THE EFFECTS OF CALCIUM CARBONATE ON THE APPARENT
DIGESTIBILITY, SERUM CONCENTRATION AND APPARENT
RETENTION OF DIETARY MINERALS IN DAIRY CATTLE

by

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ABSTRACT

The effect of increasing the calcium content of a hay-grain diet fed to postparturient dairy cattle was studied. Mineral apparent digestibilities, serum concentrations and apparent mineral retentions were monitored while the animals were under the stress of peak lactation.

Nine Holstein and seven Ayrshire cows were randomly assigned to the control (0.7% Ca) or the Ca-treatment (1.5% Ca) based on calving order. The diets otherwise contained adequate nutrients. Each animal was fed to appetite for 80 days with no difference (p > .05) occurring between treatments in intake when expessed as a percentage of body weight. Daily milk production (4% FCM) was higher (p < .05) as was the average body weight (p < .01) for the control animals reflecting the disproportionate number of young animals on the calcium treatment.

After a minimum of 60 days on trial, 5 cows from each treatment were exposed to a 5 day digestibility collection period. No change in organic matter or nitrogen apparent digestibility occurred (p > .05) but there were higher (p < .05) levels of calcium and iron, increased (p < .01) levels of copper, and lower (p < .05) zinc and molybdenum apparent digestibilities for animals on the Ca-treatment. Fecal pH was higher (p < .05) in the calcium treated cows indicating a buffering effect occurred as a result of the addition of the calcium carbonate. No change (p > .05) was evident in the secretion of minerals into the milk but urinary phosphorus excretion was significantly

higher ($p < .05$) in the control group.

Milk progesterone was analyzed to correspond blood samples (average of 14 per animal) to specific regions of the estrus cycle. Serum phosphorus, iron, copper and zinc varied with reproductive cycling as phosphorus dropped ($p < .01$) at the onset of regular estrus while the other minerals fluctuated with the cycle (copper and zinc ($p < .05$), iron ($p < .01$)). In the serum of Ca-treated animals, calcium and zinc concentrations were higher ($p < .01$), copper increased ($p < .05$), and phosphorus was lower ($p < .01$) than the levels for the control animals. Breed effects were apparent as both phosphorus and copper were higher ($p < .01$) in the serum of Ayrshires than of Holsteins. Plasma glucose concentrations proved not to be different ($p > .05$) between treatments.

Calcium supplementation of the diet allowed the animals to go from a negative to a positive calcium balance ($p < .01$). It also increased ($p < .05$) the amount of phosphorus apparent retention. In all, 6 essential minerals had altered apparent digestibilities and/or serum concentrations with possible long term effects on animal metabolism.

TABLE OF CONTENTS

LIST OF TABLES

ACKNOWLEDGEMENTS

INTRODUCTION

Calcium has long been recognized as an essential component in dairy cattle nutrition. Its functions include skeletal growth and maintenance as well as many metabolic activities. In dairy cattle, milk production and fetal growth are of prime importance; both of which depend on an adequate supply of calcium. The problem most often encountered in high milk producing cows is the increased need for calcium can not be met during the peak lactation period following parturition. The maximum absorption rate of dietary calcium is insufficient to meet the drain of calcium into the milk resulting in withdrawal of calcium from the skeletal stores. If this is only a temporary condition then the animal can recover and replace the lost calcium when milk production decreases. However, if it is prolonged or if the demand is too rapid to enable the mobilization procedure to initiate, then serious health problems occur.

The minimum level of dietary calcium recommended by NRC (1978) has in the past not allowed sufficient uptake of calcium to prevent a negative calcium balance during early lactation (unpublished data). The apparent digestibility of this calcium was low when compared with the optimum level suggested by Ramberg et al. (1974). As the relationship was dependent on dietary intake levels, a study was proposed to investigate the possibility of increasing the amount of calcium absorbed by feeding higher levels of calcium to postparturient

dairy cows.

Factors other than calcium absorption may be affected when increased quantities of calcium are fed. Interactions with dietary ingredients within the digestive tract, competition for absorption sites and alterations in storage and metabolism of several minerals can occur. This presents a major difficulty in establishing minimum requirements for dietary nutrients as these influences can be quite variable. As well, homeostatic control of many of the body minerals complicates any investigation of metabolic interactions. Whether these interactions manifest within the range of increased calcium intake necessary to allow greater absorption of calcium has not yet been demonstrated.

Several non-dietary factors may also affect digestion and metabolism of the minerals. The physiological state of the animal has a major impact. In a trial such as this, the stage of lactation and the milk yield would be responsible for the calcium drain on the animals. Age, pregnancy, reproductive cycling and animal health have also been implicated in altered digestion and metabolism (Thompson et al., 1978). Such factors must be considered before any meaningful relationships can be established.

It was the prime purpose of this study to investigate the possibility of raising the amount of calcium absorbed by animals under the stress of peak lactation. Alterations in apparent uptake and serum concentrations of the related dietary nutrients were observed. Also, an investigation was undertaken of some of the non-dietary factors related to absorption and retention of the minerals.

LITERATURE REVIEW

The role of calcium in animal nutrition has long been studied and there are many well established facts. There are also many theories that remain unsubstantiated due to a lack of documentation or to ambiguities from similar experiments providing differing conclusions. In this study, the effect of increased dietary calcium on mineral absorption and the resultant concentrations of these minerals in the serum were of prime interest. Unfortunately, rarely does an experimentor in nutrition deal with a basic causal-effect relationship. Many factors that may appear incidental to the imposed treatment often influence the outcome of an experiment. In the present case these factors may be associated with the feedstuff; such as the physical form, the corresponding dietary components, or the byproducts of digestion. They could also be physiological parameters such as age and production stresses or they could result from environmental conditions. The objective of the following section is to identify some of the secondary interactions that occur with calcium and to gain insight into how these might alter the digestion and metabolism of dietary calcium.

The addition of calcium in the form of carbonate may result in an alteration in the pH in the digestive tract. Wheeler and Noller (1977) reported highly significant differences ($p < .01$) between the pH levels within the small intestine, colon and feces of cattle fed supplemental calcium carbonate versus those on unsupplemented diets.

Such an alteration in the acid/base balance could have profound effects on the availability of many of the dietary minerals. For example, Ender and Dishington (1970) proposed the alkalinity of the diet was the overriding factor in determining calcium availability. According to Smith and McAllan (1966) a precipitation of calcium phosphate occurred in the small intestine of ruminants. The degree of precipitation increased slowly with rising pH to the level of about 6.5 where it then advanced rapidly. The authors also showed an increased nonphosphate binding of calcium and magnesium in the ileal effluent with increasing pH. The exact nature of the organic constituents involved in the binding was unknown. Grace et al. (1977) were able to separate out a dilute alkali-insoluble fraction associated with magnesium binding. This fraction contained pectin, which Molloy and Richards (1971) observed to be able to bind varying amounts of magnesium and calcium. Smith and McAllan (1966) observed a competition between magnesium and calcium for part of this binding material of which calcium was generally bound more efficiently at pH 5.5 and above. Substantial pH dependent binding of cations by the non-cellulosic polysaccharides of the plant cell wall has been demonstrated by Branch et al. (1975). These authors suggested the ionization of the carboxyl groups of uronic acid might be responsible for binding appreciable amounts of dietary calcium.

Other factors which may alter calcium and magnesium availability are also interrelated with pH change. Decreasing the acidity of the abomasal digesta would cause greater dissociation of proteins,

carbohydrates and other such organic molecules. This would allow increased opportunity for the binding of calcium and magnesium ions by electrostatic attraction (Storry, 1961b). Nucleic acids have been shown by Chang and Carr (1968, cited by Braithwaite, 1976) to be potent calcium binding agents down to pH 5.5. Also, precipitation of calcium and magnesium soaps has occurred at higher pH levels especially when fat was added to the diet (Storry, 1961b; Tillman and Brethour, 1958). Early work done by Talapatra et al. (1948) discounted the influence of known calcium binding agents, such as oxalate and phytate, in ruminants. This pH dependent precipitation apparently did not occur as these complexes were metabolized by rumen microorganisms.

Whether the effect of dietary protein on calcium absorption is only pH related is inconclusive. Braithwaite (1978) suggested decreased calcium retention in protein deficient sheep may possibly be due to decreased calcium binding protein, hence a decreased rate of calcium absorption. Availability of calcium from inorganic sources tended to be higher than that from organic sources (Hansard et al., 1957). The authors suggested decreased levels of dietary protein may be partially responsible for the increased availability. Perhaps up to a certain amount of dietary protein is required for optimum absorption of calcium but further amounts tend to decrease the availability of the calcium depending on the pH of the digesta.

A change in pH within the intestinal tract may alter the availability of calcium yet have no effect on the amount absorbed.

This could happen if the alteration in acidity occurred after the area where most calcium was absorbed. Several authors (Ben-Ghedalia et al., 1975; Grace et al., 1974; Klooster, 1976) reported the major site of calcium absorption in ruminants to be the upper small intestine. Other workers showed significant calcium absorption prior to this area. For example, Cragle (1973) proposed a net absorption of calcium from the abomasum. Leibholz (1974) indicated as much as 50% of calcium absorption occurred before the digesta reached the duodenum in sheep that were in positive calcium balance. In the calf, the aboma-sum actively participated in calcium uptake (Yang and Thomas, 1965). The precipitation of insoluble calcium phosphate, according to Smith and McAllan (1966), was not appreciable until the pH exceeded about 6.5. Most reports indicated the pH of the material flowing through the upper sections of ruminant's small intestine remained acidic (Ben-Ghedalia et al., 1975; Kay, 1969; Wheeler and Noller, 1977; Wheeler, 1980; Yano et al., 1979). Only as the digesta advanced along the intestine did the pH rise above 6.5 and a concomitant decrease in calcium solubility occurred. This information would therefore suggest the calcium availability in the prime areas for absorption would not be influenced by the increase in pH resulting from the additional dietary calcium carbonate.

Similar occurrences could benefit or be detrimental to other mineral availabilities depending on their sites of absorption. Magnesium and phosphorus are the minerals most often associated with calcium in terms of availability. Phosphorus appears to be absorbed

in the same region as does calcium (Ben-Ghedalia et al., 1975; Grace
et al., 1974; Klooster, 1976) with some net absorption occurring
within the reticulo-rumen (Cragle, 1973). Reports on magnesium
absorption are conflicting. Cragle (1973) and Smith and McAllan
(1966) claimed the main site to be the upper small intestine while
Ben-Ghedalia et al. (1975) and Grace et al. (1974; 1977) proposed
absorption to take place primarily in the forestomach and colon areas.
Both magnesium and phosphorus have been reported to decrease in solu-
bility past the midpoint of the small intestine (Ben-Ghedalia et al.,
1975). Like calcium, phosphorus absorption should be little affected
by a pH change in the lower small intestine. Depending on the true
site of absorption of magnesium, a rise in alkalinity may or may not
have a profound interaction.

The modifying influence of the ratio of concentrations of
dietary calcium to phosphorus on absorption and metabolism of each of
these minerals is often cited in the literature. Various dietary
ratios of calcium to phosphorus have been fed to ruminants, ranging
from less than 1:1 to greater than 10:1. There is general agreement
that ratios lower than 1:1 decreased calcium absorption (Manston,
1967; Wise et al., 1963) but ratios between 1:1 and 7:1 produced no
altered absorption (Lomba et al., 1969; Manston, 1967; Wise et al.,
1963). These same authors concluded absorption of phosphorus was
unaffected by altering dietary ratios.

