

Long-Term Calcium-Phosphorus Studies in Confined Dairy Cows

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Modern dairy cows are under considerable stress consuming and metabolizing large quantities of feed and synthesizing large quantities of milk. The nutrient requirement for peak production in a cow's lactation usually exceeds her capacity for feed. The combination of deficit consumption and obligatory nutrient balance required for milk synthesis compounds that stress. Attempts to satisfy energy requirements result in inordinate skewing of the ration toward a large proportion of concentrate.

Combination of extreme variation among rations and the vast requirement for nutrients to support heavy lactation emphasizes the need for adequate information concerning nutrient requirements, availabilities, and interactions. Calcium and phosphorus together represent about 1.5% of milk dry matter, yet they are vital to the life of the cow and to the composition of milk. National Research Council (NRC) requirements (11) for a 600 kg cow producing 40 kg milk are 125 g calcium and 87 g phosphorus daily. We have been taught that the phosphorus requirement must be satisfied daily while the calcium can be balanced once each lactation. Neglecting calcium in the ration of high-producing cows at any time seems inefficient.

Our work reported earlier (13) compared narrow and wide calcium-to-phosphorus rations each with and without supplemental vitamin D₃, 300,000 IU/wk. Forty-six Holstein cows were assigned during three years to four treatment groups. The original 24 cows were assigned 45 days before parturition for preconditioning. Replacement animals were preconditioned on their respective rations five months before parturition. The cows were group-fed 6.8 kg alfalfa hay and concentrate ration (Table 1) to satiety, averaging 15 kg per cow daily. The groups were kept in open lots with free-stall shelter.

Calcium and phosphorus balances were determined on the cows during second and fifth months of lactation and during their dry periods. The balances are summarized in Table 2. Note the average negative balance for narrow-ratio second-month observations even with average intakes in excess of requirements. Intakes 150% of NRC requirement during fifth month did not preclude some negative balances. Negative calcium balances are accepted in early lactation but cows should be in equilibrium or better by mid-lactation. That was not the case in our

study (Figures 1 and 2). Comparison of Figures 3 and 4 shows the influence of supplemental vitamin D on calcium utilization. Cows producing more than 15 kg milk without vitamin D supplement had negative calcium balances with intakes to 250 g/day. We estimated the calcium requirement from the balances with cows supplemented with vitamin D₃ and producing in excess of 15 kg milk in Figure 4. We drew the requirement line where it included negative balances above the line equal in number to positive balances below the line (10). This estimate was nearly twice the NRC requirement (11). Some of our contemporaries ascribe the deviation to short-time balances but few longer ones are being reported.

Graphic treatment of data from Forbes, et al. (3), resulted in a like estimate of 5 g calcium required per kilogram milk produced when the roughage was alfalfa and corn silage (Figure 5). Figure 6 shows similar treatment of those workers' data from timothy hay-corn silage rations supplemented with inorganic calcium. Our estimate was 3 g calcium per kg milk with the timothy ration. This difference indicated interaction of ration with requirement. The current NRC requirement (11) is 2.6 g calcium per kg milk with 3.5% fat.

The dependence of phosphorus balance on the associated calcium balance is obvious on examination of Figures 7 and 8. Few positive phosphorus balances were associated with negative calcium balances and some would be expected near calcium equilibrium. Predominance of positive phosphorus balances concurrent with positive calcium balances is expected since there is no phosphorus-sensitive mechanism for active bone resorption. Bone accretion depends on adequate amounts of calcium and phosphorus but bone resorption, in excess of that available from passive physical-chemical equilibrium, depends on

Table 1
Ration Composition (from 13)

	Ca/P Ratio	
	Narrow	Wide
Sorghum grain - %	78.7	76.6
Soybean meal - %	19.4	18.9
Monosodium phosphate - %	1.4	-
Limestone - %	-	3.1
Dicalcium phosphate - %	-	.9
Trace mineral salt - %	.5	.5
Vitamin A - IU/kg	4400	4400

Table 2. Mean Calcium and Phosphorus Intakes, Balance, and Requirements (from 13)

Treatment ¹	Observations	Milk ±SD	Calcium			Phosphorus		
			Req. ²	Intake ±SD	Balance ±SD	Req.	Intake ±SD	Balance ±SD
(Ca/P)	(no.)	(kg/day)						
					– (g/day) –			
Second month								
1.2	22	24±6	83	95±37	-7±13(20) ³	59	80±16	-3±7(13)
1.3 + D	15	24±6	83	116±59	-4±14(9)	59	89±16	0±7(5)
2.5	21	23±7	81	176±72	10±33(9)	57	69±16	2±7(10)
2.6 + D	15	28±6	94	200±75	24±45(6)	66	77±22	2±17(9)
Fifth month								
1.3	22	17±7	65	99±39	1±20(12)	47	76±18	2±8(9)
1.4 + D	14	15±4	60	98±42	4±9(4)	44	71±14	3±8(5)
2.7	19	18±6	68	176±54	15±31(10)	49	65±13	4±8(10)
2.9 + D	15	20±4	73	212±64	29±29(3)	52	76±17	4±6(2)
Dry period								
1.6	13		37	50±17	8±5(0)	26	32±8	2±6(5)
1.9 + D	7		37	56±21	11±10(0)	26	30±8	6±7(2)
3.0	6		37	84±38	11±12(1)	26	29±12	4±4(1)
2.6 + D	12		37	88±42	14±18(3)	26	32±10	5±5(1)

¹Average Ca/P ratio in feed during balance period with vitamin D supplement (+D).

²Requirements were calculated from National Research Council (9) recommendations for 600 kg cow, producing 3.5% milk.

³Values in parentheses indicate numbers of negative balances.

calcium-sensitive physiological mechanisms.

