A fecal test for assessing phosphorus overfeeding on dairy farms: Evaluation using extensive farm data

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ABSTRACT

Managing P on dairy farms requires the assessment and monitoring of P status of the animals so that potential overfeeding may be minimized. Numerous published studies have demonstrated that for lactating dairy cows, increasing P concentrations in diets led to greater P excretion in feces. More recent work reported that inorganic P (P_i) in 0.1% HCl extracts of feces (fecal extract P_i , g/kg) closely reflects dietary P changes. This has led to the proposal that 0.1% HCl fecal extract P_i may serve as an indicator of the animal's P status (adequate or excessive) when compared with a benchmark value. Here, we present the results of an extensive evaluation of the proposed fecal P indicator test. With samples ($n = 575$) from >90 farms, fecal total P (TP, g/kg) and fecal extract P were positively correlated with dietary P (X, g/kg): TP = $1.92X - 0.17$ ($R^2 = 0.36$); fecal extract P = $1.82X - 2.54$ ($R^2 = 0.46$). Fecal extract P was responsive to dietary P changes, whereas the remaining P, calculated as TP minus fecal extract P, was not. A provisional benchmark value of fecal extract P representing near-adequate P status was set at 4.75 g/kg. Assessment of the farm data using the benchmark indicated that 316 out of 575 data points were associated with possible P overfeeding. Advantages of the fecal-based test over feed-based analysis to assess P status are discussed. The fecal extract P method is a simple and practical test that can be used as an assessment tool for helping dairy producers improve P management and reduce their environmental footprint. **Key words:** fecal P test, P overfeeding, dairy farm, assessment

INTRODUCTION

Dairy producers must strive to improve P balance on farms and reduce potential P loss to waters. A critical step in doing so is to minimize excess P in diets to reduce P excretion in feces, which in turn would limit subsequent P accumulation in soils and decrease potential P loss to waters (Powell et al., 2002; Dou et al., 2003). Traditionally, adequate or excess P feeding on dairy farms is assessed by comparing dietary P concentrations in feed against NRC (2001) recommendations. This approach has certain limitations. First, routine feed analysis measures total P (TP) only, while providing no information on P bioavailability. The latter can vary a great deal depending on feed ingredients and quality (Martz et al., 1990; Weiss and Wyatt, 2004). Second, feed analysis is typically performed using a single sample. Because of the heterogeneity of feeds as well as an animal's sorting behavior, results based on a single sample may not accurately represent the actual P intake by the animal. Furthermore, feed analysis provides an estimate of P intake but reveals nothing about the need for and the utilization of P by the animal. The latter is a function of several factors such as milk yield, stage of lactation, and dynamics of P moving to and from the body reserves of the animal (Wu et al., 2000, 2001). These limitations may be the main factors contributing to producers' resistance in reducing unnecessary excess P in dairy diets, which we still encounter in the field.

The idea of assessing P overfeeding through fecal P testing, proposed by Dou et al. (2003, 2007), may help overcome the feed testing limitations and provide a useful option for improved P management. The logic of the concept is simple: P excreted in feces is the net result of P supply and P use by the animal; therefore, a fecal-based test would reflect the relative status of P supply (intake and bioavailability) and utilization (animal characteristics). The initial concept (Dou et al., 2003) was based on findings from research feeding trials, in

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Figure 1. A schematic illustration of P partitioning in lactating dairy cows linked with fecal P excretion. Phosphorus in feed intake is partitioned into the available pool and unavailable feed residue, the latter is excreted in feces. Available P is utilized by the animal for milk production, tissue retention, salivary circulation, and microbial synthesis, plus negligible amounts of P excreted in urine. Inevitably, some available P remains unabsorbed and is excreted in feces. The essence of the fecal P indicator concept resides in the available but unabsorbed P: the greater the excess P in the available pool (through feed intake and through digestion releasing P in bioavailable forms), the greater the unabsorbed P in feces, the relative magnitude of which is measured as 0.1% HCl extract P_i , referred to as fecal extract P (where P_i is inorganic P). Reproduced with permission from the American Chemical Society.

