Utilization of Phosphorus in Lactating Cows Fed Varying Amounts of Phosphorus and Sources of Fiber

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ABSTRACT

This study was undertaken to determine the effect of dietary P content and fiber source on P utilization. Four dietary treatments were formed in a 2×2 factorial arrangement. The P content was 0.32 or 0.44%, and the fiber source was varied by substituting 10% soyhulls for 6% alfalfa hay on a dry matter (DM) basis. Diets also contained approximately 50% corn silage and alfalfa silage for all treatments. The diets were fed to 32 early to midlactation Holsteins for 10 wk. Fecal P excretion was estimated using indigestible acid detergent fiber marker determined with 12-d in situ incubation and grab sampling. Milk yield was high, averaging 43 kg/d across treatments, and 42.1 and 44.0 kg/d for the 0.32 and 0.44% P diets, respectively. Milk fat content was also high, averaging 3.68 and 4.12% for the 0.32 and 0.44% P diets, respectively. Milk protein yield averaged 1.240 and 1.323 kg/d. Differences in milk production were associated with 1.5 kg/d less DM intake for the lower P diets on average. Based on lactation performance, 0.32% P appeared inadequate for this level of production, whereas the calculated (National Research Council) requirement was 0.37%. Fecal P concentration increased linearly with P intake, and based on this relationship, reducing dietary P from 0.44 to 0.37% would reduce fecal P excretion by 12%. Partial substitution of soyhulls for alfalfa hay did not affect feed intake or milk production, but reduced fecal P excretion, partially because of increased P apparent digestibility. The reduction in fecal P excretion resulting from reduced P intake or substitution of soyhulls for alfalfa hay was apparently through reductions in the regulated portion of fecal P. Cows producing 43 kg/d of milk appeared to need >0.32% P, whereas the requirement assessed from National Research Council data was 0.37%. Using highly digestible nonforage fiber sources in place of forage fiber sources in the diet may allow less P to be fed while still meeting the requirement.

(**Key words:** phosphorus requirement, phosphorus excretion, fiber source, dairy cow)

Abbreviation key: HPAH = high P, alfalfa hay, HPSH = high P, soyhulls, LPAH = low P, alfalfa hay, LPSH = low P, soyhulls.

INTRODUCTION

The current NRC (2001) suggests that P at 0.32 to 0.37% of the diet can meet the requirement for cows producing 30 to 45 kg/d of milk. This reflects a 20 to 25% reduction from the previous edition of NRC (1989). Dairy producers have been overfeeding P. Although producers have started reducing P in diets, a recent survey (Dou et al., 2003) showed that P is still overfed by about 25% when compared with the new guidelines (NRC, 2001). One of the reasons for overfeeding is the lack of knowledge concerning feed P utilization.

Although the use of P in the digestive tract is primarily affected by P intake, other dietary factors may have an impact. At present, little is known about changes in P utilization with different feeding variables or TMR. One of the most common feeding variables is the level and type of forage used in the diet. Because forage stimulates saliva secretion and saliva contains P, the possibility exists that the amount and source of forage or fiber used in a diet may have an impact on P loss in feces. In that case, there is a need to determine if dietary P level should be adjusted according to forage characteristics. Knowledge of this could have implications because P needs to be fed precisely according to the requirement to minimize its excretion. Also, because salivary P is inorganic, whereas most P in forage and grain is organic, dietary forage could affect the form of fecal P (organic or inorganic P), and hence, the fate of manure P in soil. Inorganic and organic forms of P have different solubility characteristics and, therefore, different risks in runoff potential (Dou et al., 2002).

Wu et al. (2003) evaluated the effect of dietary forage amount on P use. Cows fed 0.33 or 0.42% P performed similarly in lactation at either 48 or 58% forage in the diet. The effect of forage proportion on fecal P excretion was small at these levels, whereas P intake had a much

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bigger impact. Based on this, it was concluded that adjusting the dietary P content according to forage proportion might not be necessary in practical situations to provide similar amounts of absorbable P.

In addition to the amount, different types of forages or fiber sources are used in diets for dairy cows. Byproducts such as soyhulls are increasingly being included in the diet as a forage substitute. Soyhulls are a nonforage fiber source. The feed source costs less and contains less P than alfalfa. These characteristics make soyhulls an ideal partial replacement for alfalfa. Because soyhulls do not have the physical roughage characteristics of a coarse feed source and, therefore, do not stimulate saliva secretion as much as alfalfa hay, it is possible that substituting soyhulls for alfalfa hay in the diet could reduce fecal P excretion, depending upon the requirement and supply of absorbable P.

The objective of this study was to determine the effect of dietary P amount and fiber source on P utilization and excretion. This study was a follow up to an earlier study (Wu et al., 2003). The previous study addressed the forage amount aspect, and the present study focused on the fiber source aspect.

