EFFECTS OF MANIPULATING DIETARY CATION-ANION BALANCE ON CALCIUM METABOLISM IN SHEEP.

by

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Dedicated to:

My lovely wife, Andree,

and my adored children,

Mariko and Miki.

Abstract

Doctor of Philosophy Animal Science (nutrition)

Hiroshi Takagi

EFFECT OF MANIPULATING DIETARY CATION-ANION BALANCE ON CALCIUM METABOLISM IN SHEEP

Experiments were conducted to investigate the effect of reducing dietary cation-anion balance (CA-balance), defined as meq [(Sodium + Potassium)-(Chloride + Sulfur)], on calcium (Ca) metabolism. Reducing the dietary CA-balance from +314 to -22 (meq kg^{-1} dry matter (DM)) did not affect plasma Ca level but reduced Ca retention mainly due to a 10-fold increase in urinary Ca excretion with similar rate of apparent absorption. It also decreased urine pH. The response to induced hypocalcemia created by the infusion of 5.6% EDTA solution revealed that reducing dietary CA-balance from +354 to +37 (meq kg^{-1}DM) did not affect the volume of the compartment, within which there was a rapid equilibration of free Ca but tended to increase the rate of Ca mobilization from it during the infusion. The Ca kinetic study with a four-compartment model indicated that reducing dietary CA-balance from +338 to -127 (meq kg^{-1}DM) during the eucalcemic period and from +429 to -147 (meq kg^{-1}DM) during an EGTA-infusion period (simulated lactational Ca loss) caused hypercalciuria and increased ionized form of plasma Ca. Increased true intestinal Ca absorption and reduced bone accretion were observed in the lowest CA-balance diet only during the EGTA-infusion period. There were no differences in the size of total exchangeable Ca pool but amount of Ca movement between them tended to increase in reduced CA-balance diets during both periods. Results indicated that feeding reduced CA-balanced diet may have a beneficial role in preventing hypocalcemic parturient paresis (milk fever) by increasing Ca flux through the exchangeable Ca pool and Ca mobilization capability.

Résumé

Docteur en Philosophie Zootechnie (nutrition)

Hiroshi Takagi

EFFETS DE LA MANIPULATION DE LA BALANCE D'ANIONS-CATIONS ALIMENTAIRES SUR LE METABOLISME DU CALCIUM CHEZ LE MOUTON.

Des expériences ont été entreprises afin d'étudier l'effet d'une réduction de la balance d'anions-cations alimentaires (balance-CA), définie en meq $((Na^+ + K^+) - (Cl^- + S^=))$, sur le métabolisme du calcium (Ca). Une réduction de la balance-CA alimentaire de +314 à -22 (meq kg^{-1} matière sèche (MS)) n'a pas influencé le Ca plasmatique mais a cependant diminué la rétention du Ca surtout à cause d'une augmentation de 10 fois l'excrétion urinaire de Ca avec un taux similaire de l'absorption apparente. Le pH de l'urine a également été réduit. La réponse à l'hypocalcémie induite par l'infusion d'une solution de 4.6% EDTA a révélé qu'une réduction de la balance-AC alimentaire de +354 à +37 (meq kg^{-1}MS) n'a pas influencé le volume du compartiment dans lequel s'établissait une équilibration rapide du Ca libre mais tendait à augmenter le taux de mobilisation du Ca de ce compartiment pendant l'infusion. L'étude cinétique du Ca utilisant un modèle à quatre compartiments a indiqué qu'une réduction de la balance-CA alimentaire de +338 à -127 (meq kg^{-1}MS) pendant le période eucalcémique et de +429 à -147 (meq kg^{-1} MS) pendant la période d'infusion de EGTA (simulant la perte de Ca pendant la lactation) causait une hypercalciurie et augmentait la forme ionique du Ca plasmatique. Une augmentation de l'absorption intestinale réelle du Ca et une diminution de l'accrétion osseuse ont été observés avec la diète ayant la balance-CA la plus

faible seulement pendant la période d'infusion d'EGTA. Les dimensions des compartiments de Ca total échangeable n'étaient pas différentes. Cependant la quantité de mouvements du Ca entre les différents compartiments tendait à augmenter avec les rations contenant une balance-CA réduite pendant les 2 périodes.

Les résultats indiquent que l'alimentation avec une ration contenant une balance-CA réduite par la supplémentation de divers sels minéraux peut avoir un rôle bénéfique dans la prévention de la fièvre vitulaire (fièvre du lait) en augmentant le flux de Ca dans le compartiment de Ca échangeable et en augmentant la capacité de mobilisation du Ca.

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I. GENERAL INTRODUCTION

Proper balancing of dietary fixed cation and anion (non-metabolizable ion by body) is important in formulating rations in ruminants (Block, 1988) as well as in other species (Mongin, 1981). Dietary cation-anion balance, defined as the summation of meq sodium (Na^+) and potassium (K^+) minus the summation of meq chloride (Cl^-) and sulfur ($S^=$) present in diet, can play an important role in ruminant nutrition through its effects on acid-base status and production parameters (Wheeler, 1981; Block, 1988). There is also a large variation that exists in the concentration of these ions among feed stuff (Wheeler, 1981). Furthermore, changes in dietary cation-anion balance can be achieved by either supplementation of mineral salts that contain both fixed anion and cation or as acids or alkali solutions that contain Na or K associated with anions that are either not fixed (i.e. metabolizable) or unavailable to animals.

The effect of changing dietary cation-anion balance on major mineral metabolism especially calcium (Ca) in ruminants has been investigated in detail only in two study with goats (Fredeen, 1984) and most of studies conducted were based only on conventional balance methods (Ender *et al.*, 1971; Lomba *et al.*, 1978). These results, however, led to develop a concept that feeding rations that contained a reduced cation-anion balance to prepartum cows during the dry period might be used as a preventative method for hypocalcemic parturient paresis (milk fever) and several studies have been conducted

to investigate its prophylactic effects againist milk fever (Ender *et al.*, 1971; Dishington, 1975; Dishington and Bjomstad, 1982; Block, 1984; Leclerc, 1986). However, very little is known about the effects of manipulating dietary cation-anion balance achieved by supplementing various mineral salts as a source of supplying fixed anions (Cl and/or S) on not only Ca metabolism but also on the interaction between metabolism of major mineral (Ca, phosphorus [P], magnesium [Mg], Na, K, Cl, and S) in ruminants. The effect(s) of reduced cation-anion balanced diets on Ca metabolism may involve the following; kidney function, gastrointestinal absorption, acid-base status, and bone metabolism.

The present study was undertaken to investigate the effect of feeding reduced cation-anion balanced diets achieved by supplementing various mineral salts to provide excess amount of anion-forming elements (Cl and S) compared to that of cation-forming elements (Na and K) to sheep in relation to;

- major minerals metabolism,
- plasma concentrations of major minerals,
- acid-base status,
- Ca mobilization rate during induced acute hypocalcemia,
- comparison with vitamin D injections,
- kinetics of Ca metabolism during eucalcemic and simulated lactational Ca loss.

II. LITERATURE REVIEW

1. INTRODUCTION

Calcium (Ca) is necessary for the integrity of cell membranes and is essential for maintaining normal function in muscle, nerve and secretory organs. For this reason, extracellular Ca is maintained within very narrow limits by endocrine systems concerned with Ca homeostasis. These regulatory systems basically involve the following three messengers; parathyroid hormone (PTH), thyroid calcitonin (CT) and vitamin D, particularly 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). These messengers are used by three target organs, namely the gastrointestinal tract, bone and kidney.

