

T2, and T3 and decreased linearly with increasing DMI. Therefore, an equation was developed by regression to predict inevitable fecal P excretion across a range of DMI: $(\text{g/d}) = [0.85 \pm 0.070 (\text{g/d})] \times \text{DMI (kg/d)} + [5.30 \pm 1.224 (\text{g/d})]$; ($R^2 = 0.90$; $P < 0.01$). This equation can be used to estimate the inevitable fecal P component of the total P requirement for maintenance of lactating cows. Because P balances were not different from zero, inevitable fecal P excretion was the fecal P portion of the total P maintenance requirement. Therefore, the P maintenance requirement (g/kg DMI) was calculated.

INTRODUCTION

Accurately estimating the total dietary P requirement of dairy cattle is important for optimal performance and to decrease excess manure P entering the environment from dairy farms. The factorial method has been used to estimate P requirements (NRC, 2001; ARC, 1980). The total dietary P requirement is the sum of the absorbed P requirements for maintenance, milk production, growth, and pregnancy divided by the absorption coefficient. The P requirement for maintenance is defined as the inevitable loss of P in feces plus endogenous urine P when an animal is fed very near or just below its true P requirement (NRC, 2001). The inevitable fecal component typically is by far the largest part of the total P requirement for maintenance. The approach to estimate and the values reported for the P requirement for maintenance vary (ARC, 1980; NRC, 1989; INRA, 1989; NRC, 2001). Previously, the daily P requirement for maintenance was expressed as g/kg of BW (ARC, 1980; NRC, 1989; INRA, 1989). The ARC (1980) suggested that the net P requirement for maintenance was 12 mg P/ kg BW per day. This was based on experimental data of fecal P excretion extrapolated to zero P intake. Based on the ARC

(1980) approach, the NRC (1989) estimated the total dietary requirement of P for maintenance as: $\text{g/d} = [0.0143 \times \text{BW (kg)}] \div 0.50$, where 0.50 was an overall estimate of the absorption coefficient for P from the diet. The Institut National de la Recherche Agronomique (INRA; 1989) suggested that the P maintenance requirement of 12 mg P/kg BW per d reported by the ARC (1980) was too low and suggested the P maintenance requirement to be 23 to 32 mg P/kg BW per d for cows producing 5 to 50 kg of milk/d. Also, the AFRC (1991) noted that previous research (ARC, 1980) estimated P maintenance requirements for growing and non-lactating animals in which BW and DMI were correlated highly. The AFRC (1991) contended that this was not an adequate representation of the maintenance requirement of P for a mature lactating cow. A mature lactating cow likely has varying feed intake through the lactating and non-lactating cycle, but BW may not change as much relative to the BW change of a growing animal. Also, a lactating cow may have a greater maintenance demand for P due to the physiological demands associated with milk production. Therefore, the AFRC (1991) estimated the P maintenance requirement as: $(\text{g/d}) = 0.693 \times \text{DMI (kg/d)} - 0.06$; in this case the requirement was equal to the daily inevitable fecal P excretion. The AFRC (1991) did not include endogenous urine P in their approach of estimating the requirement. This equation was developed using sheep, but used for lactating cattle because insufficient data from cattle were available.

Subsequently, Spiekens et al. (1993), using the AFRC (1991) approach, determined the inevitable fecal P loss of high and low production groups each with five lactating cows with similar BW but different DMI (17 vs. 11 kg/d) and MY (21 vs. 10 kg/d). The two groups were fed the same diet with 0.21% P, dry basis. Phosphorus

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intakes (37 vs. 22 g/d) differed between groups. Inevitable fecal P loss, or the fecal component of the total dietary maintenance requirement for P was estimated to be 1.20 and 1.22 g P/kg DMI for the high and low groups, respectively.

The NRC (2001) used the results of Spiekens et al. (1993) to set the fecal component of the P requirement for maintenance (1.2 g/ kg of DMI for a diet with an absorption coefficient of 0.8 for P; or 1.0g of absorbed P/ kg of DMI) for lactating dairy cows. urinary P excretion (0.002 g/d) is added to the fecal component to calculate the total dietary P requirement for maintenance. The objective of this experiment was to evaluate the estimates of the P maintenance requirement of lactating cows with a wider range in DMI and MY to test and compare with other results in the literature and evaluate the approach taken by the NRC (2001).

MATERIALS AND METHODS

Experimental Design, Cows, Experimental Treatments, and Diet

The Michigan State University All-University Committee on Animal Use and Care approved (AUF# 11/00-151-00) methods for care and use of the cows. Twenty-one lactating Holstein cows were selected from available animals at the Michigan State University Dairy Teaching and Research Center to provide seven cows for each treatment group; the treatment groups initially were classified as early (55 ± 6.5 DIM), mid (164 ± 6.0 DIM), or late (253 ± 6.0 DIM) lactation. Initially, treatments of different DIM were chosen to theoretically provide different ad libitum intakes.

A diet was formulated to supply P at or very close to the cow's true requirement to be able to measure inevitable P excretion (NRC, 2001). The basal diet met or

exceeded requirements for all other nutrients (Table 1). The diet was formulated to contain 0.24% P. The analyzed dietary P concentration was 0.26% P, dry basis. Once treatments were assigned, all cows were fed this same basal diet for the duration of the experiment (35 d). Cows were fed their diets once daily at approximately 1000 h.

The experiment lasted for 35 d and consisted of a 6-d evaluation period, a 24-d diet adaptation period (d 7 through 30), and one 5-d collection period. Samples collected included fecal grab samples, urine spot samples, milk samples, and feed ingredient samples. The first 6 d were used to determine if treatments, as defined by DIM, would meet the objective of having a wide range of ad libitum DMI

At the end of the 6-d evaluation period, it was determined that there was minimal DMI difference among treatment groups [20.6, 20.7, and 21.8 ± 0.86 kg/d for early, mid, and late lactation groups, respectively ($P = 0.53$)]. Also during the pretrial period, MY was affected by DIM [47.3, 29.1, and 25.3 ± 1.23 kg/d for early, mid, and late lactation, respectively ($P < 0.01$)]. It was desired to increase the range in DMI among the three treatment groups. Therefore, the DMI needed to supply enough NE_L to support the average MY of each cow recorded during the 6-d pretrial period was calculated for the mid and late lactation treatment groups. Once the DMI to supply adequate energy for lactation was determined for the mid and late treatment groups, the average restriction by treatment was approximately 75% of ad libitum intake determined during the evaluation period. This still did not produce the range in DMI desired because MY for the mid- and late lactation groups were numerically similar. Therefore, cows in the late lactation group were restricted an additional 25% of their 6-d evaluation period ad libitum DMI to increase the range of DMI and MY. The treatments based on targeted DMI were:

treatment 1 (T1), average DMI of 11.3 kg/d; treatment 2 (T2) average DMI of 15.3 kg/d; and, treatment 3 (T3) average DMI of 25.1 ± 0.74 kg/d when the sample collection started on d 30 (Table 2). Targeted DMI was accomplished by a stepwise reduction over d 7 through 11 of the experiment; these daily amounts were offered and completely consumed for the remainder of the experiment. Cows in the early lactation treatment (highest milk yield) were allowed to continue ad libitum intake throughout the experimental period to maximize ad libitum DMI. Therefore, DMI as the definition of the treatments expressed as a percentage of the average daily ad libitum intake of each individual cow during the evaluation period was: 50, 75 and 100%, for T1, T2, and T3, respectively. Two cows were removed from T1. One cow died of causes unrelated to the experiment during the sample collection period and a second cow was determined to be an outlier (± 3 standard deviations) in P balance. Their data were subsequently removed.

Experimental Procedures

Chromium oxide was used as an external marker to estimate daily fecal excretion. An initial dose of 45 g chromium oxide was given orally via balling gun in two equal amounts of 22.5 g at 0600 and 1800 h in lock ring gelatin capsules (Torpac Inc., Fairfield, NJ) on d 25 of the experiment. The initial dose of 45 g was given to help ensure that a steady state concentration of Cr was reached prior to fecal sample collections. On d 26 through 35, cows were given chromium oxide orally in capsules via balling gun in two equal doses of 7.5 g at 0600 and 1800 h, (Torpac Inc., Fairfield, NJ) so that a total of 15 g/d were supplied. Approximately 1 g of spelt hulls was added to the gelatin capsules to provide buoyancy to the capsule in the rumen until the gelatin capsule dissolves (Oba and

Allen, 2000). Spelt hulls contained 0.22% P and contributed only 53 mg dietary P over the entire 11-d dosing period; therefore, P supplied by spelt hulls was ignored in P-related calculations of the data.

Approximately 500 g of feces (rectal grab samples) were collected into plastic containers with snap-on lids (Sweetheart Cup Company, Inc., Chicago, IL) starting on d 31 at 0600 h. Fecal grab samples were collected every 15-h through 1500 h on d 35 to minimize diurnal variation in chromium oxide and P excretion. A total of eight samples were collected from each cow, representing every 3 h of a 24-h clock (Table 3). Fecal samples were frozen at -4°C for later processing and P, Cr, and DM analyses. Spot samples of urine were taken via manual stimulation of the area around the vulva during the same time frame as fecal samples. Approximately 100 ml of urine were collected into 150-ml graduated screw-top urine specimen containers and frozen (-4°C) for later processing and P and specific gravity analyses. Urine samples were missed occasionally because of failure to urinate after manual stimulation, but at least 6 of the 8 samples were available from each cow for compositing and analyses.

Cows were milked twice-daily at 0400 and 1400 h. Two milk samples (25 ml each) per cow were collected daily at each milking into sample vials containing a potassium dichromate pellet during the 5-d collection period. One milk sample was for analysis of milk fat % (MF), true protein % (MProt) lactose % (ML), SNF, and SCC (Michigan DHIA, East Lansing). The second milk sample was frozen at -4°C for later analysis of P concentration.

Corn and alfalfa silages were sampled twice weekly on Monday and Thursday for DM concentration determination via Koster tester (Seedburo Equipment Co, Chicago,

IL). As necessary, adjustments were made in the as-fed amounts of forages in the diet to insure the same DM proportions of the dietary ingredients throughout the experiment. Weekly alfalfa and corn silage samples also were taken and frozen (-4°C) for later DM concentration and chemical analysis. Alfalfa and corn silages were stored in upright silos. To obtain samples, approximately 100 kg of forage were unloaded from the silo and then grab samples were taken from different sections of the pile to form a 7-kg sample. A sub-sample was then taken by the roll and quartering method. The sample was rolled and quartered twice and the resulting 1-kg sub-sample was stored in a sealed plastic bag at -4°C for later analysis of DM concentration and chemical composition. Bagged feed ingredients (concentrates and beet pulp pellets) were sampled bi-weekly by taking a grab sample from 10% of the bags. These samples were stored in sealed plastic bags at -4°C for later analysis of DM concentration and chemical composition. Bulk ingredients (whole cottonseed and vitamin and trace mineral premix) were sampled weekly by taking grab samples from separate places from the pile and stored in sealed plastic bags and -4°C for later analysis.

Laboratory Procedures

Feed and Fecal Sample Analyses. After the completion of the sample collection period, feed and fecal samples were thawed at room temperature. Samples were then dried in a forced-air oven at 52°C for 48 h for DM concentration determination. Once DM concentration was determined samples were ground through 5 and 2mm screens in a Thomas-Wiley Mill (Arthur Thomas Company, Philadelphia, PA). Feed samples were composited by ingredient type by taking a 5 g aliquot from each sample and combining

them into one new sample and stored in a capped plastic container. The composite sample represented the average composition of the ingredient used throughout the experimental period. A sub-sample of the each composite was analyzed for chemical composition (Dairy One Forage Lab, Ithaca, NY). Composite fecal samples (40 g each) were made from the eight individual samples from each cow and stored in a capped plastic container.

Phosphorus concentration in feed and feces was measured by colorimetric assay adapted from AOAC (1990) method 965.17. All laboratory glassware was acid-washed before use. Duplicate 1 g sub-samples of individual feed and fecal composites were taken after thorough mixing and dry-ashed at 600 °C for 4 h. The resulting ash was digested in 20 ml of 4N HCl plus 5 drops of concentrated H₂SO₄. Molybdovanadate reagent was added to a 1-ml aliquot of the digested sample to react with P for color development. Color intensity increased with greater P concentrations. Duplicate samples were pipetted into 96-well microtiter plates and P concentration was determined by a spectrophotometer (SpectraMax 190, Molecular Devices, Sunnyvale, CA). Samples were read at a wavelength of 400 nm and compared against the standard curve (0, 0.5, 1.0, 1.5, and 2.0 mg P/dl). The standard curve solutions contained the same volumes and proportions of reagents and deionized, distilled water as the samples. The 0 mg P/ml standard also was used as a blank to adjust the spectrophotometer for any background P present in the reagents or water used in the digestion or assay processes. New sub-samples from the composite were re-digested and assayed if the coefficient of variation (CV) between the duplicate samples was greater than 3%. If the P concentration of the

unknown sample was greater than the highest concentration of the standard curve, the unknown was diluted 1-to-20 with deionized, distilled water and re-assayed.

Two known controls were included in each assay. One control was a composite fecal sample (pooled sample) made of 8 g each from 10 individual cow fecal sample composites. The pooled sample of constant P concentration was used in each run of the digestion and assay processes in order to calculate overall intra- and inter-assay CV for all plates read on the spectrophotometer. For all assays of fecal samples in the experiment, the intra- and inter-assay CVs were 0.72 and 2.22%, respectively.

A second known control was a 1.0 mg/dl purchased certified standard P solution (Fisher Scientific, Chicago, IL). This solution was assayed along with unknowns to evaluate the assay-to-assay variation of the spectrophotometer. The intra- and inter-assay variations among all assays were 0.58 and 1.20%, respectively.

Chromium concentration in feces was analyzed by flame atomic absorption spectroscopy (SpectrAA 220, Varian, Inc., Walnut Creek, CA) using a method adapted from Williams et al. (1962). Duplicate 1 g sub-samples were taken from fecal composites after thorough mixing and dry-ashed at 600 °C for 4 h. Resulting ash was then digested on a hot plate in a phosphoric acid, manganese sulfate, and potassium bromate solution at 400 °C for approximately 10 min. Chromium concentration was determined against the standard curve (1, 2, 3, 4, and 5 µg Cr/ml). The standard curve solutions contained the same volume and proportions of reagents and deionized, distilled water as the sample unknowns. A 0 µg Cr/ ml was used to adjust the atomic absorption spectrometer for any background Cr present in reagents and water used in the digestion or assay procedures. This solution contained the same reagents and volumes as the

unknowns. Unknowns were redone beginning with sub-sampling for the dry-ashing step if the CV between duplicates was greater than 5%.

Two known controls were included in each assay. A pooled fecal sample containing Cr similar to the pooled fecal sample for P analysis was made to digest and assay in duplicate along with each separate run of unknowns to obtain the intra- and inter-assay CV. The intra- and inter-assay variations were 2.26 and 4.44%, respectively.

The second known control was a 3 mg Cr/ml solution made from purchased certified chromium oxide powder (Fisher Scientific, Chicago, IL). This control was used to evaluate the assay-to-assay variation of the atomic absorption spectrometer. The intra- and inter-assay variations of the Cr control solution were 2.65 and 6.9%, respectively.

Urine Sample Analysis. After the conclusion of sample collection, urine samples were thawed to room temperature and 10 ml were taken from each urine spot sample and composited. Composite samples were acidified with 1 ml concentrated HCl and filtered through cheesecloth to remove any large particulate matter. Once filtered composite urine samples were analyzed for P concentration. Also, specific gravity of each composite was measured using an Adams® Midget Urinometer (Bristol-Myers Squibb Company, New York, NY). The specific gravity value was used to estimate daily urine excretion, $\text{kg/d} = 332.91 + 0.2885 \times \text{DMI, kg/d} - 0.2175 \times \text{MY, kg/d} - 0.083 \times \text{dietary DM \%} + 0.524 \times \text{dietary CP, \% DM} - 326.5 \times \text{urine specific gravity} + 0.2375 \times \text{apparently absorbed water, kg/d}$ (Holter and Urban, 1992).

Urine P concentration was determined by colorimetric assay adapted from Fiske and Subbarow (1925). Duplicate 1-ml sub-samples were taken from urine composites and 2 ml 20% TCA was added. The sample was then centrifuged at 4000 g for 15 min.

A 175- μ l aliquot of the supernatant was used for determination of P concentration. A molybdate plus aminonaphtholsulfonic acid reagent was used for color development, with color intensity increasing as P concentrations increased. The P concentration of urine was determined at 660 nm against the standard curve (0, 0.25, 0.5, 0.75, 1.0, and 1.25 mg P/dl) by using a SpectraMax 190 spectrophotometer (Molecular Devices, Sunnyvale, CA). The standard curve solutions contained the same volume and proportions of reagents and deionized, distilled water as the samples. The 0 mg P/ml standard also was used as a blank to adjust the spectrophotometer for any background P present in the reagents or water. Urine samples were redone if the CV between duplicates was greater than 10%. Additionally, if the P concentration of urine was greater than the highest concentration of the standard curve, an aliquot of the original urine sample composite was diluted 1-to-6 with deionized, distilled water and re-assayed.

A pooled sample of urine as a known control was made by compositing 5-ml aliquots from six individual urine composites. Duplicate 1 ml pooled urine sample aliquots were treated as unknowns in each separate assay in order to evaluate the variation in the spectrophotometer from assay-to-assay and to calculate the intra- and inter-assay variation. The intra- and inter-assay variations were 4.8 and 11.3%, respectively.

Milk Sample Analysis. After the conclusion of the sample collection period milk samples were thawed in a warm water bath (40 °C) and mixed thoroughly by inversion and shaking. A single 10-g aliquot of whole raw milk was weighed into 150 ml beakers for P analysis. Samples were dried overnight at 100 °C in a forced-air oven and DM concentration of the samples was determined. Once dried, samples were dry-ashed and

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assayed according to the same protocol as the fecal samples described above except that the standard curve for milk P concentration was 0, 0.5, 0.75, 1.0, and 1.25 mg P/dl. Once milk P% (DM basis) was computed it was converted to a wet raw milk basis. Each milk sample from each cow at each individual milking was analyzed for P concentration and then a 5-d weighted average was computed for each cow based on milk yield at each milking. A 5-d weighted average also was computed for MF, MProt, ML, SNF, and SCC for each cow. Statistics reported are based on the 5-d weighted average for each cow for these dependent variables.

Two separate milk sample pools as known controls were made to digest and assay along with the unknowns. Milk pools were made from 2 L of milk obtained from an early lactation (10 DIM) cow. A high milk P pool was a 25-ml aliquot of whole raw milk, and a low milk pool was made by diluting whole raw milk approximately 1-to-2.5 with water for a total volume of 25 ml. The milk pools were digested and analyzed in duplicate along with the unknowns to estimate intra- and inter-assay variation. The intra- and inter-assay variations were 1.73 and 6.39%, respectively for the high pool and 1.70 and 6.18%, respectively for the low pool.

A third known control was a solution containing 0.80 mg P/dl made from a purchased 1.0 mg P/dl certified standard (Fisher Scientific, Chicago, IL). This control solution was assayed with the unknowns to provide an estimate of the assay-to-assay variation in the spectrophotometer. The intra- and inter- assay variations of this control were 0.56 and 1.02%, respectively.

Statistical Analysis

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Statistical inferences were made using SAS (1999). Prior to ANOVA, the mean, standard deviation, minimum, and maximum values were calculated by the Means procedure for all dependent variables to help validate the datasets. Next, an outlier analysis was conducted using the calculated mean $\pm$ 3 standard deviations of each dependent variable. Based on this analysis it was determined that there was one outlier that should be removed from the dataset. One cow in T1 was an outlier in P balance.

The Mixed Models procedure was used to determine that the residuals of each dependent variable in the dataset were normally distributed. This was done using the linear model $X_{ijk} = \mu + T_i + P_j + T_i \times P_j + C_k (T_i \times P_j) + E_{ijk}$ where:

X_{ijk} is the expected response,

μ is the overall mean of the response,

T_i is the effect of treatment i,

P_j is the effect of parity j,

$T_i \times P_j$ is the interaction between treatment i and parity j,

C_k is the random effect of cow k and is nested within treatment i and parity j, and;

E_{ijk} is the experimental error.

There were two parity classifications for this experiment, $\leq$ second parity and $\geq$ third parity. Distribution of residuals was determined by F-test to be normal if $P > 0.001$.

Dependent variables also were tested for heterogeneity (unequal variances). This was done using the Mixed Procedure of SAS (1999). The absolute value of the residuals was computed and used to determine heterogeneity. The linear model was $X_{ik} = \mu + T_i + C_j (T_i) + E_{ik}$ where:

X_{ik} is the absolute value of the residual,

μ is the overall mean of the residuals,

T_i is the effect of treatment i .

C_k is the random effect of cow k and is nested within treatment I , and;

E_{ik} is the experimental error.

Cow was random and nested within treatment. Variances were determined by F-test to be heterogeneous (unequal) if $P < 0.05$.

For ANOVA, statistical significance was declared if $P < 0.05$, and tendencies were noted if $P < 0.10$. The least squares ANOVA was used to determine differences among treatment means of each dependent variable by the mixed model procedure of SAS (1999). The linear model used was $X_{ik} = \mu + T_i + C_k(T_i) + E_{ik}$ where:

X_{ik} is the response of the dependent variable,

μ is the overall mean of the response,

T_i is the effect of treatment i .

C_k is the random effect of cow k and is nested within treatment i , and;

E_{ik} is the experimental error.

Parity and the treatment $\times$ parity interaction were included in the original model, but were inconsequential and omitted in the final model. Orthogonal contrasts (linear and quadratic effect of treatment) were used to compare treatment mean responses.

A major dependent variable of interest relative to the experimental objective was fecal P excretion, g/d $\div$ DMI, kg/d. Regression analysis with daily fecal P excretion as the dependent variable and DMI as the independent variable was done according to SAS (1999). The fit of the model was determined by the coefficient of multiple determination

(R^2) also using the regression procedure. A scatter plot of fecal P excretion, g/d (y-axis) versus DMI, kg/d (x-axis) and the best-fit regression line was graphed.

RESULTS AND DISCUSSION

Experimental Diet. Ingredient composition and analyzed chemical composition of the basal diet is presented in Table 1. The diet was formulated to be as low as possible in P concentration and therefore, contained 25% beet pulp pellets (without molasses), comparable to corn silage and alfalfa silage in energy content, but with less P (0.08 vs. 0.21 and 0.20% P, DM basis for beet pulp pellets, corn silage, and alfalfa silage, respectively). Cornstarch in effect, replaced a portion of corn grain and soybean meal (44% CP) and supplied similar energy, but with less P (0.009%, dry basis vs. 0.30 and 0.71% P for corn grain and soybean meal, respectively). However, beet pulp pellets and cornstarch did not contain as much protein (8.4 and 0.6% CP, DM basis, respectively,) as alfalfa silage, corn silage, or corn grain (20.0, 8.5, or 9.1% CP, DM basis, respectively). Therefore, urea and biuret were added to meet the dietary crude protein requirement without adding more P. The analyzed CP concentration of the diet was higher than formulated perhaps because of mixing error or a lower value for %CP used for urea or biuret during formulation than what they actually were.

Performance Characteristics of Cows. General characteristics of the cows and performance variables are present as treatment means with statistical significance are in Table 2. Differences among treatments in BW, BCS and DIM resulted from the initial assignment of cows based on DIM and length of time cows had been lactating. Daily

DMI differences were as experimentally set and expressed as a percentage of body weight and ranged from 1.8, 2.9 and 4.2% ($P < 0.02$; quadratic effect). It is important to show differences in these variables as they were used as the criteria to define the treatments and estimate the effects of DMI on inevitable fecal P excretion and that component of P maintenance requirement.

Apparent DM digestibility averaged about 70% (Table 2) and did not differ among experimental treatments ($P > 0.10$). The reduction in milk yield and energy-corrected milk yield was caused by the combination of reduced DMI and increasing DIM. Milk fat%, MProt, ML, SNF, and SCC were not affected by treatment ($P > 0.05$).

Characteristics of Feces, Urine, and Milk. Some characteristics of feces, urine, and milk are summarized in Table 3. Fecal DM% tended to decrease slightly ($P = 0.0865$; linear effect) with increasing DMI. Fecal DM excretion increased linearly ($P < 0.01$) as DMI increased (3598, 4610, and 7630 g/d) for T1, 2, and 3, respectively. This response would be expected because apparent DM digestibility was similar among treatments. This is similar to results reported by Spiekens et al. (1993), where two groups of five cows in different stages of lactation had different DMI (17 vs 11 kg/d), and fecal excretion increased as DMI increased. In the current experiment, there was an inverse relationship between fecal P% (dry basis) and DMI, kg/d (0.43, 0.40 and 0.35% P, dry basis; $P < 0.0012$; linear effect). Total daily fecal P excretion (15.3, 18.2, and 26.3 g/d for T1, 2, and 3, respectively) increased linearly ($P < 0.01$) with increasing DMI; cows on all treatments were fed the same diet (0.26% P, dry basis). These results differed slightly from the results of Spiekens et al. (1993) who reported no difference in fecal P concentration between treatments (0.48 vs. 0.49% for high and low DMI, respectively).

