Effect of Grain Source and Exogenous Phytase on Phosphorus Digestibility in Dairy Cows

R. L. Kincaid,¹ D. K. Garikipati,¹ T. D. Nennich,² and J. H. Harrison²

¹Animal Sciences Department, Washington State University, Pullman 99164–6310

²Puyallup Research and Extension Center, Washington State University, Puyallup 98371

ABSTRACT

Two experiments were conducted to determine P digestibility in lactating dairy cows fed corn or barley as grain sources. The first experiment utilized a replicated incomplete 5×4 Latin square design with 8 lactating Holstein cows fed diets containing either corn alone or corn in combination with one of 4 barley varieties that differed in chemical composition. Total tract digestibility of P ranged from 11 to 29% for diets containing the barley varieties and was approximately 35% for the corn diet. A second experiment compared P digestibility in cows fed diets containing corn or barley when exogenous phytase was added to the diets. Lactating Holstein cows ($n = 16$) were arranged in 4 replications of a Latin square with 2 grains (barley or corn), fed separately or with added exogenous phytase (427 phytase units/kg of total mixed ration and 4 periods of 21 d. Phytate P comprised about 50% of the total P (0.46% P) in the total mixed ration. The concentration of serum inorganic P was higher in cows fed diets with exogenous phytase (5.8 vs. 6.5 mg/dL in cows fed barley diets and 5.5 vs 6.0 mg/dL in cows fed corn diets). Using acid detergent lignin as an internal marker, hydrolysis of phytate P was increased by the exogenous phytase, and total P digestibility tended to be increased. In contrast to Experiment 1, in Experiment 2 there was no effect of grain source on P digestibility and total fecal P. Dry matter intake and efficiency of milk production were not affected by exogenous phytase or grain type. Although phytase activity occurs in the rumen, physical properties of the diet and ruminal passage rates may prevent total hydrolysis of phytate in the rumen of lactating cows. Thus, exogenous dietary phytase might improve P digestibility in dairy cows in some dietary situations. (**Key words:** phosphorus, phytate, phytase, cattle)

INTRODUCTION

Although P has more known functions in the body than any other mineral nutrient (Lynch and Caffrey,

1997), it is one of the main polluting nutrients from animal agriculture. Accordingly, nutrient management plans for concentrated animal feeding operations must consider a whole farm P budget (EPA, 2002). Dietary P management is a key strategy in reducing P accumulation in dairy farms (Valk et al., 2000). Therefore, methods to increase P digestibility in cattle and to reduce P excretion are needed.

In general, digestibility of P in cereal grains and their by-products is lower than the digestibility of P in commonly used inorganic supplements (NRC, 2001). In cereal grains, phytic acid (*myo*-inositol 1,2,3,4,5,6 hexakisphosphate) typically represents 65 to 85% of total P (Reddy et al., 1982). Salts of phytic acid are deposited in the aleurone, scutellum, cotyledon, and endosperm during seed formation (Bergman et al., 2000). Phytates in cereals are not uniformly distributed within the kernel but are associated with specific morphological components in the seeds (Ravindran, 1999). Corn has 88% of its phytic acid distributed in the germ layer in contrast to barley, which has 87% of its phytic acid in the aleurone and only 13% in the germ (O'Dell et al., 1972). Whereas corn contains little phytase activity (Eeckhout and de Paepe, 1994), barley contains considerable endogenous phytase, chiefly around the protein bodies in the aleurone layer (Tronier et al., 1971).

Although phytate often is presumed completely hydrolyzed prior to fecal excretion, ruminal hydrolysis is not uniform among feedstuffs and is affected by factors such as processing methods (Bravo et al., 2000). In addition, high dietary Ca reduces the effectiveness of ruminal phytase activity (Sansinena, 1999). Thus, phytate hydrolysis is not complete within the rumen, and phytate has been shown to be present in feces of cattle fed a barley-based diet (Taylor et al., 2001).

Ruminal phytase is largely of bacterial origin and is associated with the bacterial cell pellet (Yanke et al., 1998). Grain type, processing methods, and ruminal outflow rates may affect the ability of phytases in the rumen to access and completely hydrolyze phytate in grains. Exogenous phytase, by increasing total phytase activity in the fluid portion of ruminal contents, might increase the extent of ruminal hydrolysis of phytate.

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Corresponding author: R. L. Kincaid, e-mail: rkincaid@wsu.edu.

