Influences of Calcium and Phosphorus Intakes, Vitamin D Supplement, and Lactation on Calcium and Phosphorus Balances

GEORGE WARD, R. C. DOBSON, and J. R. DUNHAM
Department of Dairy and Poultry Science
Kansas State University, Manhattan 66502

Abstract
Calcium and phosphorus balances were measured on 45 Holstein cows during the second and fifth months of lactation and during the dry period. Cows were conditioned with and continuously fed alfalfa hay-sorghum grain-soybean oil meal rations with mineral supplement to provide Ca:P ratios near 1.1:1 or 2.3:1, either with or without 300,000 IU vitamin D₃ weekly by capsule. Supplemental vitamin D improved predictability of Ca balance from Ca and P intakes and milk Ca. Calcium requirement for lactation, estimated from balances with vitamin D supplemented cows, was 5 g Ca per kilogram milk. Phosphorus requirement, estimated from P balances associated with positive Ca balances, was 2.3 g per kilogram milk. These requirements were estimated from the intakes at which equilibrium was probable for the respective element.

Introduction
The need for re-evaluation of calcium, phosphorus, and vitamin D requirements for high-producing cows has been shown (9). Greater energy requirements and changing economic conditions have forced basic changes in dairy cow rations. Adequacy of natural vitamin D in high-energy, low-roughage rations has been questioned.

Stott (22) implicated inadequate P as a cause of parturient paresis and demonstrated effective corrective effect of rations having 1:1 Ca-to-P ratios. Recently 2 to 2.5:1 Ca:P ratios have been suggested for continuous feeding (6, 12).

The vitamin D requirement of lactating cows generally has been satisfied adequately by feeding sun-cured forages or exposure of the animals to sunlight or both (18). Supplemental vitamin D improved Ca absorption (9), interacted with Ca:P ratio in its influence on packed cell volume and P in blood cells (3), and improved reproductive efficiency through more recognizable expression of estrus (2, 23). Those recent findings justify further investigation of the influence of vitamin D in lactating-cow rations, especially in the light of increasing milk production and changing management methods.

This study was to evaluate Ca and P requirements for lactation and to determine effects of supplemental vitamin D on Ca utilization. Calcium and P status of pregnant, dry cows also was investigated.

Experimental Procedure
Calcium and P balances were determined during the second and fifth months of lactation and intervening dry periods for 45 2- to 6-year-old Holstein cows, each during one to three lactations. Twenty-four cows were subjected to their respective treatments 45 days prior to anticipated parturition, and those removed through normal herd culling were replaced with cows preconditioned with their respective treatments at least 5 months prior to parturition and data collection. Treatments consisted of continuously group-fed rations with narrow (0.9 to 1.3:1) and wider (2.1 to 2.5:1) Ca:P ratios, each with and without 300,000 IU vitamin D₃ weekly by capsule. Rations included 6.8 kg alfalfa hay plus concentrate mixture to satiety, averaging 15 kg per cow. The narrow Ca:P concentrate mixture consisted of 78.7% sorghum grain, 19.4% soybean oil meal, 1.4% monosodium phosphate, 0.5% trace mineral salt, and 4,000 IU vitamin A per kilogram. The wider Ca:P concentrate mixture consisted of 76.6% sorghum grain, 18.9% soybean oil meal, 3.1% limestone, 9% diethylamino phosphate, 0.5% trace mineral salt, and 4,000 IU vitamin A per kilogram. During the first 10 months of the study the narrow Ca:P mixture included 1.5% diammonium phosphate.

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1 Contribution 824, Department of Dairy and Poultry Science, Kansas Agricultural Experiment Station, Manhattan.
Table 1. Mean calcium and phosphorus intakes, balances, and requirements.

