

# **Starch Digestion and Phosphorus Excretion in Lactating Dairy Cows**

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science  
in  
Dairy Science (Dairy Nutrition)

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August 8, 2002  
Blacksburg, Virginia

**Keywords:** Phosphorus and nitrogen excretion, phytase activity, starch digestion

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## **ABSTRACT**

The effects of starch and phosphorus (P) source on P partitioning and ruminal phytase activity were evaluated in eight lactating cows (113 DIM). Four cows were ruminally cannulated. Cows were randomly assigned to treatments in a duplicated 4x4 Latin square with four, 18-d periods. Diets included dry ground corn (DG) or steam flaked corn (SF), with a no supplemental P (low P diet; 0.34% P) or supplemental purified phytic acid (PA; 0.45% P) to provide additional P from an organic source. Total collection of milk, urine, feces, and feed were sampled each period, while rumen fluid was sampled on d 18. Excretion of feces, urine, P, and N was lower in cows fed SF than in cows fed DG. Milk yield was unaffected by diet despite a lower DMI by cows fed SF. Cows fed SF tended to have a higher feed efficiency and lower milk urea nitrogen (MUN) concentration than cows fed DG. Rumen pH was unaffected by diet, but milk fat content was lower for cows fed SF. Milk yield, DMI, and feed efficiency were not affected by PA. Cows fed PA had increased P intake and excretion, but a lower milk P as a percentage of intake compared with cows fed the low P diet. An interaction of starch source and P source was observed for ruminal phytase activity. Altering dietary sources of starch and P offers opportunity to improve P availability and reduce manure nutrient excretion.

## ACKNOWLEDGMENT

First, I would like to thank my major professor, Dr. Katharine Knowlton. I always appreciate your help and time you put into helping me. You have taught me many valuable skills for the future and you were a great advisor. Also, I would like to thank my committee members, Dr. Ronald Pearson and Dr. Carl Polan.

I want to thank Julie McKinney for all her help in the lab. Thanks Julie for always being there when I have felt down and thanks for being such a true friend. I want to thank Kristin Burkholder for all her help with my project, and letting me know that if I need anything, you are there.

Thanks to all my fellow graduate students for all their support, especially Beth Keene for all her help in the barn. I especially want to thank Tracy Jewell for making it through school with me and being such a good roommate and friend. I want to thank all the undergraduates who have helped me in collecting data; I would not have finished my project without your help.

Thanks to Pennfield feeds and Loveland Industries for the support of this project.

I want to thank my family for always being on my side and comforting me through times of trial. Also, thanks to the Shafer family for praying and keeping me in mind at all times.

I especially want to thank Brent for keeping me sane; always being there when I needed someone to listen, and making sure that I was always okay.

I cannot forget the Lord, for keeping me safe and watching over me through good times and bad times, and always knowing that I am loved no matter who I am or what I have done.

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# INTRODUCTION

Nutrient contamination of ground and surface water is the leading environmental issue facing dairy farmers in the United States. One of these nutrients that has become a serious concern to the public is phosphorus (P). Phosphorus is brought onto the farm through feed and fertilizer. Roughly 30% of feed P is captured in meat and milk, and 70% is lost in manure. The manure that accumulates on farms is usually spread onto cropland, and if manure P application is greater than crop P needs, P may accumulate in the soil.

Phosphorus that accumulates in soil may eventually run off into nearby surface water and cause algae blooms and eutrophication. Algae blooms shade aquatic vegetation and reduce photosynthesis for plants. The decomposition of the algae consumes dissolved oxygen in the water, which will reduce oxygen available for fish and may lead to fish kills. Eutrophication restricts the use of water for recreation, drinking, fishing, and industry (Sharpley et al. 1994; Sims et al. 1998; Sharpley 2000). Also, excess P in surface water may be linked to outbreaks of *Pfiesteria*, which may be harmful to humans (Sharpley and Gburek 1998).

Animal agriculture is one of the major contributors of P pollution. Smith and Alexander (2000) estimated that the median percentage contribution of animal agriculture ranged from 7 to 48 % in major watersheds. These concerns have brought about new, stricter legislative policies limiting manure P application. Under P-based regulations, farms will have to reduce manure application to cropland. This may mean that farmers will have to acquire more farmland to land apply their manure.

Nutrient management plans are developed and implemented to balance land application of nutrients and plant nutrient requirements. Much effort has been focused on manure management on the farm to reduce nutrient losses from the farm through handling nutrients once they accumulate. More recent work has focused on managing the herd and feeding programs to help minimize nutrient excretion on the farm and reduce dietary P excretion (Morse et al. 1992a; Wu et al. 2000; Wu and Satter 2000; Knowlton et al. 2001; Knowlton and Herbein 2002). Previous work suggests that feeding the more digestible starch sources (high moisture corn vs. dry corn, corn vs. barley) may also

decrease P excretion (Guyton et al. 2000). This may be due to the increase in ruminal phytase activity with the more digestible starch source.

Phosphorus from cereal grains and forages are not entirely available for absorption or utilization by livestock. About 70% of the P in cereal grains is organically bound. This form of P is called phytate P or phytic acid. Phytate P content varies in different types of cereal grains and forages. Approximately 65 to 70% of the total P in cereal grains is in the form of phytate P, while forages are very low in phytate P (Nelson et al. 1968; O'Dell et al. 1972; Lolas et al. 1976; Nelson et al. 1976; Clark et al. 1986; Morse et al. 1992b). Phosphorus in forages and cereal grains is more available to ruminants than to nonruminants, because rumen microbes possess phytase (Reid et al. 1947; Raun et al. 1956; Clark et al. 1986; Morse et al. 1992b; Yanke et al. 1998). Phytase is an enzyme that breaks the phosphate groups from the inositol ring, making the P more available for absorption in the small intestine.

Our objective was to evaluate the effect of starch source, dietary phytate P content, and their interaction on P partitioning and excretion and ruminal phytase activity in lactating dairy cows. We hypothesize that the more digestible starch source, steam flaked corn, will increase ruminal phytase activity, thereby decreasing P excretion compared with cows fed dry ground corn. This increased phytase activity will be particularly of benefit in diets with higher organic P content

# CHAPTER 1

## REVIEW OF LITERATURE

### 1.1 Environmental impact of phosphorus

Nutrient contamination of ground and surface water is the leading environmental issue facing dairy farmers in the United States. One of these nutrients that has become a serious concern to the public is phosphorus (P). Phosphorus is brought onto the farm through feed and fertilizer. Roughly 30% of feed P is captured in meat and milk and 70% is lost in manure. The manure that accumulates on farms is usually spread onto cropland, and if manure P application is greater than crop P needs, P may accumulate in the soil.

Phosphorus that accumulates in soil may eventually run off into nearby surface water and cause algae blooms and eutrophication. Algae blooms shade aquatic vegetation and reduce photosynthesis for plants. The decomposition of the algae consumes dissolved oxygen in the water, which will reduce oxygen available to fish and may lead to fish kills. Eutrophication is the term used to define a body of surface water depleted of oxygen. This restricts the use of water for recreation, fishing, drinking, and industry (Sharpley et al. 1994; Sims et al. 1998; Sharpley 2000). Also, excess P in surface water may be linked to outbreaks of *Pfiesteria*, which may be harmful to humans (Sharpley and Gburek 1998).

Animal agriculture is one of the major contributors of P pollution. Smith and Alexander (2000) estimated that the median percentage contribution of animal agriculture ranged from 7 to 48 % in major watershed areas. According to the 1998 National Water Quality Inventory, 45% of the nation's lakes and 60% of the nation's rivers and streams are polluted from agricultural sources (EPA 2000).

#### 1.1.1 Environmental regulations

These concerns have brought about new, stricter legislative policies. In 1965, the Water Quality Act was passed, requiring that all states develop ambient water quality standards for interstate water bodies. This policy was the first major federal water quality

law. Then in 1972, the Clean Water Act was passed. The goal of this act was to restore and maintain the chemical, physical, and biological integrity of the nation's waters (P.L. 92-500, 1972). The United States Environmental Protection Agency (EPA) is authorized to enforce this act. Some programs developed under this act include the Total Maximum Daily Load (TMDL) program, concentrated animal feeding operation (CAFO) policies, and state nutrient management programs.

There are approximately 300,000 river and shore miles, and 5 million lake acres across the United States that are polluted and do not meet the standards of clean, safe water (Parry, 2000). Many substances ranging from animal manure to chemicals can pollute waters. The EPA classifies these polluters as point source and non-point source polluters. Point sources are discrete, identifiable sources of pollutant. Examples include contamination from large industries and animal feeding operations that place pollutants directly into a waterway by a pipe, ditch, or tunnel. Non-point sources are more diffuse sources, and include urban runoff, agriculture, and forestry. Sources of runoff of these pollutants are rainfall or snowfall, which will move over the ground, causing pollutants to runoff into nearby surface water.

### 1.1.2 Total Maximum Daily Load Program

The TMDL program was developed to help improve bodies of water that are impaired. A TMDL specifies the amount of pollutants (sediment, nutrients, pathogens, ammonia, and metals) that can enter a body of water (stream, lake, bay, or river) in a day without affecting water quality. The TMDL program is unique in that it regulates pollutants from both point and non-point sources.

The first step involved in the development of a TMDL is identifying impaired waters. Testing waters and comparing the results to a water quality standard determines if a waterway is impaired. If the waterway does not meet safe water standards, it is added to the list of impaired waters (known as the 303d list for the section of the Clean Water Act mandating TMDL). The next step in this program is identifying the sources of pollutants and developing standards to help improve the quality of the water. The TMDL plan is developed, and a formal public notice is placed in local newspapers to inform residents of

the project. Modifications to the plan may be made in response to public comment. Then the state submits the TMDL plan to the EPA for approval. When the plan is approved, the state will adopt the plan as a regulation. Then participating parties, such as contractors, will implement the plan to improve the impaired water body.

Total Maximum Daily Load plans involve many elements. They include a description of the waterway, flow rate of the water, seasonal variation, land use around the water, weather and pollutant source. The plan involves specific detail of what the allowable levels of pollutants are and what management changes are needed to keep the water from being impaired. Examples for improving streams with high coliform levels may include fencing 90 to 100% of all cattle out of streams, or eliminating all direct pipes from dairies and houses. Some plans may identify specific farms as the pollutants of a particular stream. This requires farmers to implement best management or conservation practices to help improve the quality of the water. The plans themselves do not force farmers to participate in these practices, but the law of the state can force farmers to participate in these programs.

### 1.1.3 CAFO regulations

The EPA regulates point sources through the Clean Water Act and has developed programs to prevent runoff from large agriculture operations, also referred to as CAFOs. The EPA specifies that farms that discharge pollutants into water through a ditch, flushing system, pipe or other man made system, or that directly place pollutants into bodies of water that originate or pass through the farm and that meet size criteria are CAFOs. Animal operations with more than 1000 animal units (AU; 1000 pounds live weight) and house animals in a confined area for 45 days are considered CAFOs. This size criteria includes farms with more than 1000 slaughter animals, 700 mature dairy cattle, or 2500 swine.

The size criteria for permits vary among states, from virtually all farms (MD), to those with 300 AU (VA), 1000 AU (NY), or those units with a combination of large size and specified animal density (2 AU per acre in PA). Some states specify that nutrient management plans are required for any type of farming operation, whether it is animal

agriculture, crop operations, or land owners that apply sludge or animal manure. CAFOs are required to obtain a permit under the National Pollutant Discharge Elimination System (NPDES) to prevent point source discharge. These agriculture operations must have storage facilities for animal waste, wastewater, and pollution runoff from severe weather situations. CAFOs are allowed to apply this waste to cropland under conditions outlined in a nutrient management plan.

Under the Clean Water Act, states are allowed to implement their own programs to take the place of the NPDES permit programs. Virginia and 43 other states are approved to implement other NPDES permit programs. In Virginia, there are three types of permits that animal operations can be given. The Virginia Pollution Abatement (VPA) general permit pertains to farms that have at least 300 AU and may be considered a non-point source. This type of permit is required by operations that apply animal waste, sewage sludge, or industrial waste to land. The VPA individual permit is for farms that have greater than or equal to 300 AU, or farms that may be contaminating state waters. The Virginia Pollution Discharge Elimination System (VPDES) permit is for producers that have on farm processing and apply the discharge to the land.

#### 1.1.4 Nutrient management plans

To help decrease nutrient runoff, nutrient management plans are required for NPDES permits and most state regulatory programs. The purpose of nutrient management plans is to balance nutrients that are brought onto the farm with plant nutrient requirements. As part of their nutrient management plans, farmers have to keep records on crop yields, cropping systems, manure management, fertilizer use, and soil and manure tests.

Many states require these plans to be nitrogen based, which means that manure application is limited to the nitrogen needs of crops. The problem with this type of nutrient management plan is that manure application will exceed the P needs of crops. This causes P to accumulate in the soil, and the accumulated P may runoff into nearby surface water. Due to this, some states have passed laws mandating that farms use P based nutrient management plans.

The Water Quality Improvement Act of 1998 mandated that all Maryland agriculture operations develop a nutrient management plan to help reduce P and N contamination of the Chesapeake Bay. Under this law, nutrient management plans are required for agriculture operations with annual incomes greater than \$2500 or more than 8 AU. Crop or agriculture operations that use chemical fertilizers, sludge, or manure are also obligated to obtain a plan. In Maryland, all farms are required to have a N-based nutrient management plan written by December 31, 2001 and implemented by December 31, 2002. Phosphorus based nutrient management plans are to be implemented by 2005.

In Virginia, any nutrient management plan developed after October 2001 for the poultry industry has to be P-based. Under P-based regulations, farms will have to reduce manure application to cropland. This may mean that farmers will have to acquire more farmland to apply their manure. Bosch and Pease (1993) observed an increase in P application when manure application was calculated according to the N needs of the crops on a typical dairy farm in Rockingham County, VA. This farm scenario assumed a herd size of 80 cows, and a farm consisting of 85.3 acres of rye-corn silage, 135.8 acres of pasture, 5.3 acres of alfalfa, and 2.6 acres of corn grain. When manure was applied to crop ground according to the N-based requirements of crops, an excess of 2,021 kg of P was applied. When manure was applied to crop fields according to the N- and P- based requirements of crops, manure application was limited. Additional commercial N fertilizer was needed to supply crops with N due to the reduced application of manure under P-based limits.

Cows can excrete large amounts of manure also meaning there may be large amounts of nutrients present in the manure. Van Horn (1991) estimated that a cow milking 31.8 kg a day fed a 0.45% P diet would excrete 72.7 kg of manure a day and 0.06 kg of P a day. This is an estimate, but not all cows will excrete the same amount of manure or manure P. The accumulated manure is usually spread onto crop ground. Some crops, such as corn silage and alfalfa hay, will remove small amounts of the nutrients supplied from manure (15.9 and 18.6 kg per acre, based on estimated yield (kg) of crop harvested per acre) and multiple cropping systems may only use 22.7 kg of P per acre (Van Horn 1991). Crop uptake of P varies with the crop, crop yield, and soil type. The low uptake of manure P from crops may cause P to accumulate in the soil, which may

eventually runoff and contaminate nearby surface water. This is why it is important to know the amount of nutrients that will be applied to the soil. Estimates are not as accurate as true soil and manure tests. Correct analysis is needed for accurate application to develop a nutrient management plan, and to determine if farmers will have to acquire more land to apply their manure.

