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Effects of exogenous phytase supplementation on phosphorus metabolism and digestibility of beef cattle

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ABSTRACT: Objectives were to determine interactions between phytase inclusion and dietary P concentration on P utilization by beef cattle fed a starch-based diet. Six ruminally-fistulated steers (BW = 750 ± 61 kg) were allotted to a 6 × 6 Latin square design with a 3 × 2 factorial arrangement of treatments. Factors included phytase inclusion, at 0, 500, or 2,000 phytase units (FTU)/kg of diet DM, and dietary P concentrations, at 0.10% and 0.30% of total diet DM. Feed ingredients, fecal samples, and orts were composited within period, lyophilized and ground. Samples were analyzed for NDF, ADF, CP, fat, ash, total P, and other minerals. Data were analyzed using the MIXED procedure of SAS with animal as the experimental unit. The CORR procedure was used to compare blood and urinary P concentrations. There were no treatment interactions ($P \geq 0.30$) for any parameter measured. There were no main effects ($P \geq 0.45$) of phytase inclusion on DMI, total fecal output, apparent DM digestibility, water intake, or urinary output. Steers fed 0.10% P had decreased ($P < 0.01$) DMI and total fecal output, but increased ($P < 0.01$) apparent DM digestibility compared with steers fed 0.30% P. Although N intake and retention were not affected by treatment, steers fed the 0.10% P diet tended ($P = 0.10$) to absorb more N compared with steers fed 0.30% P; and, steers fed the 0.10% P diets excreted more N in the urine ($P = 0.02$) and less N in the feces ($P < 0.01$) compared with steers fed the 0.30% P diets. Steers fed the 0.10% P diets also consumed 70.1% less ($P < 0.01$) total P each day, and excreted 51.9% less ($P < 0.01$) P in feces and 94.6% less P in the urine ($P < 0.01$) compared with steers fed 0.30% P. Excretion of water-soluble P in the feces was greater ($P < 0.01$) on a g/d basis for steers fed 0.30% P when compared with steers fed 0.10% P. However, the proportion of total fecal P excreted as water-soluble P increased ($P < 0.05$) by 23.0% in steers fed 0.10% P compared with steers fed 0.30% P, regardless of phytase inclusion level. There was no effect of dietary phytase concentration on blood or urinary ($P \geq 0.27$) P concentrations. Blood P concentration was positively correlated ($r = 0.60$; $P < 0.01$) to urinary P concentration when steers were fed 0.10% P; however, when steers were fed 0.30% P, there was no correlation ($r = 0.36$; $P = 0.16$) between blood and urine P. Regardless of dietary P concentration, phytase supplementation did not increase calculated P absorption or retention.

Key words: beef cattle, phosphorus, phytase

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INTRODUCTION

Phytate binds 60 to 80% of total P as inorganic phosphates (PO_4^{3-}) in most cereal grains and by-product feeds (Eeckhout and De Paepe, 1994; Viveros et al., 2000), making P indigestible (Simons and Versteegh, 1990) and reducing P absorption by animals (Simons and Versteegh, 1990; Kincaid et al., 2005). The enzyme phytase liberates PO_4^{3-} from phytate to increase absorption in nonruminants (Lei et al., 1993). Most often, approximately 500 phytase units (FTU) per kg feed is used, but greater inclusion (1,500 to 2,500 FTU per kg) may improve positive effects of phytase (Walk et al., 2013). This technique is known as "superdosing".

Dairy cattle have been fed microbial phytase with mixed results (Bravo et al., 2002; Guyton et al., 2003;

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Knowlton et al., 2007). The greatest dose of microbial phytase used in beef cattle research to date has been 600 FTU per kg diet DM (Hankins-Herr et al., 2009). Rumen microbial populations produce phytase, but it has been suggested that passage rate (Kincaid et al., 2005), grain type (Eeckhout and De Paepe, 1994; Ravindran et al., 1994), processing method (Park et al., 1999; Bravo et al., 2000), and dietary concentration of Ca (Sansinena, 1999) may reduce the ability of ruminal phytases to cleave PO_4^{3-} from phytate. Feedlot cattle are often fed processed, grain-based diets that increase rate of passage. There is minimal information about effects of feeding phytase to feedlot cattle, and to our knowledge, no information about effects of superdosing of microbial phytase in diets fed to feedlot cattle has been reported.

We hypothesized that feeding increasing concentrations of microbial phytase to beef cattle would reduce the amount of P required in the diet, and decrease P excreted in manure, in steers fed a 0.10% P diet, but phytase would have no effect on P excretion in steers fed 0.30% P. Objectives were to determine interactions between phytase inclusion and dietary P concentration on P utilization by beef cattle fed a starch-based diet.

MATERIALS AND METHODS

All animal procedures were approved by the University of Illinois Institute of Animal Care and Use Committee (IACUC #15107) and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Animals and Management

Six Angus-cross, ruminally-fistulated steers (BW = 750 ± 61 kg) were used in a 6 × 6 Latin square design with a 3 × 2 factorial arrangement of treatments: 1) 0 FTU/kg phytase (Quantum Blue 5000 G; ABVista, Marlborough, United Kingdom), 0.10% dietary P, 2) 500 FTU/kg phytase, 0.10% dietary P, 3) 2,000 FTU/kg phytase, 0.10% dietary P, 4) 0 FTU/kg phytase, 0.30% dietary P, 5) 500 FTU/kg phytase, 0.30% dietary P, or 6) 2,000 FTU/kg phytase, 0.30% dietary P. One FTU is defined as the amount of enzyme that liberates 1 μmol of inorganic phosphate per minute from 0.0051 *M* Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al., 1994). Because the objectives were to determine interactions between phytase inclusion and dietary P concentration on P utilization by beef cattle fed a starch-based diet, the 0.10% P diet was formulated with corn-grain components (starch and bran), that contained very little P, while the 0.30% P diet was a typical feedlot diet consisting of mostly corn (Table 1). Using corn-grain components to formulate the 0.10% P diet was chosen to eliminate the variation among other grain sources and to have both diets be based on corn grain.