MATERIALS AND METHODS

The experiment was conducted using a protocol that was approved by the Pennsylvania State University Institutional Animal Care and Use Committee. A completely randomized block design with a 2×2 factorial arrangement of treatments was used. The factors were dietary P amount and fiber source, each having 2 levels. Their combinations formed the following 4 dietary treatments: low P, alfalfa hay (**LPAH**); low P, soyhulls (**LPSH**); high P, alfalfa hay (**HPAH**); and high P, soyhulls (**HPSH**). The P content of the diet was designed to be 0.33 or 0.42%, as used in the previous study (Wu et al., 2003). The low P diets contained no P supplement, whereas the high P diets were obtained by including 0.51% monosodium phosphate (Table 1). To formulate the soyhull diets, approximately 10% ground soyhulls were used to replace 6% alfalfa hay. Prechopped alfalfa hay from large square bales was loosened and mixed into TMR without being further chopped. Corn silage and alfalfa silage were the other forage sources. Overall, the diets were high in forage. Steam-flaked corn (370 to 410 g/L; Pennfield Corp., Lancaster, PA) was the major grain source. This form was used to minimize starch fermentation in the hindgut based on the consideration that hindgut fermentation might result in microbial P excretion, which would interfere with the determination of the effect of fiber source on P excretion. Adjustments were made primarily in corn when soyhulls were included to ensure that the diets were isoni-

Table 1. Ingredient and nutrient content of diets (DM basis).

Item	Dietary treatment ¹			
	LPAH	LPSH	HPAH	HPSH
	(%)			
Ingredient content				
Corn silage	38.70	38.52	38.24	37.80
Alfalfa silage	11.98	12.45	12.00	12.59
Alfalfa hay	9.99	3.97	9.99	3.98
Soyhulls	...	9.92	...	10.00
Corn, steam flaked ²	16.31	11.68	16.25	11.75
Soybean, roasted	8.59	8.54	8.58	8.59
Soybean meal, 48% CP	7.27	7.76	7.27	7.62
Distillers grain	4.05	4.05	4.05	4.05
Ca salt of fatty acids ³	1.00	1.00	1.00	1.00
Limestone	0.66	0.66	0.66	0.66
Magnesium oxide	0.33	0.33	0.33	0.33
Salt	0.43	0.43	0.43	0.43
Monosodium phosphate	...	...	0.51	0.51
Dolomitic limestone	0.24	0.24	0.24	0.24
Potassium chloride	0.33	0.33	0.33	0.33
Mineral and vitamin mix ⁴	0.12	0.12	0.12	0.12
Nutrient content				
CP	15.8	15.8	15.7	15.7
NDF	29.4	33.1	29.2	33.0
ADF	19.9	22.7	19.9	22.7
Total P	0.33	0.32	0.44	0.43
Available P ⁵	0.22	0.21	0.32	0.32

¹LPAH = Low P, alfalfa hay; LPSH = low P, soyhulls; HPAH = high P, alfalfa hay; and HPSH = high P, soyhulls.

²Pennfield Corp. (Lancaster, PA).

³Megalac (Church & Dwight Co., Inc., Princeton, NJ).

⁴The premix provided per kilogram of TMR: 82 mg Zn; 62 mg Mn; 14 mg Cu; 192 mg Fe; 0.6 mg I; 0.5 mg Co; 0.3 mg Se; 3,700 IU vitamin A; 1,100 IU vitamin D; and 23 IU vitamin E.

⁵Calculated using P availability of 64% for forages, 70% for grains, and 90% for NaH_2PO_4 (NRC, 2001).

trogenous and isocaloric. Based on NRC (2001) tabular values, dietary Ca content was approximately 0.82%, CP was 17%, and NE_L was 1.62 Mcal/kg for all diets.

Thirty-two multiparous Holsteins averaging 97 DIM (SD = 19) and 48 kg/d of milk (SD = 7.7) at the beginning of the experiment were used. Cows were divided into 8 replicate blocks based on similarity in milk yield and DIM. Cows within blocks were randomly assigned to one of the 4 dietary treatments. The trial lasted 10 wk following a 3-wk adaptation period. During the first 2 wk of the adaptation period, all cows were fed a common herd diet, and during the last week, cows were gradually changed to their treatment diets.

The animals were housed in a tie-stall barn and offered a TMR ad libitum at approximately 0800 h (5 to 10% refusal). The actual amounts of feed offered and refused by individual animals were recorded daily to obtain net intake. Milking was at 0500 and 1700 h, and milk yields were recorded at each milking. Cows were weighed and scored for body condition (Wildman et al., 1982) after milking at the beginning and end of the treatment. Each BW was the average of 2 consecutive

measurements on the same day, and BCS was the average of 3 evaluators. Milk samples were collected weekly from 2 consecutive milkings. The samples were preserved with 2-bromo-2-nitropropane-1,3 diol (CAS# 52-51-7), and analyzed by the Pennsylvania DHIA Laboratory (University Park, PA) for fat, protein, lactose, total solids, and urea N using infrared spectroscopy (Fossmatic 4000 MilkoScan; Foss Electric, Hillerød, Denmark), and for SCC using a cell counter (Fossmatic 400; Foss Electric); SNF was calculated as total solids minus fat.

Feed ingredients were sampled weekly; however, grain sources (steam-flaked corn, roasted soybeans, soybean meal, and distillers grain) and soyhulls were pooled every 4 wk. The TMR and Orts were sampled daily from individual cows, and then pooled by treatment and week. Dry matter content of weekly samples was determined by air-drying in an oven at 55°C for 48 h. Diet formulations (as-fed basis) were adjusted weekly for changes in DM content of the ingredients. Orts were used only for DMI calculation. The feed offered was regulated to leave no more than 10% Orts. The impact of Orts nutrient composition on nutrient intake was considered negligible. For example, the analysis of the P content of Orts during wk 9 of treatment (0.33 and 0.45% for the low and high P diets, respectively) showed a rather small difference (3%) from the P content of these diets.