Manure application, soil and manure tests may be inaccurate if manure and soil are not sampled correctly. It is very important that sampling is done correctly because not knowing the correct amount of nutrients needed or applied to the soil can cause an increase in nutrient runoff. Peters (2000) measured the variability in samples of four liquid and eight solid subsamples of poultry and dairy manure. They reported that the variability was low in the liquid samples, but the solid samples ranged from 2 to 20 times higher for total N concentrations compared to the liquid samples. They specified the importance of properly sampling different types of manure samples.

### 1.1.5 Best management practices

Farmers can implement many practices to reduce the risk of nutrient pollution in the nation's waters. Examples of these best management practices include fencing animals out of streams, using cover crops in the winter, applying manure to fields when the ground is not frozen, using nitrogen fixing plants, rotating crops, and appropriate manure storage.

Storing manure in an appropriate facility on a dairy farm is an important best management practice. Dairy farms are required by NPDES permits to have appropriate manure handling facilities. These handling facilities can be an earthen pit with an embankment or excavating pit, or a structure made of concrete or metal. The U.S. Department of Agriculture Natural Resources Conservation Services (NRCS) sets standards for manure holding structures (Wright et al. 1999). Some states require farmers to have enough storage for rainfall, and animal waste for at least 150 d and a 25-year, 24-h storm.

Manure storage is not just important to prevent runoff. It also affects manure composition. Tamminga (1992) observed that microorganisms in animal manure that has

been stored properly for several months as slurry or liquid will decompose the manure P by 80% into the inorganic P form. The decomposed manure P becomes 90 to 100% more available for crop uptake.

Implementing best management practices can be a benefit to the environment. Bosch and Pease (1993) observed ways to reduce nutrient loss to surface water when they evaluated various scenarios of manure application on a dairy and dairy/poultry farm in Rockingham County, VA. They used data from surveys and other sources to simulate a representative farm that milked 80 cows. Farm A had 241 acres and farm B had a total of 154 acres. Farm C was identical to farm A with the addition of three broiler houses and farm D was identical to farm B with the addition of three broiler houses. Three nutrient management policies were assigned to each farm to observe the effect of the policies on the loss of N and P from land applied manure. When policy 1 (unlimited manure application) was implemented, P application went above P recommendations for crop needs for all farms (2,030.5, 1,863.6, 5,828.2 and 4,224.1 kg of excess P for farm A, B, C, and D). A slight decrease in excess P application was observed when policy 2, manure application limited to N recommendations, was implemented. Under policy 2, all farms meet N needs of crops with manure, but excess P still accumulated in the soil. When policy 3 was implemented, crop needs for P and N were met. Under this scenario, whole farm P losses were minimized. Policy 2 and 3, N-based and P-based manure application limits, were environmentally beneficial but required farmers to reduce manure application. These farmers will need to find other ways to get rid of animal waste under these and similar policies.

There are other practices that farmers can use to minimize nutrient loss from manure application. One such practice involves incorporating manure into the soil after applying manure to the fields. This method decreases nutrient loss to surface water and decrease N volatilization into the atmosphere (Pease et al. 1998). Another study was conducted looking at integrating different manure management techniques to dairy farms with 60, 100, or 150 cows in Rockingham County, VA. Nutrient losses were calculated for various manure management policies, such as incorporating manure into the ground within 48 hours of application, manure application based on N needs of crops and pasture land, and manure application based on P needs of crops. Incorporating manure into the

soil decreased N losses slightly and decreased P losses by 6 to 10% on all farm types when compared with a farm not incorporating manure. Pease et al. (1998) calculated an 18 to 50% decrease in N loss and a 3 to 15% decrease in P loss when manure application was based on crop N needs. There was a decrease in N (21 to 56%) and P (21 to 43%) accumulation when manure application was based on P needs of crops. Again, applying manure to fields based on N and P requirements of crops will cause a decrease in the amount of manure that can be applied.

Implementing best management practices can be a benefit to the environment but some can be expensive for farmers to implement. Bosch and Pease (1993) observed that farmers would have to purchase commercial N fertilizer when applying manure to soils according to P requirements of crops. With each farm situation a decrease in farm income was observed on farms with P-based manure application compared with farms with no implemented policies. Net farm income was decreased by \$12,233 (81%) when N and P restrictions were implemented for medium sized farms that did not have broiler houses compared with a farm that had no restrictions. For small farms without broiler houses, a net farm income decreased by, \$15,579 (142%) compared with a farm with no restrictions. A medium size farm with broiler houses may see a decrease in income by \$17,377 (40%) and a small farm with broilers a decrease of \$14,272 (38%) compared to a farm with no restrictions. The decrease in income is due to the purchase of extra commercial fertilizer to supply crops with needed N and potassium (K), hauling manure off the farm, and renting land to apply the excess manure that cannot be applied to the farmer's land. A separate study conducted by Pease et al. (1998) observed a decrease in net farm income by 11 to 23% when the P-limit policy was implemented on a dairy farm. This was due to the purchase of commercial N fertilizer to meet the N needs of crops.

Testing soil and manure is very important to determine the quantity of nutrients that are needed by fields and supplied by manure. This can help farmers save money on purchasing fertilizer and decreasing the risk of nutrient runoff into nearby streams. Hart et al. (1997) observed that nutrient concentrations vary among cow manure. They reported that one cow can provide enough N for 1.5 acres of corn silage or 0.66 acres of grass forage cut five to six times. Testing the manure and soil will enable a farmer to determine if manure can supply the N needs of the crops. Hart et al. (1997) observed that

farmers could save \$60 to \$100 per acre if manure supplies enough nutrients without having to purchase commercial fertilizer. Pease et al. (1998) reported a 0 to 5% increase in a farmer's income when manure application was applied to meet the N needs of the crops compared to farm that does not apply manure according to nutrient needs of crops. This savings was due to the decreased purchase of commercial fertilizer.

If farmers have to export manure off the farm to meet nutrient management requirements, it will become very expensive because of hauling costs. This problem may cause farmers to find other ways to manage manure on the farm. Some solutions may involve using a mechanical solid separation or composting system. Mechanically separating manure into solids and liquids will allow the farmer to export approximately 20% of the P in manure from the farm (Wright 2000). This would help reduce P manure application, but the cost of the system can be expensive. Wright (2000) reported equipment costs for the separator to be about \$30,000. An additional \$25,000 is needed for installation of pipes and plumbing. Other costs, such as labor, electricity, maintenance, and repair costs need to be considered if a manure separator is going to be used. If farmers have solid waste, composting could be used. Composting manure requires less maintenance and is cheaper to start up compared to a mechanical separator, but a market for the compost is needed. Both processes require extra labor, space and storage on the farm.

To encourage farmers to participate in these best management practices, many services and cost share programs are available through state and federal agencies. These agencies provide money to build proper manure holding facilities and watering systems to keep animals out of streams, for instance. Also, funds have been provided to encourage farmers to plant cover crops to help reduce nutrient loss from the soil. Farmers can receive tax credits if they purchase specific equipment for improving conservation. Tax credits can also be granted to farmers who have expenses from implementing best management practices.

Phosphorus pollution is a very large concern of farmers, citizens, and politicians. Best management practices, nutrient management plans and cost share programs can help reduce P pollution in surface water.

## 1.2 Digestion of phosphorus in ruminants

Phosphorus from cereal grains and forages is not entirely available for absorption or utilization by livestock. About 70% of the P in cereal grains is organically bound. This form of P is called phytate or phytic acid. Phytate P consists of a sugar molecule, called myoinositol, with covalently linked phosphate groups. This organically bound P is not available for absorption by monogastric animals because they do not possess the enzyme phytase. Ruminants are able to digest phytate P because rumen microorganisms have the phytase enzyme. This enzyme breaks the phosphate groups from the inositol ring, making the P available for absorption in the small intestine (Reid et al. 1947; Raun et al. 1956; Nelson et al. 1976; Morse et al. 1992b).

Martz et al. (1999) observed that the true absorption of P from corn silage fed to nonlactating dairy cows ranged from 85 to 94%. The NRC (2001) specifies that the coefficient for absorption of P from forage and concentrates are 64% and 70%, with availability of inorganic sources ranging from 30 to 90%. Brintrup et al. (1993) reported a 67% apparent P absorption coefficient when cows were fed diets with 0.41% P concentration. Morse et al. (1992a) observed a 74% apparent P absorption coefficient when cows were fed diets that ranged from 0.30, 0.41 and 0.56% P concentrations in the diet and Wu et al. (2000) observed a 70% apparent P absorption coefficient of P in a diet with a 0.40% P concentration. Others have observed true absorption coefficients in inorganic P sources. Tillman and Brethour (1958) observed a 75% true absorption coefficient when cows were fed dicalcium phosphate and 90% with phosphoric acid, while sheep fed monosodium phosphate had a 90% true absorption of P. Challa and Braithwaite (1988) reported a 75% true absorption of dicalcium phosphate when fed to cows.

### 1.2.1 Inorganic phosphorus absorption

The small intestine is the major site of absorption of inorganic P. Khorasani et al. (1997) observed that P digestion in the small intestine ranged from 71 to 85% in eight

lactating dairy cows that were fed four different treatment diets of alfalfa, barley, oats or triticale silage. Phosphorus digestion was not affected by treatment diets.

The process involved in the absorption of inorganic P in the small intestine is not very well understood. The inorganic P moves across the brush border membrane in the cells lining the small intestine by way of active co-transport mechanism with  $\text{Na}^+$ . The driving force for the accumulation of P in the cells of the small intestine is the movement of the  $\text{Na}^+$  ion against the electrochemical gradient. This  $\text{Na}^+$  gradient and subsequent accumulation of P in the cell is brought about by the  $\text{Na}^+$ ,  $\text{K}^+$  adenosine triphosphatase (ATPase) pump (Murer et al. 1981; McKay 1995). The process of inorganic P movement out of the cell across the basolateral membrane to the blood stream is not understood. Other minerals such as Ca move into the bloodstream against the electrochemical gradient in exchange for  $\text{Na}^+$  across the basolateral membrane. This process is brought about by the  $\text{Na}^+$ ,  $\text{K}^+$  (ATPase) pump (Reinhardt et al. 1988; Johnson 2001). Chloride moves passively across the basolateral membrane into blood in the small intestine. The movement of iron out of the cell into blood is not understood, but is bound to transferrin, an iron-binding protein (Johnson 2001).

The availability of dietary P, vitamin D, salivary P, and the pH of the intestinal lumen contribute to the absorption of inorganic P. There are two types of processes, passive or active, that are involved in the absorption of P in the small intestine. These processes help supply P to the animal when diets are sufficient or deficient in P or when plasma P levels are low (Horst 1986; Reinhardt et al. 1988). The active process is dominant when animals are deficient in P. This active process can be influenced by the active form of vitamin D (Braithwaite 1976; Horst and Reinhardt 1983; Horst 1986; Care 1994). The active form of Vitamin D is synthesized by the photochemical conversion of 7-dehydrocholesterol in the skin or by the photochemical conversion of ergosterol in plants (Horst and Reinhardt 1983). When vitamin  $\text{D}_3$  enters the bloodstream it is converted into 1, 25- dihydroxyvitamin D ( $1,25\text{-(OH)}_2\text{D}$ ) by microsomal enzymes located in the microsome and mitochondria of the liver (Horst et al. 1983). When P is low in the blood,  $1,25\text{-(OH)}_2\text{D}$  synthesis is activated, which in turn causes an increase in efficiency of absorption of P in the small intestine (Horst and Reinhardt 1983; Horst 1986; Reinhardt et al. 1988; Yano et al. 1991; Care 1994; McKay 1995). This process was

demonstrated in calves fed diets deficient in vitamin D for 57 days (Engstrom et al. 1987). A significant increase in plasma P concentration compared to control animals was observed when 1,25-(OH)<sub>2</sub>D was injected into these calves.

The conversion of vitamin D into 1, 25-(OH)<sub>2</sub>D can also occur with the help of the 1 $\alpha$ -hydroxylase enzyme in the kidney when plasma P is low (Engstrom et al. 1987; Reinhardt et al. 1988). When Jersey bull calves were fed a vitamin D deficient diet, their renal 1 $\alpha$ -hydroxylase activity increased from 37 pmol/min per g of tissue to 479 pmol/min g of tissue, a thirteen-fold increase in enzyme activity (Engstrom et al. 1987) compared to control.

Hormones play an important role in regulating P absorption in ruminants. Parathyroid hormone (PTH) has been reported to both inhibit and activate P transport. At low concentrations of P, PTH binds to a basolateral membrane receptor and initiates phospholipase C. During high concentrations of P, PTH binds to the basolateral membrane and activates adenylate cyclase. Phospholipase C and adenylate cyclase activate phosphorylation of the Na<sup>+</sup> Pi co-transporter. Phosphorus transport can be inhibited by parathyroid hormone-related peptide (PTHrP) by activating the same receptors located on the basolateral membrane that activate phospholipase C and adenylate cyclase. Vitamin D has been reported to block PTH from inhibiting P transport (McKay 1995). Parathyroid hormone has also been shown to affect 1 $\alpha$ -hydroxylase activity. Engstrom et al. (1987) determined this effect when they injected calves fed deficient vitamin D diets with PTH. They observed an increase in 1 $\alpha$ -hydroxylase activity (37 vs. 254 pmol/min per g of tissue) when compared to calves that were not injected with PTH (Engstrom et al. 1987).

The second process of P absorption in the small intestine is the passive process. This process is dominant when there is sufficient P available for absorption in the lumen of the small intestine. This process does not involve the interaction of hormones or vitamins, as does the active process, to ensure enough P is available for absorption by the intestine. Reinhardt et al. (1988) and Horst (1986) reported that the passive process of P absorption takes place when ruminants consume normal to high amounts of P from a diet or when the amount of P in plasma is high.

The salivary gland is a major contributor to P homeostasis in dairy cows. Saliva contains 70 to 80% of the total endogenous fecal P, which mixes with dietary P before passing through the digestive tract (Reinhardt et al. 1988). Recycling of P from the blood to the GI tract via saliva increases P in ruminal fluid when diets are low in P. This is important because rumen microorganisms need P for cellulose digestion and microbial protein synthesis (Hall et al. 1961, Evans and Davis 1966; Witt and Owens 1983). Evans and Davis (1966) found that P concentrations in ruminal fluid of Jersey steers were 198, 417, 543 mg P/L of rumen fluid when they were fed diets containing 0.067, 0.123, and 0.173% P respectively. This response was also found when Witt and Owens (1983) fed steers diets of 0.04, 0.16, and 0.54% P. Ruminal P concentrations in these steers were 264, 379, 434 mg/L of ruminal fluid. In both studies, P concentrations in the rumen fluid remained near or above 200 mg P per L of rumen fluid despite the diets that were deficient in P. Chicco et al. (1965) observed that rumen microorganisms need 60 mg or more of P per L of rumen fluid for cellulose digestion.

The P in saliva is almost completely composed of inorganic P and it is absorbed in the small intestine along with dietary P (Grace 1981; Horst 1986; Reinhardt et al. 1988). Challa et al. (1989) found P absorption from saliva ranged from 75-80% in calves fitted with rumen and abomasal cannulas. Salivary P secretion was estimated from the difference between dietary P intake and flow of P in the digesta at the reticulum by using  $P^{32}$  and Cr. The calves in this experiment were fed a basal diet with the addition of P from an inorganic P source that was added to the diet or infused into the abomasum. The inorganic P source that was given to the calves ranged from 10 to 100 mg/d per kg of body weight. Challa et al. (1989) observed that only 50-60% of the P that was consumed from the unsupplemental basal diet was absorbed. Calves that were fed the diet supplemented with an inorganic P source had a greater dietary P absorption (80-90%) compared with calves fed the unsupplemental basal diet. Challa and Braithwaite (1988) observed that when P intake and absorption of P increased, P secretion in saliva significantly increased (59.9, 77.3, and 86.8 mg of P in saliva per kg live weight with low, medium and high P diets).