Lueker and Lofgreen (1961) observed no effect of the ratio

(even up to 10:1) on absorption but did find an influence upon the
excretion of metabolic fecal phosphorus. The phosphorus appeared to
be absorbed in direct relation to dietary phosphorus intake, whereas
calcium was more influenced by physiological parameters. In an
experiment with calcium deficient sheep, Braithwaite (1975) summarized
in that instance, phosphorus retention was controlled by the rate of
calcium retention. It therefore appears that within a wide range of
calcium to phosphorus ratios, neither mineral will be differentially
absorbed but phosphorus excretion may be altered.

This wide variability in the practical dietary ratios of
calcium to phosphorus levels in ruminant feedstuffs is in contrast to
the narrow proportions acceptable for monogastric animals. Most single
stomached animals require the calcium and phosphorus in the diet to be
tightly regulated around 2:1, the ratio of calcium to phosphorus in
the bone. Smith and McAllan (1966) demonstrated a direct relationship
between the concentration of inorganic phosphate and that of bound
calcium in the intestinal digesta. This was dependent on the calcium
to phosphorus ratio and the intestinal pH level. Altering the
calcium to phosphorus ratio in monogastric diets would have con-
siderably greater effect on the calcium and phosphorus availability as
the pH of the duodenal and ileal contents is higher in monogastric
animals than it is in ruminants (Wheeler, 1980).

Through recent advances in radioactive tracer techniques some
of the mechanisms of calcium absorption have been elucidated. To gain

insight into the interactions of increased calcium in the diet with other dietary minerals it is necessary to appreciate the complexities of this mineral's absorption process. The method of calcium absorption in non-ruminants proposed by Wasserman and Taylor (1973, cited by Braithwaite, 1974) was used by the author to explain the adaptations by sheep to changes in calcium intake. This concept involved two processes. The first was a non-saturable, diffusional one related to the concentration of calcium in the intestine. The second was a saturable, active process related to body needs. Braithwaite (1976) postulated the active absorption method to be the major control mechanism for calcium absorption and stated that it probably was dependent on bone calcium reserves. Earlier work by Scott (1965) and Storry (1961a) suggested only a small fraction of the calcium in the sheeps intestine was absorbed by simple diffusion of the free ion.

The active absorption of calcium has been observed by DeLuca (1976) to be dependent on vitamin D. The metabolite 1,25-dihydroxy-cholecalciferol (D.C.C.) as well as vitamin D-induced calcium - binding proteins have been implicated in the process. It is therefore understood that depending on an adequate supply of vitamin D, the absorption of calcium from the diets fed to ruminants can be strongly influenced by their metabolic requirements for calcium.

Another possible complication when considering mineral interactions with calcium is the excretion of metabolic calcium into the digestive tract. Braithwaite (1976) summarized the main losses of calcium and phosphorus into the intestine as that due to the digestive

juices. However, the amount of endogenous calcium excreted appears to be relatively stable over varied conditions. Lueker and Lofgreen (1961) reported essentially constant excretion of calcium from diets with a highly variable calcium content and a wide range of calcium to phosphorus rations (0.8:1 to 6.0:1). Similar results were produced by Hansard et al. (1954; 1957) and Lengemann (1965). The latter author suggested the amount of endogenous calcium lost into the digestive tract of dairy cattle was dependent on the level of calcium in the serum. This dependency was previously reported to occur in non-ruminants by Gran (1960, cited by Braithwaite, 1976). Ramberg et al. (1970) indicated a bias may exist such that metabolic calcium excreted into the intestine may be preferentially absorbed over dietary calcium. When comparing absorption rates from diets varying in calcium content, Ali and Evans (1967) inferred part of the reason for lower absorption from diets supplying little calcium would be due to lower calcium in the duodenal secretions and hence less available calcium.

Visek et al. (1953) determined a lack of effect on calcium excretion due to increasing age when the rates were corrected for bodyweight. This information was used by Hansard et al. (1954) to infer it was possible to measure the utilization of calcium from various dietary sources providing there was a constancy within groups of animals of known age and nutritional status. Absorption measured by apparent digestibility assumes constant endogenous excretion. Ramberg et al. (1974) concluded this assumption met if the fecal data were averaged over a week or more.

Many of the above mentioned factors may act separately or in combination such that the type of feedstuff influences the quantity of calcium absorbed. Calcium in milk was shown to be almost totally available to ruminants by Hansard et al. (1954). However, in forage diets, less than 40% availability is generally reported (Braithwaite, 1975; Braithwaite and Riazuddin, 1971; Paquay et al., 1968). When grain was added to an all forage diet, Conrad and Hibbs (1973) indicated an improved calcium availability occurred. Stevenson and Unsworth (1978) concluded the type of roughage-cereal diet fed to sheep had a significant effect on the apparent availability and retention of calcium. Calcium from inorganic sources has been demonstrated to be slightly more available than from organic sources (Hansard et al., 1957). Such results would make it necessary to utilize virtually identical diets, varying only in calcium content, to determine any true effects of calcium supplementation.

Utilizing blood mineral levels to indicate the concentration of those minerals within the body can lead to gross inaccuracies. Most minerals exist in separate forms referred to as pools or compartments within the body. For example, Braithwaite et al. (1969) proposed the existence of six compartments of calcium in sheep, one of which was serum. The concentration of calcium in any of these pools depended on metabolic needs as well as dietary intake. The main storage compartment appeared to be the bone which was under positive hormonal control. Copp (1969) indicated the parathyroid hormone increased the rate of calcium absorption into the bone while thyro-

calcitonin caused a release of calcium from the bone. The intermediate pools all acted to buffer the system such that the serum most likely did not reflect total body storage of calcium. Ramberg et al. (1976) concluded the concentration of calcium in the serum remained remarkably constant over wide ranges of calcium intake which reflected the effectiveness of the homeostatic control. Blood values do however, show enough variability to provide a reasonable estimation of dietary intake (McDonald and Belonje, 1975).

One of the more profound factors which may exert an influence on the imposed treatments of an experiment dealing with mineral balance is the physiological state of the animals. Thompson et al. (1978) stated that a meaningful quantitative relationship between diet and blood could only be determined when allowances were made for non-dietary factors which affect the blood constituents. Some of these considerations were age, stage of lactation, milk production, gestation and animal health.

Braithwaite (1975) reported the efficiency of absorption of dietary calcium to be high and relatively constant over wide ranges of calcium intakes in young growing ruminants. However, this efficiency decreased with increasing calcium intake in older animals and generally decreased with age (Braithwaite, 1976). The author stated that in young animals, "the rate of absorption is limited by the availability of dietary calcium," whereas in older animals, "the efficiency of absorption does not to any significant degree reflect the availability of dietary calcim." Here, the decrease in efficiency of absorption

with increasing age was postulated to reflect decreased calcium
requirements.

This fluctuation, as well as that of other minerals, has been
monitored in the blood by workers attempting to diagnose mineral
disorders. Thompson et al. (1978) indicated a general decrease in
calcium, phosphorus, sodium, potassium and copper with increasing age.
Inorganic phosphorus had the most dramatic decline from the under three
year group to the five to six year group. Not all researchers drew
the same conclusions. Tumbleson et al. (1973) produced similar
results for calcium and inorganic phosphorus but reported no effect of
age on sodium or potassium. Kitchenham et al. (1975) indicated no
significant relationship existed between calcium, sodium, potassium,
copper or iron and age. These authors also stated that adjustment of
inorganic phosphorus concentrations for age was perhaps unjustified
due to the small size of the reduction and the relative inconsistency
of the measurement among herds. However, incorporating the effect of
age into a study on mineral absorption and blood composition was con-
sidered necessary.

During the period of high calcium demand following the onset
of lactation, calcium absorption has been shown by Symonds et al.
(1966) to increase. The authors concluded however, this increase was
insufficient to supply the needs of lactation and the animal was soon
thrust into negative calcium balance. Ramberg et al. (1970) also
demonstrated a rise in calcium absorption from the gut early after the
onset of lactation which was independent of the dietary calcium intake.

This increase in calcium absorption after calving has been described by Braithwaite et al. (1972) and Klooster (1976) to be primarily an increase in efficiency of absorption along with some decrease in fecal endogenous calcium. This relationship was apparent in mature animals as well, which temporarily reversed the decreased efficiency of absorption due to age. Braithwaite (1976) used this information to further elucidate on the theory of decreased absorption being due to decreased requirements in older animals. Once lactation ceased and body stores were regenerated, the efficiency of absorption returned to its normally low level.

In studying the metabolic profile of dairy cattle, Manston and Rowlands (1973) reported the variation in plasma inorganic constituents among cows within the same milk yield group to be less than that among all cows within the herd. Rowlands and Pocock (1976) suggested the cows should be selected by their stage of lactation rather than their milk yield. This increased the accuracy of the test as the blood constituents appeared to be more variable the closer the date of sampling was to calving. It was therefore inferred that both stage of lactation and milk yield should be considered in any comparisons of blood constituents.

Work done on blood mineral concentrations with relationship to estrus cycling has led to implications that reproductive hormones may influence serum mineral levels. Bach and Messervey (1969) reported possible subclinical hypocalcemia after the estrus period in dairy cattle. The authors noted a drop in the diffusible calcium

fraction over estrus and postulated that estrogen enhanced production of the calcium binding protein. Braithwaite et al. (1972) demonstrated that estrogen administered to sheep increased the rate of calcium absorption, retention and bone accretion. Plasma copper in rats changed as plasma estrogen varied during the estrus cycle (Sato and Henkin, 1973). The authors considered this to be due to estrogen stimulated ceruloplasmin synthesis. However, Money et al. (1967) reported no effect on blood or liver copper levels when estrogens were administered to sheep. Plasma zinc concentrations varied little in heifers taken at various stages of the estrus cycle (Dufty et al., 1977). According to Underwood (1977), manganese metabolism was influenced by estrogenic hormones. Any influence of gestation in early pregnancy would most likely be due to an alteration in hormone levels rather than mineral demand for fetal growth.

Breed effects have been reported in two papers. Serum calcium, potassium and magnesium were analyzed by Kitchenham and Rowlands (1976) to be significantly different between Holsteins and Ayrshires. South African cattle breeds were shown by Heynes (1971) to have different blood mineral levels. While there is relatively little information on this parameter, these differences warrent further study.

Seasonal fluctuations in blood mineral levels have been tied to variations in diets due to altered climate, soil conditions and methods of management (Manston and Rowlands, 1973; Rowlands et al., 1979; Thompson et al., 1978). Such environmental factors can be excluded from a controlled experimental situation.

Blood sampling times and methods were also demonstrated to affect blood parameters. Plasma volume was influenced by water intake or by feeding on diets containing high water content (Thompson et al., 1978). This in turn had a diluting effect on component levels. The constituents returned to their normal levels within four to six hours after feeding. Dougherty (1970) concluded venipuncture could cause excitement in the animal resulting in altered glucose, calcium and possibly other blood constituents. Considering these factors, every attempt should be made to keep blood sampling times and methods as consistent as possible between sample periods.