2. Calcium metabolism

2.1. Calcium absorption

2.1.1. Site of absorption

It is generally accepted that in the ruminant the majority of dietary Ca as well as phosphorus (P) is absorbed from the upper portion of the small intestine as evidenced by a decrease of Ca concentrations in digesta found in the upper small intestine (Smith, 1969; Ben-Ghedalia *et al.*, 1975; Yano *et al.*, 1979). Phillipson and Storry (1965) reported that the epithelium of the rumen appears to be relatively

impermeable to Ca even when Ca was in concentrations six times of those normally found in the rumen. However, others suggested that these elements were absorbed in considerable amounts before reaching the duodenum, especially in young animals (Perry *et al.*, 1967; Cragle, 1973; Grace *et al.*, 1974). No Ca absorption was observed in the duodenum or in the terminal ileum (Phillipson and Storry, 1965; Ben-Ghedalia *et al*, 1975). However, this finding of no net absorption of Ca in the duodenum must be an artifact created by Ca secretions in bile and pancreatic juice (Cragle, 1973). In contrast to single-stomach species, the ruminant has a considerably high endogenous secretion of Ca from the large intestine resulting in masking, quantitatively, net absorption of Ca when measured by a balance study (Kenny, 1981a).

2.1.2 Mechanism of absorption

As proposed by Wasserman and Taylor (1969), Ca absorption from the small intestine occurs by two processes; the first process is a saturable transmural movement in nature and is subject to physiological (Pansu *et al.*, 1983b) and nutritional regulation (Armbrecht and Wasserman, 1976); the second process is thought to be a linear function of the luminal concentration of Ca and appears to be independent of age and/or Ca intake (Pansu *et al.*, 1983a). The above mentioned processes (saturable active transport and non-saturable diffusive) were observed in non-ruminants but probably also

operate in ruminants (Braithwaite, 1976). It has been well established that there is an active absorption process in all of the major segments of the intestine (Kimberg *et al.*, 1961; Urban and Schedl, 1969; Pansu *et al.*, 1981; Schachter and Kowarski, 1982). This process is exhibited to the greatest extent in the duodenum and decreases as the distance from the pylorus increases (Kimberg *et al.*. 1961). Thus, no detectable active absorption of Ca was observed in the distal ileum (Pansu *et al.*, 1983b). Walling and Rothman (1969) conducted a kinetic study and concluded that an active transport system became saturated at an intestinal luminal concentration of Ca at approximately 3 to 5 mmol.

This active transport process involves a carrier protein, termed Ca-binding protein (CaBP), discovered by Wasserman and Taylor (1966) that preferentially binds to Ca. CaBP has a molecular weight of 28,000 dalton and is located in the goblet cells and in the surface coat-microvillar region of all intestinal epithelial cells ; its existence in ruminants was also confirmed (Wasserman and Taylor, 1968: Fullmer and Wasserman, 1973). The amount of CaBP found in the different segment of the intestinal mucosa reflected the site of active absorption of Ca (duodenum>jejunum>ileum) (Taylor and Wasserman, 1967) and is thought to be a direct reflection of the amount of circulating $1,25(OH)_2D_3$ (Behar and Kerstein, 1976), which is the hormonally active daughter metabolite of the vitamin D_3, thus a reflection of vitamin D_3 status of animals (Bronner, 1982).

The second absorption process of Ca, the diffusive

process, is found to act along all segments of the intestine (Pansu *et al.*, 1983b). There seems to be species differences in the nature of saturation of this process measured *in vivo*. There was no saturation level in rat and chicken (Wassermann and Kallfelz, 1962) whereas saturation at 1.2mM in the dog (Cramer and Duck, 1962) and at approximately 40mM in sheep (Cramer and van'tKlooster, 1965) were observed using chronic *in vivo* preparation (Thiry-Vella loop).

Therefore, a relationship of the two absorption processes would be that at lower intraluminal Ca concentrations saturable transfer is relatively greater than the diffusion process. The proportion absorbed by diffusion becomes greater as luminal Ca concentration in the intestinal segments is increased.

The amount of Ca absorbed by the intestine decreases with an increase in age (Hansard *et al.*, 1957; Braithwaite and Riazuddin, 1971; Ghishan *et al.*, 1980; Kenny, 1981a; Pansu *et al.*, 1983a). Hansard *et al.* (1954) found in cattle that the percent of Ca absorption decreased with advancing age being 95% at one month and declining to a value of 23% at 12 to 16 years of age. Absorptive efficiency of Ca did not change with pregnancy but during the latter part of pregnancy the intestinal absorption of Ca became much more important quantitatively than the resorption of the maternal bone in meeting the high demands for Ca (Ramberg *et al.*, 1970; Hove and Hilde, 1984). Lactation studies showed an increase in both amount of Ca absorption and efficiency of

absorption; efficiency rose from 22.6% prepartum to 34% during 8 to 14 days postpartum and as lactation proceeded the intestinal response declined as bone resorption takes over the role of meeting increased demand of Ca (Mayer *et al.*, 1969b; Ramberg *et al.*, 1970).

2.2. Calcium excretion

Caicium is lost from body tissues by excretion into the intestine and in the urine. Losses into the intestine occur largely via pancreatic juices and bile and should be absorbed at the same efficiency once mixed with dietary Ca. But lack of correlation between endogenous loss of Ca in the feces and Ca intake or total fecal Ca suggests that some discrimination may exist (Braithwaite, 1974; 1979). Although the rate of excretion of endogenous Ca was found to increase with increasing age, when corrected for body weight (BW) its value remained remarkably constant between 10 and 20 mg d^{-1} kg^{-1}BW (ARC, 1980) and was affected neither by the amount of Ca ingested or absorbed (Braithwaite, 1974; 1979; 1981). Recently, Braithwaite (1982) re-examined the relationship of endogenous Ca loss and feed intake in sheep and demonstrated an existence of a highly significant linear relationship. Endogenous Ca loss increased by the rate of 0.64 mg d^{-1} kg^{-1}BW for each 1 g d^{-1} kg^{-1}BW increase in feed intake.

Urinary excretion of Ca in ruminants is generally low (ARC, 1980) compared to that of non-ruminants who tend to

absorb more Ca than needed and consequently excretes the excess in the urine (Schyver *et al.*, 1974). The apparent transport maximum for Ca of 1.65 mmol L^{-1} glomelular filtration (GF) observed in ruminants (Hove *et al.*, 1983) was similar to that for man (1.88 mmol L^{-1} GF) calculated by Sutton and Dirks, (1978). However, the Km value for reabsorption, which indicates the Ca concentration in ultrafiltrate that is required to half-saturate the transport mechanism, was much lower than the corresponding value in man leading to the high rate of Ca reabsorption that characterizes the ruminant kidney (Hansard *et al*, 1954).

Urinary excretion of Ca in ruminants was not affected by changes in the rate of Ca intake or absorption. However, Braithwaite (1979) reported that there was a tendency towards high values with increases of P intake. This hypercalciuria associated with P depletion was also observed in man as well as in experimental animals (Lotz *et al.*, 1968). Riborich and DeLuca (1975) suggested that an unidentified hormonal factor that may be present in both blood and kidney may be contributing to the impairment of Ca reabsorption in phosphate depletion. Hove *et al.* (1983) demonstrated a direct positive correlation of urinary excretion of Ca to the total Ca level in the plasma. They calculated that an elevation of plasma Ca by 2.2 to 2.7 mmol would cause a 20-fold increase in urinary Ca excretion (ranged from 1 to 2 g d^{-1}). Urinary Ca excretion is influenced by the secretion rate or circulating blood level of PTH (Toverud and Boass, 1979) and vitamin D (Sutton and Dirks, 1978). Mayer *et al.*,

(1966) reported a decrease in urinary Ca excretion rate when cows were hypocalcemic and had an increased level of circulating PTH.