Table 1. Ingredient composition of TMR fed to lactating cows (Experiment 1, n = 8).

	Dietary treatment and barley variety				
	Corn	Baroness	Idagold	Steptoe	Harrington
Alfalfa hay, %	19.3	19.1	19.1	19.1	19.1
Grass silage, %	22.7	22.7	22.7	22.7	22.7
Whole cottonseed, %	7.7	7.6	7.6	7.6	7.6
Corn distillers grains, %	4.9	5.9	5.9	5.9	5.9
Corn, cracked, %	38.4	13.3	13.3	13.3	13.3
Barley, ¹ steam rolled, %	—	24.3	24.3	24.3	24.3
Soybean meal, %	0.77	0.76	0.76	0.76	0.76
Blood meal, %	0.71	0.70	0.70	0.70	0.70
Fish meal, %	0.79	0.79	0.79	0.79	0.79
Megalac, ² %	0.21	0.62	0.62	0.62	0.62
Urea, %	1.01	0.76	0.76	0.76	0.76
Sodium bicarbonate, %	0.75	0.74	0.74	0.74	0.74
Limestone, %	0.43	0.42	0.42	0.42	0.42
Dicalcium phosphate, %	0.52	0.51	0.51	0.51	0.51
Mineral, ³ %	1.10	1.08	1.08	1.08	1.08
MgO, %	0.24	0.23	0.23	0.23	0.23
Bloat guard, %	0.06	0.06	0.06	0.06	0.06
Vitamin premix ⁴ , %	0.39	0.38	0.38	0.38	0.38

¹Barley was one of 4 hybrids (Baroness, Idagold, Steptoe, or Harrington).

²Church & Dwight Co., Inc., Princeton, NJ.

³Mineral premix contained approximately 30% Na, 43% Cl, 12 mg/kg of Co, 720 mg/kg of Cu, 25 mg/kg of Fe, 70 mg/kg of I, 2350 mg/kg of Mn, 37 mg/kg of Se, and 1740 mg/kg of Zn.

⁴Vitamin premix contained approximately 2,100,000 IU/kg of vitamin A, 400,000 IU/kg of vitamin D, and 7550 IU/kg of vitamin E.

Therefore, 2 experiments were conducted on P digestion in lactating cows. The first experiment compared the P digestibility of corn and 4 varieties of barley. The second experiment determined whether exogenous phytase increases P digestibility of lactating cows fed concentrates containing either corn or barley.

MATERIALS AND METHODS

The protocols were approved by the Institutional Animal Care and Use Committee of Washington State University.

Experiment 1

Lactating multiparous Holstein cows (n = 8; multiparous), averaging 174 DIM at the start of the trial, were used in a double incomplete 5 × 4 Latin square design to evaluate P digestibility when corn was partially replaced with one of 4 varieties of steam-rolled barley (Baroness, Idagold, Harrington, and Steptoe) in diets. Each experimental period was 14 d. The first 10 d served as an adjustment period followed by 4 d of sample collection. Cows were housed in a tie-stall barn and were individually fed their treatment diets as a TMR once per day. Total mixed rations and orts were sampled on a daily basis, subsampled, and composited on a weight basis over the 4-d collection period. The ingredient and chemical compositions of the diets are described in Tables 1 and 2. Grass silage and alfalfa hay were

included as the main forages; therefore, grains were the primary sources of starch. Some cracked corn was included in all of the diets, and the diets with the test barley varieties contained 24.3% steam-rolled barley. Dry matter intake, orts, and milk yield (2× milking schedule) were recorded daily. Total feces were collected and subsampled on a daily basis for 4 consecutive d, and subsamples were combined to obtain a sample for each treatment period.

Experiment 2

Lactating multiparous Holstein cows (n = 16) were arranged in 4 replicates of a 4 × 4 Latin square design with 4 dietary treatments and 4 periods. Treatment periods were 28 d. The first 21 d served as an adaptation period, and the final 7 d were for data collection. The dietary treatments were 26% barley (Baroness) with no added phytase (barley); 26% barley plus 427 FTU of phytase/kg of TMR on a DM basis (barley + phytase); 26% corn with no added phytase (corn); and 26% corn plus 427 FTU of phytase/kg of TMR on a DM basis (corn + phytase). One FTU is the amount of enzyme that liberates 1 μmol of phosphate/min from 0.0051 mol/L of Na phosphate at 37°C and pH 5.5. All diets contained approximately 0.46% P, 18% CP, 35% NDF, and 19% ADF. Ingredient and nutrient compositions of the diets are given in Tables 3 and 4. The diets were fed as TMR. Of the barley or corn that was added to the diets, about

Table 2. Chemical composition of TMR fed to lactating cows (experiment 1).