Table 1. Composition of diets fed to steers

Phytase	0.10% P[1]			0.30% P		
FTU/kg diet DM	0[2]	500	2,000	0	500	2,000
Item, % DM basis						
Dry rolled corn	–	–	–	75.0	74.99	74.96
Grass hay[3]	15.0	15.0	15.0	15.0	15.0	15.0
Corn starch	59.5	59.49	59.46	–	–	–
Corn bran	13.0	13.0	13.0	–	–	–
Corn oil	2.5	2.5	2.5	–	–	–
Phytase[4]	0	0.01	0.04	0	0.01	0.04
Supplement						
Ground corn	–	–	–	4.672	4.672	4.672
Corn starch	1.700	1.700	1.700	–	–	–
Soybean meal	6.072	6.072	6.072	3.700	3.700	3.700
Urea	1.700	1.700	1.700	–	–	–
Limestone	0.400	0.400	0.400	1.500	1.500	1.500
Trace mineral[5]	0.100	0.100	0.100	0.100	0.100	0.100
Monensin[6]	0.017	0.017	0.017	0.017	0.017	0.017
Tylosin[7]	0.011	0.011	0.011	0.011	0.011	0.011
Analyzed Composition						
DM	88.23	88.23	88.23	83.87	83.87	83.88
OM	97.68	97.68	97.68	95.72	95.72	95.73
Fat	3.02	3.01	3.01	2.58	2.58	2.58
NDF	19.66	19.66	19.65	19.00	19.00	18.99
ADF	8.97	8.97	8.97	8.38	8.38	8.37
CP	11.54	11.55	11.57	9.80	9.81	9.83
Ca	0.30	0.30	0.30	0.67	0.67	0.67
P	0.09	0.09	0.09	0.27	0.27	0.27
Phytic acid	0.10	0.10	0.10	0.64	0.64	0.64

[1]Diets were formulated to contain 0.10 and 0.30% P based on book values (NRC, 1996).

[2]Denotes phytase enzyme addition to diet; 0 = 0 FTU phytase/kg diet DM; 500 = 500 FTU phytase/kg diet DM; 2,000 FTU phytase/kg diet DM. Where, 1 FTU is defined as the amount of enzyme that liberates 1 μmol of inorganic phosphate per minute from 0.0051 *M* Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al., 1994).

[3]Grass hay fed to cattle was 87.01% DM and contained 8.97% CP, 67.99% NDF, and 41.23% ADF (DM basis).

[4]Phytase (5,000 FTU/g of Quantum Blue; ABVista, Marlborough, United Kingdom) was hand-mixed at the time of feeding.

[5]Included: 8.5% Ca (as $CaCO_3$), 5% Mg (as MgO and $MgSO_4$), 7.6% K (as KCl_2), 6.7% Cl (as KCl_2) 10% S (as S_8, prilled), 0.5% Cu [as $CuSO_4$ and Availa-4 (Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN)], 2% Fe (as $FeSO_4$), 3% Mn (as $MnSO_4$ and Availa-4), 3% Zn (as $ZnSO_4$ and Availa-4), 278 mg/kg Co (as Availa-4), 250 mg/kg I [as $Ca(IO_3)_2$], 150 mg/kg Se (Na_2SeO_3), 2205 KIU/kg VitA (as retinyl acetate), 662.5 KIU/kg VitD (as cholecalciferol), 22,047.5 IU/kg VitE (as DL-α-tocopheryl acetate), and less than 1% CP, fat, crude fiber, salt.

[6]Rumensin 90 (198 g monensin/kg of Rumensin 90 DM; Elanco Animal Health; Greenfield, IN).

[7]Tylan 40 (88 g tylosin/kg of Tylan 40 DM; Elanco Animal Health).

Diets were fed once daily for ad libitum intake. Steers were housed in metabolism stalls at the University of Illinois Beef Cattle and Sheep Field Research Laboratory in Urbana, IL. Stalls (2.3 × 1.3 m) were equipped with in-

dividual feed bunks. Water was provided on an ad libitum basis via nonsiphoning, automatic water bowls equipped with flow meters and data loggers (OM-CP-PULSE 101A) to record water intake (OMEGA Engineering Inc., Stamford CT). The barn was equipped with a heating, ventilation, and air-conditioning system, providing a controlled environment at 18.3°C.

Collection of Samples

To aid in diet transition and to account for ruminal microbial differences, on d 0, all steers were weighed and partial ruminal contents (approximately 10 L) were removed from each steer, mixed among all steers, and mixed contents were then redistributed to all steers. Steers were then allotted to 1 of the 6 dietary treatments according to Patterson and Lucas (1962).

During each period, steers were fed for a 14 d adaptation period and samples were collected for 5 d after that. Thus, each period of the square lasted 19 d. Feed samples, (100 g, as-is basis), were collected each day on d 1 through 5 of the collection period. Feed ingredient samples were analyzed for DM (24 h at 105°C) at the initiation of each period to adjust for dietary inclusion. If there were orts present on a collection day, orts were weighed back and subsampled. Feed and orts samples were stored at –20°C until analysis.

On d 1 of the collection period, fecal bags were attached on each steer to determine fecal output for 120 h. Feces were collected in canvas bags secured by a leather harness attached around the heart girth and under the neck. Feces were weighed 3 times daily, 0600, 1200, and 1800 and a 5% subsample of the total weight was saved at each collection. Fecal subsamples were combined as collected and stored in a -20°C freezer until analysis.

Urine collection funnels were attached to steers on d 2 of collection and used to collect urine for 72 h. During the 72 h of urine collection, steers were under continuous observation by trained personnel to ensure the apparatus remained in place and collections accurately represented total urine output. Funnels were connected to 18.9 L plastic collection tanks via plastic hoses (0.64 cm thick with a 1.9 cm opening). Urine was acidified using 200 mL of 6*N* HCl in the urine collection containers. This amount of acid was determined by sampling urine during the first period and measuring pH to calculate the correct volume of 6*N* HCl needed to keep urinary pH under 3 at all times. Every 24 h, urine volume was measured using a 2,000 mL graduated cylinder. A 1% subsample of acidified urine was collected and stored at 4°C until analyzed.