2.3. Skeletal metabolism

The skeleton contains 98% of the body's Ca where it is held in two forms within the soft and fibrous organic matrix; a crystalline form of Ca phosphate that resembles the mineral hydroxyappatite and a non-crystalline or amorphous Ca phosphate form (Koo and Tsang, 1984). It appears that the amorphous material is predominant in early life and is superseded by crystalline appatite in later life (Kunkel *et al.*, 1986). The rate of accretion of Ca into bone is high in young animals but decreases markedly in the first few months of life to a low level at maturity (Braithwaite and Riazuddin, 1971). There is a small amount of Ca carbonate present, which probably lies on the crystal mineral surface of the appatite phase (Barone, 1981). A change in skeletal retention of Ca, which is a combination of the rate of bone resorption and bone accretion, reflects changes in total body Ca retention. Since bone accretion remains relatively constant in animals of a given age, it is bone resorption that is largely responsible for Ca homeostasis (Braithwaite and Riazuddin, 1971; Braithwaite, 1975). At the onset of lactation, there is an increase in bone resorption to meet the additional Ca requirement for milk production often resulting in net loss of Ca (Edner *et al.*, 1971). Kinetic

studies, however, indicate that the increase in bone resorption at parturition occurs only when Ca intake during the prepartum period is low, and an increase in intestinal absorption is used to meet the additional Ca requirement only when animals are fed a high Ca diet (Ramberg *et al.*, 1970). Only a small fraction of the stored Ca in the body is rapidly exchangeable with the ionized form of blood and soft tissue Ca; the size of the exchangeable Ca pool of ruminants decreases markedly with increasing age and appears to be related to the rate of bone accretion (Braithwaite and Riazuddin, 1971). Ramberg *et al.*, (1970) reported that there was a temporary decrease in the size of this Ca pool at the onset of lactation, which indicated that this pool must have some part in supplying the additional demand for Ca in cows at parturition.

3. Calcium regulation by calcitropic hormone

3.1. Vitamin D and its metabolites

As early as 1950, documentation exists showing that vitamin D is an essential factor for the optimal absorption of Ca from the intestine (Nicolaysen *et al.*, 1953). In the past decade, technological advances have led to a new understanding of vitamin D metabolism. There are two major sources of vitamin D in all animals; photochemical conversion of 7-dehydrocholesterol to vitamin D_3 and/or ergosterol from plants to vitamin D_2. Dietary supplementation with crys-

talline vitamin D is also commercially available.

Vitamin D undergoes several major important metabolic conversions and produces at least 20 vitamin D metabolites (Norman *et al.*, 1982). In ruminants, metabolism of vitamin D by rumen organisms may involve a detoxification process, particularly when a large quantity of vitamin D is administered orally (Sommerfeldt *et al.*, 1981). The first metabolic conversion of vitamin D_3 is its hydroxylation by vitamin D_3-25-hydroxylase to form 25-hydroxyvitamin D_3 ($25(OH)D_3$) (Horst and Reinhardt, 1983). This process usually occur in the liver, although a small amounts of the enzyme was detected in other organs (Olson *et al.*, 1976). This conversion takes place in the microsomes as well as in the mitochondria of the hepatic cells. Fukushima *et al.* (1978) proposed that a fast, high affinity (Km=5.6 mmol) and low capacity microsomal enzyme reaction was responsible for 25-hydroxylation of physiological levels of vitamin D (<2.5 mmol) and that superphysiological or toxic levels triggered a low affinity (Km=1.000 mmol) and high capacity mitochondrial enzyme reaction. The activity of hepatic vitamin D_3-25-hydroxylase seems to be loosely controlled, at least under chronic conditions (Kenny, 1981b) with species differences being observed (Hollis *et al.*, 1977; Horst and Reinhardt, 1983). Hollis *et al.* (1981) observed the inhibitory action of vitamin D_3-25-hydroxylase by vitamin D_3 in the cow by injecting 15×10^6 IU of vitamin D_3 whereas no such control action was observed in pigs.

After another hydroxylation by $25(OH)D_3$-1-hydroxylase,

$1,25(OH)_2D_3$ is formed mainly in the kidney from $25(OH)D_3$ (Marx *et al.*, 1982). This enzyme was found almost entirely located in mitochondria in the renal cortex, although 1-hydroxylase activity was found elsewhere (Koo and Tsang, 1984).

Another enzyme $25(OH)D_3$-24-hydroxylase was also located in kidney , which forms 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) from $25(OH)D_3$ (Horst and Reinhardt, 1983). There seems to be a reciprocal relationship between 1-hydroxylase and 24-hydroxylase products; when one is being synthesized and released, production of the other is suppressed (Boyle *et al.*, 1971). The biological function of $24,25(OH)_2D_3$ is not well understood but Smith *et al.* (1982) reported an inverse relationship between serum Ca and $24,25(OH)_2D_3$ levels in hypocalcemic dairy cows.

Metabolic conversion of vitamin D must occur before it becomes functionally active and at least several vitamin D_3 metabolites ($25(OH)D_3$, $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$) may exert biological effects. However, it is generally agreed that $1,25(OH)_2D_3$ is the major hormonally-active metabolite at physiological concentrations (Koo and Tsang, 1984). The major target organs for $1,25(OH)_2D_3$ are intestine, bone and kidney but receptors for $1,25(OH)_2D_3$ have been found in other tissues such as the parathyroid gland (Wecksler *et al.*, 1980). The biogenesis of $1,25(OH)_2D_3$ is regulated by the strong feedback control by the need for Ca (Friedlander *et al.*, 1977; Green *et al.*, 1981; Pansu *et al.*, 1981) or by the need for P (Fox *et al.*, 1978). In states of Ca and P deple-

tion and under the stimulation of PTH, the activity of the 25(OH)D_3-1-hydroxylase in the kidney is increased and 1,25$(OH)_2D_3$ production is facilitated (Fraser and Kodicek, 1973).

There is a modest yet significant increase in 1-hydroxylase activity under dietary P depletion. However, P depletion may play a more important role in the actual binding of 1,25$(OH)_2D_3$ to the intestinal mucosa tissue since phosphate depletion elevates the intestinal levels of CaBP even in the presence of exogenous 1,25$(OH)_2D_3$ (Putkey and Norman, 1982). On the other hand, in states of Ca and P sufficiency, 24,25$(OH)_2D_3$ is produced predominantly (DeLuca, 1979). A sharp rise in the enzymatic capacity for production of 1,25$(OH)_2D_3$ is associated with a reduced blood Ca level and this increase in the activity of 25(OH)D_3-1-hydroxylase may be via the secretion of PTH; the parathyroid gland perceives hypocalcemia and secrets PTH to stimulate 25(OH)D_3-1-hydroxylase production in the kidney, among its other functions (Koo and Tsang, 1984).