Calcium and phosphorus balances measured on dry cows averaging 250 days pregnant are shown in Figure 9. The NRC requirements, 30 g calcium and 28 g phosphorus daily for 650 kg cows in late gestation, appear adequate from those data.

Concern with the disparity between our estimate and the NRC calcium requirement for lactation led to examination of the site and chemical form of that element in alfalfa. Leaves, petioles, and stems of alfalfa were examined by energy-dispersive x-ray. Calcium is most prevalent in leaves with potassium less concentrated in leaves and more prevalent in stems.

Residue from leaves suspended in a nylon bag in the rumen for 24 hours was examined by scanning electron microscope. The vascular structure survived nearly intact (Figure 10). The vascular bundles are surrounded by a one-cell-thick parenchymatous layer, each cell containing a distinctive crystal. Examination of digesta from the lower, more fluid portion of the rumen of an alfalfa-fed fistulated cow revealed that the leaf vascular bundles survive ruminal digestion with most of the crystals intact in the bundles.

Examination of feces revealed the extent of digestive destruction of the vascular bundles. Some bundles were broken up and some remained nearly intact but with most of the crystals "shelled out" (Figure 11). The calcium map produced from the same field by x-ray analysis is shown in Figure 12.

Free crystals were isolated from feces of alfalfa-fed cows by sieving, repeated washing, and sedimentation and, finally, selective sedimentation from bromoform-ethanol. Figure 13 shows crystals isolated in that manner. The calcium map from the same field is shown in Figure 14. The element scan from that

field (Figure 15) indicates that calcium is the principal cation with some silicon present. Carbon and oxygen are not recorded in the scan.

The principal anion in the crystals was identified as oxalate (14) on the Raman microprobe (12). The insolubility of calcium oxalate is well known. Some potassium oxalate was also present both in separate crystals and in crystals with calcium oxalate. Some of the isolated particles were partially eroded, indicating partial solution of the calcium-containing particles in the digestive tract. Analysis of samples of alfalfa hay, leaves, and stems for both calcium and oxalate indicated oxalate equivalence ranging from 19 to 33 percent of the calcium (Table 3).

Hansard, et al. (6), reported true digestibilities of calcium from 15 selected sources. The digestibility of calcium from three roughages including alfalfa averaged lower than that from any of the mineral sources tested. Those authors played down the importance of that finding in their report because of variability of their results. Our experiences with alfalfa hay lend credence to their results. Some calcium is available from alfalfa as indicated by survival and normal lactation by cows kept four years on alfalfa hay and sorghum grain in the experiment reported here.

We chose to include only 60% of alfalfa calcium in calculating its contribution to the ration. That figure is a compromise based on the oxalate content of alfalfa and the high requirement for calcium which we found using alfalfa hay as the primary calcium source. The net result of that discount is some change in qualitative and quantitative calcium-phosphorus supplements required for specific dairy cow rations.

Two groups of 15 two-year-old Holstein cows were group-fed one half alfalfa hay and one half rolled

Table 3
Calcium and Oxalate Contents
of Alfalfa (Dry Basis)

Sample	Calcium	Oxalate	Ox/Ca
	- % -		eq/eq
Alfalfa hay			
A	1.14	.54	.21
B	1.60	.88	.24
C	1.38	1.02	.33
D	1.76	.79	.20
Alfalfa leaves			
A	1.90	1.34	.31
B	2.03	1.00	.22
Partitioned sample			
Whole	1.98	.96	.22
Leaves	2.60	1.09	.19
Stems	1.09	.48	.20

Table 5
Lactation Means by Treatments

	Sodium Phosphate		Calcium Phosphate	
	-D	+D ¹	-D	+D
Cows - No.	10	9	9	11
Lactations - No.	31	20	24	27
ME Milk - kg	7660	6950	7302	7090
±SD	±1250	±1150	±990	±920
ME fat - kg	250	232	228	246
±SD	±43	±34	±37	±35
Fat - %	3.26	3.33	3.13	3.47

¹300,000 IU vitamin D₃ weekly supplement.

Table 4. Mean Analyses of Feeds and Composite Rations (Dry Basis)

	EE	CF	Prot.	Ash	NFE	Ca	P	Mg	Cl	Fe	Mn	Zn
	%											
	ppm											
Alfalfa	2.1	30.8	18.9	10.0	38.2	1.38	.27	.22	.24	330	34	27
Grain A	3.2	2.2	10.8	3.6	80.2	.18	.70	.12	.10	140	14	24
Grain B	3.3	2.5	10.9	3.5	79.8	.35	.74	.13	.07	200	19	26
Ration A ¹	2.6	16.5	14.8	6.8	59.2	.78	.48	.17	.17	240	24	26
Ration B ¹	2.7	16.6	14.9	6.8	59.0	.86	.50	.18	.15	260	26	26

¹Half alfalfa hay + half grain.

Table 6. Milk Test-Day Data: Least Square Means and Probabilities of Differences

Treatment	Mineral			Vitamin		
	A	B	p=	-D	+D	p=
Milk - kg/day	21.3	21.2	.93	21.8	20.6	.31
Fat - %	3.40	3.35	.52	3.30	3.45	.043
Protein - %	3.11	3.10	.85	3.02	3.18	.016
Ash - %	.74	.74	.75	.74	.73	.31
Lactose - %	4.67	4.70	.64	4.61	4.76	.068
Total solids - %	11.91	11.89	.93	11.67	12.13	.009
Calcium - %	.109	.110	.43	.109	.111	.04
Phosphorus - %	.086	.085	.69	.084	.087	.18

sorghum grain supplemented with 4400 IU vitamin A per kg and 1.5 percent monosodium phosphate or 1.7% of a mono-di-calcium phosphate mixture. Mean analyses of feeds and composite rations are shown in Table 4. Plain salt was available *ad libitum*. Alternate cows in each treatment group were assigned to vitamin D₃ supplement, 300,000 IU weekly by capsule. The treatments were continuous through lactation and dry periods until individual cows were culled from the herd for one of the usual causes—open with low production, mastitis, or physical ailment. Culled cows were replaced with two-year-old heifers to maintain the integrity of the treatment groups. Milk production was measured by Dairy Herd Improvement records. Group means for 102 305-day mature-equivalent lactations by 39 cows are in Table 5.