Phosphorus concentration in saliva can be influenced or regulated by 1, 25-(OH)<sub>2</sub>D. Riad et al. (1987) observed a decrease in salivary P concentrations and

secretions from 13.9 to 0.18 mmol per minute and an increase of 1, 25-(OH)<sub>2</sub>D in plasma, when four heifers were injected with 1 $\alpha$ -OH-D<sub>3</sub>. This caused the heifers to be hyperphosphataemic. Manas-Almendros et al. (1982) reported similar results when goats and sheep were injected with 1,25-dihydroxycholecalciferol. Riad et al. (1987) reported an increase in salivary Pi concentration and secretion and a decrease in 1, 25-(OH)<sub>2</sub>D in the plasma of four heifers that were intravenously injected with Pi. When ruminants are deficient in P, vitamin D can be converted into 1, 25-(OH)<sub>2</sub>D, which causes an increase in efficiency and absorption of P in the small intestine. Riad et al. (1987) concluded, that 1, 25-(OH)<sub>2</sub>D can help regulate salivary P secretion when animals are deficient or sufficient in P. Ruminants have been observed to be efficient at P homeostasis with the help of vitamin D, 1, 25-(OH)<sub>2</sub>D and PTH.

Phosphorus absorption in the small intestine can be affected by the pH of the small intestine and rumen. Witt and Owens (1983) found that solubility of inorganic P sources such as sodium phosphate, mono-dicalcium phosphate, and defluorinated rock phosphate varies when emerged in ruminal buffer and abomasal fluid of varying pH. In this study, ruminal buffer was maintained at a pH of 5, 6, or 7, to evaluate the effect of pH on solubility. The abomasal fluid used in this experiment was collected from an abomasally cannulated Hereford steer, and had a pH of 2.5. Solubility of each inorganic P source was higher in abomasal fluid than in ruminal buffers at any pH. This increased solubility makes the P more available for absorption, and may increase P recycling to the rumen.

The pH of the small intestine in ruminants is lower than the pH of small intestine in monogastrics. Braithwaite (1976) and Challa and Braithwaite (1988) reported that the lower pH of the duodenum in ruminants will prevent P from precipitating, which will allow the mineral to be more available for absorption. An in situ experiment compared P release in nonlactating Holstein cows fed a control diet (alfalfa plus ruminal infusion of 3 L/d of distilled water), an acid diet (alfalfa plus ruminal infusion of 3 L/d of 1N HCL), an energy supplemented diet (alfalfa and ground corn plus infusion of distilled water), and an energy supplemented, acid diet (alfalfa and ground corn plus infusion of acid). When the pH of the rumen was reduced with acid infusion, P release was significantly increased compared to the water infusion treatment (87.8 vs. 90.2%). There was no change in P

release with the energy supplemented diets compared to the control (Emmanuele and Staples 1994). Similarly, when twelve ruminally cannulated sheep fed an orchardgrass hay diet were ruminally infused with a buffered solution (7.00 pH) or an unbuffered solution (6.40 pH), P solubility increased in sheep infused with the lower pH, unbuffered solution (Giduck et al. 1988).

### 1.2.2 Dietary phosphorus excretion

Dairy farmers have been overfeeding P for many years (Shaver and Howard 1995; Sink et al. 2000). Published research indicates that when ruminants are fed P in excess of their requirements, P excretion in feces and urine increases. A study conducted at the University of Florida evaluated the effects on P excretion when twelve Holstein cows were fed diets containing three different P concentrations (0.30% P, 0.41% P, and 0.56% P). They found that cows fed the high P diet excreted 100.4 g/d of P, significantly more than the cows fed the low P diet (60.0 g/d; Morse et al. 1992a). Other studies have also shown an increase in fecal P and urinary P excretion with an increase of dietary P (Call et al. 1986; Wu et al. 2000; Wu et al. 2001; Knowlton and Herbein 2002).

Phosphorus excretion in urine is usually very low, averaging less than 1 g/d or 1% of P intake (Morse et al. 1992a; Knowlton et al. 2001; Knowlton and Herbein 2002). However, when dietary P increases, more P is secreted through urine. Morse et al. (1992a) observed that cows fed the high P diet excreted two to three times more urinary P than cows on the low P diet (linear effect of diet,  $P < 0.01$ ). Wu (2001) and Knowlton and Herbein (2002) also observed increased urinary P with increased dietary P. Ruminants fed higher concentrate diets excreted more P in urine when compared to roughage diets (Scott 1972; Harmon and Britton 1983). Terishma et al. (1978) observed similar results with an increase in urinary P when wethers were infused with lactate. Scott et al. (1984) observed that sheep infused with P into the jugular vein and fed a pelleted grass diet excreted more urine than sheep fed the pelleted hay diet.

Urinary P excretion has been observed to vary between animals in the same species. Field et al. (1984) reported that two out of three sets of sheep triplets did not excrete any urinary P with any of the twelve diets that were fed. Field (1983) observed

differences in urinary P excretion in sheep fed low P diets. They found that most of the sheep excreted very little P in urine. Some sheep, however, had higher efficiencies of dietary P absorption, and excreted more urinary P than the other sheep. The sire breed of sheep has been reported to affect urinary P excretion (Field et al. 1986). Lambs with the sire breed of Texel had significantly higher urinary P excretion (525 mg/d) compared to Blackface (251 mg/d), East Friesland (200 mg/d), Finnish Landrace (126 mg/d) and Suffolk (123 mg/d). The lambs with the sire breed of Texel excreted 77 % more P in urine compared to the lambs with the sire breed of Suffolk.

Many farmers overfeed P because they fear a drop in milk production with lower P diets. The NRC (2001) specifies that a cow producing 54.4 kg/d of milk needs a dietary P content percent of 0.38% or 114 g/d, but a cow producing 25 kg/d of milk requires 0.32 % P in the diet or 65 g/d. The NRC (1989) specified that a cow producing 30 kg of milk requires 0.37% P in the diet or 78 kg/d of P. The NRC (2001) values have lowered by 17% compared with the 1989 NRC. Dietary P requirements of dairy cattle (g or %) depends on dietary ingredients because of varying P availability in the feed. Wu et al. (2000), Wu and Satter (2000) and Wu et al. (2001) observed that diets in the range of 0.33 and 0.37% dietary were sufficient for lactating dairy cows, and did not cause a decrease in milk production. Impaired milk production was only observed at dietary P contents of less than 0.30%.

Fecal P is composed of several P fractions (Spiekers et al. 1993). One fraction of fecal P is undigested feed P, or P that is not available for absorption. A second fraction is P of endogenous origin, including sloughed cells from the digestive tract and microbial cell walls. The final fraction is salivary P, which is secreted to maintain P homeostasis. Salivary P is excreted into the feces if excess P is available for absorption in the small intestine.

Braithwaite et al. (1985) used  $P^{32}$  to evaluate P metabolism in sheep and found that 73% of the dietary P was absorbed by the small intestine and 27% was excreted as endogenous fecal P. They reported that endogenous fecal P was significantly increased with an increase in P intake. Other experiments have come to the same conclusion, that endogenous P excretion in feces increased significantly with an increase in P intake (Braithwaite 1983; Challa and Braithwaite 1988; Challa et al. 1989; Brintrup et al. 1993;

Martz et al. 1999; Wu et al. 2001). Braithwaite et al. (1983) observed a linear relationship between P intake and endogenous fecal P excretion in pregnant and lactating ewes fed diets deficient in P compared to ewes fed diets sufficient in P concentrations. These studies included salivary P secretion into the endogenous P excretion in feces. These studies also observed an increase in P intake and increased endogenous fecal P excretion, but did not observe a relationship between dry matter intake (DMI) and P excretion. This does not rule out DMI as a cause of an increase in endogenous fecal P excretion as seen by Spiekens et al. (1993). Spiekens et al. (1993) divided ten cows into a high and low milk producing group and fed each group the same diet (0.20% P). The cows that consumed more feed excreted more P in feces (20.3 vs. 13.3 g/d P). They concluded that endogenous P excretion in feces is related to DMI.

Total P excretion increases with an increase in P intake (Call et al. 1986; Morse et al. 1992a; Wu et al. 2000; Knowlton and Herbein 2002; Wu et al. 2001). Maintaining high P dairy cattle diets can be very expensive with the increase of P supplements added to the diet. Wu et al. (2001) concluded that P excretion in feces could be reduced by 25-30% when farmers decrease dietary P concentrations and follow P recommendations for feeding dairy cattle. This will help farmers save \$10 to \$15 per cow per lactation by decreasing the amount of P supplements in the diets.

### 1.2.3 Dietary phytate phosphorus

Phytate P content, also called phytic acid, varies in different types of cereal grain, forages, and protein sources that are fed to lactating dairy cows. Approximately 65-70% of the total P in cereal grains is in the form of phytate P. Forages fed to dairy cows are very low in phytate P. Stems and leaves of forages have been observed to contain small amounts of phytate P compared with seeds (Nelson et al. 1976). Clark et al. (1986) reported that corn silage contained 0.13% phytate P prior to ensiling. The corn silage sampled after fermentation contained 0.0012% phytate P. They concluded that microorganisms involved in the fermentation of silage might decrease the phytate P in forages. Protein sources that are fed to dairy cattle also contain a significant proportion of phytate P.

**Table 1. Phytate P content of various feeds.**

<b>Feed</b>	<b>Phytate P, % of P content</b>	<b>Citation</b>
<b>Corn</b>	66%	Nelson et al. (1968)
	66%	Nelson et al. (1976)
	63.8%	Morse et al. (1992b)
	80%	O'Dell et al. (1972)
<b>Barley</b>	56%	Nelson et al. (1968)
	66.1 - 69.6%	Lolas et al. (1976)
<b>Wheat</b>	67%	Nelson et al. (1968)
	61.7 - 79.9%	Lolas et al. (1976)
	68%	Morse et al. (1992b)
<b>Oats</b>	56%	Nelson et al. (1968)
	56.7 - 65.4%	Lolas et al. (1976)
<b>Corn Silage</b>	0.0012%	Clark et al. (1986)
<b>Alfalfa Hay</b>	0 %	Nelson et al. (1976)
<b>Bermuda grass</b>	0 %	
<b>Brome grass</b>	0 %	
<b>Orchard grass</b>	0 %	
<b>Fescue</b>	0 %	
<b>Sudan grass silage</b>	0 %	
<b>Dried distillers grain</b>	43%	Nelson et al. (1968)
	32%	Morse et al. (1992b)
<b>Soybean meal</b>	67%	Nelson et al. (1968)
	60%	Nelson et al. (1976)
	68.6%	Morse et al. (1992b)
<b>Corn gluten meal</b>	62%	Nelson et al. (1968)
<b>Cottonseed meal</b>	71%	Nelson et al. (1968)

Distribution of phytate P within the kernel varies in different cereal grains. O'Dell et al. (1972) observed that 88% of the phytate P in corn was located in the germ of the kernel, 3.20% in the endosperm and 0.4% in the hull. In wheat, however, the majority of phytate P (87%) is located in the aleurone layer with, 12.9% in the germ, 2.2% in the endosperm, and none in the hull (O'Dell et al. 1972). Lolas et al. (1976) compared wheat mill bran of five different wheat varieties and observed that a large percentage of phytate P is located in the wheat bran (87.1% phytic acid P as % of total P) when compared to the whole-wheat seed.

#### 1.2.4 Phytate phosphorus digestion

Ruminants are able to utilize the phytate P in feedstuffs because of the microbial enzyme phytase (meso-inositol hexaphosphate phosphohydrolase, EC 3.13.8). Phytase breaks the phosphate groups from the inositol ring, which makes the P available for absorption in the small intestine. Reid et al. (1947) observed that phytate P in different feedstuffs can be digested by sheep. In this study four sheep fed different rations were slaughtered and the contents from the rumen, reticulum, abomasum, small intestine, large intestine and rectum were removed. The contents from these areas of the digestive tract were analyzed for phytate P content. No phytate P was detected in the rumen or reticulum, implying that rumen microorganisms are able to hydrolyze phytate P rapidly and completely. They also found no phytate P in the rumen or reticulum of a sheep that was slaughtered eight hours after feeding, again suggesting that phytate hydrolysis is very rapid.

An in vitro experiment conducted by Morse et al. (1992b) used rumen fluid from a lactating Holstein cow to determine the rate of disappearance of phytate P from several feedstuffs. Approximately 90% of the P in phytate disappeared in 6 to 8 hours in wheat middlings, rice bran, hominy, soybean meal and dried distillers grain. The phytate P in cottonseed meal disappeared within 12 to 24 hours. Hydrolysis of phytate P for all feedstuffs, except for cottonseed meal, was complete within 12 hours of incubation. Studies conducted on sheep also found that ruminants are able to hydrolyze phytate P (Tillman and Brethour 1958; Ellis and Tillman 1961). Tillman and Brethour (1958)

observed that 91.8% of the phytate P was digested when sheep were fed diets that were supplemented with calcium phytate.

Most studies indicate that hydrolysis of phytate P is very efficient in lactating dairy cows and calves. Thirteen Holstein cows consuming an average of 38.3 and 42.6 g/d of phytate P hydrolyzed 98% of the phytate P into inorganic P (Clark et al. 1986), calculated based on analysis of grab samples of grain, silage, orts, and feces for phytate P. Morse et al. (1992b) reported that 99% of the phytate P was hydrolyzed in vivo in lactating cows, based on phytate intake and excretion (Cr as a marker) in cows fed diets containing 0.38% phytate P (of total dietary DM; 69% phytate P as % of total dietary P). Nelson et al. (1976) measured phytate hydrolysis in the intestinal tract of nine-month old steers, ten-month old steers and 56-day old bull calves. The nine-month old steers were fed diets that contained 0.23% phytate P (49% phytate P as percent of total dietary P). They observed no phytate P in the feces of these calves, indicating that phytate P was completely hydrolyzed before excretion. The ten-month old steers consumed 6.2 g of phytate P of a diet that contained 0.29% phytate P (58% phytate P as a percent of total dietary P). When these animals were slaughtered, no phytate P was present in contents of rumen, abomasum, or small, and large intestine. These researchers concluded that phytate hydrolysis must have occurred in the rumen. Finally, the younger bull calves in this study were fed milk, and had access to the same diet as the ten-month-old steers (0.29 % phytate P). Less than 1% of the consumed phytate P was excreted in the feces, suggesting that complete phytate P hydrolysis occurs at a young age.

Others studies have reported low values for phytate P hydrolysis. Raun et al. (1956) used an artificial rumen to analyze the hydrolysis of calcium phytate in a 72 hour incubation period and reported that rumen microorganisms hydrolyzed 27% of the phytate P when 1 mg of phytate P was added to 20 ml of rumen fluid collected from a Shorthorn steer. When 1.58 mg of phytate P was added to the medium they saw an increase in inorganic P release, but a lower percent of phytate P hydrolysis (4.9%). They concluded that the artificial rumen system is not completely accurate and that phytate P hydrolysis could have been inhibited by an excess amount of substrate. Ellis and Tillman (1961) observed low digestibility of total P when sheep were fed wheat bran (25.49%). They felt that the particle size of the wheat bran containing phytate P was small and it

may have by-passed the rumen, which would not allow it to be digested by rumen microorganism.

