

Milk Production, Estimated Phosphorus Excretion, and Bone Characteristics of Dairy Cows Fed Different Amounts of Phosphorus for Two or Three Years¹

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ABSTRACT

Diets containing 0.31, 0.39, or 0.47% P on a DM basis were fed to 10, 14, and 13 multiparous Holstein cows, respectively, for a full lactation. Most of the cows (33) were fed similar amounts of P in the previous one or two lactations. The objective was to obtain information on bone integrity after prolonged feeding of different amounts of P. At the end of the experiment, a section (~20 cm) of the 12th rib was surgically removed. The bone was tested for strength and analyzed for ash and P content. The shear strength and the energy required to deform the bone to the point of fracture did not differ among treatments. Bone specific gravities were 1.50, 1.57, and 1.55 for the three treatments. Ash and P content of the bone, measured in wet weight, dry weight, and wet bone volume, were similar for the 0.39 and 0.47% P treatments, but tended to be lower with the 0.31% P treatment. Milk production of cows in all groups was high, averaging >11,900 kg for the 308-d lactation. Feeding P at 0.31% of dietary DM over two to three lactations appeared to decrease P concentration of bone, but the decrease was not severe enough to affect bone strength. Dietary P at 0.39% did not affect bone P content or strength. Dietary P content of 0.31% appears to be borderline deficient for cows producing >11,900 kg/308 d.

(Key words: phosphorus, dairy cow, reproduction, bone)

INTRODUCTION

Several surveys (Sansinena et al., 1999; Satter and Wu, 1999) have revealed that dairy producers feed 0.45

to 0.50% dietary P. This is in excess of the recommendations by NRC (1989 and 2001) and in excess of the needs of lactating cows (Wu and Satter, 2000b; Wu et al., 2000). A reduction in dietary P to more closely match the cow's requirement can result in 25 to 30% less manure P and a savings of \$10 to 15/cow per year in P supplementation costs (Wu et al., 2000).

Although the data relating milk production to dietary P content are substantial and convincing (Brintrup et al., 1993; Satter and Wu, 1999; Valk and Ebek, 1999; Wu and Satter, 2000b; Wu et al., 2000), more information is needed on the status of bone as a function of dietary P. Bone serves as an important reservoir of P that can be mobilized to meet P requirements (Ter-nouth, 1990; Von Koddebusch and Pfeffer, 1988). A significant amount of bone P is made available in the first weeks of lactation when Ca is mobilized from bone to support lactation.

The objective of this study was to obtain information on bone strength and bone P content of lactating cows that had been fed low P diets for two to three lactations. Lactation performance of cows in this third year of experiment and in the previous one or two years with specified dietary P concentrations is also reported to show long-term effects of dietary P on animal performance.

MATERIALS AND METHODS

Thirty-seven multiparous Holstein cows were used in a 308-d lactation trial. Diets (Table 1) containing 0.31, 0.39, or 0.47% P (DM basis) were assigned to groups of 10, 14, and 13 cows at parturition. Uneven group size resulted in part from attrition of cows from treatment groups initiated 1 to 2 yr before this part of the long-term study. Molasses and beet pulp were included as diet ingredients because of their low P content; this enabled formulation of a basal diet containing 0.31% P. Diets containing 0.39 and 0.47% P were obtained by adding monosodium phosphate to the low P

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¹Trade names and the names of commercial companies are used in this report to provide specific information. Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the USDA or an endorsement over products not mentioned.

Table 1. Ingredients and nutrient content of diets.

Item	Dietary P (% of DM)		
	0.31	0.39	0.47
Ingredient, %			
Alfalfa silage	30.00	30.00	30.00
Corn silage	20.00	20.00	20.00
High moisture ear corn	25.25	25.25	25.25
Soybeans, roasted ¹	10.00	10.00	10.00
Blood meal	2.00	2.00	2.00
Molasses	5.00	5.00	5.00
Beet pulp	7.00	6.63	6.28
Salt ²	0.40	0.40	0.40
Mineral and vitamin mix ³	0.10	0.10	0.10
Yeast culture ⁴	0.25	0.25	0.25
Monosodium phosphate ²	...	0.37	0.72
Chemical composition			
CP, %	17.4	17.4	17.3
ADF, %	25.4	25.4	25.3
NDF, %	29.6	29.5	29.3
P, %	0.31	0.39	0.47

¹Roasted soybeans were rolled as they exited the roaster and broken into sizes ranging from one-eighth to one-half bean pieces.

²Added Na from NaCl and NaH₂PO₄ accounted for 0.16, 0.24, and 0.32% of the total dietary DM for the 0.31, 0.39, and 0.47% P diets, respectively.

³Each kilogram of the mix contained 0.32 g of Se, 0.43 g of Co, 1.03 g of I, 13.35 g of Cu, 23.99 g of Fe, 51.00 g of Mn, 62.01 g of Zn, 7,000,000 IU of vitamin A, 2,222,000 IU of vitamin D, and 17,630 IU of vitamin E.

⁴Diamond V XP Yeast Culture, Diamond V Mills, Cedar Rapids, IA.

diet. The experimental protocol was approved by the Animal Care Committee of the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

This trial is the third in a series of P feeding trials with lactating cows. The first two trials have been reported (Wu and Satter, 2000b; Wu et al., 2000). The 37 cows used in the present trial included 14 and 19 cows that were fed similar dietary P concentrations for one or two previous lactations, respectively (Table 2). The remaining four cows (three in the 0.31% P group and one in the 0.39% P group) were new to this trial. During dry periods dietary P content averaged between 0.35 and 0.40%.

Cows were housed in a tie-stall barn and offered a TMR once daily ad libitum (5 to 10% refusal). Actual amounts of feed offered and refused by individual animals were recorded daily to obtain feed intake. Milking was at 0500 and 1700 h; milk yields were recorded at each milking. Cows were weighed after milking early in lactation (22 DIM, SD 16 d) and again at the end of lactation. All cows were administered bST (Posilac; Monsanto Co., St. Louis, MO) every 2 wk beginning at wk 9 of lactation.

