]	Nel (Mcal/lb)	CP (%DM)	UIP (%CP)	NDF (%DM)	Ef \NDF (%DM)	ADF (%DM)
Feed								
Alfalfa Haylage	5.50	38.40	0.67	22.30	19.70	42.40	42.40	31.20
Alfalfa hay, early bloom	12.52	87.50	0.66	24.50	28.00	37.30	37.30	31.20
Corn Silage	17.92	43.90	0.61	9.60	40.00	50.90	50.90	31.00
Corn grain ground	26.97	88.00	0.90	10.00	50.00	9.00	2.30	3.00
Corn gluten feed, wet	16.34	40.00	0.87	23.00	35.00	45.00	11.30	14.00
Whole cottonseed w/ lint	13.16	92.00	1.01	23.00	45.00	44.00	22.00	34.00
Soybean meal	3.64	89.00	0.91	55.00	35.00	10.00	2.50	6.00
Mineral mix[2]	3.96	96.90	0.00	0.00	0.00	0.00	0.00	0.00
Totals (wt)	100.00							

[1] DM = dry matter, NEl = net energy, lactation, CP = crude protein, UIP = undegraded intake protein, NDF = neutral detergent fiber, Ef NDF= effective neutral detergent fiber, ADF = acid detergent fiber.

[2] DM = 96.9%, calcium = 21.21%, phosphorus = 3.08%, magnesium = 2.74%, potassium = 0.09%, sodium = 4.74%, chlorine = 3.43%, sulfur = 0.19%, cobalt = 5.6 ppm, copper = 296 ppm, iron = 5735 ppm, iodine = 14.2 ppm, manganese = 1226 ppm, selenium = 18.23 ppm, zinc = 2462 ppm, vitamin A = 58 kilo-International Units (kIU)/#, vitamin D = 12.5 kIU/#, and vitamin E = 311kIU/#.

Collection

Rumen samples were collected from each heifer every third day beginning April 30, 2001. April 30, 2001 corresponded to d -14 and d -29 for cows 1847 and 1854, respectively. The sampling period was d -14 to d 28. Rumen fluid was also sampled and in vitro incubation conducted in a post-transition period. Post-transition samples were first collected on d 64 for 1847 and d 48 for 1854. These collections were conducted to compare the transition period and a post-transition period. On each collection day, rumen fluid samples were collected prior to the morning feeding, at approximately 0700 h. Rumen fluid samples from each cow were collected in a 7.57 L Rubbermaid thermos that was preheated by filling with warm water. Immediately prior to collection, the water was emptied into a bucket. The fistula of the cow was removed from the canula by gently pushing in, twisting sideways and

then removing the canula from the rumen. The fistula was then placed in the bucket of warm water to maintain flexibility. Using a gloved hand, a small plastic cup was inserted into the rumen and lowered beneath the rumen mat to the liquid portion. This liquid was removed and placed in the warmed thermos. This method of collection was repeated until at least 500 ml of rumen fluid was obtained. The fistula was gently placed back in the canula, double checked to ensure proper placement and rinsed. The thermos was transported to the laboratory for rumen fluid incubation.

Incubation

In vitro procedures of McDougall's (1948) were followed. Buffer solution contained:

9.8 g/L $NaHCO_3$
9.3 g/L Na_2HPO_4 $7H_2O$
0.57 g/L KCl
0.12 g/L $MgSO_4$
0.04 g/L $CaCl_2$

All ingredients were combined and diluted to 1 L volume with deionized water (McDougall, 1948). The solution was capped with parafilm and left to stir overnight. Carbon dioxide was bubbled through the buffer solution for 2 h prior to use. Twenty-five ml of buffer solution was added to each Nalgene tube 30-45 min prior to rumen fluid collection and placed in the 39° C water bath to warm (Marten and Barnes, 1980).

The pH of the rumen fluid from each cow on each collection day was determined prior to incubation with a pH meter (model 250 Denver Instruments, Arvada CO). A sample of rumen fluid from each cow was stored each day, for future alkalinity analysis. Alkalinity was determined for each sample of rumen fluid using a titration method (APHA et al., 1998), by adding 0.1 N hydrochloric acid to 50 ml of rumen fluid until the pH reached 4.5 (method # 2320 B, APHA et al., 1998). Alkalinity was calculated as follows:

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times N \times 50{,}000}{\text{ml sample}}$$

where:

A = ml standard acid used and
N = normality of standard acid (APHA et al., 1998).

$$50{,}000 = \frac{5 \text{ mg } (100;\ \text{atomic wt of } CaCO_3)}{1 \text{ mL} \times 0.1 \text{ N}}$$

The collected rumen fluid was blended in a Waring blender (model 51BL31, New Hartford, CT) for 15 sec to assure even distribution of microbes, feed particles and other rumen fluid contents. The rumen fluid was filtered through four layers of cheese cloth into a 200 ml Erlenmeyer flask. Carbon dioxide (CO_2) was bubbled through the rumen fluid. Five ml of rumen fluid was added to each Nalgene tube containing the feed samples and the warmed buffer solution. Five tubes were filled at a time to avoid over-oxygenating the microbes. Carbon dioxide was bubbled through the tubes to maintain anaerobic conditions. Tubes were immediately capped with a stopper (#5 ½) fitted with a one-way gas release valve. Racks of tubes were placed in a 39° C water bath incubator. Tubes were incubated for either 15, 30, 60, or 120 min. At the end of each period and at 90 min, the tubes were gently swirled, careful to avoid getting solids on the stopper or upper-wall of the tube. A single incubation was conducted to determine phytate concentration of each feed, TMR and phytic acid at time zero.

At the end of the designated time the tubes were removed from the water bath incubator, dried with a paper towel, and the stopper was removed. Each tube was weighed and the pH recorded (model 250 Denver Instruments, Arvada CO). Next, hydrochloric acid (12.1 N) was added drop-wise until the tube contents reached a pH of <2 (Morse et al.,

1992). The tube was weighed again and the contents transferred into 50 ml conical storage tubes, capped and placed in a refrigerator for future phytate phosphorus analysis.

Phytate Phosphorus Determination

Following incubation, each tube was centrifuged (approximately 1912 *g*) for 10 min (model 225 Fisher Scientific, Bedford MA; Figure 3.1). The three tubes relating to a particular feed, incubation time, sample date, and cow were amalgamated by filtering through Whatman 54 filter paper. Inositol-phosphates should be retained in the solids due to the attachment of the inositol-phosphates to cations, proteins or other substances that bind them to the solids. Filtered solids were rinsed once with deionized water and once with acetone. The filtrate was placed back in the 50 ml tube and stored. The filter paper and filtered solids (containing inositol-phosphates) were loosely rolled and placed in a 15 ml conical storage tube for drying. Once dry, 10 ml of a 1.2% hydrochloric acid, 10% sodium sulfate solution was added. The tubes were capped and placed on a wrist action shaker (Burrell Corporation, Pittsburg PA) for 2 h. The hydrochloric acid in the solution allowed the inositol-phosphates to be released into the liquid medium. The samples then settled overnight in the solution to further hydrolyze the di- through hexaphosphates from the solids. Next, samples were centrifuged for 10 min at approximately 1762 *g*. The supernatant was transferred to a 50 ml Nalgene in vitro tube and the di- through hexaphosphates were precipitated by adding 5 ml of a 0.4% ferric chloride, 0.6% hydrochloric acid, and 5% sodium sulfate solution to the supernatant sample. They were then vortexed and placed in a 100° C oven for 1 h (Morse et al., 1992). At the end of the hour the samples were immediately placed on ice for 30 min. The white precipitate that formed contained the di- to hexaphosphates (Early, 1944). The samples were centrifuged for 7 min at approximately

2550 *g*, to collect the precipitate as a pellet in the bottom of the tube. The supernatant was decanted and the pellet was rinsed with a 0.6% hydrochloric acid, 5% sodium sulfate solution to wash away any free phosphates that might still be in the sample. Centrifugation and rinsing was repeated three times. Liquid was decanted and the pellet removed and placed into a 30 or 50 ml beaker to be dried overnight at 100° C for total P analysis. Beaker contents were ashed in a muffle furnace for 4 h at 550° C (Earley, 1944; Morse et al., 1992).

Ash was digested to hydrolyze all phosphates from the ferric-inositol precipitate. Ash was digested by adding 5 ml hydrochloric acid (4 N) to each beaker along with two to three drops of nitric acid (15.8 N). By covering the beaker with a watch glass and heating on a stir plate, phosphate bonds were broken to release free phosphates into the solution. Once cooled, all liquid in each sample was brought to volume with deionized water in a 25 ml volumetric flask. The sample was then mixed and placed in a vial for storage.

A 1 ml aliquot of the stored sample was transferred to a 10 ml volumetric flask. Two ml of a molybdovanadate reagent was added and the sample was brought to volume with deionized water and mixed. The P in the digest combined with molybdovanadate reagent, producing a yellow color that was read photometrically at 400 nm on a spectrophotometer (model DR/4000 Hach, Loveland, CO; A.O.A.C., 2001). The spectrophotometer reported the absorbance from the yellow color in each sample. A standard curve of known concentrations of P plotted against absorbance was utilized to determine mg of P present in each sample. The molybdovanadate reagent was created by heating 10 g of ammonium molybdate in 100 ml of deionized water and heating 0.5 g of ammonium

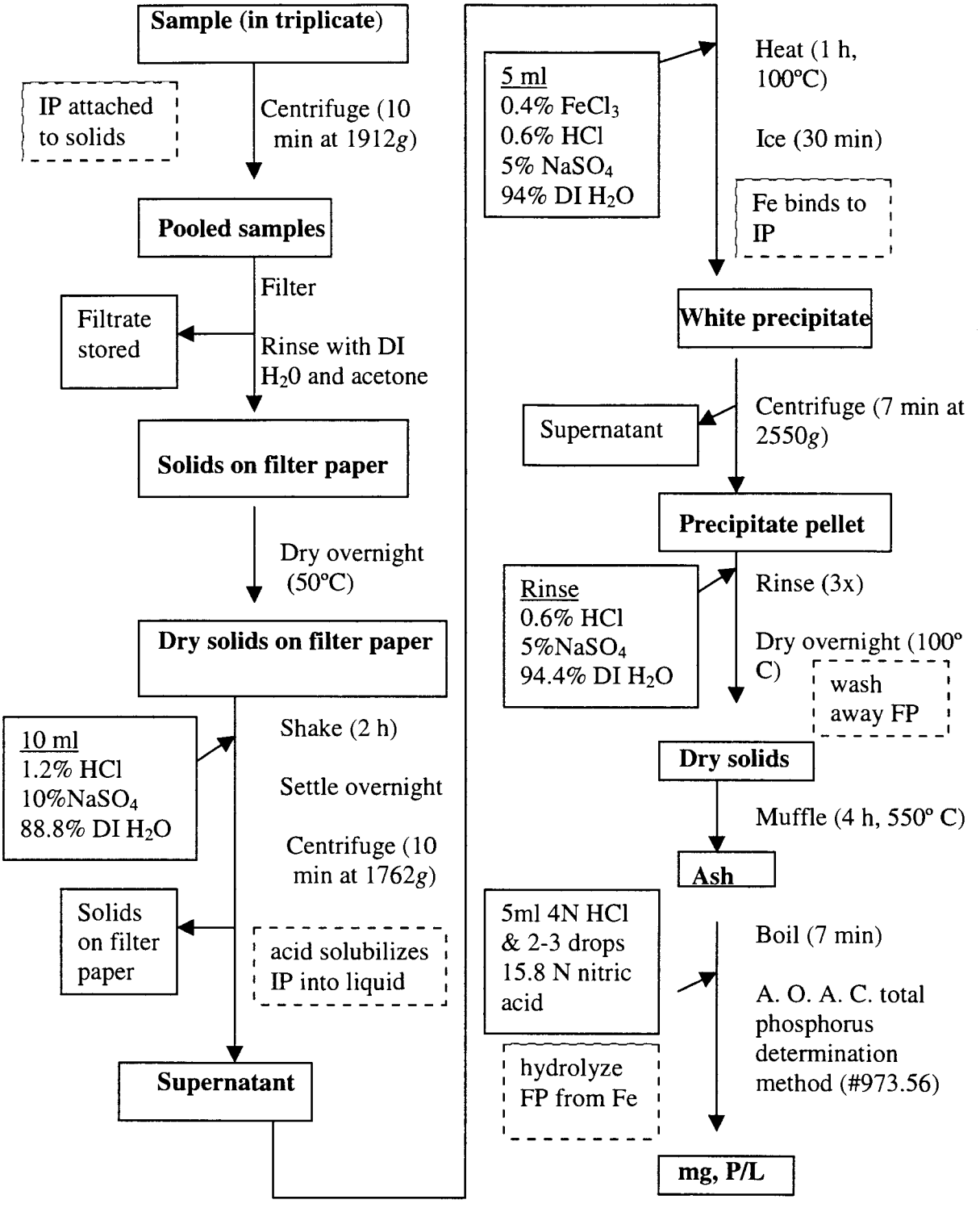

Figure 3.1. Laboratory procedure flow diagram to determine phytate-P present in previously incubated in vitro rumen fluid sample. IP = inositol phosphates, FP = free phosphates.

metavanadate in 150 ml of deionized water. Perchloric acid was added (112.5 ml) to the metavanadate when cooled. Following, the ammonium molybdate was added slowly. This was brought to a final volume (1 L) and stirred for 1 h.

Corn, whole cottonseed, soybean meal, and the total mixed ration were analyzed for phytate-P content. The same batch of each feed used in the in vitro incubations was analyzed. Samples were weighed out in triplicate (0.5 g) into 50 ml conical tubes. Samples were analyzed for phytate as was described previously (Figure 3.1), starting at the shaking step. A separate set of similar samples were analyzed for the solubility of the phytate-P in water. Samples were weighed out in triplicate into 50 ml conical tubes and 20 ml of deionized water was added. Samples were shaken for 1.5 h. Then the water was decanted and the solids and the water were individually analyzed for phytate-P, as was previously described.

