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## Prediction of phosphorus output in manure and milk by lactating dairy cows

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### ABSTRACT

Mathematical models for predicting P excretions play a key role in evaluating P use efficiency and monitoring the environmental impact of dairy cows. However, the majority of extant models require feed intake as predictor variable, which is not routinely available at farm level. The objectives of the study were to (1) explore factors explaining heterogeneity in P output; (2) develop a set of empirical models for predicting P output in feces ( $P_f$ ), manure ( $P_{Ma}$ ), and milk ( $P_m$ , all in g/cow per day) with and without dry matter intake (DMI) using literature data; and (3) evaluate new and extant P models using an independent data set. Random effect meta-regression analyses were conducted using 190  $P_f$ , 97  $P_{Ma}$ , and 118  $P_m$  or milk P concentration ( $P_{MilkC}$ ) treatment means from 38 studies. Dietary nutrient composition, milk yield and composition, and days in milk were used as potential covariates to the models with and without DMI. Dietary phosphorus intake ( $P_i$ ) was the major determinant of  $P_f$  and  $P_{Ma}$ . Milk yield negatively affected  $P_i$  partitioning to  $P_f$  or  $P_{Ma}$ . In the absence of DMI, milk yield, body weight, and dietary P content became the major determinants of  $P_f$  and  $P_{Ma}$ . Milk P concentration ( $P_{MilkC}$ ) was heterogeneous across the treatment groups, with a mean of 0.92 g/kg of milk. Milk yield, days in milk, and dietary Ca-to-ash ratio were negatively correlated with  $P_{MilkC}$  and explained 42% of the heterogeneity. The new models predicted  $P_f$  and  $P_{Ma}$  with root mean square prediction error as a percentage of observed mean (RMSPE%) of 18.3 and 19.2%, respectively, using DMI when evaluated with an independent data set. Some of the extant models also predicted  $P_f$  and  $P_{Ma}$  well (RMSPE% = 19.3 to 20.0%) using DMI. The new models without DMI as a variable predicted  $P_f$  and  $P_{Ma}$  with RMSPE% of 22.3 and 19.6%, respectively, which can be used in monitoring P excretions at farm level. When evaluated with an independent data set, the new model and extant models based on milk protein content predicted  $P_{MilkC}$  with

RMSPE% of 12.7 to 19.6%. Although models using P intake information gave better predictions, P output from lactating dairy cows can also be predicted well without intake using milk yield, milk protein content, body weight, and dietary P, Ca, and total ash contents. **Key words:** modeling, phosphorus, lactating cow, manure

### INTRODUCTION

Phosphorus is an essential element for dairy cows with well-documented functions. A primary role of P is development and maintenance of the skeletal system. Phosphorus is also involved in cellular energy transfer through ADP and ATP. Phosphorus is intimately involved in acid-base buffer systems of blood, a part of casein, and associated with a small fraction of milk fat. It is also involved in enzyme systems and a constituent of saliva, and thereby assists in digestive functions (NRC, 2001; Vitti and Kebreab, 2010). Increased environmental concerns and regulations have drawn attention to P amounts in dairy rations. A major source of environmental pollution has been overfeeding P to dairy cows, caused by the safety margins added to diets to ensure that health and production of animals is not compromised (Kebreab et al., 2008). Although several studies have demonstrated no effect of feeding P above NRC (2001) recommended levels on production and health (Wu et al., 2001; Dou et al., 2003; Reid et al., 2015), the average dietary P concentration is still about 30% greater than NRC (2001) recommendations (Dou et al., 2003). Excess P is excreted by dairy cattle and contributes to eutrophication of water bodies (Smith et al., 2001). In the United States, the livestock sector is responsible for about 33% of P load into freshwater resources (FAO, 2006). Moreover, P overfeeding primarily using inorganic supplements could result in economic losses (Knowlton and Herbein, 2002) because P accounts for more than 50% of the cost of typical vitamin-mineral mixes used in dairy farms (Chandler, 1996).

In dairy cattle, P is primarily excreted in feces and secreted in milk. Urinary P excretion ( $P_u$ ) is negligible under practical feeding conditions; therefore, P balance

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of lactating dairy cows is determined primarily by P intake ( $P_i$ ) with feed, P excreted in feces ( $P_f$ ), and P secreted in milk ( $P_m$ ; Valk et al., 2002). Measuring daily  $P_f$  can be laborious and expensive; therefore, several mathematical models have been developed for predicting  $P_f$  or P excreted in both feces and urine ( $P_{Ma}$ ). The majority of the extant models (e.g., Van Horn et al., 1994; Wu et al., 2001; Weiss and Wyatt, 2004a; Nennich et al., 2005) require DMI of individual cows as an input, which may not be routinely available in dairy farms. Phosphorus secretion in milk is generally predicted assuming P concentration in milk ( $P_{MilkC}$ ) is constant at 0.90 g/kg (NRC, 2001). However, Klop et al. (2014) reported that  $P_{MilkC}$  is variable and it is affected by differences in milk protein and lactose contents, which in turn is a function of nutrient composition in the diet.

Efficient use of dietary P by dairy cows has considerable economic and environmental implications, and developing and evaluating mathematical models that predict P balance are needed. A body of literature has been building that encompasses studies with wide range of P sources, intake, nutrient concentrations, milk production potentials, and stages of lactation. Potentially, the data can be used to identify variables that are required to predict  $P_f$  or  $P_{Ma}$  and  $P_m$ . The objectives of the current study were to (1) explore factors explaining heterogeneity in  $P_f$ ,  $P_u$ ,  $P_{Ma}$ , and  $P_m$  of modern lactating dairy cows using literature data published after 2000, (2) develop a set of empirical models for predicting  $P_f$ ,  $P_{Ma}$ , and  $P_m$  using those factors, and (3) evaluate the new and extant models for predicting P output by dairy cows using an independent data set.

## MATERIALS AND METHODS

### Data Sources

Literature searches of the ScienceDirect (<http://www.sciencedirect.com/>) and *Journal of Dairy Science* (<http://www.journalofdairyscience.org/>) online databases were conducted using the combination of search terms “phosphorus excretion,” “fecal phosphorus,” “urinary phosphorus,” and “dairy cows.” The period covered was from 2000 to 2015. The searches were refined to include only scientific research published in journal articles in English. The searches resulted in 357 articles: 199 from ScienceDirect and 158 from *Journal of Dairy Science*. Abstracts of the 357 articles were examined for 2 major inclusion criteria: (1) studies should be in vivo involving lactating dairy cows, and (2) availability of information on measured  $P_f$ ,  $P_u$ , or  $P_m$ . A further screening was carried out based on (1) availability of information on sample size ( $N$ ) and information on un-

certainty of the P excretion measures, such as standard deviation ( $SD$ ), standard error of the mean ( $SEM$ ), or standard error of the difference ( $SED$ ); and (2) availability of description on diet composition, milk yield and composition, DIM, and BW. The second screening resulted in 38 articles meeting the criteria including 190  $P_f$ , 118  $P_m$ , and 97  $P_u$  treatment means (g/d). Descriptions and summary statistics of relevant measured dietary and animal variables are provided in Table 1. The mean and variances of variables in the complete and reduced data sets containing means of  $P_f$ ,  $P_m$ , and  $P_u$  were similar. Therefore, summary statistics of only the complete data set is given in the Table 1.