Data related to reproduction and health were recorded. Estrus was monitored by the farm crew during the day and while cows were in the holding area before

milking, using signs such as standing while being mounted and mucus discharge. Cows were inseminated at the first estrus after 52 d postpartum. The semen source and inseminator were not the same for all cows but were equally distributed between treatments. Pregnancy was confirmed by rectal palpation. The conception rate at the first AI was the percentage of cows that conceived on first AI. Cows were identified as nonbreeders when they developed severe health problems, whether the problem was related to reproduction or not, or failed to become pregnant by 260 DIM. These cows were not used in the calculation of reproductive measures.

Cows were dried off or removed from the experiment following completion of 44 wk of lactation. As a normal herd management practice, cows on occasion were dried off earlier than wk 44 to assure an 8-wk dry period before the next lactation or were removed from the experiment when they developed significant health problems. Consequently, 14 cows were removed from the experiment between wk 17 to 43 (Table 3). To obtain 308-d lactation performance for these cows, milk yields from the last five weekly averages were used to extrapolate by linear regression to generate estimates for the missing weeks.

Feeds offered and refused were recorded daily. Alfalfa silage, corn silage, and Orts were sampled daily, frozen, and composited weekly. Concentrates (high moisture corn, roasted soybeans, blood meal, molasses, and beet pulp) were sampled weekly. Dry matter content of weekly samples was determined by oven-drying at 60°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 calculations.

Table 2. History of P feeding for cows assigned to the third year (current) trial¹.

Yr 1	Dietary P (% of DM)		Number of cows in the current study having previous assignment to P trials
	Yr 2	Yr 3 (current trial)	
0.38/0.31 ²	0.38/0.31 ²	0.39	7
0.48/0.44 ²	0.48/0.44 ²	0.47	7
...	0.31	0.31	7 ³
...	0.40	0.39	6
...	0.49	0.47	6

¹During preceding dry periods, no supplemental P was fed, and dietary P averaged 0.30%.

²The first number is the percentage of dietary P during confinement feeding in the first two thirds of the lactation and the second number is the percentage of dietary P during grazing in the last one third of the lactation.

³Included four cows that were fed 0.38% P during confinement feeding and 0.31% P during grazing in yr 2. All cows in that group were fed 0.31% P in yr 3.

Table 3. Number of cows that left the experiment before completing 44 wk of lactation.

Dietary P (%)	Week of lactation									Total
	17	20	30	32	33	40	41	42	43	
0.31	1 ^m	1 ^r	1 ^d	...	1 ^d	4
0.39	1 ^h	1 ^m	...	1 ^h	2 ^d	2 ^d	7
0.47	1 ^m	...	1 ^h	1 ^m	3

^{d,g,m,r}Reasons for termination: d = dried off to allow for a 56-d dry period; h = health problems; m = mastitis; r = reproduction failure.

Milk samples were collected biweekly from two consecutive milkings and analyzed by AgSource Milk Analysis Laboratory (Menomonie, WI) for fat, CP, lactose, total solids, and SCC with an infrared spectrophotometer with a B filter (Fossmatic 605; Foss Technology, Eden Prairie, MN); SNF was calculated as total solids minus fat. Aliquots of the two samples obtained during each of the sampling weeks were pooled according to milk volume and frozen until analyzed for P content.

Sampling times for urine and blood fell within the following weeks of lactation: 2 to 4, 5 to 7, 9 to 11, 14 to 16, 19 to 21, 24 to 26, 29 to 33, 35 to 37, and 40 to 42; the times for feces were wk 9 to 12, 14 to 17, 19 to 22, 24 to 27, 29 to 32, 34 to 37, and 39 to 42. Samples of urine were obtained during urination. Approximately 10 ml of blood was obtained from coccygeal vessels 3 h after feeding. Blood samples were centrifuged at 2200 × g for 15 min to obtain serum. Feces were sampled from the rectum on four consecutive days in each of the sampling weeks at 0930 to 1100 h on d 1 and 3 and 1400 to 1530 h on d 2 and 4. These samples were pooled across days during a week for individual cows and dried at 60°C.

All dried feed and fecal samples were ground through a Wiley mill with a 1-mm screen (Arthur H. Thomas, Philadelphia, PA). Ground concentrate samples were composited every 4 wk. The composite concentrate samples and the ground weekly samples of alfalfa silage and corn silage were analyzed for DM (105°C), CP (LECO FP-2000 Nitrogen Analyzer, Leco Instruments, Inc., St. Joseph, MI), and NDF (heat stable α -amylase and Na₂SO₃ were used) and ADF (Robertson and Van Soest, 1981). The ANKOM²⁰⁰ Fiber Analyzer incubator (ANKOM Technology, Fairport, NY) was used for NDF and ADF analyses. Analyses for NDF and ADF in roasted soybeans and blood meal were made only on one 4-wk composite sample.

For analysis of P, ground samples of alfalfa and corn silages were composited approximately every 4 wk and concentrate samples approximately every 12 wk. These composites and the ground fecal samples were processed as described by Nelson and Satter (1992).

All samples of feeds, feces, urine, and milk were analyzed for P content by direct current plasma emission

spectroscopy by adapting the procedure described by Combs and Satter (1992). A certified commercial P solution (VHG Labs, Inc., Manchester, NH) was used as a calibration standard. Accuracy of the analysis was assured by referring to additional commercial standards (Standard Reference Material 1570a, spinach leaves, and 8436, durum wheat flour; National Institute of Standards and Technology, Gaithersburg, MD). Serum was analyzed for inorganic P concentration by the Marshfield Laboratories (Marshfield, WI) according to AOAC (1980).

Chemical analyses of feeds and feces were based on DM measurements made at 105°C. The nutrient content of the TMR was computed from the average nutrient content of the individual diet ingredients analyzed with the aforementioned composite samples.