Nutrient digestibility was determined during wk 9 of treatment. Feces were sampled from the rectum after each milking for 4 d using the marker technique with indigestible ADF. This protocol resulted in 8 samples for each cow. The 8 samples were pooled and dried at 55°C. A previous study (Wu et al., 2000) showed apparent variation in fecal P content during the day, particularly when the dietary P content was high (0.48%); the variation appeared much smaller when dietary P was lower (0.32 and 0.40%). Nevertheless, the sampling protocol used in the present study may not have enabled as accurate an estimation of fecal P excretion as would a more frequent schedule with varied collection times.

Dried feed and fecal samples were ground through a Wiley mill with a 1-mm screen (Arthur H. Thomas, Philadelphia, PA). Ground samples were analyzed for DM (102°C), CP using the Kjeldahl digestion system (Kjeltec Tecator 2020; Höganäs, Sweden) and flow injection colorimetry (QuikChem method 15-107-06-2-F, QuikChem FIA+ 8000 Series; Lachat Instruments, Milwaukee, WI) according to AOAC (1990), and NDF (heat-stable α -amylase and Na_2SO_3 were used) and ADF according to Robertson and Van Soest (1981). The ANKOM²⁰⁰ Fiber Analyzer incubator (ANKOM Technology, Fairport, NY) was used for NDF and ADF analyses. Feed and fecal samples were analyzed for P using

the preparation for Kjeldahl N by flow injection colorimetry (QuikChem Method 15-115-01-2-C; Lachat Instruments). Certified commercial standards for N (LC17940-1) and P (LC18590-1) (LabChem Inc., Pittsburgh, PA) were used to ensure accuracy in the analyses using flow injection colorimetry. Fecal samples and the TMR samples in wk 9 of treatment were analyzed for indigestible ADF (ADF remaining after 12-d ruminal in situ incubation) to estimate nutrient apparent digestibility. Samples were weighed (0.35 g) into 5×10 cm Dacron bags with 50- μm pores (No. R510; Ankom Technology) and incubated in the rumen of a cannulated cow fed a diet consisting of 60% forage and 40% concentrate (Luchini et al., 1996).

Chemical analyses of feeds and feces were based on DM measurements made at 102°C. Nutrient content of the TMR was computed from the average nutrient content of the individual diet ingredients analyzed using the aforementioned composite samples.

Two models were used for analyses of data with SAS (SAS Institute, 2000). Model 1 was used for DMI, milk yield, and milk composition by the mixed model procedure. Weekly averages of the measurements were used as repeated measures, for which a combination of autoregressive covariation on cow within dietary P, fiber, block, and random effect between animals was structured. For milk yield and DMI analyses, a covariate term was included in the models using the average measurements obtained during the 3-wk adaptation period. Repeated measures were not obtained for BW, BCS, and nutrient excretion, and these variables were evaluated using model 2 by the GLM procedure according to a completely randomized block design. The 2 models are as follows:

$$Y_{ijkl} = \mu + B_i + P_j + F_k + W_l + (P \times F)_{jk} + (P \times W)_{jl} + (F \times W)_{kl} + (P \times F \times W)_{jkl} + E_{ijkl} \quad [1]$$

$$Y_{ijk} = \mu + B_i + P_j + F_k + (P \times F)_{jk} + E_{ijk} \quad [2]$$

where Y_{ijkl} , Y_{ijk} = observation; μ = overall mean; B_i = block effect; P_j = dietary P amount effect; F_k = dietary fiber source effect; W_l = week effect; $(P \times F)_{jk}$ = interaction between dietary P and fiber source; $(P \times W)_{jl}$ = interaction between dietary P amount and week; $(F \times W)_{kl}$ = interaction between dietary fiber source and week; $(P \times F \times W)_{jkl}$ = interaction among dietary P, fiber source, and week; and E_{ijkl} , E_{ijk} = residual error.

Results are presented as least square means. Differences were considered significant at $P < 0.05$ and as a trend at $P < 0.10$, unless otherwise indicated.

Table 2. Analyses of dietary ingredients.

Ingredient	DM	CP	NDF	ADF	P
	(%)				
Corn silage (n = 11)					
Average	39.2	7.4	37.3	21.5	0.209
SD	1.0	0.7	3.3	2.5	0.014
Alfalfa silage (n = 11)					
Average	39.0	18.9	46.2	40.9	0.326
SD	2.1	1.0	2.3	2.1	0.017
Alfalfa hay (n = 11)					
Average	89.9	17.2	42.0	36.0	0.269
SD	2.7	1.4	4.8	4.0	0.067
Soyhulls (n = 3)					
Average	92.6	10.8	65.7	49.4	0.136
SD	0.3	0.4	1.7	1.6	0.011
Corn, steam-flaked (n = 3)					
Average	87.2	7.0	9.0	3.3	0.245
SD	0.6	0.3	0.6	0.5	0.019
Soybeans, roasted (n = 3)					
Average	93.1	37.0	20.9	13.9	0.621
SD	1.5	2.7	3.8	2.1	0.028
Soybean meal (n = 3)					
Average	90.4	50.1	10.0	8.7	0.794
SD	0.2	1.5	1.0	1.6	0.039
Distillers grain (n = 4)					
Average	92.6	23.5	29.6	18.7	0.724
SD	0.5	2.1	1.1	1.1	0.109