Treatment means and associated  $SD$  and  $N$  were of primary interest for the analysis. Additionally, ingredient and nutrient composition of experimental diets, including DM, CP, NDF, ADF, starch, gross energy ( $GE$ ), ether extract ( $EE$ ) contents (% of DM), DMI (kg/d), milk yield (kg/d), milk composition, BW (kg), and DIM were also retrieved. Any missing nutrient composition values of the experimental diet were calculated using the ingredient composition and nutritive value tables in NRC (2001). For studies only reporting  $SEM$ ,  $SD$  was calculated as  $SD = SEM\sqrt{N}$ . For studies only reporting  $SED$ ,  $SD$  was calculated as  $SD = (SED/\sqrt{2})/\sqrt{N}$ . Phosphorus concentration in milk (g/kg of milk) was calculated by dividing  $P_m$  by milk yield. The  $SD$  for  $P_{MilkC}$  was calculated with the  $SD$  of  $P_m$  and milk yield using the online error propagation calculator (<http://laffers.net>; Stella et al., 2013). Phosphorus output in manure was calculated by adding  $P_f$  and  $P_u$ . The corresponding  $SD$  were calculated as for  $P_{MilkC}$ .

### Statistical Analysis

A meta-regression analysis was conducted to construct a set of empirical equations to predict P excretions in manure and milk from dairy cows using R statistical software (version 2.12.2, R Foundation for Statistical Computing, Vienna, Austria). Random effect models were first used to estimate heterogeneity of the treatment means and extended to mixed effect meta-regression models, potentially explaining much of the heterogeneity as described in Viechtbauer (2010). Random effect models are given by

$$y_i = \mu + u_i + e_i,$$

where  $y_i$  = mean  $P_f$ ,  $P_u$ ,  $P_{Ma}$ ,  $P_m$ , or  $P_{MilkC}$  of lactating cows in  $i$ th treatment group;  $\mu$  = overall mean  $P_f$ ,  $P_u$ ,  $P_{Ma}$ ,  $P_m$ , or  $P_{MilkC}$  of lactating cow;  $u_i$  = random deviation of the mean response (e.g.,  $P_f$ ) of cows in the  $i$ th

treatment from the overall mean, which is assumed to be normally distributed with a mean of 0 and variance of amount of residual heterogeneity ( $\tau^2$ ) representing heterogeneity of the excretion [ $u_i \sim N(0, \tau^2)$ ]; and  $e_i$  is the sampling error [ $e_i \sim N(0, v_i)$ ], the variance ( $v_i$ ) of which is assumed to be known and calculated using the SD of the treatment means. The sampling error remained fixed during estimation and served to weight the individual treatment means (Viechtbauer, 2010). Besides estimating  $\tau^2$ , the random effect models were also used to conduct an influence diagnosis to identify outliers. The influence diagnosis conducted using the *influence* function in the *metafor* package of R (version 1.6-0) revealed all P excretion measurements were associated with standardized residual values ranging between -3.0 and 3.0, so no observation was excluded from the database.

The estimated heterogeneity was expressed as a percentage of total variability ( $\tau^2$  plus  $v_i$ ), yielding  $I^2$  statistics. An  $I^2$  value greater than 50% indicates considerable heterogeneity. In the presence of considerable heterogeneity, the random effect models are extended to mixed effect models including fixed effects of variables

potentially able to explain the heterogeneity (Viechtbauer, 2010). The mixed effect models are given by

$$\theta_i = \beta_0 + \beta_1 x_{i1} + \dots + \beta_p x_{ip} + u_i,$$

where  $\theta_i$  is the true effect size in the  $i$ th treatment group;  $\beta_0$  is the overall true effect size;  $x_{ij}$  is the value of the  $j$ th explanatory variable ( $j = 1, 2, \dots, p$ ) for the  $i$ th treatment mean;  $\beta_{1-p}$  is the change in the response for a unit increase in each of the explanatory variables; and  $u_i$  is the remaining variability that could not be explained by the explanatory variables [ $u_i \sim N(0, \tau^2)$ ]. In the present analysis, the parameters in the mixed effect models ( $\beta_0, \dots, \beta_p$ ) were estimated via weighted least squares with weights equal to  $1/(v_i + \tau^2)$ .

### Explanatory Variables

The candidate explanatory variables for the mixed effect models were DMI (kg/d), P intake ( $P_i$ ; g/d), dietary CP, NDF, ADF, EE, total ash (all in % of DM), and GE (MJ/kg of DM) contents, milk yield (kg/d), milk fat and protein percentages, DIM, and BW. Mixed

**Table 1.** A summary data (n = 190) retrieved from 38 studies<sup>1</sup>

Variable	Mean	SD	Minimum	Maximum
Phosphorus output (g/cow per day)				
Feces	48.6	19.0	15.5	99.2
Urine	0.67	0.73	0.033	3.66
Manure	49.8	20.0	15.7	103
Milk	31.3	7.11	12.3	50.3
Phosphorus intake (g/cow per day)	81.8	24.3	28.1	142
DMI (kg/cow per day)	21.5	3.60	8.7	29
Diet composition (% of DM)				
Forage	53.6	14.1	12.3	100
CP	16.6	2.1	10.2	21.6
NDF	34.5	7.2	19.6	55.0
ADF	21.6	4.1	14.3	36.1
Starch	21.1	8.1	1.5	37.2
Ash	6.1	1.5	2.1	9.8
Ether extract	3.4	1.1	0.5	6.0
Phosphorus	0.38	0.09	0.2	0.58
Calcium	0.65	0.23	0.12	1.18
Calcium:ash	0.11	0.05	0.01	0.30
Gross energy (MJ/kg)	18.3	1.64	11.1	19.0
Milk yield (kg/cow per day)	31.2	8.1	15.4	52.8
Milk protein (%)	3.1	0.2	2.7	4.1
Milk fat (%)	3.8	0.6	2.9	5.4
Phosphorus in milk (g/kg)	0.96	0.18	0.56	1.49
DIM	137	74.1	15	280
BW (kg)	608	54.0	463	754