Milk was analyzed monthly for fat, ash, protein, total solids, calcium and phosphorus. Lactose was calculated from those results. Analysis of variance among the results from 570 samples indicated only insignificant differences between mineral treatments but significantly greater percentages of fat, protein, total solids and calcium in milk were associated with

vitamin D₃ supplement than with no supplement (Table 6).

We analyzed 428 monthly blood samples for dry matter, red blood cell volume, total phosphorus and cell phosphorus in blood. Plasma was analyzed for dry matter, calcium and total phosphorus. Cell phosphorus was calculated. Of the listed measures only plasma dry matter varied significantly between mineral treatments (Table 7). Higher calcium in plasma of cows not supplemented with vitamin D₃ (p=0.07) followed the results of our previous study (2) in which cows having less calcium in their ration had greater concentration of plasma calcium. In that experiment vitamin D₃ supplementation elicited more efficient use of feed calcium. Apparently, increased mobilization of bone calcium results in increased plasma calcium concentration.

Reproductive data for the treatments are summarized in Table 8. No significant difference was noted among treatments for days to conception, services per pregnancy, length of gestation, or calving interval due to the variations among cows. Some individual cows were consistently good breeders and

Table 7. Blood Data: Least Square Means and Probabilities of Differences

Treatment	Mineral			Vitamin		
	A	B	p=	-D	+D	p=
Blood						
DM - %	17.7	17.7	.71	17.7	17.7	.96
Cell vol. - %	31.1	31.7	.38	31.5	31.3	.83
P - mg/100ml	17.4	17.5	.87	17.1	17.8	.32
Cell P - mg/100ml	8.53	8.75	.56	8.50	8.79	.46
Plasma						
DM - %	9.76	9.38	.007	9.61	9.53	.64
Ca - mg/100ml	10.5	10.6	.38	10.8	10.4	.07
P - mg/100ml	13.0	13.0	.93	13.0	13.0	.97
Cells						
P - mg/100ml	27.3	27.6	.78	27.0	27.9	.38

Table 8. Summary of Reproduction: Means and Standard Deviations

Treatment	A ¹ -D	A+D ²	B ¹ -D	B+D	A	B	-D	+D
Cows - no.	7	7	8	9	14	17	15	16
Pregnancies - no.	20	12	17	19	32	36	37	31
Conception - days postpartum	103	134	114	116	115	115	108	123
±SD	±45	±79	±51	±56	±65	±52	±47	±65
Services/pregnancy	2.3	2.9	2.6	2.3	2.5	2.4	2.4	2.5
±SD	±1.6	±2.0	±1.6	±1.3	±1.8	±1.4	±1.6	±1.6
Gestation - days	276	279	280	280	277	280	278	280
±SD	±4	±6	±5	±3	±5	±4	±5	±4
Calving interval - days	379	413	394	396	392	395	386	402

¹A - monosodium phosphate supplement. B - calcium phosphate supplement.

²+D - 300,000 IU vitamin D₃ supplement weekly.

Table 9. Summary of Reproduction and Production (from 15)

Treatment	A-D	A+D	B-D	B+D	-D	+D	A	B
Observations	17	10	14	17	31	27	27	31
Uterine invol. (adj.) (days postpartum)	43	47 ²	43	39 ³	43	42	45	41
							P<0.05	
First ovulation (days postpartum)	18	22 ²	14 ³	14 ³	16	17	20	14
							P<0.005	
First estrus (days postpartum)	46	34	48	29	47	31	41	37
					P<0.06			
Conception (days postpartum)	133	98	136	97	134	97	120	115
					P<0.025			
Services per pregnancy	2.5	2.3	2.4	2.3	2.5	2.3	2.4	2.3
120-day FCM (kg/day)	22.6	21.8	22.3	22.6	22.5	22.3	22.3	22.5
305-ME								
Milk (kg)	6695	6368	7208	6595	6926	6511	6574	6872
Fat (kg)	235	211	244	215	239	214	226	227

¹Treatments: A, low calcium; B, high calcium; -D, no vitamin supplement; +D, 300,000 IU vitamin D supplement per week.

² and ³Values on the same line with different references are significantly different (P<0.05).

others were consistently hard breeders. Results of our earlier study (15) with much greater difference in calcium-phosphorus ratios (Table 9) indicate advantage of the wider ratio for uterine involution and early ovulation. Estrus and conception occurred earlier with vitamin D₃ supplementation. Fewer heats were missed and/or were "silent" in the cows supplemented with vitamin D₃.

Summary of 43 calcium and phosphorus balances is in Table 10. Those results corroborate our earlier findings (13) that an alfalfa hay-sorghum grain ration

does not satisfy the calcium requirement for lactation as expected from NRC (11) estimates. That most of the cows were in positive balance in the sixth month was not so much a matter of stage of lactation as that milk production was low enough to be satisfied by the calcium intake. Calcium consumption was relatively stable from the second to the sixth month of lactation and milk yield decreased, thus allowing for calcium adequacy at the latter stage.