1,25$(OH)_2D_3$ acts in the small intestine to increase Ca absorption, particularly by the active transport process (Omdahl and DeLuca, 1977; Pansu *et al.*, 1981; DeLuca *et al.*, 1982; Yeh and Aloia, 1984). There is considerable evidence to support the claim that vitamin D_3 also enhances passive transport by increasing permeability of the intestinal mucosa cell to Ca (Bronner, 1982; Yeh and Aloia, 1984). The stimulative actions of 1,25$(OH)_2D_3$ on intestinal active transport of Ca are suggested as follows: 1,25$(OH)_2D_3$ binds

to a cytosolic receptor of intestinal cells and the $1,25(OH)_2D_3$-receptor complex moves intranuclearly where DNA-directed mRNA synthesis is initiated; the mRNA or mRNAs move to the endoplasmic reticulum where polyribosomal translation and protein synthesis are initiated (Wasserman, 1981; Pike, 1985). It is assumed that protein or proteins synthesized are directly involved in Ca transport and CaBP, is one of these proteins (Kenny, 1981a). At a steady state, the relationship between intestinal levels of CaBP and the circulating levels of serum Ca and P have been endocrinologically established (Putkey and Norman, 1982). Vitamin D_3 also has an influence on Ca-dependent ATP and alkaline phosphate activity, which may be involved in Ca transport across membrane, located in either the brush border and/or basal lateral membrane of the intestinal cells (Kumar, 1984). Regardless of the mechanisms, without vitamin D_3 the intestinal absorption of Ca as well as P diminish (Harrison and Harrison, 1961; Wasserman and Taylor, 1973; Napoli *et al.*, 1982).

Vitamin D_3 metabolites are also known to influence the regulation of both bone resorption and mineralization, particularly $1,25(OH)_2D_3$, which is a potent stimulator of bone Ca resorption (Holick *et al.*, 1976) and also $24,25-(OH)_2D_3$ (Tam *et al.*, 1986). Bone resorption is necessary for skeletal growth and remodeling and for the regulation of blood Ca and P concentrations. Holtrop *et al.* (1981) reported that $1,25(OH)_2D_3$ increases the number and activity of osteoclasts, which is the characteristic cell localized in regions of ac-

tive bone resorption in the absence of PTH. Marie (1982) reported that the injection of $1,25(OH)_2D_3$ resulted in increased the rate of efflux of Ca from bone and caused to stimulate the activity of existing osteoclasts without increasing serum Ca. He also found that Ca released from bone was immediately utilized for new bone mineralization. Ramp *et al.* (1980) reported that vitamin D deficiency reduced net Ca efflux and increased net magnesium (Mg) efflux from the bone. Recently Chambers *et al.* (1985) suggested that $1,25(OH)_2D_3$ acts via action of prostaglandin (PG).

In contrast to the above findings, Braithwaite (1980) suggested that the major action of $1,25(OH)_2D_3$ was to increase the rate of bone accretion and to decrease the rate of bone resorption since animals injected with vitamin D_3 showed an increase in total body retention of Ca reflecting an increase in skeletal retention. This increased rate of bone accretion has been reported in man and in lactating ewe treated with 1-alpha-OH-D_3 (Pierides *et al.*, 1976; Braithwaite, 1978). Recently, Kurihara *et al.* (1986) reported that $1,25(OH)_2D_3$ had a direct specific anabolic effect on osteoblastic cells *in vitro* during the growth phase and that this effect was related to receptor concentrations.

In the kidney, 99% of filtered Ca is normally reabsorbed in the absence of vitamin D. The remaining 1% is usually under the direct control of vitamin D (DeLuca, 1978). Thus, vitamin D contributes a small but significant component in renal Ca absorption. Receptors for $1,25(OH)_2D_3$ were found in both proximal and distal nephrons in the rat and the hormone

enhanced the tubular reabsorption of Ca as well as sodium (Na) and P (Puschett *et al.*, 1972). However, Sutton and Dirks, (1978) suggested that the effects of vitamin D_3 on renal Ca handling are variable and may depend, in part, on the prior vitamin D status of the animals. Tubular Ca reabsorption has been shown to increase 48h after the administration of vitamin D to vitamin-D deficient rat. In vitamin-D repleted humans, $1,25(OH)_2D_3$ has been shown to increase Ca excretion before any change is observed in plasma Ca or PTH level (Brickman *et al.*, 1981). Also $25(OH)D_3$ was found to enhance Ca reabsorption independent of both Na reabsorption in the distal nephron and the presence of PTH (Puschett *et al.*, 1972; Sutton *et al.*, 1977).

3.2. Parathyroid hormone (PTH)

Physiologically, PTH is the most important regulator of extracellular Ca concentration (Koo and Tsang, 1984). Secretion of PTH in response to a lowering of serum Ca concentration is rapid , and together with the short half life (20 to 30 min) of circulating PTH this hormone is thought to be of major importance in the minute to minute regulation of blood Ca concentration (Ramp and Waite, 1982).

PTH indirectly stimulates intestinal absorption of Ca and P through stimulation of 25(OH)-1-hydroxylase activity and in an elevation of $1,25(OH)_2D_3$ production (Goff *et al.*, 1985b). Pang *et al.* (1980) also suggested that potent vasoactive properties of PTH that influence hemodynamics

requires consideration of the possibility that PTH may influence intestinal Ca absorption by influencing blood flow through the intestine.

PTH also enhances the bone resorption process, "osteocytic osteolysis", which may be responsible for a rapid movement of Ca from bone to extracellular fluid (Vaes, 1968), and may also inhibit bone mineralization directly by its action on osteoblasts (Silve *et al.*, 1981); both of these result in an increase of Ca concentrations in blood. Garabedian *et al.* (1974) suggested that PTH and $1,25(OH)_2D_3$ probably function together as physiologic synergists in stimulating bone resorption by acting at separate receptor sites in the bone resorbing cells. Thus, he concluded that the mobilization of Ca and P from bone requires both $1,25(OH)_2D_3$ and PTH.

PTH inhibits the proximal tubular reabsorption of Ca, P, bicarbonate and Na (Puschett, 1978). However, the ultimate urinary concentrations of these ions revealed a decrease in Ca excretion , an increase in P excretion and minimum to negligible effects on Na and bicarbonate excretion (Ramp and Waite, 1982). Therefore, reabsorption of these ions at sites distal to the proximal tubules in response to PTH causes different total excretion output. While Na and bicabonate does not respond to PTH, PTH inhibits reabsorption of P (Puschett, 1978); the reabsorption of Ca which is inhibited by PTH in the proximal tubes is stimulated by the PTH at distal sites resulting in a decreased loss of Ca in the urine (Ramp and Waite, 1982).

The net effect of PTH is the elevation of serum Ca and lowered P concentrations. Major physiological stimuli for increasing the secretory rate of PTH are Ca, Mg as well as beta-adrenergic agents and cyclic-AMP (Ramp and Waite, 1982). Among the regulators of PTH, Ca is the most potent, particularly in its ionized form in blood (Fisher *et al.*, 1973). Mayer *et al.* (1975) reported that the secretory rate of PTH was only modestly influenced by changes in blood Ca levels above 10 mg dL^{-1}, however, below 10 mg dL^{-1} decrements in blood Ca profoundly influenced PTH secretion. Goff *et al.* (1985a) reported that the continuous infusion of synthetic bovine PTH stimulated renal conservation of Ca, renal 1-alpha-hydroxylation of $25(OH)D_3$ and bone resorption in pregnant cows even in the presence of slight hypercalcemia. Mayer and Hurst (1978) reported that the PTH secretion was most pronounced when plasma Ca was in the mildly hypocalcemic range with a relatively constant basal secretion of PTH in normocalcemia and little or no additional PTH secretion occurred when plasma Ca was less than 7.5mg dL^{-1}, thus resulting in a sigmoidal type of PTH secretion response to plasma Ca concentrations. Lack of effects of dietary P on PTH secretion was demonstrated by Sherwood *et al.* (1968).

3.3. Calcitonin (CT)

Another calcitropic hormones is calcitonin (CT) produced by the thyroid gland C-cells in response to hypercalcemia (Black *et al.*, 1973a). A 100-fold increase in circulating CT

was observed following an increase in blood Ca level from 10 mg to 14 mg dL^{-1} (Cooper *et al.*, 1971). Secretion of CT is also stimulated by gastrin, glucagon and cholecystokinin along with several other structural analogues of these hormones (pentagastin and cerulein) (Koo and Tsang, 1984).