However, they reported an increase in daily fecal P excretion due to increased DMI and fecal DM excretion.

Predicted urine excretion was 11.4, 14.3, and 21.0 kg/d for T1, 2, and 3, respectively, increasing linearly ($P < 0.01$) with increasing DMI. Urine P concentrations on a wet basis, were 0.0024, 0.0005, and 0.0003% for T1, 2, and 3, respectively, but not different ($P = 0.50$). Total daily urine P excretion was not different among treatments. These results are similar to those of Wu et al. (2000) in which there was no difference in urine P excretion of cows fed diets with 0.31 to 0.49% P. In other studies, with dietary P from 0.30 to 0.67% P, urinary P excretion increased with increasing dietary P concentration (Knowlton and Herbein, 2002; Morse et al., 1992b). Because urine P concentration was low, total urinary P excretions across all treatments were only 1.8 and 1.7 g/d for Knowlton and Herbein (2002) and Morse et al. (1992b), respectively.

In the current experiment, milk P concentration was not different among treatments and averaged about 0.081% (Table 3), which is similar to results reported by Knowlton and Herbein (2002), Wu et al. (2000), and Brintrup et al. (1992). Milk P concentration was not different between treatments when dietary P ranged from 0.31 to 0.67%. Average milk P% for these three studies was 0.087% and ranged from 0.085 to 0.091% P in these three studies. Due to differences in milk yield (Table 2), milk P secretion increased linearly for T1, 2, and 3, respectively ($P < 0.01$).

Phosphorus Excretion and Balance. Phosphorus balance [P intake, g/d – (fecal P excretion + urinary P excretion + milk P secretion (g/d))] was important in identifying whether or not the treatments as defined would allow appropriate evaluation of inevitable fecal P excretion and that component of the P maintenance requirement of lactating dairy

cows. Table 4 summarizes P intake, excretion, secretion, and balance by treatment. P intake increased linearly (29.4, 39.8, and 65.2 g/d for T1, 2, and 3, respectively; $P < 0.01$) as DMI increased. Additionally, total P excretion (fecal P + urinary P) increased linearly ($P < 0.01$; 15.6, 18.3, and 26.4 g/d for T1, 2, and 3, respectively). Feces is the major route of P excretion (NRC, 2001) and urinary P excretion constituted only between 0.04 and 1.6% of total P excretion across treatments (Table 4).

Maintenance Requirement for Phosphorus. Phosphorus balance was 1.5 g/d for T1, 2, and 3 (Table 5). Phosphorus balances were not different from zero ($P > 0.05$) providing support for the idea that cows were fed very close to their true requirements for P. Because the cows consumed amounts of P that resulted in P balance very near zero, the P excretion in feces and urine was considered inevitable P loss and the requirement for maintenance was calculated from these data (NRC, 2001). Two assumptions are made when determining the P maintenance requirement in this way. Firstly, because P is fed at a low dietary concentration, the consumed P will be utilized with high efficiency. AFRC (1991) and NRC (2001) assumed that the coefficient of absorption was 0.8. That is, most of the P consumed from the diet will be absorbed from the digestive tract. Secondly, because most of the dietary P is absorbed, most of the P found in feces and all of that in urine is of endogenous origin. Phosphorus of endogenous origin in feces means that dietary P was absorbed across the digestive tract, secreted back into the rumen via saliva, and subsequently excreted in feces. As mentioned previously, a small amount of P is excreted through urine and is considered as part of the P maintenance requirement because it is part of the absorbed P fraction. Inevitable fecal P excretion includes endogenous fecal P plus the unavailable dietary P excreted in feces. The AFRC (1991)

acknowledged that urine P theoretically should be added to inevitable fecal P for determining the maintenance requirement, but ignored it in their final recommendation due to the low amount of P generally excreted in urine by cattle. However, NRC (2001) added an additional 0.002 g urine P/kg BW to their final recommended maintenance requirement.

In the current experiment, inevitable fecal P excretion/kg of DMI declined with increasing DMI (1.36, 1.19, and 1.04 g of P/kg of DMI for T1, T2, and T3, respectively; linear effect of treatment, $P < 0.01$). Using a similar experimental approach, Spiekens et al. (1993) found that inevitable fecal P excretion was 1.20 and 1.22 g/kg of DMI for lactating cows consuming 17 and 11 kg DM/cow per day. Additionally, the NRC (2001) reported that as much as 50% of the endogenous fecal P loss is associated with microbial cells excreted in feces. Because T3 cows had the highest DMI, more microbes would be present in their rumens. Therefore, these cows will have more endogenous fecal P excretion associated with microbial cells.

Using a constant (e.g., 1.2 g of P/ kg of DMI; NRC, 2001) as an estimate of inevitable fecal P component of the maintenance requirement does not allow for the difference found as DMI changed in the current experiment. The estimate of NRC (2001) could be modified to account for decreasing inevitable fecal P (component of the maintenance requirement) as DMI increased. To assess modification of the NRC (2001) approach for estimating the fecal fraction of the P maintenance requirement regression analysis was done. Inevitable fecal P excretion (g/d) was regressed on DMI (kg/d) (Figure 1) using all of the data collected in this experiment. The regression analysis indicated a linear relationship between fecal (inevitable) P excretion and DMI ($P < 0.01$).

The equation that described this relationship was: inevitable fecal P excretion, g/d = $[0.85 \pm 0.070 \text{ (g/d)}] \times \text{DMI, kg/d} + [5.30 \pm 1.224 \text{ (g/d)}]$, where 0.68 is the slope of the regression line and 5.3 is the y-intercept (Figure 1). This means that for every 1 kg increase in DMI an additional 0.85 g P was excreted in feces and that if a cow could have a DMI of 0 kg then she would still excrete 5.30 g P in her feces. This relationship explained 90% of the variation associated with fecal P excretion and had a root mean square error (RMSE) of 1.75 g/d. Additionally, the y-intercept was different from zero and the slope was different from 1. There was more variation in inevitable fecal P excretion at greater DMI (cows on T3 with ad libitum intake). Because cows were allowed to consume ad libitum intake there was less control over their P intake relative to the DMI of intake-restricted cows in T1 and T2. Therefore, the opportunity was greater for T3 cows to consume more dietary P than their true requirement, increasing fecal P excretion. When more dietary P is consumed than needed the additional P will be excreted in feces and potentially urine (Knowlton and Herbein, 2002; Wu et al., 2001; Wu et al., 2000; Brintrup et al, 1993; Morse et al., 1992b; Martz et al, 1990). When DMI was restricted (T1 and 2) there was a tighter relationship between fecal P excretion, g/d and DMI, kg/d because there was less potential for variation in DMI and P intake in excess of requirement for milk production plus maintenance. Therefore, feeding cows closer to P needs resulted in a tighter relationship between inevitable fecal P excretion and DMI and presumably allowed for a more accurate determination of the fecal component of the P maintenance requirement in T1 and T2.

The total dietary P maintenance requirement for a 700 kg cow consuming 22 kg DMI and producing 40 kg milk/d accounting for endogenous urinary P loss, would be

9.8, 20.0, 19.3, 15.2, and 23.4 g P/d for the ARC (1980), NRC (1989), INRA (1989), AFRC (1991), and the NRC (2001), respectively. As a comparison, the P requirement for maintenance of this same cow would be 24.1 g/d based on the model [inevitable fecal P excretion (g/d) = $0.85 \times \text{DMI (kg)} + 5.30 \text{ (g/d)}$] developed in the current experiment. This example P maintenance requirement includes an additional 0.002 g P/kg BW to account for the endogenous urinary P loss part of the total maintenance requirement.

In the current research, cows with greater DMI utilized P more efficiently (Table 5) based on apparent P digestibility. Apparent P digestibility increased with increased DMI (linear effect of treatment; $P < 0.01$). More efficient use of ingested P may be explained by MY. Cows with higher milk had increased demands for P relative to the other two experiments; therefore P would be more efficiently used to support the higher MY.

CONCLUSIONS

The relationship of inevitable fecal P excretion (g/d) $\div$ DMI (kg/d), the approach taken by the NRC (2001) to express the P maintenance requirement, could be evaluated because P balance was not different from zero ($P > 0.05$) so inevitable fecal P excretion was equal to that portion of the P requirement for maintenance of lactating cows. The NRC (2001) set the P requirement for maintenance to be constant at 1.2 g P/kg DMI. However, in the current research this relationship decreased linearly with increased DMI. It is unknown whether or not these are statistically different from the NRC (2001) recommendation, but due to low variation (0.040 g P/kg DMI) we were able to detect a difference in inevitable fecal P related with DMI. Therefore, the approach taken by the

NRC (2001) was modified to account for increased inevitable P excretion with decreased DMI. In this experiment, inevitable fecal P excretion (g P/kg DMI) decreased linearly with DMI (Table 4). Therefore, regression analysis was used to determine the relationship between inevitable P excretion and DMI. The relationship was: inevitable fecal P excretion (g/d) = $[0.85 \pm 0.070] \times \text{DMI (kg/d)} + [5.30 \pm 1.244 \text{ (g/d)}]$; (RMSE = 2.75 g/d; $R^2 = 0.90$; $P < 0.01$). However, this new approach needs to be evaluated by further research to determine whether or not it is valid.

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Table 1. Formulated ingredient and analyzed chemical composition of the basal diet.

Item	Percent of dietary DM
Ingredient	
Alfalfa silage	11.4
Corn silage	19.3
Beet pulp pellets, dehydrated without molasses	25.0
Whole cottonseed	11.0
Corn starch	10.9
Corn, dry ground	13.4
Soybean meal, 44% CP	3.9
Blood meal	1.3
Corn Oil	0.1
Limestone	0.8
Salt (NaCl)	0.4
Urea	0.7
Biuret ¹	0.8
Vitamin and mineral premix ²	1.0
Chemical composition³	
DM	58.9
CP	20.3
NE _L ⁴ , Mcal/kg	1.69
ADF	21.7
NDF	39.0
Ash	3.8
Ca	0.94
P	0.26
Mg	0.22
K	0.95
Na	0.39
Cl	0.36
S	0.19
Cu, mg/kg	20
Fe, mg/kg	346
Mn, mg/kg	82
Zn, mg/kg	83

¹Graciously provided by Moorman's Manufacturing Company, Quincy IL.

²Trace mineral and vitamin base mix was 71.4% dry ground corn, 25.5% vitamins and minerals, and 3.1% corn oil. The concentrations of the vitamins and minerals were: 0.05% Ca, 0.28% P 0.60% Mg, 3.3% K, 1.4% S, 0.01% Na, 332 mg/kg Cu, 699 mg/kg Mn, 894 mg/kg Zn, 90 mg/kg Fe, 4 mg/kg Co, 7 ppm Se, 8 mg/kg I, 24 KIU/kg vitamin A, 5 KIU/kg vitamin D, 0.09 KIU/kg vitamin E, dry basis.

³Nutrient composition was determined by Dairy One Forage Lab (Ithaca, NY) except for P and ash.

⁴Energy value estimated based on computation per NRC (2001).

Table 2. Performance characteristics of cows as influenced by experimental treatments.¹

Item	Treatment ²			SEM	P-value ³		
	T1	T2	T3		Treatment	Linear	Quadratic
Number of cows	7	7	5	--	--	--	--
BW, kg	640	530	603	25.3	NS ⁴	NS	<0.01
BCS	3.3	2.5	2.1	0.19	<0.01	<0.01	0.04
DIM	285	169	85	6.6	<0.01	<0.01	<0.01
DMI, kg/d	11.3	15.3	25.1	0.74	<0.01	<0.01	NS
DMI, %BW	1.8	2.9	4.2	0.13	<0.01	<0.01	<0.01
Apparent DM digestibility, %	68.1	70.0	69.7	0.91	NS	<0.01	NS
MY, kg/d	15.7	23.9	47.0	1.56	<0.01	<0.01	NS
ECM ⁵ , kg/d	15.7	23.5	50.6	1.87	<0.01	<0.01	NS
Milk fat, %	3.69	3.51	4.17	0.254	NS	NS	NS
Milk protein (true), %	2.94	2.88	3.04	0.062	NS	NS	NS
Milk lactose, %	4.69	4.65	4.72	0.064	NS	NS	NS
SNF, %	8.60	8.49	8.78	0.118	NS	NS	NS
SCC, 1000 cells/ml	271	286	191	74.3	NS	NS	NS

¹Values are least squares means.²Treatments; T1 = 50% ad libitum intake, T2 = 75% ad libitum intake; and, T3 = 100% ad libitum intake.³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).⁴NS = Not significant ($P > 0.10$).⁵ECM = Energy corrected milk yield (kg/d) = $[(0.3246 \times \text{MY (lb/d)}) + (12.86 \times \text{milk fat yield (lb/d)}) + (7.04 \times \text{milk protein yield (lb/d)})] \div 2.2 \text{ (kg/lb)}$ (Dairy Record Management Systems, 1999).

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Table 3. Characteristics of feces, urine, and milk as influenced by experimental treatments.¹

Item	Treatment ²			SEM	P-value ³		
	T1	T2	T3		Treatment	Linear	Quadratic
Fecal DM, %	18.3	17.6	16.6	0.58	NS ⁴	0.09	NS
Fecal DM excretion, g/d	3599	4610	7631	331.6	<0.01	<0.01	NS
Fecal P, % of DM	0.43	0.40	0.35	0.01	<0.01	<0.01	NS
Inevitable fecal P excretion, g/d	15.3	18.2	26.5	1.16	<0.01	<0.01	NS
Urine excretion ⁵ , kg/d	11.4	14.3	20.2	0.93	<0.01	<0.01	NS
Urine P, % (wet basis)	0.0024	0.0005	0.0003	0.003	NS	NS	NS
Urine P excretion, g/d	0.26	0.07	0.06	0.14	NS	NS	NS
Milk P, % (wet basis)	0.080	0.084	0.079	0.003	NS	NS	NS
Milk P secretion, g/d	12.3	20.1	37.3	1.23	<0.01	<0.01	NS

¹Values are least squares means.

²Treatments; T1 = 50% ad libitum intake, T2 = 75% ad libitum intake; and, T3 = 100% ad libitum intake.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

⁵Estimated from equation (Holter and Urban, 1992).

Table 4. Phosphorus excretion and balance as influenced by experimental treatments.¹

Item	Treatment ²			SEM	P-value ³		
	T1	T2	T3		Treatment	Linear	Quadratic
P intake, g/d	29.4	39.8	65.2	1.93	<0.01	<0.01	NS ⁴
Total P excretion ⁵ , g/d	15.56	18.27	26.41	1.17	<0.01	<0.01	NS
Fecal P excretion, g/d	15.30	18.20	26.35	1.16	<0.01	<0.01	NS
Urine P excretion, g/d	0.26	0.07	0.06	0.14	NS	NS	NS
Milk P secretion, g/d	12.3	20.1	37.3	1.23	<0.01	<0.01	NS
P balance ⁶ , g/d	1.5	1.5	1.5	1.51	NS	NS	NS
Inevitable fecal P loss ⁷ , g P/kg DMI	1.36	1.19	1.04	0.040	0.0002	0.0001	NS

¹Values are least squares means.

²Treatments; T1 = 50% ad libitum intake, T2 = 75% ad libitum intake; and, T3 = 100% ad libitum intake.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

⁵Total P Excretion, g/d = the total maintenance requirement for P.

⁶P balance, g/d = P intake (g/d) - [total P excretion (g/d) + milk P secretion (g/d)]; P balance was not different from zero ($P > 0.05$).

⁷Inevitable fecal P loss, g P/kg DMI = fecal P excretion (g/d) / DMI (kg/d), which is the major part of the total maintenance requirement for P.

Tabel 5. Dietary P utilization as influenced by experimental treatments.¹

Item	Treatment ²				P-value ³		
	T1	T2	T3	SEM	Treatment	Linear	Quadratic
Apparently absorbed P, g/d	14.1	21.6	38.9	1.15	<0.01	<0.01	NS ⁴
Apparent P digestibility, %	47.0	54.1	59.7	1.54	<0.01	<0.01	NS
Total P excretion, % P intake	53.0	45.9	40.3	1.36	<0.01	<0.01	0.0401
Fecal P, % P intake	52.2	45.7	40.2	1.54	<0.01	<0.01	NS
Fecal P, % total P excretion	98.4	99.6	99.8	0.85	NS	NS	NS
Urine P, % P intake	0.79	0.16	0.09	0.412	NS	NS	NS
Urine P, % total P excretion	1.6	0.4	0.2	0.85	NS	NS	NS
Milk P, % P intake	41.9	50.7	57.6	3.38	0.02	<0.01	NS
Milk P, % total P output	43.4	52.3	58.8	2.33	0.0012	<0.01	NS

¹Values are least squares means.

²Treatments; T1 = 50% ad libitum intake, T2 = 75% ad libitum intake; and, T3 = 100% ad libitum intake.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

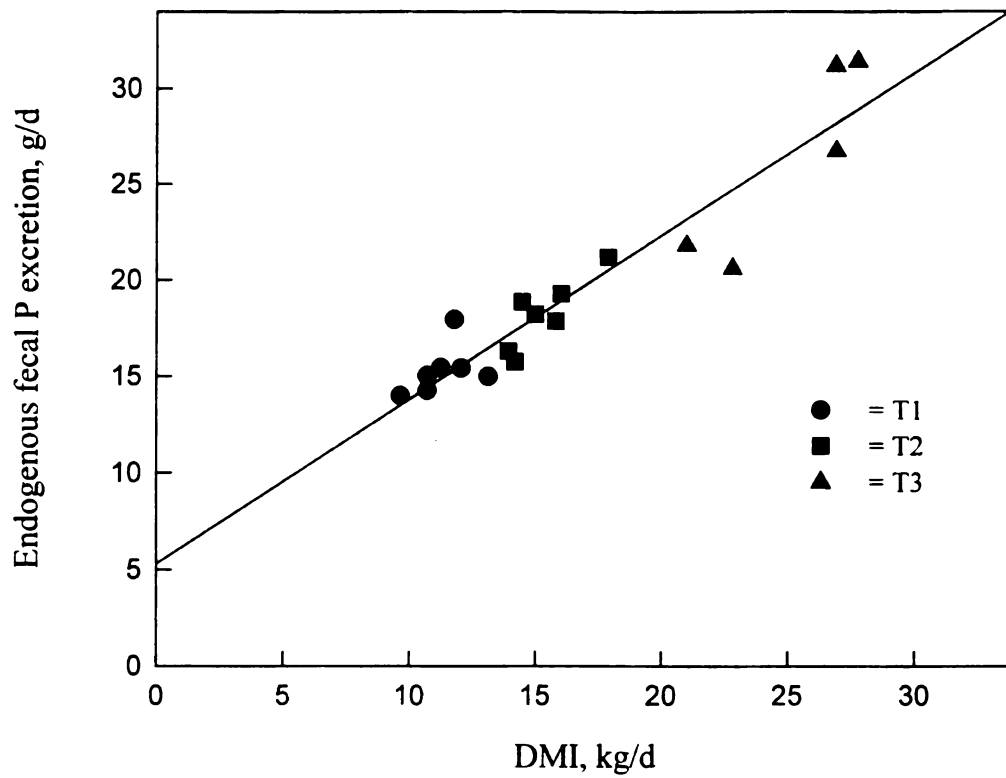


Figure 1. The relationship between inevitable fecal P and daily DMI: inevitable fecal P (g/d) = $[5.30 \pm 1.224 \text{ (g/d)}] + [[0.85 \pm 0.70] \times \text{DMI (kg/d)}]$ (RMSE = 2.75; y-intercept different from zero; slope different than one; $R^2 = 0.90$; $P < 0.01$).

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CHAPTER 4

INCREASING DIETARY PHOSPHORUS: UTILIZATION OF PHOSPHORUS BY DAIRY CATTLE AT DIFFERENT POINTS OF THE LACTATION CYCLE

ABSTRACT

The objectives were to evaluate the effect of varying dietary P concentration on P balance and utilization of lactating and non-lactating Holstein cattle and to construct a dataset for future research to evaluate P excretion prediction models. A series of seven 30-d experiments were conducted. Experiments were designed so that P balance data were collected at various points of the lactation cycle.

Experiments 1 and 2 were conducted with 21 pregnant nulliparous heifers at 57 and 28 ± 1.0 d prior to parturition, respectively. Experiments 3 and 4 were conducted with 21 pregnant non-lactating primi- and multiparous cows at 59 and 30 ± 1.0 d prior to parturition, respectively. Experimental treatments in Experiments 1 through 4 were diets with 0.19 (low; LP), 0.28 (moderate; MP), and 0.35% P (high; HP), dry basis. Experiments 5, 6, and 7 each consisted of 35 lactating primi- and multiparous cows. In Experiment 5 cows were 97 ± 10.0 DIM, in Experiment 6 cows were 183 ± 6.2 DIM, and in Experiment 7 cows were 294 ± 11.6 DIM.

Experiments 5, 6, and 7 were designed to have different DIM so that DMI and MY theoretically would be different, so that P intake, utilization and excretion would differ for future modeling work. However, the DMI range desired did not occur. Therefore, DMI was restricted in Experiment 7 to the NE_L intake required to support MY

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measured during the first 21 d of the experiment for any cow producing less than 25 kg/d. Cows producing greater than 25 kg/d were allowed to consume ad libitum. Dry matter intakes during the 5-d collection period for Experiments 5, 6, and 7 were 19.8, 19.3, and 17.8 ± 0.59 kg/d, respectively. Treatment groups for Experiments 5, 6, and 7 were fed diets with 0.27 (T1), 0.36 (T2), 0.42 (T3), 0.46 (T4), and 0.52% P (T5), dry basis. In Experiment 7 because of the DMI restriction of some cows, dietary P treatment and DMI were confounded because the distribution of the cows with unrestricted DMI was not equal across all treatments. Therefore, ANOVA were not conducted on this dataset.

In all experiments, fecal P concentration and fecal P excretion increased with increased dietary P concentration and ranged from 0.43 to 2.21% P, respectively, for Experiments 1 through 4 and 0.40 to 1.54, respectively, for Experiments 5 and 6. The effect of dietary P concentration on urine P concentration and excretion was small but inconsistent and averaged 0.0012% P and 0.82 g P/d for Experiments 1 through 4 and 0.0063% and 1.65 g P/d for Experiments 5 and 6. In general, P balance decreased with increased dietary P concentration in all experiments. Milk yield was not affected by dietary P in Experiment 5 or 6 ($P > 0.05$). Three datasets were constructed from the data collected in these experiments to be used in subsequent research to evaluate published models for predicting P excretion.

INTRODUCTION

There are more known biological functions involving P than any other element (NRC, 2001). Therefore, it is important to understand how dietary P is utilized and what

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happens to P consumed in excess of the P needed to sustain the body and production. Fecal excretion is the main route of P removal from the body consisting of 95 to 98% of total P excretion (NRC, 2001; Morse et al., 1992b) with the remaining 2 to 5% in urine. During lactation, the second major route of P removal from the body is milk secretion. Milk P concentration ranged between 0.085 to 0.10% (Knowlton and Herbein, 2002; Wu et al., 2000; Brintrup et al., 1993; Spiekens et al., 1993; Brodison et al., 1989; Flynn and Power, 1985).

Increasing dietary P concentration increased fecal P concentration and excretion when cows were fed diets with 0.30 to 0.67% P (Kohn et al., 2002; Knowlton and Herbein, 2002; Wu et al., 2002; 2001; 2000; Brintrup et al., 1993; Morse et al., 1992b; Martz et al., 1990). Increasing dietary P also increased urine P excretion when cows were fed diets with 0.30 to 0.67% P (Knowlton and Herbein, 2002; Morse et al., 1992b). Urine P excretion ranged from 0.3 to 4.1 g/d in these three experiments. No effects on milk P concentration was observed when cows were fed diets with P concentrations ranging from 0.24 to 0.67% P (Knowlton and Herbein, 2002; Wu et al., 2000; Brintrup et al., 1993; Brodison et al., 1989; Call et al., 1987; Forar et al., 1982). Additionally, Knowlton and Herbein (2002), Wu et al. (2000), and Brintrup et al. (1993) reported that milk P yield was not affected by dietary P fed at 0.31 to 0.67%. Phosphorus excretion data from pregnant non-lactating data was not found.

The first objective of this research was to characterize the effects of dietary P concentration on P balance and routes of excretion from non-lactating and lactating dairy cattle. The second objective was to assemble a dataset from all P balance and excretion data to be used in subsequent research to evaluate models used to predict P excretion.

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MATERIALS AND METHODS

Experimental Design, Cows, and Diets

All procedures for care and sampling of animals were approved by the Michigan State University All-University Committee on Animal Use and Care (AUF# 11/00-151-00). A total of four experiments with non-lactating and three lactating Holstein animals were conducted to address the effect of increasing dietary P concentration P balance, P excretion, and performance.