The cows whose ration was supplemented with monosodium phosphate became elevated in the

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Table 10. Calcium and Phosphorus Balances: Means and Standard Deviations

Treatment (Ca/P)	Observations (no.)	In lact. (days)	Milk ±SD (kg/day)	Calcium			Phosphorus		
				Req. ¹	Intake ±SD	Balance ±SD - (g/day) -	Req.	Intake ±SD	Balance ±SD
Second month									
1.32 ²	7	58	30±3	99	127±20	-8±7(6) ³	70	96±17	0±10(3)
1.38 + D ⁴	5	46	29±4	96	130±17	-6±9(4)	68	94±12	3±4(2)
1.54	4	50	29±3	96	142±19	-8±21(2)	68	92±13	1±8(2)
1.46 + D	6	54	26±7	89	154±24	11±12(2)	63	105±20	14±6(0)
Sixth month									
1.36	6	170	23±1	81	141±13	12±6(0)	57	104±6	10±5(0)
1.39 + D	4	172	17±4	65	121±17	3±8(1)	47	87±19	-3±10(2)
1.60	4	170	20±6	73	170±17	22±5(0)	52	106±6	11±4(0)
1.53 + D	7	180	18±4	68	159±32	10±15(2)	48	104±21	7±11(2)

¹NRC requirement for 600 kg. cow.²Mean Ca/P ratio in consumed feed.³() number of negative balances.⁴300,000 IU vitamin D₃ weekly supplement.

chine, tucked up in the flank, and slab-sided with some crampiness in the rear legs and some abnormal rear hoof growth (Figures 16 and 17). The cows supplemented with calcium phosphate were not affected (Figures 18 and 19). The little difference in ration composition between treatments appears to eliminate calcium as the causal agent. The monosodium supplemented group consumed very little salt even though it was available continuously. The sodium in their supplement apparently satisfied their appetite for salt and indicated the sodium dependence of salt appetite.

Sodium requirements are established for lactating and nonlactating cows (reviewed by NRC) (11). However, those authors indicated the chlorine requirement is "less than 0.28% (equivalent to 0.46% salt) in the dry matter." Our cows consumed about 60% of that amount in their ration. Chlorine deficiency in cattle has not been described. However, Coppock and Fettman (1) discussed that possibility in a recent review of chlorine as a required nutrient for lactating cows. Several reports have delineated the chlorine requirement for broiler chicks (5,7,8) and turkey poults (4,9), varying between 0.07 and 0.145% of the ration. Our rations averaged about 0.16% chlorine and the drinking water at 60 ppm increased the effective chlorine content to about 0.18% of the dry matter consumed. Even though salt was available *ad libitum*, little was consumed by the monosodium phosphate-supplemented cows. Lactation appeared to aggravate the condition. Lactation is a significant drain on body chlorine since milk contains about 0.1% of that element.

Complete occlusion of the pancreatic duct with calculi was found at necropsy of three cows supplemented for three to four years with vitamin D₃. Analysis of air-dry calculi from one cow revealed 30% calcium, 0.09% magnesium, and 0.21% phosphorus. Treatment of the calculi with 1 N sulfuric acid produced effervescence indicating the presence of carbonate. Calcium carbonate appeared to be the principal constituent of the calculi.

No reports of vitamin D toxicity at the moderate level in this experiment have been published. Many of the studies were conducted with irradiated yeast as the source of vitamin D₂, ergocalciferol. We used vitamin D₃, cholecalciferol. Hint of possible toxicity in cattle with vitamin D₃ was found in one editorial but not in a research report.

Summary

Calcium balance can be kept near even or positive in early lactation if calcium and phosphorus intakes and vitamin D consumption are adequate. Adequate calcium consumption promotes more rapid uterine involution and earlier ovulation.

Phosphorus consumption must be adequate from day to day. There is no phosphorus-sensitive mechanism to elicit phosphorus resorption from bone. That mechanism is only calcium-sensitive. Inadequate phosphorus intake depresses appetite as well as preventing bone accretion. Phosphorus balance, with adequate phosphorus intake, largely depends on calcium adequacy since the calcium-to-phosphorus ratio in bone is constant.

Vitamin D₃ supplementation increased the frequency of positive calcium balance. The frequency of silent heats was less in the vitamin D-supplemented cows. Calculi in the pancreatic ducts of three cows were associated with 300,000 IU vitamin D weekly supplement for three years or more.

Alfalfa calcium is less available than calcium from inorganic sources for lactating cows. The calcium is in crystals in tissue surrounding the alfalfa vascular bundles and those crystals tend to stay intact during ruminal digestion. The crystals mostly "shell out" during post-ruminal digestion, but many crystals remain intact in the feces. Crystals isolated from feces were predominantly calcium oxalate with some potassium oxalate. Some crystals are partially eroded, thus providing calcium to the animal. We estimate that 60% of the alfalfa calcium is available for utilization.

Our study comparing monosodium phosphate with

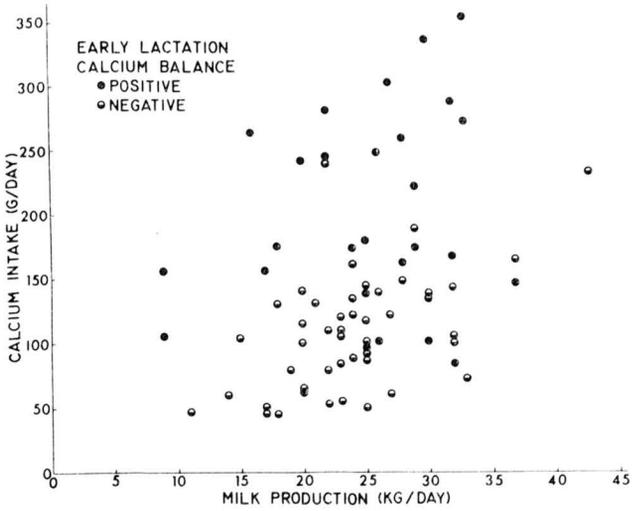


Figure 1. Incidence of negative calcium balance in early lactation at various calcium intakes and milk production.