<table>
<thead>
<tr>
<th>Treatmenta (Ca:P)</th>
<th>Observations (no.)</th>
<th>Milk Intake (kg/day)</th>
<th>Calcium Intake ± SD</th>
<th>Calcium Balance ± SD</th>
<th>Phosphorus Intake ± SD</th>
<th>Phosphorus Balance ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second month</td>
<td></td>
<td></td>
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<tr>
<td>1.2:1</td>
<td>22</td>
<td>24 ± 6</td>
<td>96 ± 5 × 37</td>
<td>-7 ± 13 (20)c</td>
<td>72 ± 10 ± 16</td>
<td>-3 ± 7 (13)</td>
</tr>
<tr>
<td>1.3:1 + D</td>
<td>15</td>
<td>24 ± 6</td>
<td>96 ± 5 × 59</td>
<td>-4 ± 14 (9)</td>
<td>72 ± 10 ± 16</td>
<td>0 ± 7 (5)</td>
</tr>
<tr>
<td>2.5:1</td>
<td>21</td>
<td>23 ± 7</td>
<td>93 ± 5 × 72</td>
<td>10 ± 33 (9)</td>
<td>70 ± 10 ± 16</td>
<td>2 ± 7 (10)</td>
</tr>
<tr>
<td>2.6:1 + D</td>
<td>15</td>
<td>28 ± 6</td>
<td>107 ± 20 ± 75</td>
<td>24 ± 45 (6)</td>
<td>79 ± 10 ± 22</td>
<td>2 ± 17 (9)</td>
</tr>
<tr>
<td>Fifth month</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1.3:1</td>
<td>22</td>
<td>17 ± 7</td>
<td>78 ± 5 × 39</td>
<td>1 ± 20 (12)</td>
<td>58 ± 10 ± 18</td>
<td>2 ± 8 (9)</td>
</tr>
<tr>
<td>1.4:1 + D</td>
<td>14</td>
<td>15 ± 4</td>
<td>73 ± 5 × 42</td>
<td>4 ± 9 (4)</td>
<td>54 ± 10 ± 14</td>
<td>3 ± 8 (5)</td>
</tr>
<tr>
<td>2.7:1</td>
<td>19</td>
<td>18 ± 6</td>
<td>81 ± 5 × 54</td>
<td>15 ± 31 (10)</td>
<td>60 ± 10 ± 13</td>
<td>4 ± 8 (10)</td>
</tr>
<tr>
<td>2.9:1 + D</td>
<td>15</td>
<td>20 ± 4</td>
<td>86 ± 212 ± 64</td>
<td>29 ± 29 (3)</td>
<td>64 ± 10 ± 17</td>
<td>4 ± 6 (2)</td>
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<tr>
<td>Dry period</td>
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<tr>
<td>1.6:1</td>
<td>13</td>
<td></td>
<td>34 ± 50 ± 17</td>
<td>8 ± 5 (0)</td>
<td>26 ± 10 ± 8</td>
<td>2 ± 6 (5)</td>
</tr>
<tr>
<td>1.9:1 + D</td>
<td>7</td>
<td></td>
<td>34 ± 56 ± 21</td>
<td>11 ± 10 (0)</td>
<td>26 ± 10 ± 8</td>
<td>6 ± 7 (2)</td>
</tr>
<tr>
<td>3.0:1</td>
<td>6</td>
<td></td>
<td>34 ± 84 ± 38</td>
<td>11 ± 12 (1)</td>
<td>26 ± 10 ± 12</td>
<td>4 ± 4 (1)</td>
</tr>
<tr>
<td>2.6:1 + D</td>
<td>12</td>
<td></td>
<td>34 ± 88 ± 42</td>
<td>14 ± 18 (3)</td>
<td>26 ± 10 ± 10</td>
<td>5 ± 5 (1)</td>
</tr>
</tbody>
</table>

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

b Requirements were calculated from National Research Council (18) recommendations for 600-kg cow producing 3.5% milk.

c Values in parentheses indicate numbers of negative balances.
rather than monosodium phosphate, and the other mixture included .7% urea, each with commensurately less soybean oil meal and more sorghum grain on basis of nitrogen content. Cows were fed in outdoor bunks and had free access to outside lots and shelter.

Cows were removed from their respective groups for balance trials in their second and fifth months of lactation and in their dry periods. Calcium and P balances were determined for 7-day periods following 14-day preliminary periods. Cows were in stanchions, each fitted with a rubber mat extending over the edge of the pan in which urine and feces were collected together. Chopped alfalfa hay was fed once daily, and concentrate for the cow's treatment was fed twice daily. Softened, fluorinated, municipal water was metered to each cow. Feeds were sampled each feeding and composited each balance period. The feces-urine slurry was weighed twice daily, and an aliquot was frozen for subsequent aggregation and analysis. Milk was weighed, and a sample from each milking was preserved with formaldehyde, refrigerated, and composited by cow at the end of each trial. Calcium and phosphorus were determined in feed and excreta dried at 100°C, in milk ashed according to the procedure for milk, and in water by AOAC procedures (1).