In contrast, one experiment conducted by Duskova et al. (2001) measured detectable concentrations of phytic acid (8, 477, and 275 mg of phytic acid per kg DM) in grab samples of feces from 1, 6 and 13 week old calves. The 1 to 6 week old calves were consuming approximately 0.83 kg/d of calf starter and 4 l/d of milk until weaning at 6 weeks of age. The 13-week old calves were consuming approximately 6.05 kg/d of grain. They observed that phytic acid is decomposed in the ruminant digestive tract, but phytate P is not completely hydrolyzed in calves.

Phytase activity has been reported to vary depending on the P source, inorganic P or organic P source, present in the rumen. Godoy and Meschy et al. (2001) observed an increase in phytate P activity in a high and low P diet when an organic P buffer (corn sodium phytate) was compared with an inorganic buffer (monosodium phosphate) in a semi-continuous culture system (RUSITEC) that used rumen fluid from goats. The goats were fed either a high or low forage based diet. A buffer effect was observed, but no effect of diet on phytase activity was reported. They concluded that phytase activity increases when there is a larger supply of phytate P.

The enzyme phytase has been detected in several strains of rumen microorganisms. *Schwanniomyces castellii*, a strain of yeast, hydrolyzed 90 to 95% of the phytate P in soft wheat bran and glandless cottonseed flour (Sequeilha et al. 1993). Yanke et al. (1998) observed ruminal phytase activity in strains of the ruminal microorganisms *Selenomonas ruminantium*, *Megasphaera elsdenii*, *Prevotella ruminicola*, *Mitsuokella multiacidus*, and *Treponema spp.* Strains of *Selenomonas ruminantium* showed the most activity. Yanke also reported an increase in phytase activity occurred with higher grain diets in beef steers. Yanke et al. (1998, 1999) and D'Silva et al. (2000) observed that phytase activity occurs on the outer membrane and not in the cell of rumen microorganisms.

Recent research has focused on ways to improve phytate utilization by ruminants and other livestock species. Hatzack et al. (2000) reported varieties of grains being modified to reduce the phytate P concentration in the grain, offering potential to improve P availability with low phytate P grain varieties. They observed that two barley low-

phytate P mutant genes decreased in phytate P by 25% and 66% compared to the parent lines of the modified barley grain. Phytate P availability also varies with grain treatments and preservatives. Park et al. (1999) reported that soybean meal and rapeseed meal treated with formaldehyde had decreased phytate P digestion compared to soybean meal and rapeseed meal not treated with formaldehyde in an in situ experiment that used three crossbred sheep.

### 1.3 Starch Digestion

#### 1.3.1 Ruminant starch digestion

One way to meet the significant energy requirements of lactating dairy cows is by adding starch to the diet in the form of cereal grains. Starch makes up approximately 60-80% of the grain. The rumen microorganisms have the first opportunity to utilize the starch from the diet. Starch is broken down and fermented in the rumen into volatile fatty acids (VFA). Rumen fermentation of starch can range from 40-90% depending on many factors, including the structure of the grain, genotype, the availability of starch for enzymatic attack, grain type, processing affects, and environmental conditions of the rumen.

Starch is made up of two polymers, amylose and amylopectin, that consist of  $\alpha$  1-4 linked glucose residues. Amylose is a linear chain molecule with  $\alpha$  1-4 linked glucopyranosidic chains. Amylopectin is a branched polymer that has  $\alpha$  1-4 linked glucose chains joined by  $\alpha$ 1-6 bonds at every 20 to 25 glucose residues. Amylose and amylopectin content vary among plant species and cultivars. Starches from waxy cultivars contain less than 1% amylose, while non-waxy grains contain 14-34% amylose (Kotarski, 1992). Amylopectin is more digestible in the rumen than amylose, which will cause waxy cultivars to have higher starch digestibility in the rumen (Rooney and Pflugfelder 1986; Huntington 1997; Wester et al. 1997; Akay et al. 2002).

Genotype and grain source can alter the site of starch digestion in ruminants. In a comparison study, Philippeau et al. (1999b) discovered a significant difference in starch digestion between dent (60.8%) and flint corn genotypes (34.8%). Hull-less barley tended to have a higher rumen starch digestibility when compared to a diet with the hull (75.0 vs.

64.9%) still attached to the barley grain (Beauchemin et al. 1999). Philippeau et al. (1999b) looked at rumen starch digestibility of wheat- and corn-based diets. The wheat-based diet increased ruminal starch digestion when compared to the corn-based diets (86.6 vs. 47.8%). Research conducted by McCarthy et al. (1989) and Casper et al. (1990) showed that barley-based diets have a higher ruminal starch digestibility than corn-based diets. Herrera-Saldana et al. (1990) also demonstrated that some cereal grains are more available for rumen degradation than others. They compared five cereal grains using in vitro and in situ methods for analyzing digestibility of starch. They found that oats are more available for starch digestion in the rumen, followed by wheat, barley, corn and then milo. Sorghum-based diets were found to have a significantly lower ruminal starch digestion (75%) when compared to a corn- (84%) or barley- (88%) based diet (Spicer et al. 1986).

Understanding the structure of the kernel of cereal grains is necessary to understanding these differences in starch digestion. There are three main components that make up the grain kernel. The first layer is the pericarp, or outer protective layer. The second component is the embryo, and the last component is the endosperm. The endosperm consists of four sections, including the aleurone layer, and the peripheral, corneous, and floursy endosperm. A protein matrix surrounds the starch granules in the peripheral and corneous endosperm. The aleurone layer contains enzymes and inhibitors, but does not contain any starch granules. The floursy endosperm contains the largest amount of starch; these starch granules are not bound in a protein matrix. This causes the floursy endosperm to be more susceptible to processing effects (Kotarski et al. 1992; Zinn et al. 2002).

The protein matrix that surrounds the peripheral and corneous endosperm creates a challenge for rumen microorganisms attacking the kernel for starch fermentation (Kotarski et al. 1992; McAllister et al. 1993). Various processing techniques disrupt this protein matrix, making the starch more available for rumen fermentation and digestibility. Processing methods include both physical and chemical modification. Physical processes include breaking, cracking, grinding, or rolling grains. Chemical modification processes involve water, heat, and pressure (Nocek and Tamminga 1991). Grinding corn and barley from an average particle size of 3 mm to a particle size of 2 mm

increased starch digestion in the rumen with both grain types (McAllister et al. 1993). Callison et al. (2001) reported that processing grain to a finer particle size affects the digestibility of starch in the rumen. They compared coarse ground corn (4.8 mm), medium ground corn (2.6 mm), and finely ground corn (1.2 mm). As the particle size decreased, a quadratic effect was observed on true ruminal digestibility (49.8, 46.5, and 87.0%). Yang et al. (2001) observed that ruminal starch digestion increased from 37.8 to 50.1% when eight lactating dairy cows were fed flatly rolled barley (average particle size of 1.36 mm) compared with coarse grain barley (1.60 mm).

The application of heat, moisture, and mechanical action can also help degrade the protein matrix and allow starch to be more available for rumen fermentation. Steam flaking grain involves these techniques, making the starch in the steam flaked grain very digestible in the rumen. Huntington (1997) compared dry rolled corn and sorghum diets to steam processed corn and sorghum diets, and found that starch digestibility increased from 75 to 85% with steam flaking corn and from 52 to 78% when steam flaking sorghum. Other studies found that steam processed corn increased ruminal starch digestion when compared to dry rolled corn (Zinn 1990; Zinn et al. 1995; Barajas and Zinn 1998). Starch digestion in the rumen increased when steam flaked sorghum was compared to dry rolled sorghum (Oliveira et al. 1993; Theurer et al. 1999a).

Extent of steam processing affects starch digestibility. Oliveira et al. (1993, 1995), Yu et al. (1998), and Santos et al. (1999) all found that steam flaked corn or sorghum (flake density of 309 and 360 g/L) had a significantly higher starch digestibility compared with steam rolled corn or sorghum (flake density of 472 and 490 g/L). Grains that are steam flaked can be further processed to a smaller flake thickness or density (Zinn et al. 2002). When flake density was decreased, a linear increase in ruminal starch digestion occurred (Zinn 1990; Plascencia and Zinn 1996; Swingle et al. 1999; Theurer et al. 1999b). Steam flaking has a greater influence on starch digestion when compared to unprocessed grains than does reducing the particle size by coarse or fine grinding (Dhiman et al. 2002).

High moisture ensiling can also have an effect on starch digestion in the rumen. High moisture corn diets were found to increase starch digestion in the rumen of Holstein cows (Knowlton et al. 1998) and Angus-Hereford heifers (Streeter et al. 1989) compared

with dry rolled corn. An experiment comparing diets with 90% dry rolled corn, 90% high moisture corn or 90% steam flaked corn showed that ruminal starch digestion increased by 19% with the high moisture corn or steam flaked corn diet in steers when compared with the dry rolled corn diet (Cooper et al. 2002). Ensiling may not always significantly increase ruminal starch digestion when different grain sources are compared. Axe et al. (1987) saw an increase in ruminal starch digestion when dry rolled wheat replaced high moisture ground sorghum feed to steers. The wheat used in this experiment was dry rolled after harvesting, which may have increased the ruminal starch digestion compared with the sorghum diets.

An increase in starch availability can change the environment of the rumen. With faster rates of starch fermentation due to grain type and grain processing methods, the pH of the rumen usually declines (Axe et al. 1987; Zinn et al. 1995; Philippeau et al. 1999b; Yang et al. 2001). Other studies also found that rumen pH was numerically, but not significantly lower with a more digestible starch source (Oliveira et al. 1993; Zinn et al. 1995; Plascencia and Zinn 1996; Joy et al. 1997; Crocker et al. 1998; Knowlton et al. 1998). In several studies with Holstein cows, steam flaked corn or sorghum diets increased ruminal propionate concentration, and decreased acetate to propionate ratio compared with dry rolled corn or sorghum (Oliveira et al. 1993; Plascencia and Zinn 1996; Joy et al. 1997; Crocker et al. 1998). Steam flaked corn decreased acetate to propionate ratio in Holstein cows compared with coarse or finely ground corn (Dhiman et al. 2002). When wheat replaced high moisture sorghum in steers, the molar proportion of acetate and the acetate to propionate ratio both decreased (Axe et al. 1987). Knowlton et al. (1998) confirmed that the more digestible starch source tended to decrease molar concentration of acetate and acetate to propionate ratio in Holstein cows and Philippeau et al. (1999a) reported the same results in an in situ experiment that used non-lactating Jersey cows.

The presence of protozoa in the rumen can also affect rumen fermentation of starch. Rumen protozoa are very large in size and are able to store and digest the starch-digesting bacteria and starch granules (Kotarski et al. 1992; McAllister et al. 1993; Huntington 1997). Protozoa can engulf 130 to 21200 bacteria per protozoan per hour at bacterial densities of  $10^9$  cells per ml of rumen fluid (Russell and Hespel 1981). Starch

fermentation may be inhibited or reduced when starch granules and starch fermenting bacteria are engulfed. Mendoza et al. (1993) observed a 9% increase in rumen starch digestion in deaunated sheep when compared to sheep that were not deaunated.

Increasing feed intake in ruminants has been reported to decrease ruminal starch digestion. Increasing the intake of corn by steers from 8.7 to 10.6 kg/d decreased ruminal starch digestion numerically, but not significantly (81.4 vs. 76.0%; Russell et al. 1981b). It has been observed by several studies, that restricting feed intake did not affect starch digestibility in the rumen of sheep (Hart and Glimp 1991; Hatfield et al. 1993) or steers (Murphy et al. 1994; Zinn et al. 1995).

### 1.3.2 Ruminal starch digestion and dry matter intake and milk yield

Increased starch digestion in the rumen has been observed to decrease DMI in ruminants. Decreased DMI was observed in steers fed steam flaked corn compared with those fed diets containing a less digestible starch sources such as dry rolled corn (Owens et al. 1997; Barajas and Zinn 1998). Plascencia and Zinn (1996) observed an increase in DMI when lactating cows were fed steam flaked corn compared with dry rolled corn. Other studies have found an increase in DMI when cows were fed diets that were ground. Knowlton et al. (1998) observed an increase in DMI when cows were fed dry corn or high moisture corn that was ground compared with cows fed dry corn or high moisture corn that was rolled (22.5 vs. 21.3 kg/d). A low rumen-available nonstructural carbohydrate diet containing dry ear corn fed to lactating dairy cows caused an increase in DMI compared with cows fed a high rumen-available nonstructural carbohydrate diet containing high moisture shelled corn (Aldrich et al. 1993). Others have reported no significant change in DMI when lactating dairy cows were fed steam flaked corn or steam flaked sorghum replacing dry rolled corn or sorghum in the diet (Theurer et al. 1986; Oliveira et al. 1993; Oliveira et al. 1995; Joy et al. 1997; Crocker et al. 1998; Yu et al. 1998; Santos et al. 1999; Dhiman et al. 2002). Huck et al. (1998), Theurer et al. (1999b), and Cooper et al. (2002) observed similar results when steers were fed diets of steam flaked corn or sorghum compared with dry rolled corn or high moisture corn. Cows fed

barley-based diets had decreased in DMI compared with cows fed corn-based diets (McCarthy et al. 1989; Casper et al. 1990).

Starch sources that increase ruminal starch digestion have been observed to increase milk yield in lactating dairy cows. Plascencia and Zinn (1996) reported an increase in milk production when cows were fed steam flaked corn compared with cows fed dry rolled corn (25.5 vs. 22.9 kg/d). Theurer et al. (1999a) compared nineteen lactation studies and reported a significant increase in milk production when cows were fed steam flaked corn compared with dry rolled corn. Cows fed steam flaked corn increased milk production compared with cows fed coarsely ground corn (Dhiman et al. 2002), and also compared with cows fed steam rolled corn (Santos et al. 1999). Some experiments observed no change in milk production when cows were fed the more digestible starch source. Casper et al. (1990) reported no increase in milk production when cows were fed a barley-based diet compared with a corn-based diet. Cows fed steam flaked corn or sorghum did not increase milk production compared with cows fed steam rolled corn or dry rolled corn (Oliveira et al. 1993; Oliveira et al. 1995; Joy et al. 1997) and cows fed high moisture shelled corn had similar milk yield to cows fed ear corn (Aldrich et al. 1993).

### 1.3.3 Small intestinal starch digestion

Starch fermentation in the rumen may be incomplete due to many factors, which will then allow starch to be more available for digestion and absorption in the small intestine. When Owens et al. (1986) compared 40 different cattle experiments, they found that between 18 and 42% of dietary starch from corn or sorghum diets reached the small intestines for digestion. When starch reaches the small intestine it is broken down to glucose. Carbohydrases from the pancreas and in the intestinal mucosa allow degradation of starch to glucose. Amylose is broken down by pancreatic amylase into the oligosaccharides maltotriose and maltose. Amylopectin is broken down into maltose and isomaltose by isomaltase, which breaks the  $\alpha$  1-6 bond. Maltose, isomaltose, and maltotriose are then degraded to glucose by maltase, which is located on the brush border

membrane of the intestinal microvilli (Owens et al. 1986; Kreikemeier et al. 1991; Nocek and Tamminga 1991; Huntington 1997).

The small intestine has been reported to be more efficient at converting starch to energy compared with ruminal starch digestion. Owens et al. (1986) observed that the small intestine is 70% more efficient at converting starch to energy for gain compared with starch digested in the rumen of steers fed grain. Huntington (1997) pointed out, though, that there is a limit to starch digestion in the small intestine. They determined that only 45% of the starch digested in the small intestine into glucose is absorbed. This may be due to several factors, such as processing effects, grain type, increase in starch flow to the small intestine, intake, and enzyme activity.