Experimental Similarities. Each experiment consisted of a 30 d experimental period. The first 25 d were used for diet adaptation and the last 5 d were the sample collection period. Samples collected included feed, fecal grab samples, and urine spot samples for non-lactating animals, with milk samples collected in addition for lactating cows. Lactating cows were milked twice-daily at 0400 and 1400 h. Milk samples were collected for each milking during the 5-d collection period for a total of 10 milk samples. Body weight and BCS were recorded 1 d prior to sample collection and 1 d after sample collection to obtain an average BW and BCS for the collection period. Because BCS determination is subjective, BCS was recorded by four separate technicians and averaged for each cow. All results reported are from data collected during the 5-d collection period.

Four experiments with non-lactating (nulliparous and multiparous) animals had three dietary P treatments each with 7 cows/treatment. Dietary treatments were formulated according to NRC (2001) recommendations for all nutrients except P (Table

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1). A basal diet was formulated to contain 0.16% P. This represented the low P dietary treatment (LP). Moderate (0.28% P; MP) and high (0.40% P; HP) P diets were formulated by replacing urea and cornstarch with monoammonium phosphate in the basal diet. The analyzed dietary P concentrations were 0.19, 0.28, 0.35% P (dry basis) for LP, MP, and HP, respectively (Table 1). Non-lactating animals were assigned to one of the three dietary treatments in a completely randomized design and fed respective diets once-daily at approximately 1000 h. Feed refusals were weighed every day prior to delivering that days ration for feed intake determination.

Three experiments with lactating cows (primiparous and multiparous) had five dietary P treatments each with 7 cows/treatment. Dietary treatments were formulated according to NRC (2001) recommendations for all nutrients except P (Table 2). A basal diet was formulated without supplemental P to contain 0.24% P. This treatment represented Treatment 1 (T1). Treatments 2 through 5 were formulated to contain 0.34, 0.44, 0.54, and 0.64% P, dry basis. This was accomplished by substituting monoammonium phosphate for urea and cornstarch in the basal diet. Analyzed dietary P concentrations were 0.27 (T1), 0.36 (T2), 0.42 (T3), 0.46 (T4), and 0.52% (T5), respectively. Lactating cows were assigned to one of the five dietary treatments in a completely randomized design and fed once-daily at approximately 1000 h. Feed refusals were weighed every day prior to delivering that days ration for feed intake determination. All dependent variables either measured or estimated in the seven experiments with lactating and non-lactating animals are defined in Table 3.

Experimental Differences with Non-lactating Animals. Experiment 1 consisted of 21 nulliparous animals. Experiment 1 was started at an average of 57 ± 1.0 d prior to

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parturition and continued until approximately 28 ± 1.0 d prior to parturition. Experiment 2 consisted of 21 nulliparous animals and began at 38 ± 1.0 d prior to parturition and continued until 9 ± 1.0 d prior to parturition. Experiment 3 consisted of 21 non-lactating multiparous cows and began at 59 ± 1.0 d prior to parturition and continued until approximately 30 ± 1.0 d prior to parturition. And, Experiment 4 consisted of 21 non-lactating multiparous cows and began at 40 ± 1.0 d prior to parturition and continued until 11 ± 1.0 d prior to parturition. However, one cow in each LP treatment of Experiments 3 and 4 developed a displaced abomasum and was removed from the experiment.

Experimental Differences with Lactating Cows. Experiment 5 consisted of 35 primiparous and multiparous cows with average DIM of 97 ± 10.0 and average DMI of 21.8 ± 2.47 kg/d for the first 21 d of the experiment. Experiment 6 consisted of 35 primiparous and multiparous cows with average DIM of 183 ± 6.2 and average DMI 21.6 ± 2.16 kg/d for the first 21 d of the experiment. However, two cows were removed from the experiment. One cow in T1 contracted mastitis and data from one cow in T5 were determined to be outliers (± 3 standard deviations) in fecal P excretion and P balance and they were removed from the dataset used for statistical analyses. Experiment 7 consisted of 35 primiparous and multiparous cows with average DIM 294 ± 11.6 and average DMI 23.0 ± 2.42 kg/d for the first 21 d of the experiment. However, one cow was removed from the study because she quit consuming feed for 48 hours during the 5-d collection period.

Experiments 5, 6, and 7 followed each other sequentially, but each was conducted with a separate set of cows. The purpose of designing the experiments based on DIM,

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was to get a wide range of ad libitum DMI and presumably P utilization and excretion. However, DMI (21.8, 21.6 and 23.0 kg/d, respectively) did not vary as much as expected based on DIM among these three experiments. Therefore, 4 d prior to the sample collection period during Experiment 7, DMI was restricted so that NE_L intake was equal to the energy needed to support measured MY of each cow during d 1 through 21 of the experiment. Restrictions were made using the energy requirements recommended by the NRC (2001). However, any cow producing greater than 25 kg milk/d was allowed to continue consuming ad libitum intake. A total of 11 cows were allowed to consume ad libitum.

Because cows producing greater than 25 kg milk/d were allowed to continue ad libitum intake in Experiment 7, the overall DMI average still did not vary much from that of cows in Experiments 5 and 6. When DMI was compared across treatments for the 5-d collection period, least square means for DMI were 19.8, 19.3, and 17.8 ± 0.59 kg/d, respectively for Experiments 5, 6, and 7. Even though not all cows in Experiment 7 had restricted feed intake, the restriction did reduce the overall DMI. Restriction of feed offered for part (11 of 34) of the cows in Experiment 7, resulted in a confounding of DMI and dietary P treatments. Therefore, ANOVA was not conducted for this experiment. However, the highest priority objective was to gather some data of P excretion at lower DMI for future modeling work.

Experimental Procedures

Experimental procedures were identical for all seven experiments except where noted. Chromium oxide was used as an external marker to estimate daily fecal excretion.

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An initial dose of 45 g chromium oxide were given orally via balling gun in two equal amounts of 22.5 g at 0600 and 1800 h in lock ring gelatin capsules (Torpac Inc., Fairfield, NJ) on d 20 of the experimental period. The initial dose of 45 g was given to help ensure that a steady state Cr concentration was reached prior to fecal sample collections. On d 21 to 30 of the experiment, cows were given chromium oxide in two equal amounts of 7.5 g at 0600 and 1800 h in lock ring gelatin capsules for a total of 15 g daily. Approximately 1 g of spelt hulls was added to each gelatin capsule to provide buoyancy to the capsule in the rumen until the capsule dissolved (Oba and Allen, 2001). Spelt hulls contained 0.22% P, DM basis, which contributed only 53 mg dietary P over the entire 11-d dosing period; therefore, P supplied by spelt hulls was ignored in P-related calculations of the data.

Approximately 500 g of feces (grab samples) were collected into plastic containers with snap-on lids (Sweetheart Cup Company, Inc., Chicago, IL) starting on d 26 at 0600 h. Fecal grab samples were collected at 15-h intervals through 1500 h on d 30 to minimize diurnal variation in Cr and P excretion. A total of eight fecal samples were collected, with one sample representing every 3 h of a 24-h clock. Fecal samples were frozen at -4°C for later processing and P, Cr, and DM analyses.

Eight urine samples were taken via manual stimulation of the area around the vulva during the same time fecal samples were collected. Approximately 100 ml of urine were collected into 150-ml graduated urine specimen containers with screw-on lids (Life Sciences Products, Inc., Denver, CO) and frozen (-4°C) for later processing and P and specific gravity analyses. Urine samples were occasionally missed, but at least 6 of the 8 samples were available for compositing and analyses.

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Lactating cows in Experiments 5, 6, and 7 were milked twice-daily at 0400 and 1400 h. Two milk samples (25 ml each) per cow were collected daily at each milking during the 5-d collection period into sample vials containing a potassium dichromate pellet. One milk sample was for analysis of milk fat % (MF), true protein % (MProt), lactose % (ML), SNF, and SCC (Michigan DHIA, East Lansing). The second milk sample was frozen at (-4°C) for later analysis of P concentration.

Corn and alfalfa silages were sampled twice weekly on Monday and Thursday for DM concentration determination via Koster tester (Seedburo Equipment Co, Chicago, IL). As necessary, adjustments were made in the as-fed amounts of forages in the diet to insure the same DM proportions of dietary ingredients throughout the experiment. Weekly alfalfa and corn silage samples were taken for later DM concentration and chemical determination. Alfalfa silage was stored in bunker and upright silos and corn silage was stored in bunker silos. For upright silos, approximately 100 kg of silage was unloaded from the silo and then grab samples were taken from different sections of the pile to form a 7-kg composite sample. An 1-kg sub-sample was then taken by the roll and quartering method. The sample was rolled and quartered twice and the resulting sub-sample was stored in a sealed plastic bag (-4°C) for later analysis of DM concentration and chemical composition. For bunker silos, grab samples from the freshly exposed face were taken from random spots across the entire face of the silo until a 7-kg composite sample was obtained. The sample was then processed the same as previously mentioned. Bagged feed ingredients (premix ingredients and beet pulp pellets) were sampled bi-weekly by taking a 100-g grab sample from 10% of the bags. These samples were stored in sealed plastic bags (-4°C) for later analysis of DM concentration and chemical

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composition. Bulk ingredients (whole cottonseed and vitamin and trace mineral mix) were sampled weekly by taking 100-g grab samples from separate places from the pile; they were stored in sealed plastic bags at -4°C for later analysis of DM concentration and nutrient composition.

Laboratory Procedures

Feed and Fecal Sample Analyses. After the completion of the sample collection period, feed and fecal samples were thawed at room temperature. Samples were then dried in a forced-air oven at 52°C for 48 h for DM concentration determination. Once DM concentration was determined for each sample, they were ground through 5 and 2 mm screens in a Thomas-Wiley Mill (Arthur Thomas Company, Philadelphia, PA). Feed samples were composited by ingredient type by taking a 5 g aliquot from each sample, which was then stored in a capped plastic container. The composite sample represented the pooled composition of the ingredient used throughout the experimental period. An approximate 5-g sub-sample of the each composite was used for determination of chemical composition (Dairy One Forage Lab, Ithaca, NY).

Composite fecal samples were made from the eight individual samples from each cow. Approximately 5 g from each individual sample were taken and combined into one sample and stored in a capped plastic container. Each composite sample was the pooled diurnal representation of total fecal excretion of the 5-d period for each cow represented total daily excretion because feces were collected over a 5-d period to theoretically reduce diurnal variation in chromium oxide and P excretion.

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Phosphorus concentration in feed and feces was measured by colorimetric assay adapted from AOAC (1990; method 965.17). All laboratory glassware was acid-washed prior to each use. Duplicate 1 g sub-samples of individual feed and fecal composites were taken after thorough mixing and dry-ashed at 600 °C for 4 h. The resulting ash was digested in 20 ml of 4N HCl and five drops of concentrated H₂SO₄. A molybdovanadate reagent was added to a 1-ml aliquot of the digested sample for color development. The molybdovanadate reagent reacted with P to form a phosphomolydenum yellow complex. Color intensity increased with greater P concentration. Two aliquots from each digested duplicate were pipetted into 96-well microtiter plates and P concentration was determined by a spectrophotometer (SpectraMax 190, Molecular Devices, Sunnyvale, CA). Samples were read at a wavelength of 400 nm and compared against the standard curve (0, 0.5, 1.0, 1.5, and 2.0 mg P/dl). The standard curve solutions contained the same volumes and proportions of reagents and water as the unknowns. The 0 mg P/ml standard also was used as a blank to adjust the spectrophotometer for any background P present in the reagents or deionized, distilled water used in the digestion or assay processes. Samples (composite feed and fecal samples) with unknown P concentrations were re-digested and assayed if the coefficient of variation (CV) between the original duplicate samples was greater than 3%. The volume in the microtiter plate represented a 1-to-10 dilution of the original sample. Therefore, if the P concentration of the unknown was greater than the highest standard, the unknown was diluted 1-to-20 with deionized, distilled water and re-assayed.

Two known controls were included in each assay. An assay was defined as the P determination of all the samples digested at the same time. Therefore, each assay may

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have included more than one microtiter plate of unknown samples. One control was a pooled composite fecal sample made from 8 g of feces from the composites of 10 individual cows. The pooled sample was treated as an unknown throughout the digestion and assay processes in order to calculate overall intra- and inter-assay variations for all plates read by the spectrophotometer. For all assays of fecal samples in the experiment, the intra- (within plate) and inter-assay (plate-to-plate) variations were 0.72 and 2.22%, respectively.

The second known control was a 1.0 mg/dl purchased certified standard P solution (Fisher Scientific, Chicago, IL). This solution was assayed along with the unknowns to evaluate the assay-to-assay variation of the spectrophotometer. The intra- and inter-assay variations across all assays were 0.58 and 1.2%, respectively.

Chromium concentration in feces was analyzed by flame atomic absorption spectroscopy (SpectrAA 220, Varian, Inc., Walnut Creek, CA) adapted from the procedure of Williams et al. (1962). Duplicate 1-g sub-samples were taken from fecal composites after thorough mixing and ashed at 600 °C for 4 h. The resulting ash was digested on a hot plate in a phosphoric acid, manganese sulfate, and potassium bromate solution at 400 °C for approximately 10 min. Chromium concentrations were determined by against the standard curve (1, 2, 3, 4, and 5 mg Cr/ml). The standard curve solutions contained the same volumes and proportions of reagents and deionized, distilled water as the unknowns. A standard with just reagents and deionized, distilled water and no added Cr was used to adjust the atomic absorption spectrometer for any background Cr present in reagents or deionized, distilled water used in the digestion or assay procedures. Unknowns were redone if the CV between duplicates was greater than 5%. If samples

read greater than the highest concentration standard, the original sample was diluted 1-to-12 and re-assayed.

Two known controls were included in each assay. A pooled fecal sample containing Cr similar to the pooled fecal sample for P analysis was made to digest and assay in duplicate along with each separate digestion of unknowns to obtain the intra- and inter-assay variation. The intra- and inter-assay variation was 2.26 and 4.44%, respectively.

The second known control of 3 mg Cr/ml solution made with the same reagents and proportions as the unknowns from a purchased certified chromium oxide powder (Fisher Scientific, Chicago, IL). This control was used to evaluate the assay-to-assay variation of the atomic absorption spectrometer. The intra- and inter-assay variations of the Cr control solution were 2.65 and 6.90%, respectively.

Urine Prediction and Sample Analysis. After the conclusion of the experimental period, urine spot samples were allowed to thaw and equilibrate to room temperature. Ten ml of urine were taken from each urine spot sample and composited. Composited samples were then acidified with concentrated HCl to dissolve any salts that may have formed during storage and filtered through four layers of cheesecloth to remove any large particulate matter. Urine sample specific gravity was measured using an Adams® Midget Urinometer (Bristol-Myers Squibb Company, New York, NY). Once filtered, composite urine samples were analyzed for P concentration.

Urinary P concentration was determined by colorimetric assay adapted from the procedure of Fiske and Subbarow (1925). Duplicate 1-ml sub-samples were taken from urine composites and treated with 20% TCA. The sample was then centrifuged at 4000 g

for 15 min. A 175- μ l aliquot of the supernatant was then assayed for determination of P concentration. A molybdate aminonaphtholsulfonic acid reagent was used for color development. The reagents formed a phosphomolybdenum blue complex with P. The color development intensified with greater P concentrations. The P concentration of urine was determined at 660 nm against the standard curve (0, 0.25, 0.5, 0.75, 1.0, and 1.25 mg P/dl) by using a SpectraMax 190 spectrophotometer (Molecular Devices, Sunnyvale, CA). The standard curve solutions contained the same volumes and proportions of reagents and water as the unknowns. The 0 mg P/ml standard also was used as a blank to adjust the spectrophotometer for any background P present in the reagents or deionized, distilled water used in the assay. Urine samples were redone if the CV between original duplicates was greater than 10%. The total volume in the microtiter plate represented a 1-to-3 dilution of the original sample. Additionally, if urine P concentration was above the range of the standard curve, original samples were diluted 1-to-6 and reread.

A pooled urine sample as a known control was made by compositing 5-ml aliquots from six individual urine composites. Duplicate 1-ml pooled urine sample aliquots were treated as unknowns in each assay in order to evaluate the variation in the spectrophotometer from assay-to-assay and to calculate the intra- and inter-assay variation. The intra- and inter-assay variations were 4.77 and 11.3%, respectively.

Total urine excretion (kg/d) was predicted to use in the calculation of total urine P excretion (g/d). Two equations were used to determine daily urine excretion, one for non-lactating and one for lactating cows. The equation to predict urine excretion for non-lactating cows was: $(\text{kg/d}) = 212.1 + 0.8822 \times \text{DMI (kg/d)} - 0.03452 \times \text{dietary DM (\%)}$

$+ 1.001 \times \text{dietary CP (\% DM)} - 216.4 \times \text{urine specific gravity (mg/ml)} + 0.1414 \times$
 apparently absorbed water (kg/d) ($R^2 = 0.92$; $P < 0.005$; Holter and Urban, 1992), where
 apparently absorbed water (kg/d; AA) = [feed water intake (kg/d; FWI) + drinking water
 intake (kg/d; DWI)] – fecal water excretion (kg/d). Drinking water intake was predicted
 by the equation: $\text{DWI (kg/d)} = -10.34 + 0.2296 \times \text{dietary DM (\%)} + 2.212 \times \text{DMI (kg/d)}$
 $+ 0.03944 \times \text{dietary CP (\% DM)}^2$ ($R^2 = 0.64$; $P < 0.001$; Holter and Urban, 1992). The
 equation to predict urine excretion for lactating cows was: $(\text{kg/d}) = 332.91 + 0.3885 \times$
 $\text{DMI (kg/d)} - 0.2175 \times \text{MY (kg/d)} - 0.0830 \times \text{dietary DM (\%)} + 0.524 \times \text{dietary CP (\% DM)}$
 $- 326.5 \times \text{urine specific gravity (mg/ml)} + 0.2375 \times \text{AA (kg/d)}$ ($R^2 = 0.70$; $P <$
 0.0001 ; Holter and Urban, 1992). Again, DWI was predicted to determine AA and the
 equation used was: $\text{DWI (kg/d)} = -32.39 + 2.47 \times \text{DMI (kg/d)} + 0.6007 \times \text{milk (kg/d)} +$
 $0.6205 \times \text{dietary DM (\%)} + 0.0911 \times \text{Julian Day} - 0.000257 \times (\text{Julian Day})^2$, where
 Julian Day is the day of the year when January 1 = Julian Day 1 and December 31 =
 Julian Day 365 ($R^2 = 0.69$; $P < 0.0001$; Holter and Urban, 1992). Feed water intake and
 fecal water for both types of cows were determined by: $\text{FWI (kg/d)} = \text{as-fed feed intake}$
 $(\text{kg/d}) - \text{DMI (kg/d)}$ and; $\text{fecal water (kg/d)} = [\text{fecal DM excretion (kg/d)} \div (\text{fecal DM}$
 $(\%) \div 100)] - \text{fecal DM excretion (kg/d)}$.

Milk Sample Analysis. After the conclusion of the sample collection period milk
 samples were thawed in a warm water bath (40 °C) and mixed thoroughly by inversion.
 A single 10-g aliquot of each whole raw milk sample was weighed for P analysis.
 Samples were dried overnight in a 100 °C forced-air oven. Once dried, samples were
 dry-ashed and assayed according to the same protocol as the fecal samples described
 previously. The standard curve developed for determination of P concentration in milk

was 0, 0.5, 0.75, 1.0, and 1.25 mg P/dl. Once milk P% (DM basis) was determined it was converted back to a whole raw milk basis. All milk samples taken (10/cow) were analyzed separately for P concentration and then a 5-d weighted average P concentration was computed for each cow. A 5-d weighted average also was computed for MF, MProt, ML, SNF, and SCC for each cow. Values from statistical analyses are based on the 5-d weighted average for each cow.

Two separate milk sample pools as known controls were made to digest and assay along with the unknowns. Milk pools were made from 2 L of milk obtained from an early lactation (10 DIM) cow. A high milk P pool was made by pipetting a 25-ml aliquot of the milk into a vial, and a low milk pool was made by adding water (approximately 1.0-to-2.5) with water for a total volume of 25 ml. The pools were digested in duplicate along with each digestion of unknowns.

By making milk pools in this manner a check of the assay was created. Completing an analysis of the high and low known control duplicate samples allowed the intra- and inter-assay variations to be calculated. The intra- and inter-assay variations were 1.73 and 6.39%, respectively for the high pool and 1.70 and 6.18%, respectively for the low pool. The overall intra- and inter-assay variations were 1.72 and 6.29%.

A third known control was a 0.80 mg P/dl solution made from a purchased 1.0 mg P/dl certified standard (Fisher Scientific, Chicago, IL). This control solution was assayed with the unknowns to provide an estimate of the assay-to-assay variation of the spectrophotometer. The intra- and inter- assay variations were 0.56 and 1.02%, respectively.

Statistical Analyses

Data from each experiment were analyzed separately. Statistical inferences were made using SAS (1999). Prior to conducting ANOVA for each dependent variable, the arithmetic mean, standard deviation, minimum, and maximum values were calculated to assist in verifying the data sets. Next, an outlier analysis was conducted using the calculated mean $\pm$ 3 SD for each dependent variable. Based on this analysis it was determined that one cow on the T5 diet in Experiment 6 was an outlier in fecal P excretion and P balance and all her data were removed from analysis.

The mixed models procedure was used to determine if the data for each dependent variable in each dataset were normally distributed. This was done using the linear model

$X_{ijk} = \mu + T_i + P_j + T_i \times P_j + C_k (T_i \times P_j) + E_{ijk}$ where:

X_{ijk} is the expected response;

μ is the overall mean of the response;

T_i is the effect of treatment i ;

P_j is the effect of parity j ;

$T_i \times P_j$ is the interaction between treatment i and parity j ;

C_k is the random effect of cow k and is nested within treatment i and parity j ; and,

E_{ijk} is the experimental error.

Within each experiment, cow was random and nested within treatment and parity. The distribution of dependent variable residuals was determined by F-test to be normal if $P > 0.001$. No skewed distributions were detected in the dataset.

Data of dependent variables in each dataset also were tested for heterogeneity. The absolute value of the residuals was computed and used to determine heterogeneity

using the Mixed procedure of SAS (1999). The linear model was $X_{ik} = \mu + T_i + C_k (T_i) + E_{ik}$ where:

X_{ik} is the absolute value of the residual;

μ is the overall mean of the residuals;

T_i is the effect of treatment i ;

C_k is the random effect of cow k and is nested within treatment i ; and,

E_{ik} is the experimental error.

Variances were determined by F-test to be heterogeneous (unequal) if $P < 0.05$.

Variances for each dependent variable were determined to be equal.

A difference among treatment means was significant if $P < 0.05$. A difference with a probability value > 0.05 but < 0.10 , was denoted as a tendency. The least squares method of ANOVA was used to determine the significance of a dependent variable by the mixed model procedure of SAS (1999). The linear model used for Experiments 1 through 7 was $X_{ik} = \mu + T_i + C_k (T_i) + E_{ik}$ where:

X_{ik} is the response of the dependent variable;

μ is the overall mean of the response;

T_i is the effect of treatment i ;

C_k is the random effect of cow k and is nested within treatment i ; and,

E_i is the experimental error.

RESULTS AND DISCUSSION

Non-lactating Animals

Experiment 1. Performance descriptions for pregnant non-lactating heifers in Experiment 1 are in Table 4. Body weight, BCS, DMI, and DMI (% of BW) were not affected by treatment. Apparent DM digestibility decreased linearly with increasing dietary P concentration ($P = 0.03$). The diet was exactly the same except P concentration so the linear decrease was not expected and cannot be explained. Dietary P concentration should not affect apparent DM digestibility in similar diets unless the concentration was very low (NRC, 2001).

Phosphorus balance and characteristics of P excretion are found in Table 5. Phosphorus intake linearly increased with increased dietary P concentration. Which was expected because there were no effects of P concentration on DMI. Fecal DM% was not different among treatments. However, Fecal DM excretion increased linearly (0.03) with increased dietary P%. Fecal P concentration and excretion increased with increased dietary P. Because apparent DM digestibility decreased with increased dietary P concentration, fecal P % and excretion was should increase due to the undigested DM excreted in feces. This is consistent to results reported by Valk et al. (2002). Fecal P excretion increased when non-lactating cows were fed dietary P of 0.16, 0.19, and 0.26%. In the current experiment fecal P excretion (g/kg DMI) increased linearly with increasing dietary P concentration.

Total urine collections were not considered necessary due to the relatively small amount of P excreted in urine. Estimated urine excretion was not affected by treatment. However, urinary P% (0.0009, 0.0008, and 0.0017% for LP, MP, HP, respectively) was linearly ($P = 0.04$) influenced by increased dietary P. Therefore, urinary P excretion (0.11, 0.10, and 0.19 g/d) tended ($P = 0.08$) to increase with increasing dietary P.