On d 5 of the collection period, fecal bags and urine funnels were removed and ruminal fluids were collected for 24 h. Ruminal contents were strained through 2 layers of cheesecloth to measure pH of ruminal fluid at 0, 3, 6, 9, 12, 15, and 21 h post-feeding (Metler Toledo FE20; Metler Toledo Inc., Columbus, OH).

Blood was collected via jugular venipuncture at the conclusion of each collection period. Blood samples were collected in BD Vacutainer Trace Element K_2 ETDA 10.8 mg plastic tubes (Catalog Number 368381; Thermo Fisher Scientific; Waltham, MA). Blood was stored at room temperature for 3 h. Following blood collection, rumen contents from all steers (approximately 4 L) were collected and mixed and then redistributed to steers fed the corresponding diets in the following period to reduce potential carryover effects on rumen microorganisms and facilitate diet transition.

A 200-mL sample of water from the water cups in the metabolism barn was collected one time during the experiment to analyze for P in the water and results indicated that the water contained 3.201 μg P/mL.

Laboratory Analysis

After collection, fecal samples were thawed, thoroughly mixed by hand and a 454-g subsample was used for analysis. Feed ingredients, fecal samples, and orts were composited within period and lyophilized (FreeZone, Labconco, Kansas City, MO), and then ground through a Wiley mill (1-mm screen, Arthur H. Thomas, Philadelphia, PA). Ground feed samples were analyzed for NDF and sequential ADF [using method 5 and 6 (Ankom Technology, 2014), respectively; Ankom^{200} Fiber Analyzer, Ankom Technology, Macedon, NY], N (Leco TruMac, LECO Corporation, St. Joseph, MI), fat [method 2 (Ankom Technology, 2014); Ankom Technology], total ash (500°C for 12 h, HotPack Muffle Oven Model: 770750, HotPack Corp., Philadephia, PA), and total P (Miles et al., 2001). The resulting individual values were used to calculate nutrient composition of the diets. Dietary ingredient composites were subjected to perchloric acid digestion and inductively coupled plasma atomic emission spectroscopy analysis of complete minerals at a commercial laboratory (method 975.03: AOAC, 1988; STAR Lab, Wooster, OH); however, P was also analyzed separately using the colorimetric method of Miles et al. (2001) for more precise comparison among samples.

Blood plasma samples were deproteinated following the procedures of Miles et al. (2001). Total P was analyzed using the colorimetric method of Miles et al. (2001) using a spectrophotometer (Syndergy HT, BioTek, Winooski, VT) where absorbance was measured at 660 nm. Results for total P analysis were deemed acceptable at a coefficient of variation (CV) of ≤ 5% within duplicates of feed, feed refusals, blood, urine, and total fecal P. The inter-assay CV for feed, feces, blood, and urine P determination was 5.4%. To ensure minimal P contami-

nation, all glassware was soaked in 10% HNO_3 for 3 d and rinsed 3 times in deionized water prior to use.

Urine was analyzed for N and total P. Apparent DM digestibility was calculated by subtracting the weight of feces (DM basis) from the weight of feed consumed (DM basis) and dividing the resulting value by weight of feed consumed (DM basis). This value was converted to a percent basis by multiplying by 100.

Dietary P was calculated by multiplying the weight of feed offered (DM basis) by the percent P in the diet. Orts were analyzed for P as described above and the weight of orts (DM basis) was multiplied by the P content of the orts to determine P refused. Intake P was calculated by subtracting the P in the orts from the P in the feed offered. Feces were analyzed for P to determine P output.

The water-soluble portion of total P in feces was analyzed using a method adapted from Kleinman et al. (2007). In quadruplicate, representative wet fecal samples containing 0.5 g solids were mixed with distilled water at a 100:1 solution to solids ratio for 60 min while on an orbital shaker (New Brunswick Scientific Classic C2 Platform Shaker, Edison, NJ). Samples were then centrifuged at a speed of 1,500 × *g* and temperature of 4°C for 10 min (Thermo Scientific Sorvall Legend XFR, Waltham, MA) and the supernatant was analyzed for P concentration using the same colorimetric procedure (Miles et al., 2001) as described above. Results for fecal water-soluble P analysis were deemed acceptable at a CV of ≤ 10% within quadruplicates. The inter-assay CV was 4.11% for the water-soluble P results.

Phytic acid content in each of the feed ingredients used in this trial were analyzed by a commercial lab (Enzyme Services and Consultancy; Memphis, TN). In this assay, samples were extracted with HCl to solubilize the attached phosphate groups. They were then treated with a phytase that is specific to phytic acid and its lower myo-inositol phosphate forms. Subsequent phosphatase treatment was done to ensure all phosphate groups were removed from the inositol ring. Then, the total phosphate released was measured colorimetrically given as g of phosphorus per 100 g of sample from which the phytic acid concentration was calculated.

Statistical Analysis

The experimental design was a 6 × 6 Latin square with a 3 × 2 factorial arrangement of treatments. Data were analyzed using the PROC MIXED procedure and correlations were analyzed using the PROC CORR procedure of SAS (SAS Inst. Inc., Cary, NC). Repeated measures were used to analyze ruminal pH using the Toeplitz covariance structure. Individual animal was the experimental unit. Contrast statements were used to test the linear and quadratic effects of phytase inclusion. There were no linear or quadratic effects ($P \geq 0.11$) of phytase inclusion; therefore, only treatment main effects and interactions are reported. Significance was declared at $P \leq 0.05$. Trends were discussed at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

There were no interactions between P concentration and phytase inclusion ($P \geq 0.30$) on any parameters measured. We had hypothesized that feeding steers 0, 500, or 2,000 FTU per kg diet DM when they consumed 0.10% P would linearly increase total P absorption and retention in cattle, whereas feeding steers phytase and a 0.30% P diet would have no effect on P absorption or retention. In other words, we expected cattle fed 0.10% P would have a greater response to released P due to their borderline P deficiency. However, this was not the case and no interactions were in feedlot cattle fed for ad libitum intakes in this metabolism trial. Therefore, the main effects of phytase inclusion and dietary P concentration will be discussed.