which water-extractable P (P_i) in feces of lactating cows (Y, g/kg) was correlated with dietary P concentrations (X, g/kg): $Y = 1.37X - 2.80$ ($R^2 = 0.95$, $n = 8$). However, the approach of using water-extractable P as an indicator was somewhat confounded when a subsequent study found other factors (pH and Ca content as well as sample handling method) also affected P extractability in water (Chapuis-Lardy et al., 2004). Further pursuing the fecal P indicator concept, Dou et al. (2007) reasoned that the effects of pH, Ca, and sample handling on water extractable fecal P were most likely chemical in nature; such effects could be overcome by using a dilute acid solution as the extractant instead of water. After testing a series of acid solutions, they found 0.1% HCl to be the most suitable extractant. Using samples from 25 commercial dairies, 0.1% HCl extract P_i (fecal extract P) closely reflected dietary P changes ($Y = 1.74X - 1.76$, $R^2 = 0.69$, $n = 25$) and was independent of pH, Ca, or sample handling (Dou et al., 2007). They also found that although P_i in 0.1% HCl extract increased linearly with increasing dietary P concentrations, remaining P components, including organic P (P_o) in 0.1% HCl extracts and P in residues after extraction, did not respond to dietary P changes. Based on these findings as well as current understanding of P

metabolism in dairy cattle, Dou et al. (2007) delineated a diagram of P partitioning (Figure 1). They contended that the greater the excess of P in feed compared with the animal's needs, the greater the "unabsorbed" P in feces; the relative magnitude of the latter is measured as 0.1% HCl extract P_i . Therefore, a fecal test of 0.1% HCl extract P_i would have the potential to be used as a management tool for assessing P overfeeding on dairy farms, when coupled with a benchmark representing adequate P status.

The objectives of the present work were to 1) evaluate the fecal extract P method using a much larger data set ($n = 575$) with samples collected from >90 farms, 2) derive a provisional benchmark of fecal extract P indicating adequate P status, and 3) assess P overfeeding on the farms using the benchmark.

MATERIALS AND METHODS

Farm Description and Sample Collection

Data used in the current study were part of a large project conducted on farms in New York, Delaware, Pennsylvania, and Maryland from 2002 to 2005. Herd size, milk yield, and farm distribution in the 4 states

Table 1. Distribution and description of farms participating in the project, in reference to relevant information in the states

State	Dairy farms (n)		Herd size ¹			Milk yield (kg/cow per day)		
			Project		State mean ³	Project		State mean ¹ (lb/cow per yr)
	Project	State total ²	Mean/median	Range		Mean	Range	
Delaware	3	120	312/300	165–470	75	30.1	24.1–36.5	15,000
Maryland	23	900	108/85	25–180	90	30.2	18.2–41.2	16,083
New York	29	7,100	488/460	49–1,684	95	34.6	20.5–47.9	17,378
Pennsylvania	51	9,900	178/92	10–600	59	33.2	21.2–42.7	18,081

¹Based on statistics data, USDA-NASS (2003).

²Number of milk cows only.

³State average derived by dividing milk cow inventory by number of milk cow operations (USDA-NASS, 2003).

are listed in Table 1. All herds in the study were 100% Holstein with total confinement and were fed rations formulated by independent nutrition consultants or feed company representatives. During the course of the project, the farms were “doing business as usual” in all aspects of management. No intervention was made intentionally by the researchers involved in the project, except for periodic farm visits for sample and data collection. Across the selected farms, dietary P concentrations varied widely at the beginning and throughout the sampling period.

Feed and fecal samples were obtained every 3 mo from lactating cows. At each sampling time, feed samples were obtained from multiple locations along the feed bunk at the time of feed delivery. Feed subsamples totaling approximately 2 kg were mixed by hand; a 400-g composite was sent to a commercial laboratory (Cumberland Valley Analytical Services, Maugansville, MD; <http://www.foragelab.com>) for feed analysis using established procedures. Fecal samples, taken on the same day as corresponding feed samples, were rectal grabs from randomly selected healthy lactating cows (5–17 per farm depending on number of cows and feeding groups). Bulk samples of approximately 1 kg per cow were mixed by hand; then, 200-g subsamples were stored frozen at –20°C before laboratory analysis, and handled individually throughout laboratory analysis.

