Effects of Diet on Phosphorus Digestion in Dairy Cattle

Tzu-Hsuan Yang

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

Katharine F. Knowlton, Chair
R. Michael Akers
Duane Berry
Michael L. McGilliard
Chao Shang

August 8, 2006
Blacksburg, Virginia

Keywords: Beet pulp, dairy cow, non-fiber carbohydrate, phosphorus digestion, phytic acid

Effects of Diet on Phosphorus Digestion in Dairy Cattle

Tzu-Hsuan Yang

ABSTRACT

Two studies were conducted to evaluate the effects of diet on phosphorus (P) digestion in dairy cattle. The objective of the first study was to evaluate the effects of forage and non-fiber carbohydrate (NFC) content on total P (TP) and inositol phosphates-P (IPs-P) digestion. Samples of feed, duodenal digesta and feces from a previously conducted study were analyzed for TP and IPs-P. In this study, eight lactating Holstein cows were fed diets containing either 60 or 35% forage and either 30 or 40% NFC in a 2 × 2 factorial with replicated 4 × 4 Latin square design. Dietary TP content (% DM) was 0.35, 0.36, 0.36, 0.36 and dietary IPs-P content (%DM) was 0.08, 0.13, 0.06, 0.11 for diets with forage: NFC ratio 35:30, 35:40, 60:30, and 60:40, respectively. Increasing dietary forage content decreased IPs-P and TP intake, fecal TP excretion, and total tract IPs-P digestibility (72.4 vs. 61.4%). Fecal IPs-P excretion tended to decrease as increasing forage content. Duodenal IPs-P and TP flow and apparent TP digestibility were unaffected by forage content. Increasing dietary NFC content increased IPs-P and TP intake, duodenal IPs-P flow, fecal IPs-P excretion, total tract IPs-P digestibility (61.4 vs. 72.4%), and apparent TP digestibility (32.8 vs. 41.6%). Dietary forage and NFC content affected IPs-P and TP digestion. The second study was to evaluate the effects of increasing dietary beet pulp (BP) content to replace high moisture corn (HMC) on ruminal and post-ruminal digestion of TP and IPs-P. Eight lactating Holstein cows were fed diets containing 0, 6.1, 12.1 or 24.3% BP in a replicated 4 × 4 Latin square design. Samples of rumen contents, duodenal digesta, and feces from this previously conducted study were analyzed for TP and IPs-P content. Linear and quadratic effects of BP content were analyzed using Proc Mixed of SAS. Dietary TP and IPs-P content were reduced linearly with increasing BP (0.59, 0.58, 0.57, 0.56% TP and 0.15, 0.14, 0.13, 0.11% IPs-P). Intake, ruminal content, and rumen pool size of TP decreased with increasing BP content. Digestion of TP and duodenal flow and fecal excretion of IPs-P and TP were not affected. With increasing dietary BP content, IPs-P intake was reduced, ruminal IPs-P

pool size was reduced, and rumen turnover time (h) of IPs-P was increased. Apparent ruminal IPs-P digestibility (36.5, 31.8, 24.6, 13.6 %) and apparent total tract IPs-P digestibility (85.3, 82.7, 82.1, 79.1%) decreased linearly with increasing BP. Fecal excretion of IPs-P averaged 5.2 g/d. Replacing HMC with BP reduced digestion of IPs-P. The majority of IPs-P disappearance occurred post-ruminally. In conclusion, dietary BP, forage, and NFC content affected IPs-P digestion in dairy cows.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

REVIEW OF LITERATURE

1. Impacts of P excretion from dairy cattle on environment

Excess nutrient input to water bodies stimulates undesired algae bloom which subsequently causes the depletion of dissolved oxygen with the decomposition of these plants. Hence, other aquatic organisms die and the biodiversity in the water is greatly reduced (Carpenter et al., 1969). Among the nutrient sources, nitrogen and carbon can be exchanged between water and the atmosphere while phosphorus (P) is mainly obtained from surface water. Thus, P has been identified as the key element leading to excessive growth of algae and subsequent eutrophication.

Excess P runoff into streams, lakes, and estuaries from livestock farms contributes a great portion of the nonpoint source pollution of water and accelerating eutrophication (Sharpley et al., 1994; Correll, 1998). Kilmer and Taylor (1980) and Sims et al. (1998) concluded that agricultural animal waste accounts for a significant amount of P accumulated in soil and leaching to surface water. Depending on watershed, up to 48% of P loaded into the surface water can be from livestock farms (Smith and Alexander, 2000). In the past twenty years, the number of animals per dairy farm in the USA increased substantially (USDA-NASS, 2002) and this intensification is directly associated with the increase in P losses to surface water from manure.

Dou et al. (2002) characterized fecal P excreted from dairy cattle fed P supplements in different amounts. Excess P supplements in diets led to a higher total P concentration in feces and increased the proportion of water soluble P. This soluble fraction is susceptible to loss in runoff water and can be readily used by aquatic organisms. Reducing the P supplement to accurately meet animal requirements is an important nutritional strategy to minimize P loss from dairy farms.

2. Phosphorus compounds in feedstuffs

The P compounds present in feedstuffs include orthophosphate, phytic acid, phospholipids, and other organophosphate compounds (Bieleski, 1973; Toor et al., 2005).

Among these different P components, orthophosphate and phytic acid (Figure 1-1) are the main two forms. In general, cereals and legumes contain higher amounts of phytic acid whereas forages, such as grass and hay, have a greater proportion of orthophosphate. The variation in P fractions between forages and other grain by-products may affect P bioavailability and play an important role in P digestion and excretion in dairy cows.
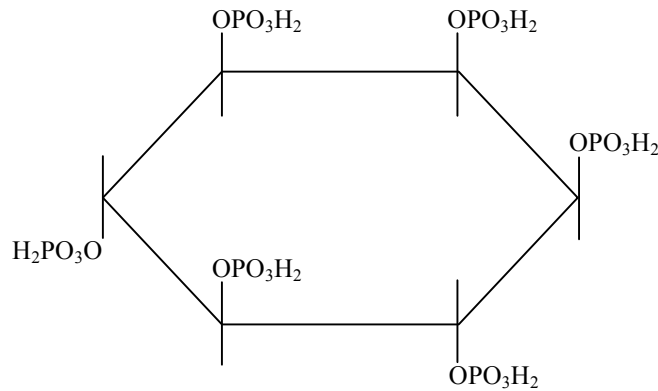
Figure 1-1. Structure of phytic acid (Adapted from Anderson, 1912).

2.1 Phytic acid and phytase

Phytic acid, *myo*-inositol hexakisphosphate (Figure 1-1), is the predominant storage form of P in cereals and legumes (Table 1-1). It is commonly present as salt, such as calcium phytate or sodium phytate, and plays an important role in various physiological functions in plants, especially seed germination (Reddy et al., 1989). Phytic acid accumulates during seed development, and reaches its highest level at seed maturity (Reddy and Sathe, 2002). Because of the characteristics of its molecular structure, phytic acid can directly react with starch or protein. Phytic acid can also chelate divalent cations such as Ca^{2+}, Cu^{2+}, Mg^{2+}, and Zn^{2+} forming insoluble complexes referred to as phytin (Cheryan, 1980; Reddy et al., 1989; Reddy and Sathe, 2002). Complex phytic acid compounds are less likely to be absorbed from the animals' digestive tracts.

The distribution of phytic acid in seeds is different among the types of plants (Reddy and Sathe, 2002). For example, the phytic acid in wheat and rice is concentrated in the germ and pericarp of the kernel, while in corn most phytic acid (88%) is in the germ. On the other hand, in legumes phytic acid is mainly present in cotyledons (mostly

within protein bodies). Therefore, it is difficult to develop one method which can effectively isolate phytic acid from different kinds of seeds.

Phytase is an enzyme which can hydrolyze phytic acid to orthophosphate and other intermediates of *myo*-inositol phosphate. The sources of phytase can be plants or microorganisms. The plant phytase (EC 3.1.3.26) preferentially hydrolyzes the phosphate at the 4 or 6 position whereas the microbial phytase (EC 3.1.3.8) first cleaves phosphate at position 3 (Tomlinson and Ballou, 1962; Irving and Cosgrove, 1972). The plant phytase has been found in some seeds, such as wheat and barley, and in other feedstuffs (Cosgrove, 1980; Eeckhout and Paepe, 1994). This endogenous capability for phytic acid hydrolyzation not only releases the inorganic P from the plant's P reservoir during seed germination, but also enables phytic acid degradation in animals and increases the P bioavailability of these grains (Reddy et al., 1989).

In most feed grains and grain by-products which contain relatively higher amounts of phytic acid, however, the phytase activity is varied and has no relationship with the total P content or phytic acid content (Table 1-1; Eeckhout and Paepe, 1994; Viveros et al., 2000).

2.2 Total phosphorus

The total P content of feedstuffs is important to dietary P management in dairy farms. It is believed that reducing the total P content in diets is the key to decreasing P excretion from dairy cows and achieving P balance in dairy farms. Unlike in monogastric animals, phytic acid can be hydrolyzed in ruminants via the microbial phytase in the rumen. Hence, most of the P compounds in feed are considered available to the ruminants. In order to meet the dietary P requirement and, at the same time, minimize P excretion from dairy cows, the total P content in different feed ingredients has been evaluated (Table 1-2).

Table 1-1. Total P, phytic acid-P content, and phytase activity in common feed grains and grain by-products, dry matter basis (Eeckhout and Paepe, 1994).

Feedstuff	Total P, %	Phytic acid-P, % of total P	Phytase[1], unit / kg
Wheat bran	1.16	84	2957
Wheat	0.33	67	1193
Barley	0.37	60	582
Corn distillers	0.90	21	385
Soybeans (heated)	0.57	46	55
Oats	0.36	59	42
Soybean meal,44% CP	0.66	53	40
Sorghum	0.27	70	24
Corn grain	0.28	68	15
Beet pulp	0.10	0	3

[1]Phytase unit defined as the amount of inorganic P released, μ mol min^{-1}, from a 0.0015M Na-phytate solution at pH 5.5 and 37 $^\circ$C.

Table 1-2. Total P content in common feeds (Eeckhout and Paepe, 1994; National Research Council, 2001).

Feedstuff	Total P, % of DM
Forages	
Legume hay	0.26
Grass hay	0.23
Bermudagrass	0.27
Grains and grain by-products	
Barley	0.37
Beet pulp	0.10
Brewers grains	0.67
Citrus pulp	0.12
Corn distillers grains	0.90
Corn grain	0.28
Cotton seed hulls	0.12
Cotton seed meal, 41% CP	1.15
Oats	0.36
Sorghum	0.35
Soybean meal, 44% CP	0.66
Wheat bran	1.16
Animal products	
Blood meal	0.30
Fish meal	2.69
Meat and bone meal	4.73

On average, the total P content in grain and other concentrates ranges from 0.11 to 1.20% while, in legume-grass forage, it ranges from 0.07 to 0.74% (Adams, 1975; Eeckhout and Paepe, 1994; National Research Council, 2001). It is noticeable that the some of the common grain by-products fed to dairy cows, such as brewers grains and corn distillers grains, contain higher amounts of total P, whereas beet pulp has a relatively lower P content.

Animal products such as blood meal and fish meal are also commonly fed to dairy cows as sources of high-quality protein. These animal products not only supply essential amino acids to the ruminants, but may also contribute substantial amounts of P. Depending on the materials origin and production process, the P content of these feedstuffs varies greatly (Table 1-2). The higher P animal products are those that contain appreciable amounts of bone.

The amounts of P in forage and other feeds may be influenced by many factors, including genus, species or strain of plant, type of soil, the stage of plant maturity, the climatic or seasonal conditions, and the application of fertilizers or soil amendments (Underwood, 1966; White et al., 1981). Normally, the content of P reduces markedly as plants mature, especially when the seed is shed. Moreover, a hot dry period may hasten the maturation of the plants. Therefore, in a dry season, the mature pasture is low in P and more P supplements are needed to the grazing animals. On the other hand, the use of phosphate fertilizers significantly raises the total P content in cereal grains and seeds by increasing the amounts of inorganic P and, subsequently, improves the P bioavailability to monogastric animals (Underwood, 1966). As the P supply from the fertilizer or other supplements, such as manure, exceeds the crop requirement, no yield advantage is found (Berrada and Westfall, 2005) and the surplus P may remain in the soil (Kihanda et al., 2005).

2.3 Other P compounds in feeds

The P components in feedstuffs can be divided into two large groups: inorganic P and organic P. The inorganic P compounds are mainly present in the form of orthophosphate. Other relatively minor inorganic P compounds include pyrophosphate which is contributed by ATP, and long chain polyphosphate, a storage form of P

compound occurring widely in lower plants (Bieleski, 1973; Toor et al., 2005). Although about half of the P in grains and grain by-products is in the organic form, the inorganic orthophosphate is the predominant P compound in most forages. The transported form of P in sieve tubes appears to be inorganic phosphate, which can also be stored in the vacuole of plant cells. Inorganic phosphates are important to plant nutrition and physiology as either a substrate or a product of most key biochemical reactions (Bieleski, 1973).

The organic P compounds in plants are mainly composed of DNA, RNA, phospholipids, and orthophosphate monoesters, particularly phytic acid. Except for phytic acid, the content of the other three organic P fractions is relatively low and the turnover rates fairly high (half-lives ranging from 2 sec to 5 hr; Bieleski, 1973). In a multi-farm study, the average proportion of these organic compounds in dairy diets was 1.0, 2.3, and 32% of total P for DNA, phospholipids, and phytic acid, respectively (Toor et al., 2005). Therefore, it is suggested that these minor P compounds are relatively unimportant to the animal nutrition in terms of P sources.

3. Phosphorus digestion and absorption in ruminants

In ruminants, the major route of endogenous and exogenous P excretion in dairy cows is feces, which accounts for 62 to 75% of total P excretion (Morse et al., 1992a; Bravo et al., 2003b; Bravo et al., 2003d). The factors affecting fecal P excretion by ruminants include the activity of endogenous ruminal phytase, the P content in diets, P secretion in saliva, and P absorption from digestive system. It was reported that the excess P excretion from dairy cattle was mainly due to the excess P input to the animals (Morse et al., 1992a; Tamminga, 1996; Rotz et al., 2002), indicating that the easiest way to reduce P loss to the environment is to reduce the supplemental P to precisely meet the animal's requirements.

3.1 Role of endogenous ruminal phytase in P digestion on dairy cattle

In contrast to monogastrics, ruminants can use the phytic acid in the feed as a P source because of the presence of the microbial phytase (Raun et al., 1956). Several studies have determined the digestibility of phytic acid in ruminants. Tillman and Brethour (1958) reported that the true P digestibility was 63% and 69%, respectively, in

sheep supplemented with calcium phytate and monocalcium phosphate. On the other hand, Lofgreen (1960) found that the true P digestibility was 33% and 50%, respectively, in mature wethers supplemented with calcium phytate and monocalcium phosphate. It was reported that the true P digestibility of high wheat bran diets, which contained large amount of phytic acid, was 25% for sheep, suggesting incomplete hydrolysis of phytic acid (Ellis and Tillman, 1961). However, less than 1% of ingested phytic acid was found in feces from the calves fed with corn-based and sorghum grain-based diets (Nelson et al., 1976). In lactating cows treated with diets containing different proportion of phytic acid, more than 99% of ingested phytic acid was hydrolyzed (Morse et al., 1992b). The variation in reported digestibilities of phytic acid may be due to the varying diet composition which affects the ruminal microbial ecosystem and, subsequently, changes the production of microbial phytase. The different phytic acid analysis methods used in these studies may also contribute to the varied results.

In the rumen, several strains of anaerobic bacteria produce microbial phytase to hydrolyze the phytic acid and release the free inorganic P (Yanke et al., 1998; Lan et al., 2002b). Yanke et al. (1998) found that the ruminal phytase activity was higher in steers fed high barley grain diets compared to steers fed 100% hay diets. Using qualitative plate-screening and quantitative phytase activity analysis, Yanke et al. (1998) found that the ruminal phytase activity was mainly from ruminal bacteria, such as *Megasphaere elsdenii*, *Prevotella ruminicola*, and *Selenomonas ruminantium*. Among the phytase-producing bacteria, *S. ruminantium*, one of the major ureolytic, acid-using, and soluble carbohydrate-using species (Stewart et al., 1988), had the highest phytase activity in the rumen, especially in steers fed high grain diets.

The structure and enzyme activity of *S. ruminantium* phytase and its mechanism for sequential phytic acid hydrolysis have been characterized recently. Similar to other bacterial phytase, the *S. ruminantium* phytase is smaller in size (46 kDa) and simpler in structure compared to the most extensively used and studied phytase found in soil fungus *Aspergillus* (Yanke et al., 1999). This phytase activity was produced late in the bacterial growth and required neither the presence of phytic acid for induction nor the limitation of phosphate for depression. While the fungal phytases from *Aspergillus* species have higher activity around pH 5.0 and 55°C, this phytase activity was optimal in the pH range of 4.0-

5.5 and at the temperature of 50-55°C. Through crystallography, Chu et al. (2004) found that the phytase produced by *S. ruminantium* appeared to be an open-pocket structure. When the substrate, phytic acid, approached and bound to the phytase, the conformation of phytase changed to a closed-pocket structure, favoring a mechanism of sequential dephosphorylation. The active site of phytase first attacked the position 5 phosphate of the phytic acid and proceeded to the hydrolysis cycle until the end product, inositol 2-monophosphate, was formed.

Another ruminal bacterial species, *M. jalaludinii*, with high phytase production was newly isolated from the rumen of cattle in Malaysia (Lan et al., 2002b). The optimum pH and temperature for the phytase produced by *M. jalaludinii* were 7 and 39°C, respectively (Lan et al., 2002a). While high phytase activity of this bacterial strain was detected *in vitro*, more studies are needed to identify the characteristics of this phytase.

3.2 Effects of diet on P digestion in dairy cattle

3.2.1 Effects of dietary P concentration on P digestion

The concentration of P in diet is the most important factor that influences the P digestion and fecal P excretion in dairy cattle. In the study conducted by Morse et al. (1992a), a 36.6% increase in dietary P responded to a 36.5% increase in total P excretion. While it was reported that a high P diet raised the apparent P digestibility in high-producing cows (Valk et al., 2002), most studies showed that P absorption (g/d) increases with an increase in dietary P, but the digestibility of P (% of intake) decreased (Wu et al., 2001; Knowlton and Herbein, 2002) or remained unaffected (Wu et al., 2003). Variation in P digestibility has not been observed in cows fed different source and amount of dietary fat (Rahnema et al., 1994), type of forage (Khorasani et al., 1997), and source of P (Knowlton et al., 2001), indicating that the amount of P excreted in feces is primarily correlated to the amounts of P consumed by dairy cattle. Dou et al. (2002) found that the proportion of soluble P in feces increased as the mineral P supplementation increased in diet. From a data set involving 39 diverse dietary treatments in dairy cattle, it was concluded that the intake of P varied mostly due to the variation in DM intake which was mainly affected by the differences in milk yield (Weiss and Wyatt, 2004).

