EFFECTS OF SUPPLEMENTAL VITAMIN D, CALCIUM:PHOSPHORUS RATIO,

AND INTAKE ON CALCIUM AND PHOSPHORUS BALANCES IN DAIRY COWS

by 602

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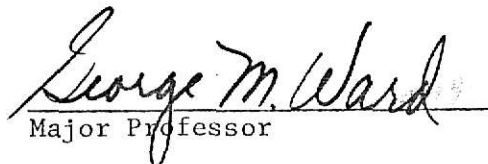
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INTRODUCTION

Researchers have indicated and it is widely accepted that ruminants
can tolerate calcium and phosphorus from a wider calcium-phosphorus (Ca:P)
ratio than non-ruminants. Even so, maximum production is dependent on an
optimal ratio. Therefore, the object of much research has been to elucidate
this relationship of calcium and phosphorus in the ration in the dairy cow.
Current recommendations regarding calcium and phosphorus in the cow's diet
largely reflect results of research done from 1910 to 1930. Disagreement
exists in the literature regarding recommended ratios. With the large
increases in milk production levels since then, applicability of these
recommendations becomes uncertain.

Along with Ca:P ratio, vitamin D influences absorption of calcium and
phosphorus. Calcium and phosphorus balances in cows are closely correlated
with absorption. Maintenance of positive balances is desirable but frequently
difficult. Vitamin D, Ca:P ratio, and other influential factors need to be
optimal to maintain positive balances.

In view of high level milk production, some workers currently recommend
Ca:P near 1:1 for the dairy cow. This recommendation comes primarily from
studies concerned with prevention of parturient paresis. Because of this,
the 1:1 ratio is of somewhat doubtful merit when applied to cows with
no previous history of the syndrome. Vitamin D supplementation in the
ration is also recommended by some, even though no conclusive evidence of
its requirement in excess of that contributed by natural rations for healthy
cattle has been presented. Because of its expensive and laborious nature,

research has lagged behind the need for accurate calcium, phosphorus, and vitamin D recommendations for the rations of high-producing dairy cows.

This research was conducted to compare two Ca:P ratios, each with and without supplemental vitamin D, on calcium and phosphorus retention by dairy cows fed alfalfa hay and sorghum grain high energy rations.

REVIEW OF LITERATURE

Early Balance Studies

An interrelationship between calcium and phosphorus metabolism was noted early in this century (Hart et al., 1909b). In some of the earliest research in this area, Forbes et al. (1916) used balance trials to study calcium and phosphorus metabolism in dairy cattle. Cows were found to be nearly always in negative calcium and phosphorus balance during the first half of lactation. Cows remained in negative balance during the first half of lactation in spite of rations higher in calcium and phosphorus (Forbes et al., 1917). Even when the high calcium and phosphorus rations were supplemented with calcium carbonate and bone flour, calcium balances remained negative but phosphorus balances became positive. In a later study, Forbes et al. (1918) found that of precipitated bone flour, calcium lactate, and calcium chloride, only the calcium of bone flour was utilized when alfalfa was used as the sole source of roughage. Even so, all calcium and phosphorus balances were negative.

Forbes et al. (1922) then studied cows during the entire reproductive cycle rather than exclusively during the first half of lactation as had been previously done (Forbes et al., 1916, 1917, and 1918). The cows were paired according to reproductive status. One of each pair was fed the alfalfa hay basal ration while the other was fed that ration plus calcium carbonate and bone flour. It was concluded that a cycle of calcium and phosphorus loss during early lactation and subsequent replenishment during late lactation

and the dry period was normal. Forbes et al. (1935) compared alfalfa with timothy hay, with and without supplements of limestone and/or bone flour, and concluded from this and the previous studies that, because of its abundance in the body, calcium dominated mineral metabolism and that dry cows fed dry feed store calcium and phosphorus at a rate comparable to the losses during early lactation. Turner et al. (1929) concurred that cows are in negative calcium and phosphorus balance early in lactation and are in positive balance in late lactation and the dry period. In this study, it was established that exercise did not alter calcium and phosphorus balance.

Huffman et al. (1930a, 1930b) disagreed with Forbes. They found that positive calcium and phosphorus balances could be maintained in cows milking as much as 36 kg per day by supplementing a timothy hay, corn silage, and grain ration with bone flour. Even with no mineral supplementation, positive calcium balances were maintained on an alfalfa, corn silage, and grain ration during early lactation. Cows in heavy production and cows fed low levels of calcium utilized calcium more efficiently than did dry cows or cows fed high calcium rations. They concluded that levels of calcium and phosphorus intake were more important than Ca:P ratio for maintaining positive balance.

Extensive calcium and phosphorus balance studies by Ellenberger et al. (1931) corroborated the calcium-phosphorus depletion-replenishment cycle theory. From weekly balances it was determined that all cows ended their lactations with positive overall balances. The negative balance portion of lactation was shortened considerably, but not eliminated, by mineral

supplementation. Ellenberger et al. (1932), from the results of later balance experiments with and without mineral supplementation, reported only one cow in negative calcium and phosphorus balance for the total lactation. Supplementation changed the balance, the calcium and phosphorus retained, Ca:P ratio from 0.24:1 to 1.24:1. They concluded from body analyses of cows on "normal" and low calcium and phosphorus rations that the cow fed the low calcium and phosphorus ration was in a negative over-all calcium balance (Ellenberger et al., 1936). Three cows were used, one on each ration, and all had lactation averages near 646 kg milk per year. Two cows were on the experiment for six lactations and one for two and one-half. Meigs et al. (1935) also used slaughter analyses to ascertain effects of low levels of calcium on calcium and phosphorus body content. They found the percentage of calcium and phosphorus in the fat free body of a cow quite constant in both the whole body and in bone. Conversely, Ellenberger et al. (1932) indicated that the ratio of calcium and phosphorus storage was influenced by the levels of calcium consumed. These workers concluded that cows fed low levels of calcium utilize about 50% of the ration calcium for milk and calf development.

Metabolism

Since approximately 99% of the body's calcium and 50-90% of the body's phosphorus is found in the skeleton (Heiss, 1876 and Katz, 1896), these reserves must always be considered in a study of calcium and phosphorus metabolism. Parathyroid hormone and thyroid calcitonin are intimately linked

with control of tissue levels of these minerals. Bone metabolism, blood levels, and control mechanisms will be discussed where they are especially pertinent to understanding calcium and phosphorus ratio or effect of vitamin D on calcium and phosphorus balance.