	Dietary treatment and barley variety				
	Corn	Baroness	Idagold	Steptoe	Harrington
CP, %	20.2 (0.56) ¹	20.9 (0.86)	19.8 (0.30)	20.4 (1.14)	21.3 (0.71)
NDF, %	38.0 (1.69)	40.6 (1.85)	40.1 (1.04)	41.5 (1.72)	41.7 (1.43)
ADF, %	21.6 (0.67)	23.0 (1.23)	22.5 (2.0)	23.6 (1.98)	23.7 (1.53)
Ca, %	1.04 (0.08)	1.16 (0.09)	1.13 (0.12)	1.17 (0.08)	1.07 (0.13)
P, %	0.58 (0.01)	0.55 (0.02)	0.50 (0.04)	0.57 (0.02)	0.54 (0.04)
Phytate P, %	0.18 (0.02)	0.20 (0.01)	0.19 (0.02)	0.15 (0.02)	0.20 (0.02)

¹Standard deviations are presented in parentheses.

60% was steam-rolled and 40% was ground and pelleted with other ingredients. The phytase was added to the pelleted portion of the concentrate mix.

At the start of the experiment, cows averaged 210 ± 19 DIM and had a mean BW of 702 ± 79 kg. The cows were randomly assigned to one of 4 dietary treatment groups (4 cows per treatment per period). The cows were fed individually via Calan gates and had access

to water and feed. Cows were switched to a different treatment diet at the end of each period until each cow had received all 4 treatment diets.

Cows were milked twice daily, and milk yield was recorded at each milking. Body weights were recorded initially and on d 21 of each study period. The amount of diet offered andorts were recorded daily to calculate feed intake. Samples of TMR were collected weekly and

Table 3. Ingredient composition of TMR fed to lactating cows containing barley or corn, with or without added phytase (experiment 2).

	Dietary treatments			
	Barley	Barley + phytase ¹	Corn	Corn + phytase ²
Alfalfa haylage, %	25.6	25.6	25.6	25.6
Alfalfa hay, %	22.3	22.3	22.3	22.3
Barley, steam rolled, %	15.1	15.1	—	—
Barley, pelleted, %	10.7	10.7	—	—
Corn, steam rolled, %	—	—	15.1	15.1
Corn, pelleted, %	—	—	10.7	10.7
Whole cottonseed, %	9.9	9.9	9.9	9.9
Wheat millrun, %	6.6	6.6	6.6	6.6
Peas, culled, %	5.3	5.3	5.3	5.3
Soybean meal, %	1.6	1.6	1.6	1.6
Sodium carbonate, %	0.71	0.71	0.71	0.71
Limestone, %	0.53	0.53	0.53	0.53
Trace mineral premix, ³ %	0.53	0.53	0.53	0.53
MgO, %	0.14	0.14	0.14	0.14
Molasses, %	0.89	0.89	0.89	0.89
Vitamin A premix, ⁴ %	0.018	0.018	0.018	0.018
Vitamin D premix, ⁵ %	0.018	0.018	0.018	0.018
Vitamin E premix, ⁶ %	0.0036	0.0036	0.0036	0.0036
4-plex, ⁷ %	0.035	0.035	0.035	0.035
Pellet binder, %	0.018	0.018	0.018	0.018
Phytase, ⁸ %	—	0.09	—	0.09

¹Barley + 427 FTU phytase/kg of DM. One FTU is the amount of enzyme that liberates 1 μ mol of phosphate/min from 0.0051 mol/L of Na phosphate at 37°C and pH 5.5.

²Corn + 427 FTU phytase/kg of DM.

³Consists of 97% NaCl, 0.18 Mn, 0.35% Zn, 0.2% Fe, 0.037% Mg, 0.035% Cu, 0.01% I, 0.006% Co, and 0.009% Se.

⁴Consists of 30,000 IU/g.

⁵Consists of 8810 IU/g.

⁶Consists of 500 IU/g.

⁷Contains 2.58% Zn as Zn Met, 1.43% Mn as Mn Met, 0.9% Cu as Cu Lys, and 0.18% Co as Co glucoheptonate. Zinpro Corp., Eden Prairie, MN.