There were no main effects ($P \geq 0.40$) of phytase inclusion on any parameter measured. One of the reasons we fed phytase to cattle was because phytase has beneficial effects in pigs. There have been numerous studies in pigs documenting the efficacy of 250 to 1,500 FTU phytase per kg diet DM to increase ADG, G:F, bone strength, P digestibility, and P absorption (Adeola, 1995; Harper et al., 1997; Gentile et al., 2003; Braña et al., 2006; Almeida and Stein, 2012). However, no such attributes were observed in this ruminant study.

The microbial population in the rumen synthesizes phytases (Guyton et al., 2003; Nakashima et al., 2007). Thus, Hankins-Herr et al. (2009) reported no differences in DMI, total fecal output, and DM digestibility when 600 FTU phytase per kg diet DM was added to a corn and corn silage finishing diet fed to beef steers compared with those not fed dietary phytase. In addition, Kincaid et al. (2005) observed no differences in DM digestibility in dairy cows when 427 FTU phytase/kg of diet DM compared with cows fed no phytase in either corn- or barley-based diets. These authors attributed their results to adequate rumen phytase synthesis. However, ruminal phytase may not release all of the P from phytate due to factors such as passage rate (Kincaid et al., 2005), grain type (Tronier et al., 1971; Eeckhout and De Paepe, 1994; Ravindran et al., 1994), processing method (Konishi et al., 1999; Park et al., 1999; Bravo et al., 2000), and dietary concentration of Ca (Sansinena, 1999). Nelson et al. (1971) suggested that providing atypically high doses of phytase (up to 7,600 FTU/kg of feed) in diets for broiler chickens may result in release of 94.4% of phytate-bound P. This concept is known as "superdosing," in swine and poultry diets. The added benefits observed were a 131%

increase in weight gain and a 59% increase in bone ash in chickens that were superdosed with phytase compared with chickens fed no phytase.

In poultry diets, phytase is sometimes included at levels up to 2,500 FTU/kg diet DM (Simons and Versteegh, 1990; Zhang et al., 2000; Walk et al., 2013), with some studies reporting dietary inclusions of up to 7,600 and 12,000 FTU/kg diet DM (Nelson et al., 1971; Shirley and Edwards, 2003). One FTU is defined as the amount of phytase that liberates 1 μmol of inorganic phosphate per minute from 0.0051 M Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al., 1994). In pigs, dietary inclusions in the range of 100 through 2,000 FTU/kg diet DM have been used (Jongbloed et al., 1995; Adeola, 1995; Santos et al., 2014), but also at levels as high as 15,000 FTU/kg diet DM (Harper et al., 1999; Kies et al., 2006). There is no information on superdosing phytase to beef cattle. Therefore, we aimed at exploring the effects of superdosing phytase, at 2,000 FTU/kg of diet DM, in beef cattle. The diets fed in this study were highly processed diets. Specifically, the 0.10% P diet contained largely pure cornstarch and chopped hay, ultimately resulting in a small particle size. As small feed particles are fermented in the rumen, their specific gravity increases and they sink more quickly to the bottom where rumen contents are solubilized in liquid and are more likely to pass through the reticulo-ruminal orifice (Faichney, 1986, 1993; Sutherland, 1987). The small particle size and subsequent rapid passage rate may have precluded the ability of phytase to cleave P from phytate prior to passage (Kincaid et al., 2005). In addition, the Ca:P for the diets were 3.33 and 2.48 for the 0.10% P and the 0.30% P diet, respectively. Phytate can form a chelate with excess Ca molecules in the rumen and not allow phytase access to the phytate to hydrolyze the ester bond between the inositol ring and P (Sansinena, 1999). In scenarios where cattle are fed heavily processed diets and large amounts of Ca, inclusions of phytase greater than the 2,000 FTU fed in the current trial may be warranted.

Despite the lack of effect of phytase, differences in dietary P did affect ($P < 0.01$) DMI, total fecal output, and apparent DM digestibility (Table 2). Steers fed 0.10% P consumed 16% less ($P < 0.01$) feed compared with steers fed 0.30% P. Geisert et al. (2010) reported a quadratic effect on DMI when finishing steers were fed 0.10, 0.17, 0.24, 0.31, and 0.38% P, where steers fed the 0.10% P diet consumed 11% less compared with steers fed 0.31% dietary P (Geisert et al., 2010). Phosphorus is involved in appetite regulation and feed utilization (Underwood, 1981; Berner, 1997). Researchers have observed decreased DMI in cattle fed P-deficient diets from as early as the 1930s (Riddell et al., 1934; Kleiber et al., 1936). Authors have historically attributed this decrease in DMI to decreased "appetite." One author went as far as to calculate the appetite response as ad libitum intake per day divided by the metabolic BW in kg ($BW^{0.75}$; Kleiber et al., 1936). Using this equation for appetite with the data in the current trial there was a 17% decrease in appetite in steers fed 0.10% P compared with those fed 0.30% P.