Statistical Analysis

Days pre- and postpartum were allotted into three periods. Period one was defined as d -14 through parturition, period two was defined as d 1 to d 28, and period three was a post-transition period, d 47 to d 56 and d 64 to d 73, respectively, for cows 1854 and 1847. Data were expressed as percent phytate-P loss. Days of collection within each period were treated as repeated measures. Four methods of data analysis were employed to determine if significant differences existed for phytate-P loss across the transition period for each feed. The objective was to achieve a single point that was representative of the phytate-P loss across the four incubation times. These points were examined for cow, feed, and period effects. The first and second methods used the outest function in the regression procedure of

SAS (SAS, 1999) to calculate a regression line of percent phytate-P loss over the incubation periods. The first method, the regression coefficient (slope), and the second method, the intercept of each line, were then used as single points to be analyzed for cow, feed, and period effects. The third method utilized the regression line's slope and intercept to calculate a midpoint (regpt) of the regression line with the following equation;

regpt = intercept + (slope x 60 min).

In the fourth method, a midpoint (midpt) or weighted average was calculated from the percent phytate-P loss across the incubations. To calculate the midpt the following equation was used;

(((1 min/120 min) x %P loss at time 0) + ((15 min/120 min) x %P loss at 15 min) + ((30 min/120 min) x %P loss at 30 min) + ((60 min/120 min) x %P loss at 60 min) + (%P loss at 120 min)) / 2

One min was utilized instead of time 0 in the equation. The incubation times were weighted to account for the difference of time span between the incubation times (0, 15, 30, 60 and 120 min).

The regression coefficient (slope) and the intercept of the regression line calculated by SAS, along with the midpoint calculated from the regression line (regpt), and the weighted midpoint (midpt) were analyzed using the general linear model procedure of SAS (SAS, 1999). Data were sorted by feed and days within each period were treated as repeated measures. The model statement included period, cow, and dry matter intake as the independent variables and either slope, intercept, regpt, or midpt as the dependent variable when data were analyzed. Data were then analyzed to determine significant differences between feeds. In the model statement, feed was the only independent variable and either slope, intercept, regpt or midpt was the dependent variable.

Dry matter intake data were also analyzed with days pre- and postpartum allotted into three periods. Individual intake days within a period were treated as repeated measures for each period. Periods were defined as was described previously. Dry matter intake was then reanalyzed with days pre- and postpartum allotted into four periods. In this analysis period one was d -14 through d -3, period two was d -2 through d 2, period three was d 3 through d 28, and period 4 was the post transition period or d 47 to d 56 and d 64 to d 73, respectively for cows 1854 and 1847.

Results

Cows were maintained on a lactation diet throughout the entire trial, yet the analysis of the diet offered differed slightly throughout the trial (Table 3.2). Percent moisture, crude protein (CP), net energy for lactation (NEl), net energy for maintenance (NEm), net energy for growth (NEg), calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) were not significantly different across periods. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) showed a significant period effect (Table 3.2). Average P concentration in the diets fed was 0.595 ± 0.081 (mean ± SD) percent on a DM basis (Figure 3.2). Percent P content of the analyzed diets was significantly different across period ($P = 0.0019$). Sodium, NEg and moisture content of the analyzed diet were significantly different than the formulated diet ($P = 0.0075$, 0.0487, and 0.0111, respectively).

Dry matter intake had no significant cow effect ($P = 0.2669$) and no significant period by cow interaction ($P = 0.8284$). Dry matter intake for period one was significantly different than periods two and three (Figure 3.2). When DMI was re-analyzed with four periods, significant period ($P < 0.0001$) and cow ($P = 0.0330$) effects were observed (Figure 3.3).

Periods one and two were different from all other periods, but period three and four were not different.

Table 3.2. Nutrient composition of lactating dairy cow diets, expressed on a dry matter basis. Formulated column represents values, used to formulate a total mixed ration. Analyzed columns represent least squares means of values from subsamples collected during individual periods and analyzed by proximate analysis.

	Periods [1]	Period 1	Period 2	Period 3	
Analyte[2]	Formulated	Analyzed	Analyzed	Analyzed	P > F
Moisture (%)	38.800	52.279	54.474	53.649	0.4847
CP (%)	17.490	19.966	20.533	19.250	0.0584
ADF (%)	18.950	21.657[a]	19.4857[b]	18.467[b]	0.0221
NDF (%)	32.060	36.129[a]	31.671[b]	32.000[ab]	0.0409
NEl (Mcal/lb)	0.7800	0.7457	0.7614	0.7533	0.0916
NEm (Mcal/lb)	0.8200	0.7457	0.7729	0.7600	0.0521
NEg (Mcal/lb)	0.5400	0.4700	0.4929	0.4833	0.0873
Ca (%)	1.2400	1.2843	1.4086	1.4667	0.5058
P (%)	0.5700	0.5446[a]	0.6527[b]	0.5867[a]	0.0019
Mg (%)	0.3700	0.3286	0.3100	0.3300	0.3741
K (%)	1.0900	1.5414	1.5029	1.2967	0.0836
Na (%)	0.2600	0.3647	0.3706	0.3850	0.9776

[1]Period 1 = d -14 prepartum to d 0 (parturition), period 2 = d 1 postpartum to d 28 post-partum, period 3 = d 47 postpartum to d 56 postpartum and d 64 postpartum to d 73 postpartum, respectively for cows 1854 and 1847.

[2]CP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber, NEl, NEm, and NEg = net energy lactation, maintenance, and growth, respectively. Ca = calcium, P = phosphorus, Mg = magnesium, K = potassium, Na = sodium.

[a, b] Numbers within row with different superscripts differ ($P < 0.05$).

Rumen pH was 6.207 ± 0.299 for cow 1854 and 6.416 ± 0.303 for cow 1847 (least squares mean ± standard deviation (SD), Table 3.4). There was a significant cow effect (P = 0.0260) for rumen pH, but periods were not significantly different (P = 0.1964). A significant period effect for rumen alkalinity at pH 4.5 was observed (P = 0.0005; Figure 3.5). No significant cow effect was observed for rumen alkalinity (P = 0.1334; SD for 1853 = 577.17 and for 1847 = 573.851).

Phytate-P present in the samples before incubation was: corn = 0.15% of DM, soybean meal = 0.45% of DM, total mixed ration = 0.28% of DM, and whole cottonseed =

0.44% of DM (Table 3.3). Results depicting the concentration of phytate that is potentially soluble in water are reported. Phytate-P was 41 to 68% soluble in water in the feeds analyzed (Table 3.3).

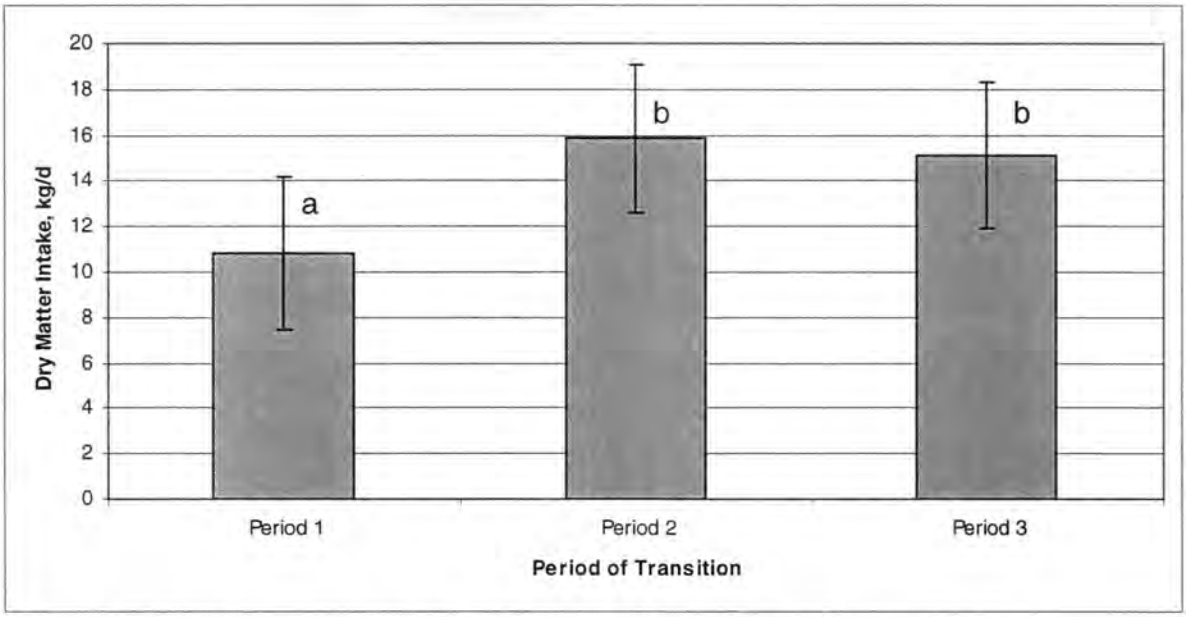

Figure 3.2. Least squares means of dry matter intake (DMI) (kg/d) sampled across the transition period. $P = 0.0001$ for period effect and $P = 0.2669$ for cow effect. Period 1 = d -14 prepartum to d 0 (parturition), period 2 = d 1 postpartum to d 28 postpartum, period 3 = d 47 postpartum to d 56 postpartum and d 64 postpartum to d 73 postpartum, respectively for cows 1854 and 1847. Bars represent standard deviations. Differences across periods ($P < 0.05$) are represented by different superscripts ($^{a, b}$).

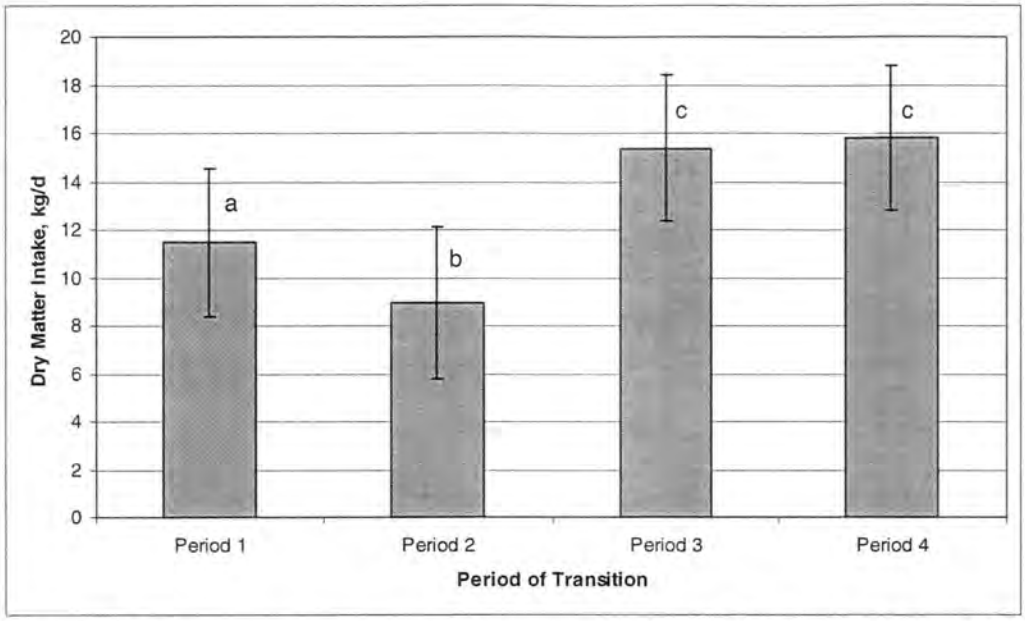

Figure 3.3. Least squares means of dry matter intake (DMI) (kg/d). $P = < 0.0001$ for period effect and $P = 0.0330$ for cow effect. Period 1 = d -14 prepartum to d 0 (parturition), period 2 = d 1 postpartum to d 28 postpartum, period 3 = d 47 postpartum to d 56 postpartum and d 64 postpartum to d 73 postpartum, respectively for cows 1854 and 1847. Bars represent standard deviations. Differences across periods ($P < 0.05$) are represented by different superscripts ($^{a, b}$).

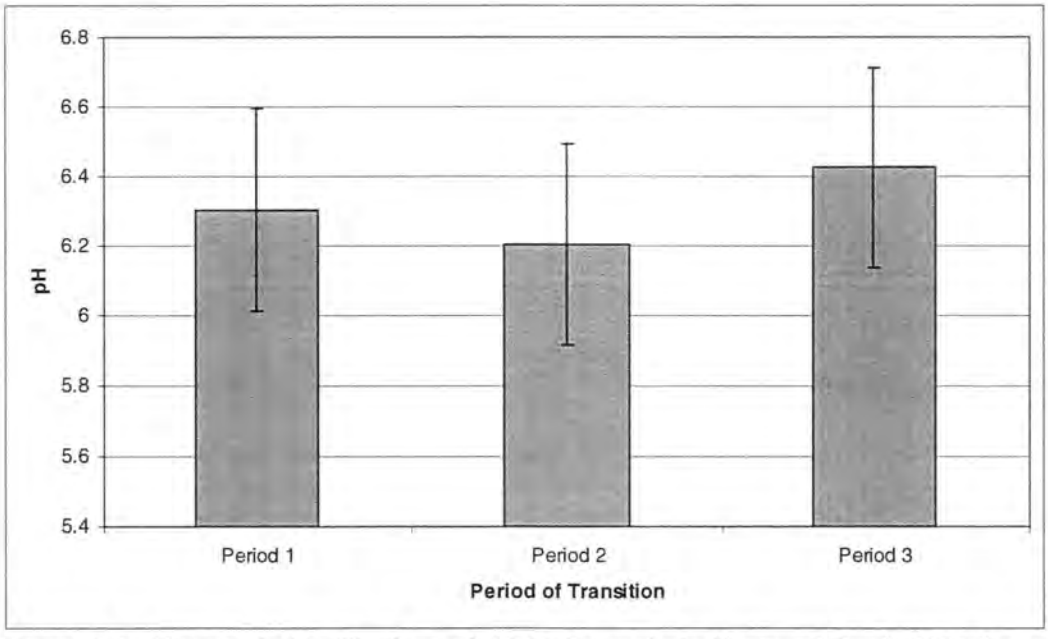

Figure 3.4. Rumen fluid pH. $P = < 0.1964$ for period effect and $P = 0.0324$ for cow effect. Period 1 = d -14 prepartum to d 0 (parturition), period 2 = d 1 postpartum to d 28 postpartum, period 3 = d 47 postpartum to d 56 postpartum and d 64 postpartum to d 73 postpartum, respectively for cows 1854 and 1847.