Processing of grain can influence the site and extent of digestion in ruminants. Steam flaking of grain will cause more starch to be digested in the rumen and causes the starch entering the small intestine to be very digestible. When Theurer compared nineteen lactating cow studies that compared steam flaked grain and dry rolled grain, he found that post-ruminal starch digestion increased with steam flaking from 61 to 93% (Theurer et al. 1999a). Other experiments have also shown that steam processed grains have greater starch digestion in the lower GI tract compared with dry rolled grain (Zinn 1994; Oliveira et al. 1995; Zinn et al. 1995; Plascencia and Zinn 1996; Barajas and Zinn 1998; Crocker et al. 1998; Theurer et al. 1999b). These studies have observed an increase in digestibility when starch entered the small intestine, but the quantity of starch digested did not increase. Knowlton et al. (1998) found that ensiling corn increased starch digestion in the small intestine compared to dry ground corn (835.5 g/d vs. 19 g/d) in lactating dairy cows. Zinn et al. (1990) found that decreasing the flake density (12.7, 10.9 and 9.1 kg /bushel) of corn increased post ruminal starch digestion, but Swingle et al. (1999) found no significant increase in starch digestion in the small intestine with a decrease in flake density (14.5, 12.7, 10.9, and 9.1 kg/bushel) of sorghum.

Grain not digested in the rumen will pass and move to the small intestine. The increase in starch flow from the rumen to the small intestine can cause an increase in starch digested in the small intestine. Grain type can influence the digestion of starch in the small intestine. McCarthy et al. (1989) explored this concept by comparing corn-based diets to barley-based diets. They found that corn starch was not as digestible in the

rumen, so more starch reached the small intestine in cows fed corn-based diets compared with those fed barley-based diets. This caused the corn-based diets to have a higher starch digestibility in the small intestine than the barley-based diets (44% vs. 20%; McCarthy et al. 1989). Also, sorghum-based diets were found to have a higher post-ruminal digestion of starch when compared to barley- or wheat-based diets (Spicer et al. 1986; Axe et al. 1987). This was due to the increase in starch that escaped rumen fermentation and entered the small intestine when cows were fed the sorghum-based diets compared with the other two diets. Conventional yellow dent corn was found to have a higher starch disappearance from the small intestine compared with waxy corn (49.7 g/d vs. 22 g/d), but the waxy corn hybrid had a higher apparent starch digestion in the small intestines (95.9% vs. 83.3% of duodenal starch flow; Akay et al. 2002).

Nocek and Tamminga (1991) found a positive correlation between ruminal starch escape and starch digested in the small intestines. They concluded that an increase in starch flow to the small intestine does not mean it is efficiently digested (Nocek and Tamminga 1991). Kreikemeier et al. (1991) drew a similar conclusion when they infused raw starch into the abomasum at 0, 20, 40, or 60 g/h. They found that when steers were infused with the 60 g of corn starch, 58% of the starch disappeared in the small intestine, while 89% of starch disappeared when 20 g/h of corn starch was infused. Also, Kreikemeier et al. (1991) concluded that when steers were infused with corn dextrin (a starch solution prepared by heating and partial acid hydrolysis to mimic the action of the abomasum) at the same rates as raw corn starch, corn dextrin had a greater starch disappearance than raw corn starch. When dextrin is processed, the granular structure of the starch is broken down, indicating that the protein matrix can inhibit starch digestion.

Pancreatic  $\alpha$ -amylase activity in ruminants is important because it helps stimulate starch digestion in the small intestine. Diet and feed intake can influence  $\alpha$ -amylase activity in ruminants. A 50% increase in pancreatic  $\alpha$  amylase activity was reported when Holstein steers were fed a 90% forage-based diet compared with a 90% concentrate diet (504 vs. 333 units/g; Kreikemeier et al. 1990). They reported an increase in pancreatic  $\alpha$ -amylase activity when Holstein steers were fed at 2X maintenance compared with 1X maintenance (508.5 vs. 327.5 units/g). Russell et al. (1981a) observed an increase in pancreatic  $\alpha$ -amylase activity when steers were fed a corn-based diet at 2X and 3X

maintenance compared with 1X maintenance, but no significant difference was observed between 2X and 3X maintenance diets (776, 1439, and 1433 units/mg of protein). They reported a significant increase in  $\alpha$ -amylase activity (1433 vs. 1120 units/mg of protein) when a corn-based diet was fed at 3X compared with an alfalfa-based diet feed at a maintenance level of metabolizable energy intake (Russell et al. 1981a).

In a study with rats, a high carbohydrate, low protein diet produced significantly more amylase when compared to a low carbohydrate, high protein diet (Johnson et al. 1977). They also discovered that when a high quality protein, casein, was added to a high carbohydrate diet, there was a significant increase in amylase synthesis compared to diets that contained the poorer quality proteins gelatin, gluten, or zein. This interaction between protein quality and starch digestion may explain why Russell et al. (1981a) saw an increase in amylase activity with alfalfa-fed steers compared with steers fed a corn diet at 1X maintenance. They concluded this was due to the increase in protein when steers were fed the alfalfa-based diet compared with corn-based diets. Alternatively, the increase in amylase activity might have been due to the increase in energy intake with the 2X and 3X corn diets compared to the 1X diet. Janes et al. (1985) reported an increase in  $\alpha$ -amylase when sheep were fed ground corn-based diets compared with dried grass-based diets. These results may be due to the increased intake of energy with the corn-based diets, but energy intake in this study is confounded with energy source.

If pancreatic enzyme activity limits starch digestion, slaframine, a compound that increases salivary activity, might increase amylase activity. Infusion of slaframine to the duodenum of steers did not affect starch digestion in the small intestine, however, these results indicate that amylase activity is not the limiting factor in starch digestion in the small intestine (Streeter et al. 1995).

Maltase and isomaltase are other important enzymes needed to break down starch in the digestive tract of ruminants. Several scientists have conducted experiments looking at the activity of these two enzymes when different diets are fed. Russell et al. (1981a) found no change in maltase activity in the small intestine of steers fed corn- or alfalfa-based diets at 1X, 2X or 3X maintenance. Maltase activity was unaffected by diet when sheep were fed equal quantities of dried, pelleted grass or a 70% corn and concentrate mixture (Janes et al. 1985). Kreikemeier et al. (1990) observed that isomaltase activity

was not affected in calves when fed a 90% alfalfa-based diet or a 90% concentrate-based diet (sorghum and wheat).

#### 1.3.4 Large intestinal starch fermentation

Starch that escapes ruminal and small intestinal digestion becomes available for fermentation in the large intestine. Starch flowing to the large intestine may be fermented into VFA. Volatile fatty acids produced in the large intestine are similar to those produced in the rumen, with acetic, propionic, and butyric produced in the highest concentration (Hoover 1978). Ruminants can use the VFA that are produced in the large intestine, but the microbial N produced cannot be absorbed. Orskov et al. (1970) found that there is a limit to starch fermentation in the large intestine. They observed that sheep fed a dried grass diet had excreted large amounts of starch and N in the feces when they were infused with over 138 g corn starch into the cecum.

Processing methods and grain type can affect starch digestion in the large intestine. Theurer et al. (1999b) found that feeding steers dry rolled corn-based diets increased starch digestion in the large intestine compared with feeding steam flaked corn-based diets (1.2 vs. 0.5% of starch intake), but there was no significant effect of diet on large intestinal starch disappearance as a percent of starch entering the large intestine. Flake density of sorghum and corn did not affect starch digestion in the large intestine in steers (Swingle et al. 1999; Theurer et al. 1999b). Ensiling corn decreased starch disappearance from the large intestine compared with dry rolled corn (260.5 vs. 1346 g/d; Knowlton et al. 1998). Fecal starch flow decreased when cows were fed a high moisture corn diet compared with a dry corn diet (262 vs. 1432 g/d). Cows fed diets that were ground had a higher starch disappearance from the large intestine compared with cows fed diets that were rolled (1071 vs. 535 g/d). Knowlton et al. (1998) observed that fecal N flow was not significantly affected by treatments, but the total N digestibility was higher for cows fed the high moisture corn than in cows fed dry corn. Wilkerson et al. (1997) observed an increase in fecal N excretion when eight lactating dairy cows were fed a dry corn diet than cows fed a high moisture corn diet.

When a sorghum grain-based diet was fed to steers, 280 g/d of starch (10.5% of starch intake) was digested in the large intestine, while only 20 g/d of starch (0.7% of starch intake) was digested when the wheat-based diet was fed (Axe et al. 1987). In a study that compared diets of conventional yellow dent corn (CC), NutriDense™ corn (NC), and a waxy corn (WC) variety to steers, digestibility of starch in the large intestine was affected by the diet (Akay et al. 2002). NutriDense™ corn contains 1.97% percentage units more oil and 0.7% percentage units higher protein than CC. Steers fed the CC diet had a greater starch digestibility in the large intestine than steers fed the WC diet. Steers fed the CC diet had a greater starch disappearance from the hindgut as a percent of ileal starch flow compared with steers fed the NC diet (90.4 vs. 66.6%). The CC and NC diet had significantly more starch excreted in the feces compared with the WC diet (0.46, 0.64, and 0.19 g/d). The University of Kentucky scientist did not observe an increase in N excretion when steers were fed the CC diet, in contrast to the findings of Orskov et al. (1970).

Factors that contribute to increasing starch digestion in the rumen and small intestine are very beneficial as shown by previous studies. Starch broken down in the rumen and small intestine is used for microbial growth and energy requirements. Starch digested in the rumen and small intestine is used very efficiently when compared to starch that escapes and enters the large intestine. Fermentation of starch in the large intestine is excreted into feces as microbial N, so it is important to minimize starch fermentation in the lower gut of ruminants.

#### 1.4 Starch digestion and phosphorus excretion

Altering carbohydrate digestion may reduce P excretion. Glenn et al. (1998) observed a decrease in P excretion as a percent of intake when cows were fed a more digestible starch source, high moisture corn, compared with dry corn (72.2 vs. 80%). Guyton et al. (2000) conducted a study to evaluate the effect of starch source on P excretion using samples from four previously reported digestion experiments. The first two experiments were companion studies that used a total of 14 cows. Diets of the cows were alfalfa based and they compared high moisture corn to dry corn, ground or rolled.

The next two experiments were also companion studies that used a total of eight cows. Diets contained orchard grass silage or alfalfa silage, dry ground barley or dry ground corn. They found that the more digestible starch source in each experiment (high moisture corn vs. dry corn, corn vs. barley) decreased fecal P excretion as a percent of intake.

Altering the starch source in the diet may decrease P excretion. This may help reduce P runoff into nearby surface water, which may improve the quality of our nation's waters.

## CHAPTER 2

### STARCH DIGESTION AND PHOSPHORUS EXCRETION IN LACTATING DAIRY COWS

#### 2.1 Material and Methods

##### *2.1.1 Cows*

Eight Holstein cows ( $113 \pm 46$  DIM; mean  $\pm$  SD) were randomly assigned to treatments in a duplicated 4 x 4 Latin square experimental design with four 18-d periods. Cows were grouped into two squares by calving date and previous milk yield, and then were randomly assigned within square to one of four treatment diets. Squares were balanced for carryover effects. One group of cows was ruminally cannulated (101.6 mm diameter, Bar Diamond Cannula, Bar Diamond Inc., Parma, Idaho). The experiment was conducted under approval from the Virginia Tech Animal Care Committee.

In period one, a cow from group one, fed the DG-PA diet was not collected as she became sick. A cow from group two, period four fed the SF-low P diet, was not collected because of mastitis.

##### *2.2.2 Diets and treatments*

The four treatment diets contained 61% forage, and dry ground corn (DG) or steam flaked corn (SF), with no supplemental P (low P diet; 0.34% P) or supplemental purified phytic acid (PA; 0.45% P). Diets were formulated to meet the cow's nutrient requirements according to the 1989 NRC. Phytic acid (19.8% P, Biosynth International Inc., Naperville, IL) was added to the concentrate mix to provide additional P from an organic source. Diets were formulated to contain 37% starch, 16.6 % CP, and 31% NDF. Ingredient and nutrient composition of the diets are in Table 2 and 3.

Cows were housed in freestalls with access to Calan doors (American Calan, Inc., Northwood, NH) for the first 14 d of each collection period. Cows were fed once a day at 0800 h and milked at 0700 and 1900 h. Feed was offered 5-10% in excess of previous day's intake.

### *2.2.3 Sample Collection*

The first 14 d of each period were for diet adjustment. Cows were moved to individual stalls on d 15 for total collection of feces and urine. On d 15 and 18, body weight was recorded. On d 15, a sterile Foley urine catheter (22 French, 75 cc; C.R. Bard, Inc., Covington, GA) was inserted into the urethra for total collection of urine. Feed refusals were collected, weighed, and sampled on d 16-18. Feed ingredients were subsampled on d 16 of each period.

Urine, feces, and milk were collected, measured, and sampled on d 16-18. Urine was weighed every 6 h and acidified (22 ml of 6 N HCL/kg of urine). At 0800 on d 16, 17, and 18, all urine from the previous 24 h was pooled and subsampled, then stored frozen for later analysis. Feces were collected into a sealed container during each 24 h collection, then weighed, mixed, subsampled, and stored frozen for later analysis. Milk yields were recorded and samples collected at six consecutive milkings on d 16-18.

Rumen fluid samples were collected at 4-h intervals on d 17-18. Then sampling times were shifted forward by 4 h on d 17-18 so that samples collected represented every 4 h of a 24 h period. Samples were collected manually from six locations in the rumen, then filtered through four layers of cheesecloth. Filtered rumen fluid samples were acidified (0.2 ml of 3.7 N H<sub>3</sub>PO<sub>4</sub> per ml) and stored frozen for later analysis for VFA content.

Ruminal pH was measured at each rumen fluid sampling time with a portable pH meter (VWR brand pH/mV/Temperature Meter, model 2000/3000; VWR Scientific Products, South Plainfield, NJ.). The electrode (Orion Ag/AgCl Sure-Flow electrode, model 9172 BN; Orion Research Inc., Beverly, MA) was calibrated at each sampling time and inserted into the ventral sac of the rumen.

Rumen contents were collected on d 18 of each collection week at 0800 h. Rumen contents were evacuated manually and every tenth handful was subsampled into a separate container. All evacuated contents were weighed. The subsample contents were mixed, and a 500 g aliquot was stored for later analysis. The volume of the 500 g aliquot was measured with a 250 ml graduated cylinder and recorded. Rumen contents were returned to the same cow following sampling and measurement.

Rumen fluid was collected on d 17 each period at 0900 and 1300 h for analysis of phytase activity. Rumen fluid was processed in an industrial size blender (Waring) at low speed for one minute and filtered through four layers of cheesecloth; both steps were conducted under CO<sub>2</sub>.

#### *2.2.4 Laboratory Analyses*

Feed refusals, feed ingredients, feces, and rumen contents samples were dried to constant weight at 60°C in a forced air drying oven (Wisconsin Oven, Memmert; Schwabach, Germany). Samples were ground through a 1-mm screen in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA) and were analyzed in duplicate for P and N (Association of Official Analytical Chemists, 1984), organic P (Clesceri et al. 1998), and NDF and ADF sequentially with  $\alpha$ -amylase (Van Soest et al. 1991). Ash content of these samples was measured by incineration at 500°C for 6 h in a muffle furnace. Starch content was determined by a two-step enzymatic method (Holm et al. 1986) in which an YSI biochemistry analyzer (YSI Inc., Yellow Springs, OH) was used to read dextrose release (Cumberland Valley Analytical Services, Hagerstown, MD). Urine samples were analyzed for P and N (Association of Official Analytical Chemists, 1984). Milk samples were analyzed for fat, protein, MUN, total solids (Dairy Herd Improvement Association, Blacksburg, VA), and P (Association of Official Analytical Chemists, 1984).

