Disappearance of infused phytate from the large intestine of dairy heifers

P. P. Ray,^{*1} C. Shang,[†] R. E. Pearson,^{*} and K. F. Knowlton^{*}

^{*}Department of Dairy Science, and

[†]Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg 24061

ABSTRACT

The objective of this study was to investigate the disappearance of phytate from the large intestine of dairy heifers. Uncertainty about the availability of phosphorus (P) in different feeds may limit implementation of dietary strategies to reduce fecal P excretion by dairy cows. Increased understanding of the dynamics of phytate degradation and disappearance of P in the large intestine may improve prediction of intestinal P digestion and absorption. Eight ruminally- and ileally-cannulated crossbred dairy heifers were used in two 4 × 4 Latin square designs with 9-d periods, including 3 d of washout. All heifers were fed a high-forage diet containing 0.14% P throughout the study. Ytterbium-labeled corn silage and Co-EDTA were dosed to the rumen 4 times daily as particulate and liquid phase markers, respectively, to measure ileal digesta flow. On d 4 to 7 of each period, each heifer was infused ileally with 0, 5, 15, or 25 g/d of phytate (phytic acid) in solution and total fecal collection was conducted. When infusion ceased (d 8 and 9) ileal digesta was sampled to measure P flow to the ileum from the basal diet. Feed, digesta, and feces were dried, ground, and analyzed for phytate P, inorganic P, and total P using high performance ion chromatography, inductively coupled plasma atomic emission spectroscopy, and the molybdovanadate yellow method, respectively. Phytate degradation in the large intestine was observed but was not complete, and the amount of infused phytate did not influence the degradability of phytate. Fecal excretion of total P increased with increasing total P infused. The slope coefficient for ileal P flow (dietary only) to feces was 0.56 ± 0.26 (mean $\pm$ SE), whereas the slope coefficient for infused P was 0.75 ± 0.13 . These indicate net absorption of P from the large intestine and greater disappearance of P from dietary P flowing to the ileum than from the infused pure phytate (44 vs. 25%). This data will support mechanistic modeling efforts to im-

prove prediction of P digestion, allowing more accurate estimation of P bioavailability in feeds.

Key words: phytate digestion, phosphorus absorption, large intestine, dairy heifer

INTRODUCTION

In high-producing dairy cows, P plays an important role in maintaining production and performance. Absorbed P is constantly secreted in milk irrespective of dietary P intake (Cohen, 1980; Knowlton et al., 2001). More than 80% of the total body P is present in bone. Bone mineral resorption plays a key role in regulating blood P, and replenishment of bone P is critical to maintain animal performance and production (Cohen, 1980; Wu et al., 2001; Peterson et al., 2005). The salient physiological characteristic of salivary P recycling in ruminants demands continuous and sufficient dietary P supply (Horst, 1986; Valk et al., 2002). In ruminants offered P-deficient diets, feed intake was depressed and ruminal digestion impaired (Komisarczuk et al., 1987; Durand and Komisarczuk, 1988). Reduced ATP production and transfer in the rat myocardium during P depletion reflects the importance of the role of P in energy production and transfer, and similar relationships were observed in ruminants fed P-deficient diets (Riddell et al., 1933; Louw, 1979; Brautbar et al., 1982).

Phosphorus losses from farms, a problem exacerbated by excess dietary P, contribute to eutrophication. For this reason and because of increasing cost due to depletion of natural sources of phosphate (Leng, 2008), the use of inorganic P (**Pi**) supplements has been reduced for last few decades. Dietary P content in dairy rations in the United States typically exceeds the amount recommended by the NRC (Dou et al., 2003), making P supplementation unnecessary. But, care needs to be taken in formulation of low-P diets to be sure sufficient P is supplied both for the rumen microbes and the cow. Current NRC (2001) recommendations for dietary P supply are based on the calculation accounting for the requirement of absorbed P for milk production, maintenance, growth, and reproduction. Specific absorption coefficients were assigned for each source of Pi, but fixed values were assigned for forages (0.64) and con-

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¹Corresponding author: drray@vt.edu

concentrates (0.70) and these are based on relatively sparse research. The single availability coefficient for forages and for grains ignores the observation that ruminal P availability is greatly influenced by type of feed or feed processing (Bravo et al., 2003; Kincaid et al., 2005). Using these fixed absorption coefficients may limit accurate calculation of dietary P supply.