Laboratory Procedures

For laboratory analysis, frozen fecal samples were thawed at room temperature, homogenized by hand and then split into subsamples for 1) DM determination in a convection oven at 65°C; 2) microwave-assisted acid digestion (Walter et al., 1997), digest TP measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES); and 3) fecal extract P measurements following the procedure described by Dou et al. (2007). Briefly, 2 g of wet fecal sample was mixed with 98 mL of 0.1% HCl solution, agitated on a reciprocal shaker for 1 h, and gravity-filtered through Whatman 42 paper.

The extracts were analyzed for P_i by an adaptation of Murphy and Riley's (1962) colorimetric molybdenum-blue method (John, 1970) on a spectrophotometer at 882 nm wavelength.

To verify that commercial laboratory analyses were comparable to those performed in our laboratory, selected feed samples from subsamples returned to us by Cumberland Valley Analytical Services for archiving were analyzed for TP. The correlation coefficient, *r*, was 0.86 (*n* = 73).

Provisional Benchmark

As part of the efforts to identify a benchmark value of fecal extract P that represents adequate P status, we used samples from 3 previously reported research trials. Each of the trials fed groups of cows with diets that differed in P concentrations; cow performance data such as milk yield, DM intake, and so on, were recorded. In each trial, the diet with the lowest P concentration but no impaired animal performance was designated as apparently adequate. Detailed information on the feeding trials can be found elsewhere (Wu et al., 2001; Knowlton and Herbein, 2002; R. A. Kohn, Univ. Maryland, College Park, MD; personal communication). For the present study, fecal extract P corresponding to the apparently adequate P diets was taken into consideration for deriving a provisional benchmark.

In addition to data obtained from the research trials, fecal TP and fecal extract P concentrations corresponding to NRC (2001) recommendations for a range of milk yield (20 to 60 kg/cow/day) were estimated using the following models:

$$\text{Fecal TP (g/kg of DM)} = [\text{dietary P intake (g)} \\ - \text{milk P (g)}] \div \text{fecal DM (kg)}, \text{ and}$$

$$\text{Fecal extract P (g/kg of DM)} = \\ a \times \text{fecal TP (g/kg)} + b.$$

Figure 2. Distribution of dietary P concentrations grouped into 3 categories: near adequate (<4.0), moderately high (4.0 to 4.5), and high (>4.5 g/kg) across 12 sampling times during 2002 to 2005 on >90 commercial dairy farms.

In the first model, milk P = milk yield $\times$ 0.9, and fecal DM = DMI $\times$ (1 – digestibility). Digestibility was determined using a subset of paired feed and fecal samples ($n = 136$), measuring the lignin contents and calculating digestibility = 1 – (feed lignin/fecal lignin). Parameters in the second model were derived from data in the present study as a linear regression of fecal extract P against fecal total P with slope a and intercept b .

Data Analysis

Concentrations of P in feed and fecal samples were reported on a DM basis. Dietary P data were for individual feed samples; fecal P data were means of multiple cow fecal samples per farm per quarterly sampling time. Least squares ANOVA and t -tests when appropriate were performed in SAS (SAS Institute, 2003). Linear regression parameters were generated in Microsoft Excel (Microsoft, Redmond, WA).

In addition, to determine if within-farm variability had an effect on the regression relationships, data were blocked by farm as random effects in a mixed model (St-Pierre, 2001) using PROC MIXED in SAS. Simple and mixed model results were analyzed for homogeneity of regression coefficients with an unpaired t -test at the 5% level of significance. The farm effect was not consistent; no significant differences between simple and mixed model regression coefficients were found in 3 of the 5 cases tested. Therefore, simple regression equation parameters were used in data presentation and interpretation.