Urinary P is generally regarded as minimal since urine is not a major route of excretion and is usually assumed to be 2 to 5% of total P excretion (NRC, 2001). Total P excretion [fecal P + urinary P (g/d)] increased linearly with increased dietary P concentration.

Urine excretion was estimated; therefore, estimated urine P may be inaccurate. However, if the largest average urine excretion across Experiments 1 through 6 is doubled ($22 \text{ kg/d} \times 2 = 44$; Table 11) and multiplied by the average urine P% (0.001%P) associated with that treatment average, only 0.44 g/d would be excreted. Even if urine excretion were under-estimated, the small amount of P in urine would not increase P excretion in urine much.

Phosphorus balance for non-lactating animals equals P intake – total P excretion (Table 5). Phosphorus balance decreased linearly with increased dietary P%.

Additionally, P balances were negative and not different from zero for LP and were different from zero for MP and HP. Salivary P can supply twice as much P to the digestive tract than dietary P (NRC, 2001). Therefore, daily P excretion can be greater than daily intake because P turnover rate can be decreased due to salivary recycling of P.

Dietary P concentration effects on fecal and urinary P excretion (% of P intake) and fecal and urinary P excretion (% of total P excretion) are in Table 6. Total P excretion (% of P intake) was linearly affected by treatment and greater than 100%. Fecal P excretion (% of intake) increased linearly with dietary P concentration and again, is probably due to salivary recycling of P absorbed in excess of P requirements. Urine P excretion (% of P intake) decreased with increased dietary P (quadratic effect; $P = 0.09$) and was a small percentage of P intake as expected. Fecal P excretion (% of total P excretion) tended to increase (99.6, 99.8, and 99.8%, respectively; linear tendency; $P =$

0.09) with increased dietary P concentration. As expected, urine P excretion (% of total P excretion) tended to decrease (0.40, 0.20, 0.18%, respectively; linear tendency; $P = 0.09$) with increased dietary P% because feces tended to increase with increased dietary P. This is contradictory to the NRC (2001) that suggested fecal P excretion is 95 to 98% and urine is 2 to 5% of total P excretion.

Experiment 2. Performance descriptions for pregnant non-lactating heifers in Experiment 2 are in Table 7. Body weight, BCS, DMI, and DMI (% of BW) were not different among treatments. However, apparent DM digestibility tended to be affected quadratically by dietary P concentration. Again, this was not expected and cannot be explained. Dietary P concentration should not affect apparent DM digestibility in similar diets unless the concentration was very low (NRC, 2001).

Phosphorus balance and characteristics of P excretion are found in Table 8. Dietary P concentration linearly affected P intake. Dietary P concentration did not affect fecal DM% and excretion. Fecal P% increased with increased dietary P concentration; therefore, fecal P excretion also increased with increased dietary P. Again this is consistent with the results of Valk et al. (2002). Inevitable fecal P excretion (g/kg DMI) was linearly decreased with increased dietary P.

Estimated urine excretion tended to be quadratically affected ($P = 0.08$) by treatment. Urinary P% increased linearly ($P = 0.03$) with increasing dietary P. Therefore, urinary P excretion (0.04, 0.10, and 0.14 g/d) increased linearly ($P = 0.01$) with increasing dietary P%.

Phosphorus balance was -1.13 , 1.38 , and -7.66 g/d for LP, MP, and LP, respectively and tended to be quadratically ($P = 0.10$) affected by treatment. Phosphorus

balance was not different from zero for LP and MP; P balance for HP was different than zero indicating they were over-fed P.

Total P excretion (% of P intake), fecal P (% of P intake), and urine P (% of P intake) were not different among treatments (Table 9). Fecal P excretion (% of total P excretion) was about 99.7% among treatments and was not affected by dietary treatments. Urinary P excretion (% of total P excretion) was about 0.35% and not affected by dietary P concentration.

Experiment 3. Performance descriptions for pregnant non-lactating cows are reported in Table 10. Body weight, BCS, and DMI (% of BW) were not different among treatments. However, DMI was linearly different and decreased with increasing dietary P. However, the DMI difference was more likely due to randomization of cows to treatments because 30 d is a short period of time to cause a DMI different due to treatment. Apparent DM digestibility was not different between treatments.

Phosphorus balance and characteristics of P excretion are found in Table 11. Phosphorus intake was linearly influenced by dietary P. The HP treatment, despite the lowest DMI, had the greatest P intake. Fecal DM% and excretion were not different between treatments despite the difference in DMI mentioned previously. However, fecal P% and excretion increased linearly with increased dietary P. Which again is similar to results of Valk et al. (2002). Inevitable fecal P excretion (g/kg DMI) was linearly ($P < 0.01$) affected by increased dietary P.

Estimated urine excretion decreased linearly ($P = 0.02$) with increased dietary P and cannot be explained by dietary P concentration. Urinary P% and excretion were not affected by dietary treatments. Phosphorus balance was linearly affected by dietary P

concentration. Additionally, P balance was different from zero in HP, tended to be different for MP, and was not different for LP. The results from this experiment, like previously mentioned experiments, indicate that the cows on all three treatments were overfed P relative to their requirements because P balance was different from zero.

Total, fecal, and urine P excretion (% of P intake) were not different among treatments. Fecal P and urine P excretion (% of total P excretion) also were not different among treatments.

Experiment 4. Performance descriptions for pregnant non-lactating cows are found in Table 13. Body weight tended to be quadratic ($P = 0.10$) that is, the cows on MP tended to weigh more than the cows on the LP and HP treatments. This probably occurred during random assignment of cows to treatments. Body condition score, dry matter intake, DMI (% of BW), and apparent DM digestibility did not differ between treatments.

Results relative to P balance and excretion are in Table 14. Phosphorus intake increased linearly with dietary P concentration. Fecal DM% and fecal DM excretion were not different between treatments ($P = 0.15$ and 0.23 , respectively). Fecal P% and excretion increased linearly as dietary P concentration increased, and is similar to the results of Valk et al. (2002). Inevitable fecal P excretion (g/kg DMI) was linearly affected by increased dietary P.

Estimated urine excretion decreased linearly as dietary P increased and cannot be explained by dietary treatments. Dietary P concentration did not affect urine P%. And, despite the linear decrease in urine excretion, urinary P excretion (0.08, 0.06, and 0.07 g/d) was not different among treatments. Phosphorus balance tended to decrease linearly

as dietary P was increased. Additionally, P balance was not different from zero for LP and MP. P balance tended to be different from zero in HP ($P = 0.07$).

Phosphorus excretion results are reported in Table 15. Fecal P (% of P intake) increased linearly with increased dietary P concentration and urine P (% of P intake) decreased linearly with increased dietary P concentration. Total P excretion (% of P intake) followed fecal P (% of P intake) and tended to increase linearly with dietary P concentration. Fecal P excretion (% of total P excretion) tended to quadratically increase with increased dietary P concentration; urinary P excretion (% of total P excretion) tended to quadratically decrease with increased dietary P concentration.

Experiment 5. Performance descriptions for lactating cows (106 ± 10.0 DIM) are in Table 16. Body weight, BCS, DMI, DMI (% of BW), and apparent DM digestibility were not different among treatments. Days in milk tended to be quadratic ($P = 0.07$) between treatments. This occurred when cows were randomly assigned to treatments. Despite the quadratic relationship of DIM, spread was not sufficient for MY or energy corrected milk (ECM) to be different among treatments. Milk fat concentration was different between treatments (quartic effect; $P = 0.05$). This does not agree with other studies that reported the effect of dietary P concentration on MF. Dietary P concentration ranging from 0.24 to 0.67% P had no effect on milk fat percentage or yield (Wu et al., 2002; Wu et al., 2001; Knowlton and Herbein, 2002; Wu et al., 2000; Valk and Šebek, 1999; Call et al., 1987; Carstairs et al., 1981; Steevens et al., 1971). Milk protein% tended to be quadratically ($P = 0.09$) affected by dietary P concentration. Again, this is different from previous research. When cows were fed dietary P concentrations ranging from 0.24 to 0.67% P, MP was not affected (Wu et al., 2002; Knowlton and Herbein,

2002; Knowlton et al., 2001; Wu et al., 2000, Valk and Šebek, 1999; Brintrup et al., 1993; Brodison et al., 1989).

Dietary P had a quartic ($P = 0.03$) effect on Milk lactose%. Again, this was different than other studies. When dietary P ranging from 0.31 to 0.49% P was fed to lactating cows, ML was not affected (Wu et al., 2002; Wu et al., 2001; Knowlton and Herbein, 2002; Knowlton et al., 2001; Wu et al., 2000; Wu and Satter, 2000). Milk solids-not-fat concentration was not affected by dietary treatments. This was similar to other studies. Dietary P ranging from 0.31 to 0.60% P did not affect SNF% (Wu et al., 2002; Wu et al., 2001; Knowlton and Herbein, 2002; Knowlton et al., 2001; Wu et al., 2000; Wu and Satter, 2000; Steevens et al., 1971).

Results relative to P balance and excretion are reported in Table 17. Phosphorus intake increased with increased dietary P concentration. Fecal DM% and fecal DM excretion were not different between treatments. Fecal P% and excretion increased with increased dietary P concentration when cows were fed dietary P ranging from 0.24 to 0.67% P (Kohn et al., 2002; Knowlton and Herbein, 2002; Wu et al., 2002; 2001; 2000; Valk et al., 2002; Brintrup et al, 1993; Morse et al., 1992b; Martz et al, 1990). The results from this experiment support these observations. Fecal P concentration tended to increase with increased dietary P concentration (quadratic effect; $P = 0.09$). This difference led to a tendency for increase fecal P excretion (cubic effect; $P = 0.09$). Inevitable fecal P excretion (g/kg DMI) tended to quadratically decrease with increased dietary P concentration.

Estimated urine excretion was not different between treatments. A cubic relationship tended to occur in urine P%. However, urine P excretion did not follow this

same trend and was not affected by dietary P concentration. This disagrees with previous research that measured urine P excretion; as dietary P concentration increased (0.30 to 0.67%), urine P excretion also increased (Knowlton and Herbein, 2002; Morse et al., 1992b). Total P excretion increases linearly as dietary P concentration was increased. Which would be expected because the major component of total P excretion (fecal P excretion) was linearly decreased with increased dietary P%.

Milk P concentration tended to be affected by dietary P concentration (quartic effect; $P = 0.10$). This disagrees with other studies in which feeding dietary P from 0.24 to 0.67% did not affect milk P% (Knowlton and Herbein, 2002; Wu et al., 2000; Brintrup et al., 1993; Brodison et al., 1989; Call et al., 1987; Forar et al., 1982). Milk P concentration was found to range between 0.085 – 0.10% (Knowlton and Herbein, 2002; Wu et al., 2000; Brintrup et al., 1993; Spiekers et al., 1993; Brodison et al., 1989; Flynn and Power 1985). The quartic tendency may be partially explained by the quartic effects reported for MF and MProt. Approximately 32% of P in milk is associated with milk fat and protein (Walstra and Jenness, 1984). Therefore, increased milk fat or protein concentrations potentially will increase milk P concentration. Treatments with higher MF and MProt had higher milk P%, most notably T3 had 10% higher MF and 6% higher milk P% (3.52 and 0.088%, respectively) than the average of the other four treatments (3.16 and 0.083%, respectively). However, this slight difference in milk P% was not enough to influence milk P yield ($P = 0.30$). Phosphorus balance ($P \text{ balance (g/d)} = P \text{ intake (g/d)} - [\text{fecal P (g/d)} + \text{urinary P (g/d)} + \text{milk P (g/d)}])$ had a cubic relationship with dietary P excretion. Phosphorus balance for T1 and 2 was not different from zero (t-test; $P = 0.20$ and 0.38) and P balance for T3 through 5 was different from zero ($P < 0.01$

for T3 and $P < 0.01$ for T4 and 5). A P balance different from zero indicates that dietary P was overfed.

Results relative to P excretion are presented in Table 18. Fecal P (% of P intake) tended to decrease with increased dietary P. This means that fecal P decreased with increased dietary P%. However, urine P (% of P intake) tended to increase cubically with P intake. Since fecal P (% of P intake) decreased and urine P (% of intake) tended to increase, some of the excess P was shifted to urine. As a result total P excretion (% of intake) increased linearly with dietary P concentration. Dietary P concentration tended to have a cubic effect on fecal and urine P excretion (% of total P excretion). Overall, these values were within the ranges assumed by the NRC (2001). Fecal and urine P excretion (% of total P excretion) averaged 96.7 and 3.3%, respectively. Milk P (% of P intake) decreased linearly with increased dietary P concentration. This would be expected since milk composition is relatively constant. Milk P % tended to increase (quartic effect; $P = 0.10$), but milk P secretion was not different among treatments (Table 17). Milk P (% of total P output) tended to decrease with dietary P concentration (quartic effect; $P = 0.09$).

Experiment 6. Performance descriptions for lactating cows (183 ± 6.2 DIM) are in Table 19. Body weight, DIM, DMI, and DMI (% of BW) were not different among treatments. Body condition score had a quartic (0.0248) relationship between treatments. This was unexpected and occurred when cows were randomly assigned to treatments. Apparent DM digestibility was different between treatments (quadratic effect; $P = 0.04$). This was not expected because all cows were fed the same diet except for P concentration; therefore, there should be no digestibility difference unless the dietary P concentration was low (NRC, 2001).

Milk yield tended to be different and ECM had a cubic relationship with dietary P concentration (quartic tendency and cubic effect; $P = 0.08$ and $P = 0.02$, respectively) among treatments. This MY and ECM difference may be partially explained by DMI. Although DMI was not significant, T2 cows had numerically less DMI than the other treatments and subsequently less MY. Dry matter intake is directly related to MY (NRC, 2001). Milk fat concentration tended to be affected by dietary treatment (quadratic effect; $P = 0.07$). This disagrees with other studies that reported MF as previously mentioned (Wu et al., 2002; Wu et al., 2001; Knowlton and Herbein, 2002; Wu et al., 2000; Valk and Šebek, 1999; Call et al., 1987; Carstairs et al., 1981; Steevens et al., 1971). Milk protein, lactose, and solids-not-fat concentrations were not affected by dietary P concentration ($P = 0.94, 0.57$, and 0.87 , respectively). This agrees with other research mentioned previously where dietary P concentration did not influence these milk components (Wu et al., 2002; Knowlton and Herbein, 2002; Knowlton et al., 2001; Wu et al., 2000, Valk and Šebek, 1999; Brintrup et al., 1993; Brodison et al., 1989; Steevens et al., 1971).

Results relative to P balance and excretion are in Table 20. Fecal DM concentration was different between treatments (quartic effect; $P < 0.01$). This was unexpected and cannot be explained by dietary concentration. However, this did not translate to a difference in fecal DM concentration, which was not different between treatments ($P = 0.98$). Phosphorus intake (56.0, 64.0, 80.1, 90.3, and 102.1 g/d, respectively for T1, 2, 3, 4, and 5) was linearly ($P < 0.01$) affected by dietary P concentration. Fecal P concentration and excretion increased with increased dietary P concentration (quadratic effect; $P = 0.04$; and, linear effect; $P < 0.01$). Again, this is

similar to the results reported previously (Wu et al., 2002; 2000, Knowlton and Herbein, 2002; Valk et al., 2002).

Estimated urine excretion increased quadratically between treatments. Dietary P concentration tended to increase urine P concentration linearly and did increase urine P excretion linearly. Which is supported by results reported by Knowlton and Herbein (2002) and Morse et al. (1992b) per previously mentioned.

Milk P concentration was not affected by dietary P concentration ($P = 0.61$). This agrees with the other studies that reported no affect on milk P concentration mentioned previously (Knowlton and Herbein, 2002; Wu et al., 2000; Brintrup et al., 1993; Brodison et al., 1989; Call et al., 1987; Forar et al., 1982). Dietary P concentration tended to affect milk P yield (cubic effect; $P = 0.07$). This may be partially explained by the quartic difference between MYs. The treatment with the lowest MY (T2) had the lowest milk P yield. Additionally, because T5 had numerically the lowest milk P concentration, less milk P was secreted in milk.

Phosphorus balance decreased with increased dietary P (cubic affect; $P = 0.04$). Additionally, P balance was not different from zero for T1, T2, and T3, but was different from zero in T4 and T5. Again, P balance different from zero indicates that P was overfed relative to P requirements.

Results relative to P excretion are presented in Table 21. Fecal P (% of P intake) increased linearly with increased dietary P concentration, and urine P (% of P intake) tended to increase linearly with increased dietary P. Meaning that more of the intake P was being excreted rather than utilized. Milk P (% of intake) and increased linearly with increased P intake. Again, this would be expected because milk composition is relatively

constant. Milk P (% of total P output) tended to decrease quadratically. However, this may be partially due to a tendency for decreased milk yield with higher dietary P concentration. Dietary P concentration did not affect fecal or urine P excretion (% of total P excretion) and was not in the range (average of 99.1 and 0.9%) of what the NRC (2001) assumed fecal and urine P excretion (% of total P excretion) to be (2 to 5%).

Experiment 7. Due to the method of intake restriction mentioned previously, treatment effects are confounded with DMI. Intake was restricted based on milk production and cows with MY greater than 25 kg/d were not intake restricted. Therefore, cows consuming ad libitum DMI were not evenly distributed across treatments. Eleven of 34 cows were allowed to consume ad libitum intake, three in T1, five in T2, two in T3, one in T4, and zero in T5. Therefore, ANOVA of dependent variables were not conducted on this dataset. However, these data were very valuable for evaluation of models to predict P excretion.

CONCLUSIONS

Throughout this series of experiments there are several dependent variables that were affected by dietary P concentration. In general, DMI and MY were not affected by dietary P excretion. This is supported by other studies. Seven studies reported that feeding lactating cows diets containing 0.30 to 0.60% P had no influence on MY (cows producing 25 to 40 kg milk/d; Wu et al., 2002; 2001; 2000; Kohn et al., 2002; Wu and Satter, 2000; Brintrup et al., 1993; Forar et al., 1982). However, the duration for the lactating experiments was only 30 d which probably was not long enough to pick up a

difference in DMI or MY. This was not a problem because the priority objective was to collect P excretion data. Phosphorus excretion differences will occur more quickly because DM excretion is correlated with DMI and rumen turnover is approximately 48 h.

All experiments showed increased fecal P concentration and excretion when dietary P concentration increased. This is supported by several studies where dietary P concentration was increased from 0.30 to 0.67% (Kohn et al., 2002; Knowlton and Herbein, 2002; Wu et al., 2002; 2001; 2000; Valk et al., 2002 Brintrup et al, 1993; Morse et al., 1992b; Martz et al, 1990). In general, increased dietary P also increased urinary P concentration and excretion. Similarly, reports from studies that reported urine P excretion showed that as dietary P concentration increased (0.30 to 0.67%), urine P excretion also increased (Knowlton and Herbein, 2002; Morse et al., 1992b). However, Wu et al. (2000) found that there was no difference in urine P excretion when cows were fed diets with 0.31, 0.41, and 0.49% P. Urinary P excretion was small, which is typical for ruminants. Urine P excretion in the current experiments generally represented less than the 2 to 5% of total P excretion generally assumed when urine P excretion is estimated (NRC, 2001).

Phosphorus balance is an important indicator of P status. For Experiments 1 through 4, P balance was negative and different from zero when animals consumed the HP treatment diet. For Experiments 5 and 6, treatments 3, 4, and 5 had negative P balances that were different from zero. Furthermore, Wu et al. (2000) suggested that apparent P digestibility $\leq 40\%$ for lactating cows was indicative of overfeeding P. In experiments 5 and 6, apparent P absorption was $\leq 40\%$ in all treatments except T1 [0.27% P (DM basis)].

Data generated from these seven experiments construct an invaluable dataset that will be used in subsequent research to evaluate models used to predict P excretion.

Table 1. Formulated ingredient and analyzed chemical composition of the basal diet for Experiments 1, 2, 3, and 4.

Item	Treatment ¹		
	LP	MP	HP
Percent of dietary DM			
Ingredients			
Alfalfa silage	17.5	17.5	17.5
Corn silage	27.0	27.0	27.0
Beet pulp pellets, dehydrated without molasses	27.0	27.0	27.0
Corn starch	9.1	9.1	9.1
Corn, ground	9.0	9.0	9.0
Mineral-vitamin mix ²	3.3	3.3	3.3
SoyChlor 16-7 ^{TM 3}	2.1	2.1	2.1
Rice hulls	1.8	1.3	0.9
Blood meal	1.1	1.1	1.1
Biuret ^{TM 4}	0.9	0.9	0.9
Urea	0.8	0.7	0.5
Ammonium chloride	0.4	0.4	0.4
Monoammonium phosphate	0	0.7	1.2
Chemical composition⁵			
DM	58.1	58.1	58.1
CP	14.6	14.1	14.7
NE _L ⁶ , Mcal/kg	0.72	0.72	0.72
ADF	25.8	25.6	25.3
NDF	39.6	39.4	39.2
Ash	5.0	5.2	5.4
Ca	0.62	0.61	0.61
P	0.19	0.28	0.35
Mg	0.33	0.33	0.34
K	1.1	1.1	1.1
Na	0.1	0.1	0.10
Cl	0.1	0.1	0.10
S	0.24	0.24	0.25
Cu, mg/kg	22.4	21.9	21.6
Fe, mg/kg	299	328	355
Mn, mg/kg	85.3	82.6	79.7
Zn, mg/kg	44.68	41.6	42.0

¹Treatments: LP = 0.19, MP = 0.28, and HP = 0.35% dietary P.

²Composition (DM basis): 91.4% soybean hulls; 8% magnesium sulfate; 0.14% manganese sulfate; 0.11% zinc sulfate; 0.06% copper sulfate; 0.06% Se; 0.001% cobalt sulfate; 0.001% ethylenediaminodihydroiodide (EDDI) 488 KIU vitamin A/kg; 71 KIU vitamin D/kg; and, 2.5 KIU vitamin E/kg.

³Graciously provided by West Central® Soy, Ralston, IA.

⁴Graciously provided by Moorman's Manufacturing Company, Quincy, IL.

⁵Chemical composition was determined by Dairy One Forage Lab (Ithaca, NY) except for P and ash.

⁶Energy value estimated based on computations per NRC (2001).

Table 2. Formulated ingredient and analyzed chemical composition of the basal diet for Experiments 5, 6, and 7.

Item	Treatment ¹				
	T1	T2	T3	T4	T5
Percent of dietary DM					
Ingredient					
Alfalfa silage	11.4	11.4	11.4	11.4	11.4
Corn silage	19.3	19.3	19.3	19.3	19.3
Beet pulp pellets, dehydrated without molasses	25.0	25.0	25.0	25.0	25.0
Whole cottonseed	11.0	11.0	11.0	11.0	11.0
Corn starch	10.9	10.6	10.3	10.0	9.7
Corn, dry ground	13.4	13.4	13.4	13.4	13.4
Soybean meal, 44% CP	3.9	3.9	3.9	3.9	3.9
Blood meal	1.3	1.3	1.3	1.3	1.3
Corn oil	0.1	0.1	0.1	0.1	0.1
Limestone	0.8	0.8	0.8	0.8	0.8
Salt (NaCl)	0.4	0.4	0.4	0.4	0.4
Urea	0.7	0.6	0.5	0.4	0.3
Monoammonium phosphate	0.0	0.4	0.8	1.2	1.6
Biuret ^{TM 2}	0.8	0.8	0.8	0.8	0.8
Vitamin and mineral premix ³	1.0	1.0	1.0	1.0	1.0
Chemical composition⁴					
DM	59.7	59.7	59.8	59.8	59.8
CP	20.8	20.4	20.2	20.2	20.2
NE _L ⁵ , Mcal/kg	0.76	0.76	0.76	0.76	0.76
ADF	22.1	22.1	21.9	21.9	21.9
NDF	37.3	37.5	37.2	37.0	37.2
Ash	4.1	4.4	4.8	5.0	5.3
Ca	0.94	0.94	0.94	0.95	0.95
P	0.27	0.36	0.42	0.46	0.52
Mg	0.23	0.24	0.24	0.24	0.25
K	1.1	1.1	1.1	1.1	1.1
Na	0.39	0.39	0.39	0.39	0.40
Cl	0.36	0.36	0.36	0.36	0.36
S	0.21	0.21	0.22	0.22	0.23
Cu, mg/kg	19	18	18	18	19
Fe, mg/kg	325	364	404	446	534
Mn, mg/kg	80	82	83	84	87
Zn, mg/kg	82	82	82	82	83

¹Treatments: T1 = 0.27, T2 = 0.36, T3 = 0.42, T4 = 0.46, and T5 = 0.52.

²Graciously provided by Moorman's Manufacturing Company, Quincy IL

³Trace mineral and vitamin premix was 71.4% dry ground corn, 25.5% vitamins and minerals, and 3.1% corn oil. The concentrations of the vitamins and minerals were: 0.05% Ca; 0.28% P; 0.60% Mg; 3.3% K; 1.4% S; 0.01% Na; 332 mg/kg Cu; 699 mg/kg Mn; 894 mg/kg Zn; 90 mg/kg Fe; 4 mg/kg Co; 7 mg/kg Se; 8 mg/kg I; 24 KIU vitamin A/kg; 5 KIU vitamin D/kg; and, 0.09 KIU vitamin E/kg, dry basis.