To conclude, it is evident that there are many factors which might affect the results of the present experiment. These concerns range from the type of feedstuff fed to the physiological state of the animals used on the trials. To minimize any confounding interactions, the experimental design should include animal parameters such as age, stage of lactaction, milk production, reproductive cycling and breed. Treatment rations should be as chemically and physically similar as possible containing sufficient nutrients to allow optimum health, growth and production. Uncontrollable parameters such as altered gastro-intestinal acidity should be monitored as closely as possible to gain inference to any resulting complications. Finally, blood sampling procedures should be consistent throughout the trial.

MATERIALS AND METHODS

a)   Data Collection and Analysis:

The experiment was carried out between September 1979 and April 1980 on the U.B.C. campus dairy herd. Both Holstein and Ayrshire cows due to calve during this period were used. There were approximately equal numbers of each breed. One third of the available animals were heifers, the oldest cows were between 6 and 7 years at calving and the average age was 42 months. The animals from each breed were randomized across 2 treatments depending on order of calving. Of the 22 animals expected to calve, 2 did not calve during the trial period and 4 others were removed from the trial due to problems associated with abnormal calving. This resulted in 8 cows per treatment, equal numbers of each breed on the control diet but 5 Holsteins and 3 Ayshires on the calcium treatment. Each animal remained on the trial for 80 days postpartum. This period was chosen to enhance any interactions due to production stress.

The diets were formulated to include recommended minimun levels of all nutrients as suggested by the National Research Council (1978). A commercial dairy concentrate containing 14% crude protein (as fed basis) and a vitamin-mineral pre-mix was fed along with chopped, orchardgrass hay at a ratio of 60% hay:40% grain. Agricultural grade limestone ($CaCO_3$) was added to the grain fraction of the calcium treatment to raise the concentration of calcium from 0.7% to 1.5% of the diet. All components of the diets were collected in advance of the trial to reduce the variability in the ingredients.

Loose cobalt-iodized salt was made available to the cows.

Dry cows were brought into maternity stalls approximately one week prior to calving where the orchardgrass hay was introduced to their diet. After parturition, the trial rations were fed in increasing quantities until ad libitum intakes occurred. Feed intakes reached upwards of 3.5% of body weight per day for most cows by one week postpartum. The animals were moved to a free-stall barn where each cow was fed individually using the Calan door system. Feed levels were adjusted periodically to ensure the animals consumed all that was weighed out for them. Small quantities of unspoiled feed left in a bunk was removed and mixed with the next feed. Weighbacks were collected and stored for analyses when larger volumes of feedstuffs remained. Occasional checks of intake levels were made by removing some of the animals from the free-stall area and placing them in stanchions where closer observations were made. Throughout the trial, feeding occurred twice daily coinciding with milking.

Daily records were kept of individual cow feed intakes and milk production. Once the milk was analyzed for milk fat percentage[+], the total milk production was adjusted to the standard 4% fat corrected milk. Water intakes were not monitored although samples were collected periodically for mineral analyses. Weekly feed samples were collected

[+] Infra-Red Milk Analysis, Provincial Government Dairy Testing Laboratory, Burnaby, B.C.

and stored for future testing. From approximately 10 days after calving, milk stripping samples were taken every second morning. Progesterone levels were determined on these samples by the method of Shelford et al. (1979).

Based on observed heats and progesterone predicted heats, blood samples were taken from the jugular vein at various stages of the reproductive cycle. The average number of samplings per cow was 14 with a range of 9 to 16. Blood samples were collected between the afternoon milking period and the subsequent feeding period. All cows were weighed after they were bled.

Approximately 30 ml blood was drawn for serum mineral analyses as well as 10 ml for plasma glucose determination. Hematocrit readings were undertaken on random samples to ensure plasma volume did not alter greatly. After coagulation, the samples were centrifuged then filter separated. Any samples which exhibited visible hemolysis were noted as this condition can grossly influence serum magnesium and potassium levels (Christian and Feldman, 1970). The serum was divided into 2 storage vials which were kept frozen (-18°C) until the end of the trial.

Five cows from each treatment were confined in stanchions where data was collected to determine apparent digestibilities of the feed minerals. The animals chosen for this trial were ones that had been consuming the experimental ration for a minimum 60 days and who were not expected to be in estrus during the collection period. Each

cow was placed in a stanchion for one week to allow it to become fami-
liar with the area. Feed intakes were closely monitored over this
period to ensure near total consumption. During the 5 day collection,
feed intakes, fecal and urinary excretions, milk production and body
weights were recorded for each animal. The cows were confined totally
to their stanchions and were milked with portable milking units.

Feed samples collected from each feeding were mixed together
then subsampled for future chemical analyses. Fecal grab samples were
composited and subsamples were taken for dry matter determinations.
Values for pH were measured on random fresh fecal samples. Urine,
collected via a catheter, was not sampled until the final 2 days of
the collection period due to blood clots appearing during the initial
part of the trial. As suggested by Christian and Feldman (1970), the
urine was made acidic by adding 1 to 2 ml glacial acetic acid per 100
ml. This was done to prevent the precipitation of calcium phosphate
which could entrap other mineral ions as well. Milk samples from the
evening milking period were kept separate from those of the morning.
Blood samples were collected twice during the digestibility trial.
Rumen fluid was sampled by means of a hose connected to a vacuum pump.
All samples were stored frozen (-18°C) for future analyses.

Samples of the feedstuffs and feces were dried for 48 hrs.
at 65°C in a mechanical convection oven then were ground to pass
through a 0.8 mm mesh stainless steel screen. These were subsampled
and stored in polyethylene bags. Digestion involved the wet acid

technique of Parkinson and Allen (1975). Nitrogen and phosphorus were subsequently analyzed on a Technicon AutoAnalyzer II. Calcium, potassium and magnesium were read on a Perkin Elmer 560 Atomic Absorption Spectrophotometer. Iron, copper, manganese and zinc were also read by atomic absorption spectroscopy but after a dry ash method as described by Heckman (1967). Dry ashing also yielded the total organic matter by difference. The turbidimetric method of Ferrara et al. (1965) was used to measure sulfur while organic extraction was required for both molybdenum (Stupar et al., 1974) and selenium (Michie et al., 1978).

Most serum minerals were analyzed by atomic absorption spectroscopy. The calcium method was that of Cali et al. (1973), magnesium and potassium were read in diluted samples. Copper and zinc were measured by the method of Parker et al. (1967), iron by Olson and Hamlin's (1969) technique and manganese was read by methods of additions as suggested by Mahoney et al. (1969). Colorimetric determination of phosphorus (Little et al., 1971), the turbidimetric measurement of sulfur (Kennedy and Milligan, 1978) and the florometric analysis of selenium (Michie et al., 1978) were used. Molybdenum was not determined due to the low levels in the serum and the inadequacies of the present techniques. The plasma was analyzed for glucose using the Sigma Technical method No. 510 (1978).

The minerals in urine and milk were analyzed using similar methods as those for serum. Milk samples were first deproteinized according to the technique of Brooks et al. (1970).

Three other organic constituents of the feedstuffs were measured as well. The first was digestible energy, the difference between the gross energy of the feed and that of the feces, as determined on the Gallenkamp Adiabatic Bomb Calorimeter. Acid detergent fibre was analyzed using the modified method of Waldern (1971). Lastly, ether extract was measured after extraction on a Goldfisch unit (Labconco).

(b)   Statistical Analysis:

The treatment effect was of prime interest in this experiment but there were other parameters which also had to be accounted for. The effect of breeds on the variables measured was unknown. Work done by Kitchenham and Rowlands (1976) was of sufficient interest to include breeds in the model. Both treatments and breeds were viewed as fixed effects in a 2 x 2 factorial experiment. Four covariables were initially considered important. These were; feed intakes expressed as a percentage of body weight, age at calving (months), daily milk production (kg 4%FCM) and days fresh. The first three applied to the apparent digestibility data while all but the first one were used in the analysis of the serum constituents. The incorporation of categories of reproductive cycling into the model for the analysis of serum traits resulted in these being nested within the factorial arrangement of the feeding experiment.

The model used to determine the effects on apparent digestibility was:

$$Y_{ijk} = u + T_i + B_j + TB_{ij} + I_{ijk} + A_{ijk} + M_{ijk} + E_{ijk}$$

where

u	= overall mean common to all samples.
T_i	= the effect of the ith treatment.
B_j	= the effect of the jth breed.
TB_{ij}	= the interaction of the jth breed within the ith treatment
I_{ijk}	= the covariable feed intake expressed as a precentage of body weight.
A_{ijk}	= the covariable age at calving (months).
M_{ijk}	= the covariable daily milk production recorded in kg 4%FCM.
E_{ijk}	= the unexplained residual error associated with each sample.

The model used to determine the effects on serum constituents
was:

$$Y_{ijkl} = u + T_i + B_j + C_k + TB_{ij} + TC_{ik} + A_{ijkl} + M_{ijkl} + F_{ijkl} + E_{ijkl}$$

where

u = overall mean common to all samples.

T_i = the effect of the ith treatment.

B_j = the effect of the jth breed.

C_k = the effect of the kth category of reproductive cycle.

TB_{ij} = the interaction of the jth breed within the ith treatment.

TC_{ik} = the interaction of the kth category with in the ith treatment.

A_{ijkl} = the covariable age at calving (months).

M_{ijkl} = the covariable daily milk production recorded in kg 4%FCM.

F_{ijkl} = the covariable days fresh or number of days from onset of lactation.

E_{ijkl} = the unexplained residual error associated with each sample.

Analyses were done by least-squares techniques using UBC BMD 10V for ANOVA with unequal numbers of observations per cell. The Newman-Keuls test was used for the comparison of means.

Other factors related to the total feed and energy intakes were analyzed with the package program MFAV, using the following model:

$$Y_{ijk} = u + T_i + B_j + TB_{ij} + A_{ijk} + F_{ijk} + W_{ijk} + E_{ijk}$$

where

u = overall mean common to all samples.

T_i = the effect of the ith treatment.

B_j = the effect of the jth breed.

TB_{ij} = the interaction of the jth breed within the ith treatment.

A_{ijk} = the covariable age at calving (months).

F_{ijk} = the covariable days fresh or number of days from the onset of lactation.

W_{ijk} = the covariable body weight (kg).

E_{ijk} = the unexplained residual error associated with each sample.

The parameters measured were feed intakes, milk production, body weight change and plasma glucose concentrations.

RESULTS AND DISCUSSIONS

Chemical analyses of the feedstuffs were undertaken on samples collected over the period of the trial. Results for the components of prime interest are presented in Table 1 along with the minimum levels suggested by NRC (1978). Nutrients of secondary importance to this experiment but ones which could affect the performance of the cows, appear in Table 2.

As can be seen, all nutrients except possibly energy, were sufficient to meet the requirements of the lactating dairy cattle. Potassium, iron and manganese were considerably higher in concentration than the recommended minimum levels. While both iron and manganese were well within the safe limits for consumption (Underwood, 1977), the high levels of potassium may have interacted to depress magnesium absorption (Suttle and Field, 1967). Also, due to contamination of the limestone used to supplement the treatment diet, the concentrations of iron and copper were higher in the treatment ration than in the control one. These factors will be dealt with later in the discussion.

Loose, cobalt-iodized salt was offered free-choice throughout the experiment. The amount of salt intake was not monitored and therefore any possible effect of increased dietary calcium carbonate on sodium, cobalt and iodine remain undetermined. According to NRC (1978), the level of sodium required by milking dairy cattle is 0.18%. While the amount present in the trial rations was higher than this, it was judged best not to limit the amount of salt available to the animals.