RESULTS AND DISCUSSION

Dietary P

Dietary P concentrations on the >90 farms in the study averaged between 4.0 and 4.4 g/kg across the 12 quarterly sampling times. At any given sampling time, however, the range of dietary P concentrations among farms was large, from around 3.0 to >5.0 g/kg. When the farm data were grouped into 3 categories, adequate (≤ 4.0), moderately high (>4.0 –4.5), and high (>4.5 g/kg), there appeared to be no consistent increase or decrease in the group distribution across the 12 sampling times (Figure 2). Percentage of diets falling in the adequate category at different sampling times varied from 29 to 60%, the moderately high group from 25 to 40%, and the high group from 11 to 32%.

Taking the farms individually, there were several notable observations: 1) many farms had sporadic spikes or drops in dietary P concentrations during the 3-yr period; 2) a few farms that were feeding a very high level of P in the beginning of the study gradually decreased P concentrations in the diets. For example, one particular farm was feeding 6.6 g/kg of P at the first sampling but had lowered dietary P gradually and was feeding 3.5 g/kg of P by the end of the project; 3) the majority of the farms showed no consistent change in dietary P concentrations during the project period.

Results from the 3-yr study indicated that higher dietary P concentrations were not associated with greater milk yield or better reproductive performance such as conception rate or pregnancy rate (Dou et al., 2003; additional data not presented). These farm-based results

support the findings of previously published research trials that have consistently demonstrated the lack of response in milk production or reproductive parameters to dietary P exceeding 4.0 g/kg (e.g., Valk and Šebek, 1999; Wu et al., 2000). Meanwhile, our results indicate that it is practically feasible to prepare diets containing adequate but not excessive P, because many farms were feeding cows with diets containing <4.0 g of P/kg of DM. On the other hand, a large number of farms were still feeding excess P (>4.5 g/kg) to their cows. For such farms, a fecal test that reflects the relative status of P supply and utilization would be useful management tool.

Digestibility, Fecal P, and the Relationship with Dietary P

Feed DM digestibility varied a great deal among the selected subset of samples. The minimum was 0.42, the maximum 0.75, and the mean 0.595 with a standard deviation of 0.0717.

Fecal TP concentrations ranged from 5.25 to 13.80 g/kg ($n = 575$), and fecal extract P ranged from 1.92 to 10.55 g/kg ($n = 575$). The 2 fecal P parameters were significantly correlated ($P < 0.0001$) but the slope of the linear regression was significantly ($P < 0.0001$) less than 1 (Eq. [1] below). The implication is that fecal extract P as a proportion of fecal TP is not uniformly distributed among the samples. In other words, fecal samples containing the same TP differed considerably in 0.1% HCl extract P_i , as shown in Figure 3. This can be attributed to variations across farm samples in terms of feed ingredients and digestibility, P bioavailability, and animal characteristics such as milk yield. Conceivably, fecal samples from 2 cows might contain the same amount of total P, but one sample could have proportionally less “unabsorbed” P (and thus less fecal extract P) and more “unavailable feed residue” P if 1) feed DM digestibility was poorer than the other, or 2) P utilization efficiency was higher by one cow than the other. This hypothetical scenario, not unrealistic in the field, leads to an important point; that is, fecal TP concentrations may not be as effective an indicator of P supply-utilization status of the animal as fecal extract P.

$$\text{Fecal extract P} = 0.6593\text{TP} - 0.1359; \\ R^2 = 0.6254 \quad (n = 575). \quad [1]$$

Both fecal TP and fecal extract P (g/kg of fecal DM) were positively correlated to dietary P concentrations (X, g/kg of feed DM) but the relationship was stronger for fecal extract P:

Figure 3. Relationship between fecal extract P (0.1% HCl extractable P_i , where P_i is inorganic P) and acid digest fecal total P for the farm-based data set ($n = 575$).

$$\text{Fecal TP} = 1.921X - 0.1679; R^2 = 0.3556 \quad (n = 575); \quad [2]$$

$$\text{Fecal extract P} = 1.8182X - 2.5352; \\ R^2 = 0.4571 \quad (n = 575). \quad [3]$$