Ruminants are more efficient than nonruminants in extracting P from phytate, the major form of P in grains and cereals. Although the expression of the enzyme phytase by ruminal microorganisms allows ruminants to hydrolyze phytate (Nelson et al., 1976; Clark et al., 1986; Morse et al., 1992), phytate degradation by ruminants may be incomplete in certain dietary situations (Park et al., 1999; Park et al., 2000; Bravo et al., 2002). Grain type, processing of feed ingredients, and supplementation with exogenous phytase have also been shown to influence ruminal phytase activity (Yanke et al., 1998) or phytate hydrolysis (Park et al., 2000; Bravo et al., 2003; Kincaid et al., 2005). In lactating cows, high phytate intake with feeding of high-concentrate diets and fast rate of digesta passage due to high DMI may result in flow of phytate P to the lower digestive tract. The large intestinal degradation of phytate has been widely investigated in monogastrics (Williams and Taylor, 1985; Matsui et al., 1999; Marounek et al., 2003) but data describing the amount and fate of phytate flowing to the large intestine in ruminants are scarce. Large intestinal phytate degradation is attributed primarily to microbial phytase activity. Though the bacterial population in the large intestine is not exactly same as the rumen, some species are in common (Maki and Picard, 1965; Yanke et al., 1998) and the total viable count of bacteria per gram of wet weight in cecal and colon digesta collectively was the same as in ruminal digesta (Edwards et al., 2005). Therefore, phytate digestion in the large intestine seems likely. In sheep fed a 50:50 forage:concentrate diet, daily passage of inositol phosphates (IP) through the upper large intestine was less than through the small intestine, likely due to microbial phytase activity in sheep large intestine (Park et al., 2002). The release of Pi due to phytate hydrolysis is of nutritional significance only if P is absorbed from the large intestine. Although the extent is highly variable, disappearance of P from the ruminant large intestine has been reported (Scharrer, 1985; Breves and Schroder, 1991).

We hypothesized that phytate entering the large intestine of ruminants can be hydrolyzed, and the released Pi absorbed. The objective of this study was to quantify phytate P degradation in the large intestine of dairy heifers and to estimate the disappearance of P from the large intestine.

MATERIALS AND METHODS

Animals and Experimental Design

Eight crossbred [Swedish Red or Brown Swiss × (Holstein × Jersey)] heifers, averaging 420 kg of BW, were fitted surgically with a half T-type Tygon ileal cannula (5 to 6 cm anterior to ileocecal junction) and a ruminal cannula (Bar Diamond Inc., Parma, ID) 60 and 21 d before the experiment, respectively. All surgeries were conducted aseptically using local anesthesia. Ileal cannulas were checked daily to ensure fixed position and cleaned twice per week to prevent local infection.

Heifers were housed in a freestall barn with constant access to water and fed once daily using Calan doors. A high-forage diet (91% forage; Table 1) was formulated to contain very low total P (0.14% of dietary DM) to minimize the amount of organic P reaching the ileum, allowing unconfounded measurement of disappearance of the infused phytate. Diets were formulated to meet NRC (2001) recommendations for heifers weighing 420 kg for all nutrients except P. Feed was offered ad libitum during a 21-d diet acclimation period. To maintain constant DM proportions of forage and concentrate in diet, the DM content of corn silage was measured weekly and the amount of forage adjusted accordingly. After 21 d of diet acclimation, heifers were moved to individual tie-stalls in a metabolism unit for infusion and sampling.