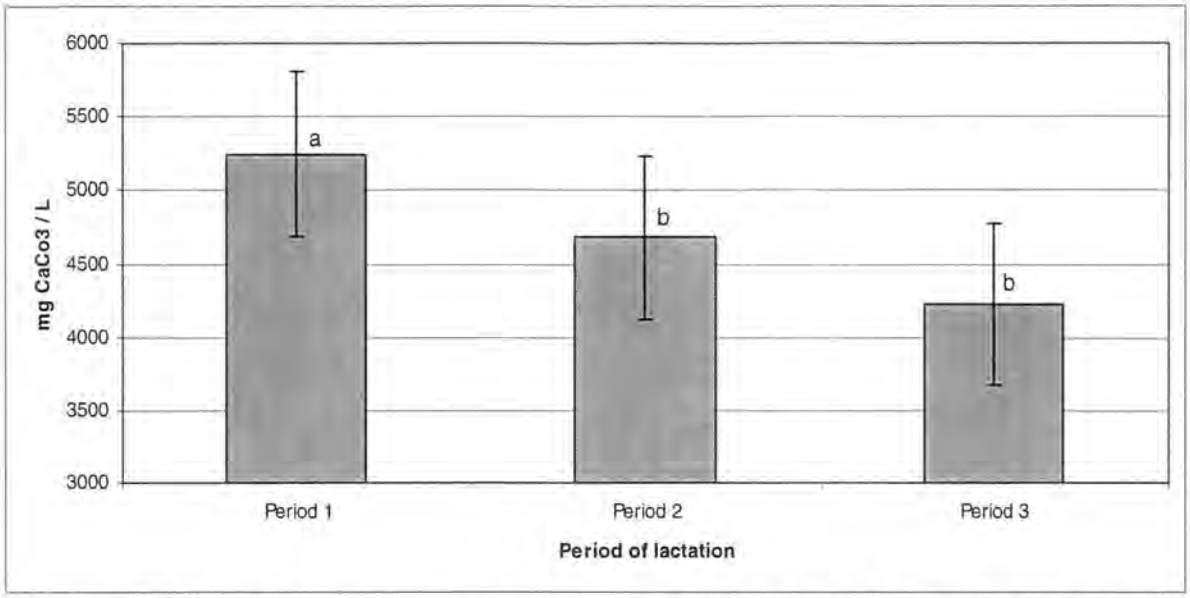

Figure 3.5. Rumen alkalinity to pH 4.5 measured as mg $CaCO_3$ / L. $P = < 0.0005$ for period effect and $P = 0.1334$ for cow effect. Period 1 = d -14 prepartum to d 0 (parturition), period 2 = d 1 postpartum to d 28 postpartum, period 3 = d 47 postpartum to d 56 postpartum and d 64 postpartum to d 73 postpartum, respectively for cows 1854 and 1847. [a, b] Periods with different superscripts differ ($P < 0.05$).

Table 3.3 Concentration of phytate-phosphorus in corn, soybean meal, total mixed ration and whole cottonseed before and after exposure to water.

	% Phytate-P	% of total
Corn	0.1474	
Water Sol[1]	0.1107	68.321
Water Ins	0.0006	0.426
SBM[2]	0.4550	
Water Sol	0.3270	67.123
Water Ins	0.1858	40.831
TMR	0.2802	
Water Sol	0.1203	41.248
Water Ins	0.1578	56.328
WCS	0.4410	
Water Sol	0.2426	59.008
Water Ins	0.2197	49.810

[1] Water sol = water analyzed for phytate-P after feed was mixed with water. Water insol = feeds analyzed for phytate P after being mixed with water.

[2] SBM = soybean meal, TMR = total mixed ration, WCS = whole cottonseed

Least squares means for disappearance of phytate-P from incubated solids were expressed as the percentage of initial phytate-P present in each feed. Data were first analyzed using the calculated regression coefficient (slope) for each regression line. Regression lines were determined for each feed on individual collection days. No significant period (Table 3.4) or cow effect was observed.

The intercepts of the calculated regression lines were then analyzed. A significant period effect was observed for phytic acid ($P = 0.0428$; Table 3.4) and a significant cow effect was observed for phytic acid and WCS ($P = 0.0094$, and 0.0001 respectively).

When data were also analyzed using regression points (regpt) phytic acid samples had a significant period effect ($P = 0.0196$). A significant cow effect was observed for phytic acid ($P = 0.0046$), WCS ($P = 0.0001$), and SBM ($P = 0.0166$).

When the midpoint data were analyzed a significant period effect was observed for phytic acid ($P = 0.0370$). There was also a significant cow effect for phytic acid ($P = 0.0080$) and SBM ($P = 0.0360$).

Discussion

The primary objective of the in vitro study was to determine the ability of the rumen microbial population present during the transition period to degrade dietary phytate P. Four incubation times were measured during the in vitro analysis (15, 30, 60, and 120 min) with the intention of selecting a single incubation time to analyze. The point analyzed needed to be post-lag time but prior to complete phytate P hydrolysis. This assumes that complete hydrolysis would have occurred. Unfortunately, no one time clearly met those requirements. Therefore, other methods of data analyses were used.

Table 3.4. Least squares means of percent phytate phosphorus loss analyzed as either the slope of the regression line (slope), the intercept of the regression line (interc.), the midpoint of the regression line (reg. pt.) or a weighted midpoint of the in vitro incubation times (mid. pt.) for cow and period effects.

		Period 1	Standard Deviations	Period 2	Standard Deviations	Period 3	Standard Deviations	P > F (period)	P > F (cow)
Corn	Slope	0.0574	0.1693	0.0428	0.1543	0.0158	0.1489	0.8539	0.6300
	Intercept	89.024	15.1540	91.892	13.8083	91.410	13.3272	0.8921	0.9229
	Reg. Pt.	92.469	9.4156	94.457	8.5796	92.356	8.2806	0.7717	0.4502
	Mid. Pt.	88.479	7.7699	89.610	7.1550	89.474	6.8705	0.9337	0.0981
Phytic acid	Slope	0.0060	0.1136	0.0221	0.1044	0.0004	0.1009	0.8494	0.4439
	Intercept	87.197[ab]	18.6776	75.234[a]	17.1646	91.760[b]	16.5661	0.0428	0.0094
	Reg. Pt.	87.554[ab]	15.2126	76.560[a]	13.9803	91.782[b]	13.4928	0.0196	0.0046
	Mid. Pt.	83.237[a]	13.3471	72.617[b]	12.3930	83.810a	11.9013	0.0370	0.0008
SBM	Slope	0.0782	0.1303	0.0126	0.1196	0.0250	0.1163	0.4334	0.9644
	Intercept	84.645	13.1248	87.616	12.0510	83.804	11.7160	0.6859	0.1198
	Reg. Pt.	89.335	8.4531	88.367	7.7618	85.305	7.5458	0.5225	0.0166
	Mid. Pt.	84.662	7.5360	83.105	6.9940	81.818	6.7590	0.7055	0.0036
TMR	Slope	-0.0221	0.2372	0.0484	0.2130	-0.0362	0.2140	0.6220	0.3442
	Intercept	80.646	26.3658	84.222	23.6685	101.51	23.7863	0.2216	0.7690
	Reg. Pt.	79.324	19.2280	87.127	17.2606	99.338	17.3465	0.1378	0.2729
	Mid. Pt.	78.532	15.5896	83.564	14.1373	88.540	14.1330	0.4751	0.1609
WCS	Slope	0.0307	0.1236	0.0738	0.1136	0.0816	0.1096	0.6035	0.2264
	Intercept	79.945	12.8725	77.552	11.8298	69.320	11.4173	0.1433	0.0001
	Reg. Pt.	81.785	9.6706	81.986	8.8873	74.217	8.5774	0.0890	0.0001
	Mid. Pt.	76.038	10.0955	77.834	9.3741	70.802	9.0020	0.1835	0.1704

[a, b] Numbers within a row with different superscripts differ ($P < 0.05$)

[1] Period 1 = d -14 prepartum to d 0 (parturition), period 2 = d 1 postpartum to d 28 postpartum, period 3 = d 47 postpartum to d 56 postpartum and d 64 postpartum to d 73 postpartum, respectively for cows 1854 and 1847.

The most accurate point does not need to show what happened across times of incubation. It does not need to express how quickly phytate was hydrolyzed. It only needs to depict or represent how much phytate P was hydrolyzed at a certain point in time. Use of the intercept was best suited to meet these criteria. The intercept's accuracy of representation is increased by the fact that no difference between slopes of the regression lines within each

feed was observed. Therefore, intercept is not changed across periods by changing slopes. A period effect can be explained by a difference of percent phytate P loss, not the rate of loss, as depicted by the slope.

Data analysis using the intercept of the regression line demonstrated a significant period effect for phytic acid and a significant cow effect was observed for phytic acid and WCS (Table 3.4).

These results do not make it possible to make a generalization for all feeds about how well phytate P is hydrolyzed by the microbes that are present in rumen fluid collected over the transition period. It appears that some phytate is hydrolyzed more readily in some feeds than others. For example, percent phytate hydrolyzed in corn was not different between incubation periods, with approximately 90% of phytate hydrolyzed in all periods. Phytic acid demonstrated period effects. Therefore, we must look at each feed and phytic acid individually.

In the current study 85 to 100% of phytate P in the corn was hydrolyzed with up to 2 h incubation. Morse et al. (1992) analyzed similar feedstuffs in an in vitro study. One of the feedstuffs they analyzed was hominy, which is hulled corn with the bran and germ removed. With 90% of phytate located in the germ of corn (Raboy, 1990), it seems that hominy should contain less phytate than corn where the germ is attached. The NRC (2001) lists hominy as containing 0.65% P, while corn grain (cracked, dry) only has 0.30% P. Morse et al. (1992) reported phytate P to be 73.4% of total P in hominy and 63.8% of total P in corn. Therefore, hominy contains a greater concentration of phytate P compared to corn. Morse et al. (1992) observed that 52.5% of phytate P in hominy had hydrolyzed after 2 h and 99.8% by 24 h. In the Morse study, a 1:4 rumen fluid to buffer ratio was used, whereas the current study used a

rumen fluid to buffer ratio of 1:5. Morse also used 12 times more feed than was used in this study, with only 5 times the rumen fluid/buffer mixture (Morse et al., 1992). The much larger amount of feed with an unequal increase in rumen fluid may explain why the hydrolysis of phytate P was slower in the Morse study compared to the current study.

In the current study 72 to 95% of phytate P was hydrolyzed from soybean meal (SBM) with up to 2 h incubation. Morse et al. (1992) also looked at the in vitro hydrolysis of phytate P from SBM and observed that 36.3% of the original phytate P in SBM had hydrolyzed after 2 h, and 100% of phytate P had hydrolyzed by 24 h. Han and Wilfred (1988) cultivated *Aspergillus Ficuum* phytase and hydrolyzed approximately half of soybean phytate during a 1 h treatment. At the end of 24 h incubation, approximately 85% of phytate in soybean meal was hydrolyzed (Han and Wilfred, 1988). Results from the current study concur with the phytate P hydrolysis of SBM from other studies.

In the current study 63 to 95% of phytate from whole cottonseed (WCS) was hydrolyzed with up to 2 h incubation. Morse et al. (1992) found that 35.2% of phytate P in cottonseed meal had hydrolyzed after 1 h and 99.9% after 24 h. Han and Wilfred (1988) observed that one third of phytate P in cottonseed meal hydrolyzed following a 1 h treatment and approximately 70% at the end of 24 h incubation. It appears that phytate P was hydrolyzed more readily in the current study after 1 h.

In the current study, an inconsistent pattern of phytate P loss occurred across the four incubations within a collection day. The inconsistent patterns within each day were not consistent across the transition period. Phytate P loss over the incubation period seemingly should increase as a linear or asymptotic function, yet this was not consistently observed. Morse et al. (1992) also reported some inconsistencies. For example, they reported the

following: after 1 h of incubation 90.6% of hominy phytate P was hydrolyzed, but after 2 h only 52.5% was hydrolyzed; after 2 h of incubation 48.7% of phytate P was hydrolyzed from cottonseed meal, but after 4 h only 17.8% of phytate P was hydrolyzed. These findings are similar to some of the inconsistent findings within this study. Percent phytate loss after a shorter incubation time would occasionally be greater than the percent phytate loss at a longer incubation time. This is counterintuitive. Theoretically, the percent of phytate P hydrolyzed should increase with the amount of time the phytate is exposed to the phytase enzyme within the rumen fluid. It is possible that a percentage of inositol-phosphates may be soluble in water. This may have attributed to the inconsistencies because the concentration of phytate that solubilized in the buffer may differ in different tubes. Many assumptions are made throughout the analysis of phytate-P (Figure 3.1) that may lead to inconsistencies. When the incubated solids are filtered it is assumed that the inositol-phosphates remain in the solid fraction; this may not be so. When the solids on the filter paper are exposed to an acid medium it is assumed all of the inositol-phosphates are hydrolyzed out of the solids and when the ferric-chloride precipitate is rinsed to remove free phosphates it is assumed the inositol-phosphates remain in the precipitate. These assumptions may be only partially true and lead to unknown errors in phytate analysis. Also, the concentration of phytate that is bound to other nutrients may have played a role in an inconsistent degradation. As those particles release the phytate due to different enzymes more phytate comes in contact with the phytase, thus releasing the phosphates.

Morse et al. (1992) suggested these inconsistent findings might be explained by variation in mixing of cottonseed meal prior to weighing it into flasks or inadequate mixing by swirling of fluid and concentrate. These may have attributed to the inconsistent findings

in this study as well. Ground whole cottonseed rather then cottonseed meal was used in this study, yet obtaining a homogeneous mixture while weighing out samples was a challenge. Inadequate mixing by swirling could also contribute to inconsistencies, yet careful attention was paid to the mixing. Another possible source of inconsistency may be the transferring of the incubated samples from the incubation tube to the storage tubes. This was performed carefully and consistently throughout the trial by empting each sample back and forth, three to four times between the in vitro tube and the storage tube. Then a small spatula was used to scrape any remaining solids from each in vitro tube. Yet, when transferring between tubes with no rinsing, not all solids will be transferred.

Another potential source of variation may be an inconsistent dispersion of the different microbial populations. Not all microbes produce phytase, therefore not all microbes cause phytate P to hydrolyze. It is possible that individual tubes may have had a sub-sample of the rumen fluid that did not contain as great a concentration of microbes that produce phytase as other in vitro tubes. This may lead to the inconsistencies that were observed.