⁸Phytase (*Aspergillus niger*) 600 PAK manufactured (ADM Animal Health & Nutrition Division, Des Moines, IA). Contains 600 FTU/g.

Table 4. Chemical composition of TMR for lactating cows (experiment 2).

	Dietary Treatment				SE	P<
	Barley	Barley + phytase ¹	Corn	Corn + phytase ²		
CP, %	18.0	18.1	18.2	18.2	0.18	0.70
NDF, %	34.9	34.7	34.8	35.1	0.30	0.48
ADF, %	19.4	19.1	19.6	18.9	0.55	0.30
Ca, %	1.16	1.14	1.11	1.18	0.04	0.39
P, %	0.45	0.47	0.46	0.45	0.15	0.53
Phytate P, %	0.235	0.237	0.236	0.238	0.02	0.42
ADL, %	3.20	3.14	3.30	3.36	0.24	0.17

¹Barley + 427 FTU phytase/kg of DM. One FTU is the amount of enzyme that liberates 1 μ mol of phosphate/min from 0.0051 mol/L of Na phosphate at 37°C and pH 5.5.

²Corn + 427 FTU phytase/kg of DM.

composited (wt/wt) by each period for each treatment. Approximately 90 min after the a.m. feeding, blood samples were collected from the coccygeal vein into non-heparinized vacutainers. Serum was harvested after centrifugation at $3000 \times g$ for 20 min and stored at -20°C until further analysis. Milk samples were collected in plastic bottles and immediately taken to the laboratory where they were frozen until further analysis. Subsamples of milk also were sent to the regional DHIA laboratory (Burlington, WA) for analysis of major components. Fecal samples (approximately 150 g) were collected from the rectum of cows during one sample collection that took place at 0900 h. Fecal samples were placed into plastic cups, sealed immediately, and taken to the laboratory where they were dried at 60°C in a forced-air oven and stored until further analysis.

Analyses. Composite feed and fecal samples were dried (60°C for 48 h) and ground to pass a 1-mm screen (Wiley mill; Arthur H. Thomas Co., Philadelphia, PA). A subsample of ground feed and feces were dried at 100°C for 24 h for calculation of absolute DM. Samples of feed and feces were analyzed in duplicate for CP, NDF, ADF, ADL, Ca, P, and phytate P. Blood and milk samples were analyzed for Ca and P. Crude protein was determined using a Leco 528 Protein Analyzer (AOAC, 1995), NDF and ADF were determined using an Ankom Fiber analyzer (Ankom Technology, Macedon, NY; AOAC, 1995), and ADL was analyzed according to the AOAC (2001). Concentrations of ADL in the TMR and feces were used to estimate P digestibility and fecal output. Calcium was determined by atomic absorption spectrophotometry (Robinson, 1975), and P was determined by colorimetry (AOAC, 2001). Concentrations of phytate P in feed and fecal samples were determined by the ferric precipitation method (Raboy et al., 1984).

Statistical Analyses

Experiment 1. Statistical analysis was done using PROC MIXED of SAS (2001). The statistical model was

a double incomplete Latin square with 5 treatments and 4 periods. The statistical model used to analyze the data was

$$Y_{ikj} = \mu + \alpha_i + b_j + c_k + e_{ijk}$$

where

Y_{ikj} = observed response,
 μ = overall mean,
 α_i = fixed effect of treatment,
 b_j = random effect of cow,
 c_k = random effect of period, and
 e_{ijk} = residual error.

Results are reported as least squares means. A protected LSD test was used to separate treatment means. Differences between observations were considered significant at $P < 0.05$.

Experiment 2. Data were analyzed by PROC GLM of SAS (2001). The statistical model used was

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \gamma_k + \rho_l + e_{ijkl}$$

where

Y_{ijkl} = observed response,
 μ = overall mean,
 α_i = fixed effect of grain source (barley or corn),
 β_j = fixed effect of enzyme treatment (no phytase or added phytase),
 $(\alpha \times \beta)_{ij}$ = effect of interaction between grain and enzyme treatments,
 γ_k = fixed effect caused by period,
 ρ_l = fixed effect caused by cows in the group, and
 e_{ijkl} = residual error.

Significance was declared at $P < 0.05$, and trends were declared at $P < 0.10$.

Table 5. Chemical composition of barley varieties fed to lactating cows (experiment 1).