Heifers were randomly assigned to 1 of 4 treatment sequences in a duplicated Latin square design with four

Table 1. Composition of basal diet fed to 8 crossbred heifers

Composition	% of dietary DM
Ingredient	
Barley straw	38.3
Corn silage	14.7
Beet pulp, dried	38.6
Cane molasses, dehydrated	4.98
Slow release NPN ¹	2.72
Trace mineral mix ²	0.11
Selenium 90 ³	0.42
Vitamin ADE mix ⁴	0.18
Nutrient	
CP	12.6
NDF	54.4
ADF	34.7
P	0.14
Phytate P ⁵	0.005

¹Optigen (Alltech Inc., Springfield, KY).

²Contained 37% Na, 60% Cl, 0.03% K, 0.3% Mg, and 14% S.

³Contained 90 mg of Se per kilogram of DM.

⁴Contained 26,400 KIU of vitamin A; 8,800 KIU of vitamin D; and 44,000 IU of vitamin E per kilogram of DM.

⁵Phytate P was 3.8% of dietary total P.

9-d periods. Treatments were infusion of 0, 5, 15, or 25 g/d of phytate (phytic acid) in solution (0, 1.41, 4.22, and 7.04 g of P/d, respectively) in a total of 960 mL/d (40 mL/h) using an infusion pump (Ardus Medical Inc., Cincinnati, OH). Solutions of varying phytate P concentrations were prepared using 50% (wt/wt) phytic acid solution (Sigma-Aldrich Co., St. Louis, MO). The pH of infusion solutions were adjusted to neutral or mildly alkaline (7 to 8.1) before infusion.

A 7-d adjustment period to the metabolism stall was allowed before the start of the first period and then each period included 3 d of washout, 4 d of infusion, and 2 d of ileal sampling. During the washout periods, heifers were fed at 0600 and 1800 h. During infusions, heifers were fed 4 times daily at 0600, 1200, 1800, and 2400 h. On d 3 of each period, heifers were fitted with urinary catheters to prevent mixing of feces and urine. Heifers were observed for signs of illness and rectal temperatures were recorded daily.

Ytterbium-labeled corn silage and Co-EDTA were dosed daily as particulate and liquid-phase markers, respectively, to measure ileal digesta flow. Dried corn silage retained on the bottom sieve of the Penn State Particle Separator was labeled using YbCl₃ solution by the method described by Harvatine et al. (2002). Lithium Co-EDTA was prepared by the method of Udén et al. (1980). Markers were mixed with rumen digesta 4 times daily throughout the study at a rate of 0.11 g of Yb or Co/heifer per day. All protocols and procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee (Blacksburg).

Sample Collection and Preparation

Total mixed ration and feed refusals were sampled daily for the last 6 d of each period. Total fecal collection was conducted during the 4 d infusion (d 1 to 4 of each period). Every 24 h, water was added (50% of total feces weight) to increase feces homogeneity, and feces from each heifer was blended and sampled. When phytate infusion ceased (d 8 and 9), ileal digesta was collected through the ileal cannula 4 times per day (0600, 1200, 1800, and 2400 h) to quantify P flow to the ileum from the basal diet. All samples were stored at -20°C immediately following collection.

Frozen samples were later thawed, TMR samples composited by period, refusals composited by heifer × period, and ileal samples composited by heifer × day. Samples were oven dried (Wisconsin Oven; Memmert GmbH & Co. KG, Schwabach, Germany) at 55°C until constant dry weight was achieved. Dried samples were ground through Wiley mill (1-mm screen; Arthur H. Thomas Co., Philadelphia, PA) and then through a Z grinder (0.2-mm screen; ZM 100 USA; Retsch GmbH,

Haan, Germany). Ground samples were stored in airtight containers.

Sample Analysis

Dried ground feed samples were analyzed for N, total P (AOAC, 1984), and NDF and ADF sequentially (Van Soest et al., 1991). Dried ground fecal samples were analyzed for N and total P (AOAC, 1984), and ileal digesta samples were analyzed for total P (AOAC, 1984). Ileal digesta samples were analyzed for Yb and Co using inductively coupled plasma atomic emission spectroscopy (Ciros Vision model; Spectro Analytical Instruments Inc., Mahwah, NJ).