**Results and Discussion**

One hundred eighty-one Ca and P balances, two to eight for each of 45 cows, were determined during 3 years (Table 1). Balances accurately measure Ca and P adequacy for body functions, including lactation (15); yet, interrelationships among Ca and P metabolism, vitamin D status, lactation, and unidentified factors complicate interpretation of results, with proper Ca metabolism dependent on the availability of adequate vitamin D. Calcium and P balances reflect the Ca and P buffering capacity of the skeleton, and, to a lesser extent, of the soft tissue pool. Bone accretion depends on adequate amounts of Ca and P but bone resorption, in excess of that related to passive physicochemical equilibrium between bone mineral and extracellular Ca and P, depends on Ca-sensitive mobilizing mechanisms, which in turn depend on parathyroid hormone and vitamin D (16). No P-sensitive mechanism has been described for active bone resorption. Parathyroid hormone, triggered by low blood Ca, promotes active bone resorption and kidney excretion of P (19); vitamin D, required for active intestinal Ca absorption (20), probably through its influence on synthesis of Ca binding protein (24), speeds bone turnover (7).

Interrelationship among P intake, milk production, and Ca status is evident in Figures 1.
and 2. Predominance of negative P balances associated with negative calcium balances (Fig. 1) indicates the interrelationship of metabolism of those two elements and reemphasizes the need to estimate the requirement for one in the presence of adequate amounts of the other (15). The estimated P requirement for lactation, indicated (Fig. 2) by the line constructed including the NRC (18) maintenance requirement for 600 kg cows, emphasizes adequacy in the presence of adequate Ca for heavy production. The requirement was estimated at the intake resulting in probable P equilibrium—equal chance for positive and negative balances (13). The estimated requirement, 2.3 g P per kilogram milk in addition to the maintenance requirement, is about 20% greater than the NRC (18) allowance.

Multiple linear regression of milk P, P intake, and Ca balance accounted for 41% of the variance among P balances associated with positive Ca balances. Milk P contributed the greatest influence on P balance, followed by P intake and Ca balance, as indicated by standard partial regression coefficients—.64, .49 and .38. The importance of the dependence of P status on that of Ca, unidentified factors, and possible nonlinear relationships is obvious from this poor accountability.

The influence of supplemental vitamin D on Ca utilization becomes apparent by comparing Figures 3 and 4. Negative Ca balances for cows producing more than 15 kg of milk were common in second- and fifth-month trials with Ca intakes up to 250 g per day without vitamin D supplement (Fig. 3). Those animals had access to sunshine except when they were confined for balance trials. Balances from cows given vitamin D supplement and producing more than 15 kg milk per day were used to estimate the Ca requirement for lactation (Fig. 4), as described for P. The estimated requirement, 5 g per kilogram milk, is about twice the NRC (18) allowance, which was based on 45% Ca availability from feed and 1.23 g Ca per kilogram milk having 4% milk fat.

Further evidence of the effect of supplemental vitamin D on Ca metabolism is in Table 2. The
Fig. 3. Incidence of negative calcium balance with cows not supplemented with Vitamin D, at various calcium intakes and milk production.

A larger proportion of Ca balance variance explained by regression, $R^2$, in the vitamin D supplemented than in the unsupplemented group indicates that, with vitamin D, Ca utilization was more consistent with Ca and P intake and milk production than without it. Calcium intake from concentrate contributed about 10 times as much to prediction of Ca balance as did Ca from hay. Improved correlation, considering Ca intakes from hay and concentrate separately, indicates predictability rather than average utilization. The inconsistent utilization of hay Ca probably was related to multiple factors not measured or not identifiable in this analysis.

Graphic analysis of data from Forbes et al. (4) (Fig. 5) led to the same estimate of Ca requirement for lactation. The data are from a continuous balance study in which 6 cows were fed approximately equal portions of dry matter in alfalfa hay, corn silage, and concentrate. Vitamin D was not supplied, but the ration contained considerably more roughage than was fed in our study. Negative Ca balances were associated with high production inherent with early lactation. Calcium consumption varied little with production, thus, providing no evidence for obligate negative Ca balances with early lactation. Positive Ca balances are probable in the second month of lactation if ample Ca is consumed and adequate vitamin D is available (Fig. 4). Forbes et al. (4) attained positive Ca balances with much lower Ca intake in cows fed timothy hay-corn silage-concentrate rations than was supplied by rations containing alfalfa. The Ca requirement estimated from Figure 6, 3 g per kilogram milk, still exceeded the NRC (18) allowance.