VFA content of rumen fluid samples were measured via gas chromatography in a Hewlett Packard model 5890 Series II fitted with an autosampler and a flame ionization detector (Agilent Technologies Inc., Wilmington, DE). Individual rumen VFA were separated using a DB-WAXetr column (30 m x 0.53 mm ID, 1  $\mu$ m film thickness). An internal standard of 1 ml 30 mM 4-methylvaleric was prepared prior to the preservation

of the sample. A split injection (51:1) of 0.5 ul was used. Injector and detector temperatures were 250°C. Initial oven temperature of 125°C was held for 5 min and increased to 180°C at a rate of 15°C/min., and held for 6 min. The total run time was 14.6 min per sample. High purity helium was the carrier gas with a flow rate of 4.2 ml/minute. Inlet pressure was held constant at 4.4 psi. A ChemData Station was used for integration and quantification of individual VFA (Agilent Technologies, Wilmington, DE).

Processed, unacidified rumen fluid was centrifuged at 1000 x g for 5 minutes at 15 to 20°C to remove feed particles and protozoa. The supernatant was decanted and centrifuged for a second time at 1000 x g. The supernatant was incubated with a 9 mM phytic acid solution (0.1 ml of rumen fluid in 3 ml of phytic acid solution). The PA solution was comprised of phytic acid in a 0.25 M acetate buffer at pH 5.5. The phytase activity was measured as inorganic P released, umol per min per ml of rumen fluid, measured using Inductively Coupled Plasma Atomic Emission Spectrometry (Virginia Tech Soil Testing Lab, Blacksburg, VA).

### 2.2.5 Statistical Analysis

All data were analyzed using PROC MIXED procedures of SAS (SAS Institute, 1999), with the following model:

$$Y_{ijkl} = \mu + G_i + C_j(G)_i + P_k + S_l + P_m + (S * P)_{lm} + e_{ijklm}, \text{ where}$$

$\mu$  = overall mean,

$G_i$  = random effect of square (i = 1 to 2),

$C_j(G)_i$  = random effect of cow within square (j= 1 to 4),

$P_k$  = fixed effect of period (k = 1 to 4),

$S_l$  = fixed effect of starch source (l = 1 to 2),

$P_m$  = fixed effect of P source (m = 1 to 2),

$(S * P)_{lm}$  = effect of interaction of  $S_l$  and  $P_m$ , and

$e_{ijklm}$  = residual error.

Residual error was used to test main effects and interactions. Differences were declared significant at  $P < 0.05$  and trends at  $P < 0.10$ . Results are reported as least square means.

## RESULTS AND DISCUSSION

### 2.1 Diet composition

Treatment diets were formulated to differ in dietary P content (Table 2). Dietary ash content increased in the PA diet compared with the low P diet. This was due to the addition of phytic acid to the diet. The SF diet and the low P diet was lower in P content compared with the DG and PA diet. The PA diet had a slightly higher Ca content than the low P diet. Also the DG and PA diets were higher in organic P content than SF and low P diet.

### 2.2 Nutrient intake and digestion

#### 2.2.1 *Effect of starch source*

Cows fed SF had a lower DMI compared with cows fed DG (Table 4). Others have also observed that steam flaking corn decreased DMI compared with diets containing less digestible starch sources usually dry rolled corn (Owens et al. 1997; Barajas and Zinn 1998; Cooper et al. 2002). In contrast, Plascencia and Zinn (1996) reported an increase in DMI when cows were fed steam flaked corn compared with cows fed dry rolled corn. Others have reported no significant change in DMI when steam flaked corn or steam flaked sorghum replaced dry rolled corn or sorghum in the diet (Oliveira et al. 1993; Oliveira et al. 1995; Joy et al. 1997; Crocker et al. 1998; Huck et al. 1998; Yu et al. 1998; Santos et al. 1999; Theurer et al. 1999a; Theurer et al. 1999b; Dhiman et al. 2002).

An increase in VFA concentrations in the rumen, especially propionate has been reported to cause a decrease in DMI. Sheperd et al. (1998) reported an 8.6% decrease in DMI when eight Holstein cows were ruminal infused with propionate. Deetz et al. (1981) observed a decrease in DMI when sheep were infused with insulin and propionate or glucagon and propionate. There was no significant change in DMI when propionate was infused alone. Bhattacharya et al. (1975) reported a decrease in DMI when wethers were intraruminally infused with propionate. Quigley et al. (1991) reported no change in DMI when ewes, equipped with venous and arterial catheters, were infused with propionate. Cows fed SF had increased ruminal propionate concentrations (Table 10), which may be the cause of decreased DMI compared with cows fed DG.

Urine and feces excretion were both decreased when cows were fed SF (22.9 and 7.2 kg/d vs. 26.0 and 8.5 kg/d). These decreases were probably due to the decrease in DMI. Streeter et al. (1989) observed an increase in DMI and fecal excretion when Angus-Hereford heifers were fed high moisture corn compared with dry ground corn diets. Axe et al. (1987) reported an increase in fecal excretion when steers were fed a sorghum-based diet compared with steers fed a wheat-based diet, but there was no reported effect of DMI. There are no reported studies relating urinary excretion and starch source, but Woodford et al. (1984) observed a significant increase in water consumption (35.6 to 65.2 L/d) when DMI increased in Holstein cows (9.6 to 16.2 kg/d). Murphy et al. (1983) also reported a positive relationship between DMI and water consumption, and Paquay et al. (1970) reported that urinary water excretion is positively related to water absorbed.

Steam flaking corn increased apparent DM digestibility (Table 4) compared with DG. Cooper et al. (2002) found that total tract DM digestibility increased when steers were fed a diet containing SF corn when compared with a dry rolled corn diet. Santos et al. (1999) also saw an increase in DM digestibility when Holstein cows were fed SF corn when compared with diets containing steam rolled corn or steam flaked sorghum diets.

Total tract starch digestibility was significantly increased when cows were fed SF compared with DG (99.0 vs. 97.6 %; Table 2). Steam flaking of cereal grains disrupts the protein matrix, allowing the rumen microorganisms increased access to the starch granules (McAllister et al. 1993), explaining the increase in DM and starch digestibility when cows were fed the SF diet.

Starch excreted in feces of cows fed SF was significantly lower than cows fed DG (60.6 vs. 169.2 g/d). Others have reported a significant decrease in starch measured in feces of cows fed steam flaked corn or sorghum compared with dry rolled corn or sorghum (Oliveira et al. 1995; Zinn et al. 1995; Barajas and Zinn 1998). Zinn et al. (2002) reported that measuring starch concentration in feces of dairy cows could be an indicator of the quality of steam flaking of corn. There are many factors that can affect the quality of steam flaked corn, such as steam chest temperature, steaming time, roll gap and roll tension. Zinn et al. (2002) observed a relationship between fecal starch concentration and total tract starch digestion. They summarized 64 trials and observed a decrease in fecal starch excretion in cows when starch digestion increased.

Fiber intake, fiber digestibility and digestible DM were not affected by the starch source, but fiber intake was numerically lower in cows fed SF than cows fed DG. Grain processing of corn did not affect fiber digestion in steers (Joy et al. 1997; Santos et al. 1999), but Oliveira et al. (1993) observed lower ADF digestion in Holstein cows when sorghum was steam flaked compared with steam rolled corn. They also observed that NDF digestion was lower when steam flaked sorghum was fed in place of dry rolled corn. In the current experiment, cows fed the SF decreased ADF and NDF excreted in feces compared with the DG diet (2487 and 3734 g/d vs. 2658 and 4197 g/d). The decreased ADF and NDF in the feces of cows fed SF was due to a decrease in DMI rather than to any effect on digestibility. Zinn et al. (1995) saw a decrease of ADF in feces of Holstein steers fed restricted DMI diets to allow for 0.64 kg/d of weight gain compared to steers fed restricted DMI diets for 1.28 kg/d of weight gain.

### *2.2.2 Effect of phytic acid supplementation*

Supplementation of the diet with PA did not affect DMI (Table 4). Similarly, others have observed no effect of P supplementation on DMI in dairy cattle (Wu et al. 2000; Knowlton and Herbein 2002). Dry matter intake tended to decrease when wheat bran was fed as the P supplement in a lactating dairy cow diet compared with a diet that was supplemented with mono- and di-calcium phosphate (Knowlton et al. 2001). These

researchers suggested that this was due to the increased rumen fill that may have occurred when wheat bran was fed. A decrease in DMI was observed when lactating dairy cows were fed a diet deficient in P over two lactations and two dry periods (Valk and Sebek 1999). Ternouth (1990) suggested that P-deficient diets may affect microbial activity, which in turn would decrease DMI.

Cows fed the PA diet excreted less urine (-1.9 kg/d) than the cows fed the L diet, but no significant effect of P source was observed on feces excretion. No other experiments have reported a change in feces or urine excretion due to the P source in the diet. Perhaps cows on the PA diet in this study consumed more water causing an increase in urine excretion; no measurements were taken of water consumption. Although DMI was not significantly affected by PA supplementation, cows on the PA diet had a numerically higher DMI, which may have caused an increase in water consumption. Woodford et al. (1984) and Murphy et al. (1983) observed a significant increase in water consumption when DMI increased in Holstein cows, and Paquay et al. (1970) reported that urinary water excretion is positively related to water absorbed.

There was no significant effect of PA on DM and starch digestion, digestible DM, fiber digestion, fecal fiber, and starch excretion. No effect of the interaction of starch source and PA supplementation was observed on DMI, urinary excretion, fecal excretion, apparent DM digestibility, fiber digestion, starch intake and digestion, and fecal starch and fiber excretion.

## 2.3. Milk yield and composition

### 2.3.1 *Effect of starch source*

Starch source did not affect milk yield, protein %, protein yield, lactose %, lactose yield, SNF%, or SNF yield (Table 5). Other studies have also observed no effect on milk yield or milk composition with grain processing (Oliveira et al. 1995; Knowlton et al. 1998; Callison et al. 2001). In contrast, Theurer et al. (1999a) reported that SF corn increased milk production and milk protein yield compared with steam rolled corn and dry rolled corn and sorghum, but Oliveira et al. (1993) and Santos et al. (1999) observed

an increase in lactose % and SNF % with SF sorghum and SF corn compared with dry rolled sorghum and steam rolled corn.

Cows fed SF had decreased milk fat percent and milk fat yield compared with cows fed DG (3.43% and 1.15 kg/d vs. 3.66% and 1.24 kg/d). Previous studies also reported a decrease in milk fat yield and milk fat percent when cows were fed SF corn compared with cows fed dry rolled corn or dry ground corn (Plascencia and Zinn 1996; Crocker et al. 1998; Dhiman et al. 2002). A decrease in milk fat content may be associated with an increase in rumen starch fermentation. An increase in rumen starch fermentation decreases the acetate to propionate ratio, which is associated with milk fat depression (Yu et al. 1998; Crocker et al. 1998; Dhiman et al. 2002).

Milk fat depression has been observed to be caused by accumulation of the fatty acid *trans* C<sub>18:1</sub> in the rumen, which is then absorbed in the small intestine and used to synthesize milk fat in the mammary system (Kalscheuer et al. 1997). Kalscheuer et al. (1997) observed that Holstein cows fed a low forage diet without an added buffer, for example NaHCO<sub>3</sub> or MgO, had higher amounts of *trans* C<sub>18:1</sub> in milk fat than cows fed the higher forage diet with or without added buffer or low forage diet with buffer (5.8 % vs. 3.0%). They reported that cows fed the low forage diet had lower milk fat percent than cows fed the high forage diet (3.67 % vs. 4.16 %). Cows fed the diets with buffer tended to have higher milk fat percent than cows fed diets without buffer (3.76 % vs. 4.07 %). Gaynor et al. (1995) reported an increase in *trans* C<sub>18:1</sub> in milk fat when cows were fed the high concentrate diets compared with the control diet, high forage. These cows were reported to have depressed milk fat percent. They observed a decrease in *trans* C<sub>18:1</sub> in milk fat when cows did not show depressed milk fat percent. Dhiman et al. (2002) reported an increase in proportions of *trans* C<sub>18:1</sub> and a decrease in milk fat percent when cows were fed steam flaked corn compared with cows fed finely ground corn. In the current study, milk fat was depressed when cows were fed SF, but there was no decrease in pH. The milk fat depression that was observed in this study may be due to an increase in *trans* C<sub>18:1</sub> in the rumen of cows fed SF.

Steam flaking corn tended to increase feed efficiency (milk yield per kg DMI;  $P < 0.07$ ) compared with DG (Table 5). In many studies steam flaking corn or sorghum has been found to improve feed efficiency. In diets fed to steers (Owens et al. 1997; Barajas

and Zinn 1998; Huck et al. 1998), feed to gain ratio decreased with SF corn diets compared with dry rolled corn diets. Lykos et al. (1997) did not observe an increase in feed efficiency (milk/DMI) when cows were fed diets that varied in total nonstructural carbohydrates.

### *2.3.2 Effect of phytic acid supplementation*

Supplementation with PA had no effect on milk yield or milk composition (Table 5). Several studies have similarly reported no effect of dietary P concentration on milk yield or milk composition (Brintrup et al. 1993; Wu and Satter 2000; Knowlton and Herbein 2002). Knowlton et al. (2001) observed no effect of milk yield or milk composition when diets were supplemented with wheat bran (an organic P source) or mono- and di- calcium phosphate (an inorganic P source). Valk and Sebek (1999) and Wu et al. (2001) reported a decrease in milk production and milk fat yield when cows were fed diets deficient in P.

### *2.3.3 Effect of interaction on starch source and phytic acid supplementation*

There tended to be an interaction of starch source and P source for SNF percent ( $P < 0.08$ ; Table 5); the direction of the response to P source differed in cows fed DG or SF diets. Within the DG diets, SNF percent was numerically greater in cows fed PA than in cows fed the low P diet, while within the SF diet, PA supplementation decreased SNF percent. No effect of the interaction of starch source and PA was observed on milk yield, milk fat %, protein %, lactose %, milk fat yield, protein yield, lactose yield, and SNF yield.

## 2.4 Phosphorus partitioning and excretion

### 2.4.1 *Effect of starch source*

Cows fed SF had lower P intake (86.5 vs. 102.1 g/d) compared with cows fed DG (Table 6). Excretion of fecal P and total P was lower in cows fed SF than in cows fed DG (50 g/d and 50.4 g/d vs. 60.7 and 61.6 g/d). These reductions are due to the significant decrease in P intake in cows fed SF compared with those fed DG. Guyton et al. (2000) also observed that cows fed diets with a more digestible starch source, high moisture corn, had decreased fecal P as a percent of intake compared with those fed diets containing dry ground corn. Other studies have observed an increase in fecal and urinary P excretion with an increase in dietary P intake (Morse et al. 1992a; Knowlton et al. 2001; Knowlton and Herbein 2002). In the current study, the more digestible starch source was found to be the SF diet. The more available starch source increases rumen fermentation, which may increase phytase activity. Rumen microbes possess the enzyme phytase, which breaks down the organic P in feed. This allows the P to be more available for absorption in the small intestine (Reid et al. 1947; Raun et al. 1956; Tillman and Brethour 1958; Ellis and Tillman 1961). Increased phytase activity would explain the decrease in total P excretion and fecal P excretion when cows were fed the more digestible starch source, SF corn. However, starch source did not affect ruminal phytase activity or apparent P digestibility in this study.