Ileal and fecal samples were analyzed for inositol hexaphosphate (IP6) and lower inositol phosphates (IP5, IP4, IP3) following the method of Ray et al. (2012) except that a different high performance ion chromatography (HPIC) quantification approach was used. In brief, dried ground (0.2-mm) samples were extracted using 0.25 M NaOH-0.05 M EDTA and then alkaline extracts were acidified with HCl-HF acid solution (500 µL of 6 M HCl and 1.2 M HF added to 5 mL of sample extract). Acidified extracts were stored overnight at 4°C and centrifuged at 30,000 × g for 20 min at 4°C. Clear supernatants were collected and passed through a methanol-conditioned C₁₈ column (Sep-Pak Plus; Waters Corp., Milford, MA) and then through a 0.2-µm IC membrane (PTFE filter; IC Millex-LG, Fisher, PA) into Dionex sample vials (Dionex Corp., Sunnyvale, CA) for HPIC analysis.

Chromatographic quantification of phytate (IP6) in clear sample extracts was achieved by HPIC (Dionex ICS 3000 with a Dionex 4 × 50 IonPac AG7 guard column and a 4 × 250 mm IonPac AS7 analytical column; Dionex Corp.) using pH 4 elution and postcolumn reaction detection (Rounds and Nielsen, 1993; Kwanyuen and Burton, 2005; Leytem et al., 2008). Treated sample extracts (50 µL) were injected into the column with an autoinjector. Phytate was eluted at 11.8 min with gradient elution (0.01 M methylpiperazine and 0.01 M NaNO₃-0.01 M methylpiperazine) at a flow rate of 1 mL/min. Eluted phytate was mixed with Wade's reagent (0.015% FeCl₃ + 0.15% sulfosalicylic acid solution) in a postcolumn reactor and detected using a UV-visible detector by monitoring absorbance at 500 nm. Three IP6 standards (6, 30, and 60 µg of P/mL) were prepared using sodium salt of phytic acid (phytic acid, sodium salt hydrate powder; Sigma, St. Louis, MO).

The combination of alkaline extraction and HPIC quantification (Ray et al., 2012) makes possible quantification of phytate and the lower IP (IP3, IP4, and IP5) in ruminant digesta and fecal samples in a single HPIC run using the same sample extract after a com-

mon postextraction cleanup process. The elution times for IP5, IP4, and IP3 were 10.5, 8.9, and 6.8 min, respectively. Calculation of concentration of these lower IP used 2 secondary standards (mixtures of inositol phosphates), prepared by hydrolyzing 50 mg of sodium phytate in 5 mL of 6 M HCl at 140°C for 1 and 2 h, respectively. Pure standards of IP3, IP4, and IP5 were used to standardize the concentration-to-peak area ratio in the 2 secondary standards for lower IP and IP6. This ratio was used to calculate the concentrations of lower IP in unknown samples. The following equation was used to calculate lower IP concentration in samples:

$$C_s = \frac{C_{ss}}{A_{ss}} \times A_s,$$

where C_s = concentration of lower IP in sample extract, C_{ss} = concentration of lower IP in secondary standard, A_{ss} = area of lower IP peak in secondary standard, and A_s = area of lower IP peak in sample extract.

Statistical Analysis

All intake, excretion, and disappearance data were analyzed using PROC GLIMMIX in SAS (SAS Institute, 2002). The model included square, infusion dose, and period as fixed effects. For excretion and disappearance data, basal ileal flow was used as covariate. Data for 1 cow were excluded from the second period, as the cow was suffering from ruminal stasis. Polynomial contrasts were used to test the effect of infusing increasing quantities of phytate (5, 15, and 25 g/d) on large intestinal phytate disappearance and fecal Pi excretion. Statistical significance was declared at $P < 0.05$. Treatment means were separated using a multiple comparison test following the Tukey-Kramer method.

RESULTS AND DISCUSSION

Diet Composition, Intake, and Digestibility

As intended, quantified total P concentration in the diet averaged 0.14% (DM basis) across all periods (Table 1). Similarly, observed dietary phytate P concentration was very low (3.8% of dietary total P).