⁴Chemical composition was determined by Dairy One Forage Lab (Ithaca, NY) except for P and ash.

⁵Energy values estimated based on computation per NRC (2001).

Table 3. Description of all variables used to evaluate experimental objectives.

Variable	Abbreviation, units	Comments	Calculated or Measured (M)
Body weight	BW, kg	Animal descriptor, used in calculating P excretion from prediction models	M
Body condition score	BCS, 1 to 5 scale	descriptor	M
Dry matter intake	DMI, kg/d	descriptor and treatment for Maintenance Experiment	M
Dry matter intake as % percent of body weight	DMI, %BW	descriptor	$DMI, \%BW = (DMI, \text{kg/d} \div BW, \text{kg}) \times 100$
Days in milk	DIM	descriptor	M
Apparent DM digestibility %	AppDMdig, %	Diet evaluation	$AppDMdig, \% = ((DMI, \text{kg/d} - (FecDM, \text{g/d} \div 1000)) / DMI, \text{kg/d}) \times 100$
Milk yield	MY, kg/d	descriptor, used to calculate milk P concentration and used in calculating P excretion in prediction models	
Milk fat ¹ , %	MF, %	descriptor	M
Milk protein ¹ , %	MProt, %	descriptor	M
Milk lactose ¹ , %	ML, %	descriptor	M
Milk solids-not-fat ¹ , %	SNF, %	descriptor	M
Somatic cell count ¹	SCC, 1000 cells/ml	descriptor	M
Dietary P, % of dietary DM	DietP, %	Treatment for most experiments and used in calculating P excretion from prediction models	M
P intake	PI, g/d	Used in calculating P excretion from prediction models	$PI, \text{g/d} = DMI, \text{kg/d} \times (DietP, \% \div 100)$
Fecal DM excretion	FecDM, g/d	Used to calculate daily fecal P excretion	$FecDM, \text{g/d} = (Cr \text{ fed, g/d} \div Cr \text{ in fecal sample, \%}) \times 100$ [Harris, 1970]
Fecal P, %	FecP, %	Used to calculate daily fecal P excretion	M
Fecal P excretion	FecPE, g/d	Used to calculate daily P balance	$FecPE, \text{g/d} = FecDM, \text{g/d} \times (FecP, \% \div 100)$
Urine excretion	UE, kg/d	Used to calculate daily P excretion	$UE^2, \text{kg/d} = 332.91 + 0.2885 \times DMI - 0.2175 \times MY - 0.083 \times \text{dietary DM \%} + 0.524 \times \text{dietary CP, \% DM} - 326.5 \times SG + 0.2375 \times AppAbsP$ [Holter and Urban, 1992]
Urine P, %	UP, %	Used to calculate daily P excretion	M
Urine P yield	UPY, g/d	Used to calculate daily P balance	$UP, \text{g/d} = (UE, \text{kg/d} \times (UP, \% \div 100)) \times 1000$

Milk P ¹ , %	MP, %	Used to calculate daily milk P yield	M
Milk P yield	MPY, g/d	Used to calculate daily P balance	$MPY, g/d = MY, kg/d \times (MP, \% \div 100) \times 1000$
P balance	Pbal, g/d	Evaluation of P status	$^3Pbal = PI, g/d - (MPY, g/d + FecPE, g/d + UPY, g/d)$
Fecal P excretion, g/kg DMI	PMR, g P/kg DMI	Estimate of the truly absorbed P maintenance requirement	$PMR, g P/kg DMI = PI, g/d \div DMI, kg/d$
Total P excretion	PE, g/d	Estimate of total daily P excretion	$PE, g/d = FecPE, g/d + UPY, g/d$
Fecal P, % of total P excretion	FecPexc, %	P utilization estimate	$(FecP, g/d \div PE, g/d) \times 100$
Urine P, % of total P excretion	UrPexc, %	P utilization estimate	$(UrP, g/d \div PE, g/d) \times 100$
Fecal Cr	FecCr, %	Used to calculate daily fecal DM excretion and AppDMdig%	M
Fecal DM, %	FecDM, %	Used to calculate fecal water	M
Fecal water	FecW, kg/d	Used to calculate AppAbsW	$FecW, kg/d = (FecDM, g/d \div (FecDM, \% \div 100)) - FecDM$
Feed water intake	FWI, kg/d	Used to calculate total water intake	$FWI, g/d = As Fed Feed Intake, kg/d - DMI, kg/d$
Drinking water intake	DWI, kg/d	Used to calculate Total Water Intake	$^4DWI, kg/d = -32.39 + 2.47 \times DMI, kg/d + 0.6007 \times MY, kg/d + 0.6205 \times Dietary DM, \% + 0.0911 \times Julian Day - 0.000257 \times (^5Julian Day)^2$ [Holter and Urban, 1992]
Total water intake	TWI, kg/d	Total amount of water consumed per day	$TWI, kg/d = FWI, kg/d + DWI, kg/d$
Apparently absorbed water	AppAbsW, kg/d	Used to calculate urine excretion, kg/d	$AppAbsW, kg/d = TWI, kg/d - FecW, kg/d$
Urine specific gravity	SG, mg/ml	Used to calculate urine excretion, kg/d	M

¹Component weighted averages calculated as: (a.m. component, % $\times$ a.m. MY, kg + p.m. component, % $\times$ p.m. MY, kg) $\div$ (a.m. MY $\times$ p.m. MY, kg).

² For dry cows, the following equation was used: $UE, kg/d = 212.1 + 0.8822 \times DMI, kg - 0.03452 \times dietary DM, \% + 1.001 \times dietary CP, \% DM - 216.4 \times SG, mg/ml + 0.1414 \times AppAbsP, g/d$ [Holter and Urban, 1992].

³ For dry cows, the following equation was used: $Pbal, g/d = PI, g/d - (FecPE, g/d + UPY, g/d)$.

⁴ For dry cows, the following equation was used: $DWI, kg/d = -10.34 + 0.2296 \times Dietary DM, \% + 2.212 \times DMI, kg/d + 0.03944 \times (Dietary CP, \% DM)^2$ [Holter and Urban, 1992].

⁵ Julian Day is the day of the year when January 1 = Julian day 1 and December 31 = Julian day 365.

Table 4. Performance characteristics of nulliparous heifers (57 to 28 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 1).¹

Item	Treatment ²			SEM	P-value ³		
	LP	MP	HP		Treatment	Linear	Quadratic
n	7	7	7	--	--	--	--
BW, kg	693	700	669	18.5	NS ⁴	NS	NS
BCS	3.6	3.7	3.7	0.09	NS	NS	NS
DMI, kg/d	12.3	12.9	12.7	0.78	NS	NS	NS
DMI, %BW	1.8	1.9	1.9	0.13	NS	NS	NS
Apparent DM digestibility, %	70.6	66.1	61.9	2.60	0.08	0.03	NS

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

Table 5. Phosphorus balance and characteristics of feces and urine for nulliparous heifers (57 to 28 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 1).¹

Item	Treatment ²			SEM	P-value ³		
	LP	MP	HP		Treatment	Linear	Quadratic
P intake, g/d	23.3	36.1	44.5	2.41	<0.01	<0.01	NS ⁴
Fecal DM, %	15.8	15.6	15.7	0.44	NS ⁴	NS	NS
Fecal DM excretion, g/d	3551	4373	4823	397.4	0.10	0.03	NS
Fecal P, % of DM	0.97	1.42	2.21	0.117	<0.01	<0.01	0.10
Fecal P excretion, g/d	34.1	63.5	107.5	9.56	<0.01	<0.01	NS
g fecal P/kg DMI	2.9	4.8	8.4	0.59	<0.01	<0.01	NS
Urine excretion ⁵ , kg/d	12.3	12.9	11.6	1.02	NS	NS	NS
Urine P, %	0.001	0.001	0.002	0.0003	0.04	0.04	NS
Urinary P excretion, g/d	0.11	0.10	0.19	0.030	0.09	0.08	NS
Total P excretion ⁶ , g/d	34.2	63.6	107.7	9.57	<0.01	<0.01	NS
P balance ⁷ , g/d	-10.91 ⁸	-27.50 ⁹	-63.3 ⁹	8.17	<0.01	<0.01	NS

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

⁵Estimated from urine excretion prediction model (Holter and Urban, 1992).

⁶Total P excretion (g/d) = fecal P + urinary P (g/d) excretion.

⁷P balance (g/d) = P intake (g/d) - total P excretion (g/d).

⁸Mean is not different from zero ($P > 0.10$).

⁹Mean is different from zero ($P < 0.05$).

Table 6. Phosphorus excretion by nulliparous heifers (57 to 28 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 1).¹

Item	Treatment ²				P-value ³		
	LP	MP	HP	SEM	Treatment	Linear	Quadratic
Total P excretion, % of P intake	150.8	172.2	241.4	21.60	0.02	<0.01	NS
Fecal P, % of P intake	150.3	172.0	241.0	21.62	0.02	<0.01	NS
Fecal P, % of total P excretion	99.6	99.8	99.8	0.09	NS	0.09	NS
Urine P, % of P intake	0.46	0.29	0.43	0.074	NS	0.09	NS
Urine P, % of total P excretion	0.40	0.20	0.18	0.090	NS	0.09	NS

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

Table 7. Performance characteristics of nulliparous heifers (38 to 9 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 2).¹

Item	Treatment ²			SEM	P-value ³		
	LP	MP	HP		Treatment	Linear	Quadratic
n	7	7	7	--	--	--	--
BW, kg	715	723	692	17.2	NS ⁴	NS	NS
BCS	3.7	3.8	3.8	0.10	NS	NS	NS
DMI, kg/d	8.0	10.0	9.2	0.94	NS	NS	NS
DMI, %BW	1.1	1.4	1.3	0.14	NS	NS	NS
Apparent DM digestibility, %	53.3	65.0	56.2	4.18	NS	NS	0.07

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

Table 8. Phosphorus balance and characteristics of feces and urine for nulliparous heifers (38 to 9 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 2).¹

Item	Treatment ²				P-value ³		
	LP	MP	HP	SEM	Treatment	Linear	Quadratic
P intake, g/d	15.3	27.9	32.1	2.76	<0.01	<0.01	NS
Fecal DM, %	16.5	16.9	16.3	0.52	NS ⁴	NS	NS
Fecal DM excretion, g/d	3718	3357	3931	395.9	NS	NS	NS
Fecal P, % of DM	0.44	0.81	1.03	0.04	<0.01	<0.01	NS
Fecal P excretion, g/d	16.4	26.5	39.6	2.64	<0.01	<0.01	NS
g fecal P/kg DMI	2.1	2.8	4.4	0.31	<0.01	<0.01	NS
Urine excretion ⁵ , kg/d	8.1	11	9.2	0.97	NS	NS	0.08
Urine P, %	0.0006	0.0009	0.0015	0.0003	0.08	0.03	NS
Urinary P excretion, g/d	0.04	0.10	0.14	0.94	0.04	0.01	NS
Total P excretion ⁶ , g/d	16.4	26.6	39.8	2.64	<0.01	<0.01	NS
P balance ⁷ , g/d	-1.1 ⁸	1.5 ⁸	-7.5 ⁹	2.88	0.10	NS	0.10

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

⁵Estimated from urine excretion prediction model (Holter and Urban, 1992).

⁶Total P excretion (g/d) = fecal P + urinary P (g/d) excretion.

⁷P balance (g/d) = P intake (g/d) - total P excretion (g/d).

⁸Mean is not different from zero ($P > 0.10$).

⁹Mean is different from zero ($P < 0.05$).

Table 9. Phosphorus excretion by nulliparous heifers (38 to 9 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 2).¹

Item	Treatment ²			SEM	P-value ³		
	LP	MP	HP		Treatment	Linear	Quadratic
Total P excretion, % of P intake	108.7	100.9	127.2	10.50	NS	NS	NS
Fecal P, % of P intake	108.4	100.5	126.7	10.47	NS	NS	NS
Fecal P, % of total P excretion	99.7	99.6	99.7	0.10	NS	NS	NS
Urine P, % of P intake	0.28	0.40	0.46	0.12	NS	NS	NS
Urine P, % of total P excretion	0.27	0.44	0.34	0.10	NS	NS	NS

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

Table 10. Performance characteristics of non-lactating multiparous cows (59 to 30 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 3).¹

Item	Treatment ²			SEM	P-value ³		
	LP	MP	HP		Treatment	Linear	Quadratic
n	6	7	7	--	--	--	--
BW, kg	784	800	748	27.2	NS ⁴	NS	NS
BCS	3.8	3.9	3.7	0.11	NS	NS	NS
DMI, kg/d	20.4	19.6	17.0	1.06	0.09	0.04	NS
DMI, %BW	2.6	2.4	2.3	0.16	NS	NS	NS
Apparent DM digestibility, %	66.8	69.8	65.6	1.82	NS	NS	NS

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

Table 11. Phosphorus balance and characteristics of feces and urine for non-lactating multiparous (59 to 30 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 3).¹

Item	Treatment ²			SEM	P-value ³		
	LP	MP	HP		Treatment	Linear	Quadratic
P intake, g/d	38.8	54.8	59.4	3.16	<0.01	<0.01	NS
Fecal DM, %	15.2	16.3	15.5	0.37	NS ⁴	NS	0.07
Fecal DM excretion, g/d	6730	5868	5899	473.6	NS	NS	NS
Fecal P, % of DM	0.77	1.18	1.65	0.13	<0.01	<0.01	NS
Fecal P excretion, g/d	50.3	70.9	98.3	10.87	0.02	<0.01	NS
g fecal P/kg DMI	1.61	3.09	4.36	0.87	NS	NS	NS
Urine excretion ⁵ , kg/d	22.0	20.6	17.2	1.24	0.04	0.02	NS
Urine P, %	0.001	0.005	0.001	0.003	NS	NS	NS
Urinary P excretion, g/d	0.17	1.29	0.14	0.30	NS	NS	NS
Total P excretion ⁶ , g/d	50.52	72.13	98.40	11.07	0.03	<0.01	NS
P balance ⁷ , g/d	-11.7 ⁸	-17.3 ⁸	-39.0 ¹⁰	8.54	0.08	0.05	NS

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

⁵Estimated from urine excretion prediction model (Holter and Urban, 1992).

⁶Total P excretion (g/d) = fecal P + urinary P (g/d) excretion.

⁷P balance (g/d) = P intake (g/d) - total P excretion (g/d).

⁸Mean is not different from zero ($P > 0.10$).

⁹Mean is different from zero ($P < 0.05$).

Table 12. Phosphorus excretion by non-lactating multiparous cows (59 to 30 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 3).¹

Item	Treatment ²				P-value ³		
	LP	MP	HP	SEM	Treatment	Linear	Quadratic
Total P excretion, % of P intake	130.8	127.5	162.6	13.63	NS	NS	NS
Fecal P, % of P intake	130.4	125.5	162.4	12.84	NS	NS	NS
Fecal P, % of total P excretion	99.6	99.0	99.9	0.50	NS	NS	NS
Urine P, % of P intake	0.44	1.97	0.23	1.07	NS	NS	NS
Urine P, % of total P excretion	0.36	1.03	0.15	0.50	NS	NS	NS

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

Table 13. Performance characteristics of non-lactating multiparous cows (40 to 11 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 4).¹

Item	Treatment ²			SEM	P-value ³		
	LP	MP	HP		Treatment	Linear	Quadratic
n	6	7	7	--	--	--	--
BW, kg	817	855	762	30.1	NS ⁴	NS	0.10
BCS	3.9	4.0	3.7	0.13	NS	NS	NS
DMI, kg/d	15.8	15.7	13.1	1.24	NS	NS	NS
DMI, %BW	1.9	1.8	1.7	0.17	NS	NS	NS
Apparent DM digestibility, %	62.6	60.1	60.1	2.91	NS	NS	NS

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

Table 14. Phosphorus balance and characteristics of feces and urine for non-lactating multiparous cows (40 to 11 $\pm$ 1.0 d prior to estimated parturition) as influence by experimental treatments (Experiment 4).¹

Item	Treatment ²			SEM	P-value ³		
	LP	MP	HP		Treatment	Linear	Quadratic
P intake, g/d	30.0	44.0	46.0	3.83	0.02	<0.01	NS
Fecal DM, %	15.4	16.2	16.1	0.29	NS ⁴	NS	NS
Fecal DM excretion, g/d	5947	6062	5086	428.0	NS	NS	NS
Fecal P, % of DM	0.43	0.76	1.08	0.04	<0.01	<0.01	NS
Fecal P excretion, g/d	25.62	46.31	54.04	3.72	<0.012	<0.01	NS
Urine excretion ⁵ , kg/d	17.0	16.4	13.3	1.19	0.08	0.05	NS
Urine P, %	0.0008	0.0004	0.0005	0.0001	NS	NS	NS
Urinary P excretion, g/d	0.08	0.06	0.07	0.02	NS	NS	NS
Total P excretion ⁶ , g/d	26.65	46.37	54.11	3.94	<0.01	<0.01	NS
g fecal P/kg DMI	1.60	3.10	4.40	0.39	<0.01	<0.01	NS
P balance ⁷ , g/d	4.4 ⁸	-2.4 ⁸	-8.2 ⁸	2.52	NS	0.08	NS

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

⁵Estimated from urine excretion prediction model (Holter and Urban, 1992).

⁶Total P excretion (g/d) = fecal P + urinary P (g/d) excretion.

⁷P balance, g/d = P intake (g/d) - total P excretion (g/d).

⁸Mean is not different from zero ($P > 0.10$).

Table 15. Phosphorus excretion by non-lactating multiparous cows (40 to 11 ± 1.0 d prior to parturition) as influenced by experimental treatments (Experiment 4).¹

Item	Treatment ²				P-value ³		
	T1	T2	T3	SEM	Treatment	Linear	Quadratic
Apparently absorbed P, g/d	4.38	-2.31	-8.09	2.915	NS ⁴	0.08	NS
Apparent P digestibility, %	14.7	-10.3	-24.8	13.20	NS	0.06	NS
P retained/P intake, g/d	0.2	-1.0	-0.2	0.130	NS	0.06	NS
P retained/apparently absorbed P, g/d	1.0	1.0	1.0	0.03	NS	NS	NS
Total P excretion, % of P intake	85.3	110.3	124.8	13.20	NS	0.06	NS
Fecal P, % of P intake	84.7	110.2	124.6	12.38	NS	0.04	NS
Fecal P, % of total P excretion	99.7	99.9	99.8	0.03	<0.01	<0.01	0.08
Urine P, % of P intake	0.3	0.2	0.2	0.03	0.05	0.03	NS
Urine P, % of total P excretion	0.3	0.1	0.1	0.03	<0.01	<0.01	0.08

¹Values are least squares means.

²Treatments: LP = 0.19%; MP = 0.28%; and, HP = 0.35% dietary P, dry basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

Table 16. Performance characteristics of early lactation cows (106 ± 10.0 DIM) as influenced by experimental treatments (Experiment 5).¹

Item	Treatment ²					SEM	P-value ³				
	T1	T2	T3	T4	T5		Treatment	Linear	Quadratic	Cubic	Quartic
n	7	7	7	7	7	--	--	--	--	--	--
BW, kg	598	578	617	610	592	23.1	NS ⁴	NS	NS	NS	NS
BCS	2.7	2.7	2.9	2.6	2.7	0.19	NS	NS	NS	NS	NS
DIM	119	119	82	99	115	10.0	0.05	NS	0.073	0.09	NS
DMI, kg/d	18.9	19.3	19.3	20.6	19.0	0.85	NS	NS	NS	NS	NS
DMI, %BW	3.2	3.5	3.2	3.5	3.3	0.14	NS	NS	NS	NS	0.08
Apparent DM digestibility, %	67.0	69.6	68.3	68	68.2	1.77	NS	NS	NS	NS	NS
MY, kg/d	35.0	37.0	38.1	38.3	33.6	2.66	NS	NS	NS	NS	NS
ECM ⁵ , kg/d	32.0	33.7	37	34.9	31.5	2.60	NS	NS	NS	NS	NS
Milk fat, %	3.16	3.10	3.52	3.13	3.26	0.15	NS	NS	NS	NS	0.05
Milk protein (true), %	2.69	2.62	2.67	2.64	2.76	0.05	NS	NS	0.10	NS	NS
Milk lactose, %	4.83	4.7	4.83	4.68	4.74	0.05	NS	NS	NS	NS	0.03
SNF, %	9.96	8.17	8.37	8.16	8.38	0.71	NS	NS	NS	NS	NS
SCC, 1000 cells/ml	73	134	127	81	155	51.9	NS	NS	NS	NS	NS

¹Values are least squares means.

²Treatments: T1 = 0.26%; T2 = 0.35%; T3 = 0.41%; T4 = 0.45%; and, T5 = 0.51% dietary P, DM basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

⁵ECM = Energy corrected milk yield (kg/d) = $[(0.3246 \times \text{MY (lb/d)}) + (12.86 \times \text{milk fat yield (lb/d)}) + (7.04 \times \text{milk protein yield (lb/d)})] \div 2.2 \text{ (lb/kg)}$ (Dairy Records Management Systems, 1999).

Table 17. Phosphorus balance and characteristics of feces, urine, and milk of early lactation cows (76 to 106 ± 10.0 DIM) as influenced by experimental treatments (Experiment 5).¹

Item	Treatment ²					SEM	P-value ³				
	T1	T2	T3	T4	T5		Treatment	Linear	Quadratic	Cubic	Quartic
P intake, g/d	49.4	70.3	80.5	94.3	98.0	4.40	<0.01	<0.01	NS	NS	NS
Fecal DM, %	16.8	16.3	17.2	16.3	16.8	0.49	NS ⁴	NS	NS	NS	NS
Fecal DM excretion, g/d	6010	6085	6247	6715	6148	512.7	NS	NS	NS	NS	NS
Fecal P, % of DM	0.40	0.68	0.94	1.27	1.49	0.05	<0.01	<0.01	0.09	NS	NS
Fecal P excretion, g/d	23.7	41.9	58.2	85.2	90.4	5.18	<0.01	<0.01	NS	0.09	NS
g fecal P/kg DMI	1.3	2.1	3.0	4.1	4.7	0.20	<0.01	<0.01	0.09	NS	NS
Urine excretion ⁶ , kg/d	16.0	17.9	16.4	17.0	16.0	1.02	NS	NS	NS	NS	NS
Urine P, %	0.003	0.016	0.007	0.010	0.024	0.006	NS	0.07	NS	0.08	NS
Urine P excretion, g/d	0.52	2.93	1.27	1.72	3.85	1.14	NS	NS	NS	NS	NS
Total P excretion ⁵ , g/d	24.23	44.83	59.45	86.88	94.22	2.23	<0.01	<0.01	NS	NS	0.10
Milk P, %	0.082	0.078	0.088	0.085	0.09	0.003	0.07	NS	NS	0.05	0.10
Milk P yield, g/d	28.5	28.9	33.8	32.6	28.5	2.22	NS	NS	NS	NS	NS
P balance ⁷ , g/d	-5.4 ⁸	-3.4 ⁸	-12.8 ⁹	-25.1 ⁹	-24.7 ⁹	3.83	<0.01	<0.01	NS	0.04	NS

¹Values are least squares means.

²Treatments: T1 = 0.26%; T2 = 0.35%; T3 = 0.41%; T4 = 0.45%; and, T5 = 0.51% dietary P, DM basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

⁵Total P excretion (g/d) = fecal P + urine P excretion (g/d).

⁶Estimated from urine prediction model (Holter and Urban, 1992).

⁷P balance (g/d) = P intake (g/d) - [total P excretion (g/d) + milk P yield (g/d)].

⁸P balance is not different from zero ($P > 0.10$).

⁹P balance is different from zero ($P < 0.05$).

Table 18. Phosphorus excretion by early lactation cows (76 to 106 ± 10.0 DIM) as influenced by experimental treatments (Experiment 5).¹

Item	Treatment ²					SEM	P-value ³				
	T1	T2	T3	T4	T5		Treatment	Linear	Quadratic	Cubic	Quartic
Total P excretion, % of P intake	51.2	63.5	73.7	91.8	96.7	4.47	<0.01	<0.01	NS	NS	NS
Fecal P, % of P intake	50.1	59.3	72.0	90.2	92.9	4.58	<0.01	<0.01	NS	0.09	NS
Fecal P, % of total P excretion	97.9	93.7	97.8	98.4	95.9	1.87	NS	NS	NS	0.05	NS
Urine P, % of P intake	1.1	4.2	1.6	1.6	3.8	1.36	NS	NS	NS	0.06	NS
Urine P, % of total P excretion	2.1	6.3	2.2	1.6	4.1	1.87	NS	NS	NS	0.05	NS
Milk P, % P intake	58.5	41.4	41.8	34.5	29.0	2.45	<0.01	<0.01	NS	NS	NS
Milk P, % total P output	54.4	39.5	36.4	27.5	23.3	1.97	<0.01	<0.01	NS	NS	0.09

¹Values are least squares means.