RESULTS AND DISCUSSION

Diet Composition

The nutrient content of the major feed ingredients used was relatively consistent during the experiment (Table 2). Compared with the values listed in NRC (2001), the CP content was low for all ingredients. For example, the analyzed CP for soybeans was 37%, whereas the average listed in the NRC is 43%. The content of NDF and ADF of corn silage was low relative to the NRC values. Soyhulls contained approximately 66% NDF, compared with 60% listed in the NRC, which also lists 2.5% lignin for this feedstuff. The P content of corn silage, soybeans, and distillers grain was lower, and the content of soybean meal was higher, than the values listed in the NRC. The content was similar for other ingredients. Overall, the analyzed P content was reasonable compared with the values listed in the NRC.

Computed from ingredients, the total P content was 0.32 and 0.44% for the low and high P diets (Table 1), respectively, similar to the formulated amounts. The available P content was approximately 0.22 and 0.32%, as estimated from the ingredients using NRC (2001). Thus, the P availability was 68 and 73% for the low and high P diets, respectively. The dietary P requirement for the cows (43 kg/d of milk during treatment) was calculated to be 0.37% or 97 g/d based on NRC (2001) and using the average DMI of 26 kg/d during

treatment. Therefore, 0.32% P was slightly insufficient (14 g/d deficient), and 0.44% was moderately excessive (17 g/d in excess). According to a recent survey (Dou et al., 2003), dairy producers feed 0.44% P on average, with a range of 0.39 to 0.47% in most cases. These levels were lower than the levels (0.45 to 0.50%) obtained from surveys conducted a few years earlier (see Wu et al., 2001), suggesting that producers have reduced P recently. Accordingly, 0.44% P was good representation of the amount currently used by producers. The dietary NDF content was approximately 29 and 33%, and the ADF content 20 and 23% for the alfalfa hay and soyhull diets, respectively (Table 1), with the differences resulting from the partial substitution of soyhulls for alfalfa hay. As a proportion of total NDF, the NDF supplied from forage, however, was lower for the soyhull diets than for the alfalfa diets (66 vs. 82% of total NDF), resulting in similar amounts of NDF supplied from forage (22 vs. 24% of the diet). The diets contained approximately 16% CP, lower than formulated (17%), resulting from lower CP content of all ingredients (Table 2) than expected. Of the total protein, 38% was RUP based on NRC (2001).

Lactation Performance

The DMI was 1.5 kg/d lower for the low P diets than for the high P diets on average (Table 3). Decreased feed intake has been one of the major consequences of P deficiency in beef cows (Bass et al., 1981), whose diets are usually much lower in P than diets for lactating dairy cows. In other studies (Call et al., 1987; Wu et al., 2000, 2001), feed intake was not affected by similarly low P, but reduced when the diet contained 0.24% P (Call et al., 1987).

Milk yield was high, averaging 43 kg/d across treatments (Table 3). The yield was numerically lower for the low P diets than for the high P diets, averaging 42.1 and 44.0 kg/d; most of the difference occurred between the soyhull diets. However, neither the P effect nor the P and fiber source interaction was significant. Milk fat content was high, too, for all of these high forage diets, but lower for the low P diets than for the high P diets, averaging 3.68 and 4.12%. The differences in milk yield and fat content resulted in a trend for lower 4% FCM for the low P diets. Although Brodison et al. (1989) reported reduced milk fat percentage when cows received approximately 0.35 compared with 0.42% P in the diet in the first year of a 3-yr study, and Wu et al. (2003) observed a numerical reduction in milk fat with 0.33 compared with 0.42% P, most of the recent studies have shown no changes in milk fat concentration with dietary P amount. Interestingly, NRC (2001) discarded milk fat as a variable in calculating the P

Table 3. Performance of cows fed diets containing different amounts of phosphorus and sources of fiber.

Item	Treatment ¹				SEM	P ²		
	LPAH (n = 8)	LPSH (n = 8)	HPAH (n = 8)	HPSH (n = 8)		P	F	P × F
DMI, kg/d	25.8	24.2	26.9	26.1	0.7	0.04	0.11	0.57
Milk, kg/d	42.5	41.7	43.4	44.5	1.6	0.26	0.93	0.55
Milk fat								
%	3.67	3.68	4.04	4.20	0.13	0.01	0.55	0.58
kg/d	1.542	1.564	1.753	1.827	0.102	0.03	0.64	0.80
FCM, kg/d	40.1	40.4	43.7	44.9	2.3	0.10	0.76	0.86
Milk protein								
%	3.02	2.89	3.04	3.06	0.07	0.19	0.40	0.28
kg/d	1.264	1.215	1.314	1.332	0.048	0.10	0.75	0.50
Milk lactose, %	4.49	4.60	4.74	4.68	0.06	0.01	0.62	0.15
Milk SNF, %	8.29	8.29	8.57	8.53	0.08	0.01	0.82	0.86
Milk SCC, 1000/mL	611	256	194	439	21	0.56	0.78	0.14
Milk urea N, mg/dL	9.1	10.1	10.2	10.5	0.8	0.39	0.45	0.68
BW								
Initial, kg	694	634	637	668	19	0.57	0.47	0.03
End, kg	709	648	680	678	22	0.96	0.17	0.19
Change, kg/d	0.216	0.196	0.611	0.155	0.167	0.31	0.17	0.21
BCS ³								
Initial	3.12	3.17	3.27	2.97	0.12	0.83	0.28	0.16
End	3.27	3.22	3.10	2.95	0.06	0.05	0.34	0.63
Change	0.15	0.05	-0.17	-0.02	0.10	0.06	0.79	0.24