Heifers infused with saline or assigned phytate solution consumed an average of 7.7 kg of DM/d, and DMI, fecal DM, and apparent DM digestibility was similar for all treatment groups (Table 2). Average DM digestibility was 68.8%, which is similar to the DM digestibility reported by others in growing heifers fed high-forage diets (Moody et al., 2007). Total P intake (average 10.8 g/d) was similar for all infusion groups,

but fecal P increased with increased infusion of phytate P (Table 2).

Intake, Ileal Flow, and Disappearance of Phytate from the Large Intestine

Phytate P intake did not differ with treatment (Table 3). Dietary phytate P reaching the ileum was similar for all treatment groups (Table 3). On average, 68% of dietary phytate P was degraded in the rumen and small intestine. Several researchers have reported that ruminants hydrolyze the majority of feed phytate (78–100%), with the rumen being the major site of phytate degradation (Clark et al., 1986; Morse et al., 1992; Kincaid et al., 2005). The digestion of phytate P in the rumen (and upper intestine) was comparatively low in this study (68%). Park et al. (1999) reported 84% ruminal effective digestibility of phytate P when ruminal passage rate was 0.02/h. Ruminal degradability of phytate P decreased to 69 and 57% when ruminal passage rate increased to 0.05 and 0.08/h, respectively. The high-forage diet in this study would not seem to suggest a passage rate fast enough to reduce ruminal phytate P digestibility (Park et al., 1999). The relatively low ruminal phytate degradation in this study may instead be due to the effect of the type of diet (high forage) and dietary phytate content on ruminal phytase activity. Lower ruminal phytase activity was reported in steers fed all-hay diets than in those fed high-grain diets (Yanke et al., 1998), and lower phytase activity was observed in vitro (semicontinuous culture fermentor) with low-phytate than high-phytate diets (Godoy and Meschy, 2001). High dietary phytate is often the result of feeding a high-concentrate diet and phytate P hydrolysis was greater in dairy cows fed an 80% grain (0.38% phytate P) diet as compared with cows fed a 52% grain (0.24% phytate P) diet (99% vs. 78–85% phytate P hydrolysis; Morse et al., 1992; Kincaid et al., 2005). The low rumen pH resulting from high-grain diets creates an environment favoring efficient phytase activity (Yanke et al., 1999; Lan et al., 2011), and the endogenous phytase activity in many cereal grains contribute to phytate hydrolysis in high-grain diets (Viveros et al., 2000; Selle et al., 2003). The relatively low phytate hydrolysis in the rumen and upper intestine in this study supports these general observations that diet affects ruminal phytate hydrolysis, and also contradicts the conventional wisdom that phytate is completely hydrolyzable in the rumen.

A linear increase ($P = 0.02$) was observed in the amount of phytate that disappeared from the large intestine when the infused phytate level increased from 5 to 25 g/d, and daily fecal phytate P output increased

Table 2. Dry matter intake, digestibility, total P intake, and fecal excretion in 8 crossbred heifers infused with different amounts of phytate P

Item	Infusion dose ¹				SEM	P-value
	0	×	3×	5×		
DMI, kg/d	7.48	7.60	7.36	8.28	0.54	0.65
Fecal DM, kg/d	2.12	2.37	2.33	2.65	0.24	0.52
DM digestibility, %	70.1	67.8	69.1	68.4	1.54	0.67
P intake, g/d	10.5	10.7	10.5	11.6	0.76	0.72
Fecal P, g/d	8.60 ^a	11.6 ^{ab}	13.7 ^{bc}	16.2 ^c	1.12	<0.0001

^{a-c}Values within a row with different superscripts differ significantly ($P < 0.05$).