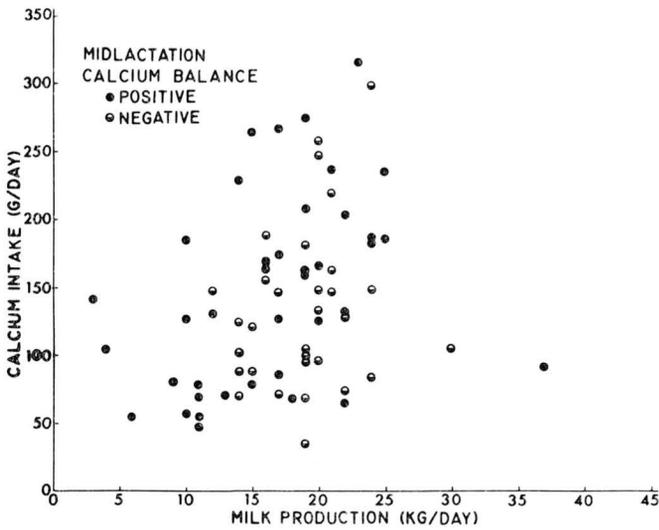


Figure 2. Incidence of negative calcium balance in midlactation at various calcium intakes and milk production.

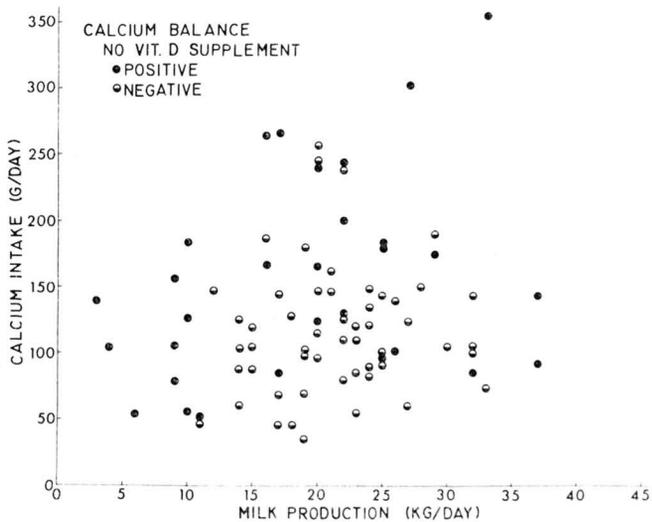


Figure 3. Incidence of negative calcium balance associated with no vitamin D supplement. From (13).

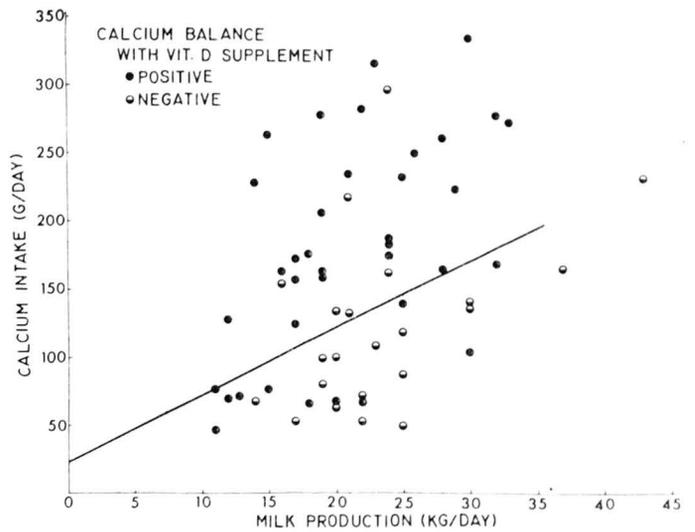


Figure 4. Relationship among calcium balance, milk production, and calcium intake with suggested requirement based on an alfalfa hay-concentrate ration. From (13).

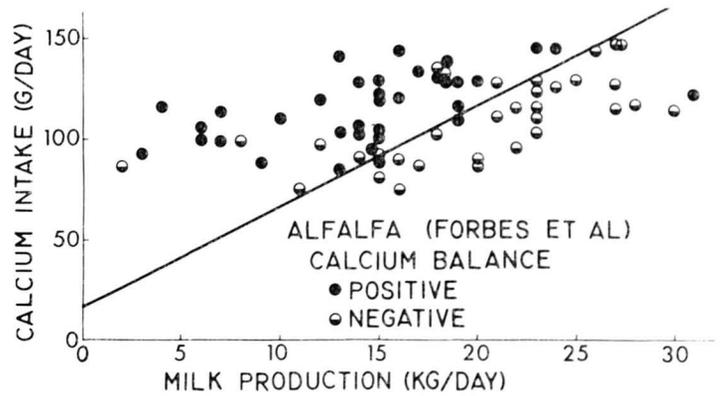


Figure 5. Relationship among calcium balance, milk production, and calcium intake from an alfalfa-corn silage concentrate ration. Data from (3).

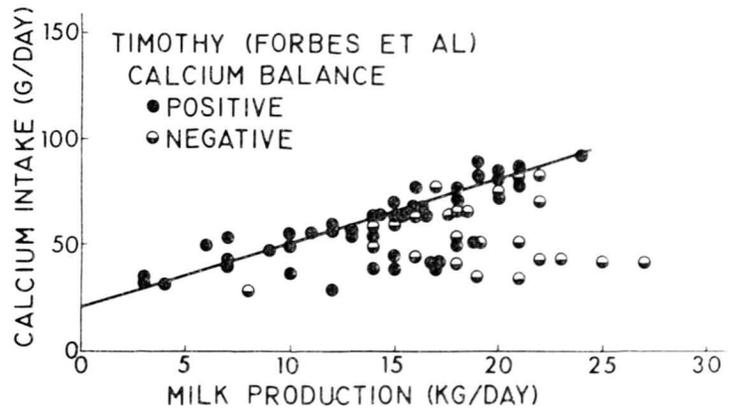


Figure 6. Relationship among calcium balance, milk production, and calcium intake from a timothy hay-corn silage-concentrate ration. Data from (3).