At the end of lactation, or in some cases after cows were removed from the experiment but still milking and receiving the same diet (320 DIM, SD 26 d), surgery was conducted to remove part of the 12th rib bone. The surgery area on the right side of the cow was clipped with a number 40 blade, scrubbed with Betadine solution, and rinsed with water. The area then was rinsed with 70% alcohol, followed by a final rinse with 7% strong tincture of iodine. Local anesthesia was applied, and an incision (~25 cm) was made over the 12th rib through the skin, fascia, and muscle down to the bone. A periosteum elevator was used to separate periosteum from the rib. A gigli wire was used to saw proximally and distally to remove a piece of rib measuring ~20 cm long. After the bone was removed, the periosteum and the muscle layers were sutured separately with catgut, and the skin edges were closed by interlocking sutures. A sulfa-urea powder was sprinkled on the opening, followed by a final suture. The closed wound was sprayed with an antibiotic solution.

Immediately after removal, bone samples were trimmed of adhering pieces of soft tissues, wrapped in damp paper towels, and placed in air-tight Ziploc plastic bags (within 3 min of removal); bone samples in the bags were kept on ice and later frozen at -20°C. Before testing for strength, bone was thawed and brought to room temperature (22°C). Bone strength was tested mechanically (MTS Universal Testing Machine, model 5/

G, MTS Systems Corp., Raleigh, NC) by a double shear block apparatus, according to the ASAE standard (1998) and the procedure described by Combs et al. (1991). The force was loaded at 5 mm/min over a section at each end of the bone (~5 cm from the end); an average result was used for one bone sample. The test resulted in maximum sheer force, shear stress, and fracture energy, which were electronically recorded. After the test, the wall thickness of the bone was measured at three places by a digital caliper with a precision of 0.025 mm, and the measures were averaged to obtain an overall wall thickness. The unfractured part of the bones was sawed into approximately 4-cm chunks, and separated chunks were used to determine DM (100°C) and total ash content (at 600°C until a consistent white ash was obtained). The ash was analyzed for P content. Bone pieces were weighed in air and water for the calculation of specific gravity.

Daily milk yield, milk component percentages and yields, and DMI were reduced to 4-wk means. These means and the data on blood serum P, urinary P, and fecal P concentrations were analyzed as repeated measures in time for effects of treatment and treatment \times time interaction by the mixed procedure of SAS (1992). Other data were analyzed by the general linear models procedure of SAS (1985) according to a completely randomized design. These data include 308-d milk yield, BW, and reproductive measures. For both types of analyses, treatment effects were tested for linear and quadratic relationships. Nonorthogonal mean comparisons were also generated in the statistical analysis by requesting probability for difference along with least squares means for specific evaluation. Pooled SEM is presented for simplicity. Categorical data on conception and pregnancy rates were analyzed for treatment effects using the FREQ procedure of SAS (1985) with a chi-square test.

RESULTS AND DISCUSSION

Nutrient content of ingredients (Table 4) was relatively consistent within the experiment. Dietary P content was computed from P content of the ingredients, and was 0.31, 0.39, and 0.47% of dietary DM for the three diets (Table 1). Molasses and beet pulp contributed 10 and 14% of the ADF and NDF, respectively. Based on NRC (1989) tabular values, Ca was 0.65% and NE_L was 1.65 Mcal/kg for all the diets.

Results pertaining to reproductive performance are presented in Table 5, but insufficient animal numbers preclude drawing conclusions about a relationship between dietary P and reproductive performance. Wu and Satter (2000b) estimated that a minimum of 250 cows per treatment would be needed to detect a 10% differ-

Table 4. Composition of dietary ingredients.

Ingredient	DM	CP	(% of DM)		
			NDF	ADF	P
Alfalfa silage					
\bar{x}	37.5	22.6	43.0	39.1	0.339
SD	4.4	1.2	3.6	3.9	0.020
Corn silage					
\bar{x}	35.3	7.7	39.9	26.0	0.245
SD	3.0	0.7	3.5	2.8	0.023
High moisture corn					
\bar{x}	71.6	7.7	8.3	3.8	0.303
SD	2.3	2.2	1.8	2.4	0.011
Soybeans, roasted					
\bar{x}	97.0	41.0	25.1	8.3	0.614
SD	1.0	1.3	0.030
Molasses					
\bar{x}	97.3	7.6	27.7	21.7	0.092
SD	1.2	0.9	1.6	1.0	0.010
Beet pulp					
\bar{x}	91.4	9.4	39.2	23.6	0.113
SD	1.2	1.6	2.4	1.4	0.025
Blood meal					
\bar{x}	90.1	100.5	0.215
SD	1.5	3.1	0.073

ence in reproductive measures at a 95% confidence level. With the small number of cows, reproductive performance for the lowest P group appeared poorer than for the other groups. Most of the cows on the low P treatment were on low P treatments (0.38 to 0.31%) in the previous two lactations, and these amounts of P did not affect reproductive performance in those years (Wu and Satter, 2000b; Wu et al., 2000). Wu and Satter (2000b) summarized eight studies on dietary P concentration involving 785 dairy cows in the literature, and obtained no evidence that dietary P from 0.32 to 0.40% of dietary DM reduced reproductive performance compared with 0.39 to 0.61%. Valk and Ebek (1999) fed dietary P of 0.24, 0.28, or 0.33% for two lactations and observed no difference in reproductive performance.

The recorded health problems (Table 5) showed no apparent association with treatments. The incidence of off-feed, which occurred more times for the 0.31% P group, was the result of repeated occurrences with two individual cows within 1 mo after calving. The number of cases of foot rot appeared higher with the high P treatments. The low number of cows per treatment precludes drawing any conclusions regarding the incidence of health problems.