Absorption-Excretion

Knowledge of calcium and phosphorus absorption is required to understand the effects of Ca:P ratio and vitamin D on calcium and phosphorus balances. According to Crampton and Lloyd (1959), both calcium and phosphorus are absorbed in the small intestine; calcium at a pH near 6.5 and phosphorus near pH 7.5. If more calcium is introduced to the intestine than it can absorb, the remaining free calcium will be present at the area of phosphorus absorption. Phosphorus and the excess free calcium combine to form insoluble tricalcium phosphate. In essence, this is the way excess calcium and wide Ca:P ratios interfere with phosphorus absorption.

Smith (1969) confirmed this and added that, although mineral absorption from the ruminant intestine is approximately equal to that of non-ruminant animals, differences occur due to the nature of the diet and the alterations occurring before the ingesta reach the abomasum. In Smith's experiment, studies with everted sacs of intestine and in vitro perfusion experiments demonstrated that calcium absorption is an active process and therefore absorption rate increases with increasing concentration. The most efficient calcium absorption occurs in the proximal duodenum where vitamin D also exerts its main influence. Efficiency of calcium absorption is also affected

by hormones other than those previously mentioned, including estrogens, thyroxines and adrenal corticoids, and by dietary factors, such as lactose and certain amino acids.

Visek et al. (1952) used Ca^{45} in a study of calcium metabolism following oral and intravenous administration in lactating goats. They found that the level excreted was proportional to blood activity and the amount of calcium absorbed was closely related to the animals' requirements for milk production. Radioisotope procedures were used in dairy cattle by Hansard et al. (1952) to study calcium metabolism. Fecal excretion of calcium was $85\pm17\%$ of the oral intake. Hansard concurred with Wallace et al. (1951) that endogenous calcium is secreted along the entire gastrointestinal tract, but the major area is in the small intestine. Furthermore, Hansard observed that 15% of an intravenous injection of Ca^{45} was excreted in the feces, 0.3% in the urine, and therefore about 85% reached the skeleton. Circulating calcium is in equilibrium among the gastrointestinal tract, bone and the soft tissue. These observations are in accord with those of other workers in this area.

Cows responded to changes in dietary calcium and phosphorus levels by altering absorption and metabolic fecal excretion (Luick et al., 1957). According to Luick, the changes in absorption were much greater than in excretion, thus corroborating earlier findings that lactating cows utilize calcium more efficiently than non-lactating cows and pregnant cows utilize calcium more efficiently than non-pregnant cows (Huffman et al., 1930a).

Hansard et al. (1954) conducted a study confirming the observation that as ages of cattle increase, efficiency of calcium utilization decreases.

Calcium absorption (or true digestibility) decreased from 99% in ten-day-old calves to 22% in aged cows. Retention dropped from 98% to 16% for the same age range. This is somewhat misleading since the ten-day-old calves were on a complete milk diet and calcium from milk is more highly digestible than that from hay and grain. A more accurate comparison was made between a six-month-old calf and an aged cow in which the true digestibility was 41% and 22%, respectively. Little change was observed among one-year-old, two-year-old, and mature animals (true digestibilities of 34%, 36%, and 34%, respectively).

The decrease observed in the calcium phosphate:calcium carbonate ratio in bone (Neal et al., 1931) with increasing age may be either the cause or the result of this variation in absorption with age. More likely, another aging factor is responsible for both decreasing absorption and change in ratio of bone salt. These workers also reported that phosphorus deficiencies decreased the calcium phosphate:calcium carbonate ratio.

Hormones

Parathyroid Hormone. In a review, Care (1969) stated that 30% of the total body calcium capable of maintaining plasma calcium is under endocrine control. The majority of this control lies with parathyroid hormone (PTH). Collip (1925) conducted some of the original work indicating PTH regulation of calcium and phosphorus metabolism. Calcium blood levels were found to be maintained by direct dissolution of the bone, as stated by Thompson and Collip (1932) and Jaffe (1933).

Conclusions from his own and others' work were compiled by Rasmussen (1961). PTH increases the calcium and decreases the phosphate in the milieu interieur. Rasmussen and others established that PTH acts on the kidney, bone, mammary gland, and gastrointestinal tract to accomplish this. That is, nonexchangable bone is converted to exchangable bone, in turn increasing plasma calcium. When calcium is mobilized from bone, phosphate, magnesium, and other mineral ions are liberated concomitantly with calcium. Circulating calcium and phosphate ions are generally regarded to be in simple equilibrium with exchangable bone consisting of small growing hydroxyapatite crystals as opposed to dense relatively dehydrated, solid non-exchangable mature bone. In the adult mammal, only about 1% of the bone is exchangable. Hence, except for absorption, exchangable bone is the first source of calcium ions for maintenance of circulating calcium.

If phosphorus can be considered under endocrine control, it is only as an indirect result of PTH activity on calcium since no direct hormonal control of phosphorus has yet been demonstrated. However, in order to maintain phosphorus levels constant relative to those of calcium, phosphorus excretion from the kidney is enhanced by PTH while kidney tubule calcium resorption is increased. In much the same way, gastrointestinal calcium absorption is also increased. Intestinal and kidney effects are rapid, sensitive to small amounts of PTH, and of limited reserve capacity. Bone activity is slower, sensitive only to larger amounts of PTH, and nearly unlimited in reserve capacity.

Vitamin D limits excretion of both calcium and phosphate, thus maintaining the necessary critical amount of calcium and phosphate ions required for bone accretion. Without vitamin D, total calcium and phosphate can decline to the point where no calcification occurs. This condition manifests itself as rickets or osteomalacia depending upon the age of the animal. PTH and vitamin D act synergistically to maintain proper calcium and phosphorus levels and ratio. Vitamin D must be present for PTH to act, but vitamin D can exert its influence in the absence of PTH. PTH is also responsible for increasing the citrate content of the skeleton (Ganong, 1969). This increase may be the way in which PTH aids in dissolution of bone either subsequent to or proceeding osteoclastic activity.