	Barley variety			
	Baroness	Idagold	Steptoe	Harrington
CP, %	13.1	12.5	11.5	12.4
NDF, %	28.1	30.0	33.5	30.4
ADF, %	8.4	6.9	12.4	8.7
Starch, %	47.9	46.2	45.6	46.9
Ca, %	0.05	0.05	0.06	0.06
P, %	0.43	0.42	0.39	0.45

RESULTS AND DISCUSSION

Experiment 1

The 4 barley varieties differed in their chemical composition; Baroness contained the highest CP content, and Steptoe contained the highest fiber content (Table 5). The P concentration in the barley varieties ranged from 0.39% (Steptoe) to 0.45% (Harrington). This difference in the chemical composition of the barley varieties contributed to the variation in the chemical composition of the TMR, which ranged from 20 to 21% CP, 38 to 42% NDF, 21.6 to 23.7% ADF, and 0.50 to 0.58% P (Table 2). The high dietary P resulted from inclusion of several high P feedstuffs (corn distillers grains, fish meal, and dicalcium phosphate), which are typically included in diets on commercial dairies in western Washington. Phytate P ranged from 26 to 38% of total dietary P; the lowest percentage of phytate P was found in the TMR containing Steptoe barley, and the highest percentage was observed in the TMR containing the Idagold barley.

Dry matter intake, BW, milk yield, and milk composition were unaffected by grain type and barley variety in the diet (Table 6). However, P intake, fecal output of P, and apparent total tract digestibility of P were affected by the variety of barley that was included in the TMR (Table 7). Total tract P digestibility in lactating Holstein cows ranged from 11 to 28% for diets containing one of the 4 barley varieties and was about 37%

for corn. Cows fed the Steptoe barley variety, which had the smallest percentage of phytate P of all barleys (Table 2), had substantially greater total tract P digestibility than did cows fed the Baroness and Idagold varieties. Accordingly, fecal excretion of P was less when cows were fed corn compared with barley and was less for the Harrington barley than for the Baroness barley (Table 7). Although the P concentration in the TMR ranged from 0.50 to 0.58%, the high dietary concentration of P in study 1 did not appear to depress P digestibility measurably.

Experiment 2

The treatment TMR differed only in their content of corn, barley, and added phytase (Table 4). The mean concentration of dietary P was 0.46%, which, although greater than the minimum P recommended by the NRC (2001), is representative of many diets containing commonly used by-product feeds. The TMR ingredients, including whole cottonseeds, wheat mill run, culled peas, and soybean meal, contributed to the comparatively high dietary P. Of the total amount of P in the TMR, 26% of the P was supplied by the corn or barley. Barley had slightly more phytate P than corn (0.22 vs. 0.20%), and the phytate in barley and corn is distributed differently within the kernel (O'Dell et al., 1972).

There were no treatment effects on DMI, milk yield, and milk composition of cows (Table 8). Although differences in DMI and milk yield in cows fed diets containing either corn or barley have been reported (Casper and Schingoethe, 1989; Yang et al., 1997), generally, differences occur only when the cereal grains constitute 50% of the diet (Kincaid and Harrison, 2002). The more extensive ruminal starch digestibility of barley than of corn (77 vs. 55%; McCarthy et al., 1989) can lead to higher concentrations of lactic acid in the rumen (Tamminga et al., 1990). In experiment 2, cereal grain was only 26% of the TMR, and the NDF content was relatively high (35%), which minimized possible differences in ruminal VFA concentrations between the corn and

Table 6. Dry matter intake, BW and milk yield of cows fed corn or known variety of barley in TMR (experiment 1).

Item	Dietary treatment and barley variety					SE	P^1
	Corn	Baroness	Idagold	Steptoe	Harrington		
BW, kg	597.1	606.4	610.1	601.8	614.8	27.2	0.42
DMI, kg/d	19.2	19.5	19.4	19.4	18.6	1.2	0.69
Milk yield, kg/d	19.5	20.1	21.2	20.6	21.0	3.3	0.47
Milk fat, %	4.19	3.85	4.17	3.84	3.98	0.25	0.69
Milk CP, ² %	3.33	3.34	3.54	3.50	3.37	0.17	0.09

¹Probability of F ratio from ANOVA.

²Milk protein values are reported as a percentage of CP in milk.

Table 7. Intake, fecal output, and digestibility of P for lactating cows fed diets containing corn or known barley variety (experiment 1).