3.2.2 Effects of grain type on P digestion

Due to their high total P and varied phytic acid P content, the type of grains may have influence on ruminal P degradation and duodenal P flow in ruminants and subsequently affect P digestibility (Table 1-3). In dry cows and lactating goats, rumen P solubility as measured by loss from porous nylon bags suspended in the rumen was different among cereals, cereal by-products, and meals (Bravo et al., 2000; 2002). In an *in vivo* study with sheep, Bravo et al. (2003a) reported that the apparent P digestion was highest in soybean meal and lowest in sunflower meal (23.0% and 4.1%, respectively), and that linseed meal, groundnut meal, and rapeseed meal were intermediate. On the other hand, Knowlton et al. (2001) found no differences in apparent P digestibility in lactating cows fed with mono- and dicalcium phosphate or wheat bran as supplemental P sources. Ekelund et al. (2003) used monosodium phosphate, rapeseed, sunflower seed/palm kernel, and wheat middlings/bran as main P sources for dairy cows and found no difference in apparent P digestibility. While the grain type may alter P digestibility, the discrepancies between studies may also arise from the DM intake, dietary P content, and milk P production.

Table 1-3. Effects of grain type and formaldehyde treatment on P digestibility in ruminants (Adapted from Bravo et al., 2003a; Ekelund et al., 2003).

P source	P digestibility[1], %		Citation
	Apparent	True	
Groundnut meal	12.7[d]	62.2[cd]	Bravo et al (2003a)
Linseed meal	14.9[bc]	63.8[c]	Bravo et al (2003a)
Rapeseed meal	12.6[d]	60.5[d]	Bravo et al (2003a)
Soybean meal	23.0[ab]	67.9[ab]	Bravo et al (2003a)
Sunflower meal	4.10[e]	59.7[d]	Bravo et al (2003a)
FT[2]-Rapeseed meal	19.3[b]	67.4[ab]	Bravo et al (2003a)
FT-Soybean meal	24.9[a]	66.5[bc]	Bravo et al (2003a)
FT-Sunflower meal	18.2[b]	68.4[ab]	Bravo et al (2003a)
Monosodium phosphate	49.8	NA	Ekelund et al (2003)
Rapeseed meal	51.9	NA	Ekelund et al (2003)
Sunflower meal	47.4	NA	Ekelund et al (2003)
Wheat middlings/bran	52.2	NA	Ekelund et al (2003)

[1]Among the first eight feedstuffs (those from Bravo et al., 2003a), values within a column within different superscripts differ significantly ($P < 0.01$).
[2]FT = Formaldehyde treated.

3.2.3 Effects of feedstuff processing on P digestion

The technical processing of feedstuffs may also affect ruminal P degradation and duodenal P flow. Heat treatment is an extensively used feed processing method to decrease the protein solubility in the rumen and, hence, increase flow of intact feed protein from the rumen. In most feedstuffs, phytic acid interacts with protein and forms stable complexes (Saio et al., 1967; Reddy et al., 1989). Therefore, the heat treatment not only decreases the protein availability to the ruminal microbes, but may also lower the amounts of phytic acid hydrolyzed by ruminal bacteria. It has been reported that heat treatment of soybean meal and rapeseed meal decreased the degradable fraction of phytic acid-P, increased the phytic acid in duodenal flow of sheep, and decreased dietary phytic acid digestibility (Konishi et al., 1999; Park et al., 2000). In the nylon bag study conducted by Park et al. (2000), 22, 37, and 55% of dietary P was recovered in the form of inositol phosphate at the duodenum of sheep fed untreated rapeseed meal and rapeseed meal heated at 133°C and 143°C, respectively. The majority of the inositol phosphates reaching to the duodenum were considered unavailable to the ruminants as it is to the monogastric animals.

As with heat treatment, formaldehyde treatment has been used for decreasing the ruminal degradability of protein in oilseed meals to improve protein utilization in ruminants. Hence, formaldehyde treatment may affect P digestion in ruminants as well. In a nylon bag study in dry cows, Bravo et al. (2000) concluded that formaldehyde treatment of cereal and meals decreased rumen P availability. It was also demonstrated that rumen P solubility of rapeseed meal and soybean meal decreased after formaldehyde treatment in both lactating cows and in goats (Bravo et al., 2002). In an *in vivo* sheep study of Park et al. (1999), formaldehyde-treated soybean meal and rapeseed meal had significant lower ruminal phytic acid digestibility than untreated soybean and rapeseed meals. In contrast, formaldehyde treatment had no effect on apparent P digestibility of soybean meal but increased apparent P digestibility of rapeseed and sunflower meals in sheep (Table 3; Bravo et al., 2003a). The authors in this last study suggested that the phytase activity of colonic microbes and homeostatic regulation due to the deficiency of P may explain the increase of apparent P digestibility. The sheep fed with formaldehyde-

treated soybean meal or rapeseed meal may have severe P depletion which triggered P absorption in large intestine and cecum.

3.2.4 Supplementation with exogenous phytase

Fungal phytase supplementation has been widely used in poultry and swine to improve the utilization of phytic acid from feedstuffs (Jongbloed et al., 1992; Augspurger and Baker, 2004). In lactating goats and dry cows, Bravo et al. (2002) found that fungal phytase supplementation to high concentrate diet increased P release of rapeseed meal and soybean meal while it had no effect when supplemented to high forage diet. It was suggested that the lower ruminal pH resulting from high grain diet (5.77) may be beneficial to the fungal phytase with an optimum pH at 5.5 (Reddy and Sathe, 2002) whereas the pH induced by high forage diet (6.47) may reduce efficiency of exogenous phytase. Similarly, the activity of ruminal endogenous phytase produced by the predominant phytase-producing bacteria is optimal at pH 4.0 to 5.5 (Yanke et al., 1999). This may also explain the higher endogenous phytase activity in the rumen of steers fed high concentrate diets (Yanke et al., 1998). Moreover, the increased rumen retention time caused by high forage diet may extend the reaction time for the ruminal exogenous phytase to hydrolyze phytic acid (Bravo et al., 2002). Therefore, there was no effect of fungal phytase supplementation on P digestion in high forage diet.

In lactating cows, Knowlton et al. (2003) found no effect of an exogenous phytase enzyme blend supplementation on P intake, milk P production, and urinary and fecal P excretion. On the other hand, apparent P digestibility tended to be higher with exogenous phytase. In the dairy cows treated with fungal phytase, Kincaid et al. (2005) also found increased IPs-P hydrolysis (%) and decreased fecal IPs-P excretion (g/d). Similarly, apparent P digestibility tended to increase with the phytase supplementation. Bravo et al. (2003a) reported that fungal phytase addition increased P digestibility of formaldehyde-treated soybean meal and formaldehyde-treated sunflower meal, but had no effect on formaldehyde-treated rapeseed meal. More data is needed on the effects of various doses of fungal phytase supplementation in various grain diets on P digestion.

3.3 Phosphorus absorption from the digestive system

Through ruminal and small intestinal cannulation techniques, some P fluxes were found across the rumen epithelium at ruminal inorganic P (phosphate) concentration above 4.3 mM (Breves et al., 1986; Beardsworth et al., 1989), but the major site of P absorption in ruminants was the small intestine (Khorasani and Armstrong, 1992; Care, 1994), especially in duodenum and jejunum (Pfeffer et al., 1970; Ben-Ghedalia et al., 1975; Wasserman, 1981).

Huber et al. (2002) isolated the duodenal and mid-jejunal segment of the goat small intestine and used immunohistochemistry method combined with radioactively labeled probes to characterize the phosphate transport mechanism. The H^+-dependent, Na^+-sensitive and Na^+-dependent, H^+-sensitive phosphate transport systems were found across the duodenal and jejunal brush border membranes, respectively, which are distinct from monogastric animals. Phosphate absorption in the small intestine of monogastric animals is less complex and primarily accomplished by a secondary active Na^+-coupled phosphate cotransporter located in the brush-border membrane of enterocytes. In the large intestine, net P absorption was observed and was significantly higher in sheep with high P intake (Grace et al., 1974). Höller et al. (1988) found slight net secretion of phosphate with the perfusion of P-free fluid into cannulated sheep colon. On the other hand, net phosphate absorption was observed when the P concentration of perfusion fluid ranged from 2.5 to 6.5 mM. Phosphate absorption from the proximal descending colon was also determined in the lamb when perfused with an electrolyte solution containing 1.2mM phosphate (Scharrer, 1985). This indicates that majority of P absorption occurs in small intestine, but some may occur in large intestine with high P intake. However, more data of net phosphate absorption from large intestine under practical conditions are still needed.

Net absorption of P in the intestine of lactating Holstein cows fed with alfalfa silage, whole-crop cereal grain silage, or high-fat diets ranged from 55.2 to 85.0 g/d which was unaffected by dietary treatment (Rahnema et al., 1994; Khorasani et al., 1997). On the other hand, the total tract P absorption was increased by the alfalfa silage and fat-supplemented diets due to decreased salivary P secretion. It was also found that the P absorption (g/d) was increased with increasing P content of diets, but the digestibility of

P was not affected (Khorasani et al., 1997; Knowlton et al., 2001). In the study of Khorasani and Armstrong (1992), the P flow at the proximal duodenum and terminal ileum was correlated linearly to P intake, however, the apparent absorption coefficient of P rose moderately as P intake increased and moderately declined with further increases in P intake.

The secretion of endogenous P from saliva is important to the P balance in ruminants and is the main origin of fecal P excretion. Up to 80% of endogenous P entering to the gastrointestinal tract is contributed by saliva (Care, 1994; Bravo et al., 2003c). In a summary of research with common diets, 77% of salivary P and 72% of ingested (dietary) P were absorbed (Bravo et al., 2003c). In the study comparing different forages (Khorasani et al., 1997), it was found that the salivary P secretion decreased with the increase of ingested P; thus, the P homeostasis was maintained which may explain the unaffected P digestibility and net P absorption with various dietary treatments in this study.

4. Methods of phytic acid analysis

Before the 1980s, quantitative analysis of phytic acid (*myo*-inositol hexakisphosphate; IP_6) was based on its reaction with ferric ion which resulted in precipitation of the iron (III)-phytate complex (Oberleas, 1971). However, this method is too insensitive for analyzing samples with a low phytic acid content. Recently, several advanced techniques have been developed to quantify phytic acid in various samples.

4.1 Anion exchange column chromatography

Determination of phytic acid content by anion exchange columns has been the standard for more than a decade. Latta and Eskin (1980) developed a simple and rapid method for phytic acid determination with the use of anion exchange resin (AG1-X8) to separate phytic acid from inorganic P. This separation is followed by the colorimetric assessment of the phytic acid concentration based on the reaction between ferric chloride and sulfosalicylic acid (i.e. Wade's reagent). However, this method is limited to analysis of fresh or dried plant materials which contain only small amounts of the lower inositol phosphates (IPs; Xu et al., 1992).

A different anion-exchange method for phytic acid determination was published by Harland and Oberleas (1977). In this method, samples were first extracted by 2.4% HCl, and the phytic acid in the extract was separated by an anion exchange column containing AG1-X4 resin. Inorganic phosphate and phytic acid were separated by elution with 0.1M and 0.7M sodium chloride respectively due to their different ionic affinities to the resin. The final eluent of phytic acid was then hydrolyzed by acid digestion and its P content was measured colorimetrically. In order to decrease the interference of plant proteins, minerals, and metal ions which naturally bound with phytic acid, Ellis and Morris (1983) further improved this procedure by adding EDTA to the extract and adjusting pH to 7. This modified method for phytic acid determination was tested in a collaborative study and finally accepted by Association of Analytical Chemists (Harland and Oberleas, 1986). Compared to the earlier iron-precipitation method, the anion exchange column method is more accurate for samples containing low amounts of phytic acid, and is capable of detecting phytic acid concentration as low as 0.1 mg/g. The disadvantages of this method are that it is rather laborious, and it may overestimate the phytic acid content because lower IPs, such as tris-, tetra*kis*-, or penta*kis*phosphates (IP_3, IP_4, IP_5) may co-elute with phytic acid (Phillippy and Johnston, 1985; Phillippy et al., 1986).

4.2 High performance liquid chromatography (HPLC)

Numerous studies have reported the application of HPLC to determine phytic acid content in dietary or digesta samples. HPLC is capable of separating the different fractions of IP compounds from IP_3 to IP_6 (Sandberg and Ahderinne, 1986; Sandberg et al., 1989). However, a standard method for analyzing phytic acid by HPLC has not yet been developed. Therefore, the acids used for extracting and purifying samples before column separation, column separation systems, and post column detection of IPs varied among current procedures.

For sample extraction, trichloroacetic acid, H_2SO_4, and HCl are the reagents commonly used (Tangendjaja et al., 1980; Camire and Clydesdale, 1982; Graf and Dintzis, 1982). These acids may interfere with detection of IPs, so need to be removed or

neutralized before loading extracted samples into the column separation system. Anion exchange columns have also been used for sample purification (Graf and Dintzis, 1982).

After sample extraction and purification, various chromatographic approaches (i.e. anion exchange, reverse phase, micellar, and ion chromatography) can be utilized to separate IPs. Xu et al. (1992) concluded that weak anion exchange or reverse phase ion-pair chromatography appeared to be the most promising separation systems. The principle of anion exchange chromatography is the same as the one discussed in previous section (4.1). Because the affinity of IPs to the resin increases with the number of phosphate groups on the molecule and the pH, different fractions of IPs can be separated by high ionic strength eluent with a concentration gradient. Due to the inconsistency of the post column eluate, the off-line detection of the individual IP fraction (from IP_1 to IP_6) is needed after the weak anion exchange chromatography. This detection approach is a time-consuming process.

As the individual fractions of IPs are collected after column separation, the quantity of each fraction can be measured by on-line or off-line detection methods. For on-line detection, a color reagent, such as pentahydroxyflavone (Meek and Pietrzyk, 1988) or Wade reagent (Wade and Morgan, 1955), is introduced to the eluate prior to the detector, thus, the IPs content can be quantified automatically. Although several phosphate or inositol assays can be used as off-line detection methods, these procedures are more laborious and not recommended for routine analysis of large sample sets (Xu et al., 1992).

4.3 Other analytical methods

4.3.1 Phosphorus-31 nuclear magnetic resonance spectroscopy

Phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P NMR) has been used for P characterization in food, feedstuffs, animal manure, and sewage sludge (O'Neill et al., 1980; Hinedi et al., 1989; Kemme et al., 1999; Turner, 2004; Toor et al., 2005). By measuring the number of P nuclei bound on the molecules at different positions, ^{31}P NMR is able to quantify IP_6 and other P forms, such as orthophosphate and orthophosphate diesters (phospholipids and DNA). The addition of EDTA and adjustment of pH are necessary for this method to extract P compounds and eliminate

interference of paramagnetic ions on the resonance signal (Xu et al., 1992). For IP_6 detection, four resonance peaks are produced because of its molecular symmetry characteristic. Due to its high resolution and distinction from orthophosphate and other IP signals, C-2 phosphate peak is specifically used for IP_6 determination (O'Neill et al., 1980). Although ^{31}P NMR characterizes P compounds and provides the P profiles of various samples, the main disadvantage of this method is that it is insensitive to detect lower IPs, especially for IP_1 to IP_4 (Kemme et al., 1999).

4.3.2 Near-infrared reflectance spectroscopy

Parrish et al. (1990) reported that near-infrared reflectance spectroscopy can be a simple and reliable method to determine the phytic acid content of cottonseed. However, because this method is not specific to phytic acid, the calibration curves must be derived from the correlation between near-infrared reflectance and the phytic acid content measured by standard methods, such as ion chromatography. Thus, the application of near-infrared reflectance spectroscopy would be limited to detect the phytic acid content of samples within the same matrix, such as routine determination of phytic acid in feedstuffs for quality control.

5. Effects of dietary carbohydrate on ruminal fermentation

As with all the other nutrients, P digestion in rumen and total tract are linked with the population of ruminal microorganisms. According to Yanke et al. (1998; 1999), starchy diets may promote phytic acid degradation by increasing ruminal bacterial phytase activity. Hence, dietary carbohydrate affecting ruminal fermentation and ruminal microbial ecosystem are also critical to P digestion.

5.1 Dietary starch

5.1.1 Effects of amount of starch on ruminal fermentation

Because starch is highly fermentable by ruminal microorganisms, increasing concentration of dietary starch increases total VFA concentration which leads to a lower ruminal pH and increased risk of ruminal acidosis (Robinson et al., 1986; Sutton et al., 1987; Stokes et al., 1991; Sievert and Shaver, 1993). With increased dietary starch, the

ruminal microbes produce relatively more propionate (Robinson et al., 1986; Sievert and Shaver, 1993) and the concentration of acetate remains the same (Robinson et al., 1986) or is slightly decreased (Sutton et al., 1987; Sievert and Shaver, 1993; De Visser et al., 1998). Among the ruminal VFA, propionate is the major gluconeogenic precursor taken up by the liver (Seal and Reynolds, 1993), thus, it is believed that increased concentration or molar proportion of propionate in the rumen results in a greater glucose supply to the animals.

Increased dietary starch also often increases the DM intake which may due to the higher digestibility of starch (Sievert and Shaver, 1993; De Visser et al., 1998). Robinson et al. (1986) concluded that the changes in the amount of ruminal VFA with increased dietary starch was mainly caused by the increased DM intake while the effect of starch concentration in diets was minor.

The type of forage fed to the animals may also play an important role on the production of ruminal VFA when different amounts of dietary starch were used. Visser et al. (1998) reported that the ruminal VFA was affected by the amount of supplemental starch only in diets containing early cut grass silage, compared to diets containing late cut grass silage.

The synchronization of starch and protein supply in the rumen is also an important nutritional strategy to optimize ruminal fermentation, promote microbial protein synthesis, and, subsequently, maximize the production of dairy cattle (Herrera-Saldana and Huber, 1989; Herrera-Saldana et al., 1990; Stokes et al., 1991). With synchrony between the ruminal fermentability of starch and protein, the ruminal microbes can utilize energy and N more efficiently for cell growth and protein synthesis. These strategies can minimize the loss of nutrients and benefit postruminal digestion and efficiency of energy and N utilization for milk production (Oldham, 1984; Huntington, 1997). Stokes et al. (1991) found that when the nonstructural carbohydrate content of the diet was greater than 24% and ruminally degradable protein was greater than 9% of DM, microbial protein synthesis was enhanced and more protein flowed to the abomasum. However, Cameron et al. (1991) reported no changes in ruminal VFA and microbial N with the supplementation of urea and starch in diets.

5.1.2 Effects of grain processing on ruminal fermentation

Different methods of processing grains, such as grinding, rolling, and steam-flaking, have been used to increase the proportion of starch digested in the rumen and the total tract digestibility of starch (Theurer, 1986; Ekinci and Broderick, 1997; Huntington, 1997). The improved starch digestibility in the rumen with processing is caused by breaking the outer coat of the kernel and disrupting the protein structure surrounding the starch granules in the endosperm to allow ruminal microbes and microbial enzymes to attach and hydrolyze the starch particles (Rooney and Pflugfelder, 1986; McAllister et al., 1990). This subsequently affects ruminal fermentation.