The DMI over the entire lactation was similar among treatments (Table 6), indicating that varying P from 0.31 to 0.47% of the diet had no effect on ad libitum feed intake. Cows in all groups had high milk yield, averaging >11,900 kg/308 d. Milk production for the 0.31% P group was higher ($P < 0.10$) than that of the 0.39% P group, and appeared to result in the highest

Table 5. Reproductive measures and health records of lactating cows fed diets differing in P content.

Item	Dietary P (% of DM)			SEM	P	
	0.31	0.39	0.47		Linear	Quadratic
Number of cows	10	14	13
Days to first estrus	74.8	70.9	83.2	12.7	0.66	0.60
Days to first AI	89.9	76.7	93.9	9.1	0.77	0.18
Days open ¹	160.2 ^a	108.5 ^b	128.1	18.6	0.25	0.14
Conception rate at first AI, %	25.0	44.4	38.5
Number pregnant ³						
120 DIM, %	25.0	50.0	30.8
260 DIM, %	75.0	80.0	84.6
Services per conception ¹	2.4	1.9	1.8	0.5	0.42	0.71
Incidence of foot rot ⁴	0	2	4
Incidence of off feed ⁴	5	4	2
Mean mastitis incidence ⁵	1.7	1.6	1.2

^{a,b}a > b ($P < 0.09$).

¹Includes only the cows that became pregnant before 260 DIM; numbers of cows excluded were 4, 6, and 2 for the three treatments, respectively. All of these cows were identified as nonbreeders, except for one which was removed due to mastitis.

²Chi-square, $P > 0.5$.

³Excluded in the calculation were cows that were identified as nonbreeders before the first AI. These were cows that developed health problems, that may or may not have been related to reproduction.

⁴Total number of occurrences were used. If one animal had multiple occurrences, the actual occurrences were counted.

⁵Average incidence per cow for the entire lactation. Drug treatment was used to qualify for an occurrence of mastitis. Multiple treatments within a period of 1 mo were counted as one occurrence.

milk yield of the three groups (Figure 1). Interpretation of the milk production data from this trial needs caution. This trial was a continuation of the trials carried

Table 6. Performance of cows fed diets differing in P content.

Item	Dietary P (% of DM)			SEM ^a
	0.31	0.39	0.47	
Number of cows	10	14	13	...
DMI, kg/d	25.0	25.0	24.6	0.6
Milk, kg/308-d	13,038	11,909	12,126	407 ^b
Milk, kg/d	42.4	38.7	39.4	1.4 ^b
3.5% FCM, kg/d	43.4	39.4	40.3	1.4 ^b
Milk fat				
%	3.64	3.50	3.64	0.12
kg/d	1.54	1.38	1.43	0.06 ^b
Milk protein				
%	3.16	3.13	3.10	0.05
kg/d	1.32	1.21	1.22	0.038 ^c
Milk lactose, %	4.73	4.69	4.70	0.07
Milk SNF, %	8.74	8.66	8.63	0.10
Milk SCC, 10 ³ /ml	642	832	465	180
BW during lactation				
Initial ¹ , kg	663	623	609	20 ^d
Ending ¹ , kg	735	718	701	22
Change, g/d	277	345	320	76

^aNo treatment by month interaction for lactational measurements ($P > 0.10$).

^b0.31% P > 0.39% P ($P < 0.10$).

^cLinear effect ($P < 0.06$).

^d0.31% P > 0.47% P ($P < 0.10$).

¹Initial BW taken at 15 DIM (SD 9 d) and ending BW at 290 DIM (SD 10 d).

out in the previous 1 or 2 yr, and cows remained on similar levels of dietary P without being randomly allocated to treatments. We did this to evaluate bone characteristics after long-term feeding of different amounts of P. In a previous trial (Wu et al., 2000), milk yields were similar for cows fed 0.40 or 0.49% P, but milk yield was reduced during the latter part of the lactation with 0.31% dietary P. Based on the results from several studies (Brintrup et al., 1993; Call et al., 1987; Dhiman et al., 1995; Kincaid et al., 1981; Steevens et al., 1971; Valk and Ebek, 1999; Wu et al., 2000), we (Wu et al., 2000) suggested that dietary P < 0.31% is marginally

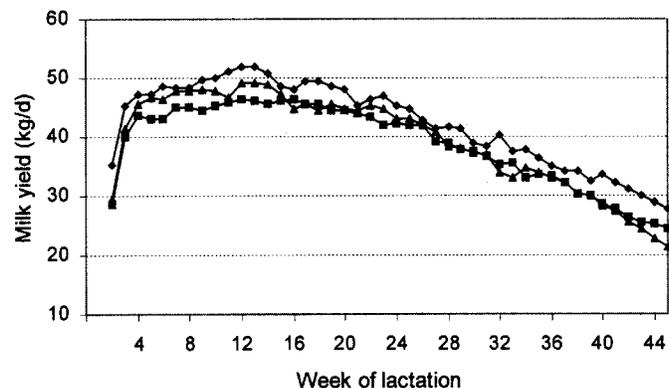
**Figure 1.** Milk yield of cows fed diets containing 0.31 (◆), 0.39 (■), or 0.47% P (▲).

Table 7. Milk production of cows fed different amounts of P for 2 to 3 yr.

Dietary P (% of DM)	n	Year 1	Year 2	Year 3	SEM
		(kg/308-d)			
0.31–0.38	7	9590 ^a	11,421	12,094	477
0.44–0.48	7	8202 ^b	10,700	12,648	477
0.31	7	...	11,222	13,008 ^a	550
0.39–0.40	6	...	10,692	11,698	595
0.47–0.49	6	...	10,900	11,516 ^b	595

^{a,b}Numbers with different superscripts in the same column differed at $P < 0.05$ for yr 1 and at $P < 0.10$ for yr 3.

deficient for cows producing about 9000 kg per lactation, but feeding 0.38 to 0.40% dietary P should be adequate for high producing cows (>10,000 kg per lactation).