Item	Dietary treatment and barley variety					SE ¹	P<
	Corn	Baroness	Idagold	Steptoe	Harrington		
P intake, g/d	117 ^a	107 ^{bc}	98 ^d	111 ^{ab}	103 ^{cd}	6.6	0.01
Fecal P, g/d	70 ^c	87 ^a	84 ^{ab}	81 ^{ab}	77 ^{bc}	7.0	0.01
P digestibility, %	34.9 ^a	19.5 ^{cd}	11.4 ^d	28.3 ^{ab}	21.2 ^{bc}	4.52	0.01

^{a,b,c,d}Means in rows with different superscripts differ ($P \leq 0.05$).

¹Reflects the greatest individual treatment error.

barley treatments. Body weights of the cattle did not differ among treatments (Table 8). The initial mean BW of the cattle was 702 ± 79 kg, and the final BW was 706 ± 77 kg.

Milk production efficiency, expressed as milk yield per kilogram of DMI, was unaffected by the dietary treatments (Table 8). A similar result was reported by Yang et al. (1997), who compared barley-, hull-less barley-, and corn-based diets of multiparous lactating dairy cattle and found no difference in efficiency of milk production. However, in primiparous cows, they found a higher ratio of milk yield to digestible DM when those cows were fed hull-less barley as the grain source vs. corn or barley.

Concentrations of P and Ca in milk. The percentage of P in milk was 0.09% and was not affected by dietary treatment (Table 8). The percentage of P in milk is primarily a function of milk protein percentage and is not affected by dietary P level (Forar et al., 1982; NRC, 2001). Similarly, the percentage of Ca in milk was 0.13%, and there was no effect of dietary treatment (Table 8).

Concentrations of inorganic P and Ca in serum.

The addition of exogenous phytase to the diets increased serum inorganic P in cows fed both the diets containing barley (5.8 vs. 6.6 mg/dL) and corn (5.5 vs. 6.0 mg/dL; Figure 1). Plasma inorganic P is known to fluctuate diurnally in cows and peaks at about 2 h post-feeding (Forar et al., 1982). In study 2, blood samples were taken about 90 min after feeding, which might have maximized the likelihood of detecting differences among treatments in serum inorganic P. There was no difference in serum inorganic P between cows fed corn or barley as the grain source. Because blood inorganic P is affected by dietary P intake (Read et al., 1986), the addition of phytase to the diets apparently increased the amount of absorbable P in the small intestine of the cows.

In contrast to P, the concentrations of Ca in serum were not affected either by the exogenous phytase or by the grain source. The serum Ca levels for the barley, barley + phytase, corn, and corn + phytase diets were 9.5, 10.0, 9.6, and 10.1 mg/dL, respectively, and were

Table 8. Effect of grain type and added phytase on BW, feed intake, and milk yield/kg feed intake (DM basis; experiment 2).

	Dietary treatment					<i>P</i> <		
	Barley	Barley + phytase ¹	Corn	Corn + phytase ²	SE	Grain	Phytase	G × P ³
BW, kg	704.4	699.5	702.7	705.9	79.0	0.69	0.62	0.15
DMI, kg	28.3	28.8	29.0	28.2	0.88	0.45	0.35	0.24
Milk yield, kg/d	44.5	43.5	43.5	43.0	4.5	0.29	0.25	0.57
4% FCM, kg/d	40.1	40.2	39.6	39.0	4.7	0.39	0.40	0.64
Milk protein, %	3.05	3.08	3.03	3.09	0.01	0.91	0.13	0.73
Milk fat, %	3.35	3.51	3.40	3.38	0.09	0.92	0.88	0.70
Milk Ca, %	0.13	0.13	0.13	0.13	0.01	0.84	0.80	0.92
Milk P, %	0.09	0.09	0.09	0.09	0.008	0.89	0.89	0.88
PE ⁵	1.6	1.5	1.5	1.5	0.11	0.26	0.50	0.69

¹Barley + 427 FTU phytase/kg of DM. One FTU is the amount of enzyme that liberates 1 μ mol of phosphate/min from 0.0051 mol/L of Na phosphate at 37°C and pH 5.5.

²Corn + 427 FTU phytase/kg of DM.

³G × P is the response caused by grain and phytase interaction.

⁴Milk protein values are reported as the percentage of true protein in milk.

⁵PE = Milk production efficiency, calculated as kg of milk/kg feed intake (DM basis).