Thyroid Calcitonin. Thyroid calcitonin (TC) is secreted primarily by cells found in the thyroid and, to a lesser extent in some species, the parathyroids and the thymus. Bone resorption is inhibited by TC, and the effects are greater in animals with a high bone turnover rate. Obviously then, young animals and mature animals under calcium stress are most affected, but the effects are minor in comparison to those of PTH (Care, 1969). Some researchers doubt the existence of TC, but a preponderance of information indicates its existence. Quite likely the relative unimportance of TC in mature animals without severe calcium demands has confused the issue.

Other Hormones. Blood and tissue calcium levels are controlled also by other hormones. Specifically, thyroxine and cortisone exert influence on calcium metabolism. Care and Gitelman (1968) established that thyroxine

and tri-iodothyronine increase bone calcium turnover rate in sheep but not calcium absorption. Cortisone also was found to decrease calcium absorption and produce a mild hypocalcemia in sheep (Keynes and Care, 1967). Insulin, sex hormones, and some other hormones of the adrenal cortex also affect bone metabolism. Research regarding the effects of these hormones on mineral metabolism of cattle is lacking.

Factors Affecting Utilization

Optimal calcium and phosphorus nutrition is dependent primarily upon three intimately interrelated factors; i.e., intake levels of calcium and phosphorus, Ca:P ratio, and vitamin D in the ration. Nearly all other nutritional factors involved in some degree exert their influence through alteration of one of these factors.

Availability

Calcium. As in the case of most nutrients, disparity occurs between calcium and phosphorus contents of feeds and their biological availability. In 1926, Meigs et al. (1926) reported that calcium from hay was 20% better assimilated than from bone meal, and calcium from fresh green material was better assimilated than from hay. Calcium assimilation, when most of the calcium was from poor quality hay, was only 15-20% of the total in the ration. Cunningham et al. (1932) found equal utilization of calcium and phosphorus from the high and low quality roughages investigated (alfalfa and cane hays). Digestibility of alfalfa hay calcium by rats was 76% (Sur et al., 1952) and 85% (Armstrong et al., 1952).

Phosphorus and Phytin Phosphorus. Ammerman et al. (1957) found that dicalcium phosphate, Curacao Island phosphate, soft phosphate, calcined deflourinated phosphate, and bone meal were of equal value in maintaining blood phosphorus levels in yearling steers. Hansard et al. (1957) also found equal availability of phosphorus from steamed bone meal, Curacao Island phosphate, and dicalcium phosphate when fed to dairy heifers. Similar results were reported by Long et al. (1957).

Phosphorus from plant sources is often less available for non-ruminants than calcium (Krieger et al., 1941). This is because 70% of the phosphorus in plant products is in the form of phytin (NRC 1954). However, as early as 1909, Hart et al. (1909a) reported that ruminant animals utilized phytin phosphorus much more efficiently than man or rats. Reid et al. (1947) reported that phytates were completely hydrolyzed in sheep, mostly in the rumen. Lofgreen et al. (1953) with lambs ascertained that while apparent digestibility of phosphorus in hays averaged 22%, true digestibility was nearly 91%. They concluded and Mather (1953) concurred, that phytin phosphorus utilization was much more sensitive to phosphorus level, calcium level, and Ca:P ratio in the ration than is utilization of inorganic phosphorus. Tillman and Brethour (1958) reported equal availability of both calcium and phosphorus from calcium phytate and monocalcium phosphate for sheep.

Ca:P Ratio

It has long been recognized that ruminant animals can tolerate wider Ca:P ratios than non-ruminant animals. Even so, efficient utilization is seriously impaired by extremely wide or narrow ratios, although much

disagreement exists in the literature regarding their effects. Categorization of calcium and phosphorus between levels and ratios is difficult. Frequently, researchers examining one do not adequately consider the other.

Meigs et al. (1926) recognized that a Ca:P ratio in excess of 2:1 interferes with phosphorus absorption. Better calcium assimilation was noted by Turner et al. (1927) with a ratio of 1.25:1 compared to 2.5:1. It was also suggested that calcium intake should not exceed phosphorus by over 50%. In a review, Bohstedt (1942) stated that cows can tolerate wider ratios without impairment if adequate vitamin D is present. In some work, variations in calcium and phosphorus availability and vitamin D content of the rations may account for some of the differences in results. This is especially true of experiments conducted before vitamin D was discovered. Since most of the calcium and phosphorus in the animal is contained in the skeleton and the ratio of Ca:P in the bone is approximately 2:1, Underwood (1961) suggested it is reasonable to assume that a 2:1 ratio is optimal. Underwood also pointed out that, due to a variety of factors, the Ca:P ratio available to the tissue is not necessarily the same ratio as that in the ration.

Dowe et al. (1957) compared Ca:P ratios from 1.3:1 to 3.7:1 for beef calf rations and found that feed intake and blood calcium levels were not influenced by Ca:P ratio but that plasma phosphorus was depressed by the wider ratios. Weight gain was depressed by the 9.1:1 and 13.7:1 ratios.

Wise et al. (1963) conducted a factorial experiment with steers with three levels of calcium (0.27%, 0.34%, and 2.43%), three levels of phosphorus (0.17%, 0.34%, and 0.68%), and, hence, nine Ca:P ratios from 0.4:1 to 14.3:1.

Severe impairment of performance and feed conversion occurred at ratios less than unity. Statistically significant but less severe impairment occurred with ratios wider than 7:1. Using these criteria for evaluating performance, these workers detected no significant difference among the Ca:P ratios from 1:1 to 7:1. Luick et al. (1957) also reported cows were unable to maintain positive calcium balance on Ca:P ratios less than 1:1.

In a long time experiment with pregnant heifers, Manston (1967) reported better absorption of both minerals from a 2:1 Ca:P ratio diet than from a 1:1 ratio.

Blood phosphorus (usually inorganic) levels are often used as indices of the state of phosphorus metabolism. Because phosphorus has less sensitive blood level control mechanisms than calcium, it reflects more accurately the state of phosphorus nutrition in the animal. On the other hand, severe metabolic disorders must exist before calcium blood levels are altered significantly, even with a poor state of calcium nutrition.