Once CT has been secreted, the hormone acts on bone to modulate the rate of Ca and phosphate (PO_4) flux into and out of bone storage pools. Phosphate and CT are two physiological inhibitors of bone resorption and they function synergistically, probably by different mechanisms (Raisz and Niemann, 1969). CT powerfully inhibits osteoclastic bone resorption (Holtrop *et al.*, 1974) which may result in decreased mobilization of Ca and PO_4 from bone. In addition, it has been proposed that CT inhibits the efflux of Ca from the extracellular fluid (ECF) of bone into the circulation (Talmage *et al.*, 1980).

Action as well as target site of CT compared to that of PTH or antidiuretic hormone in kidney tissue are different (Marx and Aurbach, 1975). CT decreases renal tubular resorption of Ca and P as well as that of Na, potassium (K) and Mg (Koo and Tsang, 1984). The overall effects of CT in plasma is a reduction of Ca and P concentration. Recently, Mastui *et al.* (1984) reported in sheep that the infusion of CT stimulated Ca and P excretion via bile and P excretion via saliva. Thus, they concluded that hypophosphatemia induced by CT was largely due to the increment of salivary excretion of P.

4. Nutritional aspects affecting calcium metabolism

4.1. Dietary calcium

The efficiency of Ca absorption increases when animals have been maintained on a Ca deficient diet mainly via an increase in the saturable vitamin D-dependent active transport. It was demonstrated that if animals were fed Ca-deficient diets, CaBP content of their intestinal mucosa cells would increases as much as 100% if adequate supply of vitamin D is present (Pansu *et al.*, 1981b). This increase in the efficiency of absorption, which adapts to changes in luminal Ca concentration, would require at least several hours because it involves the synthesis of new protein (CaBP)(Allen, 1982). In addition to the Ca concentration of the luminal contents, studies indicate that the habitual Ca intake (Ca stasis) affects the efficiency of absorption so that a long-term adaptive mechanism is also operative (Benson *et al.*, 1969). Braithwaite (1979) reported using sheep that an increased intake of Ca from 40 to 100 mg d^{-1} kg^{-1} BW resulted in a reduction of efficiency of Ca absorption by intestine from 31.6 to 10.6%. Black *et al.* (1973b) demonstrated that feeding elevated levels of Ca (150 g d^{-1}) to prepartum dairy cows resulted in a reduction in efficiency of Ca absorption from the intestine accompanied by a reduction in the size of microvilli of absorptive cells as well as a reduction of length and surface areas of microvill compared to that of animals fed a recommended level of Ca

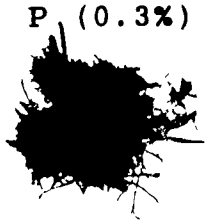
(25 g d^{-1}).

The intestine can adapt to habitually low Ca intake, therefore, it is commonly believed that dietary Ca deficiency *per se* does not lead to a dramatic increase in skeletal resorption (Allen, 1982). However, there seems to be a lower limit to Ca intake, below which Ca absorption even with an increased efficiency plus bone resorption cannot adapt sufficiently to maintain Ca balance leading to a negative Ca balance (Braithwaite, 1983a).

4.2. Dietary phosphorus

The close relationship between the skeleton and the metabolism of Ca and P was demonstrated by the findings that the amounts of Ca and P in the skeleton decreases when a diet low in P is fed (Kemm, 1976; Braithwaite, 1983b) and that P retention is controlled by the rate of Ca retention, which is itself directly related to Ca absorption. The depletion of P by feeding diets low in P leads to enhanced Ca transport by the intestine (Abdel-Hafeez *et al.*, 1982) possibly by an increased ability by the intestinal mucosa cells to accumulate circulating $1,25(OH)_2D_3$ (Horst *et al.*, 1978b; Sommerville *et al.*, 1978). Although, these studies did not definitively demonstrate an affect of P on $1,25(OH)_2D_3$ receptor concentrations, their results corresponded consistently with hypothetical increases in intestinal $1,25(OH)_2D_3$ receptors (Horst and Reinhardt, 1983). Feeding excess P (1.2% DM) with a relatively normal amount

of Ca (0.6% DM) to rats resulted in both an accelerated bone resorption and a increase in urinary Ca excretion compared to feeding a diet low in P (0.3%) to rats (Bell *et al.*, 1977).



4.3. Dietary magnesium

The effect of Mg on Ca metabolism is not consistent and depends upon many factors (Wasserman and Taylor, 1969). However, both Ca and Mg have similar effects on the regulatory hormone of Ca absorption as well as bone mineralization. Acute increases in plasma Mg stimulates CT secretion, although the effect of Mg on both hormones is less potent than that of Ca (Pento *et al.*, 1974; Habener and Potts, 1976). In contrast, chronic Mg deficiency may impair the secretion and release of PTH (Anaat *et al.*, 1976) and may be associated with PTH target organs resistance (McManus *et al.*, 1971). Contreras *et al.* (1982) reported that cows with subclinical hypomagnesaemia induced by feeding a diet with a low Mg (1.1 g d^{-1}) content, showed a reduction in their ability to mobilize Ca in response to a hypocalcemia artificially induced by means of continuous intravenous infusion of EDTA. This result was confirmed by Samson *et al.* (1983).

In both the kidney and the intestine, Ca and Mg may be absorbed at a common site (Alcock and MacIntyre, 1962). Ca does not interfere with Mg absorption particularly in the intestine, although Mg depresses Ca absorption to a modest

extent (Rude and Singer, 1981). Hypomagnesaemia may also cause a reduction in Ca absorption through decreased $1,25(OH)_2D_3$ production that is secondary to a decreased PTH secretion (Morehead and Kessner, 1969).

5. Acid-base status and calcium metabolism

The pH value of digesta from different segments of intestine varies; it is the lowest in abomasum and increase as it moves down the digestive tract. Lee (1977) reported that digesta taken from the abomasum, proximal duodenum, jejunum, ileum, cecum and terminal colon of sheep fed lucerne alfalfa hay had pH values of 3.49, 5.14, 7.55, 7.66, 6.96, and 7.23. Storry (1961) reported that practically all of the Ca in the abomasum was ultrafiltrable because of a low pH. Since the solubilities of dietary Ca salts increases as pH declines and the observation that pH of the digesta in the upper intestine is relatively low: The upper intestine would be the major site of Ca absorption in ruminants (Smith, 1969; Ben-Ghedalia *et al.*, 1975; Wheeler and Noller, 1977). It was also hypothesized that the solubility and concentration of Ca in the supernatant fluids of digesta may increase with a decrease of pH in digesta when an adequate amount of dietary Ca is fed, thus leading to an increase in Ca absorption rate (Yano *et al.*, 1979). However, with *in vivo* studies on duodenal loops in dogs, it was demonstrated that there were no differences in Ca absorption rates when the pH of the contents of the loop were altered to 3.3, 5.2, and 7.4

(Cramer, 1968). This lack of pH effect was also demonstrated in duodenal loops of chickens (Wasserman, 1963).

Aside from the pH value of digesta, Girndt *et al.* (1979) reported in rats that a link exists between plasma Ca concentrations and acid-base metabolism; a direct linear relationship was shown between concentration of plasma Ca and standard bicarbonate. They also observed a negative linear relationship between plasma pH and plasma ionized Ca expressed as a proportion of total Ca concentration. Bronsnan and Bronsnan (1982) calculated that a decrease of 0.1 pH unit increases the ionized form of plasma Ca by approximately 0.04 meq L^{-1}. Phosphate, ammonium chloride (NH_4Cl) and sulfate (SO_4) are compounds that form acids upon digestion and could create acidic conditions in animals. Metabolic acidosis induced by the ingestion of ammonium Chloride (NH_4Cl) in sheep produced an increased Ca absorption (g d^{-1} and percentage) and an increased urinary Ca excretion (Braithwaite, 1972). Ender *et al.*, (1971) also demonstrated an increase in Ca absorption as well as in Ca retention by dairy cows offered a diet composed of mineral acid supplemented silage.