Mean milk fat percentage did not differ among treatments (Table 6), and thus fat yield and FCM yield reflected milk yield. Milk protein percentages were similar among treatments. Occasionally, lowered milk protein percentages have been observed when a low-P diet was fed (Call et al., 1987; De Boer et al., 1981; Wu et al., 2000), but the majority of the studies (Brintrup et al., 1993; Brodison et al., 1989; Carstairs et al., 1981; Valk and Ebek, 1999; Wu et al., 2000) have shown no difference in concentration of milk protein due to dietary P content. The content of lactose, SNF, or SCC of milk did not differ among treatments. Changes in BW during lactation were similar.

Milk yield for cows that had been fed similar amounts of P for 2 to 3 yr is in Table 7. The yields for yr 1 and 2 were obtained from previous reports (Wu and Satter, 2000b; Wu et al., 2000) and those for yr 3 were from the current trial. Compared with 0.44 to 0.49% dietary

P, feeding 0.31 or 0.31 to 0.38% P did not reduce the cow's milking capacity after 2 to 3 yr.

The concentration of inorganic P in blood serum was lower ($P < 0.01$) for cows fed the lowest P diet than for those fed the other diets (Figure 2); the overall means during the lactation were 5.7, 6.1, and 6.5 mg/dl (SE 0.1) for the 0.31, 0.39, and 0.47% P groups, respectively. The concentrations were similar toward the end of lactation. Serum P concentration can reflect P intake in ruminants, but it is not always a good indicator of P status (Wu et al., 2000). Only extremely low serum concentrations (<4 mg/dl) may indicate deficiency of P (Forar et al., 1982). In a previous paper (Wu et al., 2000), we showed that the concentration of total P in milk appears to be related to milk protein concentration. A trend for this relationship again was observed in the present study with cows from all treatments ($R^2 = 0.21$, Figure 3). The average concentration of P in milk from the data that included 705 measures was 0.094% (SD 0.009). Extrapolation of the regression in Figure 3 to 0% protein indicates that P content of protein-free milk is 0.0487%, about half of the P present in milk containing 3.00 to 3.25% CP. This agrees with other observations that about half of the total P in milk

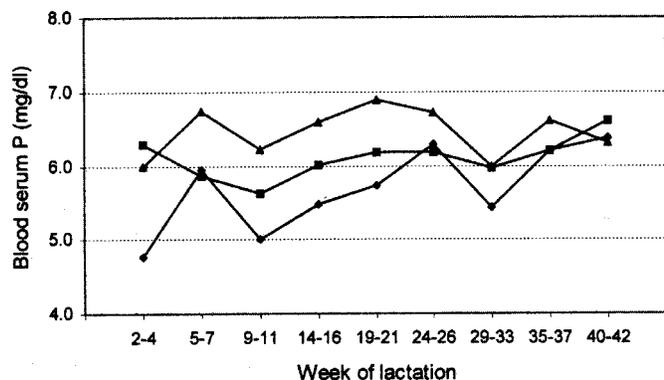


Figure 2. Concentration of inorganic P in blood serum of cows fed diets containing 0.31 (◆), 0.39 (■), or 0.47% P (▲) for the complete lactation. Means for the respective treatments during lactation were 5.7, 6.1, and 6.5 mg/dl (SEM 0.1, $P < 0.01$) with an effect of treatment by sampling period interaction ($P < 0.05$).

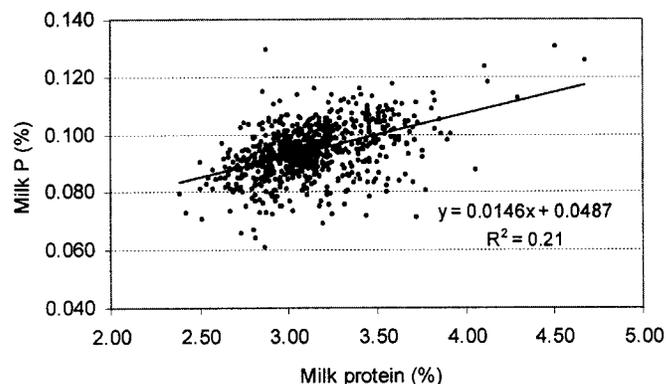


Figure 3. Relationship between concentrations of milk P and milk CP.

is complexed with casein and the other half exists as diffusible ions or in milk serum (Farrell, 1988; Jenness, 1985). A cow having milk with 4 versus 3% CP would have about 0.0146 percentage units more P in the milk, or approximately 16% more total P (Figure 3). Because approximately 60% of the P requirement for a cow milking 40 kg/d is partitioned to milk production, and assuming the maintenance requirement of P for the cow is independent of milk protein content, then a cow producing milk with 4% CP should require about 10% more dietary P than a cow producing milk with 3% CP. Our modern feeding standards need to reflect this.

The concentration of P in urine reflected dietary P concentrations (Figure 4); the overall means from all of the sampling times were 1.6, 2.8, and 3.6 mg/dl (SEM 0.7, $P = 0.17$) for the 0.31, 0.39, and 0.47% P groups, respectively, with wide variation among cows within treatment. Some cows will spill small amounts of P in urine when they absorb more P than required. We arbitrarily designated urinary P of 2.5 mg/dl as a spilling threshold. This level corresponds to a daily excretion of ~1 g/cow. The NRC (2001) considers that a 600-kg cow would excrete 1.2 g of urinary P per day. This is close to the spilling threshold used in our calculations. With a spilling threshold of 2.5 mg/dl, then 9, 25, and 35% of the urine samples exceeded the threshold for the 0.31, 0.39, and 0.47% P treatments. Also, the concentration of P in urine appeared to increase in the latter part of the lactation, a reflection of diminished P requirement for milk production. Ruminants have a well-developed capacity for conserving P, and normally very little P is excreted in the urine, even with very high dietary intake of P (Ternouth, 1990). Spilling of P in the urine is a reliable sign that the animal has ade-