Dry matter intake (DMI) was included in the model as a covariate because changes in DMI may have an effect on the rumen's microbial population health and the cow's ability to hydrolyze phytate P from feed. Analysis in the following discussion will relate to data that were analyzed using four periods of sample collection. These data would seem to be more relevant, versus the three periods, because it gives a clearer picture of what happened right around calving (d -2 to d 2). It is clear that DMI increases after calving (Figures 3.2 and 3.3). This trend has been well documented (Clark et al., 1986; Forbes, 1971; Kunz et al., 1985; Stanley et al., 1993). Data demonstrate a decrease in DMI prior to parturition. This decline is similar to other studies. Goff and Horst (1997) reported a sharp 30% decrease in DMI on d

-1 and -2. In the current study, a 22% decrease in DMI was observed with a 41% decrease for cow 1847 (data not shown). Bertics et al. (1992) reported a decrease in DMI that began a week prepartum with an overall decrease of 28% at d -1 compared to the DMI on d -17. The larger decline observed in cow 1847 versus other studies (Bertics et al., 1992; Goff and Horst, 1997; Grummer, 1995) may be explained by the large amount of grain in the diet. The cows in the current study were maintained on a lactation diet throughout the transition period (36% forage, 64% concentrates). Prepartum DMI depression may be greater for cows consuming high grain diets than for those consuming high forage diets (Coppock et al., 1972). Dry matter intake did not significantly decrease prepartum for cow 1854 (data not shown). This is not the typical scenario, but others have observed similar findings. Stanley et al. (1993) did not observe a decrease in DMI prior to calving, in beef cows, but did observe a small reduction on the day of calving.

Other aspects of the transition period may be responsible for the reduction in DMI prior to calving. Changing levels of hormones observed during the transition period may cause a decrease in DMI near calving (Grummer, 1995). Other variables such as environment (temperature, feed quality, and other cows), cow size, or cow health may also influence DMI.

Speculating why DMI decreased the week before parturition is difficult when one cow experienced the decrease while the other did not. The literature is not clear on why a decline is typically observed, how severe the decline is on average, or what effects a decreased DMI has on the cow before and after she calves. It seems likely that with so many factors contributing to a possible decrease in DMI, the occurrence and severity of a decrease in DMI depends on the individual cow.

Rumen alkalinity may have an effect on the health of certain microbe populations. Rumen alkalinity is defined as the rumen's ability to neutralize acid. Alkalinity is the capacity of a water specimen to react with hydrogen ions to reach pH 4.5 (Harris, 1991). Alkalinity is the measure of bicarbonates, carbonates and hydroxides, where pH is the measure of hydrogen ions (Harris, 1991). Alkalinity is what controls the ability to maintain pH. Very little research has been published on an optimal rumen alkalinity. The Standard Methods for the Examination of Water and Wastewater states that properly operating anaerobic digesters typically have supernatant alkalinities in the range of 2000 to 4000 mg $CaCO_3$ / L (APHA et al., 1998). The rumen alkalinities calculated in the current study were slightly higher than the 2000 to 4000 range, with period one alkalinity equal to 5249.8 mg $CaCO_3$ / L, period two alkalinity equal to 4680.5 mg $CaCO_3$ / L and period three alkalinity equal to 4225.9 mg $CaCO_3$ / L. Greater alkalinity implies a more basic rumen environment or a greater ability to maintain pH if acid is introduced into the rumen. In a typical feeding program, where a cow changes from a dry cow diet to a lactation diet after parturition, these alkalinities would probably be different. The change from a high forage diet to a high concentrate diet would most likely reduce the alkalinity of the rumen. This lower rumen alkalinity would presumably have an effect on the rumen microbes, most likely affecting phytate P hydrolysis. In the current study, diet was maintained throughout to determine if factors other than diet change affect phytate hydrolysis.

Phytic acid was used as a control to determine phytate loss from a known concentration of phytate. Phytic acid contained 51% phytate P when analyzed at time zero incubation. This low level of phytate present in samples gives an idea of how readily phytate can be hydrolyzed or solubilized. Samples incubated for t = 0 contained phytate or

feedstuffs. In addition, buffer, rumen fluid and hydrochloric acid were added before samples were analyzed for phytate P. Once incubated for up to 2 hr, 59 to 93% of the phytate P present in the samples were hydrolyzed or solubilized. At t = 0, approximately 49% of the phytate was already removed. Han and Wilfred (1988) observed that approximately 60% of phytate in soybean meal and 50% of phytate in cottonseed meal existed as water-soluble forms and were easily extracted in water by shaking for 1 h at room temperature. deBoland et al. (1975) observed that phytate P in corn germ was 86% water soluble, and in soybean meal was 69% water soluble. Therefore it seems likely that a portion of the phytate P in the feed ingredients could have started solubilizing as soon as the buffer was added to the feed ingredients prior to incubation, which was up to an hour before incubation was initiated. This is also evident when looking at the concentration of phytate in the samples at t = 0 incubation versus when the feeds were analyzed for phytate. At t = 0 incubation TMR contained 0.022% phytate while TMR contained 0.28% phytate in un-incubated samples. Corn contained 0.15% phytate P in un-incubated samples and 0.11% phytate at time zero incubation. Soybean meal contained 0.54% phytate P in un-incubated samples and 0.32% phytate at zero time incubation. Whole cottonseed contained 0.44% phytate P in un-incubated samples and 0.28% phytate at time zero. To determine the water solubility of the feeds phytate P was analyzed in the feeds after they were exposed to water for 1.5 h on a wrist action shaker. Table 3.3 depicts that, depending on the feed, between 40 and 50% of the phytate present in the feeds was hydrolyzed in water. Most likely phytate is hydrolyzed in water to other forms of inositol phosphates (mono through pentaphosphates). Therefore the analysis procedure used in the current study may not be analyzing phytate or hexaphosphates alone. deBoland et al. (1975) analyzed the accuracy of the ferric chloride

precipitation method to determine phytate P. They showed all of the myo-inositol phosphates from the di- through the hexaphosphate form insoluble iron complexes. However, the mono-, di-, and triphosphates are noticeably soluble and may not remain in the iron precipitate. Therefore the method utilized in the current study may be analyzing not only phytate, but also tetra- through hexaphosphates.

Another way of determining the accuracy of the phytate method used in the current study would be to compare the concentration of phytate observed in the current study with concentrations observed by other researchers. The phytate concentrations reported in the feeds used in the current study differ somewhat from those reported in other studies. Leske and Coon (1999), Nelson et al. (1968b) and Ravindran et al. (1994) reported the concentration of phytate in corn to be 0.17, 0.24, and 0.22%, respectively. In the current study it was determined to be 0.15%. Phytate was analyzed by slightly different methods in all four studies. Nelson et al. (1968b) reported phytate concentration most similar to those found in the current study. Nelson et al. (1968b) and Ravindran et al. (1994) used a method similar to the one used in the current study to form a phytate-iron precipitate. Leske and Coon (1999) used an ion-exchange method to extract phytate. Leske and Coon (1999) and Nelson et al. (1968b) determined phytate concentration in soybean meal to be 0.37 and 0.40% respectively. In the current study phytate concentration in soybean meal was 0.54%. Nelson et al. (1968b) reported the phytate concentration for whole cottonseed was 0.92%. The current study reported it as 0.44%, this is less than half of that reported by Nelson et al. (1968b). The total mixed ration (TMR) utilized in the current study had 0.28% phytate. Clark et al. (1986) fed a TMR containing 0.210 % phytate P. These values are similar to those obtained from the TMR used in the current study. This is a little surprising due to the

fact that Clark et al. (1986) fed a TRM that contained 50% corn silage. The TMR used in the current study contained only 18% corn silage. The increased concentration of corn grain found in the corn silage should have increased the concentration of phytate in the diet used by Clark et al. (1986). Clark et al. (1986) observed that corn silage contained only 0.0012% phytate P. They explain that the phytate content seems extremely low because usually corn silage contains a significant amount of grain. Clark speculates that it is likely that the microorganisms that provide for fermentation in the silo may also produce phytase or other hydrolyzing enzymes that cleave the phytate that is present in corn silage. Thus, virtually all of the phytate P is converted to inorganic P in corn silage, explaining the low concentration of phytate P. The TMR used in the current study contained 17.8% alfalfa (5.5% haylage and 12.3% early bloom). Nelson et al. (1968b) reported alfalfa meal to contain < .01% phytate P. The alfalfa that was utilized as a forage to replace the corn silage that has a low concentration of phytate explains why the TMR used in the current study has a greater concentration of phytate.

The objective of the in vitro study was to determine if a change in the cow's ability to utilize phytate P exists as the cow transitions from late gestation to early lactation. This objective was met but an overall conclusion cannot be made for all the feeds presented in the current study. The phytate concentration of phytic acid and WCS was hydrolyzed at different degrees in the different periods, yet no difference was observed in SBM, TMR, and corn. The solubility of phytate in water, the occasional counterintuitive result, and uncertainty of exactly what forms of inositol phosphates are measured dictates that this matter should be studied further.

CHAPTER 4: APPARENT DIGESTIBILITY OF PHOSPHORUS DURING THE TRANSITION PERIOD

Introduction

Apparent digestibility trials are used to determine the proportion of an ingested nutrient that is absorbed from the gastro-intestinal tract. Analysis of P begins in the mouth with intake and ends as excreted fecal P. Apparent digestibility of P is reported to range from 16 to 56.3%, depending on diet, stage of lactation, and P level of the diet (Clark et al., 1986; Knowlton and Herbein, 2002; Wu et al., 2000). Digestion trials have looked at the change in digestion over the stages of lactation. Most of these studies start either at the end of the transition period or after the transition period is complete. The objective of this study was to determine the cow's ability to digest total P across the transition period.

Materials and Methods

Apparent digestibility of P was measured concurrent with the in vitro study. To determine the apparent digestibility of P the following equation was used;

Apparent digestibility of P = (g P consumed – g fecal P) / g P consumed x 100%

A total mixed ration (Table 3.1) was offered twice daily between 0700 and 0800 h and again between 1800 and 1900 h. Feed bunks were swept clean before the morning feeding each day and ort weights were recorded. A washable bench scale (Ohaus Corporation, Florham Park, NJ) was used to obtain feed and ort weights.

Apparent digestibility collections were on d -6, and d 1, 23, 29, 31, 34, 63, 66, 69, and 72 for cow 1847 where prepartum days are denoted with negative values. Apparent

digestibility collections for the second animal (1854) occurred on d -23 and -16, and d 6, 12, 15, 17, 46, 49, 52, and 55.

Tie stalls were prepared for fecal collection by removing sand bedding and replacing with straw. Fecal collection pans were placed in the gutter behind each cow's tie stall. Empty pans were weighed on a washable bench scale (Ohaus Corporation, Florham Park, NJ), then placed in the alley. Following 12 h of collection, the pans were weighed, manure was thoroughly mixed with a rake, and manure pan sub-samples were collected (in a 177 ml Whirl-pak bag filled approximately three-fourths full) for future analysis. The pans were emptied, re-weighed and replaced behind the cows. After 24 h of collection, the pans were weighed, mixed and sampled again. During milking, the cows were observed and any fecal matter excreted was collected and added to the pan. At the time of fecal pan sampling, fecal grab samples were collected from each cow in a 177 ml Whirl-pak bag filled approximately three-fourths full. During the fecal collections, a small amount of straw could potentially contribute to the pan weight. Ratios of the DM of the pan samples to the DM of the grab samples were used to correct the DM of the pans for urine and straw contributions. Corrected pan weight was used to determine fecal mass excreted. Dry matter (DM) was determined for total mixed ration (TMR) samples by weighing before and after drying in a 55° C oven for 24 h (Aerts et al., 1974).

Phosphorus content of the TMR was determined using the A. O. A. C. method (method 965.17, A.O.A.C., 2001). Dry samples were ashed in a muffle furnace at 600° C for 8 h. Samples were weighed (100 mg) in duplicate into a 150 ml beaker. Twenty ml of 4 N hydrochloric acid and five to seven drops of nitric acid (15.8 N) were added to each beaker. Beaker contents were boiled for 7 min. Each sample was brought to volume, with deionized

water, in a 100 ml volumetric flask. The samples were filtered through Whatman 41 filter paper into a 125 ml Erlenmeyer flask. A 25 ml sub-sample was collected and stored in vials for future P analysis. Samples were prepared for P analysis as described previously. Absorption of samples was determined at 400 nm using a spectrophotometer (model DR/4000 Hach, Loveland, CO). A standard curve plotted mg P against absorbance and was utilized to estimate P content (mg/L) of each sample. From this value, percent P in TMR was calculated. Percent P in TMR was then multiplied by TMR DMI (kg/d) to estimate P consumed per day. Fecal DM and P were analyzed as described previously for TMR. Percent fecal P was multiplied by kg fecal DM excreted per day to calculate grams fecal P excreted per day.

Statistical Analysis

Data from the apparent digestibility study were analyzed as percent P digested. Sampling days were allocated to three periods: period one is defined as d -14 to d 0, period two is defined as d 1 to d 28, and period three is defined as d 47 to d 56 and d 64 to d 73, respectively for cows 1854 and 1847. Days within each period were treated as repeated measures. Data were analyzed using the general linear model procedure of SAS (SAS, 1999). The model statement included period and cow as the independent variables and apparent digestibility of P (%), DMI (kg/d), fecal P (% and g/d), fecal DM (kg/d), and P consumed (g/d) were the dependent variables. A second model included total mixed ration DM and P concentration as dependent variables with period as the independent variable.

Results

Cows were maintained on a lactation diet throughout the entire trial. Percent P was the only analysis that differed significantly in the diet composition, when analyzed across fecal collection periods (Table 4.1). Means in table 4.1 differ from means in table 3.2 due to collection days differing. Table 3.2 shows collection days as every third day of the transition period, whereas table 4.1 depicts only data from fecal collection days.

No significant period ($P = 0.0510$; Figure 4.1) or cow ($P = 0.8150$; Table 4.2) effects were observed for dry matter intake (DMI). Period had a significant effect on apparent digestibility of P ($P = 0.0268$; Figure 4.2 and Table 4.2), but no significant cow effect was observed ($P = 0.4650$). No significant cow or period effect was observed for fecal P (g/d; $P = 0.8459$). Fecal dry matter (DM; kg/d), P consumed (g/d), and TMR P (%) were all significantly different across periods (P = 0.0194, 0.0494, and 0.0123, respectively; Table 4.3), but fecal DM (kg/d) and P consumed (g/d) were not significantly different between cows (P = 0.6257 and 0.8619, respectively).