Despite the reduction in intake, steers fed 0.10% P had increased ($P < 0.01$) total tract apparent DM digestibility compared with steers consuming 0.30% dietary P. This difference is likely attributed to the nature of the diets fed. The objective in formulating the diets for this study was to have a P-deficient and a P-adequate diet. To accomplish this objective and have both diets being "starch-based," the 0.10% P diet was formulated by adding corn grain components without P: purified cornstarch, corn bran, and corn oil. The diets were formulated to have constant NDF and fat, and to differ only in P concentration. Murphy et al. (1994) reported that increased processing of grains increased digestibility when limit-

Table 2. Effects of the interaction between phytase concentration (0, 500, 2000 FTU/kg diet DM) and dietary P (0.10% P or 0.30% P) on steer DM digestibility

	0.10% P[1]			0.30% P				*P*-value[3]		
Item	0[2]	500	2,000	0	500	2,000	SEM	P	E	P × E
n	6	6	6	6	6	5	–	–	–	–
DMI, kg/d	10.64	11.57	9.84	12.34	12.68	13.22	0.82	< 0.01	0.64	0.32
Fecal output, kg/d	2.80	3.08	2.70	4.14	4.08	4.37	0.27	< 0.01	0.91	0.41
Apparent DM Digestibility[4], %	73.90	73.51	72.66	66.77	68.07	66.85	1.11	< 0.01	0.61	0.94
Water intake, L/d	52.29	55.83	62.36	77.38	60.42	52.17	13.52	0.53	0.80	0.38
Urine output, L/d	19.05	15.62	25.40	40.66	22.16	18.06	9.79	0.35	0.45	0.30

[1]Diets were formulated to contain 0.10 and 0.30% P based on book values (NRC, 1996).

[2]Denotes phytase enzyme addition to diet; 0 = 0 FTU phytase/kg diet DM; 500 = 500 FTU phytase/kg diet DM; 2,000 FTU phytase/kg diet DM. Where, 1 FTU is defined as the amount of enzyme that liberates 1 μmol of inorganic phosphate per minute from 0.0051 *M* Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al., 1994).

[3]*P* = the main effect of dietary phosphorus (0.10% or 0.30% diet DM); E = the main effect of phytase enzyme inclusion (0, 500, 2,000 FTU phytase/kg diet DM); P × E = the interaction of dietary P inclusion and concentration of enzyme addition.

[4]Calculated as [(Feed DM Fecal DM)/Feed DM] × 100.

fed to cattle, which may be the reason for the increased digestibility of DM by steers fed the 0.10% P diet.

Feedlot cattle diets contain elevated concentrations of P due to the nature of the feedstuffs used. For example, corn and corn-byproducts contain 0.31% and up to 0.95% P on a DM basis, respectively (NRC, 1996). These feeds are the primary feed ingredients in feedlot cattle diets in the Midwestern region of the U.S. By comparison, grazed forages contain 0.20 to 0.30% P (DM basis; NRC, 1996). Even though feeding elevated P has become common practice as we feed starch-based diets, data have shown that these elevated dietary concentrations may not be necessary to improve cattle growth performance. Erickson et al. (2002) have fed growing cattle P "deficient" diets, according to the NRC (1996), and concluded that less than 0.16% dietary P can be fed with no adverse effects on growth performance. However, there is a dearth of information regarding P metabolism at these lower dietary inclusions.

Similar to NDF and fat, diets were also formulated to be isonitrogenous; however, when analyzed, the 0.10% P diet contained 15% more CP than the 0.30% P diet. This difference in CP is attributed to mixing error at the feed mill of one of the ingredients in the supplement. But both CP concentrations were above the level assumed to decrease intake in feedlot steers, 6 to 8% of the diet DM (NRC, 1987). Even though dietary N concentration differed, there was no difference (P = 0.86) in g/d intake of N (Table 3) nor was N retention affected (P = 0.84). Nitrogen retention was unaffected because steers fed the 0.10% P diet tended (P = 0.10) to absorb more N and excreted more (P = 0.02) N in the urine and less (P < 0.01) N in the feces compared with steers fed 0.30% P. Steers in this study weighed 815 kg, on average, requiring 208 g/d N according to NRC (2016) and N-consumption in the study was, on average, 199.42 g/d. Thus, steers fed 0.10% P and 0.30% in the current study were not N-deficient.

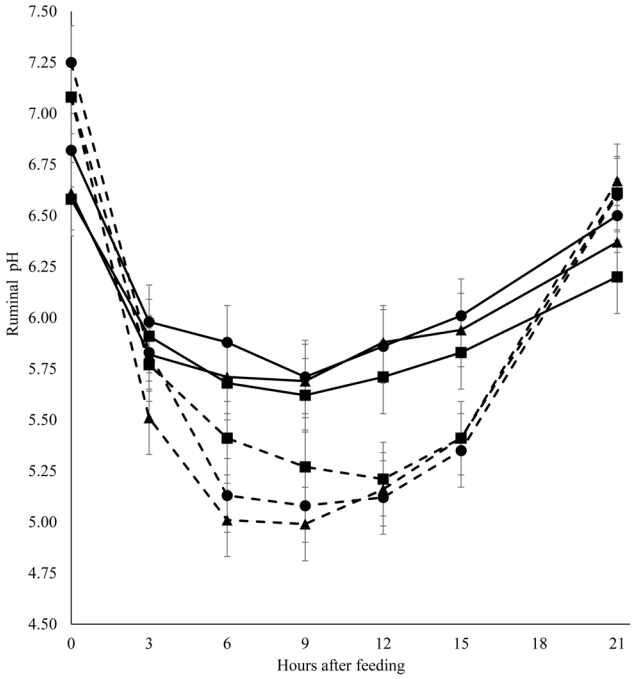

Figure 1. Effects of the interaction of phytase concentration (0, 500, or 2,000 FTU phytase/kg diet DM) and dietary P (0.10% P or 0.30% P) on ruminal pH in steers fed starch-based diets. Steers were fed P at 0.10% diet DM (- - -) or P at 0.30% diet DM (——) with varying concentrations of phytase: 0 FTU phytase/kg diet DM (●), 500 FTU phytase/kg diet DM (■), 2,000 FTU phytase/kg diet DM (▲). There was a P × h interaction (P < 0.01) and a main effect of dietary P concentration (P = 0.01) for ruminal pH. There were no other treatment effects ($P \geq 0.48$) on ruminal pH. Standard error bars depict the variation associated with the interaction of P × E × h (SEM = 0.1798).