These data exhibited the same trends as those in the previous report in which the fecal extract P method was developed (Dou et al., 2007). From equations [2] and [3], each unit increase in dietary P concentration (g/kg of feed DM) was associated with an increase of 1.92 g/kg in fecal TP or 1.82 in fecal extract P. Also, the fecal P fraction that is not measured as 0.1% HCl extract P_i , calculated as TP – 0.1% HCl extract P_i , was not associated with dietary P change (Figure 4). Clearly, the relationships that led to the fecal P indicator concept are also present with the larger data set in the present study. That is, as dietary P concentration increases beyond what is necessary for animal maintenance and milk production, the excess P is excreted in feces, mostly as available but unabsorbed P. The relative magnitude of the latter can be estimated as 0.1% HCl extract P_i . Therefore, measures of fecal extract P could serve as an assessment tool when compared with a benchmark value representing adequate P status.

Provisional Benchmark

Estimated fecal extract P corresponding to NRC recommendations for a range of milk yields is presented in Table 2. Varying milk yield from 20 to 60 kg, DMI would range from 18.6 to 31.8 kg, and the dietary P

Figure 4. Fecal extract P (determined as 0.1% HCl extract P_i , where P_i is inorganic P) is positively correlated with dietary P concentrations and reflects dietary P changes, whereas the remaining P fraction in feces, calculated as total P – fecal extract P, was not associated with dietary P changes ($n = 575$).

requirement would range from 55 to 128 g, the latter being equivalent to 2.9 to 4.0 g/kg of diet DM. Taking into account the mean DM digestibility of 0.595 and the relationship between fecal TP and fecal extract P (Eq. [1]), calculated fecal extract P for the given range of milk yields would be from 3.10 to 3.66 g/kg of fecal DM (Table 2). Factoring in variations in feed DM digestibility, calculated fecal extract P would be as low as 2.62 g/kg (mean digestibility minus standard deviation for 20 kg of milk) or as high as 4.47 g/kg (mean digestibility plus standard deviation for 60 kg of milk; Table 2).

Pooled results from the 3 research feeding trials showed a close linear relationship between fecal extract

P and dietary P concentrations ($R^2 = 0.99$; Figure 5). In trial A, the diet with 3.4 g/kg of P was designated as apparently adequate because milk yield, DMI, and other cow performance parameters did not differ from groups fed the 5.1 or 6.7 g/kg diets during the sampling period (wk 3, 5, 7, 9, and 11 postcalving; Knowlton and Herbein, 2002). In trial B, grouped cows were fed the 3.7 or 4.8 g/kg diets, and the 3.7 g/kg diet was apparently adequate based on cow performance data through 2 lactations (R. A. Kohn, Univ. Maryland, College Park, MD; personal communication). In trial C, in which cows were fed diets with 3.1, 3.9, or 4.7 g/kg of P for a complete lactation, the 3.1 g/kg diet was associated with a small reduction in milk yield in late lactation compared with the other diets; therefore, the P status was considered marginal (Wu et al., 2000). The 3.9 g/kg diet was designated as apparently adequate because cow performance did not differ from the group fed the 4.7 g/kg diet. In fact, the 3.9 g/kg diet contained perhaps slightly above what would have been considered adequate for the animals. Nevertheless, fecal extract P corresponding to the apparently adequate dietary P treatments in the 3 trials (3.4, 3.7, and 3.9 g/kg), were 3.43, 3.75, and 4.76 g/kg, respectively.

It is worth noting that the apparently adequate diets designated in the above trials were merely dietary P treatments that corresponded to near or slightly above adequate P status of the animals. The true status (adequate or excess) is a balance between P supply, which includes not only the amount of P ingested through DMI but also the amount of P released in bioavailable form through digestion (which is not readily known) and P utilization, which includes P use for milk synthesis, bone or body reserves, salivary circulation, and microbial synthesis. Clearly, P status is dynamic and animal-dependent. The absolute minimum of P required

Table 2. Fecal P characteristics (total quantity, concentrations as total P or fecal extract P) calculated based on NRC (2001; table 14–7) recommendations for a range of milk yields¹

