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 (Calamovilfa longifolia (Hook.) Scribn.), and switchgrass (Panicum virgatum L.). Cool-season grasses consisted of Kentucky bluegrass (Poa pratensis L.), smooth bromegrass (Bromus inermis Leyss.), reedtop (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.

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 firstashing 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 colorometric procedure (AOAC 1984) employing a Rapid Flow Analyzer (AOAC 1984). 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 · d⁻¹ · d⁻¹ (Table 2). Sampling day by treatment interaction was 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

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>One</th>
<th>Two</th>
<th>Three</th>
<th>Four</th>
<th>Five</th>
<th>SE*</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P intake, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fecal P, g/kg</td>
<td>10.26</td>
<td>12.39</td>
<td>14.33</td>
<td>16.06</td>
<td>18.43</td>
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<td></td>
</tr>
<tr>
<td>AM</td>
<td>2.19</td>
<td>2.58</td>
<td>2.99</td>
<td>3.45</td>
<td>4.22</td>
<td>.06</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PM</td>
<td>2.32</td>
<td>3.01</td>
<td>3.54</td>
<td>4.24</td>
<td>5.13</td>
<td>.06</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum P, g/dl</td>
<td>2.36</td>
<td>2.66</td>
<td>2.68</td>
<td>2.88</td>
<td>2.86</td>
<td>.10</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Plasma P, g/dl</td>
<td>2.86</td>
<td>3.12</td>
<td>3.16</td>
<td>3.20</td>
<td>3.30</td>
<td>.13</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>P intake g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fecal P, g/kg</td>
<td>10.05</td>
<td>12.42</td>
<td>15.32</td>
<td>20.41</td>
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</tr>
<tr>
<td>AM</td>
<td>2.31</td>
<td>2.88</td>
<td>3.07</td>
<td>4.08</td>
<td>5.17</td>
<td>.68</td>
<td>&lt;.001</td>
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<tr>
<td>PM</td>
<td>2.83</td>
<td>3.44</td>
<td>3.63</td>
<td>4.91</td>
<td>6.07</td>
<td>.68</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

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ignoring the effects of cow, period, and time of day, even though 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.

These R² values indicated that P intake alone explained a major portion of the variation of plasma P attributed to variation in P intake from Trial 1 and observations of Fecal P concentrations (*) from Trial 2.

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.

Literature Cited