<sup>1</sup>Berry et al. (2001), Bjelland et al. (2011), Borucki Castro et al. (2004), Brask-Pedersen et al. (2013), Dann et al. (2008), Ekelund et al. (2003), Ekelund et al. (2006), Elizondo Salazar et al. (2013), Feng et al. (2013), Ferris et al. (2010), Grabherr et al. (2009), Guyton et al. (2003), Haese et al. (2014), Herrera et al. (2010), Jarrett et al. (2014), Kamiya et al. (2010), Kincaid et al. (2005), Knowlton et al. (2001), Knowlton et al. (2002), Knowlton et al. (2007), Kojima et al. (2005), Moreira et al. (2009), Myers and Beede (2009), Odongo et al. (2007), O'Rourke et al. (2007), Puggaard et al. (2011), Puggaard et al. (2013), Ray et al. (2013), Reid et al. (2015), Shore et al. (2005), Stella et al. (2013), Tallam et al. (2005), Taylor et al. (2009), Valk et al. (2002), Wang et al. (2014), Wu et al. (2001), Wu et al. (2003), and Wu et al. (2000).

**Table 2.** Correlations among DMI, phosphorus intake ( $P_i$ ), dietary CP, NDF, ADF, total ash, phosphorus (P), and calcium (Ca) contents, milk protein percentage (MProt), DIM, and BW

Item	$P_i$	Milk	CP	NDF	ADF	Total ash	P	Ca	MProt	DIM	BW
DMI	0.65*	0.69*	0.24*	-0.38*	-0.39*	0.11	0.11	0.10	-0.31*	-0.06	0.31*
$P_i$		0.46*	0.37*	-0.46*	-0.48*	-0.02	0.82*	0.32*	-0.04	-0.02	0.11
Milk			0.24*	-0.40*	-0.34*	0.15	0.17*	0.11	-0.34*	-0.36*	0.18*
CP				-0.42*	-0.32*	-0.09	0.34*	0.34*	0.02	0.10	0.01
NDF					0.68*	0.04	-0.36*	-0.10	0.17*	0.13	0.06
ADF						0.15	-0.37*	-0.27*	0.08	0.22*	0.29*
Ash							-0.13	0.20*	-0.30*	0.06	0.27*
P								0.33*	0.19*	0.03	-0.15
Ca									-0.14	-0.09	-0.18
MProt										0.28*	-0.45*
DIM											0.25*

\* $P < 0.05$ .

effect models, including individual explanatory variables, were first regressed separately on the response variables. Full multivariate models were then formed including all explanatory variables having notable effects ( $P < 0.10$ ) when fitted individually. The correlated variables ( $|r| > 0.5$ ) were not included in the same model to avoid multicollinearity issues (Appuhamy et al., 2014a). For example, dietary P concentration and intake were not included together ( $r = 0.82$ , Table 2), which led to 2 full models and, thereby, 2 lines of model selection. Reduced models were formed via stepwise elimination of one explanatory variable at a time by fitting with maximum likelihood method. The best prediction model was chosen by comparing the log-likelihood value and Bayesian information criteria. The parameter estimates of the best model were obtained with restricted maximum likelihood method (Viechtbauer, 2010).

### Model Evaluation

The models were developed on treatment means of P output measurements collected from the literature, which provided data with wide range of values for several variables such as P intake, milk yield, dietary nutrient composition, and DIM potentially associated with dairy cattle P outputs. However, determination of how well the new models captured true associations requires evaluation with independent data, preferably P output measurements made daily on individual cows. Therefore, the  $P_f$  and  $P_{Ma}$  models were evaluated with a separate data set including 354  $P_f$ , 177  $P_{Ma}$ , and 43  $P_{MilkC}$  measurements (g/cow per day) from several recently conducted experiments, which were not included in model development. Two experiments conducted at the University of California-Davis provided 207  $P_f$  and 134  $P_{Ma}$  measurements. In one experiment, lactating Holstein cows were assigned to 2 dietary forage levels (37 vs. 53% of DM) and 2 dietary CP levels (16.2 vs.

19.7% of DM). In the other experiment, cows were supplemented with 2 doses of a fibrolytic enzyme (plus control), which did not affect P intake or excretion. The Institutional Animal Care and Use Committee at University of California-Davis approved all the animal procedures in the experiments. The rest of the  $P_f$  and  $P_{Ma}$  measurements and the  $P_{MilkC}$  measurements were obtained from experiments published by Weiss and Wyatt (2004b), Weiss et al. (2009, 2011), and Beckman and Weiss (2005). The fecal and manure P measurements were related to considerably large range of values for DMI (15.0 to 32.0 kg/d),  $P_i$  (53.9 to 128 g/d), dietary P concentrations (0.27 to 0.42% of DM), and milk yields (16.8 to 59.0 kg/d). Moreover, the extant models in Klop et al. (2014) for predicting  $P_{MilkC}$  were evaluated with the literature data. The Klop et al. (2014) models requiring milk lactose percentage were not evaluated as lactose percentage was not reported for more than 50% of the measurements. Moreover, data from Wu et al. (2000) were removed as they were involved in development of the models. The final data set included 100 treatment means of  $P_{MilkC}$ .

The overall agreement between model predictions and the data were determined by calculating the mean square prediction error (**MSPE**):

$$MSPE = \frac{1}{n} \cdot \sum_{i=1}^n (O_i - Pr_i)^2,$$

where  $n$  = number of observations;  $O_i$  = observed response of  $i$ th study treatment;  $Pr_i$  = corresponding predicted response. As the square root of MSPE (**RMSPE**) carries the same unit of observed values, RMSPE was expressed as a percentage of average observed value (**RMSPE%**). The RMSPE% quantifies overall agreement between predicted and observed values, but does not explain consistency of this agreement throughout the data range in question. For example, models can

over-predict the response at a constant degree (the presence of only mean bias) or the over-prediction tendency increases as predicted value increases (the presence of both mean and slope bias). Therefore, MSPE was decomposed into mean bias, slope bias, and bias due to random variability of data. Additionally, the Nash-Sutcliffe efficiency index (**NSE**; Nash and Sutcliffe, 1970) was used to compare  $P_{\text{MilkC}}$  predictions from models to the constant  $P_{\text{MilkC}}$  (0.90 g/kg of milk) used in NRC (2001). The NSE ranging between  $-\infty$  and  $\infty$  can be calculated using following equation, where  $R$  = the reference value:

$$\text{NSE} = \frac{\sum (O_i - P_i)^2}{\sum (O_i - R)^2}.$$

Positive NSE values indicate that the model predictions are better than the reference constant.