Steers fed the 0.10% P diet had a more rapid decrease (P = 0.01) in rumen pH (Fig. 1) by 6 h post-

Table 3. Effects of the interaction of phytase concentration (0, 500, 2000 FTU phytase/kg diet DM) and dietary P (0.10% P or 0.30% P) on N metabolism in steers fed starch-based diets

	0.10% P[1]			0.30% P				P-value[3]		
Item	0[2]	500	2,000	0	500	2,000	SEM	P	E	P × E
Intake, g N/d	197.31	214.77	183.15	193.53	198.46	209.28	15.47	0.86	0.68	0.33
Fecal output, g N/d	67.27	71.25	62.33	79.61	79.79	85.30	6.59	< 0.01	0.93	0.49
Urinary output, g N/d	104.12	107.37	97.27	86.39	88.72	92.18	7.22	0.02	0.86	0.54
Retention[4], g N/d	25.92	36.15	23.56	27.53	29.95	31.80	7.83	0.84	0.63	0.61
Retention, % of intake	11.32	15.56	9.80	14.09	15.05	15.59	3.70	0.34	0.67	0.66
Absorbed[5], g N/d	130.04	143.52	120.83	113.92	118.67	123.98	9.87	0.10	0.52	0.31
Retention, % of absorbed	16.99	22.99	14.89	23.79	24.68	25.53	5.63	0.14	0.73	0.69

[1]Diets were formulated to contain 0.10 and 0.30% P based on book values (NRC, 1996).

[2]Denotes phytase enzyme addition to diet; 0 = 0 FTU phytase/kg diet DM; 500 = 500 FTU phytase/kg diet DM; 2000 FTU phytase/kg diet DM. Where, 1 FTU is defined as the amount of enzyme that liberates 1 mmol of inorganic phosphate per minute from 0.0051 *M* Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al., 1994).

[3]P = the main effect of dietary phosphorus (0.10% or 0.30% diet DM); E = the main effect of phytase enzyme inclusion (0, 500, 2000 FTU phytase/kg diet DM); P × E = the interaction of dietary P inclusion and concentration of enzyme addition.

[4]Calculated as Intake N (g/d) – Fecal N (g/d) – Urinary N (g/d).

[5]Calculated as Intake N (g/d) – Fecal N (g/d).

feeding than steers fed 0.30% P, which is likely due to the rapid fermentation of the corn starch in the rumen. This decrease in pH at 6 h post-feeding in steers fed the 0.10% P diet was below a pH of 5.5 and remained less than 5.5 until 15 h post-feeding. In contrast, ruminal pH of steers fed 0.30% P diet throughout all time points measured was 5.6 or greater. A reduction in ruminal pH from 7 to 4.9 reduces protein degradation in grain-based diets (Cardozo et al., 2000; Cardozo et al., 2002). Despite the fact that ruminal pH in steers fed 0.10% P dropped to the point at which protein degradation is decreased, steers on that diet were able to absorb more N compared with steers on the 0.30% P diet.

Steers fed 0.10% P consumed less ($P < 0.01$) P and excreted less P in feces ($P < 0.01$) and urine ($P < 0.01$) than steers fed 0.30% P (Table 4). In steers consuming the 0.10% P diet, excretion of P was greater than intake of P. Therefore, both retention of P ($P < 0.01$) and absorption of P ($P < 0.01$) were negative. Steers fed 0.30% P excreted 51.9% more fecal P/d compared to steers fed 0.10% P. A portion of the fecal losses would be endogenous P losses regardless of dietary P concentration. Braithwaite (1984) demonstrated in lambs fed varying levels of P that fecal endogenous losses of P are increased when dietary P increases regardless of whether or not the requirement is being met. Additionally, fecal endogenous losses from cattle fed a P-free diet were equal to 10 mg/kg BW per day as demonstrated by the Agricultural Research Council (1980). Although fecal endogenous losses of P were not measured in our study, steers fed 0.10% P in the current trial would have an estimated 8 g of fecal endogenous losses each day (815 kg BW with 10 mg P excretion per kg of BW) and measured total fecal P losses averaged 11.19 g/d. In addition, Pfeffer et al. (2005) estimated that about 66 to 75% of fecal P in ruminants is of endogenous origin. Thus, steers fed 0.10% P would have been close to the published estimations, with an estimated 71% of fecal P being as endogenous losses. However, steers fed 0.30% P using the same 8 g estimate for endogenous losses, would have had just 35% fecal P as endogenous losses. The discrepancy between the 2 may be due to P requirements. Geisert et al. (2010) cited that steers continued to gain and grow well when fed only 0.17% dietary P. These authors suggested that 0.17% P was adequate for steers and any P above that may be fed in excess. Excess nutrients that are not used by the animal are simply excreted. Thus, the increased P excretion in steers consuming 0.30% may be due to the decreased need for P. Typically, an increase in P intake leads to an increase in endogenous losses of P in both lambs (Preston and Pfander, 1964; Braithwaite, 1984; 1985) and steers (Challa et al., 1989; Coates and Ternouth, 1992; Bortolussi et al., 1996). The difference between P-deficient and adequate diets also corresponded to changes in blood P.

There was no effect of dietary phytase concentration on blood P ($P \geq 0.45$) or urinary P ($P \geq 0.27$), but, steers fed 0.30% P had 46.2% greater ($P < 0.01$; Fig. 2) circulating concentration of plasma P and 94.6% greater ($P < 0.01$; Table 4) urinary excretion of P than steers fed 0.10% P. Blood P concentrations were correlated ($r = 0.60$; $P < 0.01$; data not shown) to urinary

Table 4. Effects of the interaction of phytase concentration (0, 500, or 2000 FTU phytase/kg diet DM) and dietary P (0.10% P or 0.30% P) on P metabolism in steers fed starch-based diets

	0.10% P[1]			0.30% P				*P*-value[3]		
Item	0[2]	500	2,000	0	500	2,000	SEM	P	E	P × E
Intake[4], g P/d	9.82	10.69	9.18	33.45	33.53	34.99	1.57	< 0.01	0.93	0.60
Fecal output, g P/d	11.90	11.62	10.05	22.02	25.02	22.71	2.01	< 0.01	0.57	0.64
Fecal water-soluble P										
g water-soluble P/d	8.80	8.66	7.64	12.74	13.35	13.13	1.88	< 0.01	0.94	0.91
% of total fecal P	74.88	77.67	73.85	56.26	58.27	60.52	8.85	0.02	0.95	0.92
Urinary output, g P/d	0.32	0.20	0.29	5.85	3.32	6.22	1.48	< 0.01	0.49	0.54
Retention[5], g P/d	-2.41	-1.12	-1.15	5.58	7.17	5.97	1.14	< 0.01	0.40	0.85
Retention, % of intake	-26.94	-12.32	-18.07	16.66	23.01	17.52	8.08	< 0.01	0.98	0.82
Absorbed[6], g P/d	-2.08	-0.93	-0.87	11.43	10.54	12.19	2.17	< 0.01	0.87	0.87

[1]Diets were formulated to contain 0.10 and 0.30% P based on book values (NRC, 1996).