In examining Ca:P ratios of 1.1:1, 3.6:1, 6.9:1, 6.9:2, and 8.2:8 (with 1 being equal to 1958 NRC requirements), Spafford (1964) determined that dairy cows fed rations with the 6.9:2 and 8.2:8 Ca:P ratios had significantly higher levels of whole blood phosphorus than those fed the other ratios. As can be seen, 8.2:8 reduces to about 1:1 and yet higher whole blood phosphorus resulted from the heavier mineral supplementation. Most likely however, this was due to the high level of phosphorus rather than the ratio. Saarinen (1950) corroborated the results that excessive phosphorus level in the ration was conducive to development and maintenance of high blood inorganic phosphorus levels. Saarinen reported that wide Ca:P ratio or high level of calcium in

feed resulted in higher blood calcium levels as long as proper rumen fermentation was maintained.

Mather (1953) observed that with increasingly wider Ca:P ratios, phytin phosphorus becomes less available because greater concentrations of calcium promote calcium phytate precipitation, thus preventing digestion. As Mather narrowed the Ca:P ratio from 3.14:1 toward 1.6:1 in dairy cow rations, a greater proportion of the phytin phosphorus was metabolized by the cows. Similarly, Barth and Hansard (1961) reported that the availability of phytin phosphorus was more sensitive to level of phytin phosphorus, calcium level, and Ca:P ratio than was inorganic phosphorus.

Boda and Cole (1954) found significantly lowered incidence of milk fever in cows with previous histories of milk fever with 1:1 Ca:P ratio and still lower with 1.3:3, again 1 being equal to requirement, when fed one month prepartum. Stott (1965) found that cows fed a 1:1 Ca:P ratio ration as few as 130-150 days before parturition had significantly lower incidence of milk fever than with the previously fed wide ratio ration. When the 1:1 ratio was discontinued after about 300 days, incidence of milk fever increased. It should be emphasized that in these and nearly all other milk fever studies, cows with predisposition to parturient paresis were used and in both of these instances, Jersey cows were studied. Of the common European dairy breeds, Jerseys have the greatest incidence of milk fever. These measures are more nearly remedial than prophylactic and the best ratio for milk fever prevention is not necessarily the best ratio for continuous feeding and for maintaining positive calcium and phosphorus balances.

Gershoff and Hegsted (1956) reported increased peristalsis in chick

intestines when fed a Ca:P ratio of 1:1 as opposed to 4:1 and 1:2. Of the three ratios, 1:1 was the least rachitic. An increase in the intestinal mucosa respiration with the 1:1 Ca:P ratio was suggested. These effects may also affect calcium, phosphorus, and other nutrient absorption.

Using calves, Chandler and Cragle (1962) confirmed some earlier observations of Huffman et al. (1930b); i.e., that calcium and phosphorus levels as well as ratio affect tissue levels of calcium and phosphorus. Daily intake varied from 1.25 g to 20 g for calcium, from 1 g to 16 g phosphorus, and from 3 IU to 30,000 IU vitamin D_3. Plasma phosphorus was highest with narrow Ca:P ratios. Calcium uptake was influenced by vitamin D level, calcium-phosphorus intake, and Ca:P ratio. Urinary excretion of calcium and phosphorus was directly dependent upon the dietary level.

Ricketts et al. (1970) reported Holstein steers receiving an 8:1 Ca:P ratio diet had lower average daily gains than animals fed either a 1:1 or 4:1 ratio. Animals fed the 1:1 Ca:P ratio had higher serum organic phosphorus, lower serum calcium, and narrower serum Ca:P ratio than the 4:1 or 8:1 ratio fed animals. No deleterious effect on feed efficiency or gain was observed from feeding the 4:1 Ca:P ratio diet.

Calcium and Phosphorus Level

If calcium or phosphorus intake is low, the level of the other becomes more critical (Eckles et al., 1926). Fitch et al. (1932) reduced calcium intake to 0.18% of the ration. Health, reproduction, and milk production remained satisfactory and the animals adjusted to this low level of intake. Palmer et al. (1935) found that reducing the calcium to 0.12% of the ration

produced no abortions, no effect on milk or milk fat production, and no
change in levels of milk constituents. However, as could be expected,
bone ash and total and unfilterable calcium of the blood plasma were
lowered. As a result of several studies, Huffman (1934) recommended intakes
of 2.2 g daily per 100 kg body weight for both calcium and phosphorus. For
milk, 1.98 g calcium and 1.54 g phosphorus were recommended per kilogram.
Kellner (1926) some years earlier had advocated slightly different values,
7.15 g calcium and 2.18 g phosphorus per 100 kg live weight and 1.8 g calcium
and 1.1 g phosphorus per kilogram of milk. Mitchell (1947) called attention
to the fact that with increased milk production, the calcium requirement
nearly always is met by increased feed consumption but the phosphorus
requirement needs to be closely watched. Mitchell stated that since no
agent such as PTH exists for phosphorus mobilization from bone, phosphorus
nutrition more than calcium is on a "hand to mouth basis."

Calcium and phosphorus supplementation to rations low in these minerals
can favorably increase urea and cellulose digestion (Burroughs et al., 1950
and Burroughs et al., 1951). In addition to this, it was concluded from rat
experiments that "a diet liberal in both calcium and protein is superior to
a diet liberal in protein alone" (Sherman et al., 1956). However, as two
groups of researchers (Chance and Loosli, 1961 and Coppock et al., 1970)
recently concluded, dramatic benefits from mineral supplementation, calcium
and phosphorus included, normally do not occur in well-balanced rations. In
looking more specifically at calcium levels and requirements, Hansard et al.
(1954) stated that maintenance requirements of cows are approximately the same

as the amounts of endogenous fecal calcium because in ruminant animals, the major portion of the calcium is lost in the feces. Furthermore, requirements for growth, pregnancy, and lactation may be estimated directly from the products involved.

No effect on birth weight of lambs, number or growth of nursing lambs was noted by Benzie et al. (1955) from feeding 1.4 g, 4.5 g, and 7.4 g of calcium to ewes during pregnancy and lactation. Bone resorption was greater in ewes fed the two lower calcium rations as indicated by bone ash measurements (Benzie et al., 1956). They noticed as have dairy cattle researchers, that calcium balances were more often positive when higher levels of calcium were fed.

In an experiment with rats, a diet formulated with calcium near requirements was compared to one twice as high in calcium (Goto et al., 1957). The rats fed the high calcium diet excreted three times as much calcium as the controls; the calcium and phosphorus contents of the bone and liver were less in animals fed the high calcium diets, while phosphorus excretion was about the same. Arrington and Davis (1955) demonstrated decreased urinary excretion and increased tissue deposition of P^{32} when calcium intake was high, indicating significant retention of phosphorus.

