Relationship between phosphorus intake and blood or fecal phosphorus in gestating cows

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Abstract

The relationship between fecal, serum or plasma phosphorus (P), and P intake was examined with 10, crossbred, 5-year-old, gestating cows (avg wt 475 kg) in an individual feeding study using 2 orthogonal 5 × 5 Latin squares. All cows received 9.07 kg of meadow grass hay which contained 7.4% crude protein (CP) with an in vitro dry matter digestibility (IVDMD) of 56%, and received .5 kg of 1 of 5 supplements which resulted in P intakes of 10.3, 12.4, 14.3, 16.1, or 18.4 g/day. Fecal and blood samples were collected for 5 days after a 14-day dietary adjustment period. Fecal grab samples were taken twice daily (hour 0800 and 2000). Blood samples were taken at 0800. Statistical analysis included analysis of variance and regression analysis. A linear response to P intake was observed for both plasma and serum P; however, with regression of P intake, the R^2 for plasma P was .06 and for serum P was .10. Evaluation of morning and evening fecal P levels with regression resulted in different equations. The morning equation (Y = .055 + .212X) had a larger intercept and a smaller slope coefficient than the evening equation (Y = -.781 + .310X). Morning and evening R^2 were .69 and .78, respectively. To examine the predictive ability of the P intake equations, a validation trial was conducted with 20 4-to-8-year-old cows individually fed (4/treatment). Daily P intakes were 10.0, 12.4, 15.3, 20.4, and 22.6 g. Management and sampling procedures were the same as used in the previous trial except blood samples were not collected. There was no difference ($P>.05$) in the equations established with regression from the morning and evening samples. The combined regression equation was (Y = .306 + .219X). This equation was not different ($P>.05$) from the equations established from either the morning or evening samples in the previous trial. These data indicate that fecal P is related to P intake; however, the extent that this relationship is influenced by the availability of dietary P may limit the usefulness of this association.

Key Words: phosphorus, feces, serum, plasma, gestating cows

Attempts to determine the phosphorus (P) status of grazing cattle have been made by many researchers (Black et al. 1943, Cohen 1972, Call et al. 1978, Little 1980, Judkins et al. 1985). Results of these studies have varied, and P requirements are still questioned (Little 1980, Call et al. 1986). One of the difficulties in studying P requirements of grazing animals is determining P intake (Cohen 1987).

Esophageal fistulated animals cannot be used to determine P content of the diet because of contamination by endogenous P from saliva (Hoehne et al. 1967, Little 1972, Mayland and Lesperance 1977), although correction for the endogenous P is possible using P^{32} (Little et al. 1977). Another approach to estimating P intake is the use of fecal P. Moir (1960) reported fecal P levels were related to P concentration in available forage. Cohen (1974) and Holechek et al. (1985) have developed equations that predict P intake of steers from the level of P excreted daily through the feces. These equations require the collection of total feces.

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The objectives of this study were to examine the relationship of blood and fecal P concentrations to P intake of gestating cows and to develop and verify equations that showed promise for predicting intake of P by the grazing animal. Fecal and blood calcium (Ca) levels were also examined to see if they were related to P intake.

Materials and Methods

Trial 1.

Ten crossbred, 5-year-old, gestating cows (avg wt 475 kg) were used to evaluate the use of fecal P or plasma or serum inorganic P level to estimate dietary P. The experimental design consisted of 2 orthogonal 5 × 5 Latin squares. Animals were randomly assigned to a square and a treatment sequence within each square. Cows were individually confined in partially covered pens with concrete floors and had ad libitum access to water. Each cow received 9.07 kg of meadow hay (7.4% CP; 56% IVDMD) and .5 kg of 1 of 5 supplements formulated to vary in P content (Table 1). Forage was a medium-quality meadow hay, consisting of both warm- and cool-season grasses, harvested in June, 1987, from a sub-irrigated meadow at the Gudmundsen Sandhills Laboratory at Whitman, Nebraska. Primary warm-season grasses were big bluestem (*Andropogon gerardii* Vitman), prairie sandreed (*Calamovifa longifolia* (Hook.) Scribn.), and switchgrass (*Panicum virgatum* L.). Cool-season grasses consisted of kentucky bluegrass (*Poa pratensis* L.), smooth bromegrass (*Bromus inermis* Leysis.), redtop (*Agrostis alba* L.), timothy (*Phleum pratense* L.), and various wheatgrasses (*Agropyron* spp.). Supplements were fed at 0600 each morning followed immediately by hay feeding. All supplements were consumed within 10 min of feeding and hay was consumed by each cow within the 24-hour period.

Table 1. Formulation of supplements used to vary phosphorus intake of cows.