TABLE 1

Nutrient Content of Rations

Component	Control	Ca-Treatment	NRC (1978)[a] minimum levels
O.M. %	90.31	90.31	-----
N %	2.72	2.72	2.08 - 2.56
Ca %	0.72	1.50	0.43 - 0.60
P %	0.50	0.50	0.31 - 0.40
K %	2.70	2.70	0.80
Mg %	0.30	0.30	0.20
S %	0.35	0.35	0.20
Fe ppm	248	275	50
Cu ppm	13.2	15.6	10
Mn ppm	110	110	40
Zn ppm	66	66	40
Mo ppb	672	672	-----
Se ppb	169	169	100
Ca:P	1.4:1	3:1	1.4:1 - 1.5:1

[a] for lactating dairy cattle between 400 and 600 kg producing between 8 and 36 kg milk daily.

TABLE 2

Other Nutrients in Rations

Component	Total Diet (both Treatments)	NRC (1978)[a] minimum levels
Digestible Energy Mcal/kg	3.08	2.78 - 3.31
Acid Detergent Fibre %	21.8	21
Ether Extract %	3.5	2
Na %	0.27	0.18
NaCl %	ad lib[b]	0.46
Co ppm	ad lib[b]	0.10
I ppm	ad lib[b]	0.50
Vitamin A I.U./kg	3425[c]	3200
Vitamin D I.U./kg	310[c]	300

[a] for lactating dairy cattle between 400 and 600 kg producing between 8 and 36 kg milk daily.

[b] ad libitum consumption of cobalt-iodized salt.

[c] calculated from grain source only.

Four parameters were measured to ensure adequate intakes of nutrients occurred. These were feed intakes, milk production (4%FCM), body weight and plasma glucose concentrations (Table 3).

Feed intakes, considered as a percentage of body weight, did not differ (p > .05) between treatments. The mean value for animals on the control ration was 3.68% (dry matter basis). Those cows on the calcium treatment consumed an average of 3.61% of their body weight in feed per day. The range of intakes of all animals once adapted to the diet was from 3.16% to 4.22% which is high when compared to the suggested maximum dry matter intakes of the NRC (1978) of 2.5% to 3.5% of body weight. The energy content of the diet (Table 2) did not meet the requirements of high milk yielding cows (NRC, 1978). However, the higher than average feed intakes would partially balance any energy deficit resulting in near optimum engery intakes.

The second variable measured was daily milk production. Once standardized to the 4% fat corrected milk level, the mean production of 24.49 kg and 23.71 kg for the control and treatment animals respectively were demonstrated to be significantly different (p < .05). This reflected the disproportionate number of young animals on the calcium treatment. In comparison with past records and those of contemporary herd mates, milk production did not appear to be affected by either treatment.

Body weight measurements were taken frequently throughout the trial corresponding with blood sampling times. Due to individual ani-

TABLE 3

Performance Parameters

Parameter	Control		Ca-Treatment	
	Mean[d]	Std. Dev.	Mean[d]	Std. Dev.
Feed Intake % of body weight	3.68	0.14	3.61	0.16
Daily Milk Production kg 4% FCM	24.49*	7.03	23.71*	5.86
Body Weight kg	522.9**	45.8	492.5**	33.7
Plasma Glucose mg/100 ml	53.18	8.06	51.35	10.68
Body Weight Change (slope)	0.036		0.063	

Significantly different *(p < .05), **(p < .01)

[d] n = 8

mal variability, an analysis of covariance was performed comparing the slopes of the lines associated with body weight change for cows on each treatment. No significant difference (p > .05) occurred as most animals, once age was accounted for, remained a constant weight (Control slope = 0.036, Ca-Treatment slope = 0.063). The mean weight of 492.5 Kg for the treatment animals was significantly lower (p < .01) than the average weight of 522.9 Kg for the control cows.

Plasma glucose has been reported to be a stable, reliable diagnostic aid for assessing metabolic carbohydrate disturbances in dairy cattle (Athanasiou and Phillips, 1978). No treatment effects (p > .05) were observed for plasma glucose concentrations. The mean glucose level for the control animals was 53.18 mg/100 ml. A value of 51.32 mg/100 ml was determined in the calcium treated animals. Other reports of glucose concentrations in dairy cattle have a range of means from 36.3 to 69.9 mg/100 ml (Manston and Rowlands, 1973; Rowlands et al., 1977; Thompson et al., 1978) with a grand mean near 45 mg/100 ml. Hunter (1977) suggested the glucose content of plasma less than 25 mg/100 ml generally was indicative of insufficient energy supply which resulted in lower non-return rates. In the current study, no sample was analyzed to have less than 38 mg/100 ml glucose concentration.

Parker and Blowey (1976) related plasma glucose to the stage of lactation. Concentrations of glucose increased from calving to nine weeks postpartum then leveled off at approximately 60mg/100 ml. While most animals in this trial had lower glucose concentrations early in

their lactation, not all animals did. This variability resulted in a lack of significance ($p > .05$) between the glucose concentration and the covariable days fresh. Hewett (1974) also reported no significant effect of stage of lactation. Time of sampling appears to be relatively unimportant when utilizing the glucose concentration to determine metabolic carbohydrate disturbances, especially after the first few weeks postpartum.

Considering the total nutrients in the diet as well as the above parameters, it is apparent that adequate levels of dietary nutrients were ingested by both groups of animals.

In an attempt to identify any alteration of the pH level within the digestive tract due to the additional calcium carbonate, samples of rumen fluid and feces were analyzed. Values for the rumen fluid ranged from a minimum of 7.5 to as high as pH 9.0. Heavy contamination with saliva and unknown sample sites within the rumen contributed to these high, random values. These data were considered unrepresentative and were therefore not recorded.

Fecal pH values were more befitting those expected. The mean for the control group was 7.35 with a standard deviation of 0.16 while that for the calcium treated animals was 7.60 ± 0.13. A significant difference ($p < .05$) existed between the two treatments implying calcium carbonate did indeed raise the pH in the digestive tract. Wheeler and Noller (1977) stated, "that a pH measurement made on a grab sample of feces is an excellant indicator of pH in the small

intestine," as there was no significant difference between the average pH of the digesta within the small intestine and that of the feces. Mean values for the small intestine contents and feces of steers fed a high concentrate diet were about 5.8 pH units. The pH level rose to 6.8 when calcium carbonate was added to the diet. Russell and co-workers (1980) also recorded increased fecal pH in steers fed high grain diets supplemented with limestone. This relationship was not consistent within sheep (Yano et al., 1979). No significant difference in pH was indicated as the calcium carbonate additions to a basal ration raised the calcium content from 0.1% to 1.2%. The fecal pH mean value was 8.3 while that in the small intestine was 5.4, 7.4 and 8.5 respectively for the upper, middle and lower portions.

It is suggested that in the present experiment the difference in pH in the feces was representative of differences within the digestive tract. Unfortunately, it cannot be known whether this alteration in acidity itself was sufficient to cause modified dietary mineral availability.

In first performing the statistical analysis of the digestibility trial data, three covariables were included in the model. These were; feed intakes expressed as a percentage of body weight, age at calving (months) and daily milk production (4% FCM). Each of these factors have been reported to affect some mineral absorptions, however, none of the covariables proved to be significant for any of the variables. This can be explained by the control imposed on the experiment. Firstly, the feed intakes were maintained at or near _ad_

libitum and when compared on a basis of body weight percentage, were

discovered to be unaffected by treatment. High intakes of feed by all

animals would produce similar rates of passage through the digestive

tract. Considerable variation in digesta passage rate could result in

altered uptakes of many of the dietary nutrients including the

minerals (Underwood, 1977). The second covariable, age, had relati-

vely little variation. The youngest animal was twenty-four months at

calving and the oldest was sixty-four months. Most reports on effects

of age on altered mineral absorptions indicate these effects occur in

very young or very old animals. For example, obvious decreased

absorption of calcium with increasing age may not appear until the

animals are over ten years old (Hansard et al., 1954). Other mineral

absorptions are either unaffected by age or the effects are negligible

as to be masked by the variability among animals (Scott, 1965). While

the average daily milk production varied widely between animals, each

animal was in its peak lactation over the trial. It can therefore be

argued that the nutrient requirements would be at their highest during

this term. For example, even if calcium absorption were at a maximum

it may not be able to supply the total amount of calcium required for

the milk (Symonds et al., 1966). The variability in milk production

during this phase would have a lesser effect than it would at a later

stage of the lactation curve.

Due to the lack of significance of the covariables, a second

analysis of variance was undertaken deleting the covariables. This

made the test more robust by increasing the degrees of freedom in

the error term which decreased the error mean square. The mean values and standard deviations of the apparent digestibilities of organic matter, nitrogen and eleven minerals are reported in Table 4.

Measurement of organic matter and nitrogen apparent digestibilities were included to ensure two things. First of all, the effect of dietary calcium, or indirectly that of dietary calcium carbonate, on the organic constituents was assessed. It was also necessary to ensure there was no obvious deficiency in the organic content which might have influenced the inorganic component apparent digestibilities. Both organic matter and nitrogen apparent digestibilities were shown to be unaffected ($p > .05$) by the increased calcium concentration. The mean values for the organic matter were 75.41% and 75.88% for the control group and calcium treated cows respectively. The nitrogen values were even closer together at 74.79% for animals on the control ration and 74.76% for those on the calcium treatment. Smith et al. (1966) fed altered quantities and ratios of calcium and phosphorus (1:1 to 4:1, 8:1 to 8:8) to dairy steers and noted no significant change in organic matter or nitrogen apparent digestibilities. The mean organic matter digestibility ranged from 67% to 71% while the means for nitrogen varied from 57% to 64%. Varner and Woods (1972) recorded digestion coefficients for organic matter of 74.2% and for protein of 71.0% for a calcium carbonate supplemented ration fed to steers. Therefore, it can be assumed there was adequate dietary organic matter and nitrogen present and that calcium did not alter the

TABLE 4

Summary of Percentage Apparent Digestibilities

Component (%)	Control Mean [e]	Std.Dev.	Ca-Treatment Mean [e]	Std.Dev.
O.M.	75.41	2.34	75.88	3.49
N	74.79	3.25	74.76	2.57
Ca	5.57*	4.53	26.60*	12.13
P	33.87	7.15	36.89	10.39
K	93.73	1.78	93.41	2.24
Mg	28.02	11.13	31.18	5.80
S	61.58	2.65	61.80	4.67
Fe	-13.61*	10.55	2.98*	12.04
Cu	15.00**	5.43	34.98**	2.70
Zn	27.46*	9.40	10.40*	4.61
Mn	13.77	9.58	11.32	5.33
Mo	20.83*	6.05	9.32*	10.44
Se	45.49	8.09	43.66	9.01

Significantly different *($p < .05$), **($p < .01$)

[e] $n = 5$

availability of these components.

The apparent digestibilities of calcium associated with the two treatments did prove to be significantly different ($p < .05$). The level of 26.60% for animals on the calcium treatment approached the upper limits for apparent absorption from adequate diets according to Braithwaite et al. (1969). Utilizing radioactive labling procedures, Braithwaite (1974) and Braithwaite and Riazuddin (1971) indicated the true absorption of calcium in sheep to be 37% to 40%. The 10% to 13% difference between the true calcium availability and that which was apparent was due to endogenous excretion. Ramberg et al. (1974) stated that 28% was about maximum apparent absorption but at lower intakes the absorption was less. This relationship was reported to be linear down to low intakes where it became curvilinear. As the mean value of apparent digestibility of the control ration calcium was only 5.57%, this would imply the ration contained less than optimum levels of calcium.