Table 4.1. Analyzed nutrient composition of the total mixed ration, expressed on a dry matter (DM) basis.

	Time [1]			
Analyte[2]	Period 1	Period 2	Period 3	P-value
DM (%)	52.71	56.23	54.55	0.1007
CP (%)	19.15	20.53	19.56	0.418
ADF (%)	23.10	19.10	18.47	0.071
NDF (%)	39.80	32.27	32.00	0.059
NEl (Mcal/lb)	0.730	0.760	0.750	0.084
NEm (Mcal/lb)	0.725	0.770	0.760	0.097
NEg (Mcal/lb)	0.450	0.490	0.483	0.132
Ca (%)	1.240	1.450	1.440	0.498
P (%)	0.545[a]	0.660[b]	0.596[a]	0.012
Mg (%)	0.320	0.320	0.330	0.758
K (%)	1.550	1.490	1.290	0.119
Na (%)	0.303	0.420	0.385	0.267

[1] Period 1 = d -14 prepartum to d 0 (parturition), period 2 = d 1 postpartum to d 28 postpartum, period 3 = d 47 postpartum to d 56 postpartum and d 64 postpartum to d 73 postpartum, respectively for cows 1854 and 1847.

[2] CP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber, NEl, NEm, and NEg = net energy lactation, maintenance, and growth, respectively. Ca = calcium, P = phosphorus, Mg = magnesium, K = potassium and Na = sodium.

[a, b] Numbers within a row with different superscripts differ ($P < 0.05$).

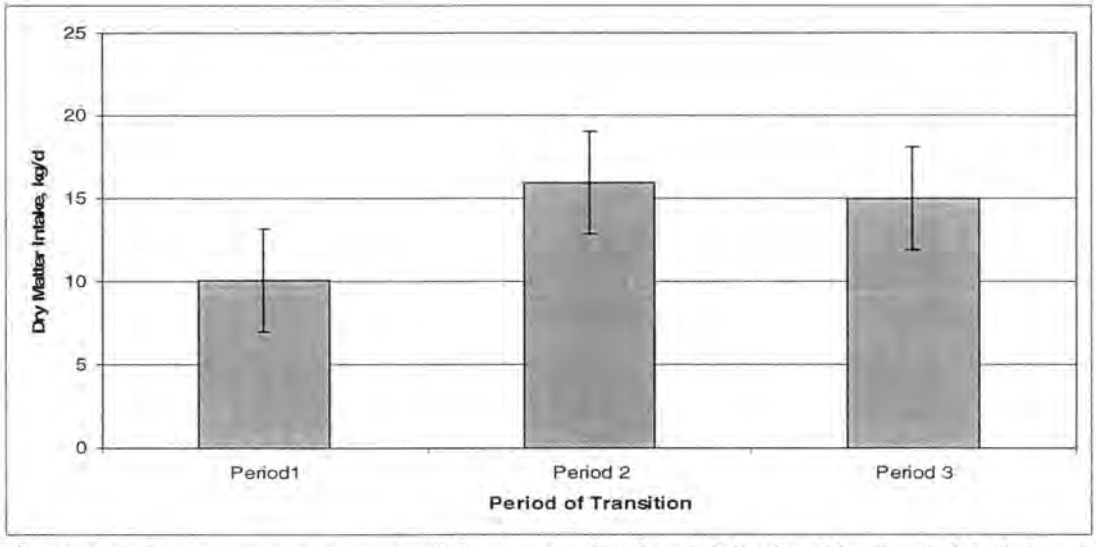

Figure 4.1. Least squares means of dry matter intake (DMI) (kg/d). $P = 0.0510$ for period effect and $P = 0.8150$ for cow effect. Period 1 = d -14 prepartum to d 0 (parturition), period 2 = d 1 postpartum to d 28 postpartum, period 3 = d 47 postpartum to d 56 postpartum and d 64 postpartum to d 73 postpartum, respectively for cows 1854 and 1847. Bars represent standard deviations.

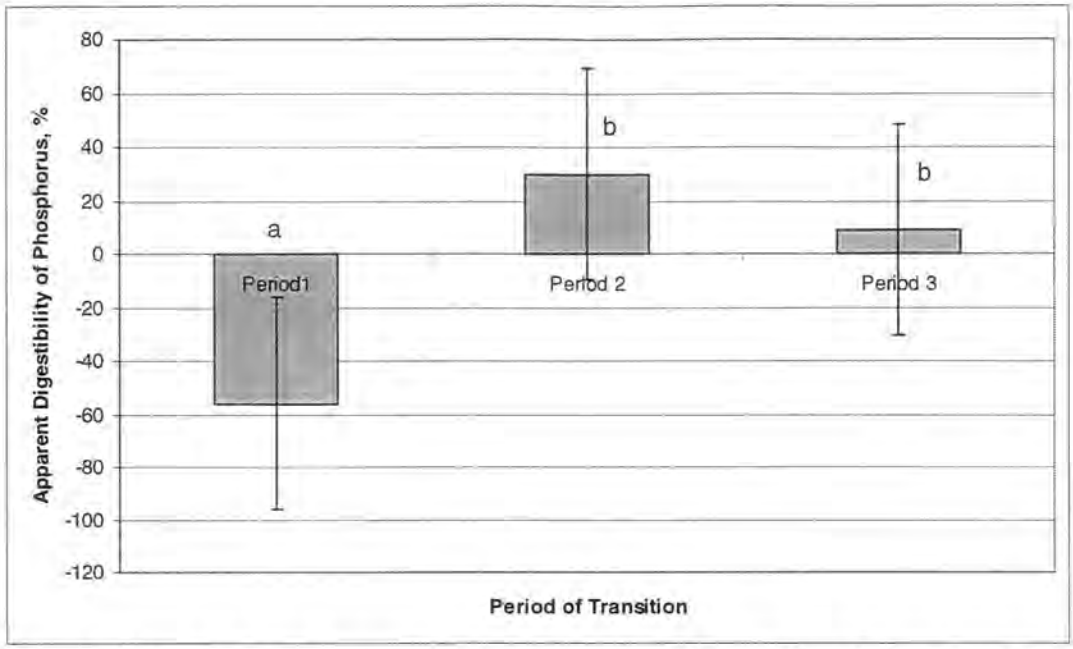

Figure 4.2. Least squares means of percent apparent digestibility of phosphorus measured across periods. $P = 0.0286$ for period effect and $P = 0.4650$ for cow effect. Period 1 = d -14 prepartum to d 0 (parturition), period 2 = d 1 postpartum to d 28 postpartum, period 3 = d 47 postpartum to d 56 postpartum and d 64 postpartum to d 73 postpartum, respectively for cows 1854 and 1847. Bars represent standard deviation. [a, b] Different superscripts differ ($P < 0.05$).

Table 4.2. Least squares means of parameters measured to calculate apparent digestibility of phosphorus during the transition and early lactation periods for dairy cows.

	Period1[1]	SD	Period 2	SD	Period 3	SD	P > F (period)	P > F (cow)
DMI, kg/d[2]	10.073	3.124	15.962	3.093	14.971	3.093	0.0510	0.8150
TMR % P	0.5446[a]	0.040	0.6596[b]	0.034	0.5956[a]	0.034	0.0123	
TMR % DM	52.279	2.160	56.235	1.870	53.649	1.870	0.1007	
P consumed, g/d	54.712[a]	25.83	104.93[b]	25.58	91.050[ab]	25.58	0.0495	0.8619
Fecal P, %	1.8165[a]	0.291	1.1481[b]	0.288	1.0876[b]	0.288	0.0074	0.0591
Fecal P, g/d	82.632	23.81	73.717	23.58	79.388	23.58	0.8459	0.3246
Fecal DM, kg/d	4.3565[a]	1.400	6.4024[ab]	1.380	7.4580[b]	1.380	0.0194	0.6257
App. Dig., %	-56.029[a]	39.92	29.815[b]	39.52	9.0520[b]	39.52	0.0286	0.4650

[1] Period 1 = d -14 prepartum to d 0 (parturition), period 2 = d 1 postpartum to d 28 postpartum, period 3 = d 47 postpartum to d 56 postpartum and d 64 postpartum to d 73 postpartum, respectively for cows 1854 and 1847. SD = standard deviation.

[2] DMI = dry matter intake, TMR P = total mixed ration % phosphorus, TMR DM = total mixed ration % dry matter, P consumed = phosphorus consumed, Fecal P = fecal phosphorus, Fecal DM = fecal dry matter, and App. Dig. = % apparent digestibility of P.

[a, b] Numbers within a row with different superscripts differ ($P < 0.05$).

Discussion

No significant cow differences were observed for apparent digestibility of P (%), fecal P (g/d), P consumed (g/d) or DMI (g/d). Apparent digestibility of P was -56.03% for days -14 through parturition, 29.85% for the days from parturition through 28 d postpartum, and 9.05% for the days in the post-transition period (d 47 to d 56 and d 64 to d 73, respectively for cows 1854 and 1847). These results differ somewhat from other published works (Clark et al., 1986; Knowlton and Herbein, 2002; Wu et al., 2000; Table 4.3).

A review of the literature revealed no estimates of apparent digestibility of P during the prepartum periods, in dairy cows. Apparent digestibility of P in period one (-56.03%) indicates that more P was excreted on collection days than was consumed by the cow. This is a counterintuitive finding. Explanations that may account for this finding will be discussed in the larger context of periods two and three.

Research has been conducted measuring apparent digestion of P in the early lactation phase. The current results are similar to the research of Clark et al. (1986), and Knowlton and Herbein (2002; Table 4.3). Clark et al. (1986) measured apparent digestibility of P at wk 4 of lactation, which would correspond with the end of period two in the current study. They observed 39.23% apparent digestibility of P, a difference of 9.38 percentage units from that observed in the current study. Knowlton and Herbein (2002) fed three levels of P (0.34, 0.51, and 0.67%) and collected data on weeks 3, 5, 7, 9 and 11 of lactation. Knowlton and Herbein's wk 3 collections best correlates to period two of the current study. In the Knowlton and Herbein (2002) study observed percent apparent digestibility of P was 53.3% when 0.34% P diets were fed, 22.7% when 0.51% P diets were fed, and 30.9% when 0.67% P diets were fed. In the current study, mean dietary P levels in period two were 0.65%, which

correlates best with the 0.67% dietary P group in Knowlton and Herbein's study. A difference of only 1.05 percentage units between the current apparent digestibility of P and Knowlton's data would indicate similar results. Wu et al. (2000) also fed three levels of dietary P (0.31%, 0.40% and 0.49%) over a complete lactation. The highest level, 0.49% P, will be discussed as it is the level most similar to that fed to the cows in the current study and Wu's wk 2 days in milk will be compared to period two of the current study. Apparent digestibility of P in period two of the current study is 17.6 percentage units less than apparent digestibility observed in wk 2 by Wu (47.7% vs. 29.8%; Table 4.3). The cows on the Wu study consumed 22.03 less g of P in wk 2 as compared to period two in the current study, and excreted 29.1 less g fecal P (Table 4.3).

Period three in the current study corresponds to Knowlton and Herbein's (2002) wk 7 data. In period three, percent dietary P was, on average, 0.58%. This was most like Knowlton's medium diet of 0.51% dietary P. In the current study, apparent digestibility of P in period three was 9.05% and Knowlton's apparent digestibility of P was 30.5%, a difference of 21.45 percentage units. Some explanations for this large difference may be the difference in DMI of the cows on the Knowlton study versus the cows on the current study. Knowlton's cows consumed 27.8 kg DM/d in wk 7 on the medium P diet, while the cows used in this study only consumed 14.9 kg DM/d in period three (Table 4.3). Different DMI led to a difference of 52.1 g P/d consumed. As the quantity of P consumed increases, the apparent digestibility of P also increases (Conrad, 1999). The greater intake in the Knowlton and Herbein (2002) study is likely due to the increased milk production reported. They reported 50.1 kg/d milk yield while milk yield in the current study was 28.3 kg/d. A decrease in DMI was observed between periods two and three in the current study. Period three (d 47

to d 56 and d 64 to d 73, respectively for cows 1854 and 1847) approaches peak lactation. It seems DMI should be increasing as milk production increases, but this was not observed. A clear explanation for this is not apparent. Wu et al. (2000) observed an apparent digestibility of P in wk 8 of lactation that was 39.15 percentage units greater than that observed in period three of the current study. In wk 8, Wu's cows consumed a greater concentration of P than the cows in the current study, yet they excreted less fecal P as compared to the current study. It is unclear why this is the case. In both the current study and Wu et al. (2000) cows digested and absorbed a greater concentration of P in period three compared to period two.

Apparent digestibility of P across the three periods differed significantly. The prepartum period was significantly different from both of the postpartum periods. Approximately 50% less P was consumed in period one than in period two, yet there was no significant difference in fecal P excretion. According to the 2001 NRC, in period one the heifer's P requirement was approximately 20 g/d. In periods, two and three, P requirements were approximately 54.5 g/d for both periods (NRC, 2001). Yet in each period the cows consumed approximately two times the level of their requirement (Table 4.2). The cows in the current study were maintained on a lactation diet throughout the trial. Therefore, concentration of P offered should not have differed as the cow's needs became greater. The cows were fed above their needs in the prepartum period, when P requirements were much less then during the postpartum period. Wu et al. (2000) reported that lower values for apparent digestibility of P occur when P is fed in excess of requirements, because the gut attempts to regulate P uptake according to body needs. Thus, the negative value observed in period one may be in part due to the reduced requirement in that period relative to P intake.

The research of Braithwaite (1983) may provide another potential explanation for the negative apparent digestibility of P in period one. He observed that sheep would resorb P from bones and soft tissue in late pregnancy in spite of a plentiful supply of dietary P. Therefore, if the cows were resorbing P from bone and soft tissue they could have been secreting more P than they were consuming, that is assuming the P resorbed was beyond the P requirements.

The level of calcium (Ca) in the diet could affect P digestibility. Clark et al. (1986) observed greater apparent digestibility of P with increased concentrations of dietary Ca. Others have observed, in high P diets, reduced P absorption when greater concentrations of Ca were fed (Field et al., 1983; Gowda et al., 1955; Mathur, 1953). Calcium levels in the current study ranged from 1.24% in period one to 1.45% in period three (Table 4.1). These values were considerably greater than the values (0.6 and 0.9%) reported by Clark et al. (1986). Therefore, if Field et al. (1983) are correct, the increase in Ca levels, could partially explain why Clark (1986) observed greater digestibility of P at wk 4 versus period two of the current study. Knowlton and Herbein (2002) and Wu et al. (2000) fed levels of Ca (0.73% and 0.65%, respectively) less than those fed in the current study. This could have contributed to the higher levels of apparent digestibility observed in those studies as compared to the current study.