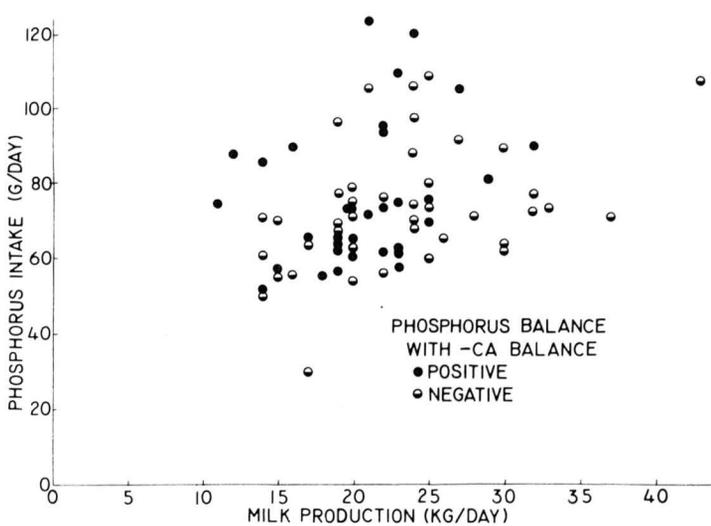


Figure 7. Incidence of negative phosphorus balance associated with negative calcium balance at various phosphorus intakes and milk production. From (13).

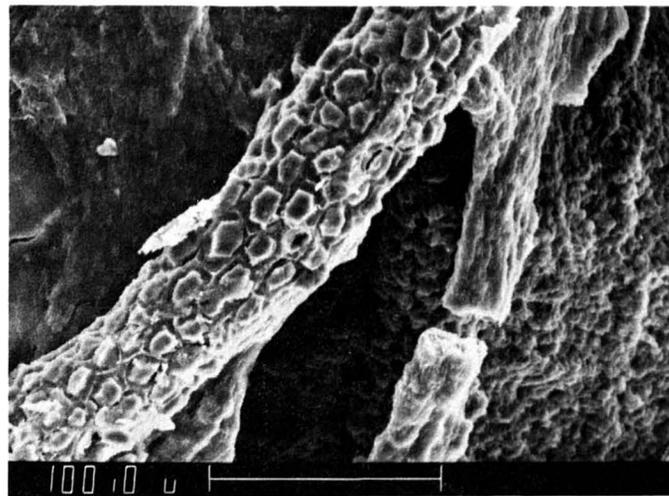


Figure 10. Scanning electron micrograph of a vascular bundle from an alfalfa leaflet after 24-hour ruminal digestion.

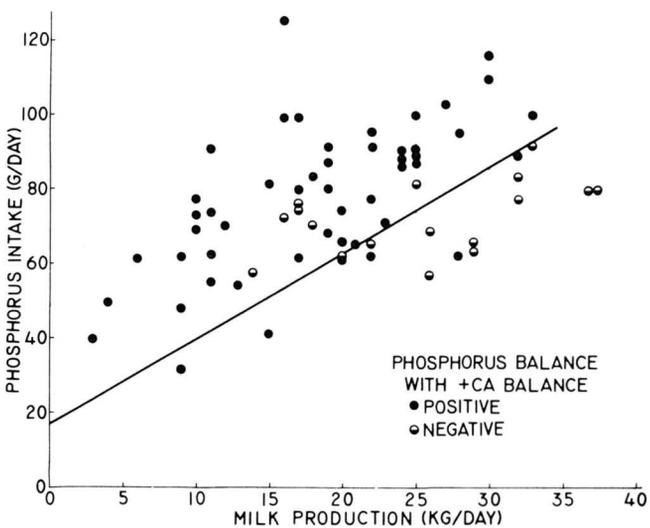


Figure 8. Relationship among milk production, phosphorus intake and phosphorus balance associated with positive calcium balance with suggested phosphorus requirement. From (13).



Figure 11. Scanning electron micrograph of a vascular bundle separated from bovine feces.

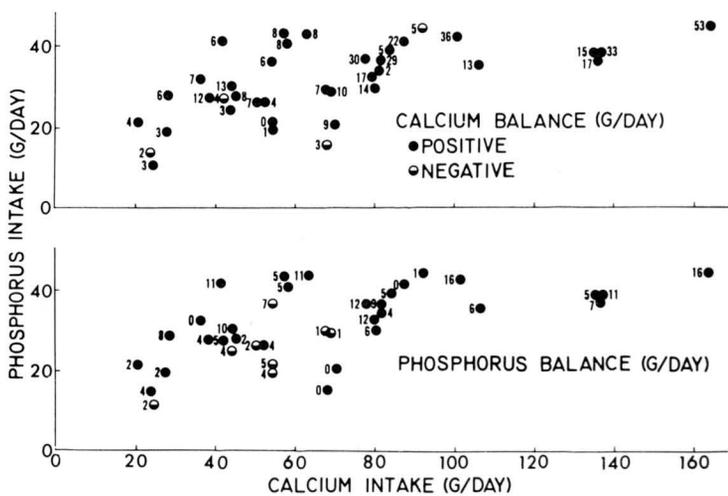


Figure 9. Dry-cow calcium and phosphorus balances arranged by calcium and phosphorus intakes. Numbers represent balances (g/day). From (13).



Figure 12. Calcium map from energy-dispersive x-ray analysis of the field in Figure 10.



Figure 13. Scanning electron micrograph of crystals isolated from bovine feces.