## RESULTS

The dependent variables,  $P_f$ ,  $P_u$ ,  $P_{\text{Ma}}$ , and  $P_m$ , and independent variables,  $P_i$ , dietary forage content, dietary P content, milk yield, and DIM, had a wide range of values (CV  $\geq 23\%$ ), allowing a model development to capture the true relationships. For example, a range of DIM values (15–280, CV = 54%) allowed models to represent the relationships of P excretions over different stages of lactation. Phosphorus intake varied more than DMI (CV = 30 vs. 17%) owing to more variable dietary P content (CV = 24%). Phosphorus intake ranged from 28.1 to 142 g/d with a mean of 81.8 g/cow per day, whereas dietary P content ranged from 0.20 to 0.58% of DM with a mean of 0.38% of DM. Total ash (CV = 24%) and calcium (CV = 39%) concentrations, and thereby dietary Ca-to-ash ratio (CV = 47%), of experimental diets varied considerably. Corn silage, alfalfa silage, and grass hay were the most frequently used forage sources (80, 50, and 25% of the diets, respectively), whereas soybean meal, ground corn grain, high-moisture corn, and cottonseed meal and roasted soybean (60, 40, 30, and 26% of the diets, respectively) were the most widely used concentrate feed ingredients for the experimental diets.

### Feces, Urine, and Manure P Models

The random-effect model analysis revealed that the average  $P_f$  of lactating dairy cows was  $47.8 \pm 1.6$  g/d, representing 58% of the  $P_i$  (Table 3). The  $P_f$  measurements were considerably heterogeneous across the study treatments ( $P < 0.001$  for  $\tau^2$ ). Between-treatment group variability or heterogeneity ( $\tau^2$ ) was responsible for

98% of the total variability of  $P_f$  ( $I^2 = 98\%$ ; Table 3), whereas within-treatment group variability accounted for only 2% of the total variability. Differences in  $P_i$  appeared to be responsible for much of the heterogeneity in  $P_f$  (Figure 1A). After the random effect model was extended to a mixed effect model (including fixed effect of  $P_i$  and milk yield),  $\tau^2$  was reduced from 340 (Table 3) to 77.6 (equation 1 in Table 4), indicating  $>75\%$  of the heterogeneity was due to the differences in  $P_i$  and milk yield among the treatment groups. Dietary ash content had also a significant association with  $P_f$  and was included in the final model along with  $P_i$  and milk yield (equation 2 in Table 4). Given all other parameters were constant, a 1.0 g/d increase in  $P_i$  would result in a  $0.72 \pm 0.03$  g/d increase of  $P_f$  (equation 2 in Table 4). Milk yield was negatively related to  $P_f$  ( $r = -0.17$ ) if expressed as a proportion of  $P_i$  (Figure 1B). Consistently, the final model included a negative relationship of milk yield with  $P_f$ , independent of  $P_i$  (Table 4). Holding all other parameters constant, a 1.0 kg/d increase in milk yield is predicted to result in a  $0.48 \pm 0.09$  g/d reduction in  $P_f$ . Independent of both  $P_i$  and milk yield, a 1-unit increase in dietary ash content (% of DM) was related to a 0.74-g increase in  $P_f$  (equation 2 in Table 4). When DMI was not taken into account, milk yield, BW, and dietary P content appeared to primarily drive  $P_f$  (equation 3 in Table 4) and explained 67% of heterogeneity (data not shown). The positive effects of milk yield and the P content collectively represent the effect of  $P_i$  given the strong positive relationship between DMI and milk yield ( $r = 0.69$ ; Table 2). The mean  $P_u$  was estimated to be  $0.35 \pm 0.027$  g/d, which was only 0.44% of the average  $P_i$  (Table 3). However,  $P_u$  was heterogeneous (CV = 109% in Table 1, and  $I^2 = 99\%$  in Table 3) across the treatment groups. Only dietary CP content and DIM had significant associations ( $P < 0.05$ ) with  $P_u$  (equation 4 in Table 4) and explained only 23% of the heterogeneity ( $\tau^2 = 0.038$  vs. 0.029).

Similar to  $P_f$ ,  $P_i$  and milk yield were significantly associated with  $P_{\text{Ma}}$ . Independent of both  $P_i$  and milk yield, dietary ash content (% of DM) and milk protein content were also positively associated ( $P < 0.005$ ) with  $P_{\text{Ma}}$  and included in the final model (equation 5 in Table 4). All 4 factors explained 87% of  $P_{\text{Ma}}$  heterogeneity ( $\tau^2 = 46.1$  vs. 354). If other parameters were constant, a 1.0 g/d increase in  $P_i$  would result in a  $0.73 \pm 0.04$  g/d  $P_{\text{Ma}}$  increase. An increase in 1.0 kg/d of milk yield would decrease  $P_{\text{Ma}}$  by  $0.30 \pm 0.11$  g/d. Dietary ash content and milk protein content were associated positively with dietary inclusion rates of soybean meal and cottonseed (data not shown), potentially incorporating more phytate-bound P to the diet. As with  $P_f$ , milk yield, dietary P, and BW were positively associ-

**Table 3.** Mean phosphorus (P) output (g/d) in feces, urine, manure, and milk, and P concentration in milk (g/kg of milk) with the number of treatment means in the data set (N), average P intake ( $P_i$ ), and heterogeneity estimate ( $\tau^2$ ) from random-effect model analyses<sup>1</sup>

Variable	N	$P_i$	Mean $\pm$ SE	$\tau^2$	$I^2$
Feces P	190	82.1	47.8 $\pm$ 1.6	340	98
Urine P	97	79.3	0.35 $\pm$ 0.027	0.038	99
Manure P	97	79.3	49.7 $\pm$ 1.9	354	99
Milk P concentration	118	81.7	0.92 $\pm$ 0.01	0.012	75
Milk P	118	81.7	31.7 $\pm$ 0.61	38.5	94