Animals can adjust to lower calcium intakes by increasing their efficiency of calcium net absorption. Ali and Evans (1967) reported that calcium increased in the tissues when calcium intake for rats was decreased. Less dietary calcium and lower pH of intestinal secretions combine to promote more efficient calcium absorption with concomitant return of less endogenous

calcium to the intestine.

Huffman et al. (1933) reported that low phosphorus rations were responsible for immediate decreases in blood phosphorus. They concluded that phosphorus requirement depended upon growth rate as well as body size. In addition to pica and low blood phosphorus as symptoms of phosphorus deficiency, Riddle et al. (1934) noted anorexia, inefficient feed conversion, and anestrus. It was reported, however, that digestion, milk production, and energy losses were normal for cows on low phosphorus rations. In contrast, Eckles et al. (1935) discerned no disturbances in estrus cycle in cows deficient in phosphorus for two or three years although lower fertility was noted. They suggested that protein deficiencies usually accompany phosphorus deficiencies and probably were the cause of anestrous conditions. In another experiment, depressed fertility was not detected in heifers fed low phosphorus rations (Palmer et al., 1941). Delayed sexual maturity and repressed evidence of estrus were noted also. Since then, numerous workers have established that ovarian dysfunction occurs with phosphorus deficiency. As Hignett and Hignett (1952) found, the ovarian dysfunctions associated with phosphorus deficiency may or may not manifest themselves as anestrus. For example, low frequency of ovulation or no ovulation was noted in some instances. The degree of the deficiency may also alter the results.

Studies conducted on heifers fed 11.8 g of phosphorus per day (2.6 g/100 kg body weight) showed no significant effect on serum inorganic phosphorus until late gestation when a decided drop in serum inorganic phosphorus did occur (Van Landingham et al., 1936). No effect on serum calcium was detected.

It was concluded that phosphorus intake, milk phosphorus, and weight changes affect serum inorganic phosphorus during lactation.

It should be recalled that both low phosphorus intake and elevated phosphorus intake are expressed in blood levels (Spafford 1964). Aylward and Blackwood (1936) found both blood organic and inorganic phosphorus increased in fasting ruminants while lipid phosphorus decreased. Both calcium and phosphorus excretion occurred during the fasting and it was shown that the phosphorus was from endogenous sources; i.e., bone and phospho-protein.

Vitamin D

Vitamin D is typically localized in four organs: liver (as free vitamin D), mucosa of small intestine, bone, and in the first third of the proximal tubule of the kidney (Kodicek, 1960 and Ganong, 1969). Of these organs, all except the liver have a higher turnover rate of phosphate, citrate, and calcium than most other tissues. Currently, it is not known which of these rates is primarily affected by vitamin D. Perhaps the primary action of vitamin D is mobilization of all three. Kodicek also noted that "the occurrence of vitamin D in the zona fasciculata of the adrenal cortex may also be of paramount metabolic significance."

In early work with dairy cattle, Wallis (1938) found large drafts on calcium and phosphorus reserves in vitamin D deficient cows. In this experiment with dry and milking vitamin D deficient cows, Wallis found that plasma calcium levels declined to one-half normal and inorganic phosphorus

declined to one-fifth normal. Immediate retention of calcium and phosphorus occurred after vitamin D administration.

Vitamin D increases calcium absorption and bone turnover. Carlsson et al. (1954b) found that after giving young rats vitamin D, a slight decrease occurred in serum citric acid followed by a much greater increase. Not only did vitamin D raise serum citric acid, but the citric acid content of bones and incisors was elevated also. In the serum, fluctuations in citric acid were paralleled by changes in calcium and phosphorus. These workers stated that "The evidence seems to be against the assumption that the effect of vitamin D on citric acid metabolism is secondary to its action on mineral metabolism. By accelerating the production of citric acid in the bones, the solvent action of the vitamin on bone salts is thought to find its explanation." Gaster et al. (1967) also reported rats supplemented with vitamin D had more citric acid per unit of fat free bone. It was concluded that vitamin D added to low calcium diets does not increase calcification but increases calcium turnover.

Active transport of Ca^{45} by the intestinal mucosa is dependent on vitamin D (Schachter and Rosen, 1959). Not only is absorption of calcium increased by vitamin D but so is utilization (Campbell, 1942). Coates and Holdsworth (1961) noted that calcium absorption was twice as great in chicks supplemented with vitamin D_3 as in rachitic controls, but no difference in effect could be distinguished between chicks fed a single oral dose of vitamin D and those given a dose before hatching. Calcium absorption was also enhanced by chick bile in vitro.

As in the case of calcification, the primary method of action of vitamin D upon calcium absorption is not well known. Quite likely, similar mechanisms are involved. As Gershoff and Hegsted (1956) observed, possible increases in intestinal mucosal respiration may in part account for action of vitamin D. A calcium binding protein (CaBP) is known to exist in intestinal mucosa. Harmeyer and Deluca (1969) investigated in rats the premise that vitamin D increased CaBP synthesis and higher CaBP resulted in increased calcium absorption. Vitamin D was found necessary for CaBP synthesis. However, the increase of CaBP synthesis and calcium absorption caused by lowering calcium intake was found parallel but unrelated. It was suggested that another factor is present in the intestinal mucosa upon which vitamin D exerts influence. Both active transport and diffusion of calcium through the mucosa were affected by vitamin D. Reviews by Wasserman (1968) and Norman (1968) summarize theories of vitamin D expression.

A study to determine the possible effect of vitamin D on inorganic phosphate absorption was conducted by Carlsson et al. (1954a). Vitamin D increased the absorption of phosphate in calcium deficient rats. They attributed this to an improvement in condition (or as previously mentioned, it could be a direct result of vitamin D or from increased citric acid content). In a diet with higher calcium level and a wider Ca:P ratio, vitamin D did not affect the absorption of phosphorus.

A precipitous decline in blood calcium level occurs in parturient paresis. Attempts to prevent this decline have involved two basic approaches.