The only explanation for this decreased absorption on the control ration would be due to lower calcium availability. Hansard et al. (1957) reported the calcium availability from ground limestone to be only marginally higher than that from orchardgrass hay. If such was the case then the increased availability of calcium probably was not due to the source. Additional limestone was thought to have adjusted the pH throughout the digestive tract as evidenced by the rise in fecal pH. However, this would be expected to have an adverse effect

by increasing the precipitation of calcium phosphate (Smith and McAllan, 1966). If this change in acidity did occur, it must not have been sufficient to cause precipitation in the first half of the small intestine where calcium is reportedly absorbed.

It therefore appears necessary to have a certain precentage calcium in the diet to overcome the calcium binding factors present in the digesta. Under the dietary conditions of this experiment, it is suggested the NRC (1978) recommended levels of 0.43 to 0.60% calcium would be too low to supply adequate calcium. Considering the needs of postparturient dairy cattle, two to three times the recommended levels would be required to reach maximum calcium absorption rates.

To further the arguement that the increased dietary calcium carbonate did not appreciably alter the pH level in the upper intestine, there was no significant difference $(p > .05)$ in phosphorus apparent digestibilities. Had the precipitation of calcium phosphate occurred within the area of absorption there would have been a decreased digestibility of both calcium and phosphorus for animals on the calcium treatment. This lack of difference agrees with Manstons' (1967) findings of no affect on the absorption of phosphorus by variation in calcium intake by dairy cattle. Toothill (1963) reported a significant decrease in the percentage of phosphorus absorbed with increased calcium levels in the diets of rats. This species differentiation may be due to the higher pH level of the material flowing through the upper section of the monogastric intestine (Ben-Ghedalia

et al., 1975).

The mean values for phosphorus apparent digestibility of
33.87% and 36.89% for the control and calcium treatments respectively,
fall within the range reported by other workers. Lomba et al. (1969)
indicated phosphorus digestibilities to be between 30 and 45% while
Grace et al. (1977) stated apparent absorption of phosphorus from many
diets fed to ruminants was usually less than 30%. As the animals on
the calcium treatment absorbed consistently high levels of phosphorus
and much more calcium than those on the control ration, the ratio of
calcium to phosphorus of 3:1 appears to be more beneficial than that
of 1.4:1.

Magnesium absorption was also unaffected (p > .05) by the
change in calcium levels in the diet. The apparent digestibilities
were 28.02% for the control and 31.18% for the calcium treated cows.
These are close to the average of 27.8% reported by Lomba et al.
(1968) for lactating dairy cattle.

There is evidence in mongastric species which relates magne-
sium utilization to the level of dietary calcium. For example, Nugara
and Edwards (1963) indicated high levels of calcium in the diet of the
chick prevented the deposition of magnesium in the bone. The authors
concluded this to be due to a common mechanism of absorption for diva-
lent ions. Toothill (1963) noted a similar result in the rat where
addition of calcium chloride to the water supply significantly reduced
the absorption of magnesium as indicated by increased fecal magnesium.
Such an interaction was also evidenced in guinea pigs. Morris and

O'Dell (1963) postulated the direct physiological antagonism between calcium and magnesium to be responsible for the decreased absorption of magnesium.

Whether this interaction ostensibly manifests itself in ruminants is undetermined. Increased calcium concentrations in the digesta reduced absorption of magnesium in calves (Smith and McAllan, 1967). This was irrespective of phosphate concentrations and pH level within the digestive tract which implies magnesium absorption occurred prior to the middle to lower small intestine. The above two factors exhibited marked reduction in magnesium ultrafiltrability in the lower sections of the small intestine (Smith and McAllan, 1966). Scott (1965) claimed, "that any competition between calcium and magnesium in absorption in the sheep is sufficiently small to be concealed in the variability which exists between experiments." Smith (1969) suggested magnesium absorption across the intestinal wall was passive. Assuming this and presuming a consistent flow of digesta, any alteration in the efficiency of absorption of magnesium would be due to altered availability. As this did not occur in the present experiment, it must be considered that any antagonism between calcium and magnesium in the gut is of a curvilinear relationship. The change in calcium content of the treatment diet was not sufficient to cause a change in the availability of magnesium.

The high potassium concentration in the diets was of concern as to its effect on magnesium absorption. Suttle and Field (1967)

concluded high potassium intakes depressed magnesium absorption in sheep. Because the apparent digestibilities of magnesium were relative to the range reported by other workers, it is suggested that the high potassium content did not appreciably affect magnesium availability.

The apparent digestibility of potassium bore no significant relationship ($p > .05$) with that of calcium. Both the apparent and true digestibilities of potassium are reported to be very high, unrelated to potassium intakes and fairly constant (Paquay et al., 1969). These authors were unable to show a significant correlation between the fate of potassium and that of calcium, magnesium or phosphorus in dairy cattle. The work reported here is in agreement with these concepts as the mean apparent digestibility for animals on the control ration was 93.73% and for those on the calcium teatment was 93.41%.

The apparent digestibilities of sulfur, copper and molybdenum will be discussed together due to their reported interactions (Underwood, 1977). In the review article of Huisingh et al. (1973), the mechanisms involved in the interactions were explained as an antagonism between sulfate and molybdate for transport systems and between copper, sulfate and molybdenum as the formation of unavailable cupric thiomolybdate.

In the present study, there was no significant difference ($p > .05$) between sulfur digestibilities yet there was a highly significant difference ($p < .01$) for copper and a significant difference ($p < .05$) for molybdenum apparent digestibilities. The value for copper

apparent digestibility rose from 15.00% on the control ration to 34.98% for animals on the calcium treatment. The sulfur digestibilities were very constant with levels of 61.58% and 61.80% for the two treatments. Molybdenum decreased in apparent digestibility as the calcium levels increased. The control ration mean was 20.83% while that of the calcium treatment was only 9.32%.

Because the dietary concentration of sulfur did not vary nor did the apparent digestibility, it is suggested the antagonism between molybdenum and sulfur was not the reason for decreased molybdenum digestibility. The other two possibilities existing are a decreased molybdenum availability allowed an increased copper absorption or an increased copper availability caused the concomitant decrease in molybdenum apparent digestibility. Dick (1954) indicated very high intakes of calcium carbonate (90 g/day) limited the storage of copper in the liver of adult sheep. However, feeding lower levels (35 g/day) to sheep did not affect the liver copper concentration (Hemingway et al., 1962). Kirchgessner (1965) reported a drop in copper retention in sheep and dairy heifers when increased levels of calcium were fed. The author recorded no effect on the copper absorption by pigs and concluded this may be due to pH differences in the digestive tract of the two species. These reports are contrary to the results of the present trial and as there are no reports on the effect of calcium on molybdenum availability, no definitive conclusion is possible.

This author does favour the concept of the increased copper availability with increased dietary calcium carbonate intake. The

first of two reasons for this opposing view concerns the amount and form of copper in the calcium treatment diet. There was a copper contamination of the limestone resulting in a slight increase in the copper levels in the treatment #2 (13.2 ppm vs 15.6 ppm). While this may be small, it could possibly have had a significant effect on the availability of the copper. The form of this extra copper was probably as carbonate as it was associated with calcium carbonate. Underwood (1977) indicated the copper in carbonate form was more easily digestible than from any other form in the diet. As a result of the contaminated limestone, the copper level in the supplemented grain portion of treatment #2 was 22% greater than in the unsupplemented grain portion of the control ration. This could have been the main reason for the increased copper apparent absorption.

To expand on the second line of reasoning regarding possible increased copper digestibility requires inclusion of another factor, dietary zinc. The amount of zinc apparent digestibility dropped significantly (p < .05) from 27.46% in animals on the control diet to 10.40% for cows consuming the extra calcium carbonate. Zinc absorption is reported to vary from a low level to greater than 80% of dietary intake depending on the source of the diet and other related factors (Miller, 1973). One of these factors was dietary calcium. Newland et al. (1958) surmised that increased calcium uptake would interfere with zinc retention in growing pigs but Suttle and Mills (1966) claimed this to be due to a lower zinc availability in calcium supplemented

rations. Other authors have concluded while there appeared to be lower availabilities of zinc when high levels of calcium were present in the diet, there was a paradoxical increase in zinc retention (Kirchgessner, 1965; Miller, 1973; Mills and Williams, 1971). According to O'Dell (1969), dietary phytate reacted to decrease the solubility of zinc. This process was accelerated upon addition of excess calcium both by mass action formation of calcium zinc phytate as well as by elevating intestinal pH. Zinc phytate was least soluble at pH 6 and above. However, Miller (1975) indicated the phytate interaction with zinc was not evident in ruminants. If high calcium diets lower zinc availability, there should be an increase in copper availability as both copper and zinc compete for the protein metal-binding sites in the intestine (Underwood, 1977).

Unfortunately, the depth of this experiment will not allow a conclusion to be drawn. Dietary calcium may have increased the availability of dietary copper causing an interference in zinc and molybdenum absorption. It may also have decreased the availability of dietary zinc and molybdenum resulting in increased copper availability. Alternatively, calcium carbonate may simply have acted as a carrier for a more highly available form of contaminant copper which in turn interacted to reduce the level of zinc and molybdenum absorption. The effect of altered pH can not be ruled out as the availability of copper and zinc were greatly influenced by the pH in the gastro-intestinal tract (Kirchgessner et al., 1977). Further work is necessary in this area.

The interpretation of the apparent digestibility of iron is confusing. A significant difference ($p < .05$) existed between the means of the two treatments yet the mean value for the animals on the control ration was negative (-13.61%). It is generally agreed that except through bleeding, iron excretion in the gut of dairy cattle is very limited (Underwood, 1977). Because of this, alterations in bodily iron contents occurs through adjustment in absorption rates. The author recorded iron absorption to be nil when body stores were adequate and only during periods of need was iron absorbed across the intestional mucosa. The low mean value for apparent absorption of iron by animals on the calcium treatment (2.98%) insinuates these animals had adequate body stores of iron. Some of these animals also were in negative absorption states as indicated by the high standard deviation (12.04%) associated with the mean. As to why the control group's mean iron digestibility value proved to be negative can only be speculated upon. Negative iron retention has been demonstrated to occur in horses by Spais et al. (1977). The values ranged from -34.9% to 59.8% with a mean retention of 9.5%. These results were influenced by copper concentration but not by zinc or manganese levels. Possible forms of contamination were removed from the area where the animals were housed and less than 1% of the total daily intake of iron could have found its way into the animals through ingestion of water. It was therefore assumed there was net excretion of iron into the gut. Also, this excretion was being affected by one or more factors that

became limited when a higher level of calcium carbonate was added to the diet.