Barzel and Jowsey (1969) demonstrated in rats that chronic metabolic acidosis induced by feeding NH_4Cl resulted in an increased bone resorption rate that occurred without affecting blood Ca and P concentrations and that long-term ingestion of sodium and potassium bicabonates to correct the acidotic condition prevented these changes. They concluded that cellular mechanisms involved with bone formation and

bone resorption may be responsive to acid-base balance, affecting the storage of alkaline bone material during the intake of excess alkali and the release of bone during periods of acidosis. Recently Arnett and Dempster (1986) demonstrated *in vivo* that by reducing pH of medium from 7.4 to 6.8 a 14-fold increase in the bone resorption process by osteoclasts occurred. The soluble Ca salts found in the labile phase of bone and in intracellular organs can be solubilized by an increase of hydrogen ion concentration according to the following equations;

$Ca_3(PO_4)_2 + 2H^+ \longrightarrow 3Ca^{2+} + 2HPO_4^{2-}$

(Hydroxyappatite)

$CaCO_3 + H^+ \longrightarrow Ca^{2+} + HCO_3^-$

In both cases, the insoluble Ca salts that buffer the increased hydrogen ion concentration is rendered soluble and at the same time generates anions (HPO_4^{2-} and HCO_3^-) that may buffer additional protons (Barone, 1981: Bronsnan and Bronsnan, 1982). Goulding and Cambell (1984) reported that a high intake of NaCl depressed the accumulation of mineral in bone. Bell *et al.* (1977) used diets that differed in their titratable ash-acidity (Ash-TA), defined and calculated as the sum of the milliequivalents of anion-forming mineral elements (sulfur (S), Cl and P) minus the sum of the milliequivalent of cation-forming mineral elements (Na, K, Ca and Mg) to establish metabolic acidosis. They, as well as Camien and Gamick (1967) found that an increase of ash-TA in the diet from alkaline to neutral or from neutral to acid was not accompanied by an increase in bone resorption rate;

instead it altered the route of Ca excretion, shifting the excretion of some endogenous Ca from feces to urine. Furthermore, Newell and Beaucheme (1975) demonstrated that no changes in bone mineral composition occurred when rats were fed diet with 2% NH_4Cl DM basis.

It was proposed that the changes in urinary Ca excretion observed in metabolic acidosis are caused by a compound involved in renal tubular Ca reabsorption situated beyond the proximal tubule, which is inhibited by chronic but not acute metabolic acidosis and enhanced by metabolic alkalosis (or bicabonate infusion) independent of PTH level (Sutton *et al.*, 1979; Marone *et al.*, 1983). Peraino and Suki (1980) and Peraino *et al.* (1980) demonstrated that effects of bicarbonate to decrease renal Ca excretion in the state of metabolic acidosis were due to changes in urine bicabonate excretion *per se* rather than the changes in systemic acid-base balance.

Sulfur is normally consumed in the diet as S-containing amino acids, thus Whiting and Draper (1981a) suggested that the variable calciuric effects of different protein was related primarily to their S-amino acid content. In rats, given high protein diets, the S-amino acids inhibited renal tubular resorption of Ca by increasing the formation and excretion of nonabsorbable calcium sulfate salts in urine (Whiting and Draper, 1981a; 1981b; Wyshak, 1981).

6. Milk fever

Milk fever (hypocalcemic parturient paresis) is a metabolic disease occurring at the time of parturition and is manifested by low concentrations of blood Ca (hypocalcemia). Appearance and diagnose of milk fever coincided with the feeding, selecting and breeding of cows for higher level of milk production. The first evidence of milk fever was mentioned in the literature in Germany by Eberhardt in 1793 (Hutyra *et al.*, 1938). Today, milk fever is categorized to be one of the most important dairy diseases with respect to economics. Payne (1966) listed the national estimate of depreciation due to milk fever in Great Britain at 161,000 pounds annually.

In the United States, the annual loss from milk fever was estimated to be approximately $10.5 million in 1965 (Littledike, 1974). Payne (1968) reported that cows that have had milk fever depreciated by an average of 16 pounds in market value and also suffered marked reductions in productive life. Block (1984) reported cows that had suffered from milk fever showed a 14% reduction in total milk production for an entire lactation period. Mullin (1975) reported that 8.79% of 5000 cows studied became severely hypocalcemia and suffered from milk fever. Recently, Curtis *et al.* (1984) using 1,983 Holstein cows from the New York Dairy Herd Improvement Cooperative records reported an incidence of 4.7%.

Losses from this disease are very difficult to estimate because of secondary complications including degeneration

and necrosis of muscle, nerve paralysis, split pelvis, ruptured gastroentenius tendons, mastitis and ketosis (Littledike *et al.*, 1981).

6.1. Biochemical and physical changes

Milk fever is usually associated with parturition and the initiation of lactation when Ca homeostasis is challenged and is most subject to failure because of the large drain of extracellular Ca into the mammary gland for the synthesis of colostrum (Payne, 1964a). Ca secreted into colostrum, which has a higher Ca content (0.26%) than milk (0.13%)(Folet and Otterby, 1978), in one hour is approximately equal to one half of the total blood Ca at any one time (Mills, 1979). As a comparison of the demand for Ca during gestation to lactation, Littledike (1974) reported that the demand for Ca during lactation exceeds that of gestation by a factor of 2 to 5. This additional Ca needed at the initiation of lactation may be met by an increased intestinal Ca absorption and/or resorption of Ca from bone (Braithwaite, 1976). Ramberg *et al.* (1970) using an isotope technique, concluded that the increase in flow of total Ca through the body at the onset of lactation was mainly a result of an increase in Ca absorption from the intestine and that bone resorption did not contribute to the total inflow until two weeks postpartum. This Ca dependency from intestinal absorption during lactation was confirmed recently by Hove and Hilde (1984).

The initial symptoms of milk fever may include excitement, tetany and fine muscle tremors. There may be loss of appetite, shaking of the head and grinding teeth. More characteristically, the animal sinks into a sternal recumbency, becomes drowsy and the head turns into the flank. The cow finally becomes paretic with "dry" eyes and dilated pupils, falls into lateral recumbency and may die in a coma or convulsions.

It is recognized that most cows, regardless of whether they develop clinical signs of milk fever or not, show some degree of hypocalcemia associated with parturition. In typical postpartum hypocalcemia, it takes 1 to 2 days to reach the lowest blood Ca level and another 2 to 3 days for to stabilize within the normal range again (Littledike *et al.*, 1981). Cows suffering from classical milk fever, however, develop a severe hypocalcemia and a reduction of both total and ionized forms of Ca in blood. Normal plasma concentration ranges for Ca are 8.5 to 11.4 mg dL^{-1} (Jorgensen, 1974) and when plasma Ca levels fall below 6 mg dL^{-1} paresis occurs (Jorgensen *et al.*, 1978). In a field study (Vagg *et al.*, 1981), clinical diagnosis of milk fever was confirmed when plasma Ca level decreased to less than 7.0 mg dL^{-1}. Paretic animals also showed marked hypophosphotaemia (Littledike *et al.*, 1969); this phenomenon is also an exaggeration of normal occurrences at parturition (Mayer *et al.*, 1969b; Littledike *et al.*, 1970).