¹Daily infusion of phytate P to ileum (g/d): 0 = 0, × = 5, 3× = 15, 5× = 25.

with increased phytate P infusion to the ileum. However, phytate P disappearance (percentage of total supplied) from the large intestine was similar for all levels of phytate infusion (Table 3). Within the range of 5 to 25 g/d, the amount of phytate reaching the large intestine did not influence phytate degradation in the large intestine. This range of infusion was chosen as representative of the range associated with diets of lactating cows. For instance, a lactating Holstein cow with 680 kg of BW, 55 kg/d of milk yield, and 30 kg/d of DMI at 90 DIM would require 0.38% (DM basis) dietary P (NRC, 2001). If 70% of the dietary P is phytate P, dietary phytate intake would be 80 g/d. Applying the foregut phytate digestibility observed in this study (68% of consumed phytate P), 25 g of phytate P/d would reach the large intestine. These assumptions are extreme in terms of dietary phytate offered (extremely high) and foregut phytate hydrolysis (probably lower than would be observed in a high-phytate diet), and still the amount of phytate reaching the large intestine was within the upper limit of infused phytate into the large intestine in this study.

Although ruminal phytate hydrolysis does appear to be influenced by type of diet and feed processing method, a fixed value for large intestinal phytate hydrolysis can probably be used in estimating P availability from phytate P. In this study, the average disappearance of phytate P from the large intestine was 15% of the total

phytate entering the large intestine (Table 3). This is similar to the 19% of phytate inflow digested between the upper and lower large intestine of sheep fed a 50% forage diet (Park et al., 2002). In that study, more than 10% of total dietary inositol phosphate was not digested. In the present study, considering 68% phytate hydrolysis in the rumen and then 15% hydrolysis of phytate entering the large intestine, total-tract phytate degradation would be 73%, rendering 27% undigested and excreted in the feces. Combined, the results from this study and from the study by Park et al. (2002) indicate that large intestinal phytate degradation contributes to the overall digestibility of phytate but ruminal phytate hydrolysis is the key regulatory factor.

Large intestinal phytate degradation in sheep is considered to be due to the microbial phytase activity in the large intestine in ruminants (Park et al., 2002) and also in rodents and other nonruminants (Wise and Gilbert, 1982; Matsui et al., 1999). The microbial population in the large intestine is similar to that in the rumen (Maki and Picard, 1965; Hoover, 1978). Moreover, various species of *Bacillus* have been detected in the cecum and colon of cows and steers, and *Bacillus* strains have phytase capability (Joseph and Raj, 2007). The phytase activity in the large intestine must be less efficient than in the rumen, as less than 20% of influent phytate was digested in the large intestine in this study and in the sheep study by Park et al. (2002). Likely,

Table 3. Effect of ileal phytate infusion on disappearance of phytate from the large intestine of 8 crossbred heifers

Item	Infusion dose ¹				SEM	P-value
	0	×	3×	5×		
Phytate intake, g/d	0.40	0.41	0.39	0.44	0.03	0.66
Phytate flow, g/d	0.13	0.11	0.16	0.11	0.02	0.23
Phytate infused, g/d	0.00 ^a	1.02 ^b	3.08 ^c	5.03 ^d	0.13	<0.0001
Fecal phytate, g/d	0.12 ^a	1.05 ^b	2.63 ^c	4.30 ^d	0.28	<0.0001
Phytate disappearance, g/d	0.05 ^a	0.15 ^{ab}	0.62 ^b	0.62 ^b	0.14	<0.01
Phytate disappearance, % of total supplied	14.9	17.3	15.8	13.7	8.80	<1.0

^{a-d}Values within a row with different superscripts differ significantly ($P < 0.05$).

¹Daily infusion of phytate P to ileum (g/d): 0 = 0, × = 5, 3× = 15, 5× = 25.

this inefficiency was due to the neutral or milk alkaline pH of the large intestine compared with the acidic pH reported ideal for the most efficient phytase activity (Yanke et al., 1999; Lan et al., 2011) and due to the very short digesta retention time in the large intestine of cows (Hecker and Grovum, 1975).