Grinding is a common processing method for corn grain in the diet of lactating cows. Compared to cracked corn, ground corn increased the ratio of propionate to acetate in the rumen and increased ruminal starch digestibility (Knowlton et al., 1996). Ruminal starch digestibility and fermentation were also improved when feeding finely ground corn to the dairy cows compared to coarsely ground corn (Ying and Allen, 1998; Ying et al., 1998). Additionally, Krause et al. (2003) reported that increasing the level of starch fermented in the rumen by replacing dry cracked corn with refined cornstarch (up to 57% of dietary starch) linearly increased the ruminal proportion and concentration of propionate but tended to decrease the ruminal concentration of acetate with no effects on the ruminal microbial yield.

Ruminal NH_3 concentration is commonly decreased by grinding corn more finely, indicating improved nitrogen utilization (Ekinci and Broderick, 1997; San Emeterio et al., 2000). While some studies reported the improvement of ruminal fermentation caused by grinding process, other researches showed no changes in the ruminal pH, VFA concentration, and microbial yield (Ekinci and Broderick, 1997; Knowlton et al., 1998; San Emeterio et al., 2000). It was suggested that the grinding process may also increase the passage rate of grain from the rumen which reduce the positive effect of grinding on ruminal fermentation (Ewing et al., 1986).

High moisture corn is commonly fed to lactating cows in the United States because the starch of high moisture corn is highly fermentable and can be rapidly digested in the rumen. Replacing dry, cracked-shelled corn with high moisture corn increased the concentration and proportion of propionate and decreased ruminal pH and

20

acetate concentration (Krause et al., 2002), indicating more energy became available to the ruminants. However, Oba and Allen (2003) found that the microbial efficiency was lower when comparing ground high moisture corn to dry ground corn (39.7 vs. 48.4 g of microbial N/kg of true ruminally degraded OM), suggesting that factors other than the energy availability may affect the microbial N production, such as the passage rate of starch and organic matter from rumen.

Steam-flaking is another extensively used processing method for corn or sorghum grain to increase the starch utilization in ruminants. Cows fed steam-flaked grains had higher proportion of ruminal propionate and higher microbial protein flow to the small intestine (Theurer, 1986; Joy et al., 1997; Crocker et al., 1998; Theurer et al., 1999). Ruminal and total tract starch digestibility increased linearly (Plascencia and Zinn, 1996; Swingle et al., 1999) or remained the same (Moore et al., 1992) as the flake density decreased. Compared to the cows fed with finely ground or dry-rolled grain, cows receiving steam-flaked corn or sorghum grain had lower concentration of ruminal NH_3 and higher ratio of propionate to acetate and apparent starch digestibility (Moore et al., 1992; Joy et al., 1997; Theurer et al., 1999; Dhiman et al., 2002). Moore et al. (1992) also reported that, as the flake density decreased from 0.40 kg/L to 0.27 kg/L, the ruminal fermentation was not affected, but DM intake and milk production decreased.

In contrast to the generally positive effect of steam flaking on digestibility of corn and sorghum, Espindola et al. (1997) reported no difference in ruminal fermentation between dry-rolled and steam-rolled wheat. Wheat is inherently more digestible than the former two grains (see below, section 5.1.3), so steam flaking is of less benefit.

The increased digestibility of steam-flaked grains does not guarantee improved animal productivity. When taken to extreme, increasing digestibility can have negative effects on ruminal fermentation. Santos et al. (1997) observed that with decreased flake density, ruminal starch digestibility was improved and the production of total VFA was also enhanced, but subclinical acidosis was induced and, subsequently, the DMI and milk production were depressed.

5.1.3 Effects of grain type on ruminal fermentation

The source of starch may affect ruminal fermentation and ruminal starch digestibility due to the inherent differences in solubility and degradability. Corn and barley are two major cereals fed to lactating cows as energy sources (Grings et al., 1992). Barley is considered more readily fermentable in the rumen due to its higher solubility (Casper et al., 1990). As corn grain replaced by barley in the diet, the ruminal concentration of propionate increased and ruminal NH_3 concentration decreased due to higher microbial N synthesis (Casper et al., 1990). In contrast, the source of starch had no effect on ruminal fermentation and animal performance (Grings et al., 1992; Petit and Tremblay, 1995). Further, several studies (Stock et al., 1987a, b; Khorasani et al., 2001) suggested that the combination of rapidly digesting starch (high moisture corn, wheat, barley) and slowly digesting starch (sorghum grain, dry corn) may have a better release of energy (more synchronized with protein degradation) in the rumen, thus, increasing the microbial N production and improving animal performance (feed intake and milk yield).

Effects of grain type, amount, and processing are not straightforward, however, as rate of starch degradation interacts with rate of passage and other microbial dynamics. For instance, Mendoza et al. (1999) found no effect of mixed high moisture corn and dry-rolled grain sorghum in varied combination on ruminal N concentration and total VFA production. One explanation is that increased ruminal protozoa by the combination of these grains engulf starch grains and altered ruminal starch fermentation.

5.2 Dietary fiber

5.2.1 Effects of amount of fiber on ruminal fermentation

Studies involved in the effect of the amount of dietary fiber on ruminal fermentation are usually linked to the ratio of forage to concentrate in diet. In general, increased forage proportion is combined with the decrease of dietary starch resulting in reduced energy content of diet. Usually, increased percentage forage decreases the concentration and molar proportion of ruminal propionate, but increases ruminal pH and the acetate concentration, thus, changing the ratio of propionate to acetate (Yang et al., 2001b; Sutton et al., 2003). This effect generally reduces the post-absorptive efficiency of energy utilization.

On the other hand, Yang and Beauchemin (2004) observed increased ruminal N digestibility with increased forage to concentrate ratio (49 vs. 60%). While the flow of microbial AA was higher with increased forage content, flow of dietary AA was decreased, thus, the flow of total AA was not changed (Yang and Beauchemin, 2004). Compared to low forage diets (35% forage), bacterial colonization to rumen particles was higher and bacterial flow to the duodenum was greater with a 55% forage diet (Yang et al., 2001a). The authors suggested higher activity of cellulolytic bacteria due to high ingestion of forage particles.

5.2.2 Effects of forage processing on ruminal fermentation

Processing of forage may change the chemical and physical characteristics of fiber and affect ruminal fermentation. Two of the most extensively used processing methods are altering chop length and ensiling, which provide physically effective fiber to stabilize ruminal environment and improve fiber preservation and digestion.

Dietary physically effectively NDF is the fiber that stimulates chewing activity and saliva secretion (Emery et al., 1960; Santini et al., 1983; Cassida and Stokes, 1986; Beauchemin et al., 1994; Mertens, 1997). This buffers ruminal pH and reduces the relative concentration of VFA (Owens et al., 1998; Calberry et al., 2003). The effectiveness of fiber is determined by the response of the ruminants (i.e. chewing activity) and varies with type of feedstuffs due to their different physical properties (Clark and Armentano, 1993; Clark and Armentano, 1997). Krause and Combs (2003) evaluated the effect of the particle size of alfalfa or corn silage on chewing behavior and ruminal fermentation in dairy cattle. With increasing forage particle size (from 2.7 to 5.3 mm in alfalfa silage and from 2.8 to 5.6 mm in corn silage), the time spent eating, chewing, and rumination was increased. This was also found in other studies (Mooney and Allen, 1997; Yang and Beauchemin, 2006) and the molar percentage of acetate was increased and of propionate reduced. In the latter two studies as well as the studies of Johnson et al. (2002b) and Yang and Beauchemin (2006) the mean ruminal pH and total VFA concentration were not affected by particle size. It was suggested that the increased saliva secretion by increasing physically effective NDF may not fully overcome the effect

of ruminal VFA production which lowered ruminal pH in dairy cows fed low fiber diets (Beauchemin and Yang, 2005; Yang and Beauchemin, 2006).

Limited work suggests that chemical processing of the forage may affect nutrient availability to the ruminants. Polan et al. (1998) found that heat treatment to forage prior to ensiling reduced the protein hydrolysis during ensiling and reduced the rate of *in situ* protein degradation. Reduced protein hydrolysis may provide a relatively higher amount of ruminal undegradable protein to the animal.

5.2.3 Effects of forage type on ruminal fermentation

Ruminal fermentation can also be affected by the type of dietary fiber. Fibers in plants of varying maturity, hybrid, and species have different physical structures and nutrient content, which influence ruminal fermentation characteristics and animal performance.

The nutritive value of forage is primarily determined by the stage of maturity at harvest (Johnson et al., 1999). In a nylon bag study conducted by Gasa et al. (1991), dairy cows consuming silage made with less mature ryegrass had higher ruminal NH_3 and butyrate concentration; ruminal pH, total VFA, acetate, and propionate concentration were unaffected. On the other hand, Johnson et al. (2002a) reported that cows fed diet containing whole plant corn silage harvested at more mature stage had higher ruminal butyrate concentration. Ruminal acetate concentration was greater and propionate concentration tended to be lower at advanced maturities (blackline compared to one-third and two-thirds milkline) due to decreased digestibility of starch as maturity of corn silage increased (Johnson et al., 2002a).

As expected, different sources of dietary fiber affect ruminal fermentation. Khorasani et al. (1996) determined the effects of alfalfa silage, whole-crop barley silage, whole-crop oat silage, and whole-crop triticale silage on ruminal digestion, fermentation, and nutrient supply to the small intestine. For cows fed alfalfa silage, ruminal pH was lower, VFA production was greater, and the ratio of acetate to propionate was higher compared to other treatments. This reflected higher ruminal digestion of alfalfa silage. On the other hand, no effect of forage source on ruminal pH was found in other studies when

replacing alfalfa silage with corn silage (Dhiman and Satter, 1997; Krause and Combs, 2003).

In contrast to alfalfa silage (Khorasani et al., 1996), the higher starch content in cereal silages contributed higher production of propionate and butyrate with the highest for cows fed barley silage and triticale silage and intermediate for cows fed oat silage. This is in agreement with Krause and Combs (2003), who reported that ruminal propionate concentration tended to increase when corn silage (higher starch content) partially replaced alfalfa silage. Within different cereal silage type, the effects on ruminal fermentation were not substantial (Khorasani et al., 1996).

Due to the higher protein content of alfalfa silage, ruminal NH_3-N concentration was higher in cows fed alfalfa silage compared to cows fed other cereal silages (Khorasani et al., 1996). However, the bacterial-N was not different among the diets. Thus, the efficiency of ruminal bacterial protein synthesis in the cows receiving alfalfa silage was the lowest, suggesting excess supply of rumen degradable protein from the diet. On the other hand, higher efficiency of ruminal bacterial protein synthesis was found in cows fed oat silage and triticale silage, indicating the utilization of additional dietary non-NH_3 N (i.e. peptides or amino acids; Hume, 1970). The authors speculated that the greater soluble fraction of triticale might have higher concentration of peptides and amino acids which stimulated bacterial growth.

Nonforage fiber sources have also been evaluated as a substitute for forage fiber in diets. In general, replacing forage fiber sources with nonforage fiber sources maintains ruminal pH (Swain and Armentano, 1994; Firkins, 1997). By partially replacing sorghum silage with soyhulls, Froetschel and Amos (1991) found that an increase in soyhulls replacement decreased ruminal pH but increased the ruminal concentration of acetate, propionate, butyrate, and NH_3 due to the higher water-holding capacity of soyhulls.

Whole cottonseed was also used to substitute for forage NDF (Harvatine et al., 2002); ruminal concentration of acetate was reduced and propionate increased with increasing level of whole linted cottonseed addition. This resulted in a decreased ratio of acetate to propionate which was also found in another study (Clark and Armentano, 1993). Also, efficiency of microbial protein synthesis decreased linearly as the level of whole linted cottonseed substitution increased, perhaps a result of the lower ruminal pH.

Depending upon their chemical composition and properties, nonforage fiber sources affected ruminal fermentation differently. More studies are still needed to investigate long-term effects of partially replacing forage with nonforage fiber.

5.3 Pectin and other non-starch non-fiber carbohydrates

High producing cows require more energy from diet, mainly from the starch of cereal grains, to increase milk yield. However, high starch diets may promote greater ruminal VFA and lactic acid production and reduce physically effective fiber which reduces salivary secretion, thus, rumen acidosis may occur. Substitution of non-starch non-fiber fibrous feedstuffs for starchy grain in low forage diets might maintain the ruminal environment without decreasing microbial yield (Strobel and Russell, 1986) or increasing the filling effect of the diet which limits intake.

Feedstuffs such as beet pulp and citrus pulp have been used as a substitution for corn grain due to their high concentration of soluble fiber. These tend to produce more acetate than propionate after ruminal fermentation without substantially increasing the level of lactic acid (Ben-Ghedalia et al., 1989). Ruminal pH and VFA production are generally unaffected but ruminal concentration of NH_3-N tended to decrease for cows fed beet pulp than for those fed starch as energy source (De Visser et al., 1991; Petit and Tremblay, 1995). Also, Leiva et al. (2000) found that cows fed diets containing citrus pulp had similar VFA production to those fed diets containing corn hominy or cornmeal. However, Voelker and Allen (2003c) reported that the substitution of beet pulp for high moisture corn quadratically increased ruminal VFA pool size (mol) to a plateau partially because the reduced ruminal dilution rate may increase the rate of VFA absorption across rumen wall. Additionally, the results in this study and the study of Clark and Armentano (1997) showed that replacing beet pulp for high moisture corn increased the molar proportion of acetate and butyrate and decreased the molar proportion of propionate.

In agreement with previous studies (De Visser et al., 1991; Petit and Tremblay, 1995), ruminal NH_3-N was reduced with beet pulp substitution, however, the efficiency of microbial protein synthesis was not improved. This may be due to the reduced ruminal turnover rate of microbial protein caused by greater passage rate of starch particles and NDF (Oba and Allen, 2003; Voelker and Allen, 2003b). On the other hand, diets

26

containing citrus pulp did not change ruminal N digestion and utilization, but the N utilization in dairy cows was improved by increasing milk protein yield (Leiva et al., 2000).

Other simple carbohydrates, such as sucrose, fructose, and glucose, can be alternatives to starch as energy sources. Microbial protein yield was the same from diets containing sucrose and starch (Strobel and Russell, 1986) and VFA production was unaffected by replacing corn starch with sucrose in continuous culture fermentors (Vallimont et al., 2004). In contrast, Heldt et al. (1999) found that the supplemental sucrose, fructose, or glucose in steer diets decreased the molar proportion of acetate and increased the molar proportion of propionate, butyrate, and branched-chain VFA, implicating higher efficiency of post-absorptive energy utilization.

Additionally, increasing sucrose decreased ruminal NH_3 concentration, enhanced ruminal microbial protein synthesis (Khalili and Huhtanen, 1991), and increased milk protein yield (Sannes et al., 2002). The effects of sugar supplementation in dairy diets on ruminal fermentation are still debatable and further investigation is needed.

In summary, the effect of dietary carbohydrate on ruminal fermentation is complex and depends on the properties of feedstuffs and the interaction between each ingredient in diets. As the ruminal fermentation is changed, ruminal environment is altered which influences rumen microbial ecosystem. This may subsequently affect P degradation in the rumen.

6. Conclusions

Dietary treatments have great impacts on P digestion in ruminants. In general, fecal P excretion increases and P digestibility decreases with increasing P content in diets. Grain types, feedstuff processing methods, and other factors including DM intake and milk P production may affect P digestion as well.

With the presence of endogenous phytase in the rumen, ruminants are capable of utilizing the phytic acid-P in feedstuffs. This ruminal phytase is mainly produced by *S. ruminantium*, a species of acid- and soluble carbohydrate-using bacteria. Previous studies have found highest ruminal microbial phytase activity in steers fed high grain diets. Although it is believed that the majority of phytic acid-P can be hydrolyzed to inorganic

phosphate in the rumen and be absorbed in the small intestine, few studies have been conducted to determine the phytic acid-P digestion in the dairy cows, especially with altered diet composition.

In this project, two studies were conducted to determine the effect of dietary composition on P digestion in dairy cattle. The objective of the first study was to evaluate the effects of forage and non-fiber carbohydrate content on duodenal and fecal flow of IPs and total P. According to Yanke et al. (1998), ruminal microbial phytase activity may increase in cows fed diets higher in non-fiber carbohydrate. Thus, we hypothesized that the ruminal phytic acid hydrolyzation would increase. Therefore, the flow of duodenal and fecal phytic acid-P would decrease and the phytic acid-P and total P digestibility increase. In contrast, increased forage content would decrease ruminal phytase activity which may reduce the phytic acid-P and total P flow in duodenum and feces.

In the second study, the objective was to evaluate the effects of replacing high moisture corn (highly digestible starch) with beet pulp (high pectin and soluble fiber) on duodenal and fecal flow of phytic acid-P and total P in lactating cows. We hypothesized that both duodenal and fecal flow of phytic acid-P and total P would decrease with increasing beet pulp due to its low phytic acid-P and total P content, and that digestibility of consumed phytic acid-P would be reduced with increasing beet pulp because of effects on ruminal fermentation.

CHAPTER 2

EFFECTS OF DIETARY FORAGE AND NON-FIBER CARBOHYDRATE CONTENT ON PHOSPHORUS DIGESTION IN LACTATING COWS

ABSTRACT

The objective of this study was to evaluate the effects of forage and non-fiber carbohydrate (NFC) content on total phosphorus (TP) and inositol phosphates-phosphorus (IPs-P) digestion in dairy cows. Eight Holstein cows were fed diets containing either 60 or 35% forage and either 30 or 40% NFC in a 2×2 factorial with replicated 4×4 Latin square design with 21-d periods. Dietary TP content (% DM) was 0.35, 0.36, 0.36, 0.36 and dietary IPs-P content (%DM) was 0.08, 0.13, 0.06, 0.11 for diets with forage: NFC ratio 35:30, 35:40, 60:30, and 60:40, respectively. Duodenal digesta and feces were collected and analyzed for IPs-P and total P content. Increasing dietary forage content decreased IPs-P and TP intake, fecal TP excretion, and total tract IPs-P digestibility (61.4 vs. 72.4%). Fecal IPs-P excretion was affected by the interaction of dietary forage and NFC content and tended to decrease as increasing forage content. Duodenal IPs-P and TP flow and apparent TP digestibility were unaffected by forage content. Increasing dietary NFC content increased IPs-P and TP intake, duodenal IPs-P flow, fecal IPs-P excretion, total tract IPs-P digestibility (61.4 vs. 72.4%), and apparent TP digestibility (33.0 vs. 41.6%). Dietary forage and NFC content affected IPs-P and TP digestion in dairy cows.

1. Introduction

To decrease excess phosphorus (**P**) runoff into streams, lakes, and estuaries and avoid the subsequent algae bloom and eutrophication (Correll, 1998), dairy nutritional strategies need to be adjusted to minimize P excretion. While decreasing P supplementation to meet animals' actual requirements reduces a substantial amount of P excreted in feces (Morse et al., 1992a; Tamminga, 1996; Wu et al., 2001; Knowlton and Herbein, 2002; Rotz et al., 2002), altering dietary grain types (Bravo et al., 2000, 2002, 2003a), feedstuffs processing method (Konishi et al., 1999; Park et al., 2000), or exogenous phytase supplementation (Kincaid et al., 2005; Knowlton et al., 2005) changes P digestion in ruminants as well.