Milk yield (kg/cow per d)	DMI (kg/cow per d)	P needed		Milk P (g/d)	Fecal total P				Fecal extract P ²	
		g/d	g/kg of diet DM		g/d	g/kg of fecal DM ³	± SD ³		g/kg of fecal DM	± SD
20	18.6	55	2.9	18.0	37.0	4.91	4.17–5.97		3.10	2.62–3.80
25	20.4	64	3.1	22.5	41.5	5.02	4.26–6.10		3.17	2.68–3.88
30	21.8	73	3.3	27.0	46.0	5.22	4.43–6.34		3.30	2.79–4.04
35	23.6	82	3.5	31.5	50.5	5.29	4.49–6.42		3.35	2.83–4.10
40	25.4	91	3.6	36.0	55.0	5.35	4.54–6.49		3.39	2.86–4.15
45	26.8	101	3.7	40.5	60.6	5.58	4.74–6.78		3.54	2.99–4.33
50	28.6	110	3.8	45.0	65.1	5.62	4.77–6.82		3.57	3.01–4.36
55	30.0	119	3.9	49.4	69.6	5.73	4.87–6.96		3.64	3.08–4.46
60	31.8	128	4.0	53.9	74.1	5.75	4.89–6.99		3.66	3.09–4.47

¹Assuming BW of 650 kg, 0.9% of milk yield, and negligible P amount in urine or for uses other than milk synthesis.

²Fecal extract P = $0.6539 \times \text{fecal total P} - 0.1359$ using data in current study ($n = 575$).

³Fecal P divided by fecal DM; the latter was based on digestibility of a subset of the samples ($n = 136$): mean = 0.595, SD = 0.07166; Z. Dou, unpublished data.

Figure 5. Fecal extract P (measured as inorganic P in 0.1% HCl extracts) responds to and reflects changes in dietary P concentration, error bars for means $\pm$ standard deviation. The remaining P fraction (acid digest total P – fecal extract P) did not respond to dietary P changes. Dietary P treatments that corresponded to apparently adequate or slightly above adequate P status were 3.4, 3.7, and 3.9 g/kg of P in the 3 research trials, respectively, and the relative fecal extract P were 3.43, 3.75, and 4.76 g/kg.

to support moderate to high levels of milk production cannot be established from the current published literature. Regardless, fecal extract P corresponding to situations in which P status was apparently close to or slightly above adequate, such as under experimental conditions in the 3 feeding trials or NRC recommendations, would provide a good approximation to be used as a benchmark.

From the NRC-based estimates and the feeding trials, fecal extract P corresponding to near-adequate P status is likely around 3.5 g/kg, with an upper boundary of 4.5 g/kg in the case of high milk yield (60 kg) and relatively high digestibility (mean + SD; Table 2). From the farm-based results in the present study, fecal extract P corresponding to dietary P of 4.0 g/kg would be about 4.75 g/kg as calculated from Eq. [3]. Considering the need for flexibility, we propose fecal extract P of 4.75 g/kg to be a provisional benchmark, which is also the measured fecal extract P for the diet P treatment in trial C that corresponded to slightly more than adequate P status as discussed above. We believe that this provisional benchmark is conservative with a built-in safety margin.

Assessment of P Overfeeding

Fecal Extract P Exceeding Benchmark. Of the 575 observations in the current study, 316 (55%) had fecal extract P >4.75 g/kg, suggesting probable overfeeding of P. Of those fecal extract P concentrations exceeding the benchmark, 75% had fecal extract P >5.23

(i.e., 10% above the benchmark), 42% had fecal extract P >5.94 (25% above the benchmark), and 11% had fecal extract P >7.13 g/kg (50% above the benchmark).