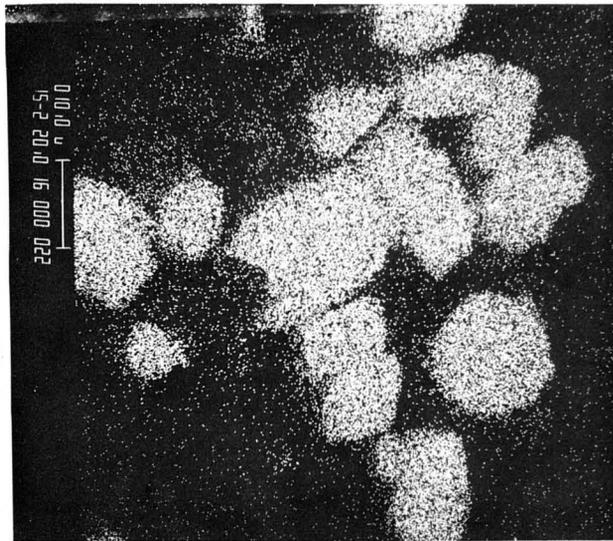


Figure 14. Calcium map from energy-dispersive x-ray analysis of the field in Figure 13.

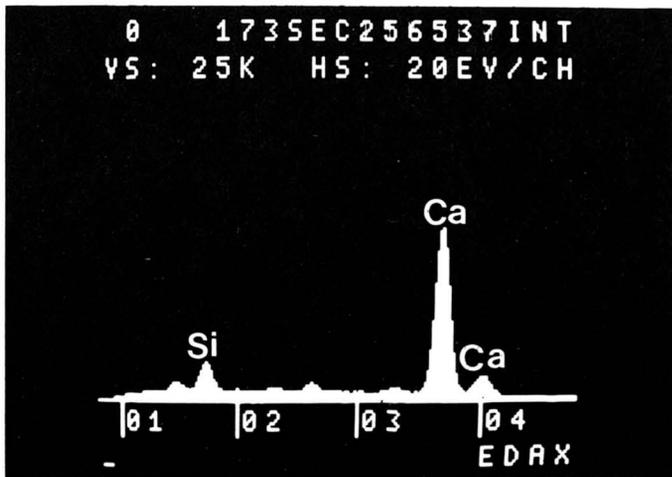


Figure 15. Energy-dispersive x-ray elemental composition scan of the field in Figure 13. Calcium is the primary element identified.

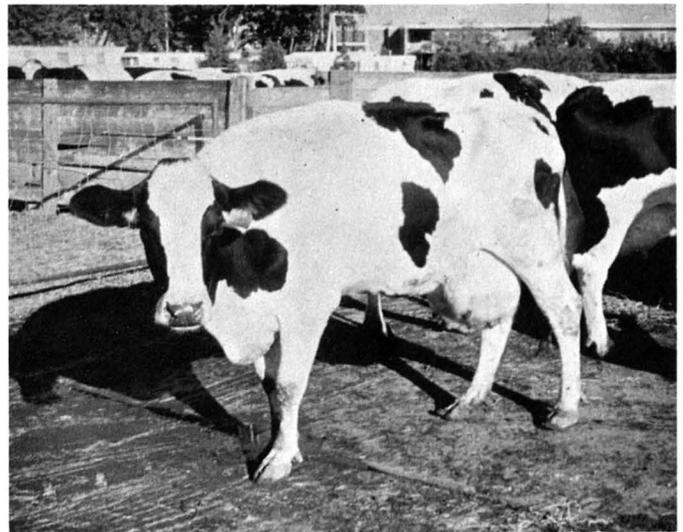


Figure 16. A cow fed an alfalfa hay-sorghum grain ration supplemented with monosodium phosphate for three years. Chlorine deficiency?

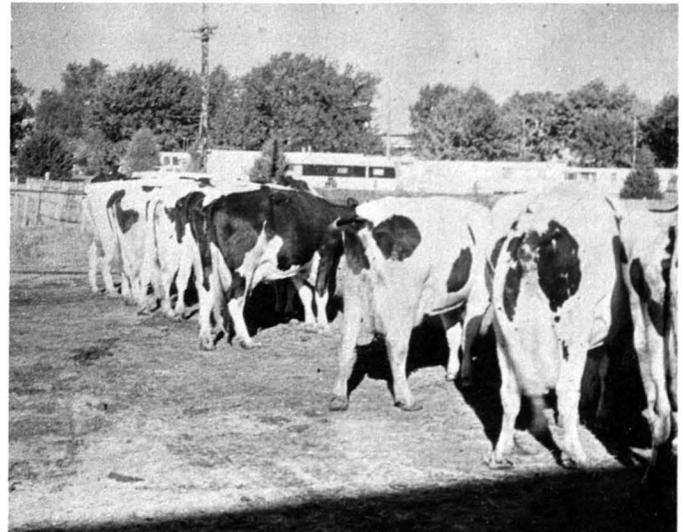


Figure 17. Group of cows including the cow in Figure 16.

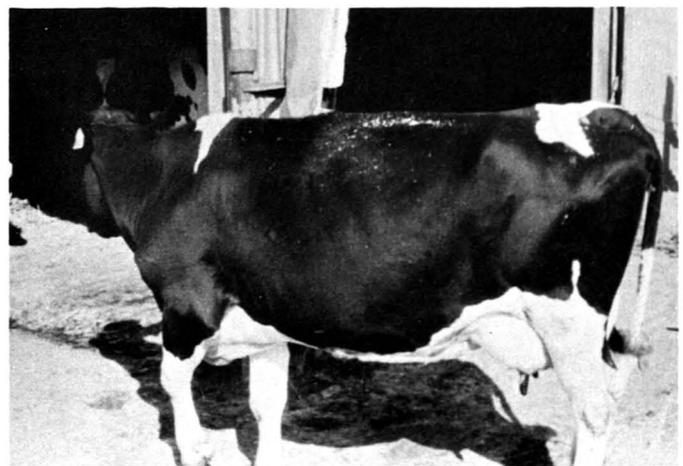


Figure 18. A cow fed an alfalfa hay-sorghum grain ration supplemented with calcium phosphate for three years.