The current study demonstrated that apparent digestibility is affected by period of transition. More research is needed to determine the apparent digestibility of P immediately prior to calving. It seems that a larger trial with more cows and more collection days may lead to a clearer conclusion of the effects of parturition on the apparent digestibility of P. When comparing apparent digestibility across studies, apparent digestibility postpartum is

typically between 30 and 40%. As cows approach peak lactation, apparent digestibility results were more variable between studies.

Table 4.3. Comparison of apparent digestibility of P from the current study with data from three other studies.

	Current Study	Clark, et al. 1986[1]	Knowlton, 2002[2]	Wu, et al. 2000[3]
P consumed, g/d				
Period 1	54.712			
Period 2	104.93	120.37	148	82.9
Period 3	91.05		143.6	117
Fecal P, g/d				
Period 1	82.632			
Period 2	73.717		101.7	44.6
Period 3	79.391		99.2	60.7
App. Dig. P, %				
Period 1	-56.029			
Period 2	29.815	39.23	30.9	47.4
Period 3	9.052		30.5	48.2

[1] Means of data across treatments for wk 4

[2] Data for period two of the current study corresponds with wk 3 at high dietary P (0.67 %P) and data for period three of the current study corresponds with wk 7 at medium dietary P (0.051 %P).

[3] Data for period two of the current study corresponds with wk 2 at high dietary P (0.49 %P) and data for period three of the current study corresponds with wk 8 at high dietary P (0.49 %P).

CHAPTER 5: PHOSPHORUS BALANCE OF THE TRANSITION COW

Introduction

Church and Pond (1998) explain that a balance trial is similar to a digestion trial except more information is required. The intent is to obtain an accurate measure of total intake and total excretion (and secretion) in order to determine if there is a net retention or loss of the nutrient in question. Researchers have reported typical P balances in the dairy cow. Values ranging from -26.2 to 22.2 g/d, depending on diet, have been reported (Knowlton and Herbein, 2002; Wu et al., 2000). These studies look at early, peak and late lactation, but neglect to analyze P balance prepartum. The objective of the current study was to determine the P balance of the transition dairy cow.

Materials and Methods

Phosphorus balance was measured concurrently with the in vitro and apparent digestibility of P studies (Chapters 3 and 4). To determine P balance for the prepartum period the following equation was used;

> P consumed, g/d = (urine P, g/d) + (fecal P, g/d) + (growth P, g/d) + (P for pregnancy, g/d).

To determine P balance for the postpartum period the following equation was used;

> P consumed, g/d = (milk P, g/d) + (urine P, g/d) + (fecal P, g/d) + (growth P, g/d).

A value of 1.4 g P/d was used as the P requirement for growth based on estimates made by the 2001 NRC model for first calf heifers with an average daily gain (ADG) of 0.88 g/d. A value of 5.1 g P/d was estimated as the P requirement for late pregnancy. The

pregnancy requirement was also calculated by the 2001 NRC model for late gestation (NRC, 2001).

Feeding methods and total mixed ration (TMR) analyses were described previously (Chapter 4, apparent digestibility of P). Total mixed ration samples collected and analyzed during the apparent digestibility study were utilized for the P balance study. Fecal collection days were d -6, and d 1, 23, 29, 31, 34, 63, 66, 69, and 72 for cow 1847, and d -23 and -16, and d 6, 12, 15, 17, 46, 49, 52, and 55 for cow 1854. Fecal samples collected and analyzed during the apparent digestibility study were used to determine P balance. Milk samples were not collected during the transition period, but at a later date, with samples from each cow and a bulk tank sample.

Urine samples were obtained once daily during each fecal collection, at the end of the first half of the 24 h period. Approximately three-fourths of a 177 ml Whirl-pak bag of urine was obtained. Urine samples were frozen for future P, dry matter (DM) and specific gravity analysis.

Urine and milk P concentrations were analyzed by the ascorbic acid, total P digestion method (HACH, 2001). In a 50 ml beaker each sample (urine or milk) was diluted 1:200 with deionized water. From this, a 5 ml sub-sample was transferred into 10 ml vials, in triplicate. Two ml of 1 N sulfuric acid and one potassium persulfate pillow (Permachem Reagents, Hach Europe, Düsseldorf, Germany) were added to each vial. Vial contents were digested for 30 min in a preheated (103-106° C) COD reactor (Hach Company, Loveland, CO). When vials were at room temperature 2 ml of 1 N sodium hydroxide was added. Next, a phosver 3 phosphate reagent powder pillow (Permachem Reagents, Hach, Loveland, CO) was added and allowed to dissolve for 30 sec. Absorbance was determined at 890 nm in the

Hach 3036 program on a spectrophotometer (model DR/4000 Hach, Loveland, CO). Phosphorus is determined when orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex, producing a molybdenum blue color (HACH, 2001). The intensity of the blue color represents the extent of absorbance. With a standard curve absorbance can be related back to total P.

Urine output was estimated using the equations of Holter (1992). The following equation predicts urine output for dry cows or heifers:

> urine output, kg/d = 212.1 + (0.8822 x DMI, kg/d) – (0.03452 x dietary DM, %) + (1.001 x dietary CP, %DM) – (216.4 x urine specific gravity) + (0.1414 x (apparently absorbed water, kg/d)); $R^2 = 0.92$ ($P < .005$).

Urine output for a milking cow is estimated as follows (R^2 =0.70, $P < .0001$):

> urine output, kg/d = 332.91 + (0.3885 x DMI, kg/d) – (0.2175 x milk, kg/d) – (0.0830 x dietary DM, %) + (0.524 x dietary CP, %DM) – (326.5 x urine specific gravity) + (0.2375 x (apparently absorbed water, kg/d));

In both equations apparently absorbed water is defined as total water intake (TWI) minus fecal water. Total water intake for both dry cows and milking cows can be predicted with the following equation ($R^2 = 0.99$, $P < .07$):

> TWI kg/d = 35.19 + (0.9823 x FWI, kg/d) – (0.011 x body weight, kg) + (1.0817 x DMI kg/d) + (1.184 x dietary CP, % DM) – (0.03881 x (dietary CP, % DM)^2) – (0.9963 x dietary DM, %) + (0.005488 x (dietary DM, %)^2);

Free water intake (FWI) can be predicted for dry cows with the following equation (R^2 = 0.64, $P < .001$):

> FWI, kg/d = -10.34 + (0.2296 x dietary DM, %) + (2.212 x DMI, kg/d) + (0.03944 x (dietary CP, % DM)^2);

and for milking cows ($R^2 = 0.19$, $P < .03$):

> FWI, L/d = -68.28 + (4.768 x dietary DM, %) - (0.03898 x (dietary CP, % DM)^2).

Fecal water for dry cows is defined by the following equations ($R^2 = 0.42$, $P < .05$):

$$\text{fecal water, kg/d} = 5.52 + (1.32 \times \text{DMI, kg/d}) + (0.0384 \times \text{dietary DM, \%});$$

and for milk cows ($R^2 = 0.74$, $P < .0001$):

$$\text{fecal water, kg/d} = 8.843 + (2.180 \times \text{DMI, kg/d}) - (0.2388 \times \text{dietary DM, \%}) - (0.2328 \times \text{milk, kg/d})$$ (Holter, 1992).

Total mixed ration crude protein was analyzed using a micro Kjeldahl procedure (A.O.A.C., 2001). Samples were dried at 55° C for 72 h and ground to 0.5 mm in a Wiley laboratory mill (model 4, Arthur H. Thomas Co., Philadelphia, PA). A 0.5 g samples of the dry TMR were digested in triplicate following addition of one st-auto Kjeldahl tablet (FistherTab, Fair Lawn, NJ) and 7 ml of concentrated sulfuric acid (36 N). Digestion tubes were placed into a block heater (Fisher Scientific, Bedford, MA) and digested at 403° C for 1 h after the heating block had reached temperature. After cooling, the samples were distilled (Kjeltec Systems, model 1002 distilling unit, Foss Tecator, Hogana Sweden). To distill, samples were diluted with 15 ml deionized water and vortexed. Next, a digestion tube and a receiver flask containing 25 ml of a receiving solution were placed in the distillation unit (Kjeltec Systems, model 1002 distilling unit, Foss Tecator, Hogana Sweden). Receiving solution contained 4% boric acid, 0.11% ethyl alcohol, 0.0017% methyl red and 0.0006% methyl blue. Sodium hydroxide (40%) solution was dispensed and the steam valve was opened. Samples were distilled for 4 min into a receiving flask containing the 4% boric acid solution, and then titrated using 0.1 N hydrochloric acid (HCl) back to the starting pink color. Percent nitrogen was calculated with the following equation:

$$\%N = \frac{(\text{ml HCl}) \times (\text{HCl normality}) \times (14.01)}{\text{Sample weight in mg}} \times 100$$

Crude protein was calculated using the following equation:

$$\% CP = \% N \times 6.25.$$

Specific gravity (SG), a variable used to estimate urine production, was determined for individual urine samples by thawing the sample in a refrigerator until samples were at 4° C. A clean beaker was weighed empty then weighed again with 50 ml of urine. Distilled water was cooled in a refrigerator until it was 4° C and then 50 ml of water was weighed. Specific gravity was determined using the following equation (APHA et al., 1998);

$SG_{T/4° C}$ = weight of urine (g)/weight of equal volume of water (g)

Where both liquids must be at 4° C for accurate measurement.

Statistical Analysis

Phosphorus balance data were reported in g/d with data allocated to three periods over the transition period. Period one is defined as d -14 prepartum to d 0 (parturition), period two is defined as d 1 to d 28 postpartum, and period three is defined as d 47 to d 56 and d 64 to d 73 postpartum, respectively for cows 1854 and 1847. Days within each period were treated as repeated measures. Data were analyzed using the general linear model procedure of SAS (SAS, 1999). The model statement included period and cow as the independent variable and P balance (g/d), DMI (kg/d), fecal DM (kg/d), milk production (kg/d), milk P (g/d), urinary P (g/d and mg/L), urine output (L/d), P consumed (g/d), and fecal P (% and g/d) as the dependent variables. A second model included total mixed ration DM and P concentration as dependent variables with period as the independent variable. Phosphorus balance data were also analyzed with day as a continuous independent variable to determine if a linear or quadratic relationship was apparent.

Results

No significant period or cow effect was observed for P balance (Figure 5.1 and Table 5.1). When data were analyzed with day as a continuous independent variable, a significant quadratic ($P = 0.0073$) effect was observed. The calculated quadratic equation to predict P balance was;

$$\text{P balance} = 1.55\ (\text{day}) - 0.026\ (\text{day})^2 - 19.44$$

Results of dry matter intake (DMI), total mixed ration (TMR) dry matter and P concentration, fecal dry matter and P concentration were described in the apparent digestibility study (Tables 4.3 and 5.1).

No significant period effects for urine P concentration (mg/L), urine excretion (L/d), or urine P excreted per day (g/d) were observed. Phosphorus excretion (mg/L and g/d) was significantly different between cows ($P< 0.0068$ and 0.0190, respectively), with cow 1854 secreting more P than cow 1847 (718.1 mg/L vs. 133.3 mg/L, mean of all three periods). A significant cow effect for milk P ($P = 0.0001$), but not for milk production, was observed (Table 5.1).

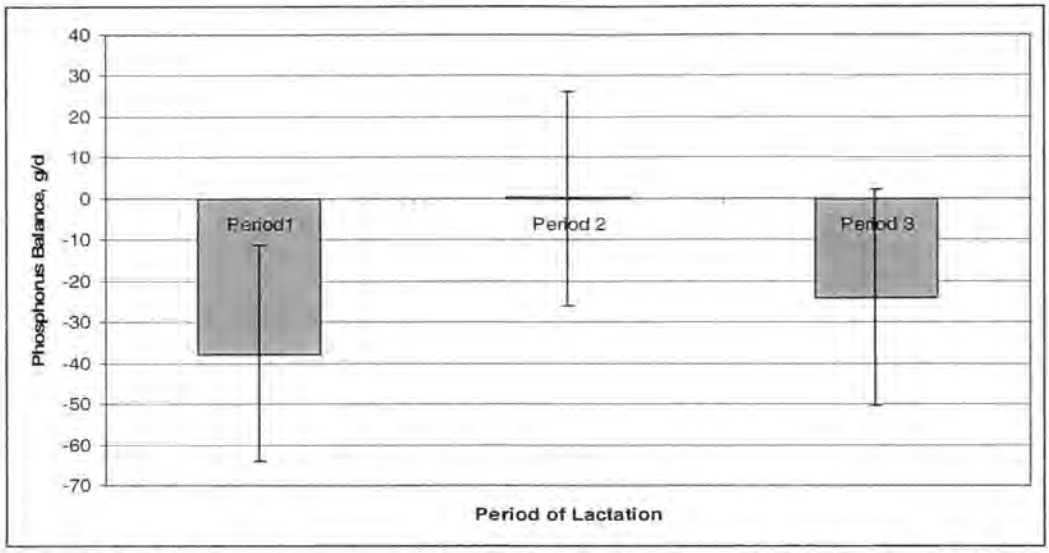

Figure 5.1. Least squares means of calculated phosphorus balance. $P = 0.1222$ for period effect and $P = 0.7069$ for cow effect. Period one = d -14 prepartum to d 0 (parturition), period 2 = d 1 to d 28 postpartum, period three = d 47 to d 56 postpartum and d 64 to d 73 postpartum, respectively for cows 1854 and 1847. Bars represent standard deviation.

Table 5.1. Least squares means of analytes used to determine phosphorus balance. Period one = d -14 prepartum to d 0 (parturition), period 2 = d 1 to d 28 postpartum, period three = d 47 to d 56 postpartum and d 64 to d 73 postpartum, respectively for cows 1854 and 1847.