Most of the 316 data points with fecal extract P exceeding the benchmark indeed had dietary P exceeding 4.0 g/kg. However, a total of 68 (22%) had dietary P concentrations <4.0 g/kg (zone I in Figure 6). These 68 data points are not necessarily false positive. Several explanations may apply. First, low P utilization by animals (low milk yield, for example) would lead to relatively high P excretion in feces even if dietary P is within the range that is generally not considered overfeeding (<4.0 g/kg). In fact, when examined closely, 46 of 68 of the data points in zone I had dietary P concentrations ranging from 3.8 to 4.0 g/kg. Such dietary P levels would be adequate for cows producing 50 to 60 kg of milk (Table 2), whereas actual milk yields associated with the group (dietary P within 3.8 to 4.0 g/kg and fecal extract P >4.75 g/kg) averaged only 31 kg (ranged from 23 to 35 kg), far below NRC (2001) target milk yields. As a consequence, excess P was excreted in feces, mostly as available but unabsorbed P. In this sense, those relevant data points would not be false positive but rather a reflection of overfeeding. Clearly, feed-based testing alone would not have identified these cases of overfeeding.

A second explanation is that P bioavailability of feeds, which is not routinely measured, can vary considerably. A diet with relatively high digestibility and P bioavailability would lead to more P in the digested but unabsorbed fraction, and thus greater fecal extract P. In this case, P supply may be more than adequate in reference to animal needs, although dietary P concentration may not exceed 4.0 g/kg. Once again, assessing

Figure 6. Distribution of data points ($n = 575$) using a grid of fecal extract P = 4.75 g/kg, a provisional benchmark, and dietary P = 4.0 g/kg.

P status based on dietary P concentration could be misleading.

Third, dietary P concentrations represented by values on the x-axis in Figure 6 are subject to experimental error. This is because each dietary P value was derived from a single TMR sample, as is commonly done in the field, or, in the case of component feeding, the value was calculated from analyses of individual feed ingredients. The heterogeneous nature of feeds and animals' sorting behavior contribute to sampling bias and subsequently test inaccuracy. Although dietary P concentrations on the x-axis may not accurately reflect the actual intake of the animals, fecal extract P on the y-axis is associated with minimal bias and experimental error because each value is the mean of at least 5 individually analyzed cow fecal samples.

Whether it is deviations from target milk yield, poor digestibility and P bioavailability, or random feed sampling bias and experimental error, each scenario is likely to occur in the field but difficult to monitor or control routinely. Individually or together, these factors affect P supply-utilization dynamics and may make it difficult to judge an animal's P status based on dietary P concentration alone. The fecal P test developed earlier and evaluated in the current study represents the net result of these and other factors; therefore, it can serve as a management tool for screening or monitoring P overfeeding when feed P analysis as a first-cut index is ineffective in preventing excess P from being used.

Fecal Extract P Below Benchmark. A total of 259 data points (45% of the data set) had fecal extract P <4.75 g/kg, suggesting that they were not associated with P overfeeding. Inevitably, some data points had fecal extract P <4.75 g/kg but dietary P >4.0 g/kg (zone III in Figure 6). The data points in zone III are not necessarily false negative. They could be caused by poor digestibility and thus a low proportion of available but unabsorbed P, high P use by the animal, or sampling bias and experimental error associated with feed samples. Nevertheless, the fecal P test is not designed to identify potential P deficiency. Based on our observations, modern dairy diets are rarely deficient in P, mainly because of the routine use of P-rich ingredients such as soybean meal (0.58 to 0.74% P), dried distillers grain (0.61 to 0.73% P), or cottonseeds (0.52 to 0.68% P; mean $\pm$ 1 SD, Table 15.3 in NRC, 2001).

Farm-to-Farm Variation. As exhibited in Figure 6, there was a great deal of across-farm variability in fecal extract P at a given dietary P concentration, and vice versa. For example, around 4.0 g/kg dietary P, fecal extract P varied from 2.56 to 7.67 g/kg on different farms or at different sampling times. Likewise, around fecal extract P of 4.75 g/kg, dietary P ranged from 3.2 to 4.9 g/kg. The large differences were perhaps a re-

sult of inherent disparity among farms in terms of feed ingredients, digestibility, P bioavailability, and animal needs and performance. These factors affect P supply-utilization status of the animal, which in turn determines the characteristics and distribution of P fractions in feces (e.g., unavailable feed residue, available but unabsorbed P; Figure 1). Once again, it illustrates the rationale behind the use of fecal extract P as a simple measure to reflect P status.