Figure 1. Effect of exogenous dietary phytase on serum inorganic P (Pi) in lactating cows fed TMR containing either barley or corn. Treatments with different letters differ ($P < 0.05$; mean SE = 0.227). Diets with added phytase contained 427 FTU phytase/kg of DM (experiment 2). One FTU is the amount of enzyme that liberates 1 μ mol of phosphate/min from 0.0051 mol/L of Na phosphate at 37°C and pH 5.5.

within the normal physiological range for cattle (NRC, 2001).

Apparent digestibility of P. The addition of exogenous dietary phytase increased hydrolysis of phytate P in lactating cows fed TMR containing either corn or barley (Table 9). The values for hydrolysis of phytate P reflect the disappearance of phytate and not phytate P digestibility, i.e., the P hydrolyzed from phytate presumably was available for intestinal absorption but was not necessarily absorbed. Total P digestibility tended to be greater in diets with exogenous phytase. The level of exogenous phytase (427 FTU/kg) was selected based on the level that resulted in the highest apparent digestibility in feedlot cattle (Hurley et al., 2002). Total excretion of fecal P tended to be decreased in cows fed the diets with added phytase (Table 9). There was no significant difference in P digestibility or phytate P hydrolysis between the barley- and corn-based diets. Wu

et al. (2000) reported diurnal variation in fecal P concentration. Based on the pattern of diurnal variation reported by Wu et al. (2000), collection of a single fecal sample at 0900 h would lead to overestimation of P digestibility. Thus, the relative P digestibility among treatments is representative of the diets, but the apparent digestibility values that are reported (Table 9) may be higher than actually occurred.

The concentration of Ca in the diet can affect phytase activity (Bedford, 2000) and ruminal phytate hydrolysis (Sansinena, 1999). Although Clark et al. (1986) observed higher apparent digestibility of P when the dietary Ca was 0.9% than when it was 0.6%, others have found reduced P absorption with higher concentrations of dietary Ca (Field et al., 1983; Mathur, 1953). Barth and Hansard (1962) reported that phytate P utilization was 100% when Ca:P was 2:1, but fell to 67% when Ca:P increased to 8:1. In the current experiment, Ca concentration ranged from 1.11 to 1.18% of the TMR (DM basis), and Ca:P in the TMR ranged from 2.4 to 2.6:1.

Fecal P, Ca, and phytate P. Fecal P excretion (g/d) tended to be less in cows fed diets containing the added phytase (Table 9). The concentration of P in the feces averaged 0.59% DM for cows fed the TMR containing barley diets and 0.55% for cows fed the TMR containing corn. Fecal excretion of phytate P (g/d) was significantly less in cows given the phytase supplement. Fecal Ca was unaffected by either the added dietary phytase or the grain source fed to cows. The Ca concentration in feces of cows fed the barley, barley + phytase, corn, and corn + phytase diets were 2.5, 2.4, 2.6, and 2.3%, respectively.

Comparison Between Experiments

The lower P digestibilities in Experiment 1 compared with Experiment 2 probably were caused by a combina-

Table 9. Effect of grain source and added phytase on P intake, P secretion in milk, digestibility of total dietary P, and phytate P in dairy cows (experiment 2).

	Dietary treatment				SE	$P <$		
	Barley	Barley + phytase ¹	Corn	Corn + phytase ²		Grain	Phytase	G $\times$ P ³
Total P intake, g/d	128	134.1	134	127.2	10.2	0.23	0.24	0.32
Phytate P intake, g/d	66.5	68.2	68.4	67.1	6.4	0.21	0.26	0.30
P in milk, g/d	39.9	39.0	39.1	38.7	2.5	0.57	0.32	0.72
P in feces, g/d	65.8	64.0	66.8	60.4	4.5	0.45	0.09	0.32
P absorbed, g/d	62.2	70.0	67.1	66.8	3.1	0.13	0.11	0.27
P digestibility, %	48.6	52.3	50.1	55.5	3.4	0.56	0.07	0.67
Phytate P in feces, g/d	14.3	9.8	13.5	9.9	0.8	0.43	0.03	0.28
Phytate P hydrolysis, %	78.4	85.4	80.3	85.1	4.9	0.56	0.02	0.43
DM digestibility, %	68.5	67.5	68.0	66.8	3.3	0.38	0.22	0.38

¹Barley + 427 FTU phytase/kg of DM. One FTU is the amount of enzyme that liberates 1 μ mol of phosphate/min from 0.0051 mol/L of Na phosphate at 37°C and pH 5.5.

²Corn + 427 FTU phytase/kg of DM.

³G $\times$ P is the response caused by grain and phytase interaction.