¹LPAH = Low P, alfalfa hay; LPSH = low P, soyhulls; HPAH = high P, alfalfa hay; and HPSH = high P, soyhulls.

²P = P level; F = fiber source; P × F = P level and fiber source interaction.

³Obtained using a scale of 1 = thin to 5 = fat.

requirement for lactation based on the consideration that only 10% of the P in milk is associated with lipids. That, however, does not mean that P does not have an effect on milk fat, because P is involved in many aspects of metabolism in addition to being a component of milk. Similar to FCM, milk protein yield tended to be lower for the low P diets, averaging 1.240 compared with 1.323 kg/d for the high P diets. Milk lactose and SNF were also lower for these diets. Dietary P amount did not affect BW change, although cows fed the low P diets gained in BCS, whereas those fed the high P diets lost BCS. Overall, based on the differences in lactation, it appeared that cows fed 0.32% P did not perform as well as those fed 0.44% P. However, the condition under which the difference appeared should be indicated. First, the number of cows per treatment used in the experiment was small (n = 8), and the variation in milk yield was large (SEM = 1.6 kg/d). Second, the difference resulted mostly from LPSH, which had the lowest milk yield, protein percentage and yield, and DMI. Third, the experimental period (70 d) was relatively short, and the reduction in lactation for the low P diets was surprising as one might expect that P would be mobilized from bone to support milk production during the treatment, unless the mobilization was approaching cessation in these midlactation cows. Lastly, the protein

content of the diets used in the experiment was low, and the metabolizable protein was likely limiting milk production. It could be questioned whether cows would have responded differently if dietary protein was closer to the requirement.

Recent studies have shown no changes in milk yield when dietary P was varied (see Wu et al., 2000). These studies included comparisons of dietary P of 0.33 and 0.39%, 0.35 and 0.44%, 0.37 and 0.55%, 0.38 and 0.48%, 0.39 and 0.65%, and 0.34, 0.51, and 0.69%. All these comparisons showed no effect of P amount on milk production. More recently, Wu et al. (2003) reported similar milk yields when cows were fed 0.33 or 0.42% P. However, cows used in these studies were lower in milk production than those used in the present study. For example, the milk yield in the study of Wu et al. (2003) averaged 36 kg/d, compared with 43 kg/d in the current study. Using this amount of milk, and the average DMI (26 kg/d), the P requirement of the cows used in the present study would be 97 g/d or 0.37% of the diet according to NRC (2001). The low P amount fed was 84 g/d or 0.32%, lower than the calculated amounts. The apparently reduced lactation performance with this amount of P supports the method of calculating P requirements used by NRC (2001).

Table 4. Apparent digestibilities of nutrients in cows fed diets containing different amounts of phosphorus and sources of fiber.

Item	Treatment ¹				SEM	P ²		
	LPAH (n = 8)	LPSH (n = 8)	HPAH (n = 8)	HPSH (n = 8)		P	F	P × F
	(%)							
DM	65.0	66.8	61.1	64.5	1.5	0.05	0.09	0.60
CP	58.2	61.6	53.5	56.9	2.3	0.05	0.15	0.99
NDF	48.3	52.3	44.9	50.6	2.0	0.23	0.03	0.69
P	34.2	43.9	23.9	33.2	2.5	0.01	0.01	0.93

¹LPAH = Low P, alfalfa hay; LPSH = low P, soyhulls; HPAH = high P, alfalfa hay; and HPSH = high P, soyhulls.

²P = P level; F = fiber source; P × F = P level and fiber source interaction.

Some studies have suggested that feeding extremely low amounts of P over a long period can result in reduced milk production. Wu et al. (2000) showed that cows fed 0.31% P decreased milk yield during the last one-third of lactation and these cows produced 10,790 kg of milk during the lactation or 35 kg/d on average. Likewise, reduced milk yields were reported when diets contained 0.24 compared with 0.32 or 0.42% P for cows milking 20 kg/d (Call et al., 1987), 0.30 compared with 0.54% P for cows milking 29 kg/d (Kincaid et al., 1981), and 0.24 compared with 0.28 or 0.33% P for cows milking 30 kg/d (Valk and Šebek, 1999). Calculated based on the NRC (2001) predictions for DMI and P availability (67%) for a 50:50 forage:concentrate diet that contains no mineral P supplements, the dietary P requirement for 20, 30, and 36 kg/d of milk would be 0.29, 0.33, and 0.35%, respectively. Clearly, the lower P amounts that resulted in reduced milk yields in all these studies were below the NRC calculated requirements. This again supports the NRC (2001) assessment for P requirements.