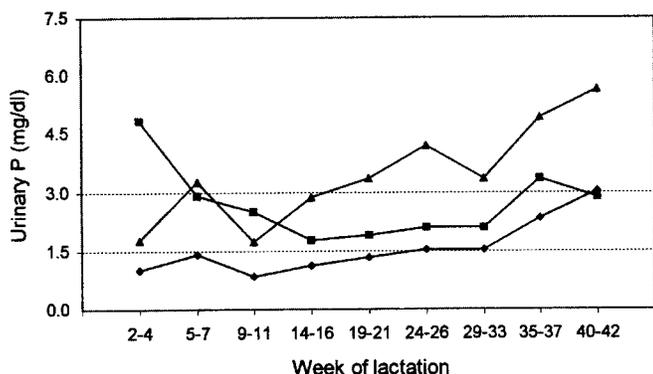


Figure 4. The concentration of total P in urine of cows fed diets containing 0.31 (◆), 0.39 (■), or 0.47% P (▲) for the complete lactation. Means for the respective treatments during lactation were 1.6, 2.8, and 3.5 mg/dl (SEM 0.7, $P = 0.17$) with a difference ($P = 0.06$) between the 0.31 and 0.47% P groups but no effect ($P > 0.10$) of treatment by sampling period interaction.

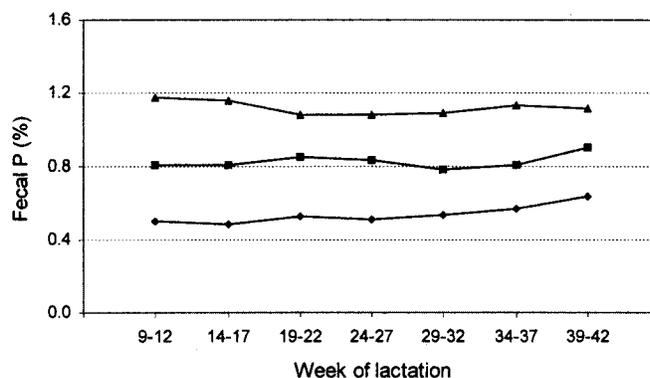


Figure 5. The concentration of P in feces of cows fed diets containing 0.31 (◆), 0.39 (■), or 0.47% P (▲) for the complete lactation. Means for the respective treatments during lactation were 0.538, 0.829, and 1.12% (SEM 0.02, linear effect, $P < 0.01$) with no effect ($P > 0.1$) due to sampling time or treatment by sampling time interaction.

quate P. Failure to spill, however, does not necessarily mean that P is deficient.

The P content of feces increased ($P < 0.01$) as dietary P content increased (Figure 5). The concentration was relatively constant throughout the lactation for all of the treatments. The P content of each treatment diet was held constant for the entire lactation, thus the intake of P was a function of DMI. Based on relatively constant concentration of fecal P, mobilization of bone P appeared able to compensate for reduced intake of P during the first weeks of lactation when DMI was low. The mobilized bone P, of course, must be restored in later lactation. Also, since milk protein content increases during the last two-thirds of lactation (Wu and Satter, 2000a), milk P content also increases. The P needed for bone deposition and the increased P requirement per unit of milk, combined with the P needs of the growing fetus and uterine mass, suggest that P content of the diet should not be decreased relative to milk production in later lactation. The relatively constant amount of fecal P (Figure 5) and only moderate increases in urinary P in late lactation (Figure 4) suggest that feeding a constant concentration of dietary P throughout lactation would result in only a moderate oversupply of P in late lactation.

Figure 6 is a plot of fecal P concentration in relation to P intake using averages for the entire lactation of cows from all treatments. It shows that fecal P concentration linearly ($r = 0.92$) increased as P intake increased. This relationship was based on the range of P fed in this experiment (approximately 70 to 130 g of P/d per cow). If amounts below the requirement also had been fed, we would expect a curvilinear relationship. Using the regression equation depicted in Figure 6 ($Y = 0.013x - 0.438$), fecal P concentrations for cows fed

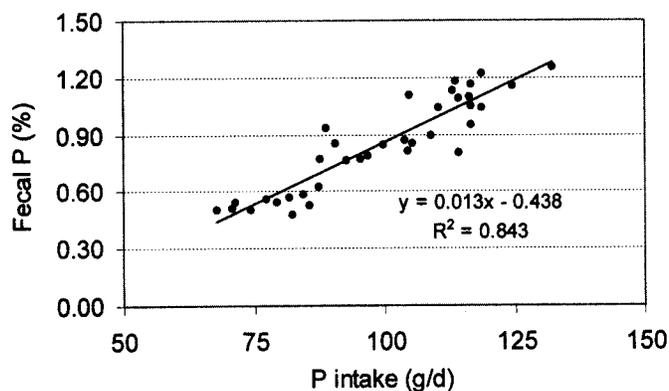


Figure 6. Relationship between P intake and fecal P concentration.

diets containing different amounts of P can be predicted. Morse et al. (1992) reported a quadratic equation relating P intake (g/d) and P excretion in urine and feces. The equation developed ($R^2 = 0.70$) was $Y = 14.67 + 0.6786 X + .00196 X^2 - 0.317 \times \text{kg/d of milk}$, where Y = excretion in urine and feces (g/d), and X = P intake (g/d).

The average concentration of fecal P from all sampling periods was 0.538, 0.829, and 1.118% (DM basis) for the 0.31, 0.39, and 0.47% P treatments, respectively. With these concentrations, DMI (Table 6), and an estimated DM digestibility of 68% for all of the diets, 43, 66, and 88 g/d of P were estimated to be excreted in feces for the three treatments, respectively (Table 8). This would result in net absorption of 33, 32, and 28 g/d. The estimated apparent digestibility of P for the three treatments would therefore be 45, 33, and 24%. We (Wu et al., 2000) previously suggested that an apparent digestibility for P of 40% or less may be indicative of excessive intake of P. According to the illustration of Wu et al. (2000), the regulated portion of fecal P, i.e., that fecal P over which the animal has discretion, was 10, 30, and 49 g/d for the three groups, with the differences between treatments (~20 g/d from one level of dietary P to a higher level) coinciding with the differ-

Table 8. Estimated apparent digestibility of P in cows fed diets differing in P content.