Figure 2. Comparison between P intake (g/d) and fecal P excretion (g/d) of cows fed corn and barley diets in experiment 1 and cows fed diets containing corn or barley and added phytase in experiment 2; values are reported according to Knowlton and Herbein (2002) and Wu et al. (2000). Barley varieties in Experiment 1 are listed. Diets with added phytase contained 427 FTU phytase/kg of DM. One FTU is the amount of enzyme that liberates 1 μ mol of phosphate/min from 0.0051 mol/L of Na phosphate at 37°C and pH 5.5.

tion of factors. First, the TMR in experiment 1 had a higher percentage of P (0.55% vs. 0.46%), and P digestibility is known to decrease as the percentage of dietary P increases (Weiss and Wyatt, 2004). In addition, milk yields were much lower for cows in experiment 1 (20 vs. 43 kg/d); thus, less P was secreted into milk. Second, the diets in experiment 1 had higher NDF concentrations (40% vs. 35%). Increased dietary NDF concentration enhance salivary flow, which increases total salivary P entering the rumen (Khorasani et al., 1997), and may reduce dietary P digestibility during P excess. Third, diets in experiment 2 contained wheat millrun, which possesses appreciable phytase activity (Cromwell, 1999). Thus, the endogenous phytase in what millrun might have hydrolyzed some phytate P in the diets in experiment 2. Accordingly, the lower P digestibilities in experiment 1 are to be expected.

Other differences between the 2 studies include the higher percentage of cereal grain in the diets of experiment 1 (37.6%) vs. experiment 2 (25.8%) and that 40% of the barley was ground in experiment 2. Guyton et al. (2003) reported an interaction of ruminal starch availability and intake of phytic acid on ruminal phytase activity. In addition, the endogenous phytases in wheat millrun in experiment 2 might have hydrolyzed

some of the phytate P in barley. In experiment 2, grinding of 40% of the barley would have exposed more of the phytate to action of phytases, enhancing phytate hydrolysis. Finally, the higher percentage of P and NDF in diets of experiment 1 might have negatively affected P solubilization from barley.

When the P excretion data of both studies are compared with the P excretion data reported by Knowlton and Herbein (2002) and Wu et al. (2000), the P excretion for cows fed corn in experiment 1 agree with predicted values (Figure 2). Whereas cows fed the barley diets in experiment 1 excreted more P than predicted, cows in experiment 2 excreted less P than predicted. However, data in Figure 2 only represent P intake and fecal P excretion, ignoring milk P secretion. Also, the collection of a single fecal sample at 0900 h from cows in experiment 2 might have led to an underestimation of fecal P output. Thus, a combination of cow, diet, and sampling factors contributed to differences in estimates of P digestibility between the 2 experiments.

The added dietary phytase increased the hydrolysis of phytate P in lactating cows. In cows not fed supplemental phytase enzymes, hydrolysis of dietary phytate P ranged from 69 to 89%. The phytate P hydrolysis in cows fed phytase ranged from 75 to 98%. The results

of the present experiment indicate that phytase supplementation tended to improve phytate P and total P digestibility in cattle fed either corn or barley as the grain source. The results are in contrast to a previous study where >95% of phytate P hydrolysis occurred in the dairy cows without any addition of phytase (Clark et al., 1986). However, neither study separated the phytate hydrolysis that occurred in the rumen from that which occurred in the lower gastrointestinal tract. The current results are supported by the findings of Hurley et al. (2002) who reported higher P digestibility in feedlot cattle fed 500 FTU of phytase/kg of DM. Similarly, Sansinena (1999) found ruminal escape of phytate was 28 to 47%, with greater escape occurring with increased dietary Ca (0.28 to 1.75% Ca, DM basis). Another contributing factor to the improvement in P digestibility with added phytase in the current experiment is that the high-producing lactating cows had feed intakes of 4% of BW. Thus, the accompanying rapid rumen turnover rate may limit phytate hydrolysis prior to the absorption sites of P in the small intestine.

CONCLUSIONS

Differences exist in P digestibility among barley varieties, and in some situations, P digestibility of barley is less than that for corn. Addition of exogenous phytase to lactating cows fed corn or barley tended to improve P digestibility. Complete hydrolysis of phytate P does not always occur within the rumen, and in these situations, the addition of dietary phytase may enhance the amount of P that is available for absorption from the small intestinal tract. There was no effect of grain source or phytase supplementation on milk yield or composition.

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