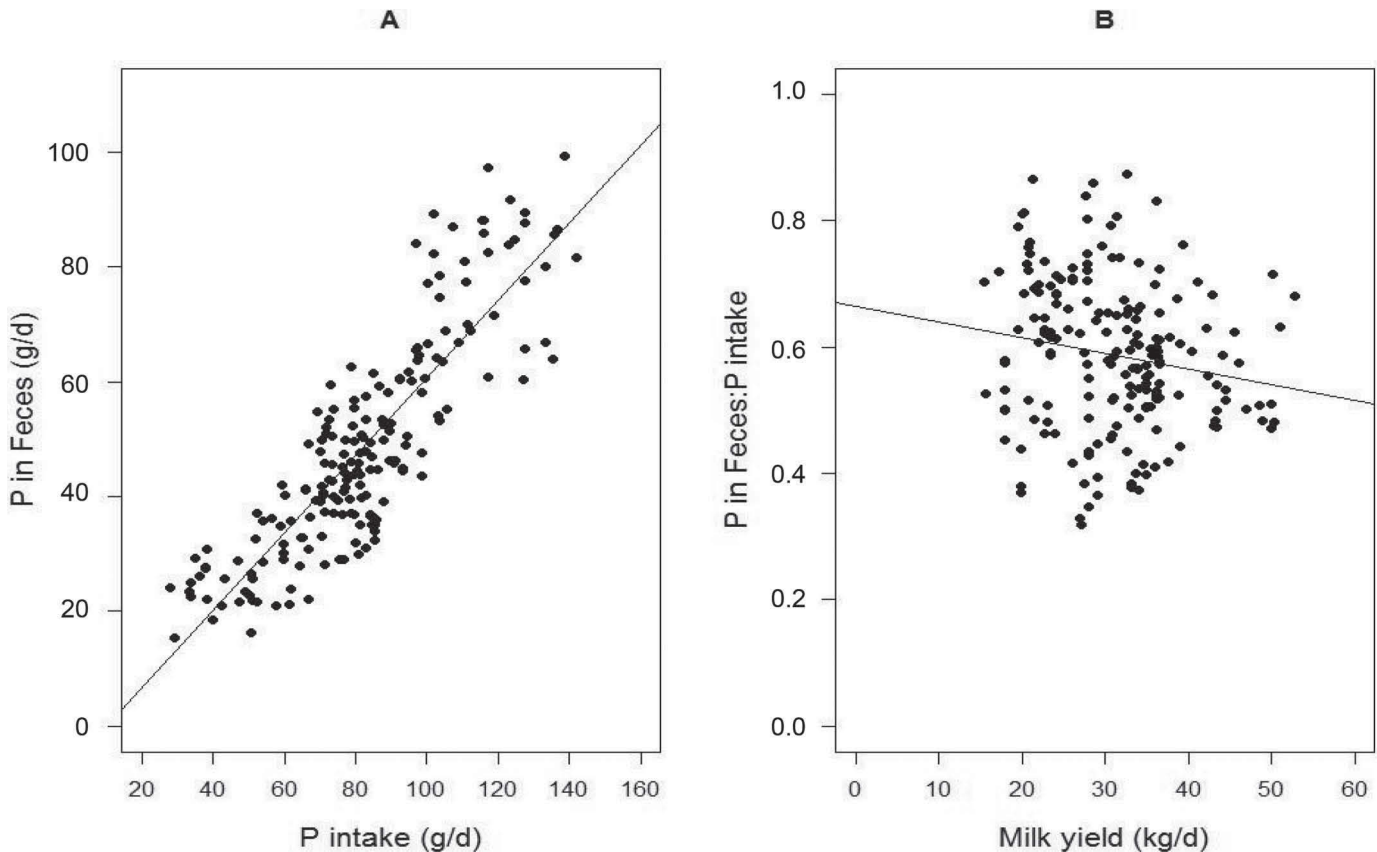
<sup>1</sup> $I^2 = \tau^2$  as a percentage of total variability.

ated with  $P_{Ma}$  in the absence of DMI (equation 6 in Table 4). Additionally, dietary CP concentration had a negative relationship with  $P_{Ma}$ , in the absence of DMI. The variables explained 80% of heterogeneity ( $\tau^2 = 354$  vs. 71.4, Table 3 and 4).

### Milk P Models

The mean  $P_{MilkC}$  was  $0.92 \pm 0.01$  g/kg of milk (Table 3). Between-treatment group and within-treatment group variability represented 75 and 25% of the total variability of  $P_{MilkC}$  in data ( $I^2 = 75$ ; Table 3), respec-

tively. Milk yield ( $P < 0.001$ ), DIM ( $P < 0.001$ ), dietary Ca concentration ( $P = 0.005$ ), and dietary ash concentration ( $P = 0.011$ ) were negatively associated  $P_{MilkC}$ . Given dietary Ca is part of dietary ash, a variable Ca-to-ash ratio was calculated expressing dietary Ca as a proportion of dietary ash. The final mixed effect model (equation 7 in Table 4) including milk yield, DIM, and Ca-to-ash ratio explained 42% of the heterogeneity in milk P concentration ( $\tau^2 = 0.012$  vs. 0.007). The mean  $P_m$  of lactating dairy cows was estimated to be  $31.7 \pm 0.61$  g/d and highly heterogeneous between the studies ( $I^2 = 94\%$ ). Variability in milk yield was



**Figure 1.** Relationship between P intake and fecal P output (A), as well as milk yield and the ratio of fecal P output to P intake (B).

**Table 4.** New models developed for predicting P output (g/d) in feces ( $P_f$ ), urine ( $P_u$ ), manure ( $P_{Ma}$ ), and milk ( $P_m$ ), and factors affecting P concentration in milk ( $P_{MilkC}$ , g/kg of milk)<sup>1</sup>

Variable	Model	$\tau^2$	LL	BIC
$P_f$	With DMI = $(0.73 \pm 0.03 \times P_i) - (0.37 \pm 0.08 \times \text{milk})$ [Eq. 1]	77.6	-547	1108
	= $(0.72 \pm 0.03 \times P_i) - (0.48 \pm 0.09 \times \text{milk}) + (0.74 \pm 0.46 \times \text{ash}\%)$ [Eq. 2]	75.6	-542	1103
$P_u$	Without DMI = $-59.4 \pm 13.0 + (0.42 \pm 0.11 \times \text{milk}) + (166.1 \pm 10.3 \times P\%) + (0.051 \pm 0.023 \times \text{BW})$ [Eq. 3]	112	-563	1151
	= $(0.038 \pm 0.006 \times \text{CP}) - (0.002 \pm 0.0006 \times \text{DIM})$ [Eq. 4]	0.08	-56.8	126
$P_{Ma}$	With DMI = $-45.6 \pm 12.1 + (0.73 \pm 0.04 \times P_i) - (0.30 \pm 0.11 \times \text{milk}) + (2.37 \pm 0.53 \times \text{ash}\%) + (10.4 \pm 3.15 \times \text{MProt})$ [Eq. 5]	46.1	-316	661
	Without DMI = $-52.9 \pm 17.7 + (202.6 \pm 13.4 \times P\%) + (0.23 \pm 0.12 \times \text{milk}) - (0.99 \pm 0.54 \times \text{CP}\%) + (0.060 \pm 0.023 \times \text{BW})$ [Eq. 6]	71.4	-227	478
$P_{MilkC}$	= $1.32 \pm 0.07 - (0.006 \pm 0.002 \times \text{milk}) - (0.0005 \pm 0.0001 \times \text{DIM}) - (1.16 \pm 0.29 \times \text{Ca:ash})$ [Eq. 7]	0.007	81	-133
$P_m$	= $15.5 \pm 2.20 + (0.67 \pm 0.05 \times \text{milk}) - (0.019 \pm 0.005 \times \text{DIM}) - (38.6 \pm 9.25 \times \text{Ca:ash})$ [Eq. 8]	11.7	-324	672