[2]Denotes phytase enzyme addition to diet; 0 = 0 FTU phytase/kg diet DM; 500 = 500 FTU phytase/kg diet DM; 2,000 FTU phytase/kg diet DM. Where, 1 FTU is defined as the amount of enzyme that liberates 1 mmol of inorganic phosphate per minute from 0.0051 *M* Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al., 1994).

[3]*P* = the main effect of dietary phosphorus (0.10% or 0.30% diet DM); E = the main effect of phytase enzyme inclusion (0, 500, 2,000 FTU phytase/kg diet DM); P × E = the interaction of dietary P inclusion and concentration of enzyme addition.

[4]Intake includes feed P and water P (3.03 mg/mg).

[5]Calculated as Intake P (g/d) – Fecal P (g/d) – Urinary P (g/d).

[6]Calculated as Intake P (g/d) – Fecal P (g/d).

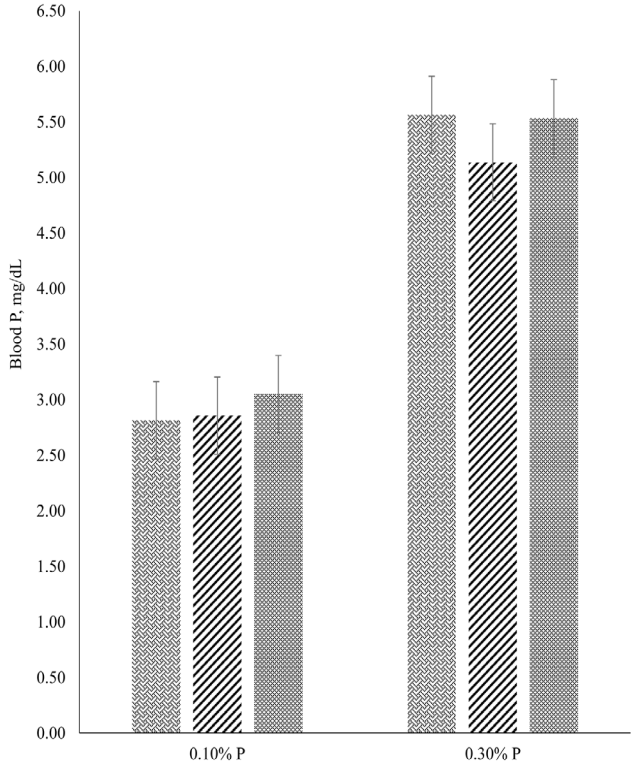

Figure 2. Effects of the interaction of phytase concentration (0, 500, or 2,000 FTU phytase/kg diet DM) and dietary P (0.10% P or 0.30% P) on blood P concentrations in steer fed starch-based diets. Steers were fed P at 0.10% diet DM or P at 0.30% diet DM with varying concentrations of phytase: 0 FTU phytase/kg diet DM (▨), 500 FTU phytase/kg diet DM (▨), 2000 FTU phytase/kg diet DM (▨) on a DM basis. There were no interactions of P × phytase inclusion ($P = 0.76$) nor main effects of phytase inclusion ($P \geq 0.45$) on blood P concentrations. Steers fed dietary concentrations of 0.30% P had greater ($P < 0.01$) blood P concentrations than steers fed dietary concentrations of 0.10% P. Standard error bars depict the variation associated with the interaction of P × E (SEM = 0.3478).

P concentrations when dietary P was 0.10%. However, when dietary P was 0.30%, there was no correlation ($r = 0.36$; $P = 0.16$). Cattle conserve P by reducing relative urinary P excretion to maintain blood P (McDowell, 2003); however, when P is adequate, cattle will excrete excess P in their urine. Thus, while plasma concentrations are controlled through multiple homeostatic mechanisms, urinary P fluctuates more with changes in dietary P concentration. Furthermore, plasma P concentrations of less than or equal to 4.50 mg/dL in cattle are indicative of deficiency (NRC, 1996). Steers fed the 0.10% P diet had an average plasma P level of 2.87 mg/dL and, based on the urinary correlations and plasma P, were P-deficient. When steers consumed 0.30% P diets, blood P concentrations exceeded 5 mg/dL, indicating they were P-adequate. Bortolussi et al. (1996) reported that when steers were fed a diet containing 0.04% P, blood plasma P concentrations were 3.50 mg/dL. Most compelling, was that due to the Latin square design, steers used throughout the entirety of this study, were adapted to a new diet every 20 d, thus blood P concentration changed within 20 d of dietary P concentration changes, despite the mature size of the steers used in the current study. This observation indicates that blood can be an accurate early indicator of P deficiency. In fact, when sheep consumed either 1.94, 4.01, or 6.03 g/d P, there was a linear increase in plasma P concentrations as dietary P intake increased, observed after 9 d (Scott et al., 1985). Also, steers consuming as little as 5.80 g/d P had plasma P concentrations of 3.12 mg/dL compared with steers consuming 27.2 g/d P, which had plasma P concentrations of 7.57 mg/dL after 14 d (Bortolussi et al., 1996).