The inconsistencies in apparent Ca requirements for lactation may be related to combinations of differences among ration and metabolic adaptation. Though Forbes et al. (4) supplemented feed of some cows in each group with limestone and some with bone meal, their Ca intakes were not influenced sufficiently to separate the influence of amount from source of Ca. Huffman et al. (11) also found that Ca from rations containing timothy hay was used more efficiently than that from alfalfa hay rations. However, those workers demonstrated, with Ca supplementation of the timothy rations, that efficiency of Ca utilization depended on intake rather than on source. Hansard et al.
(8) indicated that calcium was somewhat less available from alfalfa than from inorganic sources.

Calcium absorption appears to be a function of need for Ca in relation to Ca supply in the ration. Calcium balance tends toward equilibrium because of partial metabolic adaptation triggered by Ca deficit (5, 14, 17, 21). Smith and St-Laurent (21) reported significantly more Ca binding protein in intestinal mucosa of...

**Table 2. Summary of data and contributions to calcium balance, with and without vitamin D supplement.**

<table>
<thead>
<tr>
<th></th>
<th>Without supplemental vitamin D, N = 84</th>
<th>With supplemental vitamin D, N = 59</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (g/day)</td>
<td>Mean ± SD (g/day)</td>
</tr>
<tr>
<td>Concentrate Ca</td>
<td>52 ± 51</td>
<td>73 ± 65</td>
</tr>
<tr>
<td>Hay Ca</td>
<td>77 ± 30</td>
<td>83 ± 39</td>
</tr>
<tr>
<td>Total Ca</td>
<td>129 ± 70</td>
<td>156 ± 78</td>
</tr>
<tr>
<td>Total P</td>
<td>72 ± 17</td>
<td>79 ± 18</td>
</tr>
<tr>
<td>Milk Ca</td>
<td>24 ± 9</td>
<td>25 ± 8</td>
</tr>
<tr>
<td>P Balance</td>
<td>0 ± 2</td>
<td>2 ± 8</td>
</tr>
<tr>
<td>Ca Balance</td>
<td>0 ± 1</td>
<td>12 ± 29</td>
</tr>
<tr>
<td>Ca Balance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( R^2 ) (Ca Balance)</td>
<td>.33</td>
<td>.41</td>
</tr>
</tbody>
</table>

\( b' = \) Standard partial regression coefficient.

\( R^2 = \) Proportion Ca balance variance explained by regression of indicated independent variables.
lambs fed a Ca-deficient ration than in the mucosa of lambs fed more than adequate Ca. Whether the impetus for Ca binding protein synthesis is Ca deficit, with vitamin D required for implementation, or whether Ca binding protein synthesis can be stimulated by vitamin D per se is not clear. In either case extensive preconditioning of research subjects with the ration to be studied is indicated for Ca utilization experiments (14, 17).

Results of Ca and P balances conducted with dry cows, 250 ± 14 days pregnant, are plotted in Figure 7. Predominance of positive Ca and P balances at or below suggested allowances (18) for “maintenance and pregnancy” indicated their adequacy. Calcium:phosphorus ratios between 1:1 and 4:1 resulted in favorable balances in dry, pregnant cows.

Adequate ration Ca for positive Ca balance early in lactation makes ration P critical. Net bone resorption to compensate for inadequate Ca furnishes P, thus, relieving part of the actual P requirement. With P mobilization dependent on passive equilibrium and Ca mobilization, less-than-adequate ration P, with ration Ca sufficient to support Ca equilib-
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conium, results in rapidly decreased appetite and milk production to the point of P adequacy (10). Stott (22) reported that dairy cattle maintain positive P balances except when they lose weight. Thus, P requirements based on animal well being and determined in presence of negative Ca balance are invalid.

Conclusions

Vitamin D supplement improved Ca utilization by dairy cows fed a ration consisting of 1/3 alfalfa hay and 2/3 sorghum grain-soybean oil meal mixtures. Positive Ca balances can be maintained by cows in early lactation provided they are fed rations supplemented with vitamin D and are consuming adequate Ca. Five grams Ca per kilogram milk, in addition to the maintenance allowance, is adequate to maintain Ca equilibrium during early lactation. The phosphorus requirement for lactation was satisfied with 2.3 g per kilogram milk. The NRC (18) Ca and P allowances for “maintenance and pregnancy” are adequate.

References

(10) Huffman, C. F., C. W. Duncan, C. S. Robinson, and L. W. Lamb. 1933. Phos-

FIG. 7. Influence of calcium and phosphorus intakes on their balances in dry cows. Numbers represent balances (g/day).