In contrast, the concentration of plasma Mg in the periparturient period is generally elevated (Marr *et al.*,

1965; Littledike *et al.*, 1969; Forslund *et al.*, 1983) although in some cases hypomagnesamia develops (Barber *et al.*, 1983). Also, plasma concentration of urea nitrogen, lactic acid, pyruvic acid and Cl increase as does hematocrit (Littledike *et al.*, 1981). Blood glucose is usually not affected (Lindsay and Pethick, 1983).

Hormones regulating blood Ca are affected by milk fever and *vise versa*. The active metabolite of vitamin D_3, $1,25(OH)_2D_3$, was reported to increase in paretic cows (Kichura *et al.*, 1982) with no changes in other vitamin D_3 metabolites including $24,25(OH)_2D_3$, 25,26-dihydroxyvitamin D_3 ($25,26(OH)_2D_3$) and $25(OH)D_3$ (Horst *et al.*, 1979). However, Smith *et al.* (1982) reported increased plasma concentrations of $24,25(OH)_2D_3$ in paretic compared to normal cows. Horst and Reinhardt (1983) hypothesized that paretic cows suffered from a interference and/or a reduce sensitivity of $1,25(OH)_2D_3$ receptors at the target organs and thus concluded that $1,25(OH)_2D_3$ generally promotes a net influx of Ca into the body, however, its overproduction in paretic cows may promote a net efflux of Ca from the body to the mammary gland that is large enough to promote hypocalcemia, especially in the absence of intestinal and bone responsiveness to $1,25(OH)_2D_3$.

Horst *et al.* (1978a) reported increased parathyroid hormone (PTH) in paretic cows. Because the ruminant has a continuous flow of dietary Ca to the intestine resulting from continuous rumen emptying, it was suggested that the ruminant has little or no "exercise" of the parathyroid to

secrete PTH because of a lack of fluctuating blood Ca levels. Therefore, ruminants are unable to respond quickly with secretion of PTH in postparturient hypocalcemia (Kronfeld, 1971). However, there was no time lag observed between the induction of hypocalcemia at parturition and the secretion of PTH (Mayer *et al.*, 1969a).

Mayer *et al.*,(1975) and Hollis *et al.* (1981) reported a reduced calcitonin (CT) concentration in paretic cows. Also, reduced concentration of extractable CT in the thyroid gland and a reduction in size, number and secretory activity of parafollicular cells in paretic cows were reported (Capen and Young, 1967; Black *et al.*, 1973a).

Sasser *et al.* (1979) and Hollis *et al.* (1981) reported an increase in levels of estrogen, estrone and prolactin in paretic cows. Also, paretic cows demonstrated elevated plasma cortisol (Hayashi *et al.*, 1979; Horst and Jorgensen, 1982; Waage *et al.*, 1984), plasma and urinary hydroxyproline (Hollis *et al.*, 1981; Black and Capen, 1971), plasma cholinesterase (Forslund *et al.*, 1983) and plasma non-esterified fatty acids (NEFA) (Luthman and Persson, 1975).

6.2. Nutritional factors affecting the occurrence of milk fever

With all the factors contributing to the occurrence of milk fever, nutritional factors emerge as a top priority. Ender *et al.* (1962a; 1962b) and Edner and Dishington (1970) demonstrated that milk fever is, in fact, a typical nutri-

tional disorder. They induced milk fever in 60 to 76% of cows fed a milk-fever-inducing diet containing low P (8-10 g d^{-1}) and high Ca (100-200 g d^{-1}) when offered without interruption from three months prepartum until 10 day postpartum. Thus, they concluded that the feeding of high quantities of Ca in roughages, such as alfalfa or clover, to mature cows for three months before calving was the major cause of their experimentally-induced cases of milk fever. Recently, Block (1984) induced milk fever in 47.4% of cows fed a diet with high Ca (0.63 to 0.69% DM), low P (0.24 to 0.25% DM) and a high ratio of Ca-to-P (2.63 to 2.89) achieved by the inclusion of alfalfa hay in the diet. Jorgensen (1974) concluded from a survey of all the relevant information concerning the occurrence of milk fever that an intake of Ca above 100g d^{-1} increases the incidence of milk fever. The importance of intake of dietary Ca rather than intake of dietary P or the Ca-to-P ratio in relation to the incidence of milk fever was also reported by Mayer *et al.* (1969b).

Evidence suggests that the Ca content of the prepartum diet plays an important role in the development of milk fever and animals fed low levels of Ca prepartum are better able to maintain plasma Ca levels at parturition than those fed high Ca diets (Braithwaite, 1976; Kichura *et al.*, 1982). Ender *et al.* (1962a) and Barton *et al.* (1983) reported that cows may develop milk fever when the intake of P was high, medium or low if the Ca intake is exceedingly high. Others suggested that dietary P level itself is the critical ele-

ments in the development of milk fever (Scott, 1965).

On the other hand, Little and Wright (1975) proposed that the dietary ratio of Ca-to-P may have a greater significance in the etiology of milk fever than the total intake of each element using the evidence provided by Boda (1956) and Boda and Cole (1954). However, it was demonstrated that ratio of Ca-to-P in the diet had no effect on serum levels of either element (Bush and Steevens, 1970) and that cows can tolerate a wide range of Ca-to-P (as high as 5:1) without any visible detrimental effect (Hoar *et al.*, 1970) but the amount of Ca and P absorbed and/or retained is directly influenced by Ca-to-P in diets (Bush and Stevens, 1970; Braithwaite, 1975). Young *et al.* (1966) demonstrated that pregnant heifers had higher retention of Ca and P when diets contained a ratio of 2 than a ratio of 1. Kendall *et al.* (1970) concluded that the optimum ratio of dietary Ca-to-P is about 2.3:1 and that any ratio wider or narrower than this tended to predispose the animal to milk fever. The ratio of 2.3:1 is approximately the ratio of Ca to P found in bone. Kendall *et al.* (1970) and Gardner (1970) concluded that if animals were fed more than 100 grams of Ca per day during the dry period (requirement ranges from 23 to 40 g d^{-1}) and also were fed a prepartum ration that contains a dietary ratio of Ca-to-P at 2.3:1 the incidence of milk fever markedly increases.

7. Prevention of milk fever

The high economic losses resulting from milk fever occurring in the dairy industry has created the need for the development of the methods for reducing the incidence of this disease (Payne and Manston, 1967; Littledike *et al.*, 1981: Goff *et al.*, 1987). There are two major areas of preventative methods; the use of vitamin D_3 and its metabolites, and dietary manipulations.

7.1. Dietary manipulation

7.1.1. Dietary calcium and phosphorus levels and their ratio.

A relationship between dietary ratio of Ca-to-P and the incidence of milk fever was initially studied by Boda and Cole (1954). They observed that cows fed a large amount of Ca in relation to the amount of P for varying lengths of time during the dry period had a relatively high incidence of milk fever, whereas cows fed rations low in Ca in relation to P had a much lower incidence. However, their experimental diets were impractical from a managerial standpoint and the adverse effects of feeding such rations would likely outweigh any possible advantages to protection against milk fever (Bush and Steevens, 1970).

Going *et al.* (1974) reported a reduction in incidence of milk fever in cows fed diets low in Ca (approximately 15

g d^{-1}) for the last 2 weeks of pregnancy. The low Ca diets during the dry period would force animals to rely less on Ca absorption from the intestine and rely more on Ca mobilized from bone at parturition when the Ca homeostatic mechanism is stressed (Green *et al.*, 1981). Additionally low Ca would stimulate the mechanism involved in active absorption of Ca from the small intestine and when animals are transferred to a Ca-rich diet postpartum an increase in Ca absorption from the intestine would occur (Braithwaite, 1974), which would increase the endogenous Ca pool. Furthermore, Braithwaite (1982) reported a reduction of endogenous fecal Ca when diets were low in Ca resulting in more efficient use of the absorbed Ca even though higher endogenous fecal Ca losses have been observed during pregnancy and lactation (Braithwaite, 1983a).