Phytic acid, *myo*-inositol hexakisphosphate, is the predominant storage form of P in cereals and legumes (Eeckhout and Paepe, 1994). Unlike monogastric animals, ruminants are able to hydrolyze phytic acid into free phosphate in the rumen because of their endogenous ruminal microbial phytase. However, P digestibility ranging from 24 to 99% was observed in sheep (Tillman and Brethour, 1958; Lofgreen, 1960; Ellis and Tillman, 1961) or lactating cows (Morse et al., 1992b) fed diets containing calcium phytate. Further, Yanke and co-workers (1998) discovered higher ruminal bacterial phytase activity in steers fed with high barley diet compared to steers fed high forage diet. This implies that dietary composition may have an influence on P digestion in ruminants through affecting ruminal phytase activity.

The objective of this study was to evaluate the effects of forage and non-fiber carbohydrate (**NFC**) content on phytic acid-P and total P digestion in lactating cows. Based on Yanke et al. (1998), increasing dietary NFC would provide more starch for the predominant phytase-producing bacteria (*Selenomonas ruminantium*); thus, we hypothesized that ruminal microbial phytase activity would increase and subsequently enhance ruminal phytic acid-P degradation. This may decrease the flow of duodenal and fecal phytic acid-P and increase the digestibility of phytic acid-P and total P. In contrast, increased forage content would decrease ruminal phytase activity which may reduce the phytic acid-P and total P flow in duodenum and feces.

2. Methods and Materials

Cows, treatments, and sample collection were outlined by Schwab et al. (2006). Briefly, 4 primiparous and 4 multiparous lactating Holstein cows fitted with ruminal and duodenal cannulas were assigned to replicated, concurrently run 4 × 4 Latin squares based on similar DIM (204 ± 31 and 33 ± 11 DIM; mean ± SD). Each square contained 2 primiparous and 2 multiparous cows. Treatments were in a 2 × 2 factorial arrangement, and periods were 21d with the first 15 d for diet adaptation. Treatment diets contained 35 or 60% forage and 30 or 40% NFC on a DM basis (Table 2-1). Chromic oxide was used as a marker of digesta flow.

Soybean hulls and beet pulp were sampled with each delivery and samples of all other feeds were collected at the beginning of wk 3 of each period and dried at 55 °C for 48 h in a forced-air oven. Samples of all diet ingredients were ground with a Wiley mill (1-mm screen; Authur H. Thomas, Philadelphia, PA) and pooled by type for nutrient analysis.

Duodenal digesta and fecal samples (500 ml per sampling) were collected every 3 h from d 16 to 19, pooled by cow within period, and frozen at -20 °C. Frozen duodenal digesta samples were homogenized and lyophilized. Fecal samples were dried at 55 ºC for 48 h in a force-air oven and ground with a Wiley mill (1-mm screen; Authur H. Thomas, Philadelphia, PA). All samples were further ground through 0.2-mm screen.

Samples of feed, duodenal fluid, and feces were analyzed for total P and phytic acid-P content. Total P content was measured by a colorimetric method based on the production of yellow vanadomolybdophosphoric acid after nitric acid and perchloric acid digestion (AOAC, 1984). Phytic acid-P content was analyzed via an anion-exchange chromatography method (Harland and Oberleas, 1986). Phytic acid-P of samples was extracted and separated by an anion exchange column containing AG1-X4 resin (100-200 mesh, chloride form; Bio-Rad Laboratories). The phytic acid-P extract was then hydrolyzed by nitric acid and perchloric acid digestion (AOAC, 1984) and measured colorimetrically to quantify P content (Murphy and Riley, 1962).

All data were analyzed as a replicated Latin square using Proc MIXED of SAS (2002) according to the following model:

31

$$Y_{ijklmn} = \mu + \alpha_i + C_{(i*n)j} + \beta_k + F_l + N_m + (F*N)_{lm} + \gamma_n + (\alpha*\gamma)_{in} + (\alpha*F)_{il} +$$
$$(\alpha*N)_{im} + (\alpha*F*N)_{ilm} + \varepsilon_{ijklmn}$$

where

μ = overall mean,

α_i = fixed effect of square (i = 1 to 2),

$C(i*n)_j$ = random effect of cow within square and parity (j = 1 to 4),

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

F_l = fixed effect of forage content (l = 1 to 2),

N_m = fixed effect of NFC content (m = 1 to 2),

$(F*N)_{lm}$ = effect of interaction of F_l and N_m

γ_n = fixed effect of parity (n = 1 to 2)

$(\alpha*\gamma)_{in}$ = effect of interaction of α_i and γ_n

$(\alpha*F)_{il}$ = effect of interaction of α_i and F_l

$(\alpha*N)_{im}$ = effect of interaction of α_i and N_m

$(\alpha*F*N)_{ilm}$ = effect of interaction of α_i, F_l, and N_m, and

ε_{ijklmn} = residual error.

Differences were declared significant at $P < 0.05$ and trends at $P < 0.10$. Results were reported as least square means.

3. Results and Discussion

In this study, a subset of samples was also quantified for P containing compounds by ^{31}P nuclear magnetic resonance (NMR) spectroscopy (Turner, 2004). The results of NMR quantification indicated that the final P compounds extracted by the anion-exchange method were inositol phosphates instead of phytic acid. Therefore, the digestion of inositol phosphates-P (**IPs-P**) was discussed in this thesis.

3.1 Dietary inositol phosphates-P and total P content

Numerically, IPs-P content was less in high forage diets and low NFC diets (Table 2-1). This may be the result of low IPs-P content in forage (ranged from 0.05 to 0.24 mg/g) and relatively higher IPs-P content in grains (2.22 mg/g for fine ground corn and 3.47 mg/g for barley). Dietary total P content was balanced across the treatments and averaged 0.36%.

3.2 DM intake, duodenal DM flow, and fecal DM excretion

Treatment effects on intake, duodenal flow, fecal excretion, and apparent digestibility of DM were reported in Table 2-2. While intake, duodenal flow and fecal excretion of DM were affected by both dietary forage and NFC content, apparent digestibility of DM was not different among the treatments. This may further affect the IPs-P and total P flow.

An effect of parity (age) was observed on intake, duodenal flow, and fecal excretion of DM. It is possible that multiparous cows may have larger rumen size than primiparous cows. Thus, all of these measures were lower in primiparous cows than in multiparous cows. Similar parity effect was also found in intake, duodenal flow, and fecal excretion of IPs-P and total P (Table 2-3; Table 2-4).

3.3 Inositol phosphates-P and total P intake

IPs-P intake increased as dietary forage content decreased and as dietary NFC content increased (Table 2-3). This can be explained by the effect of treatments on DMI (Table 2-2) and the numerical trends in dietary IPs-P content (Table 2-1).

Despite the approximately equal dietary total P content among the treatments, total P intake increased with decreasing dietary forage content and with increasing dietary NFC content (Table 2-4). This may result from the treatment effect on DMI (Table 2-2; Schwab et al., 2006).

Similarly, due to the higher DMI, multiparous cows had higher intake of IPs-P and total P.

3.4 Inositol phosphates-P flow and digestion

3.4.1 Effects of parity, treatment, and lactation stage interactions

Interaction of treatment and lactation stage was found in duodenal IPs-P content, duodenal IPs-P flow, and ruminally IPs-P digestibility (Table 2-3). Interaction of parity and lactation stage was found in duodenal IPs-P flow as well (Table 2-3). Multiparous cows in early lactation had higher IPs-P flow than those in late lactation. More data are needed to investigate the biological meanings of these interactions.

3.4.2 Effects of dietary forage content

While duodenal IPs-P flow was unaffected by forage percentage in the diet, cows fed high forage diets tended to have lower fecal IPs-P excretion (g/d; Table 2-3). This indicated the occurrence of post-ruminal IPs-P digestion which might also be altered by treatment.

Despite exhibiting similar starch intakes (Schwab et al., 2006), total tract digestibility of IPs-P was higher in cows fed low forage diet. This implies that factors other than starch supply may affect ruminal phytase and other phosphatase activity and IPs-P digestion. One possible explanation could be the variation in ruminal pH. The pH for optimal phytase activity produced by *S. ruminantium* ranged from 4.0 to 5.5 (Yanke et al., 1999). Increasing dietary forage content increased ruminal pH (Schwab et al., 2006). This may further inhibit the phytic acid digestion in the rumen.

3.4.3 Effects of dietary NFC content

Cows given high NFC diets had higher fecal IPs-P content (g/kg) and fecal IPs-P excretion (Table 2-3). This may be resulted from the differences in IPs-P intake; cows fed high NFC diets ingested higher amount of IPs-P. On the other hand, the differences in fecal IPs-P excretion relative to IPs-P intake suggest altered IPs-P digestion.

Increasing NFC from 30 to 40% increased IPs-P intake by 12 and 11 g/d for low and high forage diets, respectively. However, fecal IPs-P excretion only increased by 1.1 and 2.3 g/d. This resulted in altered total tract IPs-P digestibility (Table 2-3) and indicated a higher degree of IPs-P degradation and probably higher endogenous phytase and/or other phosphatase activity in cows fed high NFC diets. In agreement with our results, Yanke and co-workers (1998) also found higher ruminal phytase activity in steers fed high grain diets. The relatively higher IPs-P digestibility caused by increasing NFC content supports our assumptions. With increasing dietary NFC content, dietary starch content increased (Table 2-1) which provided more readily usable substrate for the predominant ruminal phytase-producing bacteria (i.e. *S. ruminantium*). Therefore, increased IPs-P digestion may be contributed by increased ruminal microbial phytase activity.

Due to the nature of enzyme activity and production, it is possible that increased ruminal phytase activity and phytic acid-P digestion could be induced by increasing phytic acid supply (Greiner et al., 1997; Campbell, 1999). However, for the production of the ruminal phytase produced by *S. ruminantium*, neither the presence of phytic acid for induction nor the limitation of phosphate for depression is required (Yanke et al., 1999). Therefore, increased bacterial population induced by increasing starch supply explained the improved IPs-P digestibility.

3.4.4 Effects of the interaction of forage and NFC content

Effects of the interaction between dietary forage and NFC content were also found on fecal IPs-P content and fecal IPs-P excretion (Table 2-3). With low NFC diet, cows receiving higher amount of forage had lower fecal P excretion. This may be due to the low intake of both DM and IPs-P.

In this study, higher duodenal IPs-P flow than IPs-P intake was observed in three out of four treatments, resulting in negative ruminal IPs-P digestibility. These biologically impossible results may be explained by methodological limitations. One possible error can be attributed to sample collection and sample treatment. During sampling, pooling, and homogenizing, unrepresentative samples may be generated. Also, the use of chromic oxide may introduce experimental errors as well. While it is the most widely used external digestibility marker, limited recovery rate and inaccurate representation have been reported (MacRae, 1974; Titgemeyer, 1997). Another source of error may come from the limitation of IPs-P analysis. The anion-exchange chromatography method used in this study was first developed as routine phytic acid-P determination for food and feed samples (Harland and Oberleas, 1986). Thus, its application in determining IPs-P content of digesta samples may be restricted.

Measurable fecal IPs-P and relatively low total tract IPs-P digestibility indicated incomplete IPs-P digestion in these lactating cows. While phytic acid-P digestibility may be improved by exogenous phytase supplementation (Kincaid et al., 2005; Knowlton et al., 2005), more research is needed to investigate phytic acid and other IPs degradation in gastrointestinal system of dairy cows.

3.5 Total P flow and digestion

3.5.1 Effects of dietary forage content

Total P content of duodenal digesta increased with increasing dietary forage content, however, duodenal total P flow was unaffected (Table 2-4). Significant salivary P secretion may buffer the effect of total P intake and result in relatively constant duodenal total P flow. This was also observed by Khorasani et al. (1997). In contrast, other researchers showed that salivary P secretion increased with increased P intake (McDowell, 1992; Bravo et al., 2003d), but remained similar when the ingested P decreased (Bravo et al., 2003d). In this study, salivary P secretion was also affected by the interaction of dietary forage and NFC content (Table 2-4). Cows fed high forage diet with higher NFC content secreted larger amount of P in saliva (58.6 g/d). However, the lowest amount of P secreted in saliva (44.3 g/d) was found in the cows fed low forage diet with high NFC content. Further studies are needed to investigate varied patterns of salivary P secretion among studies.

For cows fed high forage diets, fecal total P content (% DM) was not changed by treatment, but less total P was excreted in feces (Table 2-4). Since fecal P excretion is positively correlated to P intake (Wu et al., 2000; Knowlton et al., 2001; Wu et al., 2001; Knowlton and Herbein, 2002), less P intake caused by high dietary forage content resulted in less fecal P excretion. Hence, the apparent P digestibility remained unaffected (Table 2-4) which is in agreement with other studies (Knowlton et al., 2001; Wu et al., 2003).

3.5.2 Effects of dietary NFC content

Increasing dietary NFC content decreased total P content of duodenal digesta but had no effect on duodenal total P flow (Table 2-4). As previously discussed, salivary P secretion may counter the effect of total P intake.

Fecal total P content tended to decrease in cows fed high NFC diets (Table 2-4). With higher total P intake and lower total P excreted in feces, cows fed high NFC diets had higher apparent P digestibility. This supports our hypothesis. Based on the findings of Yanke et al. (1998), the increased starch amount in high NFC diets may be beneficial to the growth of the ruminal phytase-producing bacteria and subsequently increase

ruminal phytase activity. This improves both phytic acid-P and total P digestion. Compared to our results, Guyton et al. (2003) observed that apparent P digestibility was unaffected by starch source (dry ground corn vs. steam-flaked corn). Thus, the amount of dietary starch rather than starch source may be more critical to P digestion.

In summary, while DMI and DM digestion may affect IPs-P and total P digestion in lactating cows, our results showed different patterns of IPs-P and total P digestion as the dietary forage or dietary NFC concentration altered (Table 2-5).

4. Conclusions

Inositol phosphates-P digestion was increased by both decreasing dietary forage content and increasing NFC concentration. This is consistent with our hypothesis and indicates higher ruminal phytase and/or other phosphatase activity induced by the respective diets. Total P digestibility increased with increasing dietary NFC content, but was unaffected by forage concentration.

Limited IPs-P was digested in lactating cows which implies the opportunity for exogenous phytase supplementation. More research is needed to determine the optimal combination of diet composition and phytase supplementation for P digestion.

5. Acknowledgements

TABLES

Table 2-1. Ingredient and nutrient composition of experimental diets (Adapted from Schwab et al., 2006).

	35% forage		60% forage	
	30% NFC	40% NFC	30% NFC	40% NFC
	----------------------------% of DM----------------------------			
Corn silage	17.5	17.5	30.0	30.0
Alfalfa hay	11.7	11.7	20.0	20.0
Grass hay	5.8	5.8	10.0	10.0
Corn (fine ground)	0.0	15.9	0.0	15.9
Barley (ground)	0.0	8.0	0.0	7.4
Soybean hulls	34.0	17.6	18.7	2.7
Beet pulp	17.0	8.8	9.3	1.4
Soybean meal	10.2	9.6	6.7	6.9
Blood meal	0.0	0.6	1.8	2.2
Fat[1]	1.6	1.6	1.6	1.6
Urea	0.2	0.2	0.0	0.0
Smartamine M[2]	0.07	0.06	0.08	0.07
Vitamin-mineral premix[3]	1.5	2.7	1.4	3.0
Calcium monophosphate	0.5	0.2	0.4	0.1
DM	58.5	57.5	54.3	54.3
NDF[4]	47.4	36.9	45.3	34.8
NFC[4]	29.1	39.2	29.8	38.9
Starch[4]	5.2	18.9	7.7	20.7
CP	16.4	16.2	16.6	16.3
Total P	0.35	0.36	0.36	0.36
Inositol phosphates-P	0.08	0.13	0.06	0.11

[1]Megalac, Church and Dwight Co., Inc., Princeton, NJ.
[2]Adisseo, Alpharetta, GA.
[3]Vitamin-mineral premix contained (% DM): 10% Ca, 8% Mg, 2.5% S, 15.2% Na, 0.2% Zn, 0.2% Mn, 418 mg of Cu/kg, 70.4 mg of Co/kg, 23.5 mg of I/kg, 10.5 mg of Se/kg, 65,300 IU of vitamin A/kg, 15,100 IU of vitamin D/kg, and 249 IU of vitamin E/kg.
[4]Concentrations of NDF, NFC, and starch were analyzed using wet chemistry (DairyOne Forage Laboratories, Ithaca, NY) procedures.

Table 2-2. Intake, duodenal flow, fecal excretion, and apparent digestibility of dry matter in eight lactating cows fed diets containing varying concentrations of forage and NFC[1] (Adapted from Schwab et al., 2006).

NFC content, %	35% forage		60% forage		SEM[1]	P <		
	30	40	30	40		Forage	NFC	INT[2]
DM								
Intake, kg/d[3]	21.3	22.2	18.1	19.8	0.75	0.01	0.08	0.59
Duodenal flow, kg/d[3]	15.1	15.5	12.5	14.8	0.60	0.01	0.02	0.11
Fecal excretion, kg/d[3]	8.55	8.30	7.51	8.07	0.45	0.02	0.53	0.12
Apparent digestibility, %	59.6	62.6	58.7	59.5	1.59	0.13	0.14	0.40

[1]Unequal n, largest SEM reported (n=7).
[2]INT = Interaction of dietary forage and NFC content.
[3]Significant effect of parity, $P < 0.05$.

Table 2-3. Digestion and excretion of inositol phosphates-P (IPs-P) in eight lactating cows fed diets containing varying concentrations of forage and NFC[1].

NFC content, %	35% forage		60% forage		SEM[1]	P <		
	30	40	30	40		Forage	NFC	INT[2]
IPs-P intake, g/d[3]	17.9	29.9	10.5	21.6	0.82	0.01	0.01	0.60
Duodenal IPs-P, g/kg DM[5]	1.27	1.54	1.31	1.73	0.08	0.09	0.01	0.25
Duodenal IPs-P, g/d[3,4,5]	19.4	24.0	16.4	25.5	1.08	0.51	0.01	0.06
Ruminal IPs-P digestibility, %[5]	-8.27	20.7	-56.6	-21.0	5.96	0.01	0.01	0.59
Fecal IPs-P, g/kg DM	0.68	0.82	0.63	0.88	0.05	0.71	0.01	0.05
Fecal IPs-P, g/d[3]	5.80	6.89	4.68	6.96	0.35	0.06	0.01	0.04
Total tract IPs-P digestibility, %	67.6	77.2	55.2	67.5	1.85	0.01	0.01	0.41

[1]Unequal n, largest SEM reported (n=7).
[2]INT = Interaction of dietary forage and NFC content.
[3]Significant effect of parity, $P < 0.05$.
[4]Significant effect of interaction of square (i.e. lactation stage) and parity, $P < 0.05$.
[5]Significant effect of interaction of square (i.e. lactation stage), forage, and NFC, $P < 0.05$.

Table 2-4. Total phosphorus digestion and excretion in eight lactating cows fed diets containing varying concentrations of forage and NFC[1].