	Period1	SD[3]	Period 2	SD	Period 3	SD	P > F (period)	P > F (cow)
DMI, kg/d[1]	10.073	3.120	15.962	3.090	14.971	3.090	0.051	0.815
TMR DM, %	52.279	2.160	56.235	1.870	53.649	1.870	0.1007	
TMR P, %	0.5446[a]	0.040	0.6596[b]	0.034	0.5956[a]	0.034	0.0123	
P consumed, g/d	54.712[a]	25.83	104.93[b]	25.58	91.054[ab]	25.58	0.0494	0.8619
Urine P, mg/L	260.45	425.6	430.33	421.5	520.94	421.5	0.6685	0.0068
Urine, L/d	14.981	4.000	11.867	3.970	11.017	3.970	0.3691	0.9589
Milk production, kg/d			28.909[a]	2.120	34.286[b]	2.120	0.0007	0.9956
Milk P, g/d[2]			23.847[a]	1.719	28.273[b]	1.719	0.0006	0.2941
Fecal DM, kg/d	4.3565[a]	1.400	6.4024[ab]	1.390	7.4580[b]	1.390	0.0194	0.6257
Fecal P, %	1.8165[a]	0.291	1.1481[b]	0.288	1.0876[b]	0.288	0.0074	0.0591
Fecal P, g/d	82.632	23.81	73.717	23.58	79.388	23.58	0.8459	0.3246
Urine P, g/d	3.4388	6.356	5.8215	6.300	6.0871	6.300	0.8211	0.019
P Balance, g/d	-37.717	26.50	0.1461	26.20	-24.094	26.23	0.1222	0.7069

[1] DMI = dry matter intake, TMR DM = percent dry matter in the total mixed ration, TMR P = percent phosphorus in the total mixed ration, P consumed = grams of phosphorus consumed per day in the total mixed ration.

[2] Milk P was only analyzed once and results are; 1847 = 0.8316 g/L, 1854 = 0.8609 g/L and pooled 0.8462 g/L. Cow data was not different from bulk tank samples (0.8446 g/L).

[3] SD = standard deviation.

[a, b] Numbers within a row with different superscripts differ ($P < 0.05$).

Discussion

Significant cow effects were observed only for daily urinary P excreted. Differences were not observed due to large standard deviations (Tables 5.1). A P balance of -37.72 g/d was observed in period one, 0.15 g/d in period two and -24.09 g/d in period three.

The negative P balance observed in the prepartum period indicates a greater concentration of P was excreted than was consumed. No other studies, using cattle, were located that examined P balance in the prepartum period. Braithwaite (1993) observed, in sheep, a P balance near zero at the end of gestation, approximately period one in the current study. Phosphorus resorption from bone and soft tissue, in response to a high P demand, may account for some of the excess amounts of fecal P (Braithwaite, 1983) resulting in a negative value. The prepartum collections were taken on d -21, -15, and -5, all at the point where fetal P demands are climaxing, yet it does not seem possible that resorption would account for all of the excess P excretion.

The periods of lactation and the levels of dietary P studied in the current study correspond with the work of Wu et al. (2000) and Knowlton and Herbein (2002; Table 5.2). Wu et al. (2000) measured P balance in lactating cows fed three levels of P on wks 2, 4, 6, 8, 23, and 40 of lactation. The high concentration of dietary P (0.49%) will be compared to the current study and wk 2 will be compared to period two. Wu et al. (2000) reported a P balance of 10 g/d in wk 2 of lactation. This is a difference in P balance of 9.85 g between the Wu data and period two of the current study. This difference may be due to the differences in the collection methods. Wu estimated digestibility of dry matter and P using a marker (Ytterbium) compared to the total collection of feces and measurement of DMI used in the

current study. Musimba et al. (1987) looked at the accuracy of Ytterbium as a marker to estimate dry matter (DM) intake and fecal DM output in a grazing situation. They compared the marker technique with total fecal collection reporting that the marker overestimated fecal DM and DM intake in two of three months. If Wu did overestimate fecal DM, he would have also overestimated fecal P, thus leading to an even larger difference between his data and the current study.

Knowlton and Herbein (2002) conducted a P balance study looking at balance in early lactation when cows were fed three levels of P. They collected samples on wk 3, 5, 7, 9, and 11 of lactation. Period two of the current study will be compared to wk 3 of the Knowlton study (Table 5.2) and to the high concentration of dietary P (0.67%). Similar to the current study, Knowlton used a total collection method for fecal collection. In the Knowlton and Herbein (2002) study, catheters were used to measure urine output.

Results observed in period three were considerably different than results observed in other studies that looked at P balance between wk 6 through 10 of lactation. A large difference in P balance was observed between the Wu et al. (2000) data and the current study; a difference of approximately 45 g P/d. The reasons for this large difference are unclear. Knowlton and Herbein (2002) reported results similar to the current study in period two, but not in period three (Table 5.2). The Knowlton cows consumed almost 52 more g P/d compared to the cows in the current study, but only excreted 30 g P/d more. Urine output calculated in the current study was less than measured by Knowlton and Herbein (2002). Knowlton and Herbein reported urinary P content that was similar to cow 1847's urinary P, but less than that of cow 1854 (data not shown). It appears that cow 1854 excreted atypical amounts of P in her urine (Hibbs and Conrad, 1983; Kleiber et al., 1951; Knowlton and

Herbein, 2002; Spiekers et al., 1993). Urine P (g/d) was significantly different between the two cows and 1854's urine P values were greater than those reported by Wu et al. (2000) and Knowlton and Herbein (2002). Wu et al. (2000) reported that urinary P concentration varied widely among cows, reporting values as high as 20 to 50 mg/dl (Wu et al., 2000). Cow 1854 demonstrated urine P concentrations as high as 87.9 mg/dl in period three, supporting an assumption that the cows on this trial were overfed P.

The time of fecal sampling may help explain differences observed between the studies sited and the current observations. In the current study, feces were sampled twice daily (approximately 0430 h and 1630 h). Samples from each collection were analyzed individually and grams of fecal P per 12 h were summed. Wu et al. (2000) reported a diurnal pattern of fecal P concentration, particularly with high dietary P. They observed that fecal P was highest at 1700 h and lowest at 0500 h suggesting that if sampling was conducted in the morning, fecal P would tend to be reduced; if sampling was conducted in the evening, fecal P concentration may be elevated. Similar diurnal patterns were not observed in the current study, but sampling did occur at the possible greatest and least fecal P concentrations and would therefore have been intermediate when summed. Knowlton and Herbein (2002) obtained fecal samples every 4 h, thus eliminating the possibility of diurnal patters affecting fecal P concentrations. Wu et al. (2000) reported the sampling times used in their study (between 0930 and 1100 and again between 1400 and 1530) would have underestimated the mean 24 h fecal P concentration. This would have increased the P balance they reported and the true balance may be less than reported.

There were large numerical differences across the three periods for P balance in the current study. These differences were not statistically significant. The lack of significance is

due to the large standard deviation (SD = 26.20, Table 5.1). Braithwaite (1983) reported that ewes experience an increase in P demand at the onset of lactation. Numerically, cows retained more P in period two compared to periods one and three. A significant period effect may have been observed with a greater number of cows on trial.

Endogenous fecal P is described by Kleiber et at. (1951) as the form of fecal P that reaches the intestinal contents either by diffusion from blood or interstitial tissue fluid, as part of secretions such as saliva, as a component of cells or cell fragments sloughed off from the intestinal lining, or contained in phagocytes. Kleiber et al. (1951) observed that 43% of fecal P was endogenous in a cow consuming 11 kg DM and producing 10 kg milk/d. Therefore, if approximately 43% of the 82 g of fecal P excreted in period one was endogenous P, approximately 35 g were not directly from a dietary source. Those 35 g are the difference between apparent digestibility and true digestibility. Braithwaite (1983) observed in sheep, given a plentiful supply of P and Ca, that endogenous fecal P had increased by one third from the time equivalent to period one in the current study to the time equivalent to period three, and almost double from period one to period two. In period one of the current study, the TMR that was fed had a greater concentration of P than a dry cow diet would contain. A greater concentration of dietary P may have led to greater concentration of endogenous fecal P, causing a more negative P balance.

Table 5.2. Comparison of the data from the current study with data from two other phosphorus (P) balance studies.

	Current Study	Knowlton and Herbein, 2002[1]	Wu, et al. 2000[2]
P consumed, g/d			
Period 1	54.71		
Period 2	104.93	148.00	83.90
Period 3	91.05	143.60	117.00
Urinary P, g/d			
Period 1	3.35		
Period 2	5.82	6.08	1.00
Period 3	6.09	1.13	1.00
Fecal P, g/d			
Period 1	82.63		
Period 2	73.72	101.70	44.60
Period 3	79.39	99.20	60.70
Milk P, g/d			
Period 2	23.85	45.50	28.40
Period 3	28.27	42.20	33.10
P Balance, g/d			
Period 1	-37.72		
Period 2	0.15	-4.70	10.00
Period 3	-24.09	0.80	22.20

[1] Data from the Knowlton and Herbein (2002) study. Data for period two of the current study corresponds with wk 3 at the high dietary P level (0.67 %P) and period three of the current study corresponds with wk 7 at the medium dietary P level (0.51 %P).

[2] Data from the Wu et al. (2000) study. Data for period two of the current study corresponds to wk 2 at the high dietary P level (0.49 %P) and period three of the current study corresponds to wk 8 at the high dietary level (0.49 %P).

CHAPTER 6: IN SITU DIGESTIBILITY OF PHYTATE PHOSPHORUS

Introduction

The in situ technique makes it possible to compare the rates of phytate P disappearance in feedstuffs. The in situ samples have the advantage of being exposed to the actual rumen environment. The feeds are exposed to the rumen contents, the rumen contractions, and the full scope of microbe populations, all of which may not be possible in an in vitro study. The objective of this study was to measure the rate that phytate P was released from feedstuffs commonly used in ruminant diets using the mobile bag technique.

Materials and Methods

A lactating Holstein cow (1847) was fed a total mixed ration twice daily, at approximately 1000 and 2100 h. The study included three feeds, one diet and one standard which were weighed out in triplicate. Feeds used were soybean meal (SBM), whole cotton seed (WCS) and dry cracked corn along with a total mixed ration (TMR). The standard used was phytic acid (Sigma, St. Louis, MO). Each feed was dried at 55° C for 12-18 h, and ground though a 0.5 mm screen in a Wiley laboratory mill (model 4, Arthur H. Thomas Co., Philadelphia, PA). This grind size was smaller than reported in other papers (Perrier et al., 1992; Reis et al., 2001; Sebek and Everts, 1999; Vanzant et al., 1998), however Nocek (1985) demonstrated that sample grind had no effect on dry matter disappearance rates from polyester bags.

Feed samples (60.0 ± .09 g) were weighed in triplicate into polyester bags (Ankom Technology, Fairport, NY) measuring 10 x 20 cm with a porosity of 50 (± 15) μm. A 15

mg/cm^2 ratio of sample size: bag surface area (SS:SA) was utilized, similar to that recommended by Nocek (1985). The ratio was determined using the following equation:

> SS: SA = sample size, mg / ((bag width, cm x bag length, cm) x 2) (Vanzant et al., 1998).

Bags were tied tightly with nylon cord. The bags were attached, with nylon cord, to three links of thick chain, six or seven bags to each length of chain. The chains added weight to the bags to advance sinking to the bottom of the rumen, keeping the bags under the rumen mat and in the liquid portion of the rumen contents. Bags were inserted into the rumen and the chains were attached to a rope approximately 38 cm in length and 1.3 cm wide. Plastic zip ties attached the chains to the rope. Chains and bags were attached to the rope to avoid regurgitation or passing down the gastrointestinal tract. The in situ bags were placed in the rumen for 72, 48, 24, 14, 7, 4, 2, or 0 h. Bags were removed simultaneously at h 72 (Nocek, 1985). Once bags were removed, they were rinsed in warm running tap water until the water ran clear. Bags were transported to the laboratory and rinsed a second time to ensure no rumen contents remained. Bags were placed in a 100° C oven for 96 h. When bag contents were dry, bags and contents were each weighed and the bag contents were removed. Samples were ground using a mortar and pestle until an even consistency was achieved. The samples were analyzed for phytate P concentration. A sample size of 0.50-0.55 g was used for phytate-P determination as described previously (Chapter 3, Figure 3.1), beginning at the step when samples are placed on the wrist action shaker.

Statistical Analysis

Lag times and rates of digestion of phytate P were calculated with the following equation (Nocek and English, 1986):

$$\text{Residual} = D_0 \left(e^{(k \times (z/2))} - 1\right) + R_0$$

where:

D_0 = digestible fraction
k = digestion rate constant
z = lag time – total incubation time
R_0 = residue at t = 0.

Lag times and rates of digestion were also calculated using an equation from Mertens and Loften (1980):

$$R = D_0 e^{-k(t-L)} + U$$

where:

R = residue at time t
D_0 = digestible fraction
k = digestion rate constant
t = incubation time
L = discrete lag time
U = remaining fraction (% of phytate at 72 h).

Data were analyzed using the general linear model of SAS to determine the least squares means (LSM) of percent phytate P loss for the sets of triplicate. Degradation rate constants, digestible fraction, and lag times were calculated using the nonlinear procedures of SAS (proc NLIN) based on Marquardt's model (SAS, 1999). Proc NLIN was used a second time to calculate degradation rates, potential degradability, and lag times with time zero removed.

Results

Approximately 32% of the in situ polyester bags had weak seems and broke open while inside the rumen. Phytic acid was used as a fifth sample in the in situ study, but the bags were empty upon removal from the rumen. Thus, samples of phytic acid were not included in the analysis.

The following is an example of the equations that can be derived to determine phytate degradation for each feed ingredient from the NLIN estimated degradation rates (Tables 6.1 and 6.2) calculated by SAS (SAS, 1999). The least squares means of the percent phytate P loss (Figure 6.1) were used to calculate potential degradability, lag time and degradation rate. Using the equation from Nocek and English (1986) and data from the current study, residual phytate in corn can be estimated:

$$\text{Phytate residual in corn} = 98.7547\ ((e^{(2.9222(-0.00014-inc)/2)}) - 1) + R_0$$

and with time zero removed;

$$\text{Phytate residual in corn} = 100.3\ ((e^{(0.378(-11.547-inc)/2)}) - 1) + R_0$$

where:

Residual = percent phytate-P loss
inc = incubation (hours)
R_0 = residue of t = 0 (Nocek and English, 1986).

As an example, if the incubation time was 2 h and the residue at t = 0 was 100% the residual phytate in corn would equal:

$$98.755\ ((e^{(2.9222(-0.00014-2)/2)}) - 1) + 100 = 6.559\%.$$

The LSM of phytate in corn observed in the study was 5.128%.