The difference between the two iron digestibility means may be due to similar reasons as those expressed for copper. Little is known about the availability of iron from various sources. The fact there was a higher concentration of iron in the calcium treatment diet due to contamination of the calcium carbonate cannot be ignored. Mills and Williams (1971) implied iron absorption was adversely modified by calcium. Also, as the availability of iron is reported to be greatly influenced by the pH in the gastrointestinal tract (Kirchgessner et al., 1977), the calcium treatment should have lowered the digestibility of iron due to the tacit increase in pH. Copper, zinc and manganese have all been associated with lowered iron absorption through direct competition for absorption sites (Underwood, 1977). Zinc also interfered with the incorporation into and the release of iron from ferritin. If copper was competing for absorption sites then there should have been a drop not a rise in iron absorption on the calcium treatment. It is therefore suggested the decreased zinc availability in the calcium supplemented ration probably was influential in bringing about the increase in apparent digestibility of iron.

The mean value for manganese apparent digestibility for those animals on the control treatment did not differ ($p > .05$) from that for cows on the calcium treatment. The levels were 13.77% and 11.32%

respectively. In rats, Lassiter et al. (1972) reported a major effect
of low dietary calcium on manganese metabolism both at the tissue level
and in the gut. The levels of calcium in the above experiment were
0.1% and 0.6% with the resulting manganese digestibilities of 6.3% for
the low calcium ration and 18.6% for the high calcium diet. It is
therefore understood that if this relationship does manifest itself in
dairy cattle, the concentration of calcium in the control diet was not
low enough to alter manganese metabolism. High dietary calcium and
phosphorus appeared to increase the requirement for manganese in
cattle by affecting absorption in the small intestine as well as
retention in the tissue (Vagg and Payne, 1971). Again, the calcium
level required to achieve this result was outside the range of that
imposed on the present experiment. This was especially true of the
phosphorus level. While there may be a relationship between the
amount of calcium in the diet and manganese absorption and retention,
the moderate concentrations of dietary calcium appear to bear no
consequence.

Selenium concentration in the feces was between 54 and 57% of
that in the diet for both treatments. The mean apparent digestibility
for the control animals was 45.49% which was not significantly dif-
ferent ($p > .05$) from that of animals on the calcium treatment
(43.66%). A report of selenium digestibility in hoggets (Cousins and
Cairney, 1961) concluded the feces contained roughly 40% of the quan-
tity of selenium fed. Butler and Peterson (1961) suggested this level

was low for sheep as their results showed greater than 50% dietary

selenium appeared in the feces. These values are higher than those for

monogastrics whose main route of selenium excretion is via the urine.

Formation of insoluble, unavailable forms of selenium occur in the

rumen under highly reducing conditions (Underwood, 1977) which

explains this contrast in methods of excretion. There are no reports

of calcium altering the availability or absorption of dietary selenium

in ruminants.

Milk progesterone levels were analyzed to identify the blood

sampling times with specific regions of the reproductive cycle. The

actual progesterone value was assigned to an integral category

depending on its association with respect to the samples taken before

and after it. Originally, there were nine such categories which are

defined in Table 5.

Of the eight animals on each treatment, two did not begin

cycling during the eighty day trial. These four animals were

diagnosed as having cystic ovaries, which when treated resulted in

normal cycling. All other animals varied in length of time between

calving and the onset of estrus. This ranged from 32 to 48 days. One

complete reproductive cycle occurred before insemination was attempted

and all cows exhibited at least one complete cycle within the trial

period. To determine the effect of the reproductive cycle on the

serum constituents, two separate analyses of variance were undertaken.

The first utilized the data of all cows while the second combined only

TABLE 5

Original Milk Progesterone
Category Descriptions

Category	Description	Progesterone Levels [f]
		(ng/ml)
1	Before any activity.	1 - 3
2	Random peak not associated with cycling.	7 - 15
3	First Proestrus	7 - 15
4	Estrus	1 - 3
5	Metestrus	10 - 35
6	Metestrus to Diestrus	35 - 50
7	Diestrus	50
8	Diestrus to Proestrus	50 - 35
9	Proestrus	35 - 10

[f] Maximum determinable level = 50 ng/ml

TABLE 6

Additional Hypotheses
ANOVA #1 and #2

$$Ho: \quad u_1^a = u_2^a = u_3^a = u_4^b$$

$$Ho: \quad u_3^c = u_5^d = u_9^d$$

$$Ho: \quad u_6^e = u_7^e = u_8^e$$

a, b, c, d, e   Means in the same line with different superscript letters differ significantly ($p < .05$).

those animals that cycled. Treatment and breed effects were considered as were the covariables of age at calving, daily milk production and the number of days since calving.

The first analysis of variance yielded several treatment by category interactions whereas the second did not. This was interpreted to mean the noncycling animals were influencing the results due to having all their sample values placed in the first category and should therefore not be included when attempting to determine the effects of the reproductive cycle. The second analysis showed no difference ($p > .05$) between many of the categories when the additional hypotheses listed in Table 6 were tested. It was decided to combine some of the categories to increase the number of observations in each one. These new categories are described in Table 7. The data was re-analyzed using the additional hypotheses in Table 8.

Serum inorganic phosphorus had a highly significant difference ($p < .01$) in category effects. The values associated with precycling (category P) were higher than those of the other three categories. The predicted means were 6.42 mg/100 ml for category P and 5.13, 5.34, and 5.70 for categories E, T and D respectively. None of the covariables exerted a significant effect ($p > .05$) on the serum trait. It therefore was concluded reproductive cycling caused a depression in the serum inorganic phosphorus levels.

Serum iron proved to be highly significantly different ($p < .01$) when comparing categories P and E with categories T and D. It also approached significance ($p = 0.06$) when category P was com-

TABLE 7

Combined Milk Progesterone Categories

Category	Description	Previous Progesterone Categories	Observations per category
P	Pre Cycling	1, 2, 3	47
E	Estrus	4	28
T	Transition - Includes Proestrus and Metestrus	5, 9	38
D	Diestrus	6, 7, 8	49

TABLE 8

Additional Hypotheses, ANOVA #3
- utilizing combined milk progesterone categories

$$Ho : 3u_P - u_E - u_T - u_D = 0$$

$$Ho : u_P + u_E - u_T - u_D = 0$$

$$Ho : 2u_E - u_T - u_D = 0$$

$$Ho : u_T - u_D = 0$$

pared with the others and when category E was contrasted with T and D.
The only real lack of difference occurred between category T and D.
Because of the definite difference between values associated with low
progesterone concentration and those with high progesterone con-
centration, it can be stated that the reproductive hormones exerted an
effect on serum iron concentrations. In this case the predicted means
being tested were 1.22 mg/l for category P and 1.19 mg/l for category
E versus 1.12 mg/l for category T and 1.09 mg/l for category D.

Both serum copper and serum zinc showed a significant dif-
ference ($p < .05$) when the mean of category T was contrasted with the
other category means. The predicted means tested for the four cate-
gories for copper were 0.68, 0.70, 0.67 and 0.71 mg/l and for zinc
were 0.96, 0.93, 0.89 and 0.98 mg/l. As category T contained data
from proestrus and metestrus there could possibly have been an effect
of the transition in hormone levels on serum copper and serum zinc.

The serum inorganic sulfate levels in category P were signi-
ficantly different ($p < .05$) from those in categories E, T and D.
However, there was also a highly significant ($p < .01$) effect of the
covariable, days fresh. This category difference was dismissed due
to the coincidence of category P being present only in the early post-
parturient stage.

No other serum components were found to vary with reproduc-
tive cycling. The report of Bach and Messervey (1969) regarding the
possible influence of estrogen on serum calcium could not be substan-
tiated here as the diffusable calcium fraction was not measured.

Two of the serum minerals exhibited signifcantly higher
($p < .01$) concentration in the Ayrshires than in the Holsteins. These
constituents were phosphorus and copper. The mean phosphorus con-
centration for the Ayrshires was 6.29 mg/100 ml with a standard
deviation of 1.30 mg/100 ml. The Holstein serum contained an average
of 5.19 mg/100 ml phosphourus with a standard deviation of 1.41 mg/100
ml. Copper average values were 0.74 mg/l for the Ayrshires and 0.65
mg/l for the Holsteins with standard deviations of 0.09 mg/l for each
group. As there was no breed effect in apparent digestibilities of
any of the minerals, these differences must be metabolically
controlled. Kitchenham and Rowlands (1976) did not study copper
levels but did consider phosphorus. These authors reported no signi-
ficant difference in phosphorus concentrations between Ayrshires and
Holsteins but did find lower levels of calcium, magnesium and
potassium in the blood of the Ayrshires. It is assumed there was a
nutritional effect as well which confounded the comparison of the pre-
sent study with that of Kitchenham and Rowlands (1976).

A summary of the mean and standard deviation values for the
serum constituents for the animals of each treatment appear in Table
9. Indications of the covariable effects on the serum components are
presented in Table A of the appendix.

Serum calcium levels showed a highly significant ($p < .01$)
response, increasing from 9.87 mg/100 ml for animals on the control

TABLE 9

Summary of Serum Constituents

Component	Control		Ca-Treatment	
	Mean [g]	Std.Dev.	Mean [h]	Std.Dev.
Ca mg/100 ml	9.87**	0.80	10.22**	0.70
P mg/100 ml	5.79**	1.46	5.42**	1.46
K meq/l	5.36	0.94	5.26	0.80
Mg mg/100 ml	2.39	0.35	2.41	0.22
S mg/100 ml	47.2	6.3	48.1	7.1
Fe mg/l	1.16	0.23	1.16	0.19
Cu mg/l	0.67*	0.10	0.69*	0.10
Zn mg/l	0.90**	0.21	1.02**	0.20
Se ug/l	66.1*	19.4	70.7*	20.0

Significantly different *($p < .05$), **($p < .01$)

[g] $n = 78$
[h] $n = 84$
Mo - not analyzed
Mn - grand mean 12 ug/l

ration to 10.22 mg/100 ml for those animals receiving additional

calcium. While the level of the serum calcium in the control animals

was well within the physiological range expected for dairy cattle

(8.78 - 10.38 mg/100 ml : Rowlands et al., 1977), it was apparently

being limited by the concentration of calcium in the diet and the

availability of such. This rise corresponds to the findings of other

authors that serum calcium shows a small effect of dietary conditions

(McDonald and Belonje, 1975; Payne et al., 1970; Thompson et al.,

1978).

The change in calcium levels in the serum was inversely

related to the change in serum inorganic phosphorus (p < .01). The

mean for the control group (5.79 mg/100 ml) was higher than that for

the calcium treatment (5.42 mg/100 ml). In similar work reported by

Bushman et al. (1965), Hoar et al. (1970) and Smith et al. (1966), the

level of phosphorus in serum dropped when the calcium content of the

diet was raised in relation to the constant amount of dietary

phosphorus. Smith et al. (1966) described that altering the calcium

to phosphorus ratio in the feedstuff from 1:1 to 2:1 to 4:1 lowered the

serum phosphorus but the authors reported it, "did not, at any time,

drop below the range normally expected for dairy cows." This range is

generally agreed to be between 4.6 and 7.8 mg/100 ml (Rowlands et al.,

1977) with a mean near 5.5 mg/100 ml (Hunter, 1977, based on 1157 ana-

lyses from lactating dairy cattle.) The calcium to phosphorus ratio

change did not consistently produce a change in plasma inorganic

phosphorus levels in other studies such as those by Parker and Blowey

(1976) and Swenson et al. (1962).

Several authors have reported the phosphorus level in the blood to be directly related to phosphorus intake but not to the phosphorus levels in the body (McDonald and Belonje, 1975; Payne et al., 1970; Wise et al., 1963). As there was no significant difference (p > .05) between the two treatments for apparent digestibility of phosphorus, the increased calcium can be assumed to have had an effect on phosphorus metabolism.