	35% forage		60% forage			P <		
NFC content, %	30	40	30	40	SEM[1]	Forage	NFC	INT[2]
P intake, g/d[4]	74.4	79.9	65.2	71.4	2.67	0.01	0.04	0.90
Duodenal P, %DM[5]	0.87	0.80	0.94	0.88	0.02	0.01	0.01	0.83
Duodenal P, g/d[4]	130	124	118	130	4.50	0.52	0.52	0.08
Salivary P, g/d[3]	55.6	44.3	53.0	58.6	3.07	0.08	0.37	0.02
Fecal P, % DM	0.59	0.55	0.58	0.53	0.02	0.50	0.06	0.90
Fecal P, g/d[4]	50.5	46.0	43.4	42.5	2.54	0.01	0.18	0.31
Apparent P digestibility, %	31.9	42.8	34.0	40.3	3.48	0.96	0.02	0.49

[1]Unequal n, largest SEM reported (n=7).
[2]INT = Interaction of dietary forage and NFC content.
[3]Salivary P estimated as duodenal P, g/d, less P intake, g/d.
[4]Significant effect of parity, $P < 0.05$.
[5]Significant effect of square, $P < 0.05$.

Table 2-5. Intake, duodenal flow, fecal excretion, and digestibility of DM, total P, and inositol phosphates (IPs-P) and salivary total P secretion in eight lactating cows fed diets containing varying concentrations of forage and NFC[1].

	35% forage	60% forage
30% NFC	High duodenal total P flow	Low DM, total P, IPs-P intake
	High fecal DM and total P excretion	Low duodenal DM, total P, IPs-P flow
	Low apparent total P digestibility	Low fecal DM, total P, IPs-P excretion
	Low total tract IPs-P digestibility	Low total tract IPs-P digestibility
40% NFC	High DM, total P, IPs-P intake	High salivary total P secretion
	Low salivary total P secretion	High duodenal total P, IPs-P flow
	High duodenal DM flow	High fecal IPs-P excretion
	High fecal DM, total P excretion	Low fecal DM, total P excretion
	High total tract IPs-P digestibility	High total tract IPs-P digestibility

[1]Apparent DM digestibility was not different among treatments.

CHAPTER 3

PHOSPHORUS DIGESTION IN LACTATING COWS FED DIETS CONTAINING BEET PULP

ABSTRACT

The objective of this study was to evaluate the effects of replacing high moisture corn (HMC; highly digestible starch) with beet pulp (BP; high pectin and soluble fiber) on total P (TP) and inositol phosphates-phosphorus (IPs-P) digestion in dairy cows. Eight multiparous Holstein cows fitted with ruminal and duodenal cannulae were fed diets containing 40% forage (corn silage and alfalfa silage) and 0, 6.1, 12.1 or 24.3% BP (replacing high moisture corn grain on a DM basis) in a replicated 4×4 Latin square design with 21-d periods. Rumen content, duodenal digesta, and feces were collected and analyzed for TP and IPs-P content. Linear and quadratic effects of increasing BP content were analyzed using Proc Mixed of SAS. Dietary TP and IPs-P content were reduced linearly with increasing percent BP (0.59, 0.58, 0.57, 0.56 % TP and 0.15, 0.14, 0.13, 0.11 % IPs-P, respectively). With increasing BP content, TP intake, ruminal TP content, and rumen TP pool size decreased. Digestion, duodenal flow, and fecal excretion of TP were not affected. With increasing dietary BP content, IPs-P intake was reduced, ruminal IPs-P pool size was reduced, and rumen turnover time (h) of IPs-P was increased. Apparent ruminal digestibility of IPs-P was decreased linearly with increasing BP (36.5, 31.8, 24.6, 13.6 %; $P < 0.02$), and apparent total tract IPs-P digestibility was similarly affected (85.3, 82.7, 82.1, 79.1%; $P < 0.01$). As previously reported, increasing BP was associated with reduced ruminal starch digestion and increased post-ruminal starch digestion. Fecal excretion of IPs-P averaged 5.2 g/d. Replacing HMC with BP reduced digestion of IPs-P, and the majority of the disappearance of IPs-P occurred post-ruminally.

1. Introduction

Excess phosphorus (**P**) input into water bodies has been identified as the key element leading to undesired algae bloom and eutrophication of streams, lakes, and estuaries (Correll, 1998). Depending on watershed, up to 48% of P loaded into the surface water can be from livestock farms (Smith and Alexander, 2000). For dairy cattle, excess P supplements in diets increase fecal P excretion, especially in the form of water soluble P which can be readily used by aquatic organisms (Dou et al., 2002). Reducing P supplementation to accurately meet animal requirements and enhancing P digestion are important nutritional strategies to minimize P loss from dairy farms.

Factors affecting fecal P excretion by ruminants include dietary P content, salivary P secretion, ruminal microbial phytase activity, and P digestion and absorption in the digestive system. While most studies report decreased P digestibility (% of intake) with the increase of dietary P content (Morse et al., 1992a; Tamminga, 1996; Wu et al., 2001; Knowlton and Herbein, 2002; Rotz et al., 2002), few studies have determined the effect of dietary carbohydrate composition on P digestion in dairy cows.

Because of the presence of microbial phytase in the rumen, it is believed that the P in phytic acid forms (*myo*-inositol hexakisphosphate) can be completely utilized by ruminants. However, previous studies showed varied P digestibility (24 to 99%) when supplementing sheep (Tillman and Brethour, 1958; Lofgreen, 1960; Ellis and Tillman, 1961) or lactating cows (Morse et al., 1992b) with calcium phytate. It was reported that the ruminal phytase activity was higher in steers fed high concentrate diets (Yanke et al., 1998). Among the ruminal microorganisms, the major phytase-producing bacteria was *Selenomonas ruminantium*, a ureolytic, acid-using, soluble carbohydrate using species (Stewart et al., 1988). Thus, dietary carbohydrate composition may alter phytic acid digestion via effects on the ruminal microbial ecosystem.

The objective of this study was to evaluate the effects of replacing high moisture corn (**HMC**; highly digestible starch) with beet pulp (**BP**; high pectin and soluble fiber) on inositol phosphates-P (**IPs-P**) and total P digestion in lactating cows. Our hypothesis was that due to the reduced highly fermentable starch (unfavorable for *S. ruminantium*), replacing HMC with BP would decrease the hydrolysis of ruminal IPs-P and the IPs-P digestibility.

2. Methods and Materials

Cows, treatments, and sample collection were outlined by Voelker and Allen (2003a) and Voelker and Allen (2003b). Briefly, 8 multiparous Holstein cows (79 ± 17 DIM; mean ± SD) fitted with ruminal and duodenal cannulae were randomly assigned to replicated, concurrently run 4×4 Latin squares. Treatments diets contained 40% forage (corn silage and alfalfa silage) and 0, 6.1, 12.1 or 24.3% unmolassed, pelleted BP (replacing high moisture corn grain on a DM basis; Table 3-1). The nutrient content of BP averaged 39.9% NDF, 8.9% CP, 8.0% indigestible NDF, 3.9% starch, and 0.29% free glucose on a DM basis. Treatment periods were 21 d with final 10 d for sample collection. Chromic oxide was used as a marker to estimate nutrient digestibility in the rumen and total tract.

Samples of all diet ingredients were collected daily on d 12 to d 19 and combined into one sample per period. Ruminal contents were evacuated manually through the ruminal cannula d 20 and d 21 both before and after feeding, and total ruminal content mass and volume were determined. Duodenal and fecal samples were collected every 9 h from d 12 to d 14. All samples were frozen immediately at -20 °C. Ruminal, duodenal, and fecal samples were pooled by cow and by period.

Samples of feed, duodenal fluid, and feces were dried at 55°C for 72 h, analyzed for DM content, and ground with a Wiley mill (1-mm screen; Authur H. Thomas, Philadelphia, PA). Ruminal subsamples were lyophilized. All samples were further ground through 0.2-mm screen. All samples were analyzed for total P and IPs-P content. Total P content was measured by a colorimetric method based on the production of yellow vanadomolybdophosphoric acid after nitric acid and perchloric acid digestion (AOAC, 1984). Inositol phosphates-P content was analyzed via an anion-exchange chromatography method (Harland and Oberleas, 1986). Inositol phosphates-P of samples was extracted and separated by an anion exchange column containing AG1-X4 resin (100-200 mesh, chloride form; Bio-Rad Laboratories). The IPs-P extract was then hydrolyzed by nitric acid and perchloric acid digestion (AOAC, 1984) and measured colorimetrically to quantify P content (Murphy and Riley, 1962).

Ruminal pool sizes of total P and IPs-P (g) were calculated by multiplying the concentration of each component by the ruminal digesta DM mass (g). Ruminal turnover time was calculated by the following equation:

Turnover time, h = [pool size of P, g / intake of P, g/d] x 24

All data were analyzed as a replicated Latin square using Proc MIXED of SAS (2002) according to the following model:

$$Y_{ijkl} = \mu + \alpha_i + C_{(i)j} + \beta_k + \gamma_l + \varepsilon_{ijkl}$$

where

μ = overall mean,

α_i = fixed effect of square (i = 1 to 2),

$C_{(i)j}$ = random effect of cow within square (j = 1 to 4),

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

γ_l = fixed effect of treatment (l = 1 to 4),

ε_{ijkl} = residual error.

Linear and quadratic dose-response effects of dietary BP content (0, 6, 12, and 24%) were evaluated. Effects of dietary BP content were declared significant at $P < 0.05$, and trends were declared at $P < 0.10$. Results were reported as least squares means.

3. Results and Discussion

3.1 Dietary inositol phosphates-P and total P content

Dietary total P content averaged 0.58% which was higher than NRC (2001) recommendation (0.35% for cows 90 DIM milking 35 kg/d). With BP substitution for HMC, dietary IPs-P and total P content decreased linearly (Table 3-1). This was due to the relatively high IPs-P (0.17%) and total P (0.28%) content of HMC compared to BP (0.01% and 0.13%, respectively).

3.2 Effects on inositol phosphates-P flow and digestion

Inositol phosphates-P intake decreased linearly (Table 3-2) as the BP was substituted for HMC. This was the result of dietary IPs-P content and effect of BP on DMI (Voelker and Allen, 2003a); increasing BP decreased DMI linearly.

Despite the change in IPs-P intake, increased BP content had no effect on IPs-P content (g/kg) of duodenal digesta or on duodenal IPs-P flow (g/d). Ruminal IPs-P disappearance ranged from 12 g/d to essentially zero as dietary BP increased. Ruminal IPs-P digestibility was decreased with increasing BP content (36.5, 31.8, 24.6, 13.6 %; Table 3-2). The linearly decreased ruminal IPs-P digestibility with the increasing of BP supports our hypotheses,

implying greater ruminal phytase activity in cows fed the higher corn diet. The higher BP and lower HMC content in diets provided less readily fermentable carbohydrate starch (Voelker and Allen, 2003a). Ruminal endogenous phytase is primarily produced by *Selenomonas ruminantium* (Yanke et al., 1998), an acid- and starch-using species (Stewart et al., 1988). While microbial species were not measured, this decrease in readily fermentable starch would not favor *S. ruminantium*.

The effect of BP on ruminal P digestion may also have been simply through alteration of pH, as activity of ruminal endogenous phytase is optimal in the pH range of 4.0-5.5 (Yanke et al., 1999). Thus, a more acidic rumen environment may also increase the endogenous phytase activity and subsequently increase ruminal IPs-P digestibility. However, substituting BP for HMC did not alter the mean ruminal pH or time below pH 6.0 (Voelker and Allen, 2003c), suggesting other factors are also associated with IPs-P digestion in the rumen.

Fecal IPs-P content (g/kg) increased with the increase in BP content, but fecal IPs-P excretion (g/d) was not affected (Table 3-2). Reducing dietary BP content therefore resulted in the decrease of total tract IPs-P digestibility from 85.3 to 79.1% (Table 3-2). Compared to our data, Clark and co-workers (1986) reported a relatively higher degree of phytic acid-P digestibility (ranged from 94.6 to 99.3%) in high producing early lactating cows with high grain intake. Similarly, the digestibility of phytic acid-P ranged from 79.8 to 98.8% with a mean of 94% from cows fed with high phytic acid-P diets (0.38% of dietary DM; Morse et al., 1992b). These differences in phytic acid-P digestibility among studies may result from the various diet compositions and also the different phytic acid detection methods.

In this study, although up to 85.3% IPs-P was digested by the animals, only 13.6 to 36% of the consumed IPs-P was hydrolyzed in the rumen. The majority of the IPs-P degradation occurred post-ruminally (Figure 3-1) and this post-ruminal IPs-P degradation increased with the increasing BP substitution (Table 3-2). This finding was in contrast to our previous assumption that most IPs-P was degraded in the rumen by the endogenous microbial phytase. Significant phytic acid-P digestion posterior to rumen was also found in sheep fed with diets contained different oilseed meals (Bravo et al., 2003a), suggesting the existence of post-ruminal phytase activity.

The limited IPs-P degradation in the rumen may also suggest the opportunity for exogenous phytase supplementation for dairy cows. Studies showed that P digestibility in

lactating cows tended to be higher by adding fungal phytase in diets (Kincaid et al., 2005; Knowlton et al., 2005). On the other hand, fungal phytase supplementation increased ruminal P availability in lactating goats fed high concentrate diets, but had no effect in dry cows fed high forage diet (Bravo et al., 2002). One possible explanation is that the lower ruminal pH resulting from high grain diet (5.77) may be beneficial to the fungal phytase with an optimum pH at 5.5 (Reddy and Sathe, 2002) whereas the pH induced by high forage diet (6.47) may reduce efficiency of exogenous phytase.

While the major site of P absorption in ruminants is the small intestine (Khorasani and Armstrong, 1992; Care, 1994), especially the duodenum and jejunum (Pfeffer et al., 1970; Ben-Ghedalia et al., 1975; Wasserman, 1981), phosphate absorption from the colon perfused with electrolyte solution has also been observed (Scharrer, 1985; Höller et al., 1988). Our results of post-ruminal IPs-P degradation indicated a substantial amount of free phosphate flow into the distal small intestine and large intestine. The potential of P absorption from the large intestine in ruminants under practical conditions still needs to be investigated.

3.3 Effects on total P flow and digestion

With increasing BP substitution, total P intake decreased linearly (Table 3-3). Similar to the effect on IPs-P intake, decreased total P intake may result from the decreased dietary total P content and decreased DMI (Voelker and Allen, 2003a).

Digestion, duodenal flow, and fecal excretion of total P were unaffected by treatments (Table 3-3). Salivary secretion of total P tended to increase quadratically to a plateau as increasing BP (Table 3-3). Although the dietary P content was higher than NRC recommendation, apparent digestibility of total P was within the range (45 to 50%) of other studies (Brintrup et al., 1993; Spiekers et al., 1993; Wu et al., 2000; Knowlton et al., 2001).

The unaffected P digestion indicated that the contribution of reduced IPs-P digestion caused by increasing BP to total P digestion was limited and could be countered by salivary P secretion. Large amount of salivary P flow into rumen may also explain the unaffected duodenal P flow and fecal P excretion. In ruminants, salivary P production plays a more important role in removing excess P from blood than the kidney (Care, 1994). In order to maintain P homeostasis, a large amount of P is secreted in saliva and only the needed amount is reabsorbed by small intestine (Breves and Schroder, 1991; Care, 1994). Khorasani et al. (1997) reported that P flow at

the duodenum was relatively constant; salivary P secretion decreased as ingested P increased. This is in contrast to our observation that estimated salivary P flow was independent of the varied P intake.

Apparent P digestibility (% of intake) may be affected by P intake (Wu et al., 2001; Knowlton and Herbein, 2002), milk P yield (Valk et al., 2002) which is increased with milk protein yield (Wu et al., 2000), as well as by endogenous phytase activity as we hypothesized. In this study, P intake was only modestly altered by treatment (Table 3-3) and milk protein yield was relatively constant among the treatments (Voelker and Allen, 2003a).

While fecal P excretion, apparent P digestibility, and milk production were unaffected by increasing dietary BP, it would cost $51.00/ton to replace HMC with BP on a DM basis (Ingredient market, 2006). Thus, increasing BP substitution for HMC may not be economical from the aspect of practical dairy farm management.

3.4 Effects on ruminal inositol phosphates-P and total P pool size and turnover time

Ruminal IPs-P contents (%) tended to decrease and ruminal IPs-P pool size decreased linearly with increasing BP substitution (8.52, 7.06, 7.34, 6.86 g; Table 3-4; Figure 3-2). Similarly, with increasing BP content from 0 to 24%, ruminal total P content (%) also had a tendency to decrease and rumen pool size of total P decreased 18% (Table 3-4; Figure 3-2). Voelker and Allen (2003a, b) reported that ruminal pool size of DM decreased and ruminal DM passage to duodenum (kg/d) tended to increase with increasing BP. This may also decrease ruminal pool size of IPs-P and total P. Rumen pool size of any nutrient reflects the balance between intake, absorption (if relevant), degradation and passage of that nutrient. Thus, the decreased IPs-P content and rumen IPs-P pool size can also result from the decreased IPs-P intake. Decreased ruminal phytase activity (decreased degradation) may explain the decrease in rumen IPs-P pool size as well. Similar to the effect on rumen IPs-P pool size, increasing BP content from 0 to 6% resulted in a 13% decreased rumen total P pool size, whereas further increase BP substitution from 6 to 24% caused only a 6% decrease.

Increasing BP content increased rumen IPs-P turnover time and tended to decrease rumen total P turnover time quadractically to a plateau (Table 3-4). Increased rumen IPs-P turnover time indicated that the time each g of IPs-P stayed undigested in the rumen was longer with increasing BP substitution. Assuming passage rate was unaffected, this result suggests that IPs-P

in the rumen must have been more slowly digested. This result supports our hypotheses, suggesting the substitution of BP to HMC lowers ruminal phytase activity.

4. Conclusions

The data reported in this study suggest that replacing starchy grain with BP reduces IPs-P digestion in lactating cows. With increasing BP substitution for HMC, ruminal and total tract IPs-P digestibility and ruminal pool size of IPs-P and total P decreased. Increasing BP content also increased ruminal IPs-P turnover time. The altered IPs-P digestion indicates the changes in ruminal phytase activity. More investigation is needed in the actual differences in ruminal endogenous phytase activity.

As our previous findings (see Chapter 2), limited ruminal IPs-P degradation and great post-ruminal IPs-P digestion were observed in this study. This suggests the presence of post-ruminal phytase and/or other phosphatase activities. While exogenous phytase supplementation may increase ruminal phytic acid degradation, the post-ruminal phytase and other phosphatase activities and the capacity of P absorption in the large intestine need to be evaluated.

5. Acknowledgements

TABLES

Table 3-1. Ingredient and nutrient composition of experimental diets (Adapted from Voelker and Allen, 2003a).

	\% Beet pulp in diet			
	0	6	12	24
	----------------------------*% of DM*----------------------------			
Corn silage	20.1	20.1	20.1	20.1
Alfalfa silage	19.9	19.9	19.9	19.9
Protein mix[1]	19.5	19.5	19.5	19.5
Mineral vitamin mix[2]	4.8	4.8	4.8	4.8
Dried, pelleted beet pulp	0	6.1	12.1	24.3
High moisture corn	35.6	29.5	23.5	11.4
DM	50.2	50.5	50.8	51.6
Starch[3]	34.6	30.5	26.5	18.4
NDF[4]	24.3	26.2	28.0	31.6
CP	18.0	18.0	18.0	18.1
Total P[5]	0.59	0.58	0.57	0.56
Inositol phosphates-P[5]	0.15	0.14	0.13	0.11

[1]Protein mix contained 75% soybean meal, 20% corn distillers grains with solubles, and 5% blood meal.
[2]Mineral vitamin mix contained 10.4% trace mineral premix, 0.4% vitamin D, 0.4% vitamin A, 2.7% magnesium oxide, 22.3% dicalcium phosphate, and 63.8% dry ground corn as a carrier.
[3]Starch were analyzed by an enzymatic method (Karkalas, 1985).
[4]Concentration of NDF were analyzed according to Soest et al. (1991).
[5]Significant linear ($P < 0.01$) effect of beet pulp substituted for high moisture corn as 0, 6, 12, and 24% of diet DM.