Disappearance of Lower IP from the Large Intestine

The absence of lower IP in ileal digesta indicates either that lower IP was completely degraded in the rumen and small intestine or their concentration in ileal samples was too low to be detected by HPIC. The former seems likely. In an in vitro study with pig small intestinal mucosa, phytase activity was higher toward IP3 and IP4 than IP5 and IP6 (Hu et al., 1996). In sheep fed corn- and rapeseed meal-based diets (50% forage and 50% concentrate), daily passage of lower IP from the small intestine to the upper large intestine decreased by more than 50%, indicating the ability of small intestinal mucosa to degrade lower IP. In the current study, the amount IP5 and IP4 infused daily to the ileum was different between treatment groups and fecal output of each increased with increased infusion amount (Table 4). Thus, the apparent disappearance of IP5 and IP4 from the large intestine did not differ with amount infused when expressed as percent of total supplied. The apparent disappearance of IP5 and IP4 from the large intestine averaged 34 and 64% of total supplied, respectively. No IP3 was detected in the feces due either to complete hydrolysis in the large intestine or fecal concentration below the detection limit.

This is the first experiment where large intestinal lower IP disappearance has been investigated and observed in large ruminants. In ruminants, the disappearance of lower IP from a very small large intestinal pool

may not be of nutritional significance but the quantification method may be useful to investigate lower IP disappearance from the hindgut of nonruminants.

Fate of Pi in the Large Intestine

The observed phytate disappearance from the large intestine was much lower than ruminal phytate disappearance (15 vs. 68%), but however modest, phytate degradation would increase the pool of available P (Pi) in the large intestine. Inorganic P release from phytate degradation in the large intestine has nutritional importance only if released Pi is absorbed.

As expected, basal flow of Pi to the ileum was not different between treatment groups (Table 5), representing approximately 43% of total P flow to the ileum daily. Inorganic P in infusion solutions increased with increased phytate content because infusion solutions were prepared from a phytic acid solution that contained Pi as an impurity (~18% of infused P). Despite the impurity, the amount of basal (dietary) Pi flowing to the ileum was higher than the flow of infused Pi for each treatment group.

Fecal Pi increased in a linear manner ($P = 0.05$) with increasing phytate infusion level (5 to 25 g/d) and was greater than Pi entering the large intestine (Pi in ileal flow + Pi in infusion solution; Table 5). Large intestinal degradation of IP6 and lower IP likely contribute to the large intestinal pool of Pi but this relationship is not simple. In addition, fecal Pi was greater than the amount of Pi entering the large intestine as ileal flow, even in the absence of phytate infusion. The difference between the amount of Pi in feces and the sum of Pi in basal flow and infusion solution is an estimate of net Pi release from hydrolysis of IP6 and lower IP. Net disappearance of Pi from the large intestine makes this relationship even more complex.

Table 4. Effect of different levels of lower inositol phosphate (IP) infusion on large intestinal lower IP disappearance

Item	Infusion dose ¹			SEM	P-value
	×	3×	5×		
IP5 infused, ² g/d	0.76 ^a	2.44 ^b	3.79 ^c	0.08	<0.0001
Fecal IP5, g/d	0.5 ^a	1.62 ^b	2.29 ^c	0.19	<0.0001
Net IP5 disappearance, ³ % of total supplied	29.7	36.1	36.8	5.97	0.59
IP4 infused, ⁴ g/d	0.28 ^a	0.91 ^b	1.73 ^c	0.04	<0.0001
Fecal IP4, g/d	0.13 ^a	0.35 ^b	0.45 ^c	0.05	<0.0001
Net IP4 disappearance, ³ % of total supplied	58.6	65.1	68.8	5.88	0.41

^{a-c}Values within a row with different superscripts differ significantly ($P < 0.05$).

¹Daily infusion of phytate P to ileum (g/d): × = 5, 3× = 15, 5× = 25.

²IP5 = inositol pentaphosphate.

³Net disappearance is the difference between infused and fecal flow. Unknown is the precise fate of each IP. Disappearance of an IP may indicate partial degradation (to IP4, IP3, and so on) or complete degradation.

⁴IP4 = inositol tetra phosphate.