Based on the NRC (2016) prediction model, the maintenance requirement for P in a steer weighing 815 kg is 17.42 g/d. The steers on the 0.10% P diet consumed, on average, 9.90 g P/d whereas steers on the 0.30% P diet consumed 33.99 g P/d. Thus, steers on the 0.10% P diet were not getting enough dietary P to meet their maintenance requirement, ultimately resulting in negative values for absorbed and retained P. Ruminants have a greater renal threshold for P than nonruminants (McDowell, 2003) because of their ability to store P in the kidney for times of greater need, which represents an evolutionary advantage for grazing ruminants. In situations when ruminants are required to graze poor-quality forages intake is the limiting factor at getting enough P to meet their requirement. Renal storage of P can supply blood P in times of need. Even though steers fed 0.10% P had negative P retention they excreted P in the urine, indicating they were mobilizing small amounts from body reserves of P, either from the bone or kidney. Conversely, steers on the 0.30% P diet most likely were not mobilizing from their body reserves to meet their requirement for P. They had both positive absorption and retention values, and retained on average about 60% of what was absorbed.

One justification for studying P absorption and retention in beef steers was an attempt to reduce P-causing pollution from beef steers, thus water-soluble P concentrations were measured in the feces. Water-soluble P concentration in the feces was greater ($P < 0.01$) in steers fed 0.30% P (Table 4). However, the proportion of total fecal P that was excreted as water-soluble P was increased by 23.0% in cattle fed 0.10% P compared to steers fed 0.30% P, regardless of phytase inclusion level. Not only did steers fed 0.30% P consume more ($P < 0.01$) total P but they consumed 100% more phytate-P as a proportion of total dietary P than cattle fed 0.10% P. Intake of P bound to phytate made up 32.7% and 67.7% of total P intake in the 0.10% and 0.30% P diets with no supplemental phytase, respectively (Fig. 3), potentially explaining the differences in water-soluble P.

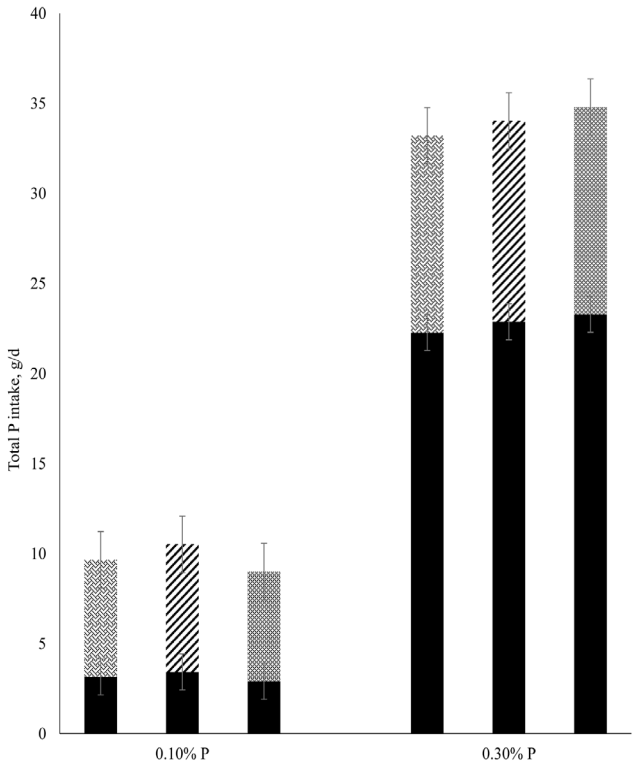

Figure 3. Effects of the interaction of phytase concentration (0, 500, or 2,000 FTU phytase/kg diet DM)and dietary P (0.10% P or 0.30% P) on the proportion of intake phytic acid-bound P in total P consumed by steers fed starch-based diets.: Steers were fed P at 0.10% diet DM or P at 0.30% diet DM with varying concentrations of phytase: 0 FTU phytase/kg diet DM (▒), 500 FTU phytase/kg diet DM (▨), 2,000 FTU phytase/kg diet DM (▓) on a DM basis with ■ denoting the proportion of phytic acid-bound P intake of total P intake within their respective treatments. There were no effects of phytase inclusion ($P \geq 0.57$) or the interaction of P × phytase inclusion ($P = 0.68$) on total P intake. Steers fed dietary concentrations of 0.30% P had greater ($P < 0.01$) total P intake compared to steers fed dietary concentrations of 0.10% P. There were also no effects of phytase inclusion ($P \geq 0.60$) or the interaction of P × phytase inclusion ($P = 0.79$) on phytic-acid bound P intake. Steers fed dietary concentrations of 0.30% P had greater ($P < 0.01$) phytic acid-bound P intake compared to steers fed dietary concentrations of 0.10% P. Standard error bars depict the variation associated with the interaction of P × E (SEM = 1.57 and 1.00, for total P intake and intake of phytic acid-bound P, respectively).

The concentration of water-soluble P in cattle manure has implications in nutrient management if that manure is going to be used as fertilizer. Water-soluble P concentration in manure is positively correlated to dissolved-reactive P on the soil surface and is a readily available source of solubilized P that is responsible for the eutrophication of fresh-water bodies (Pote et al., 1999). Besides concentration of water-soluble P, total P and N in feces used as fertilizer are also of concern in nutrient management. Commonly planted corn hybrids in Illinois uptake a N:P ratio of about 5.8 (Bender et al., 2013). Uncomposted beef feedlot manure provides an N:P ratio of 2.6 (Eghball et al., 1997), thus contains excess P compared with what the crop actually needs. Because of this, manure application to corn with beef feedlot manure to meet the requirements for N would increase soil levels of P beyond what is required. In the current trial, the N:P ratio of manure was 6.76 and 2.30 from steers fed 0.10% P and 0.30% P, respectively.

There were no interactions between phytase and P concentration in steers and there were no main effects of phytase. However, cattle consuming only 0.10% P were in negative P balance, which impacted P absorption and retention. Blood P values responded rapidly to the P deficient diets, confirming that blood can be an adequate indicator of P in cattle within 20 d of deficiency.

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