Serum potassium levels were not significantly different (p > .05) between the two treatments. The respective values for the control and the calcium treated animals were 5.36 and 5.26 meq/l. Rowlands et al. (1977) reported the range of serum potassium levels of 351 lactating dairy cows between 40 and 100 days post-calving to be 4.11 to 6.11 meq/l with a mean of 5.06 meq/l.

Magnesium levels in the serum were also unaffected by increased calcium intakes (p > .05). The mean values of 2.39 mg/100 ml for the control group and 2.41 mg/100 ml for the calcium treatment animals correspond to the published range of 2.28 to 3.08 mg/100 ml with a mean of 2.63 mg/100 ml (Rowlands et al., 1977). Parker and Blowey (1976) reported serum mangesium levels in animals fed diets with calcium : phosphorus ratios more closely aligned with those of this trial. Their mean values were 2.29 to 2.45 mg/100 ml with no significant difference due to the calcium : phosphorus ratio. There are reports (Bushman et al., 1965; Hoar et al., 1970) indicating serum magnesium was adversely affected by increased dietary calcium.

However, this occurred only when magnesium digestibility was lowered due to an interaction in the gut with the increased calcium. As there was no change in the apparent digestibility of magnesium in this experiment, and no difference in serum magnesium levels, it can be concluded there was no significant physical or metabolic interaction between calcium and magnesium.

Kennedy and Milligan (1978) monitored the level of serum inorganic sulfate in sheep. The values ranged from 32.5 to 60.0 mg/l with a mean of 45.0 mg/l. The mean values reported here are 47.2 mg/l for the control group and 48.1 mg/l for the animals receiving extra calcium. These means were not significantly different ($p > .05$) and as there was no change in apparent digestibility of sulfur, there are no obvious interactions between sulfur and calcium.

Serum iron levels were apparently equal for animals on either treatment. The mean values were the same with only a slight difference in the standard deviations (1.16 ± 0.23 mg/l for the control versus 1.16 ± 0.19 mg/l for the calcium treatment). The widest range in values reported in the literature was that of Stout et al. (1976), being 0.37 to 3.21 mg/l with a mean of 1.72 mg/l. The change in apparent absorption of iron when calcium was added to the diet did not follow through to the serum.

Copper levels in the serum of the animals on the calcium treatment were significantly higher ($p < .05$) than those on the control treatment. The means of 0.67 mg/l and 0.69 mg/l were quite similar and considering the standard deviation of 0.10 mg/l in each case, it

is questionable as to whether these means are indeed different.
Copper in serum is metabolically controlled by the liver and except in
the case of deficiency or toxicity, should be little affected
(Underwood, 1977). However, as there was a highly significant
increase in copper apparent digestibilities in the calcium treated
animals, it is possible this increased copper could have contributed
to this rise in serum copper. Hemingway et al. (1962) reported a
slight but significant reduction in serum copper levels in sheep
drenched daily with calcium carbonate. The authors did not mention
any information regarding digestibility of copper so it is impossible
to conclude as to whether this effect was due to an interference in
absorption or metabolism. The range of serum values for lactating
dairy cattle reported by Rowlands et al. (1977) was from 0.56 to 0.96
mg/l. The mean value was stated to be 0.73 mg/l.

Increased dietary calcium resulted in a significant (p < .05)
decrease in zinc absorption but concomitantly caused a highly signifi-
cant (p < .01) increase in serum zinc. This paradox was first reported
by Kirchgessner et al. (1960; cited by Mills and Williams, 1971). The
mean value of serum zinc in the animals on the control ration was 0.90
mg/l whereby that from the animals on the high calcium diet was 1.02
mg/l. Depending on the stage of estrus, Dufty et al. (1977) reported
mean serum zinc values of 1.01 ± 0.16 mg/l to 1.15 ± 0.27 mg/l. One
mechanism that could possibly account for this increase in serum zinc
would be a metabolic interaction between calcium and zinc. It is
suggested increased levels of calcium in the serum caused an increased

release of zinc from the tissues to the serum or a blockage in the deposition of zinc.

Serum manganese levels were analyzed but due to limitations in the techniques available, only an estimate could be reported. The amount present in the serum of the cows on both treatments varied about the mean of 12 ug/l. The technique was not sufficiently accurate to enable a difference to be determined between the treatments. As to the possible effect of calcium on manganese, Lassiter et al. (1972) utilized the isotope   Mn to determine a major change in manganese metabolism. This occurred both in the tissue and within the intestines when deficient levels of calcium were fed to rats. There is widespread disagreement in the literature as to the exact range of manganese in serum (Underwood, 1977). Until further methods become available, any effect of calcium on serum manganese in dairy cattle will remain unknown.

Serum molybdenum could not be analyzed due to the low levels expected and the present inadequacies of equipment.

There was a significant increase ($p < .05$) in the level of serum selenium in the animals exposed to a higher level of dietary calcium. However, there was also a significant ($p < .05$) treatment : breed interaction. On examination of the data it appeared that the low serum selenium values associated with one animal unduly affected the mean. This significant difference must therefore be rejected as a real difference due to treatment. Removing the data of this one animal from the analysis deleted the significant difference. The mean

values of the remaining animals were 72 ± 20 ug/l whereas the level

of serum selenium in the cow in question was only 44 ± 13 ug/l.

Underwood (1977) indicated the mean value of whole bood selenium in

ruminants was 80 ug/l with a range of 40 to 200 ug/l. No explanation

can be offered as to why the selenium level in the one animal was so low.

Milk and urine samples were analyzed to enable estimation of

the percentage retention of the various minerals within the cattle on

each treatment. Due to the very low levels of trace elements in the

milk and urine and the difficulty in assessing these concentrations

accurately, a delineation of less than 3% of intake values was assumed

to be negligible. As a result, only the macro mineral levels were

used in the estimation of the retention rate. The assumption there-

fore was the apparent digestibilities of the trace elements repre-

sented closely enough the percentage of the mineral intake retained.

Table 10 provides the means and standard deviations for the total

daily output of macro elements in the milk and urine of the animals on

the two treatments. The apparent percentage retention for these same

minerals are presented in Table 11.

In ruminants, the urinary excretion of calcium has been

reported to be both marginal and stable over wide ranges in intakes

(Braithwaite, 1974; Braithwaite, 1975, Ramberg et al., 1974). These

losses were not significantly affected by changes in absorption by age

(Braithwaite and Riazuddin, 1971). Ramberg et al. (1976) stated that in

general, the small variations observed in urinary calcium could have

TABLE 10

Total Daily Output of the Macro Elements
in the Milk and Urine

MILK

Component	Control		Ca-Treatment	
(g/day)	Mean [i]	Std.Dev.	Mean [i]	Std.Dev.
Ca	27.51	7.06	27.34	1.99
P	15.32	3.50	14.31	3.45
K	32.09	9.83	33.20	5.56
Mg	2.56	0.67	2.51	0.25
S	6.15	3.18	6.33	2.81

URINE

Ca	0.34	0.18	0.57	0.30
P	8.84*	3.54	6.53*	1.15
K	247.40	87.39	256.20	85.29
Mg	3.96	1.92	3.44	2.24
S	3.26	1.82	4.18	2.33

Significantly different * ($p < .05$)

[i] n = 5

TABLE 11

Apparent Percentage Retention
of the Macro Elements

Component	Control		Ca-Treatment	
(%)	Mean ^{j}	Std.Dev.	Mean ^{j}	Std.Dev.
Ca	-18.6**	6.0	14.5**	12.8
P	3.1*	10.4	10.3*	6.0
K	27.3	23.4	25.7	20.1
Mg	14.1	9.0	18.7	5.0
S	13.4	6.7	14.0	8.1

Significantly different * $(p < .05)$, ** $(p < .01)$

^{j} $n = 5$

only a trivial effect on calcium metabolism. Such was the case in the present study where there was a slight but nonsignificant ($p > .05$) difference between the means of the two treatments. The mean calcium excretion in the urine for the control animals (0.34 g/day) represented 0.31% of the average daily intake of calcium while that for the calcium treatment group (0.57 g/day) comprised 0.23%. Paquay et al. (1968) determined a highly significant correlation between digestible calcium and the calcium in the milk. No such relationship existed in this trial as there was no significant difference ($p > .05$) between treatments for calcium contents of the milk yet there was for apparent digestibilities. The levels of calcium in the milk of the animals on both treatments were consistent with the mean value reported by Ramberg et al. (1974) of 1.2 g/l. Converting the values in Table 10 resulted in means of 1.13 g/l and 1.15 g/l respectively for the control and calcium treated groups.

Phosphorus in the urine has been reported to decrease as calcium intake increases (Braithwaite, 1975; Bushman et al., 1965). The former author described the hypothetical mechanism for such action in the following manner. "When the calcium intake was increased, more calcium was absorbed and bone resorption decreased. The supply of phosphorus from bone was reduced and urinary excretion of phosphorus decreased." This would explain the significantly lower ($p < .05$) phosphorus excretion in the urine of animals fed supplemental calcium when compared with the cows on the control ration. The range of phosphorus excretion determined by Lomba et al. (1969) was from 0.3

to 30.5 g/day with a mean of 6.6 g/day in the lactating cow. The phosphorus mean excretion rates of the control group (8.84 g/day) and the calcium treated animals (6.53 g/day), were well within the normal range. There was no significant difference (p > .05) between groups for phosphorus secretion into the milk. The average amount secreted by the control group was 15.32 g/day and that of the calcium treated group was 14.31 g/day. The mean value (10.4 g/day) presented by Lomba et al. (1969) was low but the range (3.2 to 21.4 g/day) covered the levels encountered in the present trial.

The potassium levels in the milk and urine varied widely within treatments but there was no difference (p > .05) in the mean quantities of the two treatments. The range of milk potassium losses quoted by Paquay et al. (1969) vary from 10.9 to 33.0 g/day with a mean of 23.7 g/day. The average potassium losses of the control and calcium treated animals, 32.09 and 33.20 g/day respectively, were at the high end of the range most likely due to the high dietary content of potassium. As the urine is the primary route for potassium excretion (Miller, 1975) it was not surprising that almost 60% of the dietary potassium intake appeared in the urine. The average daily excretion of potassium by animals on the control diet was 247.40 g (59.18% of intake) and the amount for the calcium treated cows was 256.20 g (59.82% of intake).

The average daily secretion of magnesium into the milk for the control animals (2.56 g/day) did not differ (p > .05) from the amount secreted by the animals on the calcium treatment (2.51 g/day).

The range of values reported by Lomba et al. (1968) for magnesium

losses to the milk was 0.62 to 2.83 g/day with a mean of 1.85 g/day.

While these authors indicated there was a nonsignificant correlation

between digestible magnesium and milk magnesium, they implied a shift

in magnesium intakes most likely would influence the milk magnesium

content. The mean intake levels that were reported were half of the

average for animals on this experiment which would justify the added

secretion into the milk. The urinary magnesium was also reported to

be significantly correlated with magnesium intake. Again, the mean

daily urinary excretions of magnesium for the animals on the present

trial (3.96 g/day for the control, 3.44 g/day for the calcium

treatment) were slightly higher than that derived by Lomba et al.

(1968) of 2.9 g/day.