An equation from Mertens and Loften (1980) was also utilized, but residual sums of squares were greater with Mertens' equation (Table 6.1) and lag times were too small to calculate standard errors.

Table 6.1. In situ degradation rates of phytate P using the nonlinear procedures of SAS (SAS, 1999).

	Nocek and English (1986)[2]			Mertens and Loften (1980)[3]	
Feed	D_0	k	L	D_0	k
Corn[1]	98.7547	2.922	-0.00014	99.952	1.3362
TMR	91.8865	0.675	-0.15600	91.579	0.2452
WCS	97.2666	0.730	0.00638	99.435	0.3474
SBM	96.4527	0.424	0.20010	102.70	0.2003

[1] Corn = dry cracked corn, TMR = total mixed ration, WCS = whole cottonseed, and SBM = soybean meal.

[2] Residual = $D_0 (e^{(k \times (z/2))} - 1) + R_0$ where D_0 = digestible fraction, k = digestion rate constant, z = lag time (L) – total incubation time and R_0 = residue at t = 0.

[3] Residual = $D_0 e^{-k(t-L)} + U$ where R = residue at time t, D_0 = digestible fraction, k = digestion rate constant, t = incubation time, L = discrete lag time and U = remaining fraction or % of phytate at 72 hours.

Table 6.2. In situ degradation rates of phytate-P using the nonlinear procedures of SAS (SAS, 1999), with time zero omitted from the model.

	Nocek and English (1986)[2]			Mertens and Loften (1980)[3]	
Feed	D_0	k	L	D_0	k
Corn[1]	100.30	0.378	-11.547	11.126	0.2023
TMR	95.700	0.254	-5.2945	51.108	0.1043
WCS	97.183	0.762	0.1251	100.30	0.3502
SBM	89.565	1.030	1.6008	144.70	0.3038

[1] Corn = dry cracked corn, TMR = total mixed ration, WCS = whole cottonseed, and SBM = soybean meal.

[2] Residual = $D_0 (e^{(k \times (z/2))} - 1) + R_0$ where D_0 = digestible fraction, k = digestion rate constant, z = lag time (L) – total incubation time and R_0 = residue at t = 0.

[3] Residual = $D_0 e^{-k(t-L)} + U$ where R = residue at time t, D_0 = digestible fraction, k = digestion rate constant, t = incubation time, L = discrete lag time and U = remaining fraction or % phytate at 72 hours.

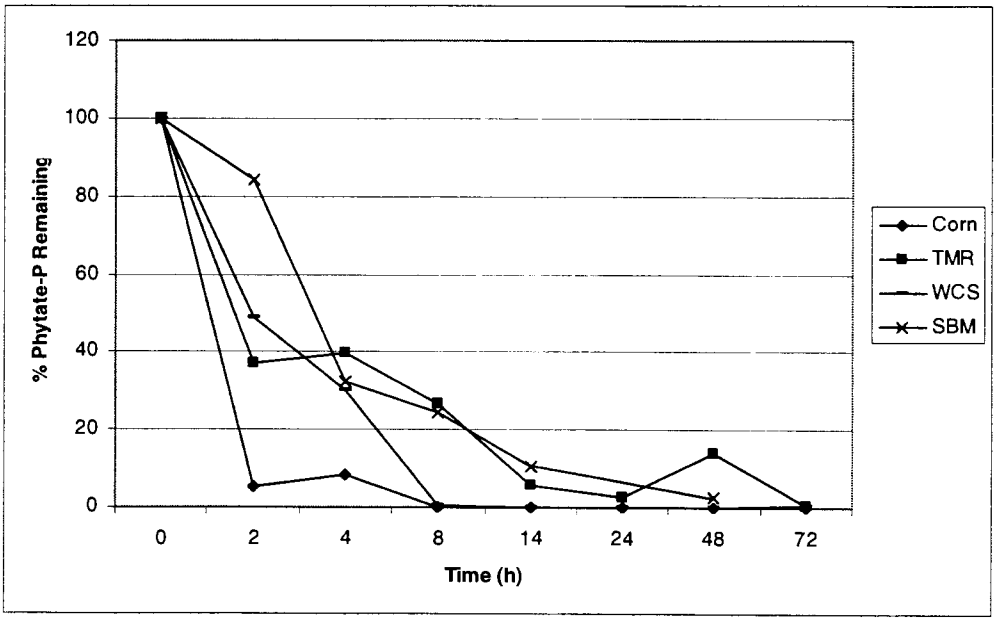

Figure 6.1. Least squares means of the percent phytate P loss during in situ incubation. Feeds included corn, total mixed ration (TMR), whole cottonseed (WCS), and soybean meal (SBM).

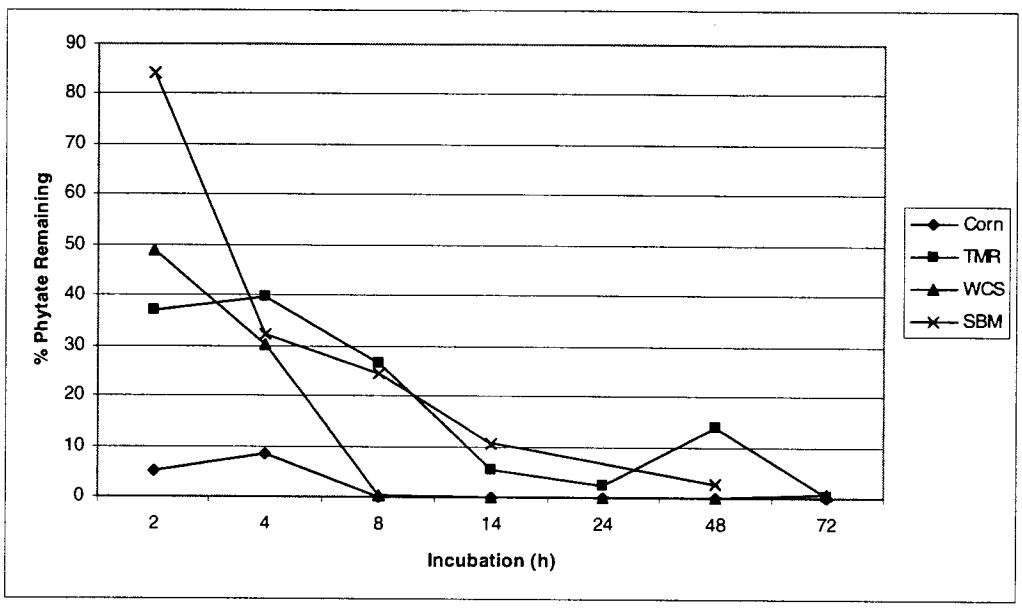

Figure 6.2. Least squares means of the percent phytate P loss during in situ incubation with time zero removed. Feeds included corn, total mixed ration (TMR), whole cottonseed (WCS), and soybean meal (SBM).

Discussion

Phytate degradation appears to be hydrolyzing from two separate pools in the feedstuffs. Nocek and English (1986) explain that the primary assumption associated with first-order kinetics is that the pool in question is homogeneous and that substrate concentration will be degraded as a linear function of time in the rumen. Thus, using first order kinetics to analyze the release or degradation of some aspect of a feed may lead to erroneous conclusions if the pool is not homogeneous. Phytate P concentration in feedstuffs may be put into three categories; water soluble, potentially degradable and undegradable (Bravo et al., 2000). The water soluble pool should not be a consideration when discussing figure 6.1 because the phytate that is water soluble should have been released either upon entering the rumen fluid or when the nylon bags were rinsed. Therefore the time zero in situ samples would not have contained water soluble phytate when analyzed. In theory figure 6.1 represents the potentially degradable and undegradable components. The rapid loss of phytate from 0 to 2 h, depending on the feed, may be considered a separate pool of P. Therefore it seems the potentially degradable pool may be separated into a readily degradable and slowly degradable pool. Bravo et al. (2000) conducted a study analyzing the total P degradability in forges, cereals and meals that were treated with formaldehyde. In their study they analyzed a rapidly degradable P pool and a releasable P pool, similar to the current study. Bravo et al. (2000) used the following equation; $Y = A + B(1-e^{-ct})$ to calculate the rapidly releasable P fraction (A), the releasable P fractions (B) and the rate constant of P release for fraction B (C). This equation possibly could have done a better job calculating a rapidly releasable and the releasable fractions compared to the method used in the current

study, yet it would not have calculated the lag time which seemed necessary when analyzing phytate.

Figure 6.2 depicts the data starting at 2 h, separating the rapidly degradable pool from the slowly degradable (releasable) pool. The fractions were divided at 2 h to be consistent across feeds even though it appears that SBM and possibly WCS continued to degrade rapidly until 4 h. Separating the data was conducted to better analyze the slowly degradable pool. When multiple pools of potentially degradable material are presented to the rumen, all pools are degraded simultaneously (Nocek and English, 1986), making it difficult to distinguish between the pools and calculate degradation rate constants for each pool. A degradation rate constant was not calculated for the time between 0 and 2 h. That analysis may have lead to an erroneous degradation rate constant for each feed, because no time points between 0 and 2 h were measured in the study.

Researchers have compared and contrasted the different model equations that best fit different digestibility rate trials (Mertens and Loften, 1980; Nocek and English, 1986). No previous in situ work was located on the degradability of phytate. A direct nonlinear least squares model presented by Mertens and Loften (1980) was utilized in this study. Also the nonlinear regression procedure presented by Nocek and English (1986) was utilized. The Nocek model was reported to calculate a first order equation with the best fit. This is evident by the sums of squares residuals, which are less for the Nocek and English model compared to the model by Merten and Loften (Tables 6.1 and 6.2). The model presented by Mertens was designed to run in Proc GLM in SAS, not Proc NLIN, which was used in the current study. Proc NLIN was used in the current study due to the benefits it offered, such as the

parameter bounds that may be set into the model to narrow the focus of the output data. Both models used the iterative method of Marquardts to find the estimates of the coefficients.

Readily degradable phytate present in corn was exhausted within approximately 2 h. It also was observed that the readily degradable pool was approximately 95% of the total phytate present in corn. Total mixed ration and whole cottonseed appeared to contain approximately 60% of the phytate concentration in a readily degradable form. Phytate P in soybean meal was observed to hydrolyze in a different rate pattern. Phytate in SBM degraded slowly for the first 2 h, then rapidly for the next 2 h. The phytate concentration in SBM may be present in one pool, yet that is difficult to speculate. In the current study phytate P concentration in corn appears to be the most readily hydrolyzed of the three feeds and TMR. Approximately 95% of corn was hydrolyzed after 2 h of incubation. After 14 h, approximately 90% of phytate present in SBM was hydrolyzed and after 8 h, approximately 98% of phytate in WCS meal was hydrolyzed. The total mixed ration hydrolyzed at an intermediate rate (approximately 95% hydrolyzed in 14 h), which seems logical as it consists of a combination of the three feedstuffs.

CHAPTER 7: CONCLUSIONS

The current study was undertaken to determine the ability of the transition dairy cow to utilize P. The transition period was defined as the period 14 d prepartum to 28 d postpartum. Four aspects of P utilization were investigated. An in vitro study was conducted to determine if the stresses of transition affected the rumen's microbe population's ability to hydrolyze phytate P. Cracked corn, soybean meal (SBM), whole cottonseed (WCS), a total mixed ration (TMR) and phytic acid were analyzed. An apparent digestibility study was conducted to determine if the digestibility of P was influenced by transition. A P balance study was conducted to determine the degree that the balance of P differed as the cow transitioned. Lastly, an in situ study was conducted to determine the rate at which phytate P was hydrolyzed from corn, SBM, WCS and TMR.

Dairy cows possess the ability to efficiently utilize phytate P. Period (one, two and three) had a feed-dependent effect on utilization. In vitro phytate hydrolysis from phytic acid varied, with period two being significantly greater than period three, yet period one was not different from periods two or three. In vitro phytate hydrolysis was not different between periods (one, two and three) in WCS, SBM, TMR, and corn. With no incubation time 27 to 93% of phytate P had hydrolyzed. Further analysis demonstrated that phytate in the feeds was 40 and 68% soluble in water. Therefore, the concentration of inositol-phosphates present in the solids following the in vitro procedure may not represent the total concentration of inositol-phosphates that were present at the conclusion of the incubation time. A portion of the phytate P may have solubilized into the liquid portion of the sample. The assumption that all phytate was retained in the solids may lead to erroneous results.

Apparent digestibility of P was affected by period. The large negative values of apparent digestibility observed in period one may be explained by: 1) the excess concentration of P in the diet offered to the cows, 2) resorbtion of P from bone and soft tissue or 3) excess dietary calcium concentrations. The many factors that control the absorption of P from the gastro intestinal tract need to be more closely examined to make solid conclusion. Some of these factors might include hormone levels, P and Ca concentrations in bone and soft tissue and mineral and nutrient interactions that might interfere with the absorption of P.

Phosphorus balance in the current study varied between cows, between periods and from other research. Though no significant difference between periods occurred, period two was numerically different compared to periods one and three. Further investigation is required to conclude why such a large negative balance was observed in periods one and three of the current study. Possible explanations include a large concentration of P resorbed from bone in period one and the increased demand for P at calving during that period. Diurnal variation in fecal P concentration may account for some of the observed values. Results from the P balance study suggest that urinary P should be measured during balance studies and not assumed to be a negligible amount. This is due to the large difference in urinary P observed between the two cows in the current study.

Phytate P exists in a readily degradable and a slowly degradable pool. Feed-specific degradation rate constants were calculated from the in situ experiment for the slowly degraded pool, taking into consideration that pools overlap, and are degraded simultaneously. When determining P availability in feeds, feeds need to be analyzed individually to ensure correct degradation rates. Some other areas that may be useful in better understanding the availability of phytate would include the rate of passage of the different feeds, to help

understand if the P that is available is being used by the cow or by the microbes and the fraction of P used by each. Also nutrient and mineral interactions and how those interactions affect the release of phytase and P availability.

Utilization of phytate P by the dairy cow is an important issue. The current study observed cows that were fed beyond their P requirements and the results would presumably be different if the cows were fed at their P requirements. Terminating the overfeeding of P should be the number one goal of nutritionists who are concerned with the environmental impact of P. Looking at the rate and efficiency of phytate P breakdown should be the second step in reducing high concentrations of fecal P. The current study has stepped in the direction of achieving this second goal.

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