Table 5. Effect of ileal phytate infusion on disappearance of inorganic P (Pi) from the large intestine of 8 crossbred heifers

Item	Infusion dose ¹				SEM	P-value
	0	×	3×	5×		
Pi flow, g/d	2.81	3.70	4.20	3.19	0.75	0.54
Pi infused, g/d	0.00 ^a	0.26 ^b	0.79 ^c	1.31 ^d	0.03	<0.0001
Fecal Pi, g/d	5.80 ^a	7.22 ^{ab}	7.69 ^{ab}	9.30 ^b	0.72	<0.03

^{a-d}Values within a row with different superscripts differ significantly ($P < 0.05$).

¹Daily infusion of phytate P to ileum (g/d): 0 = 0, × = 5, 3× = 15, 5× = 25.

Reports exist of large intestinal P absorption in ruminants and our data also suggest this. When ileal total P flow and infused total P were used as continuous variables, the slope coefficient for total ileal P flow (dietary only) to feces was 0.56 ± 0.26 (mean $\pm$ SE), whereas the slope coefficient for infused P was 0.75 ± 0.13 . Thus 44% of ileal P flow and 25% of infused P disappeared from the large intestine. Similarly, phosphate absorption was observed from the proximal colon descendens in lambs when ligated intestinal segments were filled with 1.2 mM phosphate solution ($\text{HPO}_4^{2-} + \text{H}_2\text{PO}_4^-$) for 30 min inside the abdominal cavity of anesthetized animals (Scharrer, 1985). Breves and Schroder (1991) observed that in sheep fed 1.0 to 4.1 g of P/d, net disappearance of P from the hindgut was 2 to 30% of total P entering the hindgut.

The greater relative disappearance of P from dietary P flowing to the ileum than from the pure phytate infusion (44 vs. 25%) was likely due to the higher proportion of Pi in total P flowing to the ileum than in total P infused to the ileum. In sheep, the ratio of Pi to total P decreased to less than 0.2 in the lower small intestine, indicating that most of the P was absorbed as soluble Pi (Poppi and Ternouth, 1979). In a study conducted on cannulated sheep, the flow rate (mmol/h) of total and soluble P decreased by 8 and 95%, respectively, from ileum to feces (Ben-Ghedalia et al., 1975). Therefore, the forms of P and their solubility in the digestive tract are 2 of the major factors regulating P absorption in the digestive tract. Ben-Ghedalia et al. (1975) observed that the solubility of P in sheep digesta decreased by 43% from the duodenum to the terminal ileum. The ratio of insoluble non-nucleic acid P to total P increased in the lower small intestine and proximal colon of sheep, indicating the formation of higher degree P-complex at high pH in these segments of the digestive tract (Poppi and Ternouth, 1979).

The primary mechanism of phosphate absorption differs in ruminants and nonruminants. In nonruminants, the small intestinal epithelium contains Na^+ -dependent phosphate transporter (Murer et al., 1994). In ruminants, however, the uptake of phosphate by

intestinal epithelium occurs through an electroneutral pH-dependent H^+/Pi cotransporter, and acidic extracellular pH is required to maintain phosphate uptake (Shirazi-Beechey et al., 1991). The neutral pH in the large intestine, therefore, may limit active P absorption. Therefore, passive diffusion may be the predominant mechanism of P absorption in the large intestine at high luminal concentrations of P. More research is needed to elucidate the mechanism of P absorption in the large intestine of ruminants, but this data will support mechanistic modeling efforts to improve prediction of P digestion, allowing more accurate estimation of P bioavailability in feeds.

CONCLUSIONS

Infused phytate was degraded in the large intestine of dairy heifers and this degradability was not dependent on the amount of phytate entering the large intestine. Degradation of phytate and lower IP increased the pool of large intestinal Pi; released Pi disappeared partially from the large intestine. This data will support mathematical modeling approaches of predicting P digestion, allowing more accurate estimation of P bioavailability in feeds. Further study is needed with phytate from dietary sources to evaluate its flow to and degradability in the large intestine of ruminants.

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