Table 3-2. Digestion and excretion of inositol phosphates-P (IPs-P) in eight lactating cows fed diets containing different concentrations of beet pulp[1].

| | Percent beet pulp in the diet | | | | | P < | | |
	0	6	12	24	SEM[1]	trt	linear	quadratic
IPs-P intake, g/d	34.2	32.3	28.5	23.1	1.24	0.01	0.01	0.85
Duodenal IPs-P, g/kg DM	1.28	1.16	1.12	1.16	0.08	0.43	0.33	0.21
Duodenal IPs-P, g/d	21.9	22.0	21.3	20.0	2.18	0.89	0.46	0.84
Ruminal IPs-P digestibility, %	36.5	31.8	24.6	13.6	6.61	0.11	0.02	0.99
Fecal IPs-P, g/kg DM	0.67	0.71	0.71	0.75	0.05	0.19	0.04	0.89
Fecal IPs-P, g/d	5.13	5.59	5.09	4.84	0.54	0.42	0.30	0.45
Total tract IPs-P digestibility, %	85.3	82.7	82.1	79.1	1.50	0.01	0.01	0.62

[1]Unequal n, largest SEM reported (n=7).

Table 3-3. Total phosphorus digestion and excretion in eight lactating cows fed diets containing different concentrations of beet pulp[1].

	Percent beet pulp in the diet					P <		
	0	6	12	24	SEM[1]	trt	linear	quadratic
P intake, g/d	140	139	130	122	5.68	0.06	0.01	0.87
Duodenal P, %DM	1.09	1.06	1.06	1.05	0.05	0.77	0.40	0.67
Duodenal P, g/d	185	200	200	180	12.7	0.49	0.60	0.15
Salivary P, g/d[2]	44.8	60.5	70.0	58.5	9.38	0.27	0.37	0.10
Fecal P, % DM	0.94	1.01	0.97	1.06	0.09	0.37	0.15	0.96
Fecal P, g/d	72.7	80.8	71.1	67.3	8.60	0.18	0.17	0.38
Apparent P digestibility, %	48.6	42.6	46.4	45.4	5.21	0.31	0.63	0.37

[1]Unequal n, largest SEM reported (n=7).
[2]Salivary P estimated as duodenal P, g/d, less P intake, g/d

Table 3-4. Ruminal pool size and turnover time of total phosphorus and inositol phosphates-P (IPs-P) in eight lactating cows fed diets containing different concentrations of beet pulp[1].

	Percent beet pulp in the diet					P <		
	0	6	12	24	SEM[1]	trt	linear	quadratic
IPs-P								
Rumen IPs-P contents, g/kg DM	0.76	0.70	0.72	0.69	0.04	0.13	0.08	0.41
Rumen IPs-P pool size, g	8.52	7.06	7.34	6.86	0.33	0.01	0.01	0.09
Rumen IPs-P turnover time, h	6.02	5.28	6.23	7.22	0.28	0.01	0.01	0.01
Total P								
Rumen P contents, % DM	0.76	0.73	0.70	0.70	0.03	0.20	0.08	0.29
Rumen P pool size, g	85.9	74.5	71.2	70.2	4.15	0.05	0.03	0.10
Rumen P turnover time, h	11.4	9.44	8.81	9.69	0.96	0.16	0.24	0.07

[1]Unequal n, largest SEM reported (n=7).

FIGURES

Figure 3-1. Flow of phosphorus compounds (g/d) from intake, duodenum, and feces in eight lactating cows fed diets containing different concentrations of beet pulp. (Other P estimated as total P, g/d, less inositol phosphates-P, g/d)
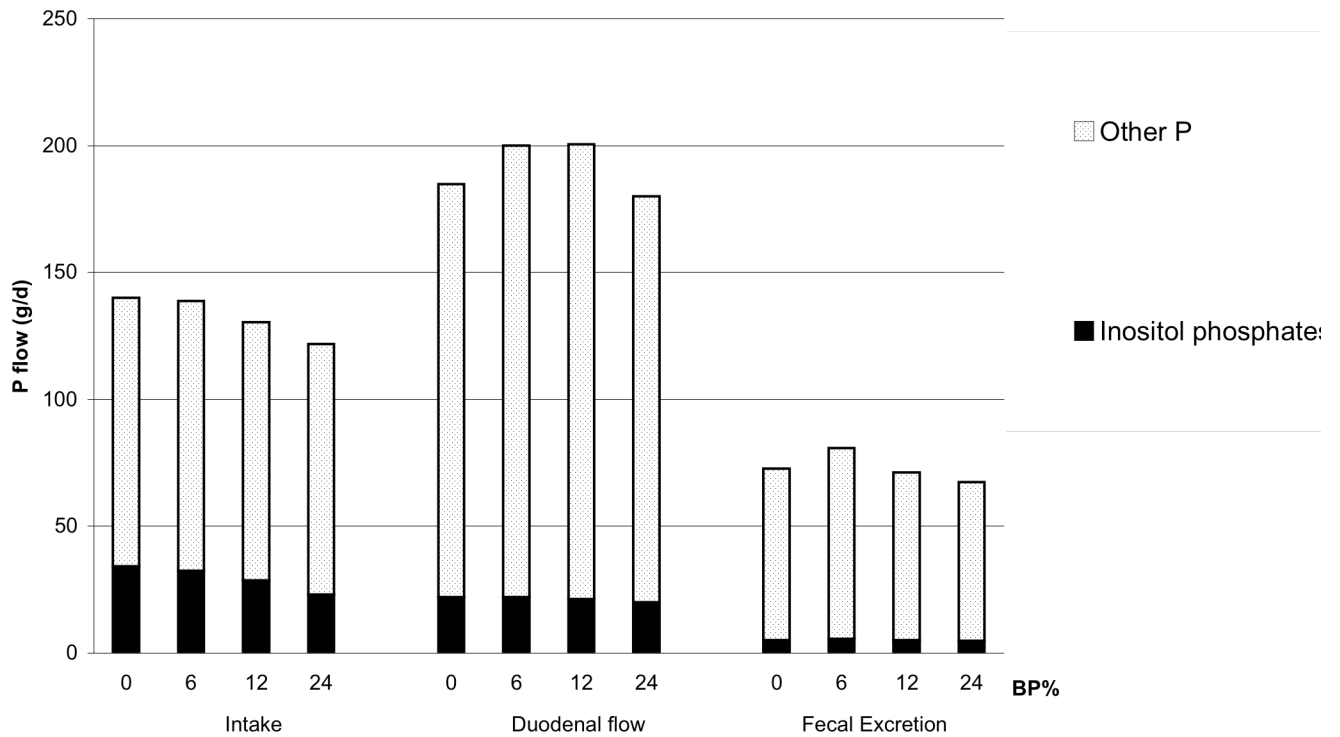
Figure 3-2. Ruminal pool size of inositol phosphates-phosphorus (IPs-P) and total phosphorus (g) in eight lactating cows fed diets containing different concentrations of beet pulp.
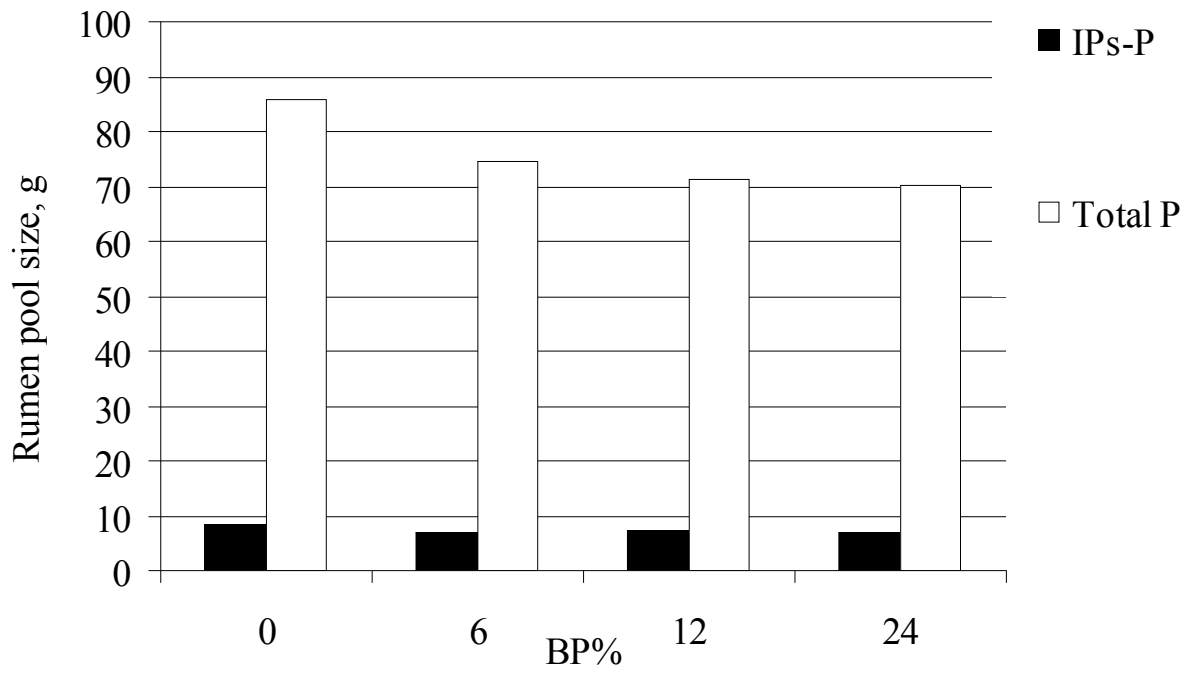
APPENDIX

METHOD EVALUATION: ANION-EXCHANGE METHOD FOR DETERMINATION OF IPS-P IN FEED AND BIOLOGICAL SAMPLES

Introduction

  Phytic acid, *myo*-inositol hexakisphosphate, is the major storage form of phosphorus (**P**) in cereals and legumes (Reddy et al., 1989). Due to the characteristics of its molecular structure, phytic acid can react with starch or protein or chelate divalent minerals such as Ca^{2+}, Cu^{2+}, Mg^{2+}, and Zn^{2+} to form insoluble complexes (Cheryan, 1980; Reddy et al., 1989; Reddy and Sathe, 2002). Thus, the presence of phytic acid in feedstuffs decreases P availability to monogastric animals.

  In general, methods for phytic acid analysis involve sample extraction followed by phytic acid or phosphorus quantification. Anion-exchange columns, following a dilute-acid extraction, have been the standard to purify phytic acid for food samples for decades (Harland and Oberleas, 1986). For quantitative analysis of phytic acid, methods are based on the reaction of phytic acid and ferric ion, which results in the precipitation of the iron (III)-phytate complex (Oberleas, 1971). However, this method is less sensitive and not specific for phytic acid due to co-precipitation of lower inositol phosphates, such as tris-, tetrakis-, and pentakisphosphates (Xu et al., 1992). While these methods have been developed for phytic acid analysis for food, limited data were reported for determining IPs-P content in digesta samples. The objective of this series of experiments was to evaluate the efficiency of the anion-exchange method developed by Harland and Oberleas (1986) for determining phytic acid content in samples of feed, rumen contents, and duodenal digesta from dairy cow.

Experiment 1

Objective

      Evaluate the efficiency of anion-exchange method for determination of IPs-Phosphorus (IPs-P) in corn grain samples by standard addition method followed by different P digestion methods.

Approach

      For IPs-P extraction, replicated samples of ground (through 0.2-mm screen) corn grain (1.000 g) were mixed with 20 ml 2.4% HCl in 50 ml centrifuge tubes and shaken for 16 hr at low speed (Eberbach Corporation, Ann Arbor, MI). After shaking, samples were centrifuged at 3000 x g, at 25ºC for 15 min to separate large solid substances from the supernatant containing IPs-P.

      Treatments for method efficiency test are shown in Table A-1. For determination of column efficiency, corn samples were also spiked with phytic acid standard solution and the recovery of phytic acid standard addition was calculated. Ten ml of each diluted sample was loaded into pre-packed (AG1-X4, 100-200 mesh, chloride form resin; Bio-Rad Laboratories) column (0.7 x 15 cm); 5 ml at a time. For each loading, sample solutions were allowed to react with the column for 10 min and the eluate was discarded. Columns were then eluted with 15 ml 0.1M NaCl (5 ml at a time) to separate orthophosphate followed by 15 ml of 0.7M NaCl (5 ml at a time) which dislodged the IPs-P. The final 15 ml eluate containing IPs-P was collected individually and hydrolyzed by different digestion methods (Table A-2) to test the digestion efficiency. After digestion, P content was quantified by a colorimetric method (Murphy and Riley, 1962).

Table A-1. Treatment description of sample extraction.

Treatment	Description
Corn	1.5 ml supernatant further centrifuged at 19400 x g, at 10ºC for 15 min. After centrifugation, 1 ml supernatant and 1 ml Na$_2$EDTA-NaOH reagent were mixed in volumetric flask and diluted to 25 ml by ddH$_2$O. Digestion and analysis was as described in Table A-2.
Spike	1.5 ml supernatant further centrifuged at 19400 x g, at 10ºC for 15 min. 1 ml supernatant, 1 ml Na$_2$EDTA-NaOH reagent, and 1 ml 286.4 µg/ml phytic acid solution were mixed in volumetric flask and diluted to 25 ml by ddH$_2$O. Digestion and analysis was as described in Table A-2.
One spin	No 2nd centrifugation. 1 ml supernatant and 1 ml Na$_2$EDTA-NaOH reagent were mixed in volumetric flask and diluted to 25 ml by ddH$_2$O. Digestion and analysis was as described in Table A-2.

Table A-2. Treatment description of IPs-P digestion.

Digestion method	Description
Autoclave	5 ml sample solution with 0.5 g ammonium persulfate and 10 ml 0.9 M H_2SO_4 was autoclaved for 60 min (120ºC; Tiessen and Moir, 1993). Released Pi was measured colorimetrically.
Digestion block	5 ml sample solution was hydrolyzed by nitric acid and perchloric acid digestion (AOAC, 1984). Released Pi was measured colorimetrically.

Results

Autoclave digestion

Table A-3. IPs-P content and relative errors for samples treated with autoclave digestion.

Treatment	IPs-P content, mg/g	Mean, mg/g	Relative error[1], %
Corn 1	0.95	0.83	13.7
Corn 2	0.70		
Spike 1	1.43	1.37	4.42
Spike 2	1.31		
One spin 1	0.76	0.89	13.7
One spin 2	1.01		

[1]Relative error calculated as differences divided by mean.

Table A-4. Recovery of standard addition for samples treated with autoclave digestion.

Treatment	Recovery rate, %	Mean, %	Relative error[1], %
Spike 1	30.1	33.8	11.1
Spike 2	37.6		

[1]Relative error calculated as differences divided by mean.

Table A-5. Relative errors of autoclave-digested samples with different extraction procedure.

Treatment	Relative error[1], %
Corn 1 vs One spin 1	10.6
Corn 2 vs One spin 2	17.7

[1]Relative error calculated as differences divided by mean.

Digestion block

Table A-6. IPs-P content and relative errors for samples hydrolyzed with digestion block.

Treatment	IPs-P content, mg/g	Mean, mg/g	Relative error[1], %
Corn 1	3.08	2.79	10.1
Corn 2	2.51		
Spike 1	5.15	5.42	4.94
Spike 2	5.69		
One spin 1	2.54	2.54	0.10
One spin 2	2.51		

[1]Relative error calculated as differences divided by mean.

Table A-7. Recovery of standard addition for samples hydrolyzed with digestion block.

Treatment	Recovery rate, %	Mean, %	Relative error[1], %
Spike 1	129	139	7.06
Spike 2	148		

[1]Relative error calculated as differences divided by mean.

Table A-8. Relative errors of block-digested samples with different extraction procedure.

Treatment	Relative error[1], %
Corn 1 vs One spin 1	9.20
Corn 2 vs One spin 2	0.58

[1]Relative error calculated as differences divided by mean.

Conclusions

For autoclave digestion, the relative errors among duplicated samples were high (>5%; Table A-3) and the recovery of the phytic acid addition was low (33.84%; Table A-4). On the other hand, the relative errors were lower (Table A-6) and the spike recovery rate was over 100% (Table A-7) for samples digested with nitric acid and perchloric acid (i.e. digestion block). Therefore, autoclave digestion with ammonium persulfate may not be strong enough to hydrolyze phytic acid compared to nitric acid and perchloric acid digestion.

The relative errors of treatment Corn and One spin were varied (Table A-5 and Table A-8). Thus, further work is needed to determine if a low speed centrifugation and Na_2EDTA-NaOH reagent addition were sufficient enough to remove the interferences, such as starch.

Experiment 2

Objective

Based on the previous experiment, nitric acid and perchloric acid digestion was used to examine the anion-exchange column efficiency.

Approach

Duplicated corn grain samples (1.000 g) were extracted with 20 ml 2.4% HCl and shaken for 16 hr. Extracted samples were centrifuged at 3000 x g, 25°C for 15 min to separate large solid substances from liquid which contains IPs-P. Supernatant was collected and centrifuged again at a higher speed, 19400 x g, 10°C for 15 min to remove interferences, such as starch or protein that often bond chemically with phytic acid. One ml supernatant of each sample was mixed with Na_2EDTA-NaOH reagent, diluted to 25 ml, and loaded into the column as previously described (Experiment 1). A standard solution of phytic acid (286.4 µg/ml) was used and treated as an individual sample in order to determine the column efficiency. Eluate of samples and each washing was collected for P content quantification. P content was determined by nitric acid and perchloric acid digestion method (AOAC, 1984) and a colorimetric method (Murphy and Riley, 1962).

Results

Table A-9. IPs-P content of each eluate from the anion-exchange column.

Treatment[1]	Eluate of sample	0.1 M NaCl eluate	0.7 M NaCl eluate
	---------------------------- P content, mg/g ----------------------------		
Corn 1	0.247	0.446	2.318
Corn 2	0.313	0.396	2.252
PA std	0.000	0.098	1.871

[1]Corn 1 and corn 2: duplicated corn grain samples; PA std: standard solution of phytic acid.

Conclusions

As this anion-exchange method developed, phosphorus compounds in the samples should bond chemically with the resin before any elution of NaCl solutions. However, measurable P was found in the eluate of corn samples before 0.1 M NaCl and 0.7 M NaCl elution indicated the loss of P directly from the anion-exchange columns (Table A-9). Thus, the content of orthophosphate (from 0.1 M NaCl elution) or IPs-P (from 0.7 M elution) in samples may be underestimated. Further work is needed to detect the chemical forms of these lost P compounds.