There was a tendency ($P = 0.11$) for DMI to be lower for the soyhull diets than for the alfalfa hay diets, primarily occurring when the P content of the diet was low (Table 3). Despite this, partial substitution of soyhulls for alfalfa hay did not affect any of the lactation measurements or BW and BCS changes. Moreover, there was no interaction between dietary P amount and fiber source found in any of the performance measurements. Ipharraguerre and Clark (2003) reviewed the literature on the use of soyhulls as an alternative feed source and concluded that including 5 to 25% soyhulls to replace corn silage and alfalfa in the diet that used 50% or more forage to provide 60 to 70% of the total NDF had no effect on DMI or milk production. Soyhulls were considered a suitable alternative feed for lactating dairy cows.

Milk urea N concentration was low for all of the diets compared with the averages reported for dairy farms

(Jonker et al., 2002), likely reflecting the use of steam-flaked corn as well as the low protein concentrations in the diets. Similar results were observed in the study of Wu et al. (2003).

Nutrient Digestibility

Fecal indigestible ADF averaged 24.0 (SD = 2.2), 21.0 (SD = 1.1), 21.7 (SD = 2.2), and 20.0% (SD = 2.9) for the LPAH, LPSH, HPAH, and HPSH diets, respectively, compared with dietary averages of 8.4 and 7.0% for the alfalfa hay and soyhull diets. Calculated apparent digestibility of DM and CP was higher for the low P diets than for the high P diets (Table 4), accompanied by the lower DMI for the low P diets. The digestibility of P was also higher for the low P diets than for the high P diets, averaging 39 and 29%, respectively. Apparent digestibility of P has been shown to decrease when P was fed above the requirement (Morse et al., 1992; Knowlton and Herbein, 2002; Wu et al., 2000, 2003), but these digestibility values appeared low, particularly for the HPAH diet. The low value resulted partially from the low DM digestibility for that treatment. The apparent digestibilities of P have ranged from 24 to 33% in reports with dietary P concentrations of 0.39 to 0.47% (Wu et al., 2001, 2003).

Apparent digestibility of DM tended to be higher, and the digestibility of NDF was higher, for the soyhull diets than for the alfalfa hay diets (Table 4). The change in CP digestibility due to the inclusion of soyhulls was insignificant, associated with large variation. Apparent digestibility of P was higher for the soyhull diets than for the alfalfa hay diets, averaging 29 and 39%, respectively, again reflecting the low value for the HPAH diet. It appeared that the increased digestibility of P for the soyhull diets was due partially to a slightly lower intake of P (Table 5), and partially to increased DM digestibility. Soyhulls are considered highly digestible because of the low lignin content (Garleb et al., 1988). Although

Table 5. Estimated P excretion in cows fed diets containing different amounts of phosphorus and sources of fiber.

Item	Treatment ¹				SEM	P ²		
	LPAH (n = 8)	LPSH (n = 8)	HPAH (n = 8)	HPSH (n = 8)		P	F	P × F
	(%)							
Intake, g/d	87.4	81.4	127.3	122.8	3.3	0.01	0.13	0.82
Fecal content, %	0.62	0.54	0.87	0.82	0.03	0.01	0.02	0.63
Fecal excretion, g/d	57.5	45.6	96.8	82.4	3.4	0.01	0.01	0.72
Absorption, g/d	29.9	35.8	30.6	40.4	3.0	0.38	0.02	0.51

¹LPAH = Low P, alfalfa hay; LPSH = low P, soyhulls; HPAH = high P, alfalfa hay; and HPSH = high P, soyhulls.

²P = P level; F = fiber source; P × F = P level and fiber source interaction.

soyhulls may have a high passage rate in the rumen because of the small particle size and high specific gravity when hydrated (Titgemeyer, 2000), the low inclusion rate (10%) and the presence of alfalfa hay (4%) as well as other forage sources (50%) in the diet may have sustained the ruminal mat to retain the potentially filterable particles of soyhulls for extended fermentation. Results are consistent with the data reviewed by Ipharraguerre and Clark (2003), suggesting that inclusion of soyhulls in the diet may result in increased nutrient digestibilities.

Phosphorus Excretion

Phosphorus intake averaged 84 and 125 g/d for the low and high P diets, respectively (Table 5), and the difference resulted primarily from the higher concentration of P in the diet and to a less degree from the higher feed intake for the latter treatment. Fecal P content averaged 0.58 and 0.85%, and the estimated fecal P excretion averaged 52 and 90 g/d for the low and high P diets, respectively. Although it is known that P excretion closely reflects P intake when P is fed above the requirement, the reduction in fecal P excretion observed in the present study is higher than that measured in other studies (Wu et al., 2000, 2001, 2003), possibly because 0.32% dietary P was inadequate for the high-producing cows used in this study, and these cows would maximize P absorption. In addition, the large excretion for the high P diets reflected the low digestibility for the HPAH diet, as discussed above. Although only about 3 g/d more P was absorbed for the high P diets than for the low P diets on average, compared with 41 g/d more in intake, most of the increased absorption occurred between the soyhull diets, coinciding with their bigger milk yield difference. This suggests that increasing the dietary P content did result in some more P absorbed in these P-deficient cows. The reason for the lower than expected P absorption for the high P diets was partially related to the lower DM

digestibility than that for the low P diets. Evidently, most of the increased P intake for the high P diets was excreted in feces, consistent with the report of Morse et al. (1992), who also determined that nearly all the intake P in excess of the requirement was excreted in feces.