Item, %	Supplement				
	One	Two	Three	Four	Five
Dry molasses	59.3	58.1	56.7	55.2	52.8
Corn	35.2	34.1	33.2	32.4	32.6
Corn oil	3.0	3.0	3.0	3.0	3.0
Ammonium phosphate		2.9	5.9	8.8	11.6
Urea	2.5	1.9	1.2	.6	

Each test period was 19 days. The first 14 days were used for dietary adjustment. Fecal and blood samples were collected during the ensuring 5 days. Supplements were sampled daily from day 13 through 17 and combined across day for each supplement. To insure representative P values for hay during day 13 through 17, samples were collected for each cow by offering an extra 500 g of hay then removing 500 g from the total amount fed after placement in the bunk. Hay samples were combined across days for each cow in each test period.

During sampling periods, cows were moved to a restraining chute at 0800 for blood and fecal collections. Sampling was within

squares with the square sampled first determined at random each day. A rectal fecal sample was collected and 2 blood samples (for serum and plasma preparation) were taken from the jugular vein of each cow. After all cows in a square were sampled, they were returned to their respective pens before animals in the other square were moved to the restraining area. Pens were cleansed at 1700, and a fecal sample was collected from the floor of each animal's pen at 2000. Care was taken to prevent contamination of the sample. Because of 2 different sampling procedures for feces, method of sampling was confounded with time of day in the analysis.

Trial 2

Data from 20, 4-to-8-year-old cows were used to validate equations developed in Trial 1. Four cows were randomly assigned to each of the 5 P supplements used in Trial 1. The hay was similar to that used in Trial 1. Two 19-day test periods with 10 different cows per period were conducted. Management and sampling procedures were similar to those described for Trial 1, except blood samples were not collected and morning fecal samples were collected from the floor of each pen at 0900 after cleaning at 0630. Fecal samples were taken in the evening as described in Trial 1.

Sample Preparation and Analysis

Blood for serum preparation was collected in an evacuated, sterile, integrated serum separator tube. An evacuated tube coated with sodium heparin was used to collect blood for plasma preparation. Tubes containing heparin were centrifuged for 15 min at 2000 × g immediately after sampling each replicate. Plasma was decanted and mixed with an equal part of 16% trichloric acid (TCA) to precipitate the protein fraction, decreasing the possibility of contamination of sample with organic P. The plasma-TCA mixture was shaken vigorously and centrifuged for 10 min at 2000 × g. The supernatant was decanted and frozen at -20° C until analyzed for P and Ca. Samples collected for serum preparation were allowed to clot at room temperature for 20 min after the sampling of each replicate. From this point, sample preparation was the same as that described for plasma samples.

Fecal samples were composited across days within treatment and a representative portion was dried at 60° C for 72 hours, allowed to air equilibrate for 24 hours, ground through a Wiley mill equipped with a 2-mm screen and then through a Udy cyclone mill equipped with a 1-mm screen. Samples were stored in airtight containers until laboratory analysis. Hay and supplements were dried at 60° C for 48 hours and processed using similar procedures. A representative portion of hay and supplement sample was also dried at 100° C for 48 hours for determination of dry matter. Dry matter of all samples and crude protein of hay and supplement samples were determined using procedures outlined by AOAC (1984). In vitro dry matter digestion was determined on hay samples (Tilley and Terry 1963). Hay, supplement, and fecal samples were prepared for P and Ca analysis by first ashing for 8 hours at 600° C. The ash was boiled in 25% hydrochloric acid for 5 min. This solution was cooled to room temperature, filtered through Whatman no. 4 filter paper, diluted to 50 ml volume, and analyzed for Ca and P. Phosphorus was determined using the molybdovanadate colormetric procedure (AOAC 1984) employing a Rapid Flow Analyzer[a]. Calcium analysis used the procedures outlined by Gitelman (1967) as modified by Moorehead and Briggs (1974) also using the auto analyzer. Denatured plasma and serum samples were thawed at room temperature and analyzed using methods identical to those used for prepared solutions of feed and feces.

Statistical Analysis

For Trial 1, data from the 2 orthogonal Latin squares were analyzed as a cross-over design with cow, period, and P intake as whole plot treatments and with 'time' as a split plot treatment (Cochran and Cox 1957). For the blood data, 'time' represented sampling day (5 days) while 'time' for the fecal samples represented time of day (AM or PM collection). For Trial 2, fecal data were analyzed as a completely randomized design with a split plot treatment arrangement. Period and P intake were the whole plot treatments and time of day (AM and PM) was the split plot treatment. For both trials, orthogonal polynomials were used to determine if linear and quadratic responses to P intake differed by time.