<sup>1</sup> $P_i$  = phosphorous intake (g/d); milk = milk yield (kg/d); BW in kg; ash% = dietary ash content (% of DM); P% = dietary P content (% of DM); Ca:ash = dietary calcium-to-ash ratio; CP% = dietary CP (% of DM); MProt = milk protein (%);  $\tau^2$  = heterogeneity estimate; LL = log-likelihood; BIC = Bayesian information criteria.

primarily responsible for the heterogeneity in  $P_m$  and explained 61% of the differences (data not shown). The final model for predicting  $P_m$  (equation 8 in Table 4) included milk yield, DIM, and Ca-to-ash, which together explained 70% of heterogeneity [38.5 (Table 3) vs. 11.7 (Table 4)].

### Model Evaluation

The model including only  $P_i$  and milk yield predicted  $P_f$  with a RMSPE% of 18.3% (Table 5). Inclusion of dietary ash content did not improve prediction accuracy, as RMSPE% remained at 18.3%. The mean and slope biases were negligible in both cases, where 98 to 99% of the prediction error were due to random variability of data. The extant models including only  $P_i$  or both  $P_i$  and milk yield (Klop et al., 2013; Weiss and Wyatt, 2004a) also performed well, although the RMSPE% were slightly greater than the new model (RMSPE% = 19.3 to 20.0%). The new model not requiring DMI data for predicting  $P_f$  was associated with an acceptable (22.3%) RMSPE%, 95% of which came from random sources (Table 5). Only one extant model (Nennich et al., 2005) was available to predict  $P_f$  without using DMI data. However, predictions were associated with RMSPE of 46.7%, about three-fourths of which was due to mean bias. The models for predicting  $P_{Ma}$  with and without DMI had an RMSPE% value 20.0%, with random error accounting for 99% of total prediction error. A  $P_i$ -based model by Weiss and Wyatt (2004a) also predicted  $P_{Ma}$  well, although the RMSPE and slope bias estimates were greater than that of the new models (Table 5). None of the extant models allow for predicting  $P_{Ma}$  without using DMI data.

When evaluated with an independent data set ( $n = 43$ ; Figure 2 and Table 6), the new model predicted  $P_{MilkC}$  with RMSPE% of 13.0%; over 77% of error was from random sources. Moreover, the positive NSE value (0.28; Table 6) indicates that new model predictions better represented the observed  $P_{MilkC}$  than the constant  $P_{MilkC}$  used in NRC (2001). The models by Klop et al. (2014) using only milk protein concentration predicted  $P_{MilkC}$  with RMSPE% of 19.1 to 19.6% when evaluated with literature data and RMSPE% of 12.7 to 13.9 when evaluated with experimental data. The positive NSE values in both cases indicate that  $P_{MilkC}$  were better represented by the models compared with the constant  $P_{MilkC}$  used in NRC (2001).

### DISCUSSION

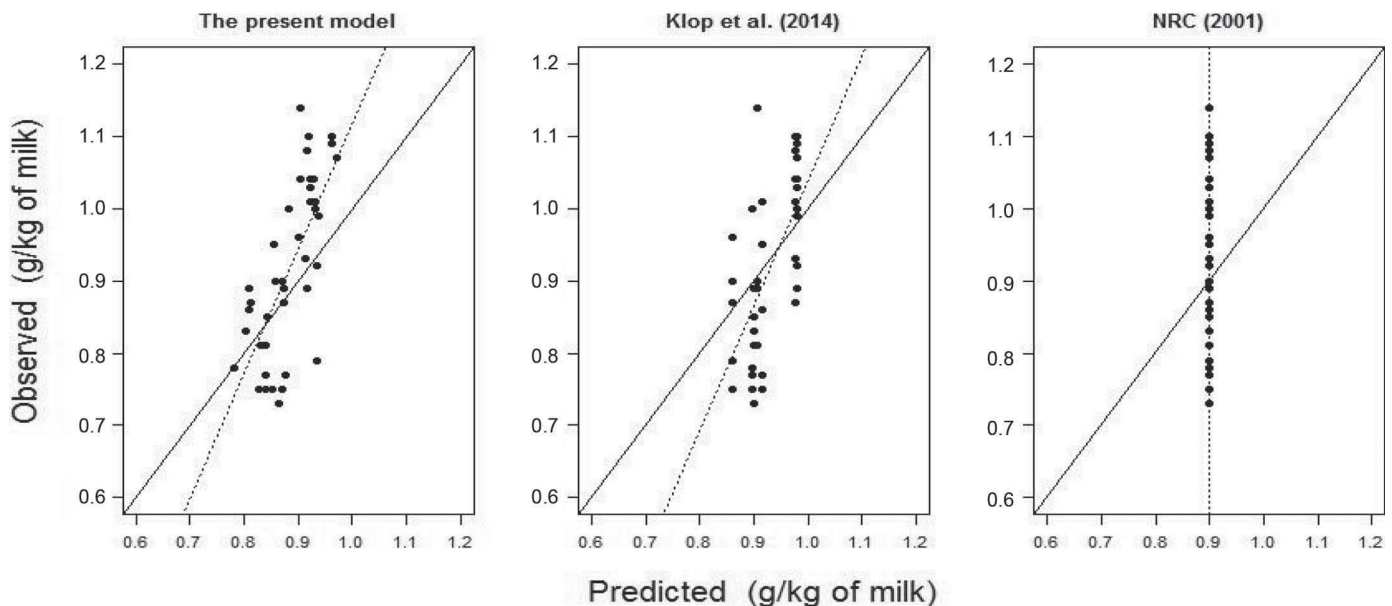
The main goal of our study was to develop a set of empirical models to predict P utilization and excretion in lactating dairy cows over a wide range of intakes and stages of lactation. Given the growing environmental concern over P excretion from livestock, numerous studies have investigated P partitioning in dairy cows with respect to P sources, dietary P and other dietary nutrient concentrations, feed intake and digestibility levels, and stages of lactation. The literature data assembled in our study included variables over a wide range that allowed the development of models that would be applicable in practical conditions. Random effect meta-analytic approaches were used to construct the models because the data are assumed to be a random sample of a population. Therefore, the relationships represented by the models can be generalized to the lactating dairy cow population. In general, the models developed in



**Table 5.** Performance of models challenged with independent data including 354 measurements of fecal P output ( $P_f$ ) and 177 measurements of manure P output ( $P_{Ma}$ )<sup>1</sup>