As covered earlier, some researchers attempt this by feeding rations high in calcium and low in phosphorus (Boda and Cole, 1954). Hibbs et al. (1946, 1947, 1955) have used the approach of feeding high levels of vitamin D. Both of these methods were proposed in order to stimulate parathyroid activity. Heretofore, parathyroid inadequacy and/or lag in activity were presumed to be the cause of not maintaining blood calcium levels. Lactation and parturition tax the parathyroid (Campbell and Turner, 1950), but Stott and Smith (1957) found no evidence of milk fever in cows calving after parathyroidectomy performed just prepartum or in mid-lactation.

Hibbs and Pounden (1955) reported good success in prevention of parturient paresis by feeding 30 million I.U. of vitamin D daily for at least three and not more than seven days prepartum. Serum levels of calcium and phosphorus during the critical prepartum and postpartum periods were maintained.

Conrad and Hansard (1957) investigated the effects of five million units of vitamin D given to cows daily for five days. Average true digestibility of calcium increased from 47.7% for the controls to 69.8% for the supplemented cows. Endogenous fecal excretion decreased from 2.27 g calcium per day for controls to 1.64 g calcium per day for vitamin D supplemented cows. The calcemic effect observed in cows was not seen in young calves but older calves retained approximately three times as much calcium with increased Ca^{45} deposition in areas of new bone growth.

In an experiment designed to ascertain the effect of vitamin D on cows' ability to mobilize calcium, Conrad et al. (1968) used ethylenediaminetetra-acetate (EDTA) to chelate blood calcium, thus challenging calcium mobilization.

With short infusion time, one-half recovery time was the same for vitamin
D supplemented and control cows, but cows with a previous history of milk
fever showed longer one-half recovery times. In a more prolonged EDTA
infusion, vitamin D fed cows mobilized calcium one and one-half times
better than controls. It was calculated that two and one-half times the
original calcium in the blood was removed with this infusion, but in the
short time infusion, only one-half of the original calcium was chelated.

Although the relationship between PTH and vitamin D has already been
discussed, it should be mentioned that vitamin D spares PTH (Campbell, 1942).
Vitamin D also increases calcium retention by the intestine and phosphorus
retention by the kidney. In this somewhat indirect manner, vitamin D does
enhance calcification. Hignett and Hignett (1953) observed that cows with
better vitamin D status were more fertile. Earlier conception after
parturition in cows given supplemental vitamin D was reported by Ward et al.
(1970). It was suggested that vitamin D supplementation may alter hormonal
level and/or expression and therefore account for the higher incidence of
observed estrus in the supplemented cows.

Colovos et al. (1951) found that vitamin D deficiency seriously impairs
calf growth, health, and feed efficiency. Digestion of protein and retention
of absorbed nitrogen were diminished by low vitamin D intake. Conversely,
ash digestion, as would be expected, was enhanced, but digestion of dry matter,
ether extract, fiber, nitrogen free extract, and energy were not significantly
affected. Utilization efficiency of both protein and energy decreased, however.
Basic metabolic rate increased, calcium and phosphorus blood levels were de-

creased, blood alkaline phosphatase levels increased, and eventually rickets were produced in vitamin D deficient calves. It was concluded that calves need vitamin D not only for normal bone growth and calcium absorption, but also for efficient digestion and utilization of certain nutrients, and to maintain normal basic metabolic rates.

Summary of Literature

It appears that wide (over 4:1) Ca:P ratios deleteriously affect calcium and phosphorus balances. Less clear are the effects of narrower ratios. Assuming calcium, phosphorus, and vitamin D levels are adequate, the optimal ratio for maintaining positive calcium and phosphorus balances lies between 1:1 and 3:1. In order to maintain positive balances in dairy cows, high levels of calcium and phosphorus, perhaps in excess of NRC recommendations, should be fed.

Vitamin D tends to improve calcium and phosphorus balance in animals on marginal planes of mineral nutrition. With higher levels of calcium and phosphorus, effects of vitamin D in excess of NRC recommendations are not understood. Although vitamin D promotes calcium absorption and calcium and phosphorus retention, cows likely have a finite storage level. Adequate levels of vitamin D and high levels of calcium and phosphorus promote positive balances in cows with depleted reserves and balances tending toward zero in cows with ample reserves.

EXPERIMENTAL PROCEDURE

General

Forty-six Holstein cows ranging in age from 2 to 6 years were randomly assigned during 3 years to treatments at least 45 days before expected parturition. Cows removed through normal herd culling were replaced. The treatments consisted of Ca:P ratios varying between 0.9:1 and 1.3:1 (A), with (A+D) and without (A-D) supplemental vitamin D, and ratios between 2.1:1 and 2.5:1 (B), with (B+D) and without (B-D) supplemental vitamin D. The cows were fed alfalfa hay and the A and B concentrate mixtures (Table 1) ad libitum by lots. The cows supplemented with vitamin D were given 300,000 IU of vitamin D_3 weekly by orally administered capsule.

Table 1. Composition of concentrate mixtures.

Ingredient	Narrow Ca:P ratio (A)	Wide Ca:P ratio (B)
	---- (%) ----	
Sorghum grain	78.7	76.6
Soybean oil meal	19.4	18.9
Monosodium phosphate	1.4	----
Ground limestone	----	3.1
Dicalcium phosphate	----	0.9
Trace mineralized salt	0.5	0.5

Both mixtures contained 4,000 IU vitamin A per kg.

For the first ten months of the study, the A ration contained 1.5% diammonium phosphate in lieu of monosodium phosphate and the B ration contained 0.7% urea. Nitrogen content was made equivalent by adding commensurately less soybean oil meal and more sorghum grain. This was changed so that possible contingent effects on results of different nitrogen sources would be averted.

Except for collection periods, the cows were kept in two lots (A and B) with access to loose housing. Softened, chlorinated, and fluoridated water was provided by automatic waterers. The cows were milked twice daily by machine.

Calcium and Phosphorus Balances

Cows were confined for balances in stanchion stalls for three weeks of each second month and fifth month of lactation and of the dry period. The first two weeks provided for establishing maximum concentrate intake and adjustment to the regimen and the third week was devoted to the balance trial. Water consumption was metered. The cows were fed as closely as possible the rations fed in the open lots. Concentrates were fed twice while hay was fed only once daily. Grain not consumed the previous day was weighed back each morning, and hay and grain were sampled each feeding and composited for subsequent analysis. Feces and urine were collected together in pans behind stalls with rubber mats extending over the edges of the pans.