Item	Dietary P (% of DM)		
	0.31	0.39	0.47
Intake, g/d	77.5	97.5	115.6
Fecal excretion, g/d ¹	43	66	88
Apparent digestibility, %	45	33	24

¹Estimated using 68% as the DM digestibility and the means for DMI (Table 6) and fecal P concentration (0.538, 0.829, and 1.118% for the three treatments, respectively, obtained from Figure 5).

Table 9. Strength measurements of the 12th rib bone from cows fed diets differing in P content.

Item	Dietary P (% of DM)			SEM
	0.31	0.39	0.47	
Number of cows ¹	9	9	11	...
Shear stress, N/mm ²	26.5	28.1	27.5	2.2
Fracture energy ² , N-m	66.6	60.5	65.0	4.2
Wall thickness, mm	5.1	5.1	5.2	0.1
Bone specific gravity ³	1.50	1.57	1.55	0.02
Ash, % of dry weight	53.9 ^c	56.2 ^a	55.6 ^{ab}	0.8
Ash, % of wet weight	46.0 ^c	47.4	48.1 ^a	0.7
Ash, g/cc, wet bone	0.69 ^c	0.74 ^a	0.74 ^a	0.01
P, % of ash	17.7	17.3	17.9	0.3
P, % of dry weight	9.5	9.7	9.9	0.2
P, % of wet weight	8.1 ^c	8.2	8.6 ^b	0.2
P, g/cc, wet bone	0.122 ^c	0.129	0.133 ^a	0.003

^{a,b,c}c < a ($P < 0.06$), c < b ($P < 0.13$).

³Linear ($P < 0.11$) and quadratic ($P < 0.14$) effects.

¹The nine cows sampled from the 0.31% P group included three cows that were fed this amount of P for 1 yr; all other cows sampled in this trial had been fed similar amounts of P for 2 or 3 yr.

²Area under the force (N) and deformation (m) curve. It is an expression of the amount of energy the bone absorbs before fracture.

ences in P intake (also ~20 g/d from one level to a higher level), suggesting that most of the unneeded intake P would be excreted in feces.

No differences were found among treatments in the shear stress the bone endured before rupture or the amount of energy required to deform the bone to the point of fracture (fracture energy) (Table 9). Wall thickness of the bone was ~5.1 mm for all treatments. Bone specific gravity tended ($P < 0.1$) to be lower for the 0.31% P treatment than for the other two treatments, with the difference being about 4%. The ash content of the bone, expressed on dry weight, wet weight, or wet bone volume, was slightly lower ($P < 0.06$ to 0.13) for the 0.31% P group. The P content of bone was similar among treatments when expressed on an ash or dry weight basis, averaging 17.6 (SE 0.3) and 9.5% (SE 0.2), respectively. When expressed on a wet weight or volume basis, however, P content was lower ($P < 0.06$ to 0.13) for the 0.31% P treatment compared with the 0.47% P treatment. The average decrease in ash and P contents (based on measurements in dry weight, wet weight, and wet bone volume) was 4.8 and 6.0%, respectively, between the 0.31% and 0.47% P treatments.

Means for the ash and P contents of dry bone were higher (48.6 and 7.5%, respectively) than those of tail bones reported by Brodison et al. (1989), who showed no differences in bone mineral measurements in lactating cows fed 0.36 or 0.44% P for 1 to 2 yr. The ash percentages on a dry basis were smaller (~59%) than those of right and left ribs (ribs 9, 10, 11, and 12) of younger cattle (6 to 36 mo of age) reported by Beighle et al. (1993). The percentages of P in ash, dry bone, or wet

bone (Table 9) were also lower than those (18.3, 10.8, and 10.3%, respectively) reported in Beighle et al. (1993). Their study also showed no differences in ash or P measurements among these rib bones. The ash percentages in our study were lower (~67%) than those of ether-extracted metacarpal bone of beef heifers reported by Williams et al. (1991), but the P percentages of bone ash were higher in our study (~16.3%) than those reported in that study.

The phosphorus status of cattle has been evaluated frequently with blood serum inorganic P concentration, which is, however, subject to the influence of recent P intake. Serum P is also influenced by P mobilization from bone. If serum P concentration begins to decline due to insufficient dietary P, P is mobilized from bone in an effort to maintain a normal serum P level. Animals deplete their bone P reserves to support normal function and production. Thus, bone characteristics should be the ultimate measure of P status.

Bone contains 80 to 85% of the total P in the body of mammals. Most of the P in bone exists in the form of calcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$], which is amorphous, and hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$], which has a crystalline form and is spatially anchored in collagen, a fibrous protein matrix. Cortical bone is composed of densely packed layers of mineralized collagen, which provides rigidity and is the major component of tubular bone. Trabecular bone is spongy, provides strength and elasticity, and constitutes the major portion of the axial skeleton.

Hydroxyapatite is the source of mobile P in bone for regulating blood P. Phosphorus is mobilized as hydroxyapatite crystals and these crystals are degraded by osteoclasts to release Ca and P (osteolysis). Bone also restores Ca and P (ossification) when surplus minerals are presented. This is fulfilled by osteoblasts, which form new bone in newly synthesized bone matrix on the surface of previously resorbed bone. If Ca and P are not present in adequate amounts, bone matrix will not be fully mineralized and osteoid tissue will form. By weight, the organic proportion of bone will increase (Shupe et al., 1988). This is the reason for measuring specific gravity of bone and expressing bone composition on a volume basis. Little and Ratcliff (1979) suggested that bone specific gravity is highly related to bone mineral storage and is a sensitive measure of P depletion.