²Treatments: T1 = 0.26%; T2 = 0.35%; T3 = 0.41%; T4 = 0.45%; and, T5 = 0.51% dietary P, DM basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

Table 19. Performance characteristics of mid lactation cows (162 to 192 ± 6.2 DIM) as influenced by experimental treatments (Experiment 6).¹

Item	Treatment ²						P-value ³				
	T1	T2	T3	T4	T5	SEM	Treatment	Linear	Quadratic	Cubic	Quartic
n	6	7	7	7	6	--	--	--	--	--	--
BW, kg	643	589	606	639	649	28.1	NS ⁴	NS	NS	NS	NS
BCS	3.0	2.9	2.6	3.4	3.0	0.23	NS	NS	NS	NS	0.02
DIM	191	187	198	189	194	6.2	NS	NS	NS	NS	NS
DMI, kg/d	20.7	17.8	19.1	19.6	19.6	1.01	NS	NS	NS	NS	NS
DMI, %BW	3.2	3.0	3.1	3.1	3.1	0.16	NS	NS	NS	NS	NS
Apparent DM digestibility, %	71.1	67.5	69.2	69.2	70.3	1.04	NS	NS	0.04	NS	NS
MY, kg/d	31.8	27.0	31.8	29.4	30.4	1.58	NS	NS	NS	NS	0.08
ECM ⁵ , kg/d	29.5	23.9	27.8	27.5	28.0	1.17	0.03	NS	0.03	0.02	NS
Milk fat, %	3.29	3.01	2.91	3.23	3.18	0.13	NS	NS	0.07	NS	NS
Milk protein (true), %	2.68	2.64	2.65	2.72	2.66	0.07	NS	NS	NS	NS	NS
Milk lactose, %	4.81	4.8	4.80	4.73	4.63	0.08	NS	NS	NS	NS	NS
SNF, %	8.50	8.41	8.47	8.45	8.28	0.15	NS	NS	NS	NS	NS
SCC, 1000 cells/ml	254	390	238	63	101	141.2	NS	NS	NS	NS	NS

¹Values are least squares means.

²Treatments: T1 = 0.26%; T2 = 0.35%; T3 = 0.41%; T4 = 0.45%; and, T5 = 0.51% dietary P, DM basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

⁵ECM = Energy corrected milk yield (kg/d) = $[(0.3246 \times \text{MY (lb/d)}) + (12.86 \times \text{milk fat yield (lb/d)}) + (7.04 \times \text{milk protein yield (lb/d)})] \div 2.2 \text{ (lb/kg)}$ (Dairy Records Management Systems, 1999).

Table 20. Phosphorus balance and characteristics of feces, urine, and milk of mid lactation cows (162 to 192 ± 6.2) as influenced by experimental treatments (Experiment 6).¹

Item	Treatment ²					SEM	P-value ³				
	T1	T2	T3	T4	T5		Treatment	Linear	Quadratic	Cubic	Quartic
P intake, g/d	56.0	64.1	80.1	90.3	102.1	3.99	<0.01	<0.01	NS	NS	NS
Fecal DM, %	643	589	606	639	648	28.1	NS ⁴	NS	NS	NS	NS
Fecal DM excretion, g/d	5982	5765	5884	6046	5812	345.9	NS	NS	NS	NS	NS
Fecal P, % of DM	0.48	0.72	1.02	1.28	1.54	0.05	<0.01	<0.01	0.04	NS	NS
Fecal P excretion, g/d	28.3	41.1	59.9	77.0	90.0	4.04	NS	NS	NS	NS	NS
g fecal P/kg DMI	1.4	2.3	3.1	3.9	4.6	0.09	<0.01	<0.01	NS	NS	NS
Urine excretion ⁵ , kg/d	19.8	15.9	18.0	18.2	20.4	1.28	NS	NS	0.02	NS	NS
Urine P, %	0.0003	0.0026	0.0016	0.0021	0.0114	0.003	NS	0.06	NS	NS	NS
Urine P excretion, g/d	0.07	0.44	0.30	0.42	1.76	0.46	NS	0.04	NS	NS	NS
Total P excretion ⁶ , g/d	28.3	41.6	60.2	77.4	91.7	3.87	<0.01	<0.01	0.10	NS	NS
Milk P, %	0.084	0.083	0.082	0.085	0.079	0.003	NS	NS	NS	NS	NS
Milk P yield, g/d	26.4	22.6	25.9	24.8	23.9	1.29	NS	NS	NS	0.07	NS
P balance ⁷ , g/d	1.2 ⁸	0.1 ⁸	6.0 ⁸	-11.9 ⁹	-13.6 ⁹	3.08	<0.01	<0.01	NS	NS	NS

¹Values are least squares means.

²Treatments: T1 = 0.26%; T2 = 0.35%; T3 = 0.41%; T4 = 0.45%; and, T5 = 0.51% dietary P, DM basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

⁵Estimated from urine prediction model (Holter and Urban, 1992).

⁶Total P excretion (g/d) = fecal P + urine P excretion (g/d).

⁷P balance (g/d) = P intake (g/d) - [total P excretion (g/d) + milk P yield (g/d)].

⁸P balance is not different from zero ($P > 0.10$).

⁹P balance is different from zero ($P < 0.05$).

Table 21. Phosphorus excretion by mid lactation cows (162 to 192 $\pm$ 6.2 DIM) as influenced by experimental treatments (Experiment 6).¹

Item	Treatment ²						P-value ³				
	T1	T2	T3	T4	T5	SEM	Treatment	Linear	Quadratic	Cubic	Quartic
Total P excretion, % P intake	51.1	65.8	74.9	86.1	89.7	3.51	<0.01	<0.01	NS	NS	NS
Fecal P, % P intake	51.0	65.1	74.5	85.6	87.7	3.60	<0.01	<0.01	NS	NS	NS
Fecal P, % total P excretion	99.8	99.0	99.5	99.4	97.9	1.61	NS	NS	NS	NS	NS
Urine P, % P intake	0.1	0.6	0.4	0.4	1.9	0.51	NS	0.06	NS	NS	NS
Urine P, % total P excretion	0.2	1.0	0.5	0.6	2.1	0.54	NS	NS	NS	NS	NS
Milk P, % P intake	47.4	35.3	32.5	27.6	23.5	1.40	<0.01	<0.01	NS	NS	NS
Milk P, % total P output	48.5	35.0	30.4	24.3	20.9	1.36	<0.01	<0.01	0.05	NS	NS

¹Values are least squares means.

²Treatments: T1 = 0.26%; T2 = 0.35%; T3 = 0.41%; T4 = 0.45%; and, T5 = 0.51% dietary P, DM basis.

³Significant effects ($P < 0.05$) and tendencies ($P < 0.10$).

⁴NS = Not significant ($P > 0.10$).

CHAPTER 5

PREDICTING PHOSPHORUS EXCRETION OF DAIRY CATTLE FED A RANGE OF DIETARY PHOSPHORUS CONCENTRATIONS

ABSTRACT

The objective of this research was to evaluate published models for accuracy (bias) and precision in predicting measured P excretion using newly generated data. The newly generated data were obtained from 20 primi- and multiparous lactating Holstein cows (Chapter 3), 73 non-lactating nulli- and multiparous pregnant Holstein cattle and 120 lactating primi- and multiparous Holstein cows (Chapter 4). Three datasets for evaluation were constructed from these data. The full dataset (FD) consisted of data of all 193 animals, the lactating cow dataset (LD) consisted of the 120 lactating cows, and the non-lactating animal dataset (ND) consisted of the 73 non-lactating, pregnant animals.

Eight models were found in the literature to evaluate. The new independent data set was used to evaluate these models. The total range in P excretion was 12.1 to 108.7 g/animal per d. Accuracy, bias, and precision were determined for each of the eight models. Phosphorus excretion was most appropriately predicted by: $(g/d) = \text{intake P} - \text{milk P (IPMP)}$ model and $(g/d) = 5.92 + 0.741 \times [\text{intake P} - \text{milk P (g/d)}]$. In contrast, the least appropriate model was the ASAE (1996).

Furthermore, to improve the IPMP model, regression analysis was conducted using each of the three datasets. Measured P excretion was regressed on the difference of intake P – milk P to develop three models, one for each the FD, LD, and ND. The P excretion model for the FD was: $(\text{g/d}) = 1.19 \pm 0.059 \times [\text{intake P} - \text{milk P} (\text{g/d})] + [1.43 \pm 2.670 (\text{g/d})]$. This relationship was linear ($P < 0.01$) and explained 68% of the variation associated with intake P – milk P (g/d). Additionally, the y-intercept was different from zero ($P < 0.05$), but the slope was not different from one ($P > 0.05$). The P excretion model for the LD was: $(\text{g/d}) = 1.22 \pm 0.055 \times [\text{intake P} - \text{milk P} (\text{g/d})] - [2.11 \pm 2.747 (\text{g/d})]$. This relationship was linear ($P < 0.01$) and explained 80% of the variation associated with intake P – milk P (g/d). The y-intercept of this regression equation was not different from zero ($P > 0.05$), but the slope was different from one ($P < 0.05$). The P excretion prediction for the ND was: $(\text{g/d}) = 1.26 \pm 0.160 \times [\text{intake P} - \text{milk P} (\text{g/d})] + [2.05 \pm 6.148 (\text{g/d})]$. This relationship was linear ($P < 0.01$) and explained 46% of the variation associated with intake P – milk P (g/d). The y-intercept was not different from zero and the slope was not different from one ($P > 0.05$). These models need to be evaluated for accuracy and precision in predicting measured P excretion using a separate independent dataset.

INTRODUCTION

Better prediction of the amount of P excreted by the dairy herd would be useful information to dairy producers and planners of Comprehensive Nutrient Management Plans (CNMPs). In an evaluation of published P prediction models, Davidson and Beede

(1999) determined that the two models (ASAE 1980; 1996) used to develop manure management systems and plan comprehensive nutrient management, were the most inaccurate and imprecise for predicting P excretion compared with other published P excretion prediction models (Morse et al., 1992b; Van Horn et al., 1994; Davidson and Beede, 1999). These two models over-predicted P excretion by 68 and 106%, respectively, compared with measured P excretion in an independent dataset. The ASAE (1980; 1996) models predict P excretion as a linear function of BW, as BW increases P excretion increases, only the slope of the linear fit is different. Neither model likely is acceptable for the development of CNMPs because the estimated P excretion is inflated excessively (Davidson and Beede, 1999). Therefore, the acreage needed to apply manure also is inflated.

Alternatively, Van Horn et al. (1994) suggested that the equation $P \text{ excretion (g/d)} = P \text{ intake (g/d)} - P \text{ output in milk (g/d)}$ might be a better and simpler estimator.

Davidson and Beede (1999) determined that this equation was the most accurate and precise model relative to the other published models (Morse et al., 1992b; Van Horn et al., 1994; Davidson and Beede, 1999) they suggested. They determined that this model over-predicted P excretion by 39% over the range of measured P excretions in an independent dataset developed from the literature. This model is interesting from its practical standpoint. Phosphorus intake and milk P yield potentially can be more easily and accurately estimated than BW or other variables found in some prediction models.

The overall hypothesis of this research was that the P excretion prediction equation, $P \text{ excretion (g/d)} = 5.92 + [P \text{ intake (g/d)} - \text{milk P yield (g/d)}] \times 0.741$ developed, but not evaluated by Beede and Davidson (1999) is the most accurate and

precise prediction equation currently available. The objective to test this hypothesis was to evaluate eight published P excretion prediction models for accuracy and precision. The objective was made possible by assembling a new set of data from P balance trials (Chapter 4) in a series of experiments designed to collect P excretion data from lactating and non-lactating Holstein cows and late gestation nulliparous Holstein heifers. This was done to represent cows at various points of the lactation cycle with a wide range in DMI, MY, and P intake.

MATERIALS AND METHODS

Phosphorus Excretion Datasets

Phosphorus intake, secretion, and excretion data were collected from balance trials with 20 lactating cows (Chapter 3) plus 82 non-lactating and 102 other lactating cows (Chapter 4) to evaluate eight P excretion prediction models published in the scientific literature. Non-lactating cows were 59 to 9 d prepartum and were fed dietary P of 0.19 to 0.35%. Lactating cows ranged from 76 to 303 DIM and were fed 0.26 to 0.52% dietary P. From these data (P balance and excretion information from 204 total animals) a dataset was constructed containing the following variables: P excretion (g/d), BW (kg), P intake (g/d), MY (kg/d), dietary P (% of dietary DM), and milk P yield (g/d). These variables were included in at least one of the eight P excretion prediction models (Table1).

An outlier analysis was conducted with the entire dataset to identify any values greater or less than ± 2 standard deviations from the mean. Based on this analysis, data

of seven cows (five non-lactating and two lactating) were determined to be outliers ($> \pm 2$ SD) and were removed from the dataset. Additionally, four cows (4 non-lactating) had missing data that did not allow calculation of total P excretion; therefore, these data also were removed from the dataset. The full dataset (FD) consisted of P intake, milk P yield, and excretion data from 193 cows (73 non-lactating and 120 lactating cows). Measured P excretion ranged from 12.1 to 108.7 g/animal per d. Two other subsets of data were constructed from these data. The lactating cow dataset (LD) consisted of the 120 lactating cows (measured P excretion ranged from 20.7 to 108.5 g/cow per d), and the non-lactating animal dataset (ND) consisted of the 73 non-lactating animals (measured P excretion ranged from 12.1 to 108.7 g/animal per d). These datasets were used to evaluate P excretion prediction models. The models evaluated were found in ASAE (1980; 1996), Morse et al. (1992b), Van Horn et al. (1994), Davidson and Beede (1999), and Wu et al. (2000) and are listed in Table 1.

Evaluation of Phosphorus Excretion Prediction Models

Accuracy. The accuracy of the eight different P excretion prediction models was evaluated using the following equation: accuracy = predicted – measured P excretion for each observation in FD, LD, and ND. This value was then determined to be different or not different from zero by t-test. Accuracy is the closeness of predicted P excretion to actual or measured P excretion.

Bias. Regression analysis was conducted on the relationship between predicted P excretion and measured P excretion to determine bias. A model was biased if the y-intercept of the regression line was different from zero (t-test) and the slope was different

from 1 (F-test). The y-intercept and slope were different from zero and one, respectively, if $P < 0.05$. The Regression procedure of SAS (1999) was used to evaluate the relationship between predicted P excretion and measured P excretion with following statistical model: $X_i = \mu + M_i + E_i$ where,

X_i = predicted P excretion;

μ = mean measured P excretion;

M_i = the effect of measured P excretion on model i; and,

E_i = the experimental error;

Bias means the predicted P excretion does not equal the measured P excretion.

Precision. The precision of prediction by models of P excretion was evaluated using the root mean square error (RMSE) and the relative over-prediction of each model in FD, LD, and ND. The relative over-prediction of each of the datasets was computed by the following equation: relative over-prediction = [absolute value of (predicted – measured) ÷ measured] × 100% for each observation in its respective dataset and the RMSE was generated as part regression analysis in the bias evaluation. Precision is how consistent a model predicts P excretion at a given measured P excretion.

RESULTS AND DISCUSSION

The eight models evaluated using the newly collected datasets are listed in Table 1. All eight models were evaluated with each of the FD, LD, and ND datasets separately to determine how well they predicted measured P excretion. The ASAE (1980; 1996) models were developed from published and unpublished data about P excretion and

composition of dairy manure. The ASAE (1996) model was developed from a maximum of 86 cow-trial observations. However, the origins, amount, and types of cattle represented in the development of this model could not be ascertained from the publications. The Morse et al. (1992b) model was developed with 93 individual cow balance datum from multiparous lactating Holstein cows. Van Horn et al. (1994) expanded the Morse et al. (1992b) dataset with 15 observations of P excretion from non-lactating, non-pregnant Jersey cows. Additionally, Van Horn et al. (1994) suggested that P excretion might be more simply estimated as P intake – milk P yield. In a review and evaluation of six P excretion models, Davidson and Beede (1991) developed the H & C model with data from Hibbs and Conrad (1983), who conducted a series of experiments from 1958 to 1967 with lactating Jersey cows. They also evaluated the intake P – milk P model (IPMP) suggested by Van Horn et al. (1994). They then developed another model, by linear regression analysis of the relationship between measured P excretion and intake P – milk P using a database of 71 treatment means assembled from the scientific literature. Wu et al. (2000) developed a prediction equation from 26 multiparous lactating Holstein cows, which simply is a linear relationship of P excretion with P intake with a slope of 0.643 and an intercept of –5.7. Because the ND contains only non-lactating cow data, the model $P \text{ excretion} = P \text{ intake} - \text{milk P}$ was simply $P \text{ excretion} = P \text{ intake}$. Therefore, the model $P \text{ excretion} = \text{intake P}$ was evaluated for accuracy, bias, and precision for the FD and LD to determine if this simple model was more accurate and precise and less biased than the eight published models. Plots of this relationship are not shown for FD and LD, however, statistics are provided for comparison to the other eight models.

Only one (Van Horn et al., 1994) of the eight models included non-lactating animals; they represented only 15 of 108 P excretion data points. Therefore, it was considered useful to determine whether or not any of the eight models could accurately and precisely predict measured P excretion of non-lactating animals as well as lactating cows. Again, accuracy is the closeness of predicted P excretion to actual or measured P excretion. Whereas, precision provides an estimate of how consistently a model predicts P excretion over a range of measured P excretion.

Evaluation of Accuracy with the Full Dataset. Plots of predicted P excretion versus measured P excretion by model are presented in Figure 1 for visualization of accuracy, bias, and precision. Relevant statistics are reported in Table 2. All milk production-related variables in all models were zero when P excretion was predicted for non-lactating animals. Of the eight models, accuracy (predicted – measured P excretion) of only one model (ASAE, 1980) was not different than zero ($P > 0.05$). The accuracies of all other models (ASAE, 1996; Morse et al., 1992b; Van Horn et al., 1994; H & C; IPMP; Davidson and Beede, 1999; Wu et al., 2000) were statistically different from zero ($P < 0.05$). To determine if P intake was an accurate predictor of P excretion, the model $P \text{ excretion} = \text{intake P}$ was also evaluated for accuracy. Accuracy for this model was 5.59 g/d, which was different from zero ($P < 0.01$).

Evaluation of Bias with the Full Dataset. The regression equation associated with the FD for all models and relevant statistics are reported in Table 3. The regression equations are plotted in Figure 1 for comparison to unity (measured P excretion = predicted P excretion). The slopes were different from one and the y-intercepts were different from zero ($P < 0.01$) for each model. Therefore, all models were biased in their

prediction of measured P excretion. Again $P \text{ excretion} = \text{intake } P$ was also evaluated for precision. This model was biased with a y-intercept of 24.2 g/d (different from zero) and slope of 0.630 g/d (different from one).

Evaluation of Precision with the Full Dataset. Relevant statistics are reported in Table 4. Precision was estimated by RMSE. The ASAE models (1980; 1996) and H & C models were relatively precise with RMSE of 6.9, 9.0, and 9.2 g/d compared to the other six models. The models of Morse et al. (1992b) and Van Horn et al. (1994) were not as precise as the previous three models with RMSE of 13.3 and 16.3 g/d, respectively. Models of IPMP, Davidson and Beede (1999), and Wu et al. (2000) had RMSE of 10.2, 15.0, and 10.4 g/d, respectively. Lastly, precision for the simple model $P \text{ excretion} = \text{intake } P$ was 11.1 g/d.

When determining the most or least accurate, biased, or precise model over-all, bias was the main factor for determination followed by accuracy then precision. Bias was weighted more heavily because without the graphs of predicted versus measured excretion, accuracy and precision can be misleading. But, with the slope and y-intercept values present the model can be compared to unity ($\text{predicted} - \text{measured} = 0$) with a slope of one and y-intercept of zero to determine how well the data points follow or do not follow unity. Accuracy was addressed secondly because this relationship addressed how close the predicted value was to the actual measured value. Precision was addressed lastly because it was an estimate of consistency in prediction of measured P excretion and represented the standard deviation of predicted versus measured P excretion around the regression line.

When evaluating the eight published models with the FD, the most accurate and precise model was IPMP. It was also to least biased (Table 3). Its accuracy was different from zero and it was biased, but its precision of prediction (RMSE = 10.2) was closer to measured P excretion relative to the other seven models across the full range of measured P excretion. The relatively small accuracy (-9.7 g/d) could be due to experimental error in measured P excretion or not accounting for P retained in bone and soft tissues (Beede and Davidson, 1999). The accuracy of the ASAE (1980) model was not different from zero. From the plot of ASAE (1980) predicted versus measured P excretion (Figure 1) it can be visualized why mean accuracy is not different from zero. It predicted P excretion within the same range (about 35 to 61 g/d) across the entire range (about 10 to over 100 g/d) of actual measured P excretion; therefore, the overall average will be close to unity (measured = predicted P excretion). The ASAE (1996) model was the least accurate and precise model evaluated (accuracy different from zero, prediction was biased, and over-prediction 82.9% with RMSE of 9.0 g/d), which is important to note because this model likely is used to determine how many head of cattle can be on a farm with a given amount of acreage. Additionally, the evaluation of the simple relationship P excretion = intake P was not an improvement over the other eight published models.

Evaluation of Accuracy with the Lactating Cow Dataset. Plots of predicted P excretion versus measured P excretion by model are presented in Figure 2 for visualization of accuracy and precision. Relevant statistics are reported in Table 5. All models evaluated with the LD were different from zero ($P < 0.05$), and thus biased in their prediction of P excretion. However, relative to the other models the IPMP model had less variation associated with accuracy of the model (Table 5). This disagreed with

results reported by Davidson and Beede (1999), in which the H & C and IPMP models were not different from zero when evaluated with an independent dataset. The simple predictor $P \text{ excretion} = \text{intake } P$ was also evaluated for accuracy. Accuracy was 16.8 g/d and different from zero.

Evaluation of Bias with the Lactating Cow Dataset. The regression equation associated with the LD for all models and relevant statistics are reported in Table 6. The regression equations are plotted in Figure 2 for comparison to unity. All models were biased in their prediction of measured P excretion. The slopes were different than one and the y-intercepts were different from zero for all eight models.

Evaluation of Precision with the Lactating Cow Dataset. Relevant statistics are reported in Table 7. The ASAE (1980; 1996), Morse et al. (1992b), Van Horn et al. (1994), and H & C, IPMP, Davidson and Beede (1999), and Wu et al. (2000) models had similar mean precisions of 5.5, 7.2, 9.1, 9.1, 7.3, 8.9, 6.6, and 7.2 g/d, respectively. The ASAE (1980) model was the most precise (RMSE = 5.5 g/d) model of the eight evaluated. Precision of $P \text{ excretion} = P \text{ intake}$ was 11.1.

The most accurate and precise and least biased model was the IPMP (Tables 5, 6, and 7). This agreed with results of Davidson and Beede (1999) when models were evaluated with an independent dataset. In that model evaluation, the IPMP model was relatively accurate (average predicted – measured P excretion = 14.5 g/d), although different from zero ($P < 0.01$) with a relative over-prediction of 39% across measured P excretion, when compared with other models (ASAE 1980; 1996; Morse et al., 1992b; Van Horn et al., 1994; H & C) evaluated with their independent dataset. Additionally, the model developed but not evaluated by Davidson and Beede (1999) by regression

analysis of the relationship between P excretion and intake P – milk P was not an improvement over the IPMP model (Tables 5, 6, and 7). The model P excretion = intake P was not an improvement over intake P – milk P.

Evaluation of Accuracy with the Non-lactating Animal Dataset. Plots of predicted P excretion versus measured P excretion by model is presented in Figure 3 for visualization of accuracy and precision. Relevant statistics are reported in Table 8. All milk production-related variables in all models were zero when evaluating models with ND. The ASAE (1980) and H & C models were not determined to be different from zero ($P > 0.05$). The accuracy of all other models was different from zero.

Evaluation of Bias with the Non-lactating Animal Dataset. The regression equation associated with the LD for all models and relevant statistics are reported in Table 9. The regression equations are plotted in Figure 3 for comparison to unity. All models were biased with slopes different from one and y-intercepts different from zero.

Evaluation of Precision with the Non-lactating Animal Dataset. Relevant statistics are reported in Table 10. Precision for all models was relatively close and ranged from 5.1 to 9.0 g/d. Van Horn et al. (1994) was the most precise at 5.1 g/d and IPMP was the least precise at 9.0 g/d.

The most accurate and precise model for the ND was IPMP. It was not as precise as the other seven models, but mean accuracy (-12.8 g/d) coupled with precision predicted P excretion closer to measured P excretion in this dataset. For non-lactating animals, P intake was the best explanatory variable for measured P excretion compared with the other variables [BW, MY, milk P yield, dietary P% (dry basis)]. There were no models found in the literature for predicting P excretion specifically by non-lactating

animals so it was useful to determine whether any of these eight models was useful for both lactating and non-lactating animals.