To obtain prediction equations for P intake, regression analysis was used to estimate the response of blood and fecal P to P intake. These regression models included P intake as the only independent variable. Cow, period, and time effects were excluded from the model since it would not be possible to specify values for these variables in most practical applications. Inverse regression (Draper and Smith 1981) was then used to construct inverse confidence intervals for P intake to estimate the precision of the prediction of P intake from blood or fecal P.

Results

Trial 1

Mean P intakes ranged from 10.3 to 18.4 $g \cdot hd^{-1} \cdot d^{-1}$ (Table 2). Sampling day by treatment interaction wsa not significant ($P>.05$) for serum or plasma P. A linear response ($P<.001$) was observed for both plasma and serum P due to P intake (Table 2); however, numerically there was little difference between Treatments 2, 3, and 4. Regression coefficients for plasma and serum P on P intake are given in Table 3. The cross-over design model (denoted

[a]Alpkem Corp., Box 1260, Clackamas, Ore. 97015.

Table 2. Effects of increasing levels of phosphorus (P) intake on blood and fecal phosphorus levels in gestating cows.

	Treatment						
	One	Two	Three	Four	Five	SE[a]	P[b]
				Trial 1			
P intake, g/d	10.26	12.39	14.33	16.06	18.43		
Fecal P, g/kg							
AM	2.19	2.58	2.99	3.45	4.22	.06	<.001
PM	2.32	3.01	3.54	4.24	5.13	.06	<.001
Serum P, g/dl	2.36	2.66	2.68	2.68	2.86	.10	<.001
Plasma P, g/dl	2.86	3.12	3.16	3.20	3.30	.13	<.001
				Trial 2			
P intake g/d	10.05	12.42	15.32	20.41	22.58		
Fecal P, g/kg							
AM	2.31	2.88	3.07	4.08	5.17	.68	<.001
PM	2.83	3.44	3.63	4.91	6.07	.68	<.001

Table 3. Equations developed from phosphorus intake for predicting fecal and blood phosphorus levels of gestating cows.

Y[c]	n	b_0	b_1	Sxy	ANOVA Model[a] R^2	Regression Model[b] R^2
			Trial 1			
Plasma	250	2.286	.059	138.4	.722	.044
Serum	250	1.782	.061	142.8	.507	.108
Feces						
AM	50	.055	.212	99.6		
PM	50	-.781	.310	145.6		
Total	100	-.363	.261	245.2	.969	.664
			Trial 2			
Feces						
AM	20	.310	.198	90.0		
PM	20	.302	.240	109.3		
Total	40	.306	.219	199.3	.950	.503

[a]R^2 calculated using P intake, period, cow and hour as classification variables.
[b]R^2 calculated using P intake as the only independent variable.
[c]Y = dependent variable, n = number of observations, b_0 = intercept, b_1 = slope, Sxy = corrected sum of products and R^2 = model SS/total SS.

ANOVA Model in Table 3) accounted for 72% of the variation of plasma P, while the regression model with P intake as the only independent variable (denoted Regression Model in Table 3) accounted for only 4% of the variation of plasma P. Such a small portion of the variation of plasma P attributed to variation in P intake indicated that P intake could not be precisely predicted from plasma P alone. Examination of the models for serum P indicated similar results (Table 3).

The regression coefficients of plasma P and serum P on P intake were used to construct inverse 95% confidence intervals for the prediction of P intake: [plasma P: (14.29 ± 23.04), based on a plasma P value of 3.13; serum P: (14.30 ± 22.67), based on a serum P value of 2.68]. Such broad confidence intervals indicated that P intake could not be precisely predicted using either plasma P or serum P.

Because of the treatment by time of sampling interaction ($P<.05$), fecal means are presented by time collected (Table 2). Both AM and PM fecal P levels increased ($P<.001$) linearly as level of P in the diet increased. Phosphorus levels in AM fecal samples increased at a lower rate ($P<.05$) than P in PM fecal samples as indicated by the slopes of the regression lines (Table 3).

A regression equation on fecal P or P intake was developed ignoring the effects of cow, period, and time of day, even though some of these effects were significant. Exclusion of these effects would increase variability of the equation and decrease the precision of the prediction; however in practical situations, the effect of a specific cow or period is usually not known and it may not be feasible to collect samples at a specific time of day. Also, the effect of any deviation of the feeding regime on the change in fecal P is not obvious.

The R^2 values from the cross-over design model (ANOVA Model in Table 3) and the regression model of fecal P on P intake (Regression Model in Table 3) were .969 and .664, respectively. These R^2 values indicated that P intake alone explained a major portion of the variation of fecal P, suggesting that fecal P might be useful for predicting P intake.