Resorption and formation of bone can occur simultaneously, but net change in bone P storage in dairy cows may vary with stage of lactation. Cows undergo a net loss of both Ca and P from bone to help supply these elements during early lactation. This is reversed in later lactation. Ternouth (1990) suggested that up to 30% of bone P can be removed during early lactation.

Based on this estimate for beef cows, a dairy cow weighing 600 kg could mobilize 600 to 1000 g of P, which also suggests that the amount of P that needs to be restored during later lactation could be large and supports the notion that the concentration of dietary P need not be reduced as the animal moves from early to late lactation (Braithwaite, 1986; Wu and Satter, 2000b). Judkins et al. (1985) and Shupe et al. (1988) reported on recovery of bone P when lactation stress was removed in beef cows, and concluded that bone P levels can be replenished following lactation without P supplementation. However, if P intake is too low over a prolonged period of time, resulting in severe loss of P from bone, the bone will eventually become weak and porous, and could be deformed or broken by stress placed on the bone. These changes are better demonstrated in vertebrae and ribs because trabecular bone is more readily resorbed than tubular bone (Simesen, 1980; Ternouth, 1990).

Shupe et al. (1988) described various signs of osteoporosis and other related changes in the bone of beef cows fed extremely low P (6 to 12 g/d) for 2 yr. The vertebrae and rib bones were demineralized, and spontaneous fractures occurred in some animals. Osteoid tissue increased and trabeculae were thin and sparse. The specific gravity of the bones was low, ranging from 1.06 to 1.34, and the bones had low P content (<0.16 g/cc). On the other hand, bones from cows fed more P had specific gravity of >1.55 and P content of 0.2 g/cc and showed no osteoporotic characteristics. Williams et al. (1991) reported that feeding 0.12 compared with 0.20% P to beef heifers 7 to 8 mo of age for 16 mo decreased the third metacarpal breaking load, breaking stress, and ash and P contents. Little (1972) showed that the ash content of fresh rib bone decreased from 50 to 41% in yearling cattle after 6 wk of P depletion. Little and Ratcliff (1979) reported that the critical bone P concentration ranged from 0.12 to 0.15 g/cc. Little (1984) further suggested that P content of less than 5% in fresh rib is indicative of a low P reserve. Ternouth (1990) suggested the following minimum bone ash and P contents for cattle, below which bone demineralization should be considered significant: 40 and 7.5% on a wet weight basis or 0.6 and 0.12 g/cc on a volume basis, respectively. In our experiment, the lowest means for ash and P contents were 46.0 and 8.1% on a wet weight basis or 0.69 and 0.122 g/cc on a volume basis. Also, the lowest mean for specific gravity was 1.50, similar to that reported by Shupe et al. (1988) for beef cows with normal bones. No bone-related abnormalities were observed in our experiment.

Considering that bone by nature is difficult to measure and measurements can vary, multiple measurements of bone strength and mineralization were made

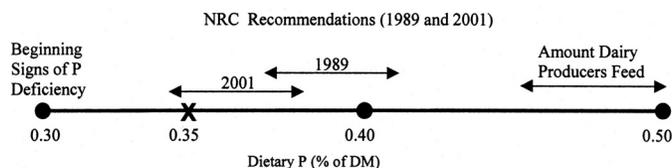


Figure 7. Current status of P nutrition of lactating dairy cows milking > 9000 kg/305 d of lactation.

in this experiment. Several of the measurements suggest some loss of P from bone for the lowest P treatment (-6.0%), although the other measurements, particularly P content of ash, would suggest that no P loss occurred with the low P treatment. Because the contents of Ca and P in bone ash should be relatively constant (36% for Ca and 17% for P), expressing P content per unit of ash content is not as descriptive as expressing it on a total weight basis. On the other hand, as mentioned earlier, specific gravity and composition measures on a volume basis are good indicators of bone changes. All things considered, we concluded that with the lowest P concentration (0.31%) fed for 2 yr that bone P content might have been slightly decreased but not to the extent of affecting bone strength. Feeding 0.38 to 0.40% for 3 yr did not affect bone P content or bone strength. We should note that, in our experiment, rib bone was removed just before cows were dried off. Had the rib been removed later in the dry period, P content of ribs from the low P treatment might have been more fully restored.

Figure 7 is a summary of what we consider the status of P nutrition of lactating dairy cows producing >9000 kg/305 d lactation. The minimum of dietary P consistent with normal or near normal animal performance is 0.30%. At this amount, deficiency may appear. At the other extreme of the continuum in Figure 7 is what most dairy producers in the United States are actually feeding. Several surveys show that dairy producers are feeding 0.46 to 0.50% dietary P. We believe the NRC (1989) recommendation are more than adequate. The most recent NRC publication (2001) has lowered slightly the suggested level of P feeding, a change we feel is fully justified. Feeding $\sim 0.35\%$ P will provide a margin of safety above what might be considered a borderline deficient diet containing $\sim 0.30\%$. If dairy producers reduce dietary P from current amounts to NRC (2001) recommended amounts, P excretion in manure will be reduced 25 to 30%, and P supplementation costs reduced by \$10 to 15 per cow per lactation.

CONCLUSIONS

This report presents data on lactation, P excretion, and bone measurements from 37 cows fed three

amounts of P (0.31, 0.39, and 0.47%) in the third year of a 3-yr P study. The 37 cows included 19 and 14 that had been fed similar amounts of P for 2 or 3 yr, respectively, upon completion of the lactation in the third and final year; the remaining four cows (three in the 0.31% and one in the 0.39% P groups) completed just one lactation for this trial. During the third year, blood serum P concentrations were slightly lower with 0.31% dietary P, but within normal ranges. Feeding 0.31 or 0.39% P for 2 or 3 yr did not reduce milk production, the cow's milking capacity, or bone strength, but P content of the bone was slightly lower with the lowest dietary P treatment.

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