Further Evaluation of Phosphorus Intake Minus Milk Phosphorus Yield. As previously mentioned IPMP over-predicted P excretion in each of the FD, LD, and ND. To offer a potentially more explanatory model involving the intake P – milk P relationship an equation to account for more variation in P excretion was developed for each newly created dataset by regressing measured P excretion on measured intake P – measured milk P (g/d). The P excretion model for the FD was: $(\text{g/d}) = 1.19 \pm 0.059 \times [\text{intake P} - \text{milk P (g/d)}] + [1.43 \pm 2.670 (\text{g/d})]$. This relationship was linear ($P < 0.01$) and explained 68% of the variation associated with intake P – milk P (g/d). Additionally, the y-intercept was different from zero ($P < 0.05$), but the slope was not different from one ($P > 0.05$). The P excretion model for the LD was: $(\text{g/d}) = 1.22 \pm 0.055 \times [\text{intake P} - \text{milk P (g/d)}] - [2.11 \pm 2.747 (\text{g/d})]$. This relationship was linear ($P < 0.01$) and explained 80% of the variation associated with intake P – milk P (g/d). The y-intercept of this regression equation was not different from zero ($P > 0.05$), but the slope was different from one ($P < 0.05$). The P excretion prediction for the ND was: $(\text{g/d}) = 1.26 \pm 0.160 \times [\text{intake P} - \text{milk P (g/d)}] + [2.05 \pm 6.148 (\text{g/d})]$, where milk P = 0. This relationship was linear ($P < 0.01$) and explained 46% of the variation associated with intake P – milk P (g/d). The y-intercept was not different from zero and the slope was not different from one ($P > 0.05$). A plot of measured P excretion plotted against intake P – milk P is provided in Figure 4 along with a plot of the regression line associated with each dataset. However, before use of any of these newly developed models, they must be

evaluated for accuracy, bias, and precision by a separate independent dataset currently not available.

Conclusions

The eight models that predict P excretion from dairy cattle in Table 1 were evaluated with three independent datasets compiled from 73 non-lactating and 120 lactating cows. The hypothesis tested in this research was that the P excretion model developed, but not evaluated by Davidson and Beede (1999): $P \text{ excretion (g/d)} = 5.92 + 0.741 \times [\text{intake P (g/d)} - \text{milk P (g/d)}]$, was the best predictor of P excretion for lactating dairy cows. However, based on evaluation using the newly collected dataset (LD) it was not an improvement over the simple difference intake P – milk P.

Over all datasets, the IPMP was more accurate and precise relative to the other seven models evaluated. The most practical equation for P excretion prediction for whole-farm nutrient management would be one equation that could be used for all mature cows. It appears that the IPMP would be a better predictor of P excretion than what is currently used for planning of nutrient management. The ASAE (1996) model is an equation likely used to predict P excretion in a given dairy operation. However, the ASAE (1980; 1996) models were overall the most inaccurate and imprecise predictors of P excretion relative to the other five. Planners of comprehensive nutrient management should not use the ASAE (1980; 1996) models.

Table 1. Phosphorus excretion models evaluated using the independent datasets.

Model	Prediction equation ¹
ASAE (1980)	$Y^1 = BW \text{ (kg)} \times 0.0724$
ASAE (1996)	$Y = BW \text{ (kg)} \times 0.094$
Morse et al. (1992b)	$Y = 14.67 + 0.6786 \times \text{intake P (g/d)} + 0.00196 \times [\text{intake P (g/d)}]^2 - 0.317 \times MY \text{ (kg/d)}$
Van Horn et al. (1994)	$Y = 9.6 + 0.472 \times \text{intake P (g/d)} + 0.00126 \times [\text{intake P (g/d)}]^2 + 0.323 \times MY \text{ (kg/d)}$
H & C model ²	$Y = -24.06 + 81.67 \times \text{diet P (\% of dietary DM)} + 0.07 \times BW \text{ (kg)} - 0.45 \times \text{milk P (g/d)}$
Intake P – milk P	$Y = \text{intake P (g/d)} - \text{milk P (g/d)}$
Davidson and Beede (1999)	$Y = 5.92 + 0.741 \times [\text{intake P (g/d)} - \text{milk P (g/d)}]$
Wu et al. (2000)	$Y = [0.643 \times P \text{ intake (g/d)}] - 5.2$

¹Y = P excretion (g/d).²The H and C model was developed from data of Hibbs and Conrad (1983).

Table 2. Accuracy of prediction of measured P excretion (g/d) of lactating and non-lactating dairy animals by various models using the full dataset.

Model	Accuracy ¹		<i>P</i> -value ²
	Mean	± SEM	
ASAE (1980)	-3.0	1.93	0.13
ASAE (1996)	11.6	1.97	<0.01
Morse et al. (1992b)	3.8	1.26	<0.01
Van Horn et al. (1994)	-4.2	1.60	<0.01
H & C model ³	-7.8	1.63	<0.01
Intake P - milk P	-9.7	1.09	<0.01
Davidson and Beede (1999)	-14.6	1.22	<0.01
Wu et al. (2000)	-20.0	1.42	<0.01
Intake P	5.59	1.27	<0.01

¹Accuracy = predicted - measured P excretion (g/d).

²Mean is different from zero if *P* < 0.05; t-test.

³Developed by Davidson and Beede (1999) from data of Hibbs and Conrad (1983).

Table 3. Bias of prediction of measured P excretion (g/d) of non-lactating and lactating dairy animals by various models using the full dataset.

Model	y-intercept	± SE	P-value ¹	Slope	± SE	P-value ²
ASAE (1980)	47.9	1.09	<0.01	0.013	0.019	<0.01
ASAE (1996)	62.1	1.43	<0.01	0.018	0.025	<0.01
Morse et al. (1992b)	26.1	2.11	<0.01	0.566	0.037	<0.01
Van Horn et al. (1994)	25.6	2.59	<0.01	0.422	0.045	<0.01
H & C model ³	32.8	1.47	<0.01	0.212	0.025	<0.01
Intake P - milk P	12.2	1.62	<0.01	0.574	0.028	<0.01
Davidson and Beede (1999)	15.0	1.20	<0.01	0.426	0.021	<0.01
Wu et al. (2000)	10.4	1.93	<0.01	0.411	0.033	<0.01
Intake P	24.2	3.00	<0.01	0.639	0.052	<0.01

¹y-intercept (g/d) is different from zero if $P < 0.05$; t-test.

²Slope (g/d) is different from one if $P < 0.05$; F-test.

³Developed (Davidson and Beede, 1999) from data of Hibbs and Conrad (1983).

Table 4. Precision of various models to predict P excretion (g/d) by lactating and non-lactating dairy animals using the full dataset.

	Mean ¹	± RMSE ²	95% Confidence Interval ³
ASAE (1980)	48.6	6.9	41.7 to 55.5
ASAE (1996)	63.1	9.0	54.1 to 72.1
Morse et al. (1992b)	55.3	13.3	42.0 to 68.6
Van Horn et al. (1994)	47.3	16.3	34.0 to 63.6
H & C model ⁴	43.8	9.3	34.5 to 53.1
Intake P - milk P	41.8	10.2	31.6 to 52.0
Davidson and Beede (1999)	36.9	7.6	29.3 to 44.5
Wu et al. (2000)	31.5	12.2	19.3 to 43.7
P intake	57.1	11.1	46.0 to 68.2

¹Mean = average predicted P excretion (g/d).

²Precision: RMSE = root mean square error of the regression of the relationship between predicted P excretion and measured P excretion (g/d).

³95% confidence interval = mean ± 2 RMSE (g/d).

⁴Developed (Davidson and Beede, 1999) from data of Hibbs and Conrad (1983).

Table 5. Accuracy of prediction of P excretion (g/d) of lactating dairy cows by various models using the lactating cow dataset.

Model	Accuracy ¹		<i>P</i> -value ²
	Mean	± SEM	
ASAE (1980)	-8.1	2.43	<0.01
ASAE (1996)	5.5	2.43	0.03
Morse et al. (1992b)	10.2	1.20	<0.01
Van Horn et al. (1994)	5.6	1.58	<0.01
H & C model ³	-13.6	1.88	<0.01
Intake P - milk P	-7.8	1.18	<0.01
Davidson and Beede (1999)	-13.7	1.41	<0.01
Wu et al. (2000)	-13.5	1.52	<0.01
Intake P	16.8	127	<0.01

¹Accuracy = predicted - measured P excretion (g/d).

²Mean is different from zero if *P* < 0.05; t-test.

³Developed (Davidson and Beede, 1999) from data of Hibbs and Conrad (1983).

Table 6. Bias of prediction of measured P excretion (g/d) of lactating dairy animals by various models using the lactating cow dataset.

Model	y-intercept	± SE	P-value ¹	Slope	± SE	P-value ²
ASAE (1980)	43.2	1.12	<0.01	0.040	0.019	<0.01
ASAE (1996)	56.1	1.45	<0.01	0.052	0.024	<0.01
Morse et al. (1992b)	29.1	1.84	<0.01	0.646	0.031	<0.01
Van Horn et al. (1994)	34.7	1.84	<0.01	0.454	0.031	<0.01
H & C model ³	24.4	1.49	<0.01	0.287	0.025	<0.01
Intake P - milk P	10.6	1.81	<0.01	0.656	0.030	<0.01
Davidson and Beede (1999)	13.8	1.34	<0.01	0.486	0.022	<0.01
Wu et al. (2000)	16.3	1.45	<0.01	0.443	0.024	<0.01
Intake P	33.4	2.25	<0.01	0.689	0.037	<0.01

¹y-intercept (g/d) is different from zero if $P < 0.05$; t-test.

²Slope (g/d) is different from one if $P < 0.05$; F-test.

³Developed (Davidson and Beede, 1999) from data of Hibbs and Conrad (1983).

Table 7. Precision of various models to predict P excretion (g/d) by lactating dairy cows using the lactating cow dataset.

Model	Mean ¹	± RMSE ²	95% Confidence Interval ³
ASAE (1980)	45.4	5.5	39.9 to 50.9
ASAE (1996)	58.9	7.2	51.7 to 66.1
Morse et al. (1992b)	63.6	9.1	54.5 to 72.7
Van Horn et al. (1994)	59.0	9.1	49.9 to 68.1
H & C model ⁴	39.8	7.3	32.5 to 47.1
Intake P - milk P	45.6	8.9	36.7 to 54.5
Davidson and Beede (1999)	39.7	6.6	33.1 to 46.3
Wu et al. (2000)	39.9	7.2	32.7 to 47.1
Intake P	70.2	11.1	59.1 to 81.3

¹Mean = average predicted P excretion (g/d).

²Precision: RMSE = root mean square error of the regression of the relationship between predicted P excretion and measured P excretion (g/d).

³95% confidence interval = mean ± 2 RMSE (g/d).

⁴Developed (Davidson and Beede, 1999) from data of Hibbs and Conrad (1983).

Table 8. Accuracy of prediction of P excretion (g/d) of non-lactating dairy animals by various models using the non-lactating animal dataset.

Model	Accuracy ¹		<i>P</i> -value ²
	Mean	± SEM	
ASAE (1980)	5.5	2.96	0.07
ASAE (1996)	21.5	3.00	<0.01
Morse et al. (1992b)	-6.8	2.18	<0.01
Van Horn et al. (1994)	-20.2	2.36	<0.01
H & C model ³	1.9	2.66	0.47
Intake P – milk P	-12.8	2.09	<0.01
Davidson and Beede (1999)	-16.1	2.23	<0.01
Wu et al. (2000)	-30.7	2.30	<0.01

¹Accuracy = predicted – measured P excretion (g/d).

²Mean is different from zero if *P* < 0.05, t-test.

³Developed (Davidson and Beede, 1999) from data of Hibbs and Conrad (1983).

Table 9. Bias of prediction of measured P excretion (g/d) of non-lactating dairy animals by various models using the non-lactating animal dataset.

Model	y-intercept	± SE	P-value ¹	Slope	± SE	P-value ²
ASAE (1980)	53.6	1.43	<0.01	0.006	0.026	<0.01
ASAE (1996)	69.5	1.85	<0.01	0.007	0.034	<0.01
Morse et al. (1992b)	26.7	1.93	<0.01	0.309	0.035	<0.01
Van Horn et al. (1994)	17.9	1.32	<0.01	0.212	0.024	<0.01
H & C model ³	44.3	1.71	<0.01	0.123	0.031	<0.01
Intake P - milk P	17.4	2.33	<0.01	0.377	0.043	<0.01
Davidson and Beede (1999)	18.8	1.73	<0.01	0.279	0.032	<0.01
Wu et al. (2000)	6.0	1.50	<0.01	0.242	0.028	<0.01

¹y-intercept (g/d) is different from zero if $P < 0.05$; t-test.

²Slope (g/d) is different from one if $P < 0.05$; F-test.

³Developed (Davidson and Beede, 1999) from data of Hibbs and Conrad (1983).

Table 10. Precision of various models to predict P excretion (g/d) by non-lactating animals using the non-lactating dataset.

Model	Mean ¹	± RMSE ²	95% Confidence Interval ³
ASAE (1980)	53.8	5.5	48.3 to 59.3
ASAE (1996)	69.9	7.2	62.7 to 77.1
Morse et al. (1992b)	41.6	7.5	34.1 to 49.1
Van Horn et al. (1994)	28.2	5.1	23.1 to 33.3
H & C model ⁴	50.3	6.6	43.7 to 56.9
Intake P - milk P	35.6	9.0	26.6 to 44.6
Davidson and Beede (1999)	32.3	6.7	25.6 to 39.0
Wu et al. (2000)	17.7	5.8	11.9 to 23.5

¹Mean = average predicted P excretion (g/d).

²Precision: RMSE = root mean square error of the regression of the relationship between predicted P excretion and measured P excretion (g/d).

³95% confidence interval = mean ± 2 RMSE (g/d).

⁴Developed (Davidson and Beede, 1999) from data of Hibbs and Conrad (1983).

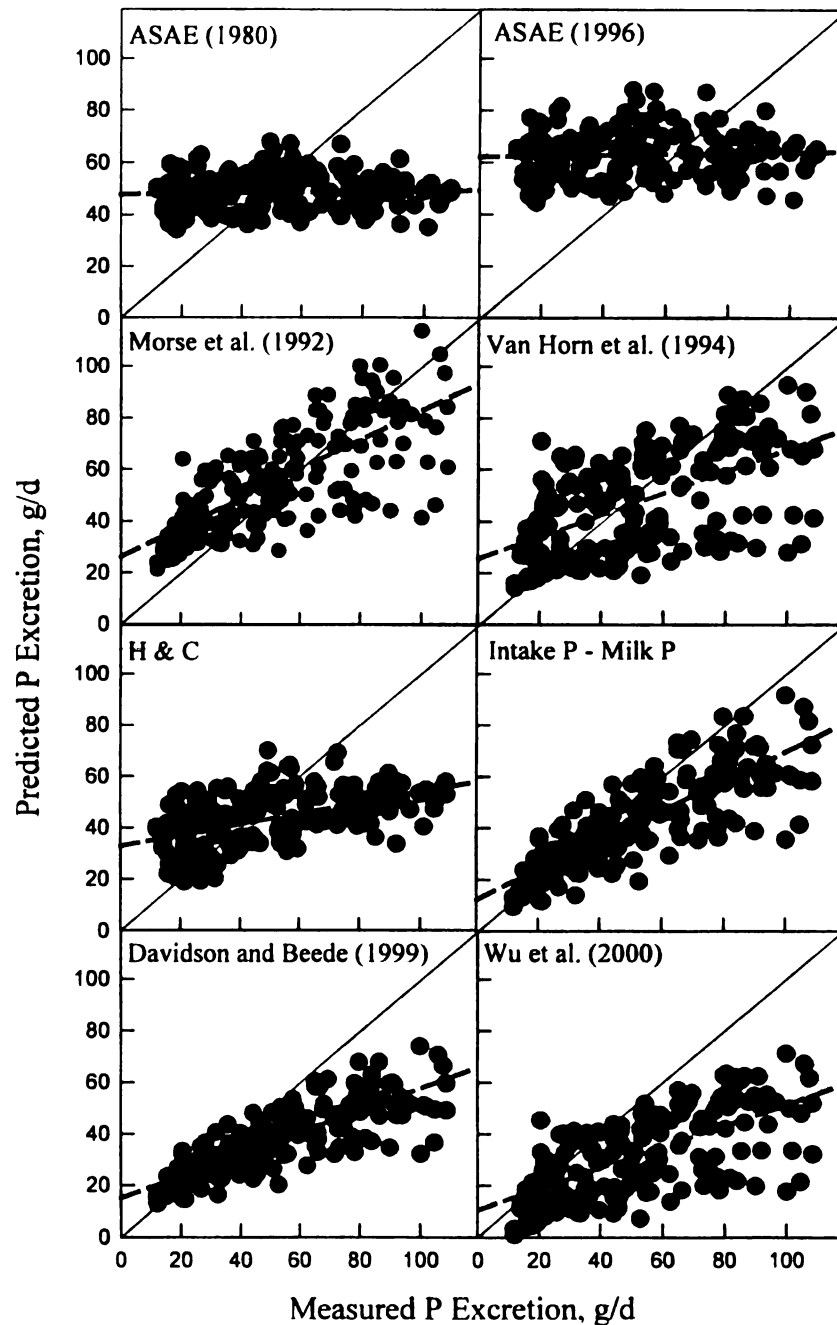


Figure 1. Predicted P excretion (•) plotted against measured P excretion in the full dataset for visualization of the precision of each model. The solid diagonal line is unity where predicted P excretion equals measured P excretion. The dashed line is the regression line of the relationship between predicted and measured P excretion.

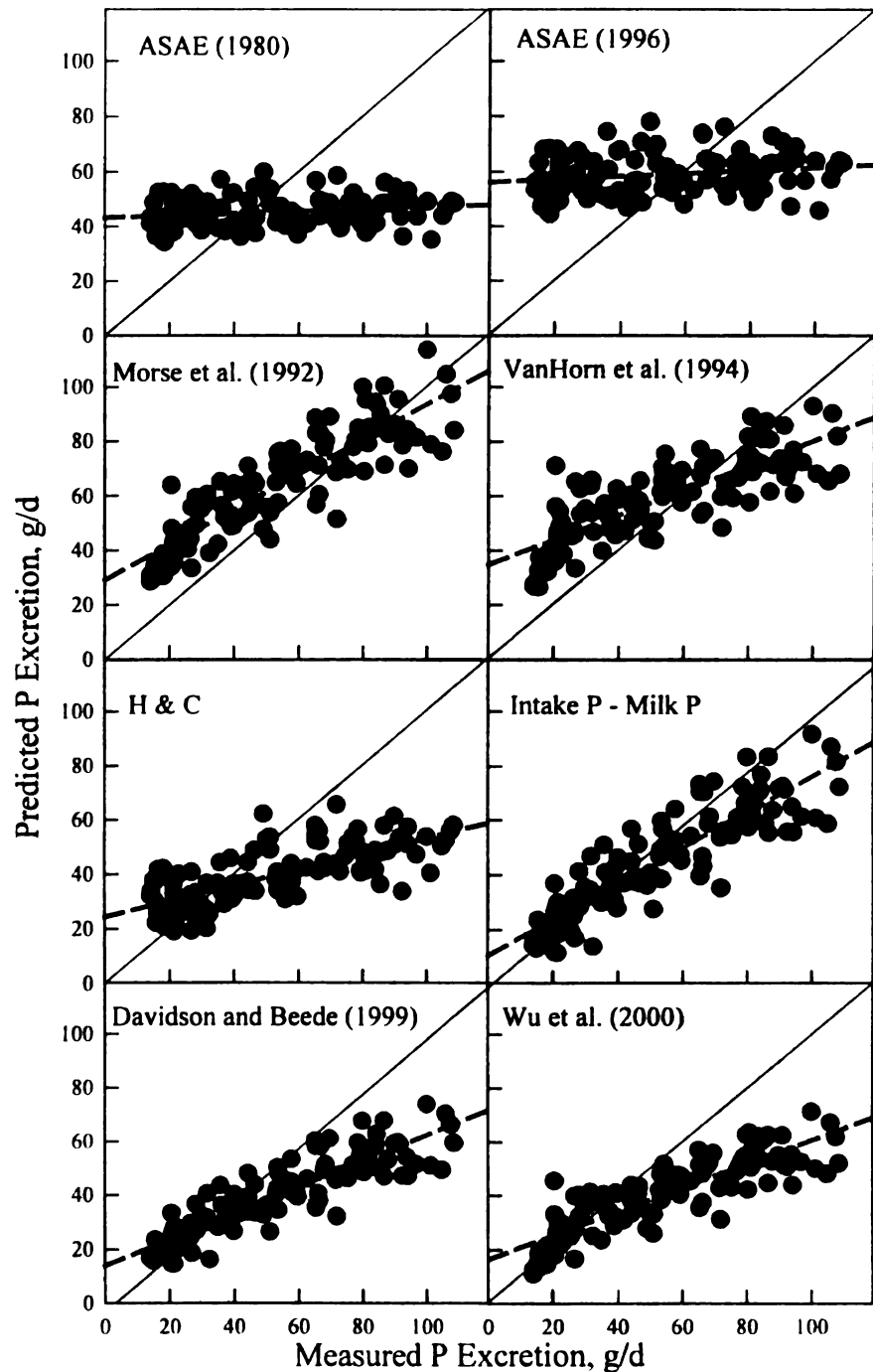


Figure 2. Predicted P excretion (•) plotted against measured P excretion in the lactating cow dataset for visualization of the precision of each model. The solid diagonal line is unity where predicted P excretion equals measured P excretion. The dashed line is the regression line of the relationship between predicted and measured P excretion.

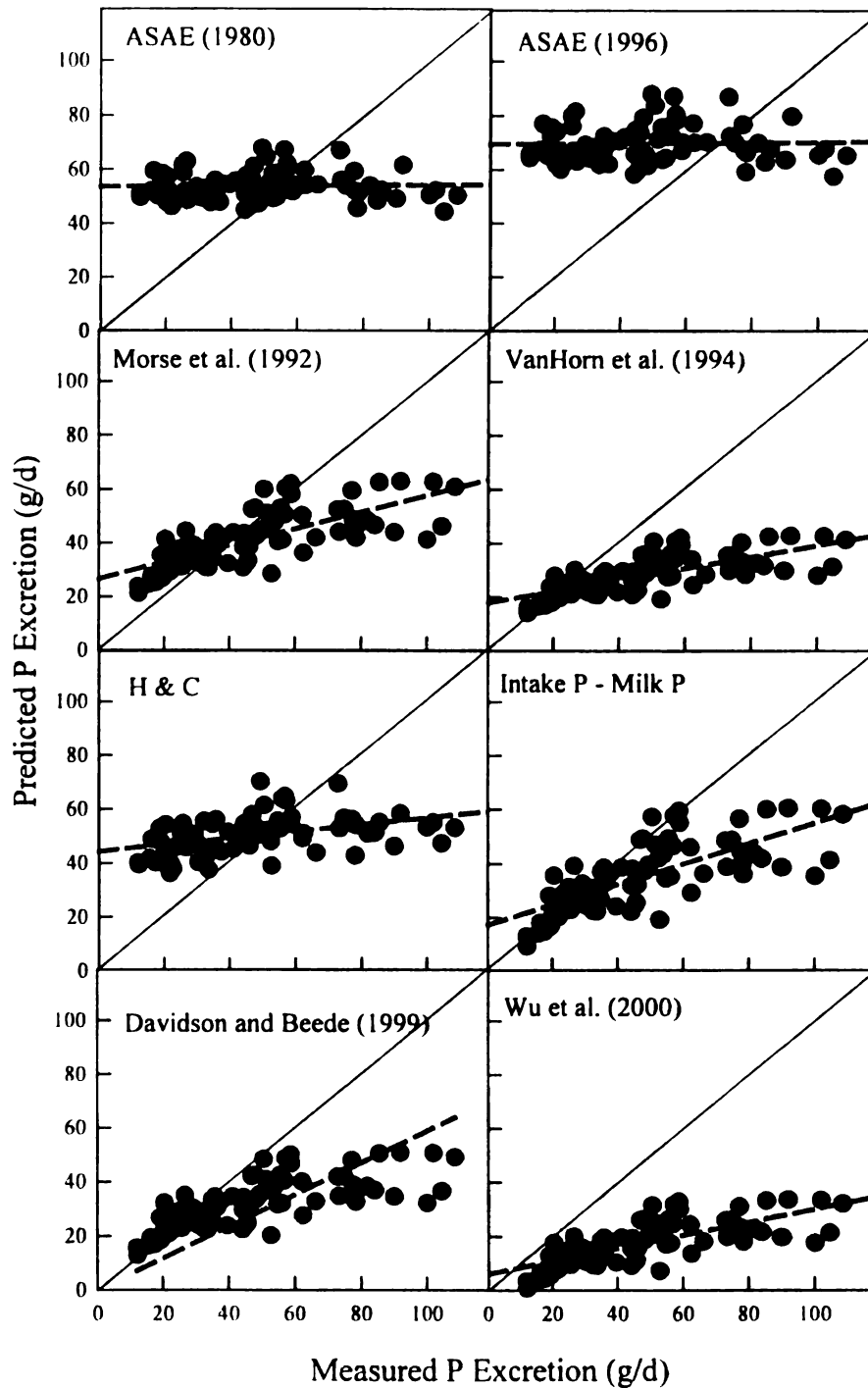


Figure 3. Predicted P excretion (•) plotted against measured P excretion in the non-lactating animal dataset for visualization of the precision of each model. The diagonal line is unity where predicted P excretion equals measured P excretion. The dashed line is the regression line of the relationship between predicted and measured P excretion.