The fact that DM digestibility was lower for the high P diets than for the low P diets could raise a concern that fecal P excretion for the high P diets might be overestimated despite the fact that these as well as the other diets were high in forage. It is true that DM digestibility was estimated using a marker (indigestible ADF), but the values obtained (Table 4) appeared reasonable for these high forage diets. The high P diets were associated with higher feed intakes, which might be more responsible for the lower DM digestibility than P intake; it is unlikely that 0.44% P would negatively affect DM digestibility compared with 0.32% P. Regardless, the difference in DM digestibility made an impact on the difference in estimated fecal P excretion. To remove this impact, the averages of DM digestibility for the low and high P diets for each fiber source (63.1% for the alfalfa hay diets and 65.7% for the soyhull diets) were used to recalculate fecal P excretion for the treatments. The adjustment slightly increased P digestibility for the high P diets, and reduced the amount of P excreted in feces. The recalculated values were 30.4, 41.9, 27.1, and 35.0% for P digestibility, and 61, 47, 93, and 80 g/d for fecal P excretion, for the LPAH, LPSH, HPAH, and HPSH diets, respectively. The recalculated P absorption was 26.5, 34.1, 34.6, and 43.0 g/d for the 4 diets, respectively, averaging 8.5 g/d more for the high P diets than for the low P diets. Using this value and the P intake difference of 41 g/d, passive absorption would be 21%, compared with 25% estimated by Wu et al. (2003). Knowlton et al. (2004) reviewed the literature and indicated that, with increased P intake, P absorption increased despite a reduction in apparent digestibility. Increased P absorption would result in more P excreted in urine if the requirement for milk production

Figure 1. Relationship between intake and fecal concentration of P: $y = 0.0049x + 0.20$ ($P < 0.01$), $r^2 = 0.51$ ($P < 0.01$).

and tissue metabolism is met. Although generally small and variable, urinary P (Wu et al., 2000; Knowlton and Herbein, 2002), and urine volume (Burkholder et al., 2004) have increased with P intake, consistent with the passive absorption concept.

Figure 1 is a plot of fecal P concentration in relation to P intake using individual cows of all treatments. The data appeared to be rather scattered around 2 fecal P concentrations, reflecting the 2 dietary P levels. Nevertheless, it shows a linear relationship between the 2 variables ($r = 0.72$), with P intake ranging from 60 to 145 g/d. The regression equation depicted ($y = 0.0049x + 0.20$) suggests that, at 100 g/d P intake, fecal P concentration would be 0.70%. Based on this equation and using the average DMI of 26 kg/d from all treatments, reducing dietary P from 0.44 to 37% (the calculated requirement amount) would reduce fecal P excretion by 12%, assuming no effect of the reduction in dietary P content on DM digestibility. Wu et al. (2001) showed a different equation ($y = 0.013x - 0.438$; $r = 0.92$) with P intake ranging from 70 to 130 g/d. Based on that equation, at 100 g/d P intake, fecal P concentration would be 0.85%. Both regressions provide a stronger relationship between P intake and fecal P concentration than that ($y = 0.0006x + 0.59$) obtained by Weiss and Wyatt (2004) based on data compiled from different experiments that involved P intakes ranging from 45 to 130 g/d. Based on the equation of Weiss and Wyatt (2004), fecal P concentration would be 0.65% at 100 g/d P intake. In the study of Wu et al. (2001), dietary P amount was the only treatment factor, whereas the current study involved fiber source as well as P amount as the dietary variables, and the studies contributing to the data summarized by Weiss and Wyatt (2004) involved various treatments. For this reason, the equation of Wu et al. (2001) is probably the most accurate.

Increased fecal P excretion as P intake increases may result from reduced rate of P absorption and increased excretion of salivary P (Braithwaite, 1985).

The regression equation depicted in Figure 1 also suggests that, at zero P intake, fecal P content would be about 0.20%. Using the average DMI of 26 kg/d and the average digestibility of DM of approximately 64% for all treatments, fecal P excretion at zero P intake would be approximately 20 g/d, or 0.8 g/kg of DMI. This is close to the amount of fecal endogenous P (0.9 g/kg of DMI) that was estimated by Spiekens et al. (1993). This value was approximated to 1 g/kg in NRC (2001) in calculating P requirements. However, caution should be taken when extrapolating fecal P concentration to zero P intake because the relationship may not be linear if P intakes below the requirement are included.