Milk and excreta were weighed and sampled twice daily and stored until the end of the collection period. Milk was preserved with formaldehyde and

refrigerated. Aliquots of excreta were taken daily and frozen. Upon completion of collections, the milk samples were aliquoted, composited, and analyzed. Feeds and excreta were oven dried at 100°C. Calcium and phosphorus were determined in oven dried feed and excreta, in milk ashed according to A.O.A.C. (1960) procedure for milk, and in water by the A.O.A.C. (1960) methods for feeds.

RESULTS AND DISCUSSION

The data from 181 calcium and phosphorus balances constituting this study are summarized in Table 2. Both calcium and phosphorus balances were more positive during the fifth month and dry period than during the second month. This agrees with earlier reports (Forbes et al., 1916, 1917, 1922, 1935; Turner and Hartman, 1929; Ellenberger et al., 1931, 1932; and Huffman et al., 1930a and b). In dry cows, poor appetite which resulted in low intake during balance periods was thought to be the primary cause of negative balances. Although negative balances were frequent, they were lower in magnitude than the positive balances. Cows tended to retain more calcium in this study than in earlier balance investigations, probably because of greater calcium consumption (Huffman et al., 1930).

The balance periods were samplings from a three year study since the same concentrate rations were fed both during and between trials. Hay quality, some psychological and sociological effects, and probable differences in feed intake were the only distinctions between the balance trials and the interim treatments. It should be emphasized that, based on requirements (NRC, 1966), more than ample calcium and phosphorus were fed during balance periods except for infrequent instances during dry periods. Dietary vitamin D intake amounted to at least 6,000 IU per day, as estimated from hay consumption. Even in dry cows, the dietary vitamin D intake was at least equal to accepted requirements (NRC, 1970). Supplementation provided about 300,000 IU of vitamin D_3 per week for those receiving it. Biosynthesis provided for even more vitamin D since the cows were confined to lots with access to solar radiation except for

Table 2. Mean calcium and phosphorus requirements, intakes and balances.

Treatment[1]	Obs.	Milk ± SD	Calcium			Phosphorus			Ca:P ratio[3]
			Req.[2]	Intake ± SD	Bal. ± SD	Req.	Intake ± SD	Bal. ± SD	
	(no.)	(kg/day)							
			--------------------------(g/day)--------------------------						
Second month									
A	22	24 ± 6	74	95 ± 37	− 7 ± 13 (20)[4]	60	80 ± 16	− 3 ± 7 (13)	1.2:1
A+D	15	24 ± 6	74	116 ± 59	− 4 ± 14 (9)	60	89 ± 16	0 ± 7 (5)	1.3:1
B	21	23 ± 7	71	176 ± 72	10 ± 33 (9)	57	69 ± 16	2 ± 7 (10)	2.5:1
B+D	15	28 ± 6	83	200 ± 75	24 ± 45 (6)	66	77 ± 22	2 ± 17 (9)	2.6:1
Fifth month									
A	22	17 ± 7	53	99 ± 39	1 ± 20 (12)	43	76 ± 18	2 ± 8 (9)	1.3:1
A+D	14	15 ± 4	49	98 ± 42	4 ± 9 (4)	40	71 ± 14	3 ± 8 (5)	1.4:1
B	19	18 ± 6	56	176 ± 54	15 ± 31 (10)	45	65 ± 13	4 ± 8 (10)	2.7:1
B+D	15	20 ± 4	60	212 ± 64	29 ± 29 (3)	48	76 ± 17	4 ± 6 (2)	2.9:1
Dry Period									
A	13		30	50 ± 17	8 ± 5 (0)	28	32 ± 8	2 ± 6 (5)	1.6:1
A+D	7		30	56 ± 21	11 ± 10 (0)	28	30 ± 8	6 ± 7 (2)	1.9:1
B	6		30	84 ± 38	11 ± 12 (1)	28	29 ± 12	4 ± 4 (1)	3.0:1
B+D	12		30	88 ± 42	14 ± 18 (3)	28	32 ± 10	5 ± 5 (1)	2.6:1

[1]Treatments were as follows: A, Narrow Ca:P ratio with no supplemental vitamin D; A+D, Narrow Ca:P ratio with supplemental vitamin D; B, Wide Ca:P ratio with no supplemental vitamin D; and B+D, Wide Ca:P ratio with supplemental vitamin D.

[2]Requirements were calculated from National Research Council (1966) recommendations for 600 kg cow producing 3.5% milk.

[3]These ratios were obtained from actual calcium and phosphorus intakes during balance trials.

[4]Value in parenthesis indicates number of negative balances.

balance trials.

Calcium:phosphorus ratios of the rations fed when the cows were not on balance periods ranged from 0.9:1 to 1.3:1 for ration A and from 2.1:1 to 2.5:1 for ration B and were somewhat wider for the rations fed during balance periods (Table 2).

Vitamin D status did not influence calcium or phosphorus balances significantly. The calcium intake and vitamin D status interaction was highly significant ($P<0.0001$) but the interaction was not a significant influence on calcium balance. Calcium balances were not influenced significantly by Ca:P ratio as shown by regression analyses, and reported previously by Hart et al. (1922, 1923, 1926, 1927a, 1927b, 1929 and 1930). The Ca:P ratios fed were within the most commonly recommended range and, therefore, were likely to give good if not maximum response. Ratio was not significant in phosphorus balance regression analyses.

Calcium intake squared and phosphorus balance were the most significant factors affecting calcium balance. Multiple regression equations containing calcium intake squared and phosphorus balance accounted for 64%, 49% and 63% of the calcium balance variance for the cows in second month, fifth month, and dry period, respectively. Substituting calcium intake for calcium intake squared, the regression accounted for 58%, 49% and 61% for the second and fifth months of lactation and the dry period, respectively. More variance among second month and dry period calcium balances was accounted for than among fifth month balances. This was probably because cows were more nearly physiologically equivalent during the second month and dry period than in

the fifth month.

Regression equations for calcium balance follow where:

Y = estimated calcium balance (g/day),

X_1 = phosphorus balance (g/day),

X_2 = calcium intake (g/day), and

X_3 = calcium intake squared.

The "t" values are in parantheses under their respective terms and their decreasing magnitude reflects the order in which the independent variables were added to maximize R^2.