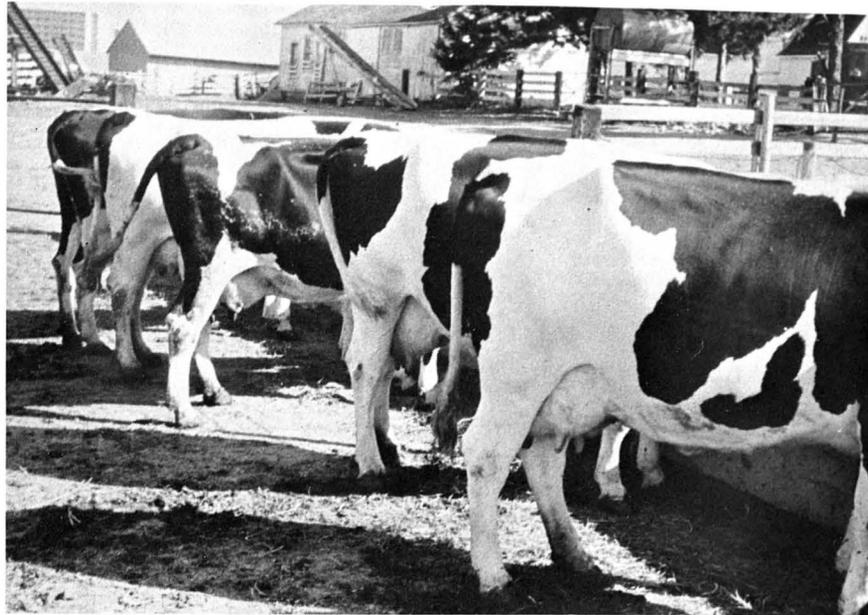


Figure 19. Group of cows including the cow in Figure 18.

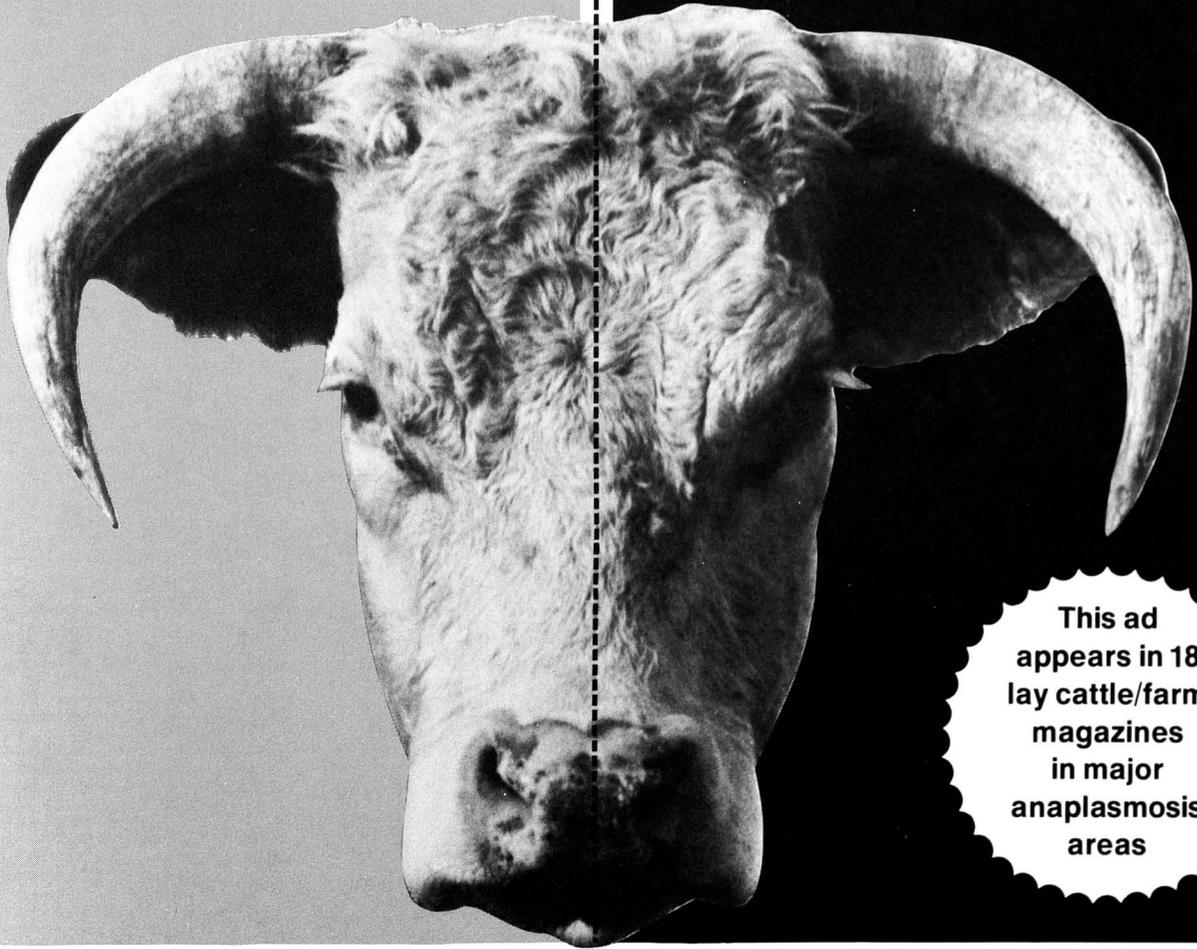
calcium phosphate as supplements for an alfalfa hay and sorghum grain ration may have shown a chlorine deficiency. Salt was omitted from the grain but was available *ad libitum*. Very little salt consumption was the unique characteristic associated with the sodium phosphate treatment. Elevated chine, slab-sidedness, and tucking up in the flank were noted in the cows which ate little salt. Salt appetite appears to be sodium-sensitive.

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