The regression coefficients of fecal P on P intake, combined over AM and PM samples, were used to compute an inverse 95% confidence interval for the prediction of P intake based on a fecal P value of 4.0: [16.7 ± 4.4 (Fig. 1)]. As with plasma and serum P, the inverse confidence interval showed that P intake could not be precisely predicted using individual observations of fecal P.

There was no difference in Ca intake among the treatments. However, since Ca intake remained the same and P intake varied,

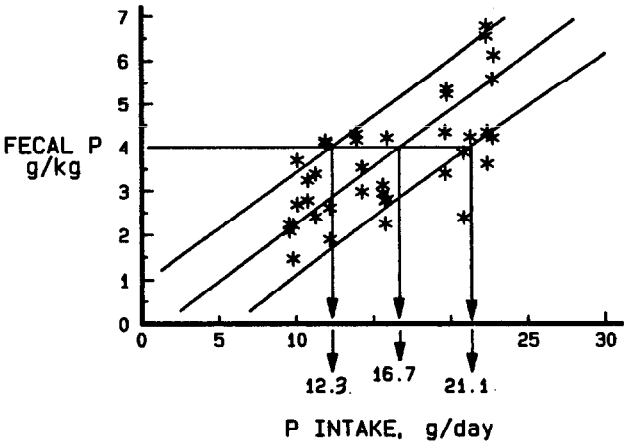

Fig. 1. Regression line (- - -) and confidence intervals (- - -) of Fecal P on P intake from Trial 1 and observations of Fecal P concentrations (*) from Trial 2.

the Ca:P ratio was different. The ratio varied from a low of 1.77 to a high of 3.23. There was no effect of P intake on fecal, serum, or plasma Ca.

Trial 2

Phosphorus intakes in Trial 2 covered a wider range than those in Trial 1 (Table 2). Mean intakes in Trial 2 ranged from 10.05 to 22.58 g • hd^{-1} • d^{-1}. A treatment by time interaction was not present ($P = .949$); however, data are presented by hour of sampling (Table 2) for ease of comparison with Trial 1. Similar to Trial 1, a linear response ($P<.001$) was observed for fecal P on intake. In addition, P level of the PM fecal sample was higher ($P<.02$) than the P level of the AM sample. Comparison of slopes (Table 3) between Trial 1 and Trial 2 indicated that there was no difference ($P>.05$) between the slopes of the overall line in Trial 1 and Trial 2. There was also no difference ($P>.05$) between the Trial 1 and Trial 2 slopes within sampling time. The R^2 was lower and the variation was higher in Trial 2 than in Trial 1. Therefore, confidence intervals were wider. Calcium values were not determined in Trial 2 since there was no effect of P intake on Ca in Trial 1.

Discussion and Conclusions

In this study, blood data from Trial 1 indicated that neither serum nor plasma P concentration predicts P intake with reasonable precision or accuracy. The differences between fecal P levels taken in the morning and the evening were anticipated. Cohen (1974) reported differences among fecal P levels when samples were taken at 0800, 1200, and 1600 in one study and differences between sampling at 0800 and 1600 in another, although the slopes of the regressions were similar. In the present study, we observed a different slope in the morning than the afternoon. Likewise, in Trial 2, we observed higher levels of fecal P in the PM than in the AM.

The regression equations for morning and evening fecal P are different ($P<.05$) than the equations reported by Cohen (1974) or Holechek et al. (1985). Re-evaluation of reported means from Holechek et al. (1985) yields a regression equation with a slope of .157 and an intercept of 2.509. Similar re-evaluation of Cohen's (1974) means gave a slope of .104 and an intercept of 1.548. The slopes we calculated from both studies were considerably lower than the slope we observed. The intercepts of the regression also differ significantly. In our study, we used mature gestating cows whereas in the studies by Cohen (1974) and Holechek et al. (1985), steers were used. Cohen (1974) suggested that when a large variation in the quality of dietary phosphorus exists, it may not be possible to estimate phosphorus intake from fecal P level and may require separate regression equations for each P source or feed

supply may be required.

Plotting the observation points from Trial 2 with the confidence intervals from Trial 1 (Fig. 1) shows that most data points fall within the bounds. However, the inverse confidence intervals indicated that the accuracy of the prediction would be questionable. Calculation of confidence intervals for means instead of observations could increase our accuracy greatly and the use of an equation to predict mean P intake of a group of animals may be more reliable (Holechek et al. 1985).

Fecal P is related to P intake. This relationship may be influenced by the availability of the P in the diet, however. The equation developed in this study from fecal P concentration may predict the P intake of gestating cows on a Sandhills meadow hay similar to that used in this study. However, verification in a production situation is needed.

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