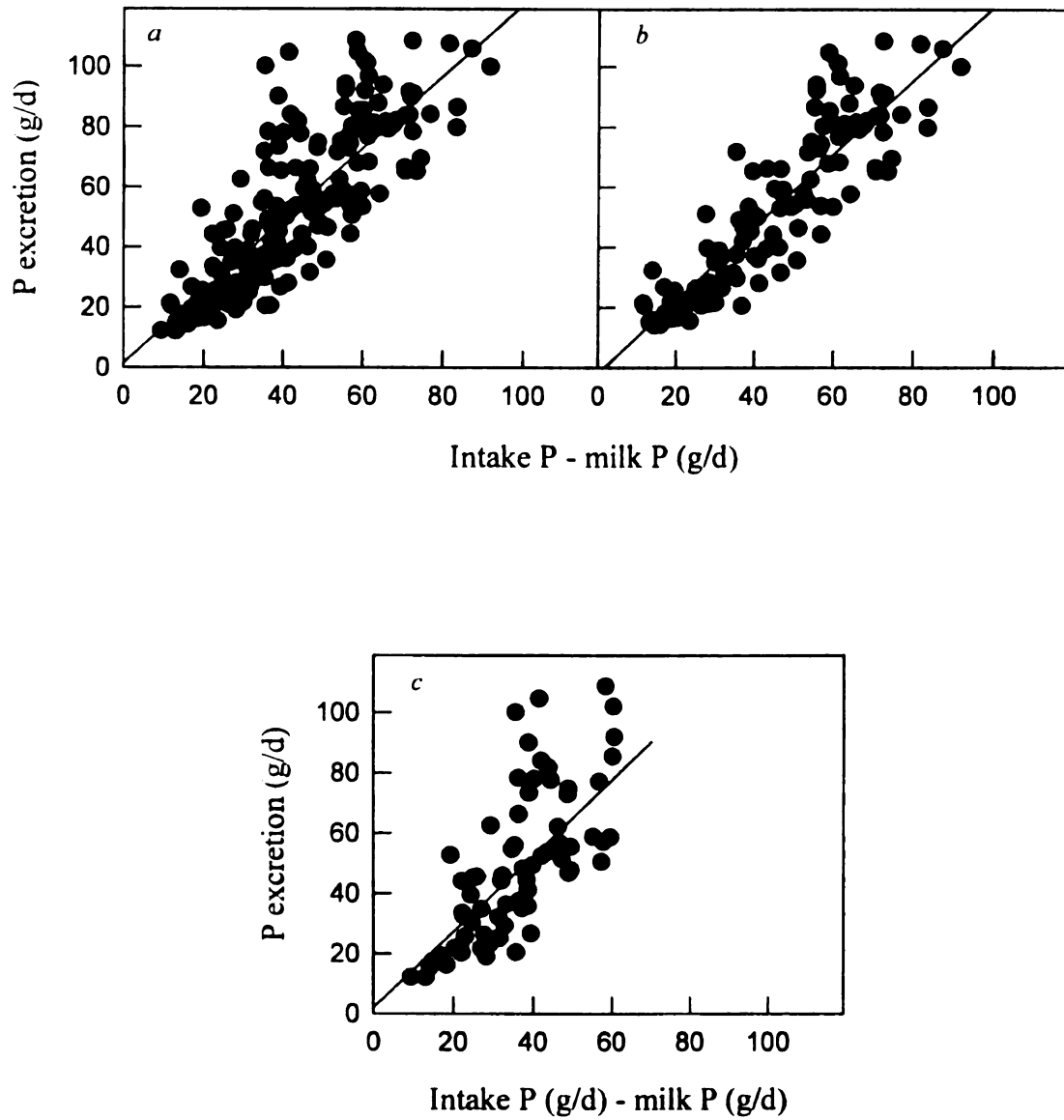


Figure 4. Phosphorus prediction models developed using linear regression of P excretion on intake P – milk P. (a) The model developed using the full dataset was: $P \text{ excretion (g/d)} = [1.19 \pm 0.059 \text{ (g/d)}] \times [\text{intake P – milk P (g/d)}] + [1.43 \pm 2.670 \text{ (g/d)}]$, (y-intercept not different from 0; slope different from 1; RMSE = 14.9 (g/d); $R^2 = 0.68$; $P < 0.01$). (b) $P \text{ excretion (g/d)} = [1.22 \pm 0.055 \text{ (g/d)}] \times [\text{intake P – milk P (g/d)}]$, (y-intercept not different from 0; slope different from 1; RMSE = 12.1 (g/d); $R^2 = 0.80$; $P < 0.01$). (c) $P \text{ excretion (g/d)} = [1.26 \pm 0.160 \text{ (g/d)}] \times [\text{intake P – milk P (g/d)}] + [2.05 \pm 6.148 \text{ (g/d)}]$, (y-intercept not different from 0; slope not different from 1; RMSE = 18.1 (g/d); $R^2 = 0.46$; $P < 0.01$).

Chapter 6

CONCLUSIONS AND IMPLICATIONS

Better defined P requirements for dairy cattle are critical to understanding how to feed lactating cows to reduce P excretion. The current total net P maintenance requirement is 1.0 g P/kg DMI (the fecal component) plus an additional 0.002 g P/kg BW to account for endogenous urine P excretion that also is part of the maintenance requirement (NRC, 2001). This estimate was computed using an absorption coefficient of 0.8 of dietary P and inevitable fecal P excretion (major part of the total P maintenance requirement) of 1.2 g P/kg DMI determined by Spiekens et al. (1993). However, based on the current experiment with higher producing cows, inevitable fecal P excretion (g/kg DMI) was 1.36, 1.19, and 1.04 and decreased with increase DMI (linear effect; $P < 0.01$). An equation was developed by regression to predict inevitable fecal P of a cow fed P close to the true requirement: $(\text{g/d}) = 0.85 \pm 0.070 \times \text{DMI, kg/d} + [5.30 \pm 1.224 (\text{g/d})]$ ($R^2 = 0.90$; $P < 0.01$). This equation accounts for decreased inevitable fecal P excretion (g/kg DMI) with increased DMI and allows an estimate of the P maintenance requirement to be calculated that potentially is closer to true P maintenance requirement.

It is not known whether or not the fecal portions of the maintenance requirement determined by this experiment are statistically different from the results reported by Spiekens et al. (1993); we suspect that they probably are not. However, this relationship may become even more important as milk production per cow increases. Dry matter intake must increase to support increased production (NRC, 2001) or efficiency of

converting nutrients to milk must increase. As average milk production increases, average DMI theoretically will increase. Because DMI affects the fecal portion of the total maintenance requirement, it may become lower (on a basis of g P/kg DMI) than observed in this study with cows consuming DMI > 25.1 kg/d but still consuming P close to their true P maintenance requirement.

According to Figure 1 (Chapter 3), when DMI was restricted (T1 and 2) there was a tighter relationship between fecal P excretion (g/d) and DMI (kg/d) because there was less potential for variation in DMI and P intake in excess of requirement for milk production plus maintenance. In future research, to help control this variation, perhaps DMI in all groups should be restricted to the NE_L intake needed to support higher milk yield. Also, type of diet may influence inevitable fecal P excretion. It would be beneficial to see how different forages or different ratios of forages-to-concentrate influence the relationship between inevitable fecal P excretion and DMI. However, the basal diet used here was quite different than that of Spiekens et al. (1993). Developing diets that are very different in nutrient composition may be a challenge because low P diets are difficult to formulate with typical ingredients.

Seven experiments were conducted using non-lactating and lactating cows at different points in the lactation cycle to determine the effects of varying dietary P concentration on P balance and excretion. Dietary P concentration affected P balance and utilization of non-lactating and lactating dairy cows. Overall, fecal P excretion increased linearly with increased dietary P concentration. In general, urine P excretion increased with increasing dietary P concentration; however, the effect on urine P excretion was

quite small and not consistent. Urine P% was low and total daily excretion generally resulted in less than 1 g P/d excreted in urine.

Phosphorus balance decreased with increasing dietary P concentrations; generally P balance was less than zero and became more negative with increased dietary P concentration. This was largely driven by increased fecal P excretion. In general, P balances of cows in the higher P dietary treatments (MP and HP for non-lactating animals; T3, T4, and T5 for lactating cows) were different from zero. This suggested that P supplied by the higher P diets was in excess of the P requirement. In general milk P%, MF, ML, MProt, and SNF were not affected by dietary P concentration. The priority objective of these seven experiments was to collect P balance data for use to evaluate models to predict P excretion.

Having a more accurate and precise model to predict P excretion by cows at different points in the lactation would be very useful important to the dairy industry. Currently, the ASAE (1996) equation is available to develop nutrient management plans for start-up and existing dairies. Beede and Davidson (1999) reported that this model was the least accurate and precise model in their evaluation with an independent dataset. The current evaluation with a separate new independent dataset agreed with Beede and Davidson (1999). The ASAE (1996) was biased (slope different from one; y-intercept different from zero) and accuracy was 12.8 g/d and different from zero. Precision was 7.8 g/d. However, it over-predicted measured P excretion by 85.3%. The range of measured P excretion was 12.1 to 108.7 g P/animal per d. This model was not adequate to be used for its current purpose. Inflated predicted P excretion also will unnecessarily inflate the calculations of land needed to apply manure to cropland.

Beede and Davidson (1999) also determined that the simple difference of intake P minus milk P suggested by VanHorn et al. (1999) was the most accurate and precise model evaluated using their independent dataset. Once this was determined they developed a model by regression: $P \text{ excretion (g/d)} = 5.92 + 0.741 \times [P \text{ intake (g/d)} - \text{milk P (g/d)}]$, using this relationship to hopefully explain more of the variation associated with P excretion. The objective of the last chapter was to determine if this new model was an improvement over intake P – milk P. The results of the current experiment concluded that this model was not as accurate or precise as intake P – milk P. We evaluated all models (Table 1; Chapter 5) reported by Davidson and Beede (1999) plus one more (Wu et al., 2000) published after their review. Intake P – milk P was the most accurate and precise predictor of P excretion for all three datasets developed using the three datasets mentioned previously. When evaluating the intake P – milk P model using all three datasets, P excretion was most accurately and precisely predicted for LD and least for ND (Tables 4, 5, 6, and 7; Chapter 5). Additionally, the accuracy and precision of this model was reduced when non-lactating animals were added to the lactating dataset (FD). It was concluded that intake P – milk P, instead of the ASAE (1996) model should be used to predict P excretion for development of nutrient management plans. For non-lactating cows the model to predict P excretion was: $(\text{g/d}) = P \text{ intake (g/d)}$. This relationship was more accurate and precise than the ASAE (1996) for non-lactating cows so should be used instead of the ASAE (1996) model until a better model for non-lactating cows is developed.

Additional model evaluation or development research would be beneficial. Intake P – milk P appears to be useful at predicting P excretion for lactating cows, but may not

be for non-lactating animals. This model was biased (slope different from one; y-intercept different from zero) with mean accuracy of -12.8 g/d, which was different from zero. Precision was 9.0 g/d and intake P – milk P over-predicted P excretion by 26.6%. Additional model development can be completed from the datasets generated. Prediction models can be developed separately for non-lactating (ND) and lactating (LD) cows or for both together (FD) to determine whether or not a more accurate and precise model, relative to intake P – milk P, can be developed.

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APPENDIX A

Digestion Procedure for Feed, Feces, and Milk Phosphorus

Reagents:

Hydrochloric Acid, 4N: Add 667ml ultra pure water to a 1L media bottle. Gradually add 333ml concentrated hydrochloric acid to the water while stirring. Cover with parafilm and cap. **BE SURE TO ADD ACID TO WATER!!!**

Concentrated Nitric Acid

Materials:

150ml beakers
100ml volumetric flasks with stoppers
125ml erlenmeyer flasks with #5 rubber stoppers
Watch glasses
Whatman 41 ashless filter paper (160 mm circles)
Funnels
Pasteur Pipette and bulb
Dispenser pipette capable of dispensing 20ml
Wash bottle filled with dd H₂O
Data sheet

Ashing Procedure:

1. Place labeled 150ml beakers in 100°C oven.
2. Let remain in oven for at least 1 hr to evaporate all water attached to beaker.
3. After 1 hr, hot weigh each beaker, one at a time, until all beakers have been weighed. Place weight (in g) in the "Beaker Wt." column of weigh sheet. Be sure to tare scale in between each beaker.
4. Weigh out fecal sample. Place beaker on scale and tare. Weigh out ~1g of fecal or feed samples into beakers(**Note: for milk digestion, add 10 g milk sample**). Record the sample weight accurately onto "Sample Wt." column of weigh sheet. Don't forget to place the cow id into the appropriate spot on the weigh sheet.
5. Place in 100°C oven for DM determination for 8 hr or overnight.
6. Hot weigh samples, one at a time, until all beakers have been weighed. Record weights accurately onto "Dry Wt." column of weigh sheet.
7. Ash at 600°C for 4 hr.
8. **Cool in muffle furnace for 1 hr or until temperature in the furnace is <150°C.** Taking glassware out too soon results in breakage.
9. Return beakers to 100°C oven and let finish cooling for 20 min.
10. Record weights accurately onto "Ashed Wt." column of weigh sheet.

Digestion Procedure (Be sure to wear goggles, lab coat, and nitrile gloves throughout digestion procedure):

1. Preheat hotplates located in rear ventilation hood. Set hot plate #1 and 2 to 400°C. Set hot plate #3 to 5.
2. Add 20ml hydrochloric acid, 4N.
3. Add 5 drops concentrated nitric acid with pasteur pipette.
4. Swirl to mix.
5. Place 4 beakers on each hotplate and cover with watch glasses.
6. Bring to boil and let boil for 7 minutes.
7. Set beaker off hotplate using beaker tongs, keeping watch glass in place. **Do not remove from hood until cool.** Vapors may be harmful if inhaled.
8. Cool for 2 min and rinse watch glass with dd H₂O into corresponding beaker. Watch glass may be reused at this time.
9. Let cool an additional 5 min.
10. Pour beaker contents into labeled 100 ml volumetric flask.
11. Rinse beaker with 5 – 10 ml dd H₂O using wash bottle, 3x, pouring contents into corresponding flask each time.
12. Dilute flasks to the mark with dd H₂O.
13. Stopper flask and invert 10x to mix.
14. Filter solution, using Whatman 41 filter paper, into labeled 125 ml erlenmeyer flask.
15. Stopper Flask.
16. Sample is ready for analysis.

APPENDIX B

Procedure for Feed, Fecal, and Milk P Assay

Reagents:

Molybdovanadate Reagent: Dissolve 40g Ammonium Molybdate • 4 H₂O in 400 ml of hot dd H₂O and cool. Dissolve 2g Ammonium meta-Vanadate in 250 ml hot dd H₂O, cool, and add 450ml Perchloric Acid. Gradually add molybdate solution to vanadate solution with stirring, and dilute to 2L with dd H₂O.

Phosphorus Standard Solution: a) STOCK SOLUTION – (2 mg P/ml) Dissolve 8.788 g KH₂PO₄ in dd H₂O water and dilute to 1L. b) WORKING SOLUTION – (0.1 mg P/ml) Dilute 50ml of stock solution to 1L with dd H₂O.

Materials

16mm X 100 mm test tubes
10ml volumetric flasks with stoppers
P 1000 pipette and tips
P 200 pipette and tips
Repeat pipette
Dispenser pipette capable of dispensing 7 ml water
Wash bottle filled with dd H₂O
96-well plate
Spectrophotometer capable of reading at 400nm

Procedure (Feed and Fecal P):

1. *Standard Curve (0.0, 0.5, 1.0, 1.5, and 2.0 mg P/ml):* From the 0.1 mg P/ml Working Solution pipette the following amounts to create the standard curve:

<u>Std. Concentration</u>	<u>ml 0.1 mg P/ml to add</u>
0.0	no addition
0.5	500µl
1.0	1000µl
1.5	1500µl
2.0	2000 µl

Note: Actual concentrations in the final 10ml volume will be the [standard]/10.

2. *Unknowns:* Mix unknowns by inversion 10x
3. Pipette 1ml of each unknown into a test tube
4. Add 2ml of Molybdovanadate Reagent to each tube, including standards, using the repeat pipette
5. Dilute Standards to mark with dd H₂O using wash bottle and mix by inversion
6. Add 7 ml dd H₂O to test tubes and mix by inversion
7. Let standards and unknown stand for 10 minutes
8. Transfer 200µl of standards and unknowns, in duplicate, to a 96-well plate (make sure to fill out "Data Sheet" to be used as a map for the plate)
9. Read plate at 400nm on spectrophotometer

10. Save file and print a copy

Procedure (Milk P):

1. *Standard Curve (0.0, 0.5, 0.75, 1.0, and 1.25 mg P/ml):* From the 0.1 mg P/ml Working Solution pipette the following amounts to create the standard curve:

<u>Std. Concentration</u>	<u>ml 0.1 mg P/ml to add</u>
0.0	no addition
0.5	500µl
0.75	750µl
1.0	1000µl
1.25	1250µl

Note: Actual concentrations in the final 10ml volume will be the standard concentration ÷ 10.

2. The rest of the procedure is the same as above.

Calculations:

$$\text{a) \% P in ash} = \frac{(\text{mg of P in aliquot}) \times (\text{dilution factor})}{\text{mg of ash in sample}} \times 100$$

$$\text{b) \% P in sample} = (\% \text{ P in ash}) \times (\% \text{ ash in sample}) \times 100$$

<u>Aliquots</u>	<u>Dilution Factor</u>
0.5 mL from 10 mL =	20
1.0 mL from 10 mL =	10

Waste Container Label

0.4 % -Ammonium Molybdate
0.02 % -Ammonium meta-Vanadate
2.5 %- Perchloric Acid (70 %)
97 % - Water

REFERENCE: Assoc. Official Analytical Chemists (1990). Method 965.17.

APPENDIX C

Digestion for Chromium Analysis

Reagents:

1. Phosphoric acid-manganese sulfate solution:
30ml of 10% w/v, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ + 970ml of 85% phosphoric acid.
2. 4.5%, w/v, potassium bromate solution.
3. 4000 ppm Ca solution: 11.09 g CaCl_2 /liter dd H_2O .
4. Chromium atomic absorption standard solution (Aldrich Chemical Co., Inc.)

Standards:

1, 2, 3, 4, 5 ppm Cr

Materials

50ml beakers

Watch glasses

100ml volumetric flasks with glass stoppers

Repeat pipette with three different tips

Wash bottle filled with dd H_2O

Whatman 41 ashless filter paper (160 mm circles)

Funnels

Digestion Procedure:

1. Weigh 1g samples into 50 ml glass beakers
2. Place samples into 100°C oven overnight for DM determination
3. Ash at 600°C for 1.5 hours
4. **Cool until furnace temperature is <150 (usually about 1 hour).** Taking beakers out too soon may cause breakage
5. Add 3ml of phosphoric acid-manganese sulfate solution; swirl to mix
6. Add 4 ml of 4.5%, w/v, potassium bromate solution; swirl to mix
7. Cover with a watch glass and digest on a previously heated hot plate (set between 2 and 3) until effervescence ceases and purple color appears (usually 5-7 min)
8. Cool for 5 min
9. Pour into labeled 100 ml volumetric flask
10. Rinse, with wash bottle, 3 times with 5 – 10 ml of dd H_2O and pour into 100 ml vol. flask
11. Add 12.5ml of a calcium chloride solution containing 4000 ppm of calcium
12. Dilute to mark with dd H_2O with wash bottle, and invert 10X to mix thoroughly

13. Filter sample with Let stand overnight to allow suspended material to settle or filter
14. Dilute digested sample 1:6 and read on an atomic absorption spectrophotometer

APPENDIX D

Urine Specific Gravity Analysis

1. Filter composited urine sample through four layers of cheesecloth into another clean, dry specimen container.
2. Rinse original specimen container with water and acetone, let dry and pour filtered sample back into original container.
3. Place ~25 ml filtered urine sample into urinometer cylinder (1" clearance from top of cylinder).
4. Measure urine temperature (°F) and record on data sheet.
5. Grasp the hydrometer at its top and slowly insert into cylinder. **AVOID WETTING FLOAT STEM ABOVE LIQUID LINE.** Excessive wetting of the stem will cause the float to sink below the true test reading.
6. Impart a slight spin to the float as it is released to release any air bubbles that may have been attached to the hydrometer.
7. Read the scale of the urinometer at the lowest portion of the meniscus of the urine at eye level. Be sure to keep float away from sides of cylinder while reading.
NOTE: If it is necessary to read the top of the meniscus, as in the case of opaque specimens, add 0.002 to the specific gravity reading to correct for viewing error.
8. Apply temperature corrections, as necessary and record on data sheet. **Note:** the urinometer was calibrated at 60°F, therefore, the SG must be corrected if the temperature of liquid is not 60°F.
 - a. $T_{\text{urine}} - 60^{\circ}\text{F} = \text{Ans}_1$
 - b. $\text{Ans}_1 / 5.4^{\circ}\text{F} = \text{Ans}_2$
 - c. $\text{Ans}_2 * 0.001 = \text{Ans}_3$
 - d. $\text{Corrected SG} = \text{Measured SG} + \text{Ans}_3$
9. Pour urine sample back into original container.
10. Rinse cylinder and hydrometer with water and then acetone and let dry between each sample.
11. The urine sample is now ready for P analysis.

APPENDIX E

Procedure for Urine Phosphorus Assay

Materials Needed:

96-well plate
P 200 pipet and tips
P 1000 pipet and tips
Repeatable pipet and tip capable of pipetting 2 ml
12 x 75 mm test tubes and rack
Sharpie
Spectrophotometer
Urine Samples

Reagents Needed:

Trichloroacetic Acid, 20% (TCA)
Molybdate Reagent
Aminonaphtholsulfonic acid
Ultra pure water (UPW)

TCA, 20%; Add 200 g trichloroacetic acid in a 1 l volumetric flask and fill to the mark w/ UPW. Label a 1 l glass container; pour solution into container, cover w/ parafilm, and close lid tightly for storage.

Sulfuric Acid, 10N; Add 250 ml concentrated sulfuric acid to 750 ml UPW. Mix. Cool. Label a 1 l container; pour solution into container, cover w/ parafilm, and close lid tightly for storage in refrigerator. Don't forget to add ACID TO WATER!

Molybdate Reagent; Dissolve 25 g ammonium molybdate in 200 ml UPW. Place 300 ml of Sulfuric Acid, 10N into a 1 l volumetric flask. Add molybdate solution and mix. Fill to the mark w/ UPW. Label a 1 l glass container; pour solution into container, cover w/ parafilm, and close lid tightly for storage. Discard if blanks show a blue color.

Aminonaphtholsulfonic acid reagent; This reagent can be purchased as 1-amino-2-naphthol-4-sulfonic acid solution (for Phosphate) from Fisher Scientific. Check expiration date prior to use.

Assay Procedure:

1. Obtain thawed, room temperature urine sample.
2. Using a Sharpie, number a test tube for each sample to be analyzed. Each sample should be done in duplicate.
3. Shake urine composite vigorously for 10 s and pipette 1 ml urine sample into labeled test tube.
4. Repeat Step 4 until all samples to be analyzed have been completed.
5. Add 2 ml TCA, 20% (1:3 dilution) to each test tube containing 1 ml of urine sample.
6. Mix by inversion and let stand for 5 min.
7. Spin sample at 4000 rpm in a centrifuge for 15 min.
8. While samples are spinning prepare plate.

Standard Curve:

Pipette 175 μ l of the following standard P concentrations into rows A and H of a 96-well plate:

0 mg/dl (TCA), A and H1;

0.25 mg/dl, A and H2;

0.50 mg/dl, A and H3;

0.75 mg/dl, A and H4;

1.0 mg/dl, A and H5;

1.25 mg/dl, A and H6.

9. Pipette 175 μ l of supernatant, being careful not to disturb pellet, into a well of a 96-well plate (do in duplicate, 175 μ l each).
10. Add 35 μ l Molybdate reagent to all wells.
11. Add 14 μ l Aminonaphtholsulfonic acid reagent to all wells, agitate the plate slightly to mix
12. Add 125 μ l UPW, agitate the plate slightly to mix, and let sit for 5 min.
13. Read standards and unknowns at 660 nm with a spectrophotometer.
14. Repeat duplicates if the CV is greater than 10%.

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