Equation	RMSPE (%)	MB (%)	SB (%)	RB (%)
<b>New models</b>				
$P_f$ (g/d)				
= $(0.73 \times P_i) - (0.37 \times \text{milk})$	18.3	0.5	0.3	99.2
= $(0.72 \times P_i) - (0.48 \times \text{milk}) + (0.74 \times \text{ash}\%)$	18.3	0.0	2.1	97.9
= $-59.4 + (0.42 \times \text{milk}) + (166.1 \times P\%) + (0.051 \times \text{BW})$	22.3	4.8	0.1	95.1
$P_{Ma}$ (g/d)				
= $-45.6 + (0.73 \times P_i) - (0.30 \times \text{milk}) + (2.37 \times \text{ash}\%) + (10.4 \times \text{MProt})$	19.2	0.1	1.1	98.8
= $-52.9 + (0.23 \times \text{milk}) + (202.6 \times P\%) + (0.060 \times \text{BW}) - (0.99 \times \text{CP}\%)$	19.6	7.1	0.6	99.2
<b>Extant models</b>				
$P_f$ (g/d)				
= $-2.3 + 0.63 \times P_i$ (Weiss and Wyatt, 2004a)	19.8	4.0	1.3	94.7
= $27.1 + 0.99 \times \text{DMI}$ (Klop et al., 2013)	26.5	16.1	3.2	80.7
= $-31.3 + 0.73 \times \text{DMI} + 16.78 \times P$ (Klop et al., 2013)	22.6	13.4	6.6	80.0
= $-3.8 + 0.64 \times P_i$ (Klop et al., 2013)	20.0	6.4	1.0	92.5
= $-15.6 + 0.82 \times \text{DMI} + 16.47 \times P - 0.096 \times \text{CP}$ (Klop et al., 2013)	22.5	13.2	8.7	78.1
= $-48.9 + 0.84 \times \text{DMI} + 16.96 \times P + 0.043 \times \text{NDF}$ (Klop et al., 2013)	22.4	22.8	11.8	65.4
= $-34.7 + 0.90 \times \text{DMI} + 17.28 \times P - 0.093 \times \text{CP} + 0.041 \times \text{NDF}$ (Klop et al., 2013)	22.4	20.7	9.6	67.7
= $19.9 + 0.79 \times P_i - 1.04 \times \text{milk}$ (Klop et al., 2013)	19.3	4.8	0.2	95.00
= $-58.3 + 1.85 \times \text{DMI} + 16.90 \times P + 0.056 \times \text{NDF} - 0.48 \times \text{milk}$ (Klop et al., 2013)	20.7	23.7	6.9	69.4
= $-43.9 + 1.72 \times \text{DMI} + 17.15 \times P - 0.082 \times \text{CP} + 0.052 \times \text{NDF} - 0.39 \times \text{milk}$ (Klop et al., 2013)	20.9	20.9	6.6	72.5
$P_{Ma}$ (g/d)				
= $(P_i \times 560.7) + 21.1$ (Nennich et al., 2005)	24.7	55.1	0.1	44.7
= $7.5 + 0.78 \times P_i - 0.702 \times \text{milk}$ (Weiss and Wyatt, 2004a)	26.7	53.6	4.0	42.3
= $-2.5 + 0.64 \times P_i$ (Weiss and Wyatt, 2004a)	21.2	11.2	0.5	88.7
= $P_i - (\text{Milk} \times 0.9)$ (Van Horn et al., 1994)	20.9	0.8	8.5	90.6
= $14.67 + 0.6786 \times P_i + 0.00196 \times P_i^2 - 0.317 \times \text{milk}$ (Morse et al., 1992)	35.4	73.8	38.6	2.23
= $(\text{milk} \times 0.781) + 50.4$ (Nennich et al., 2005)	46.7	73.4	3.0	23.6

<sup>1</sup>DMI in kg/d;  $P_i$  = P intake (g/d); milk = milk yield (kg/d); ash% and P% = dietary total ash and P, respectively (% of DM); CP% = dietary CP (% of DM); NDF, CP, and P = dietary NDF, CP, and P, respectively (g/kg of DM); MProt = milk protein (%); RMSPE% = root mean square prediction error as a percentage of observed mean; MB = mean bias; SB = slope bias; RB = random bias (all as percentage of total bias).



**Figure 2.** Observed versus predicted P concentrations when our new model, a model in Klop et al. (2014;  $0.24 + 0.022 \times \text{milk protein } \%$ ), and the NRC (2001) milk P concentration constant (0.90 g/kg of milk) were evaluated with independent data ( $n = 43$ ). The solid line represents the line of unity, whereas the dashed line represents the regression line of observed versus predicted values.

**Table 6.** Performance of models for predicting milk P concentration (g/kg of milk)

Model <sup>1</sup>	Literature data (n = 100)					Experimental data (n = 43)				
	RMSPE%	MB	SB	RB	NSE	RMSPE%	MB	SB	RB	NSE
New model = $1.32 - 0.006 \times \text{milk} - 0.0005 \times \text{DIM} - 1.16 \times \text{Ca:ash}$	15.7	3.2	3.5	93.3	0.39	13.0	9.8	12.8	77.4	0.28
Models in Klop et al. (2014) = $0.85 + 0.0278 \times \text{MProt}$ = $0.52 + 0.0138 \times \text{MPProt}$ = $0.60 + 0.0106 \times \text{MPProt}$ = $0.24 + 0.022 \times \text{MPProt}$	19.1 18.7 19.1 19.6	3.8 3.1 7.3 9.8	4.2 0.4 0.0 2.0	92.0 96.5 92.7 88.2	0.09 0.13 0.09 0.05	12.8 13.9 13.9 12.7	8.4 5.4 0.5 0.4	8.6 23.5 28.5 15.3	83.0 71.1 71.0 84.3	0.30 0.19 0.19 0.31

<sup>1</sup>NDF = dietary NDF (% of DM); MPProt = milk protein (g/kg of milk); Ca:ash = dietary Ca-to-total ash ratio; RMSPE% = root mean square prediction error as a percentage of observed mean; MB = mean bias; SB = slope bias; RB = random bias (all as percentage of total bias); NSE = Nash-Sutcliffe efficiency index.

this study performed well when evaluated using an independent data set. The prediction errors were in the acceptable range with uncertainty less than 23% of the average observed value and the majority (>95% of MSPE) of errors coming from random sources.