Potential Application and Limitations

For dairy cattle, adequate P is a state where supply meets utilization demand with minimal excess. Because P utilization demand is animal-dependent and because P supply is a function of not only total P in feed intake but also the release of P in bioavailable form through digestion, an accurate and reliable evaluation of P status for prevention of P overfeeding requires more than information on dietary P concentrations. A fecal-based test allows us to bypass the complexity of P metabolism and P supply-utilization dynamics by focusing on the net outcome.

The utility of fecal extract P as a screening tool for P overfeeding has been illustrated throughout the discussion above. For those who are interested in applying this method in the field or further testing the method, the following guidelines should be taken into consideration: 1) Use a composite of at least 5 cow fecal samples, if it is desirable to minimize sample handling and analytical cost; 2) fecal samples should be obtained from healthy lactating cows in the same feeding group by rectal grab or mid-air catch to avoid "contamination" from urine or other nonfecal materials; 3) in the case of fecal extract P >4.75 g/kg but dietary P <4.0 g/kg, check milk yield and consider repeating the sampling and analysis; 4) as mentioned above, the fecal extract P method was not intended for identifying P deficiency.

It must be pointed out that the present study as well as the relevant previous one were conducted with lactating Holstein cows in confinement only. Whether the fecal extract P method, particularly the benchmark that separates near adequacy from overfeeding, can be applied to other breeds or nonconfinement conditions remains to be investigated. In addition, several factors may influence P metabolism but were not dealt with in the present study. First, P mobilization from body reserves during early lactation and the reverse process (i.e., P replenishment) in late lactation have been reported (Wu et al., 2001). It is not clear to what extent such P movement would influence measured fecal extract P. Second, diurnal variation in fecal TP concentrations was observed in a previous study (Wu et al., 2000), the net impact of which on fecal extract P

and P status assessment deserves further investigation. Furthermore, the use of large amounts of P-rich byproducts in dairy diets such as dried distillers grains from ethanol production has been on the rise (Schroeder, 2007). How such changing practices may affect P balance, P bioavailability, and fecal extract P in particular deserves attention. Helping dairy producers improve P management to reduce their environmental footprint while maintaining or enhancing farm productivity will continue to be a challenge for the research community and the society at large. We welcome and encourage fellow researchers to rigorously evaluate the fecal extract P method with relevant data and to adapt or refine the procedures for possibly expanded application boundaries.

CONCLUSIONS

In dairy production, managing P for environmental protection without impairing animal performance requires monitoring and assessing P status of the animals on a routine basis to minimize potential overfeeding. Fecal P represents the net result of P intake through feed consumption, P release in bioavailable forms through digestion, and P utilization by the animal. A fecal-based test has the potential to reflect P supply-utilization status of the animal and to indicate if P overfeeding is occurring. Results from the current study using a large data set ($n = 575$) confirmed the findings of a previous report ($n = 25$) that 0.1% HCl extract P_i of feces responds to and reflects changes in dietary P whereas the remaining P fraction, calculated as fecal TP – 0.1% HCl extract P_i , was not associated with dietary P changes. These trends, consistent between the current and previous studies utilizing farm-based data as well as in research feeding trials, provide the basis for assessing P status through fecal P testing. A provisional benchmark of fecal extract P corresponding to near or slightly above adequate P status was established to be 4.75 g/kg. This value was based on results from previous research trials, NRC recommendations, and the relationship between fecal extract P and dietary P in the present farm-based study. The utility of assessing P overfeeding based on fecal extract P against the provisional benchmark was illustrated for the farm data.

Evaluation of P status of dairy cows will remain an inexact science, largely because of the complexity, heterogeneity, and variability of the biological and farm management systems. The fecal P indicator concept is soundly based, and the fecal extract P method is simple. When coupled with a benchmark, this simple test provides a very useful approximation for assess-

ing possible P overfeeding. Perhaps, this pragmatically useful, “quick and dirty” assessment tool will prove to be adequate for helping dairy producers improve P management and reduce detrimental effects on the environment.

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