Second month - 73 observations

$$Y = 1.22X_1 + 0.24X_2 - 30 \pm 40 \qquad R^2 = 0.58$$
$$\quad\; (5.1) \qquad (7.5)$$

$$Y = 1.21X_1 + 0.00072X_3 - 14 \pm 18 \qquad R^2 = 0.64$$
$$\quad\; (5.4) \qquad (8.8)$$

Fifth month - 70 observations

$$Y = 1.54X_1 + 0.17X_2 - 19 \pm 40 \qquad R^2 = 0.49$$
$$\quad\; (4.9) \qquad (5.1)$$

$$Y = 1.55X_1 + 0.00053X_3 - 7 \pm 21 \qquad R^2 = 0.49$$
$$\quad\; (5.0) \qquad (5.3)$$

Dry period - 38 observations

$$Y = 0.93X_1 + 0.17X_2 - 5 \pm 16 \qquad R^2 = 0.61$$
$$\quad\; (3.6) \qquad (4.3)$$

$$Y = 0.87X_1 + 0.0011X_3 + 1.0 \pm 8 \qquad R^2 = 0.63$$

These equations were selected, as were those for phosphorus balance, on the basis of containing only biologically significant terms making significant contribution to R^2. The terms tested for contribution to R^2 for calcium balances were phosphorus balance, calcium intake, phosphorus intake, Ca:P ratio, milk production, calcium content of the milk, and the product of calcium intake and Ca:P ratio; and, if significant, their squares, cubes, and logarithms were subsequently tried.

When the data were not segregated by stage of lactation, which was nearly the same as grouping by milk production, milk calcium was sometimes a significant contributor to calcium balance R^2. When both calcium intake and phosphorus balance were considered along with milk calcium, the latter was not significant. For calcium balance of all lactating cows, milk calcium contributed significantly ($P<0.025$) when considered only with calcium intake ($R^2 = 0.39$). The close correlation between milk production and feed (calcium) intake accounted for the insignificance of milk calcium contribution to calcium balance variance.

Equations including calcium balance, phosphorus intake, and milk phosphorus were most successful in accounting for phosphorus balance variance in the second and fifth months of lactation. These equations accounted for 41% and 52% of the variance for the second and fifth months, respectively. An equation including calcium balance and phosphorus intake accounted for 46% of the variance among phosphorus balances.

Regression equations for phosphorus balances follow where:

Y = estimated phosphorus balance (g/day),

X_1 = calcium balance (g/day),

X_2 = phosphorus intake (g/day), and

X_3 = milk phosphorus (g/day).

The "t" values are given in parentheses under their respective terms and their decreasing magnitude reflects the order in which the independent variables were stepwise added to maximize R^2.

Second month - 73 observations

$$Y = 0.16X_1 + 0.17X_2 + 0.17X_3 + 3.1 + 37.3 \qquad R^2 = 0.41$$

(5.1)   (2.8)   (4.3)

Fifth month - 70 observations

$$Y = 0.15X_1 + 0.22X_2 + -.052 - 5.0 + 23.9 \qquad R^2 = 0.52$$

(6.0)   (5.2)   (4.4)

Dry period - 38 observations

$$Y = 0.22X_1 + 0.16X_2 - 3.4 \pm 14.7 \qquad R^2 = 0.46$$

(3.4)   (1.9)

Phosphorus balance variances were less accountable than calcium balances. In contrast to calcium balances, regressions accounted for more variance among phosphorus balances during the fifth month than during the second month or dry period. Calcium balance, phosphorus intake, and milk phosphorus were all significant (P<0.01) contributors to phosphorus balance variance during lactation. When only calcium balance and phosphorus intake were considered, calcium balance was significant (P<0.005) for all stages but phosphorus intake was not significant for the second month. When considered with calcium balance, phosphorus intake was significant (P<0.005) for the fifth month and less so (P<0.10) for the dry period. The narrow

range of phosphorus intakes within physiological states contributed to the lower accountability for phosphorus balance variance.

The terms tested for contribution to phosphorus balance R^2 were calcium balance, calcium intake, phosphorus intake, Ca:P ratio, milk production, and phosphorus content of the milk; and, if significant, their squares, cubes, and logarithms were tried also.

SUMMARY

One hundred eighty-one calcium and phosphorus balances were conducted on 46 cows during a three-year study. Narrow and wide Ca:P rations were compared with and without 300,000 IU supplemental vitamin D_3 per week. The contribution of various factors was determined using multiple regression analyses.

A multiple regression of calcium intake and phosphorus balance best accounted for calcium balance variance, accounting for 58%, 49%, and 61% for the second and fifth months of lactation and for the dry period, respectively. Substituting calcium intake squared for calcium intake in the foregoing equation produced similar results. Calcium content of the milk was not sufficiently variable to influence calcium balance significantly. Phosphorus balance variance was accounted for best with multiple regression of phosphorus intake, calcium balance, and milk phosphorus ($R^2 = 0.41$ and 0.52 for second and *fifth months*, respectively). Calcium balance and phosphorus intake accounted for 46% of the variance in the dry period balances. Neither supplemental vitamin D nor calcium:phosphorus ratio made significant contribution to calcium or phosphorus balance variances.

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EFFECTS OF SUPPLEMENTAL VITAMIN D, CALCIUM:PHOSPHORUS RATIO,

AND INTAKE ON CALCIUM AND PHOSPHORUS BALANCES IN DAIRY COWS

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AN ABSTRACT OF

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ABSTRACT

One hundred eighty-one calcium and phosphorus balances were conducted on 46 cows during a three-year study. Narrow and wide Ca:P rations were compared with and without 300,000 IU supplemental vitamin D_3 per week. The contribution of various factors was determined using multiple regression analyses.

A multiple regression of calcium intake and phosphorus balance best accounted for calcium balance variance, accounting for 58%, 49% and 61% for the second and fifth months of lactation and the dry period, respectively. Substituting calcium intake squared for calcium intake in the foregoing equation produced similar results. Calcium content of the milk was not sufficiently variable to influence calcium balance significantly. Phosphorus balance variance was accounted for best with multiple regression of phosphorus intake, calcium balance, and milk phosphorus ($R^2 = 0.41$ and 0.52 for second and fifth months, respectively). Calcium balance and phosphorus intake accounted for 46% of the variance in the dry period balances. Neither supplemental vitamin D nor calcium:phosphorus ratio made significant contribution to calcium or phosphorus balance variances.