Prediction models requiring DMI of individual cows have limited applicability in commercial dairy farms, as it is not a routinely monitored variable. Nonetheless, the majority of extant models for predicting nutrient excretion from dairy cows, including the extant models evaluated in our study, require DMI data. Therefore, in the current study 2 sets of models were developed for estimating P excretions from dairy cows with and without using DMI as a predictive variable. The  $P_f$  and  $P_{Ma}$  models not requiring DMI appeared to be as promising as those that use DMI when evaluated with an independent data set (RMSPE% = 19.6 to 22.3%). Our model requiring DMI for predicting  $P_f$  included a positive and negative relationship with  $P_i$  and milk yield, respectively. These effects were fairly independent given the correlation between  $P_i$  and milk yield in the data was not strong ( $r = 0.46$ ). The negative relationship of milk yield with  $P_f$ , independent of  $P_i$ , indicate that  $P_i$  partitioning to  $P_f$  diminishes as milk production increases. Comparable effects have been previously reported in Valk et al. (2002) and Klop et al. (2013). Moreover, the final prediction model for  $P_f$  also included a positive relationship with dietary ash. This may be partly due to passage rates enhanced by increasing drinking water intake, which is positively related to dietary ash content (Appuhamy et al., 2014b), or secretion of more salivary P in high-forage diets usually associated with high ash contents (Valk et al., 2002). However, the effect of ash appeared to be minor, as the model including ash content had the same prediction error rate (RMSPE% = 18.3) of the model including only the  $P_i$  and milk yield effects. Therefore, the new model including  $P_i$  and milk yield is recommended for predicting  $P_f$  if DMI data are available. The Weiss and Wyatt (2004a) model using only  $P_i$  and the Klop et al. (2013) model using  $P_i$  and milk yield also predicted  $P_f$  successfully, although the RMSPE% were slightly greater (19.3 to 19.8%). The new model including linear relationships of  $P_i$ , milk yield, dietary ash content, and milk protein concentration predicted  $P_{Ma}$  better compared with extant models, so it is recommended to predict  $P_{Ma}$  if DMI data are available. Moreover, the random bias estimate of 98.8% indicates that the model parameters reasonably reflect the true relationships of  $P_i$ , milk yield, dietary ash content, and milk protein concentration to  $P_{Ma}$  within the data range. The Morse et al. (1992) model that used a quadratic  $P_i$  effect had much greater RMSPE% in predicting  $P_{Ma}$  when challenged with the experimental data (RMSPE

= 35.4%; Table 5) or the literature data (RMSPE% = 49.0%; data not shown), indicating that the relationship between  $P_i$  and  $P_{Ma}$  is most likely linear. Overall, the model performances indicate that  $P_i$  to be the main driver of P excretions in lactating dairy cows. Thus, P excretions from lactating dairy cows could be effectively regulated by managing  $P_i$ .

Our analyses demonstrated that  $P_f$  or  $P_{Ma}$  could also be predicted successfully without using DMI data (Table 5). In the absence of DMI, milk yield, dietary P concentration, and BW became primary drivers of  $P_f$  and  $P_{Ma}$  owing to their strong relationships with DMI or  $P_i$ . Besides the acceptable RMSPE (19.6 to 22.3%), the majority of prediction error (95–99%) were random. This indicates that the model parameters fairly reflect the true relationships of  $P_f$  or  $P_{Ma}$  with milk yield, dietary P concentration, and BW within the data range. Given the fact that milk yield, dietary P concentrations, and BW are more routinely available than DMI, the new models provide useful tools for developing comprehensive P management plans in commercial farms.

Previous studies reported  $P_{MilkC}$  to vary over a narrow range of 0.083 to 0.1% of milk (Flynn and Power, 1985; Spiekers et al., 1993; Wu et al., 2000). Hence, a constant milk P concentration of 0.090% (0.90 g/kg of milk) has been widely used to calculate absorbed P requirement for milk production in lactating dairy cows (NRC, 2001). In our data set,  $P_{MilkC}$  varied from 0.056 to 0.150% of milk yield with a coefficient of variation of 19%, although the mean  $P_{MilkC}$  estimate (0.092% of milk yield) was close to the constant  $P_{MilkC}$  used in NRC (2001). Unlike  $P_f$  or  $P_{Ma}$ , for which the within-treatment group variability represented just 1 to 2% of total variability ( $\bar{r}^2 > 98$ –99%), 25% of total variance of  $P_{MilkC}$  was related to cows within treatment groups. In agreement, Hidirolou and Proulx (1982) observed considerable between-animal variability in  $P_{MilkC}$  in a group of 27 beef cows. Differences in milk yield, DIM, and dietary Ca-to-ash ratio explained 30% of the between-treatment group variability of  $P_{MilkC}$ , representing 75% of the total variability. Milk yield was negatively related to  $P_{MilkC}$ , perhaps due to a dilution effect. The negative association between  $P_{MilkC}$  and DIM is consistent with the findings in Hidirolou and Proulx (1982). Moreover, dietary Ca concentration was inversely related to  $P_{MilkC}$ , whereas dietary P concentration did not have a significant effect on Ca concentration. This agrees with Braithwaite (1983), who reported that in sheep bone mobilization depended on meeting Ca requirement and was not affected by dietary P concentration. In contrast, the studies of Ekelund et al. (2006) and Knowlton and Herbein (2002) did not note dietary Ca concentration affecting bone

resorption or bone formation in lactating dairy cows. Klop et al. (2014) demonstrated a significantly positive relationship between  $P_{MilkC}$  and milk protein concentration, but it was not apparent in our analysis. Perhaps the positive relationship of milk protein concentration was confounded with the negative milk yield relationship in the new model given milk protein concentration was negatively related to milk yield ( $r = -0.34$ ). Consistently, the new model and those by Klop et al. (2014) performed equally well when evaluated using a set of independent  $P_{MilkC}$  measurements. Days in milk and dietary Ca-to-ash ratio explained 10% of heterogeneity in total  $P_m$ , whereas milk yield alone explained more than 60%. Overall, consideration of variability in milk P concentration with respect to at least stage of lactation may improve P requirement predictions of lactating dairy cows.

## CONCLUSIONS

On average, lactating dairy cows excreted 58% of ingested P in feces and 0.44% in urine, and secreted 40% in milk. Phosphorus intake was the primary driver of P in feces or manure and explained more than 60% of heterogeneity. At constant P intake, cows with higher milk production excreted less P than cows with lower milk production. Extant and newly developed models predicted P output in manure satisfactorily (RMSPE% < 23% of average observed value). The uniqueness of the study was developing models using variables available at commercial dairy farms. As such, models that do not require P or DM intake information were developed and performed as good as, if not better, models that require intake as an input variable. The average P concentration in milk was 0.092% of milk yield, which was within the range of current recommendations. However, P concentration in milk was affected by milk yield, DIM, and dietary Ca content. Therefore, consideration of variability in milk P concentration with respect to at least milk production level or stage of lactation may improve P requirement predictions of lactating dairy cows.

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