There was no difference (p > .05) in milk or urine sulfur con-

tents when expressed as a total daily output. Jenny and O'Dell (1979)

demonstrated the urinary sulfur excretion in dairy steers to be bet-

ween 13.4 and 15.1% of the dietary intake levels. The results

recorded in Table 10 amount to approximately 14% of the dietary intake

for the animals on both treatments. The major difference appears to

be in the amount of sulfur absorbed as there was about 1½ times

greater absorption of sulfur by the lactating dairy cows than by the

dairy steers studied by Jenny and O'Dell (1979). This sulfur was most

likely deposited in the milk.

Feeding higher levels of calcium to postpartum dairy cattle

resulted in a highly significant (p < .01) increase in the percentage

calcium retained. The animals on the control ration were in a defi-

cit calcium balance requiring an average of 18.6% more calcium to meet

all their physiological requirements. Presumably, these animals would

attain a positive balance once the heavy lactation stress was removed.

Braithwaite (1974) indicated calcium retention would be increased for

up to fifteen weeks after animals were able to absorb adequate calcium

such that body stores could be replenished. After this period, reten-

tion returned to near zero. The calcium treated cows were in a posi-

tive balance (14.5% apparent retention) which would infer these

animals were stressed early in their lactations and were in the pro-

cess of rebuilding body stores.

The phosphorus apparent retention also increased from a mean

of 3.1% to a mean of 10.3% (p < .05) with the addition of calcium to the

feedstuff. As the apparent absorption was not influenced, but the

serum phosphorus levels and urinary phosphorus levels were lower, this

would support the hypothesis of Braithwaite (1975). It is evident the

increased dietary calcium was sufficient to cause a net uptake

of calcium into the bone which prevented the release of phosphorus.

Potassium, magnesium and sulfur did not vary in apparent

absorption, milk secretion or urine excretion levels so the apparent

retention itself also did not change (p > .05). In this case,

increased dietary calcium had no effect on these mineral elements.

Interpretation of the trace element apparent retention values

remain gaurded as the concentrations in the milk and urine were

unattainable. Mills and Williams (1971) stated that copper, zinc and manganese urinary output represents only 1 - 2% of the total of each mineral excreted. Kirchgessner (1965) claimed the milk secretion rates were only 1 - 3% of the dietary intake and the above minerals were retained at a rate of 10 to 20%. Iron losses to the milk and urine are both less than 0.5% of dietary levels (Underwood, 1977). This author also quoted the majority of molybdenum and selenium excretion in ruminants appeared in the feces unlike monogastrics whose main route of excretion for these two minerals was the urine. While the apparent digestibility rates of the trace minerals are an overestimation of the retention of these elements, they most likely reflect a trend.

The implications from such a study as this can be extended towards dairy cattle productivity. Besides obvious parameters which affect growth, maintenance, milk production and fertility, many factors have more subtle effects depending on their association with other dietary ingredients. The slight alterations in some of the minerals apparent absorptions and serum concentration would most likely have their greatest influence on fertility. Valyuskin (1974, cited by Hideroglou, 1979) monitored feedstuffs fed to cows over a period of months. The author noted trace element levels in the feed altered those in the blood which caused a response in fertility.

Few reports consider the effect of increased calcium on reproduction. Swenson et al. (1962) added excess calcium to non-legume diets fed to dairy cattle and noticed inhibited ovulation and estrus

cycling. By adding trace elements to the diet, fertility improved. The calcium to phosphorus ratio has been studied by Littlejohn and Lewis (1960) and Parker and Blowey (1976), both of whom concluded that over a wide range the ratio appeared not to affect fertility. Moderately low (40 ppm) dietary manganese had no obvious effect on fertility unless combined with a high calcium to phosphorus ratio whereby fertility was depressed (Hignett, 1960). No definite conclusions can be drawn from this experiment as to the effects of increased calcium intakes on fertility.

Several inferences can be made on the possible alterations in fertility brought about with the change in dietary calcium levels. Hunter (1977) noted changes in the plasma inorganic phosphate and blood glucose concentrations around mating time appeared to produce the greatest effect on fertility. With lower serum phosphorus concentrations in animals on the calcium treatment, the possibility of this occurring would be greater. Both serum copper and serum zinc levels were increased with the addition of calcium carbonate. Copper levels in the blood were studied by Hignett (1960) and Littlejohn and Lewis (1960). No significant relationship was apparent between copper in the blood and reproductive performance. However, low fertility in dairy cattle indicative of delayed or depressed estrus has been shown to be due to copper deficiency (Underwood, 1977). Manickam (1977, cited by Hideroglou, 1979) reported significatly higher sera levels of copper and zinc as well as iron and manganese in regular breeder cows versus repeat breeders. These results

favour the animals on the calcium treatment. Whether the lack of additional calcium carbonate had a true influence on the level of serum selenium is unknown. Such a deficiency has resulted in impaired reproductive performance in all species studied (Underwood, 1977).

Increasing the recommended calcium content of dairy cattle rations from 0.7% to 1.5% by adding supplemental calcium carbonate may improve the fertility of the animals. This may be brought about by increasing the level of copper and zinc and possibly selenium in the serum. The higher level of dietary calcium would also adjust the calcium to phosphorus ratio closer to the range Kendall et al. (1970) recommended when considering the needs of parturient cows.

SUMMARY AND CONCLUSIONS

This study was initiated to determine if it was possible to increase the amount of calcium absorbed by postparturient dairy cattle under the stress of peak lactation. It was also of prime interest to discover any detrimental effects of such an increase. It was determined that by increasing the calcium content of the diet to approximately twice the recommended level, the apparent absorption of calcium rose from a low level to near the maximum possible. This led to an increase in calcium apparent retention such that the animals went from a negative to a positive calcium balance. The dietary interactions were confounded due to the form of the calcium added to the diet. However, in many cases it was possible to separate out the effects of the additional calcium such that conclusions could be drawn.

Firstly, there was no alteration in the ad libitum intakes of the diets or were there any change in the digestibilities of the organic constituents. Neither milk production, body weight change nor plasma glucose were affected by the treatment. Addition of calcium had no gross physical or physiological effects on the cows over the duration of the trial. The one minor physical variation which occurred as a result of the supplemental calcium carbonate was the inferred increase in intestinal pH.

Of the six minerals which exhibited differences in apparent absorption and/or serum concentration, three agree well with the documentation in the literature. The increased calcium intakes were reflected by a rise in serum calcium levels. The zinc digestibilities

were lower due to an interaction with calcium in the intestine.

However, the serum values of zinc were higher most likely because of a

metabolic interaction with the calcium at the tissue level. Although

phosphorus digestibilities were apparently unaffected by the extra

calcium, the serum concentrations were lower as was the urinary excre-

tion rate. This was a result of increased deposition of calcium which

led to a lower mobilization of phosphorus from the bone.

The remaining element interactions are less clearly defined.

The effect on copper digestibility was opposite that reported by other

workers. The apparent digestibility increased probably due to the

contamination of the limestone with copper. It is possible part of

this increase was due to the decreased availability of the zinc. The

increased copper digestibility most likely was responsible for the

rise in the serum copper levels. Similarly, iron apparent digestibi-

lity may have been affected by the contaminant iron in the limestone

or by the decreased zinc availability. The effects of the treatment on

molybdenum remains undetermined as it is unknown whether calcium or copper

was responsible for the decrease in apparent digestibility. Further

work is required to elucidate these matters.

Equally as important to the evaluation of the main effect are

the minerals which were unaffected by the treatment. Both phosphorus

and magnesium absorptions remained constant despite reports of these

elements closely interacting with calcium in the digestive tract. It

was concluded the main sites of absorption of these two minerals must

be prior to the middle to lower small intestine otherwise precipita-

tion of the calcium and magnesium phosphates would have resulted in lower apparent absorptions. Also, the implied alteration in the pH level of the digesta probably was not a major factor governing the availabilities of calcium, magnesium or phosphorus. The increased calcium to phosphorus ratio appeared not to influence the absorption of the minerals but may have been responsible for the decrease in phosphorus excretion into the urine and the lower serum phosphorus levels. The increase in dietary calcium had no effect on potassium, magnesium or sulfur. Serum selenium concentrations were higher in the animals on the treatment diet but it is doubtful whether calcium was responsible. The effects of calcium carbonate on the apparent digestibility, serum concentration and apparent retention of dietary minerals in the present experiment are summarized in Table 12.

Two secondary effects were studied as well. These were the influence of breeds and of reproductive cycling on serum constituent levels. Phosphorus and copper were in higher concentration in the serum of Ayrshire cows than in that of the Holsteins. These two minerals were also shown to fluctuate with the reproductive hormones as were zinc and iron. Any study dealing with comparisions of serum mineral levels should account for these factors.

The long term effects of such a treatment as this can only be postulated. Calcium supplementation resulted in interactions with both macro and trace minerals. As a result, it would be expected that altered metabolism may eventually show up most likely manifesting itself in the fertility and reproduction of the cows. A study on this

Table 12

Summary of the Effects of Calcium Carbonate on the
Apparent Disgestibility, Serum Concentration and
Apparent Retention of Dietary Minerals

Mineral	Apparent Digestibility	Serum Concentration	Apparent Retention
Ca	+	++	++
P	0	--	+
K	0	0	0
Mg	0	0	0
S	0	0	0
Fe	+	0	N.A.
Cu	++	+	N.A.
Zn	-	++	N.A.
Mn	0	N.A.	N.A.
Mo	-	N.A.	N.A.
Se	0	+	N.A.

0	Unaffected
+	Rose Significantly ($p < .05$)
++	Rose Highly Significantly ($p < .01$)
-	Dropped Significantly ($p < .05$)
--	Dropped Highly Significantly ($p < .01$)
N.A.	Not Analyzed

aspect should be initiated to determine whether the long term effects are beneficial or detrimental.

This study has identified the fact that the NRC (1978) recommended dietary calcium levels were too low to maintain postparturient dairy cattle in positive calcium balance. It has also exhibited it possible to increase the level of calcium absorbed by raising the calcium content of the diet by 2 to 3 times the recommended rate. A suggested more beneficial level of calcium in the ration would be 1.5% with a calcium to phosphorus ratio of 3:1. The long term effects of such a treatment require further research.

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'F' Values of Serum Constituents for Related ANOVA Model Terms[+]

Y_{ijkl}	T_i	B_j	C_k	TB_{ij}	TC_{ik}	A_{ijkl}	M_{ijkl}	F_{ijkl}	E_{ijkl}
(d.f.)	(1)	(1)	(3)	(1)	(3)	(1)	(1)	(1)	(156)
Ca	21.2**	1.2	1.2	1.9	0.2	0.9	3.8*	0.4	
P	6.8**	6.8**	4.9*	0	0.2	0	1.5	2.8	
K	0.3	0.2	0	1.3	0.8	3.0	0.3	4.9*	
Mg	2.8	1.0	1.7	0	2.4	6.2*	0.2	7.4**	
S	2.6	0	2.0	0.3	0.2	0	0.1	12.2**	
Fe	0	0	2.6	0.6	1.3	8.2**	4.2*	0.4	
Cu	4.2*	9.5**	1.7	0.4	0.4	12.4**	4.6*	30.2**	
Zn	23.4**	1.5	1.5	1.7	2.0	1.3	0.8	4.5*	
Se	4.2*	13.9**	0.2	5.5*	1.6	6.3*	7.3**	1.1	

[+]See page 24 for explanation of terms.

Significantly different *(p < .05), **(p < .01)