Milk P as a percent of intake tended to be higher when cows were fed SF corn (14.8 vs. 13%;  $P < 0.06$ ) compared with cows fed DG. The P that was consumed by cows fed SF was diverted more towards milk production than cows fed DG. This may be due to the lower intake of P of these cows. Morse et al. (1992a), Knowlton et al. (2001), and Knowlton and Herbein (2002) reported an increase in milk P as percent of P intake when cows consumed lower dietary P concentrations compared with cows that consumed higher P concentration diets.

Cows fed SF had similar absorbed P, apparent P digestibility, milk P, P balance and ruminal phytase activity compared with those fed DG.

#### *2.4.2 Effect of phytic acid supplementation*

Cows fed the higher P diet had increased fecal P excretion, urinary P excretion, and total P excretion compared with cows fed the unsupplemented P diet (49 g/d, 66.1 g/d and 0.94 g/d vs. 45 g/d, 44.6 g/d, and 0.33 g/d; Table 6). The major route of P excretion in this study was through the feces. These increases in P excretion with PA were due to the significant increase in P intake when cows were fed the PA diet compared with the low P diet (108.8 g/d vs. 79.8 g/d). Morse et al. (1992a) reported an increase in fecal P and urinary P excretion when cows were fed the higher P diet (0.56% P) compared to a medium (0.41% P) and lower P (0.30% P) diets. Milk P as a percentage of P intake decreased when cows were fed the PA diet compared with the lower P diet (11.8 vs. 16%). Knowlton and Herbein (2002) also observed a decrease in milk P as a percentage of P intake with an increase in dietary P content. Brintrup et al. (1993) and Knowlton and Herbein (2002) also saw no effect of dietary P concentration on milk P secretion. Morse et al. (1992a) observed a 13.4% increase in milk P secretion when cows were fed a high P diet compared with a low P diet. In the current experiment, absorbed P and P balance tended to be higher in cows fed the PA diet compared with cows fed the low P diet (43 g/d and 29.4 g/d vs. 35.2 g/d and 22.4 g/d). Knowlton and Herbein (2002) also observed an increase in absorbed P, but not P balance, with an increase in P in the diet.

Supplementation with PA had no effect on apparent P digestibility, milk P secretion and phytase activity. Knowlton and Herbein (2002) reported a decline from 49% to 33% in apparent P digestibility when cows were fed a higher P diet compared with a lower P diet. Cows supplemented with organic P in the form of wheat bran had similar apparent P digestibility to cows supplemented with an inorganic P source, mono- and di- calcium phosphate (mean = 45%; Knowlton et al. 2001).

#### *2.4.3 Effect of interaction of starch source and phytic acid supplementation*

An interaction of starch source and P source was observed for ruminal phytase activity (Table 6). The direction of the response to P source differed in cows fed DG or

SF diets. Within the DG diet, ruminal phytase activity was numerically greater in cows fed PA than in cows fed the low P diet, while within the SF diet, PA supplementation decreased phytase activity somewhat. Yanke et al. (1998) observed an increase in phytase activity in ruminal fluid collected from a steer fed a 90% barley diet compared to a 55% barley diet and 100 % hay diet (17.5 vs. 8.1 and 4.3 units/ml of rumen fluid). Godoy and Meschy (2001) observed an increase in phytate P availability in a high and low P diet when an organic P buffer (corn sodium phytate) was compared to an inorganic buffer (monosodium phosphate) in a semi-continuous culture system (RUSITEC) that used rumen fluid from goats. They observed a buffer effect, but no effect of diet on phytase activity. They concluded that phytase activity decreases when there is a higher level of inorganic P available for degradation by rumen microbes. This may explain the increase in phytase activity when cows were fed the DG diet supplemented with PA than cows fed the DG diet with low P concentration. However, this does not explain the increase in phytase activity when cows were fed the low P concentration diet and SF. The increase in phytase activity when cows were fed the low P concentration SF diet may be due to the partially degraded protein matrix surrounding the starch granules in SF corn. The rupture of the protein matrix of the kernel would allow more access for the enzyme phytase to break down the phytate P in the low P concentration-SF diet more efficiently than the SF diet supplemented with PA. No effect of interaction of starch source and P source was observed on P intake, fecal P excretion, urinary P excretion, milk P secretion, absorbed P, apparent P digestibility, and P balance.

## 2.5 Nitrogen partitioning and excretion

### 2.5.1 *Effect of starch source*

Starch source had no effect on N intake, apparent N digestibility, milk N, and N balance (Table 7). Similarly, Zinn et al. (1995) and Theurer et al. (1999b) reported no change in N intake when steers were fed SF corn compared with dry rolled corn.

Plascencia and Zinn (1996), however, observed an increase in N and DM intake when

cows were fed SF corn compared with dry rolled corn. An increase in N digestibility was observed when steers and lactating cows were fed diets containing SF corn in place of dry rolled corn (Zinn et al. 1995; Plascencia and Zinn 1996), but Theurer et al. (1999b) observed no change in N digestibility when steers were fed diets with SF corn in place of dry rolled corn.

Fecal and total excretion of N was higher in cows fed DG than in cows fed SF (284.6 and 431.9 g/d vs. 248.8 and 378.2 g/d vs. and). The decrease in N excretion when cows were fed SF may be due to the increased availability of nutrients to the rumen microorganisms from steam flaking grain. Grain processing, such as steam flaking, disrupts the protein matrix allowing the starch to be more available for rumen fermentation and digestibility (Kotarski et al. 1992; McAllister et al. 1993). Starch digested in the rumen and small intestine is used very efficiently compared with starch that escapes and enters the large intestine. An increase in starch fermentation in the large intestine causes an increase in N excretion. Orskov et al. (1970) found that there is a limit for starch fermentation in the large intestine. They observed that sheep fed a dried grass diet excreted N in the feces when they were infused with over 138 g corn starch into the cecum. Wilkerson et al. (1997) reported an increase in fecal N excretion when cows were fed dry ground corn compared with high moisture corn. Nocek et al. (1987) reported an increase in N digestion in cows fed high moisture ear corn compared with those fed dried ear corn, dried shelled corn or high moisture shelled corn in an in situ experiment that used a rumen fistulated Holstein cow. They concluded that dry corn had a lower N digestion because of the protein matrix decreasing the solubilization and digestion of the corn. Grain processing, such as steam flaking, improves the starch digestibility and alters the site of starch digestion (Theurer et al. 1986; Plascencia and Zinn 1996; Huntington 1997). A shift in starch digestion in the large intestine will lead to an increase in microbial N and an increase in N excretion.

Cows fed SF had lower urinary N excretion compared with cows fed DG (128.8 g/d vs. 145.1 g/d). Prigge et al. (1976) reported a decrease in urinary N excretion when lambs were fed high moisture corn compared with dry ground corn. They concluded that lambs fed high moisture corn utilized N more efficiently than lambs fed dry ground corn.

Milk urea nitrogen concentration decreased when cows were fed SF in place of DG (8.7 vs. 9.7 mg/dl). Milk urea nitrogen is a tool that can be used to measure the efficiency of protein utilization and N excretion in dairy cattle. Jonker et al. (1998) reported a positive, linear relationship between MUN concentration and urinary N excretion. Dhiman et al. (2002) reported that cows fed SF corn had lower MUN concentrations compared with cows fed finely ground corn. They determined that the dietary N was directed more towards producing milk protein instead of urine with the SF corn diet. Prigge et al. (1976) observed that steers fed dry ground corn had higher levels of plasma urea N two hours after feeding compared with steers fed high moisture corn. They concluded that steers fed high moisture corn utilized N more efficiently compared to steers fed dry ground corn.

We observed relatively low MUN values despite diets with relatively normal protein contents. Jonker et al. (1998) calculated a targeted range for MUN concentrations of 10 to 16 mg/dL when evaluating a mathematical model that included MUN and milk protein to predict urinary N and fecal N excretion, total N excretion, N intake, and N utilization efficiency. They used raw data from three studies that consisted of 10 diets, 40 cows, and 70 observations. Just recently target MUN values have been observed to be lower than they were in the past (Kohn 2002). These values are lower because lab analysis of milk has improved. They reported that a Holstein herd averaging a 9,090.9 kg rolling herd average will have a target MUN concentration of 10 to 12 mg/dL. The values reported in the current study are closer to this revised normal range than to previous standards.

### *2.5.2 Effect of phytic acid supplementation*

Supplementation with PA had no effect on N intake, fecal N, apparent N digestibility, urinary N, milk N, total N excretion, and N balance (Table 7). Knowlton et al. (2001) reported a decrease in fecal N excretion and total N excretion in cows fed diets supplemented with wheat bran, an organic P source, compared with mono- and di-calcium phosphate, an inorganic P source. This decreased N excretion was likely due to the decreased DMI in cows fed wheat bran compared with those fed mineral sources of P.

They observed no effect of P source on urinary N excretion. No effects of the interaction of the starch source and phytic acid supplementation on N intake, fecal N, apparent N digestibility, urinary N, milk N, total N excretion, and N balance were observed.

## 2.6 Organic P partitioning and excretion

### 2.6.1 *Effect of starch source*

Compared with cows fed DG, cows fed SF tended to reduce fecal organic P excretion ( $P < 0.06$ ; Table 8) and reduced absorbed organic P ( $P < 0.10$ ). These observations were due to the decrease in organic P intake in cows fed SF compared with cows fed DG (26.0 g/d vs. 35.7 g/d). Apparent organic P digestibility was not affected by starch source. Approximately 65-70% of the total P in cereal grains is in the form of phytate P (Nelson et al. 1968; O'Dell et al. 1972; Lolas et al. 1976; Nelson et al. 1976; Morse et al. 1992b). Rumen microorganisms possess the enzyme phytase, which breaks down the phytate P in grains (Reid et al. 1947; Raun et al. 1956; Nelson et al. 1976; Morse et al. 1992b).

### 2.6.2 *Effect of phytic acid supplementation*

Cows fed diets containing supplemental PA absorbed more organic P (23.6 g/d vs. 9.9 g/d; Table 8) and excreted more organic P in feces than cows fed the lower P diet (20.7 vs. 7.8 g/d). This was due to the increase in organic P intake in cows fed the PA diet compared with cows fed the lower P diet (44.2 vs. 17.5 g/d). There was no effect of P source on apparent organic P digestibility. Similarly, Clark et al. (1986) reported that Holstein cows consuming an average of 38.3 and 42.6 g/d of phytate P hydrolyzed 98% of the phytate P into inorganic P. Morse et al. (1992b) reported that 99% of the phytate P was hydrolyzed in lactating cows, based on phytic acid intake and excretion in cows fed

diets containing 0.38% phytate P. No effect of the interaction of starch source and PA supplementation was observed on organic P intake, fecal organic P excretion, and apparent organic P digestibility.

## 2.7 Rumen nutrient pool size

### 2.7.1 *Effect of starch source*

Feeding steam flaked corn diets had no effect on ruminal pool size of ADF and NDF, organic P, P or N compared with DG (Table 9). Cows fed SF tended to have a lower ruminal starch pool size compared with cows fed DG (72.7 vs. 127.5 g/d;  $P < 0.09$ ; Table 9). Grain processing, such as steam flaking, disrupts the protein matrix allowing the starch to be more available for rumen fermentation and digestibility (Kotarski et al. 1992; McAllister et al. 1993; Owens et al. 1986; Nocek and Tamminga 1991; Huntington 1997). Cows fed SF may have had a lower starch pool size than cows fed DG because they had a lower DMI. Knowlton et al. (1996) reported a decrease in ruminal pool size of DM, starch, and NDF when cows were fed ground corn diets compared with cracked corn diets. They concluded that factors such as chewing increased the starch availability when cows were fed the cracked corn diets compared with the ground corn diets. Also an increase in rate of passage may have caused an increase in ground corn to escape rumen fermentation compared with cows fed the cracked corn diets.

### 2.7.2 *Effect of phytic acid supplementation*

Rumen pool size of P and N tended to be higher in cows fed the PA diets compared with the low P diets (64.8 and 298.9 g/d vs. 57.1 and 261.1 g/d; Table 9). The larger pool size of P in the rumen could be due to the increase in P intake compared with cows fed the lower P diet. There are no published studies that report P pool size in the rumen, but Evans and Davis (1966) and Witt and Owens (1983) observed an increase of P concentration in rumen fluid of steers with diets higher in P compared with low P diets.

Cows fed the higher P diet (PA) in the current study may also have increased rumen P pool size because of an increase in recycling of P in the rumen from saliva. Challa and Braithwaite (1988) reported an increase in P secretion in saliva of calves when fed diets that increased in dietary P concentrations. The large ruminal pool size of N for cows fed PA diets could be due to the numerically, but not significantly higher in N intake compared with the low P diet. The increase in N pool size with PA may also be contributed from an increase in recycling of urea from the liver via saliva in the rumen (Stallcup et al. 1975). Cows fed the PA diet had a numerically higher DMI than cows fed the low P diet, which may be the reason for an increase in N pool size.

Cows fed PA diets had similar rumen pool size of ADF and NDF, starch, and organic P compared with low P diet. No effect of interaction on starch source and P source was observed on rumen pool size of ADF and NDF, starch, P, N, and organic P.

## 2.8 Volatile Fatty Acids and rumen pH

### 2.8.1 *Effect of starch source*

Cows fed steam flaked corn had decreased ruminal concentrations of acetate, and reduced acetate to propionate ratio concentration compared with cows fed DG. Propionate and butyrate concentration were increased with SF, but isobutyrate, isovalerate, valerate, and total VFA concentrations were not affected by starch source (Table 10).

The increase in propionate and decrease in acetate to propionate ratio is due to the microbial changes caused by the increase of ruminal starch fermentation with SF compared with DG (Hungate 1966). In other studies, steam flaked corn or sorghum diets increased ruminal propionate concentrations and decreased acetate to propionate ratio in Holstein cows compared with dry rolled corn or sorghum (Oliveira et al. 1993; Plascencia and Zinn 1996; Joy et al. 1997; Crocker et al. 1998). Crocker et al. (1998) reported an increase in ruminal butyrate concentration in Holstein cows with steam flaked corn diets compared with dry rolled corn. Other studies indicated no effect on butyrate

concentration in lactating cows fed steam flaked corn or sorghum when compared to dry rolled corn or sorghum (Oliveira et al. 1993; Plascencia and Zinn 1996; Joy et al. 1997).

Feeding steam flaked corn had no effect on rumen pH (mean pH = 6.1) compared with cows fed DG. This suggests that cows fed steam flaked corn were able to adequately buffer the pH of the rumen. Zinn et al. (1995) observed a decline in ruminal pH when Holstein steers were fed SF corn compared with dry rolled corn. Other experiments have observed a numerically, but not significantly lower rumen pH when cows were fed steam flaked corn or sorghum compared to dry rolled corn or sorghum (Oliveira et al. 1993; Plascencia and Zinn 1996; Joy et al. 1997).

### *2.8.2 Effect of phytic acid supplementation*

Supplementation with PA had no effect on total VFA concentration, concentration of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and acetate to propionate ratio (Table 10). No experiments have been reported that have measured VFA concentration with varying concentration of dietary P. No effect of the interaction of starch source and phytic acid supplementation was observed on concentrations of propionate, isobutyrate, butyrate, isovalerate, valerate, total VFA and acetate to propionate ratio.