Theoretically, the P detected in the 0.1 M NaCl eluate is the amount of orthophosphate in the corn grain. However, further work may be needed to evaluate the efficiency of this anion-exchange method for extracting orthophosphate from different samples.

The recovery of phytic acid standard solution was 116%. Although all glasswares were acid-washed to remove P contamination, sources of the excess 16% P could still be the P-contaminated glassware due to personal errors.

Experiment 3

Objective

        Evaluate the efficiency of anion-exchange method followed by nitric acid and perchloric acid digestion for IPs-P determination in corn grains by standard addition method.

Approach

        Similar to Exp 1 and 2, duplicated HCl-extracted corn grain samples (treatment Corn 1 and Corn 2) and phytic acid standard solution (treatment PA std 1 and PA std 2) were run through anion-exchange columns, respectively. The phytic acid standard solutions (286.4 µg/ml) were also used to spike the sample solutions (treatment Spike 1 and Spike 2) and the recovery of phytic acid standard addition was calculated. The efficiency of one low speed centrifugation combined with Na_2EDTA-NaOH reagent addition for removing interference, such as starch and protein, was evaluated in this experiment as well. Thus, all samples expect treatment One spin 1 and One spin 2 were centrifuged twice (3000 x *g*, 25ºC for 15 min followed by 19400 x g, 10ºC for 15 min). Treatment One spin 1 and One spin 2 were only centrifuged once at 3000 x *g*, 25ºC for 15 min. All eluate was collected, acid digested, and quantified for P content.

Results

 Table A-10. IPs-P content of each eluate from the anion-exchange column and the recovery of standard and standard addition.

Treatment[1]	Eluate of sample	0.1 M NaCl eluate	0.7 M NaCl eluate	Recovery rate, %
	----------------------- *P content, mg/g* -----------------------			
Corn 1	0.446	0.545	1.314	
Corn 2	0.529	0.545	1.364	
Spike 1	0.397	0.645	3.124	112
Spike 2	0.033	1.041	3.149	111
One spin 1	0.397	0.645	1.364	
One spin 2	0.446	0.545	1.364	
PA std 1	0.000	0.099	1.636	103
PA std 2	0.000	0.000	1.537	97

Conclusions

        Similar to our previous findings (Experiment 2), the detectable P from sample eluate before 0.1 M NaCl and 0.7 M NaCl elution indicated the loss of P directly from the anion-exchange columns (Table A-10). In this experiment, the recovery rate of phytic acid standard solution and standard addition showed high efficiency of the anion-exchange column on IPs-P extraction. The low relative error (< 2%) between treatment Corn and treatment One spin

indicated that one low speed centrifugation followed by Na_2EDTA-NaOH might be sufficient enough to remove the interference, such as starch.

Experiment 4

Objective

Evaluate the anion-exchange column efficiency for IPs-P determination in samples of rumen contents.

Approach

Similar to Exp 3, HCl-extracted rumen content samples and samples with phytic acid standard solution added were run through anion-exchange columns, respectively. One low speed centrifugation followed by Na_2EDTA-NaOH addition was used to remove the interference and no second high speed centrifugation was applied. Extracted IPs-P (i.e. eluate of 0.7 M NaCl washing) was collected, acid digested, and quantified for P content.

Results and conclusions

The recovery of the phytic acid standard addition was 104%, indicating this anion-exchange method could be applied to determine IPs-P content in digesta samples as well.

Experiment 5

Objective

      Evaluate the reproducibility of the anion-exchange method on IPs-P determination in feed and digesta samples.

Approach

      Samples of feed, duodenal digesta, and feces from the study conducted in University of New Hampshire (Schwab et al., 2006) were randomly picked to test the reproducibility of the anion-exchange method.

Results

Table A-11. IPs-P content of feed and digesta samples determined at two different times.

Sample type	Sample ID	First test	Second test	Relative error[1], %
		---------- *mg IPs-P/g* ----------		
Duodenal digesta	8	2.02	1.51	14.5
Duodenal digesta	19	1.47	1.29	6.45
Duodenal digesta	29	1.36	1.26	3.47
Feces	44	1.12	1.09	1.53
Feces	55	0.70	0.76	4.15
Feces	65	0.46	0.57	10.6
Barley	76	3.47	3.54	1.06
Soybean meal	78	6.00	6.81	6.27

[1]Relative error calculated as differences divided by mean.

Conclusions

      The relative errors of different samples were varied. This indicated that the method was not as consistent as we expected. Further trials may be needed to improve the reproducibility of this anion-exchange method, including column separation, extraction efficiency, digestion, and colorimetric analysis, on IPs-P determination in samples with different matrices.

LIST OF REFERENCES

Adams, R. S. 1975. Variability in mineral and trace element content of dairy cattle feeds. J. Dairy Sci. 58:1538-1548.

Anderson, R. J. 1912. Phytin and phosphoruc acid esters of inosite. J. Biol. Chem. 11:471.

AOAC. 1984. Official Methods of Analysis, 14th ed. Arlington VA.

Augspurger, N. R. and D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus- or amino acid-deficient diets. J. Anim. Sci. 82:1100-1107.

Beardsworth, L. J., P. M. Beardsworth, and A. D. Care. 1989. The effect of ruminal phosphate concentration on the absorption of calcium, phosphorus and magnesium from the reticulo-rumen of the sheep. Br. J. Nutr. 61:715-723.

Beauchemin, K. A., B. I. Farr, L. M. Rode, and G. B. Schaalje. 1994. Effects of alfalfa silage chop length and supplementary long hay on chewing and milk production of dairy cows. J. Dairy Sci. 77:1326-1339.

Beauchemin, K. A. and W. Z. Yang. 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. J. Dairy Sci. 88:2117-2129.

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

Ben-Ghedalia, D., E. Yosef, J. Miron, and Y. Est. 1989. The effects of starch- and pectin-rich diets on quantitative aspects of digestion in sheep. Anim. Feed Sci. Technol. 24:289-298.

Berrada, A. and D. G. Westfall. 2005. Irrigated alfalfa response to phosphorus and potassium in a calcareous soil. Commun. Soil Sci. Plant. 36:1213-1227.

Bieleski, R. L. 1973. Phosphate pools, phosphate transport, and phosphate availability. Ann. Rev. Plant Physiol. 24:225-252.

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

Bravo, D., F. Meschy, C. Bogaert, and D. Sauvant. 2002. Effects of fungal phytase addition formaldehyde treatment and dietary concentrate on ruminal phosphorus availibility. Anim. Feed Sci. Technol. 99:73-95.

Bravo, D., F. Meschy, C. Bogaert, and D. Sauvant. 2003a. Phosphorus availability of oilseed meals determined by the apparent faecal digestibility technique. Anim. Feed Sci. Technol. 108:43-60.

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

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

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

Breves, G., G. Gaebel, H. Martens, and H. Hoeller. 1986. Phosphate fluxes across the rumen wall mucosa of sheep *in vitro*. Proc. Nutr. Soc. 45:99A.

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

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

Calberry, J. M., J. C. Plaizier, M. S. Einarson, and B. W. McBride. 2003. Effects of replacing chopped alfalfa hay with alfalfa silage in a total mixed ration on production and rumen conditions of lactating dairy cows. J. Dairy Sci. 86:3611-3619.

Cameron, M. R., T. H. Klusmeyer, G. L. Lynch, J. H. Clark, and D. R. Nelson. 1991. Effects of urea and starch on rumen fermentation, nutrient passage to the duodenum, and performance of cows. J. Dairy Sci. 74:1321-1336.

Camire, A. L. and F. M. Clydesdale. 1982. Analysis of phytic acid in foods by HPLC. J. Food Sci. 47:575-578.

Campbell, M. K. 1999. Biochemistry. 3 ed. Saunders College Publishing, Harcourt Brace College Publishers, Orlando, Florida.

Care, A. D. 1994. The absorption of phosphate from the digestive tract of ruminant animals. Br. Vet. J. 150:197-205.

Carpenter, J. H., D. W. Pritchard, and R. C. Whaley. 1969. Observations of eutrophication and nutrient cycles in some coastal plain estuaries. Pages 210-221 *in* Eutrophication: Causes, consequences, correctives. Natl. Acad. Sci., Washington, DC.

Casper, D. P., D. J. Schingoethe, and W. A. Eisenbeisz. 1990. Response of early lactation dairy cows fed diets varying in source of non-structural carbohydrate and crude protein. J. Dairy Sci. 73:1039-1050.

Cassida, K. A. and M. R. Stokes. 1986. Eating and resting salivation in early lactation dairy cows. J. Dairy Sci. 69:1282-1292.

Cheryan, M. 1980. Phytic acid interactions in food system. CRC Crit. Rev. Food Sci. Nutr. 13:297-325.

Chu, H. M., R. T. Guo, T. W. Lin, C. C. Chou, H. L. Shr, H. L. Lai, T. Y. Tang, K. J. Cheng, B. L. Selinger, and A. H. Wang. 2004. Structures of *Selenomonas ruminantium* phytase in complex with persulfated phytate: DSP phytase fold and mechanism for sequential substrate hydrolysis. Structure. 12:2015-2024.

Clark, P. W. and L. E. Armentano. 1993. Effectiveness of neutral detergent fiber in whole cottonseed dried distillers grains compared with alfalfa haylage. J. Dairy Sci. 76:2644-2650.

Clark, P. W. and L. E. Armentano. 1997. Influence of particle size on the effectiveness of beet pulp fiber. J. Dairy Sci. 80:898-904.

Clark, W. D. J., J. E. Wohlt, R. L. Gilbreath, and P. K. Zajac. 1986. Phytate phosphorous intake and disappearance in the gastrointestinal tract of high producing dairy cows. J. Dairy Sci. 69:3151-3155.

Correll, D. L. 1998. The role of phosphorus in the eutrophication of receiving waters: a review. J. Environ. Qual. 27:261-266.

Cosgrove, D. J. 1980. Inositol phosphates: their chemistry, biochemistry, and physiology. Elsevier, New York.

Crocker, L. M., E. J. DePeters, J. G. Fadel, H. Perez-Monti, S. J. Taylor, J. A. Wyckoff, and R. A. Zinn. 1998. Influence of processed corn grain in diets of dairy cows on digestion of nutrients milk composition. J. Dairy Sci. 81:2394-2407.

De Visser, H., H. Huisert, and R. S. Ketelaar. 1991. Dried beet pulp, pressed beet pulp and maize silage as substitutes for concentrates in dairy cow rations. 2. Feed intake, fermentation pattern and ruminal degradation characteristics. Neth. J. Agr. Sci. 39:21-30.

De Visser, H., A. Klop, J. v. d. Meulen, and A. M. v. Vuuren. 1998. Influence of maturity of grass silage and flaked corn starch on the production and metabolism of volatile fatty acids in dairy cows. J. Dairy Sci. 81:1028-1035.

Dhiman, T. R. and L. D. Satter. 1997. Yield response of dairy cows fed different proportions of alfalfa silage and corn silage. J. Dairy Sci. 80:2069-2082.

Dhiman, T. R., M. S. Zaman, I. S. MacQueen, and R. L. Boman. 2002. Influence of corn processing and frequency of feeding on cow performance. J. Dairy Sci. 85:217-226.

Dou, Z., K. F. Knowlton, R. A. Kohn, Z. Wu, L. D. Satter, G. Zhang, J. D. Toth, and J. D. Ferguson. 2002. Phosphorus characteristics of dairy feces affected by diets. J. Environ. Qual. 31:2058-2065.

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

Ekelund, A., R. Spörndly, H. Valk, and M. Murphy. 2003. Influence of feeding various phosphorus sources on apparent digestibility of phosphorus in dairy cows. Anim. Feed Sci. Technol. 109:95-104.

Ekinci, C. and G. A. Broderick. 1997. Effect of processing high moisture ear corn on ruminal fermentation and milk yield. J. Dairy Sci. 80:3298-3307.

Ellis, L. C. and A. D. Tillman. 1961. Utilization of phytin phosphorus in wheat bran by sheep. J. Anim. Sci. 20:606-607.

Ellis, R. and E. R. Morris. 1983. Improved ion-exchange phytate method. Cereal Chem. 60:121-124.

Emery, R. S., C. K. Smith, R. M. Grimes, C. F. Huffman, and C. W. Duncan. 1960. Physical and chemical changes in bovine saliva and rumen liquid with different hay-grain rations. J. Dairy Sci. 43:76-80.

Espindola, M. S., E. J. DePeters, J. G. Fadel, R. A. Zinn, and H. Perez-Monti. 1997. Effects on nutrient digestion of wheat processing and method of tallow addition to the diets of lactating dairy cows. J. Dairy Sci. 80:1160-1171.

Ewing, D. L., D. E. Johnson, and W. V. Rumpler. 1986. Corn particle passage size reduction in the rumen of beef steers. J. Anim. Sci. 63:1509-1515.

Firkins, J. L. 1997. Effects of feeding nonforage fiber sources on site of fiber digestion. J. Dairy Sci. 80:1426-1437.

Froetschel, M. A. and H. E. Amos. 1991. Effects of dietary fiber and feeding frequency on ruminal fermentation, digesta water-holding capacity, and fractional turnover of contents. J. Anim. Sci. 69:1312-1321.

Gasa, J., K. Holtenius, J. D. Sutton, M. S. Dhanoa, and D. J. Napper. 1991. Rumen fill and digesta kinetics in lactating Friesian cows given two levels of concentrates with two types of grass silage *ad lib*. Br. J. Nutr. 66:381-398.

Grace, N. D., M. J. Ulyatt, and J. C. MacRae. 1974. Quantitative digestion of fresh herbage by sheep. 3. The movement of Mg, Ca, P, K and Na in the digestive tract. J. Agric. Sci., UK. 82:321-330.

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

Greiner, R., E. Haller, U. Konietzny, and K.-D. Jany. 1997. Purification and characterization of a phytase from *Klebsiella terrigena*. Arch. Biochem. Biophys. 341:201-206.

Grings, E. E., R. E. Roffler, and D. P. Deitelhoff. 1992. Evaluation of corn and barley as energy sources for cows in early lactation fed alfalfa-based diets. J. Dairy Sci. 75:193-200.

Guyton, A. D., J. M. McKinney, and K. F. Knowlton. 2003. The effect of steam-flaked or dry ground corn and supplemental phytic acid on phosphorus partitioning and ruminal phytase activity in lactating cows. J. Dairy Sci. 86:3972-3982.

Harland, B. F. and D. Oberleas. 1977. A modified method for phytate analysis using an ion-exchange procedure: applicattion to textured vegetable proteins. Cereal Chem. 54:827-832.

Harland, B. F. and D. Oberleas. 1986. Anion-exchange method for determination of phytate in foods: collaborative study. J. Assoc. Off. Anal. Chem. 69:667-670.

Harvatine, D. I., J. L. Firkins, and M. L. Eastridge. 2002. Whole linted cottonseed as a forage substitute fed with ground or steam-flaked corn: digestibility and performance. J. Dairy Sci. 85:1976-1987.

Heldt, J. S., R. C. Cochran, G. L. Stokka, C. G. Farmer, C. P. Mathis, E. C. Titgemeyer, and T. G. Nagaraja. 1999. Effects of different supplemental sugars and starch fed in combination with degradable intake protein on low-quality forage use by beef steers. J. Anim. Sci. 77:2793-2802.

Herrera-Saldana, R., R. Gomez-Alarcon, M. Torabi, and J. T. Huber. 1990. Influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. J. Dairy Sci. 73:142-148.

Herrera-Saldana, R. and J. T. Huber. 1989. Influence of varying protein and starch degradabilities on performance of lactating cows. J. Dairy Sci. 72:1477-1483.

Hinedi, Z. R., A. C. Chang, and R. W. K. Lee. 1989. Characterization of phosphorus in sludge extracts using phosphorus-31 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 18:323-329.

Höller, H., A. Figge, J. Richter, and G. Breves. 1988. Net absorption of calcium and inorganic phosphate from the sheep colon and rectum perfused *in vivo*. J. Anim. Physiol. a. Anim. Nutr. 59:9-15.

Huber, K., C. Walter, B. Schröder, and G. Breves. 2002. Phosphate transport in the duodenum and jejunum of goats and its adaptation by dietary phosphate and calcium. Am. J. Physiol. Regul. Integr. Comp. Physiol. 283:296-302.

Hume, I. D. 1970. Synthesis of microbial protein in the rumen III. The effect of dietary protein. Aust. J. Agric. Res. 21:305-314.

Huntington, G. B. 1997. Starch utilization by ruminants: from basics to the bunk. J. Anim. Sci. 75:852-867.

Ingredient market. 2006. Page 21 *in* Feedstuffs. Vol. 78.

Irving, G. C. J. and D. J. Cosgrove. 1972. Inositol phosphate phosphatases of microbiological origin: the inositol pentaphosphate products of *Aspergillus ficuum* phytases. J. Bacteriol. 112:434-438.

Johnson, L., J. H. Harrison, C. Hunt, K. Shinners, C. G. Doggett, and D. Sapienza. 1999. Nutritive value of corn silage as affected by maturity and mechanical processing: a contemporary review. J. Dairy Sci. 82:2813-2825.

Johnson, L. M., J. H. Harrison, D. Davidson, M. Swift, W. C. Mahanna, and K. Shinners. 2002a. Corn silage management II: effects of hybrid, maturity, and mechanical processing on digestion and energy content. J. Dairy Sci. 85:2913-2927.

Johnson, L. M., J. H. Harrison, D. Davidson, M. Swift, W. C. Mahanna, and K. Shinners. 2002b. Corn silage management III: effects of hybrid, maturity, and processing on nitrogen metabolism and ruminal fermentation. J. Dairy Sci. 85:2928-2947.

Jongbloed, A. W., Z. Mroz, and P. A. Kemme. 1992. The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. J. Anim. Sci. 70:1159-1168.

Joy, M. T., E. J. DePeters, J. G. Fadel, and R. A. Zinn. 1997. Effects of corn processing on the site and extent of digestion in lactating cows. J. Dairy Sci. 80:2087-2097.

Karkalas, J. J. 1985. An improved enzymatic method for the determination of native and modified starch. J. Sci. Food Agric. 36:1019-1027.

Kemme, P. A., A. Lommen, L. H. De Jonge, J. D. Van der Klis, A. W. Jongbloed, Z. Mroz, and A. C. Beynen. 1999. Quantification of inositol phosphates using ^{31}P nuclear magnetic resonance spectroscopy in animal nutrition J. Agric. Food Chem. 47:5116-5121.

Khalili, H. and P. Huhtanen. 1991. Sucrose supplements in cattle given grass silage-based diet. 2. Digestion of cell wall carbohydrates. Anim. Feed Sci. Technol. 33:263-273.

Khorasani, G. R. and D. G. Armstrong. 1992. Calcium, phosphorus, and magnesium absorption and secretion in the bovine digestive tract as influenced by dietary concentration of these elements. Livest. Prod. Sci. 31:271-286.

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

Khorasani, G. R., E. K. Okine, and J. J. Kennelly. 1996. Forage source alters nutrient supply to the intestine without influencing milk yield. J. Dairy Sci. 79:862-872.

Khorasani, G. R., E. K. Okine, and J. J. Kennelly. 2001. Effects of substituting barley grain with corn on ruminal fermentation characteristics, milk yield, and milk composition of Holstein cows. J. Dairy Sci. 84:2760-2769.