Table 6 illustrates the partitioning of fecal P into the components defined by Spiekens et al. (1993) using the method constructed by Wu et al. (2000) for comparing the P amounts as well as the fiber sources based on group averages. According to this partitioning, the average inevitable P was 24 g/d for both dietary P levels. Dividing this amount by the average DMI of 26 kg/d results in 0.9 g/kg, which is the same as the value obtained by Spiekens et al. (1993) using low-P diets (~0.20% P). As mentioned above, this value was approximated to 1 g/kg in NRC (2001) as the maintenance requirement for P. The estimated regulated P was 3.5 and 28.2 g/d for the 2 dietary P amounts, which are close to the values calculated by Wu et al. (2000) based on theoretical assumptions. These values show that increased fecal P excretion because of increased P intake was primarily in the form of regulated P, suggesting that cows disposed of P when intake P exceeded the requirement. It again supports the observation that 0.32% dietary P was inadequate in the present study, but only slightly. Although difficult to obtain, quantitative information on regulated P is useful for understanding the regulation of P absorption as well as for assessing the risk of manure P to the environment. Dou et al. (2002) showed that increased fecal P with P intake was primarily in the form of water-soluble P, consistent with the hypothesis that fecal P excretion is regulated via salivary P, which is inorganic. Although the principles that regulated P represents may be sound, the values obtained in the present study as well as those from the previous study (Wu et al., 2000) must be regarded as crude estimates. It is so for various reasons, including the fact that the calculated regulated P reflected accumulated errors involved in the estimation of other fecal P components during the portioning.

Substitution of soyhulls for alfalfa hay decreased estimated fecal P excretion by 18% based on the adjusted fecal P excretion values (63.5 vs. 77.0 g/d on average).

Table 6. Fecal P partitioning for cows fed diets containing different amounts of P or sources of fiber.¹

Dietary variable	Intake P	Fecal P			
		Total ²	Unavailable ³	Inevitable ⁴	Regulated ⁵
		(g/d)			
Phosphorus					
0.32%	84.4	54.0	27.0	23.4	3.5
0.44%	125.1	86.5	33.8	24.6	28.2
Fiber source					
Alfalfa hay	107.3	77.0	31.7	25.2	20.1
Soyhulls	102.1	63.5	30.1	22.9	10.4

¹Calculation was based on averages for 2 dietary treatments within each level of the dietary variables.

²Adjusted values based on the same DM digestibility for the alfalfa hay or soyhull diets that contained different amounts of P.

³Calculated from intake P and P availability of 68 and 73% for the low and high P diets, respectively, and 70.5% for both fiber source diets, according to NRC (2001).

⁴Including metabolic P and microbial P calculated according to Wu et al. (2000). This component, without counting the negligible urinary P at zero P intake, can be viewed as the maintenance requirement.

⁵Calculated as total fecal P – unavailable P – inevitable P.

Although part of the reduction was due to a lower P intake for the soyhull diets than for the alfalfa hay diets (102 vs. 107 g/d on average), the reduction is consistent with the concept that coarse forage sources have a bigger impact on chewing (Slater et al., 2000) and salivation than sources with small particles (Trater et al., 2001) and can potentially increase salivary P losses in feces. In support of this, the estimated regulated P in feces was lower for the soyhull diets than for the alfalfa hay diets (Table 6), although part of the reduction was related to the lower P intake. Scott and Buchan (1987) reported increased salivary P secretion in sheep with large particle sizes of forage. The Technical Committee on Responses to Nutrients (1991) showed that fecal endogenous P excreted in sheep increased when the proportion of hay in the diet increased or when hay was fed in a loose form compared with a pelleted form.

Although it is plausible that forage source has an effect on salivary P secretion and fecal P excretion, inconsistent results have been reported. For example, Ternouth (1989) observed that sheep fed ground straw excreted more endogenous P in feces than those fed chopped straw. Wu et al. (2003) showed that varying the forage amount from 48 to 58% in the diet for dairy cows by using more alfalfa silage did not affect fecal P excretion. Total salivary P output may not necessarily increase even if salivary volume increases, because the concentration of P in saliva may decrease as the rate of salivation increases (Cohen, 1980). Additionally, salivary P is absorbable in the small intestine, and absorption coefficients of 75 to 80% have been suggested (Challa et al., 1989). The rate of absorption, however, can vary greatly (Scott et al., 1995), apparently dependent upon the need for P (Care, 1994). For this reason, an interaction between dietary P amount and fiber

source in fecal P excretion might be expected. However, such an interaction was not found in the present study (Table 5). Similarly, Wu et al. (2003) observed no interaction in fecal P excretion between P amount and forage proportion in the diet. However, in that study, dietary forage proportion did not affect fecal P excretion, whereas in the present study, fiber source did affect fecal P excretion. Furthermore, the low P amount fed in the present study appeared inadequate; thus, cows would conserve P by reducing salivary P losses, and an interaction in fecal P excretion would be anticipated. Under this circumstance, it is remarkable that no interaction in fecal P was observed, and the observation warrants further investigation.

CONCLUSIONS

Dietary P at 0.32% appeared inadequate for cows producing 43 kg/d of milk, whereas 0.44% P was excessive compared with the calculated requirement of 0.37% according to NRC (2001). Based on the relationship between P intake and fecal P concentration, reducing P in the diet from 0.44 to 0.37% would reduce fecal P excretion by 12%. Substitution of soyhulls for alfalfa hay in the diet also reduced fecal P excretion partially through increased P apparent digestibility, suggesting that using readily digestible fiber sources in the diet may allow the P content of the diet to be reduced while still meeting the absorbable P requirement.

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