## TABLES

**Table 2. Ingredient composition of diets.**

Nutrient	Dry Ground corn		Steam Flaked corn	
	Low P	PA	Low P	PA
Diet ingredients	% diet DM			
Alfalfa silage	46.1	45.8	46.1	45.8
Corn silage	15.4	15.3	15.4	15.3
Ground corn	28.8	28.7	0.0	0.0
Steam flaked corn	0.0	0.0	28.8	28.7
Concentrate mixture	9.7	10.2	9.7	10.2
Concentrate ingredients	% of Concentrate DM			
Soybean meal, 48%	42.2	40.1	42.2	40.1
Soyplus	28.1	26.7	28.1	26.7
Mineral mix <sup>1</sup>	18.5	17.6	18.5	17.6
Sodium bicarbonate	5.3	5.0	5.3	5.0
Limestone	4.8	4.6	4.8	4.6
Salt-white	1.1	1.0	1.1	1.0
Phytate P	0.0	5.0	0.0	5.0

<sup>1</sup>Contained 2.50% Mg, 10 ppm of Co, 500 ppm of Cu, 40 ppm of I, 2250 ppm of Mn, 15 ppm of Se, 2500 ppm of Zn, 68,182 IU/kg of Vit A, 22,727 IU/kg of Vit D, and 273 IU/kg of Vit E.

**Table 3. Nutrient composition of diets.**

Nutrient	Dry ground corn		Steam flaked corn		SEm	<i>P</i> <		
	Low P	PA	Low P	PA		Starch Source	P Source	Starch X P Source
	% of diet DM							
NDF	31.7	31.5	31.7	31.4	0.46	0.91	0.61	0.90
ADF	21.5	21.2	21.8	21.6	0.48	0.51	0.66	0.89
Ash	7.7	7.9	7.8	7.8	0.08	0.31	0.05	0.81
CP	17.2	17.2	17.1	17.1	0.20	0.50	0.83	0.80
P	0.35	0.45	0.31	0.42	0.01	0.01	0.01	0.79
Ca	0.78	0.81	0.78	0.82	0.02	0.62	0.04	0.85
Mg	0.33	0.35	0.33	0.34	0.01	0.67	0.29	0.84
Organic P	0.08	0.17	0.04	0.14	0.01	0.02	0.01	0.97
Starch	24.5	24.4	25.1	24.9	0.42	0.17	0.67	0.91

**Table 4. Effect of starch source and supplemental dietary phytic acid (PA) on dry matter intake, digestibility, and manure excretion in eight lactating Holstein cows.**

	Dry ground corn		Steam flaked corn		SEm <sup>1</sup>	<i>P</i> <		
	Low P	PA	Low P	PA		Starch Source	P Source	Starch x P Source
n	8	7	7	8				
DMI, kg/d	25.1	26.4	24.2	23.7	1.0	0.03	0.63	0.25
Urine, kg/d	25.2	26.7	21.7	24.1	1.3	0.01	0.02	0.59
Feces, kg/d	8.3	8.6	7.2	7.2	0.3	0.01	0.31	0.56
Apparent DM digestibility, %	66.9	67.1	70.4	69.4	0.9	0.01	0.63	0.54
Digestible DM, kg/d	16.8	17.7	17.0	16.5	0.79	0.45	0.85	0.29
ADF intake g/d	5259	5281	4905	5067	274	0.31	0.74	0.80
% ADF digestibility	50.0	49.0	47.8	50.7	2.9	0.94	0.75	0.50
NDF intake g/d	7180	7557	6887	6890	471	0.36	0.61	0.77
% NDF digestibility	41.5	43.1	43.5	45.4	4.7	0.64	0.70	1.00
Starch intake g/d	6583	6745	6214	6302	291	0.17	0.67	0.90
% Starch digestibility	97.6	97.5	98.9	99.1	4.0	0.01	0.87	0.80
Feces ADF g/d	2616	2701	2492	2482	87	0.01	0.49	0.38
Feces NDF g/d	4134	4260	3737	3731	127	0.01	0.48	0.44
Feces starch g/d	167.7	170.6	64.5	56.7	28.7	0.01	0.93	0.85

<sup>1</sup>Unequal n, largest SEM (n=7) reported.

**Table 5. Effect of starch source and supplemental dietary phytic acid (PA) on milk yield, milk composition, and feed efficiency of eight lactating Holstein cows**

	Dry ground corn		Steam flaked corn		SEm <sup>1</sup>	<i>P</i> <		
	Low P	PA	Low P	PA		Starch Source	P Source	Starch x P Source
Milk yield, kg/d	33.8	34.0	33.1	33.5	0.94	0.50	0.74	1.0
Milk fat %	3.64	3.67	3.34	3.51	0.12	0.01	0.21	0.34
Protein %	2.93	2.98	2.98	3.02	0.09	0.28	0.15	0.96
Lactose %	4.67	4.78	4.80	4.72	0.81	0.58	0.85	0.16
Solids Non-fat %	8.60	8.73	8.75	8.54	0.20	0.82	0.69	0.08
Milk fat, kg/d	1.23	1.25	1.11	1.18	0.04	0.02	0.27	0.51
Protein, kg/d	1.15	1.20	1.15	1.18	0.03	0.69	0.18	0.68
Lactose, kg/d	1.82	1.89	1.90	1.84	0.07	0.84	0.96	0.37
Solid Non-fat, kg/d	3.34	3.47	3.40	3.30	0.10	0.53	0.89	0.25
Feed efficiency, (milk yield/DMI)	1.35	1.31	1.38	1.42	0.1	0.07	0.96	0.23

<sup>1</sup>Unequal n, largest SEm (n=7) reported.

**Table 6. Effect of starch source and supplemental dietary phytic acid (PA) on phosphorus intake and excretion in eight lactating Holstein cows.**

	Dry ground corn		Steam flaked corn		SEm <sup>1</sup>	<i>P</i> <		
	Low P	PA	Low P	PA		Starch Source	P Source	Starch x P Source
P intake, g/d	87.3	116.9	72.3	100.7	3.9	0.01	0.01	0.87
Fecal P, g/d	49.8	71.6	39.3	60.5	2.6	0.01	0.01	0.86
Absorbed P, g/d	37.5	45.7	32.9	40.2	4.3	0.25	0.08	0.91
Apparent P digestibility %	42.8	38.7	44.1	39.7	3.4	0.72	0.19	0.97
Urinary P, g/d	0.34	1.37	0.31	0.51	0.34	0.14	0.05	0.17
Milk P, g/d	25.9	25.6	24.2	25.2	1.1	0.28	0.71	0.48
Total P excretion, g/d	50.2	73.0	39.7	61.0	2.5	0.01	0.01	0.65
P balance, g/d	11.2	18.8	8.90	14.4	3.7	0.35	0.08	0.80
P balance, % of P intake	12.3	15.4	10.0	14.0	4.0	0.63	0.38	0.91
P balance, % of absorbed P	28.2	39.1	5.16	33.1	12.8	0.26	0.14	0.51
Milk P, % of P intake	30.1	22.0	33.9	25.2	1.7	0.06	0.01	0.85
Milk P, % of absorbed P	70.9	58.2	94.1	65.3	12.8	0.24	0.12	0.52
Ruminal phytase activity: P release, umol/min/ml rumen fluid								
	2.51	5.41	4.14	3.15	1.16	0.71	0.28	0.04

<sup>1</sup>Unequal n, largest SEm (n=7) reported.

**Table 7. Effect of starch source and supplemental dietary phytic acid (PA) on nitrogen intake and excretion in eight lactating Holstein cows.**

	Dry ground corn		Steam flaked corn		SEm <sup>1</sup>	<i>P</i> <		
	Low P	PA	Low P	PA		Starch Source	P Source	Starch x P Source
N intake, g/d	689.0	707.4	635.0	659.1	31.4	0.12	0.50	0.93
Fecal N, g/d	282.3	286.9	250.2	247.4	9.9	0.01	0.89	0.53
Apparent N digestibility %	59.1	59.6	59.4	62.3	2.1	0.47	0.40	0.56
Urinary N, g/d	145.1	145.0	121.7	135.9	8.3	0.02	0.20	0.52
Milk N, g/d	180.3	187.7	180.3	184.3	4.5	0.69	0.18	0.68
Total N excretion, g/d	427.4	436.3	373.0	383.3	12.1	0.01	0.35	1.0
N balance, g/d	81.2	85.1	83.1	91.5	27.6	0.88	0.83	0.94
N balance, % of N intake	11.3	11.5	11.0	13.4	3.9	0.84	0.73	0.78
Milk N, % of N intake	26.4	26.8	29.1	28.2	1.4	0.16	0.87	0.66
MUN, mg/dL	10.2	9.1	8.7	8.6	0.50	0.02	0.13	0.16

<sup>1</sup>Unequal n, largest SEm (n=7) reported.

**Table 8. Effect of starch source and supplemental dietary phytic acid (PA) on organic phosphorus intake and excretion in eight lactating Holstein cows.**

	Dry ground corn		Steam flaked corn		SEm <sup>1</sup>	<i>P</i> <		
	Low P	PA	Low P	PA		Starch Source	P Source	Starch x P Source
Organic P intake, g/d	21.5	49.8	13.4	38.5	3.60	0.02	0.01	0.65
Fecal organic P, g/d	8.65	22.5	6.91	18.9	1.69	0.06	0.01	0.47
Absorbed organic P, g/d	12.87	27.6	6.76	19.6	4.10	0.10	0.01	0.81
Apparent organic P Digestibility %	53.5	50.6	35.3	48.8	11.0	0.35	0.62	0.44

<sup>1</sup>Unequal n, largest SEm (n=7) reported.

**Table 9. Effect of starch source and supplemental dietary phytic acid (PA) on rumen pH and ruminal nutrient pool size in eight lactating Holstein cows.**

	Dry ground corn		Steam flaked corn		SEm <sup>1</sup>	<i>P</i> <		
	Low P	PA	Low P	PA		Starch Source	P Source	Starch x P Source
n	4	4	3	4				
Rumen contents, kg DM	9.2	9.6	9.0	9.8	0.9	1.0	0.32	0.74
Rumen ADF g	3567	3580	3572	3617	334	0.93	0.90	0.95
Rumen NDF g	5222	5450	4646	5314	689	0.51	0.42	0.68
Rumen starch g	112	143	74	71	37	0.09	0.63	0.55
Rumen P pool size, g	59.5	66.1	54.7	63.5	4.98	0.30	0.07	0.75
Rumen organic P pool size, g	23.5	25.1	20.1	22.3	2.69	0.23	0.43	0.92
Rumen N pool size, g	269.9	295.1	252.2	302.6	29.1	0.79	0.10	0.53

<sup>1</sup>Unequal n, largest SEm (n=3) reported.

**Table 10. Effect of starch source and supplemental dietary phytic acid (PA) on VFA concentration in eight lactating Holstein cows.**

	Dry ground corn		Steam flaked corn		SEm <sup>1</sup>	<i>P</i> <		
	Low P	PA	Low P	PA		Starch Source	P Source	Starch x P Source
n	4	4	3	4				
Rumen pH	6.01	6.00	6.10	6.15	0.08	0.24	0.60	0.51
Acetate, mol %	69.1	69.5	67.2	68.0	0.69	0.01	0.14	0.60
Propionate, mol %	16.3	16.5	18.0	17.4	0.31	0.01	0.47	0.14
Isobutyrate, mol %	0.91	0.90	0.91	0.88	0.03	0.67	0.37	0.73
Butyrate, mol %	11.3	10.7	11.5	11.5	0.59	0.02	0.18	0.10
Isovalerate, mol %	0.94	1.00	0.94	0.84	0.08	0.19	0.73	0.20
Valerate, mol %	1.43	1.44	1.50	1.41	0.06	0.47	0.36	0.18
Acetate/ Propionate	4.26	4.25	3.76	3.92	0.10	0.01	0.40	0.32
Total VFA, mM	152.1	152.4	151.0	152.2	3.9	0.85	0.85	0.90

<sup>1</sup>Unequal n, largest SEm (n=3) reported.

## CHAPTER 3

### CONCLUSIONS

The experiment that was conducted evaluated the effect of starch source, dietary phytate P content, and their interaction on P partitioning and excretion and ruminal phytase activity in lactating dairy cows. Results of this study indicate that the more digestible starch source fed to lactating dairy cows caused a decrease in urine, feces and total N and P excretion.

The reduction of manure nutrients with the steam flaked corn diet was likely due to a decrease in DMI and numerically higher efficiency of milk production compared with cows fed dry ground corn. Fecal P, urinary P, and fecal organic P excretion was reduced when cows were fed the steam flaked corn diet. This was due to a decrease in P and organic P intake compared with cows fed the dry ground corn diet. However, cows fed the steam flaked corn treatment had a greater portion of consumed P secreted into milk compared with dry ground corn treatment.

Fecal N excretion increased when cows were fed the dry ground corn diet. This may be due to the increased fermentation of starch in the large intestine instead of the rumen or small intestine. Microbial N is not absorbed from the large intestine, which may cause an increase in fecal N excretion. Grain processing, such as steam flaking, disrupts the protein matrix causing an increase in ruminal and small intestinal starch fermentation, which decreases the amount of starch to be digested in the large intestine. Cows fed the steam flaked corn diet had a higher starch digestibility and a decrease in starch excreted in the feces compared with cows fed dry ground corn diet. This demonstrates an increase in starch utilization, causing a decrease in N and starch excretion in the feces when cows are fed steam flaked corn compared with dry ground corn.

Milk urea nitrogen and urinary N excretion were also observed to be lower when cows were fed the steam flaked corn treatments compared with the dry ground corn treatments. This indicates an improved N utilization with steam flaked corn, which will help decrease environmental contamination.

With the steam flaked corn treatment, cows had decreased acetate:propionate ratio and decreased milk fat concentration, but no effect on pH of the rumen. This is probably due to the increase in total starch digestion and smaller starch pool size compared to the dry ground corn treatment. Some studies have found a decrease in pH when cows were fed a more digestible starch source. Although there was an increase in starch fermentation in the current study when cows were fed the steam flaked corn diet, the cows were able to adapt to the increase in starch fermentation in the rumen.

Increasing P in the diet with the PA diet caused cows to excrete more fecal and urinary P compared with cows fed the low P diets. Rumen microbes possess the enzyme phytase, which breaks down the organic P in feed. This allows the P to be more available for absorption in the small intestine (Reid, 1947; Raun, 1956; Morse, 1992b).

Cows fed the dry ground corn, PA diet had a numerically higher phytase activity compared with cows fed the dry ground corn, low P diet, while cows fed the steam flaked corn, PA diet had a lower phytase activity compared with cows fed the steam flaked corn, low P diet. Goody and Meschy (2001) reported an increase in in vitro ruminal phytase activity when goats were fed an organic P source compared with an inorganic P source. This may explain the increase in phytase activity when cows were fed the dry ground corn and PA diet. This does not explain the increase in phytase activity when cows were fed the steam flaked corn diet with a low P concentration. These observations may be due to the increased availability of phytate P when the corn was steam flaked. Enzymes will have a greater access to the phytate P causing an increase in phytase activity to be observed.

Ruminal phytase activity was not affected by starch source or P concentration of the diet, but some evidence is presented that diet may affect phytase activity. More work is needed to evaluate ruminal phytase activity and how it is affected by the diet. Corn processing, such as steam flaking, increased starch availability of the grain and caused a decrease in fecal and urinary P and N excretion without affecting milk yield or rumen pH. Altering starch source digestion to improve feed efficiency in lactating dairy cows may help farmers meet nutrient requirements on the farm and reduce potential P contamination of surface water.

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