Kihanda, F. M., G. P. Warren, and A. N. Micheni. 2005. Effects of manure and fertilizer on grain yield, soil carbon and phosphorus in a 13-year field trial in semi-arid Kenya. Experimental Agriculture. 41:389-412.

Kincaid, R. L., D. K. Garikipati, T. D. Nennich, and J. H. Harrison. 2005. Effect of Grain Source and Exogenous Phytase on Phosphorus Digestibility in Dairy Cows. J. Dairy Sci. 88:2893-2902.

Knowlton, K. F., M. S. Allen, and P. S. Erickson. 1996. Lasalocid and particle size of corn grain for dairy cows in early lactation. 2 Effect on ruminal measurements and feeding behavior. J. Dairy Sci. 79:565-574.

Knowlton, K. F., B. P. Glenn, and R. A. Erdman. 1998. Performance, rumen fermentation, and site of starch digestion in early lactation cows fed corn grain harvested and processed differently. J. Dairy Sci. 81:1972-1984.

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

Knowlton, K. F., J. H. Herbein, M. A. Meister-Weisbarth, and W. A. Wark. 2001. Nitrogen and phosphorous partitioning in lactating Holstein cows fed different sources of dietary protein and phosphorus. J. Dairy Sci. 84:1210-1217.

Knowlton, K. F., J. M. McKinney, K. F. Wilson, and C. Cobb. 2003. Effect of an exogenous phytase enzyme blend and dietary phosphorus content on P excretion in lactating cows. J. Dairy Sci. (Suppl. 1). 86:224.

Knowlton, K. F., C. M. Parsons, C. W. Cobb, and K. F. Wilson. 2005. Exogenous phytase plus cellulase and phosphorus excretion in lactating dairy cows. The Professional Animal Scientist. 21:212-216.

Konishi, C., T. Matsui, W. Park, H. Yano, and F. Yano. 1999. Heat treatment of soybean meal and rapeseed meal suppresses rumen degradation of phytate phosphorus in sheep. Anim. Feed Sci. Technol. 80:115-122.

Krause, K. M. and D. K. Combs. 2003. Effects of forage particle size, forage source, and grain fermentability on performance and ruminal pH in midlactation cows. J. Dairy Sci. 86:1382-1397.

Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002. Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. J. Dairy Sci. 85:1947-1957.

Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2003. Effects of increasing levels of refined cornstarch in the diet of lactating dairy cows on performance and ruminal pH. J. Dairy Sci. 86:1341-1353.

Lan, G. Q., N. Abdullah, S. Jalaludin, and Y. W. Ho. 2002a. Culture conditions influencing phytase production of *Mitsuokella jalaludinii*, a new bacterial species from the rumen of cattle. J. Appl. Microbiol. 93:668-674.

Lan, G. Q., Y. W. Ho, and N. Abdullah. 2002b. *Mitsuokella jalaludinii* sp. nov., from the rumens of cattle in Malaysia. Int. J. Syst. Evol. Microbiol. 52:713-718.

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

Leiva, E., M. B. Hall, and H. H. Van Horn. 2000. Performance of dairy cattle fed citrus pulp or corn products as sources of neutral detergent-soluble carbohydrates. J. Dairy Sci. 83:2866-2875.

Lofgreen, G. P. 1960. The availability of the phosphorus in dicalcium phosphate, bonemeal, soft phosphate and calcium phytate for mature wethers. J. Nutr. 70:58-62.

MacRae, J. C. 1974. The use of intestinal markers to measure digestive function in ruminants. Proc. Nutr. Soc. 33:147-154.

McAllister, T. A., L. M. Rode, D. J. Major, K.-J. Cheng, and J. G. Buchanan-Smith. 1990. Effect of ruminal microbial colonization on cereal grain digestion. Can. J. Anim. Sci. 70:571-579.

McDowell, L. R. 1992. Minerals in animal and human nutrition. Academic Press, Inc, Harcourt Brace Jovanovich, New York.

Meek, S. E. and D. J. Pietrzyk. 1988. Liquid chromatographic separation of phosphorus oxo-acids and other anion with postcolumn indirect fluorescence detection by aluminum-morin. Anal. Chem. 60:1379-1400.

Mendoza, G. D., R. A. Britton, and R. A. Stock. 1999. Effect of feeding mixtures of high moisture corn and dry-rolled grain sorghum on ruminal fermentation and starch digestion. Small Ruminant Res. 32:113-118.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Mooney, C. S. and M. S. Allen. 1997. Physical effectiveness of the neutral detergent fiber of whole linted cottonseed relative to that of alfalfa silage at two lengths of cut. J. Dairy Sci. 80:2052-2061.

Moore, J. A., M. H. Poore, T. P. Eck, R. S. Swingle, J. T. Huber, and M. J. Arana. 1992. Sorghum grain processing and buffer addition for early lactation cows. J. Dairy Sci. 75:3465-3472.

Morse, D., H. Head, C. J. Wilcox, H. H. V. Horn, C. D. Hissem, and B. Harris, Jr. 1992a. Effects of concentration of dietary phosphorous on amount and route of excretion. J. Dairy Sci. 75:3039-3049.

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

Murphy, J. and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27:31-36.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle.

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

O'Neill, I. K., M. Sargent, and M. L. Trimble. 1980. Determination of phytate in foods by phosphorus-31 fourier transform nuclear magnetic resonance spectrometry. Anal. Chem. 52:1288-1291.

Oba, M. and M. S. Allen. 2003. Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. J. Dairy Sci. 86:195-207.

Oberleas, D. 1971. The determination of phytate and inositol phosphates. Pages 87-101 *in* Methods of biochemical analysis. Vol. 20. D. Glick, ed. Wiley, New York.

Oldham, J. D. 1984. Protein-energy interrelationships in dairy cows. J. Dairy Sci. 67:1090-1114.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: a review. J. Anim. Sci. 76:275-286.

Park, W.-Y., T. Matsui, C. Konishi, S.-W. Kim, F. Yano, and H. Yano. 1999. Formaldehyde treatment suppresses ruminal degradation of phyatate in soyabean meal and rapeseed meal. Br. J. Nutr. 81:467-471.

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

Parrish, F. W., J. P. Madacsi, B. Q. Phillippy, A. G. Wilfred, and S. M. Buco. 1990. Determination of phytic acid in cottonseed by near-infrared reflectance spectroscopy. J. Agric. Food Chem. 38:407-409.

Petit, H. V. and G. F. Tremblay. 1995. Ruminal fermentation and digestion in lactating cows fed grass silage with protein and energy supplements. J. Dairy Sci. 78:342-352.

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

Phillippy, B. Q. and M. R. Johnston. 1985. Determination of phytic acid in foods by ion chromatography with post-column derivatization. J. Food Sci. 50:541-542.

Phillippy, B. Q., M. R. Johnston, S.-H. Tao, M. R. S. Fox, and K. D. White. 1986. Inositol phosphate derivatives of phytic acid in food products. Federation Proc. 45:375.

Plascencia, A. and R. A. Zinn. 1996. Influence of flake density on the feeding value of steam-processed corn in diets for lactating cows. J. Anim. Sci. 74:310-316.

Polan, C. E., D. E. Stieve, and J. L. Garrett. 1998. Protein preservation and ruminal degradation of ensiled forage treated with heat, formic acid, ammonia, or microbial inoculant. J. Dairy Sci. 81:765-776.

Rahnema, S., Z. Wu, O. A. Ohajuruka, W. P. Weiss, and D. L. Palmquist. 1994. Site of mineral absorption in lactating cows fed high-fat diets. J. Anim Sci. 72:229-235.

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

Reddy, N. R., M. D. Pierson, S. K. Sathe, and D. K. Salunkhe. 1989. Phytates in cereals and legumes. CRC Press, Inc, Boca Raton, Florida.

Reddy, N. R. and S. K. Sathe. 2002. Food phytates. CRC Press LLC, Boca Raton, Florida.

Robinson, P. H., S. Tamminga, and A. M. V. Vuuren. 1986. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen fermentation in dairy cows. Livest. Prod. Sci. 15:173-189.

Rooney, L. W. and R. L. Pflugfelder. 1986. Factors affecting starch digestibility with special emphasis on sorghum corn. J. Anim. Sci. 63:1607-1623.

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

Saio, K., E. Koyama, and W. T. 1967. Protein-calcium-phytic acid relationships in soybean. Part I. Effects of calcium and phosphorus on solubility characteristics of soybean meal protein. Agr. Biol. Chem. 31:1195-1200.

San Emeterio, F., R. B. Reis, W. E. Campos, and L. D. Satter. 2000. Effect of coarse or fine grinding on utilization of dry or ensiled corn by lactating dairy cows. J. Dairy Sci. 83:2839-2848.

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

Sandberg, A. S., N.-G. Carlsson, and U. Svanberg. 1989. Effects of inositol tri-, tetra-, penta-, and hexaphosphates on *in vitro* estimation of iron availability. J. Food Sci. 54:159-161.

Sannes, R. A., M. A. Messman, and D. B. Vagnoni. 2002. Form of rumen-degradable carbohydrate and nitrogen on microbial protein synthesis and protein efficiency of dairy cows. J. Dairy Sci. 85:900-908.

Santini, F. J., A. R. Hardie, N. A. Jorgensen, and M. F. Finner. 1983. Proposed use of adjusted intake based on forage particle length for calculation of roughage indexes. J. Dairy Sci. 66:811-820.

Santos, F. A. P., J. T. Huber, C. B. Theurer, R. S. Swingle, and J. M. Simas. 1997. Response of lactating dairy cows to various densities of sorghum grain. J. Anim. Sci. 75:1681-1685.

SAS Institute, I. 2002. S.A.S. Users Guide, Version 9.1.3. Cary, NC.

Scharrer, E. 1985. Phosphate absorption at different intestinal sites in the developing lamb. QJ Exp. Physiol. 70:615-621.

Schwab, E. C., C. G. Schwab, R. D. Shaver, C. L. Girard, D. E. Putnam, and N. L. Whitehouse. 2006. Dietary forage and non fiber carbohydrate contents influence B-vitamin intake, duodenal flow, and apparent ruminal synthesis in lactating dairy cows. J. Dairy Sci. 89:174-187.

Seal, C. J. and C. K. Reynolds. 1993. Nutiritional implications of gastrointestinal and liver metabolism in ruminants. Nutr. Res. Rev. 6:185-208.

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

Sievert, S. J. and R. D. Shaver. 1993. Effect of nonfiber carbohydrate level and *Aspergillus oryzae* fermentation extract on intake, digestion, and milk procdution in lactating dairy cows. J. Anim Sci. 71:1032-1040.

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

Smith, R. A. and R. B. Alexander. 2000. Sources of nutrients in the nation's watersheds. *In* "Managing nutrients and pathogens from animal agriculture". Natural Resource, Agriculture and Engineering Service (NRAES), Camp Hill, PA.13-21.

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

Spiekers, H., R. Brintrup, M. Balmelli, and E. Pfeffer. 1993. Influence of dry matter intake on faecal phosphorus losses in dairy cows fed rations low in phosphorus. J. Anim. Physiol. Anim. Nutr. 69:37-43.

Stewart, C. S., H. J. Flint, and M. P. Bryant. 1988. The rumen bacteria. Pages 10-72 *in* The rumen microbial ecosystem. P. N. Hobson, ed. Elsevier Applied Science, London; New York

Stock, R. A., D. R. Brink, R. T. Brandt, J. K. Merrill, and K. K. Smith. 1987a. Feeding combinations of high moisture corn and dry corn to finishing cattle. J. Anim. Sci. 65:282-289.

Stock, R. A., D. R. Brink, R. A. Britton, F. K. Goedeken, M. H. Sindt, K. K. Kreikemeier, M. L. Bauer, and K. K. Smith. 1987b. Feeding combinations of high moisture corn and dry-rolled grain sorghum to finish steers. J. Anim. Sci. 65:290-302.

Stokes, S. R., W. H. Hoover, T. K. MIller, and R. Blauweikel. 1991. Ruminal digestion and microbial utilization of diets varying in type of carbohydrate and protein. J. Dairy Sci. 74:871-881.

Strobel, H. J. and J. B. Russell. 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. J. Dairy Sci. 69:2941-2947.

Sutton, J. D., J. A. Bines, S. V. Morant, and D. J. Napper. 1987. A comparison of starchy and fibrous concentrates for milk production, enery utilization and hay intake by Friesian cows. J. Agric. Sci., Camb. 109:375-386.

Sutton, J. D., M. S. Dhanoa, S. V. Morant, J. France, D. J. Napper, and E. Schuller. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. J. Dairy Sci. 86:3620-3633.

Swain, S. M. and L. E. Armentano. 1994. Quantitative evaluation of fiber from nonforage sources used to replace alfalfa silage. J. Dairy Sci. 77:2318-2331.

Swingle, R. S., T. P. Eck, C. B. Theurer, M. d. l. Llata, M. H. Poore, and J. A. Moore. 1999. Flake density of steam-processed sorghum grain alters performance and sites of digestibility by growing-finishing steers. J. Anim Sci. 77:1055-1065.

Tamminga, S. 1996. A review on environmental impacts of nutritional strategies in ruminants. J. Anim. Sci. 74:3112-3124.

Tangendjaja, B., K. A. Buckle, and M. Wootton. 1980. Analysis of phytic acid by high-performance liquid chromatography. J. Chromat. 197:274-277.

Taylor, A. W. and K. J. Kilmer. 1980. Agricultural phosphorus in the environment. Pages 543-557 *in* The role of phosphorus in agriculture. ASA, CSSA, SSSA, Madison, WI.

Theurer, C. B. 1986. Grain processing effects on starch utilization by ruminants. J. Anim. Sci. 63:1649-1662.

Theurer, C. B., J. T. Huber, A. Delgado-Elorduy, and R. Wanderley. 1999. Invited review: summary of steam-flaking corn or sorghum grain for lactating dairy cows. J. Dairy Sci. 82:1950-1959.

Tiessen, H. and J. O. Moir. 1993. Characterization of available P by sequential extraction. Pages 75-86 *in* Soil sampling and methods of analysis. M. R. Carter, ed. Lewis Publishers, Chelsea, Michigan.

Tillman, A. D. and J. R. Brethour. 1958. Utilization of phytin phosphorus by sheep. J. Anim. Sci. 17:104-112.

Titgemeyer, E. C. 1997. Design and interpretation of nutrient digestion studies. J. Anim. Sci. 75:2235-2247.

Tomlinson, R. V. and C. E. Ballou. 1962. *Myo*-inositol polyphosphate intermediates in the dephosphorylation of phytic acid by phytase. Biochemistry. 1:166-171.

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

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

Underwood, E. J. 1966. The mineral nutrition of livestock. Food and Agriculture Organization of the United Nations by Commonwealth Agricultural Bureaux, Farnham Royal (Bucks.).

USDA-NASS. 2002. U.S. Dairy Herd Structure. National Agricultural Statistics Service, Agricultural Statistics Board, U.S. Department of Agriculture.

Valk, H., L. B. J. ebek, and A. C. Beynen. 2002. Influence of phosphorus intake on excretion and blood plasma and saliva concentrations of phosphorus in dairy cows. J. Dairy Sci. 85:2642-2649.

Vallimont, J. E., F. Bargo, T. W. Cassidy, N. D. Luchini, G. A. Broderick, and G. A. Varga. 2004. Effects of replacing dietary starch with sucrose on ruminal fermentation and nitrogen metabolism in continuous culture. J. Dairy Sci. 87:4221-4229.

Viveros, A., C. Centeno, A. Brenes, R. Canales, and A. Lozano. 2000. Phytase and acid phosphatase activities in plant feedstuffs. J. Agric. Food Chem. 48:4009-4013.

Voelker, J. A. and M. S. Allen. 2003a. Pelleted beet pulp substituted for high-moisture corn: 1. Effects on feed intake chewing behavior, and milk production of lactating dairy cows. J. Dairy Sci. 86:3542-3552.

Voelker, J. A. and M. S. Allen. 2003b. Pelleted beet pulp substituted for high-moisture corn: 2. Effects on digestion and ruminal digestion kinetics in lactating dairy cows. J. Dairy Sci. 86:3553-3561.

Voelker, J. A. and M. S. Allen. 2003c. Pelleted beet pulp substituted for high-moisture corn: 3. Effects on ruminal fermentation, pH, and microbial protein efficiency in lactating dairy cows. J. Dairy Sci. 86:3562-3570.

Wade, H. E. and D. M. Morgan. 1955. Fractionation of phosphates by paper ionophoresis and chromatography. Biochem. J. 60:264-270.

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

Weiss, W. P. and D. J. Wyatt. 2004. Macromineral Digestion by Lactating Dairy Cows: Estimating Phosphorus Excretion via Manure. J. Dairy Sci. 87:2158-2166.

White, L. M., G. P. Hartman, and J. W. Bergman. 1981. In vitro digestibility, crude protein, and phosphorus content of straw of winter wheat, spring wheat, barley and oat cultivars in eastern Montana. Agron. J. 73:117-121.

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

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

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

Xu, P., P. J. Aggett, and J. Price. 1992. Recent advances in methodology for analysis of phytate and inositol phosphates in foods. Prog. Food Nutr. Sci. 16:245-262.

Yang, W. Z. and K. A. Beauchemin. 2004. Grain processing, forage-to-concentrate ratio, and forage length effects on ruminal nitrogen degradation and flows of amino acids to the duodenum. J. Dairy Sci. 87:2578-2590.

Yang, W. Z. and K. A. Beauchemin. 2006. Effects of physically effective fiber on chewing activity and ruminal pH of dairy cows fed diets based on barley silage. J. Dairy Sci. 89:217-228.

Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2001a. Effect of dietary factors on distribution and chemical composition of liquid- or solid-associated bacterial populations in the rumen of dairy cows. J. Anim. Sci. 79:2736-2746.

Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2001b. Effects of grain processing, forage to concentrate ratio, and forage particle size on rumen pH and digestion by dairy cows. J. Dairy Sci. 84:2203-2216.

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

Yanke, L. J., L. B. Selinger, and K. J. Cheng. 1999. Phytase activity of *Selenomonas ruminantium*: a preliminary characterization. Lett. Appl. Microbiol. 29:20-25.

Ying, Y. and M. S. Allen. 1998. Effects of fineness of grinding and conservation method of corn grain on ruminal starch digestion kinetics in Holstein heifers before and after calving. J. Anim Sci. (Suppl.). 76:318.

Ying, Y., M. S. Allen, M. J. Vandehaar, and N. K. Ames. 1998. Effects of fineness of grinding and conservation method of corn grain on ruminal and whole tract digestibility and ruminal microbial protein production of pregnant Holstien heifers. J. Anim. Sci. (Suppl.). 76:339.

VITA

Tzu-Hsuan Yang was born in Taipei, Taiwan on July 27th, 1981. She graduated from National Taiwan University with a Bachelor of Science degree in Animal Science in June 2003. In summer of 2002, she did a research project of behavioral enrichment of ruffed lemur in Taipei Zoo as an internship. In August of 2006, she completed her Master of Science degree in Dairy Science at Virginia Polytechnic Institute and State University. She is a recipient of John Lee Pratt Graduate Fellowship in Animal Nutrition and a member of Gamma Sigma Delta, the Honor Society of Agriculture.