Animals given low Ca diets prepartum should, therefore, be ideally prepared to meet the high Ca demand at the initiation of lactation when great pertubations of Ca homeostasis and the changes of hormonal responses to their target organs occur.

Recently, Kichura *et al.* (1982) reported successful prevention of milk fever by feeding diets low in P prepartum (10 g d^{-1}) over a wide range of dietary Ca levels (9.5 or 86 g d^{-1}). This low level of dietary P did not create an elevation of plasma $1,25(OH)_2D_3$ level whereas feeding low Ca diets usually elevates this metabolite (Green *et al.*, 1981) and this higher $1,25(OH)_2D_3$ concentration would result in an increased Ca absorption from the small intestine (Omdahl

and DeLuca, 1977). However, Sommerville *et al.* (1980) and Horst *et al.* (1978b) showed that $1,25(OH)_2D_3$ accumulated in the intestinal mucosa of animals fed low dietary P irrespective of $1,25(OH)_2D_3$ concentrations in plasma, Kichura *et al.* (1982) found an increased binding of $1,25(OH)_2D_3$ to the intestinal mucosa receptors resulting in an increased efficiency of absorption of Ca and P when prepartum rations were low in P. However, Barton *et al.* (1987) could not influenced the incidence of milk fever by feeding a low level of dietary P.

Curtis *et al.* (1984), studying the epidemiology of milk fever in North America, reported that the incidence of milk fever was reduced with the feeding of high dietary protein during the early stage of the dry period along with low P and high energy "lead feeding" closer to calving. They also found that the amount of Ca in dry period was not an important factor in relation to the occurrence of milk fever when dietary P was low.

7.1.2. Acidic diets

In 1962, Ender *et al.* (1962b) reported that the incidence of milk fever was increased by feeding alkaline ration high in Ca (100 to 200 g d^{-1}) with various amounts of P for three month prepartum, whereas feeding a low alkaline ration containing A.I.V. silage (silage preserved with mineral acids) resulted in low occurrances, even though Ca content was as high as that of the high alkaline rations. Kendall *et*

al. (1969) reported a reduction in the incidence of milk fever (16.5%) in cows fed an acidic diet (NH_4Cl supplemented) compared to a 50% incidence in cows fed a basic diet ($NaHCO_3$ supplemented). Both groups received the same ratio of dietary Ca to P ratio (2.2:1).

Aside from the acidity of the diets, it was proposed that the alkali-alkalinity, defined and calculated as milliequivalents $[(Na^+ + K^+) - (Cl^- + S^=)]$ of the diets, is an overriding factor in determining Ca availability and that the beneficial effects of feeding an acidic diet can be ascribed to a negative alkali-alkalinity (Ender and Dishington, 1970; Ender *et al.*, 1971). Dishington (1975) and Dishington and Bjornstad, (1982) using this hypothesis, reported that cows supplemented with combinations of $CaCl_2$, $Al_2(SO_4)_3$ and $MgSO_4$ to reduce the alkali-alkalinity of the diet for 4 weeks prepartum showed a reduced incidence of milk fever than control cows who were supplemented with Na_2CO_3 and $NaHCO_3$. Dietary alkali-alkalinity value of the milk fever prophylactic diets varied between -255 to -1385 meq d^{-1} compared to that of control diets values ranging from +720 to +3875 meq d^{-1}.

Recently, Block (1984) demonstrated a complete prevention of milk fever by feeding a diet that had a negative dietary cation-anion balance value, defined and calculated the same as the dietary alkali-alkalinity defined by Ender and Dishington, (1970), of -128.7 meq kg^{-1}DM compared to an incidence rate of 47.4% in cows fed a control diet that had cation-anion balance value of +330.4 meq kg^{-1}DM. He included

mineral salts ($CaCl_2.2H_2O$, $Al_2(SO_4)_3.18H_2O$ and $MgSO_4.7H_2O$) to a basal diet that contained a relatively high Ca (0.69% DM) and moderate P level (0.25% DM), to obtain the negative cation-anion balance. Daily intake of dietary Ca was 3 to 4 times higher than that of suggested level for reducing the incidence of milk fever (<25 g d^{-1}).

The hypothesis of the present study was following: Manipulating dietary cation-anion balance by supplementing various mineral salts will affect Ca metabolism and that reducing dietary cation-anion balance will increase the capability of animals to elevate the concentrations of plasma Ca in the hypocalcemic state. The objectives of the study were to investigate the effect of feeding reduced and/or negative cation-anion balanced diets to sheep: (Exp. 1) on metabolism and the concentrations of plasma major mineral (Ca, P, Mg, Na, K, Cl, and S) and acid-base status of animal; (Exp. 2) on the size of the immediately mobilizable Ca pool and Ca mobilization rate from it during experimentally induced acute hypocalcemia created by the continuous infusion of 5.6% EDTA and the comparison with those of vitamin D injected; and (Exp. 3) on the kinetics of Ca metabolism using a four-compartment model in sheep during eucalcemic and simulated lactational Ca loss created by the continuous infusion of EGTA.

III. EFFECTS OF MANIPULATING DIETARY CATION-ANION BALANCE ON MAJOR-MINERAL METABOLISM IN SHEEP

Abstract

A mineral balance study was conducted to examine the effect on major mineral metabolism in sheep of dietary excess of inorganic anions (Chloride (Cl^-) and Sulfur ($S^=$)) or inorganic cations (Sodium (Na^+) and Potassium (K^+)). Cation-anion balance was calculated as meq [($Na^+ + K^+$)-($Cl^- + S^=$)]. Ten crossbred wether lambs (ave. BW 51.4 kg) were fed 2 levels of calcium (Ca) (0.82% [HC] and 0.48% [LC] of dry matter (DM)) and 5 treatments each, four of which differed in dietary cation-anion balance; control (CTR) and treatment (TRT) A, B, and C at +314, +139, +18, -22 (meq kg^{-1} DM), respectively. Additional mineral supplementation were NH_4Cl for TRT-A, $Al_2(SO_4)_3$, $CaCl_2$, and $MgSO_4$ for TRT-B, $FeSO_4$, $CaCl_2$, and $MgSO_4$ for TRT-C. A fifth treatment was injection of vitamin D_3 (16670 IU kg^{-1}BW) to lambs fed CTR. Trial design was a 2x5 factorial split-plot with five 21-day periods. Plasma Ca concentrations (mg dL^{-1}) were 8.89, 8.79, 9.04, 8.79, and 9.27 for CTR, TRT-A, -B, -C, and -D, respectively. TRT-A, -B, and -C reduced urine pH. Ca balance revealed that animals fed lower cation-anion balanced diets (TRT-A, -B, and -C) had lower Ca retention than CTR caused by a high urinary Ca excretion with a similar apparent absorption rate of Ca. No difference was observed in apparent absorption rate between HC and LC groups. Ca retention as a proportion of intake (%) for CTR, TRT-A, -B, -C, and -D were 18.2, 8.9, 11.9, 4.2 and 16.5, respectively. Mg retention as a proportion of absorbed (%) for TRT-B (10.44) and TRT-C (25.61) were smaller than

that of TRT-A (43.91) and TRT-D (40.19), although not different from CTR (36.09). Lowest dietary cation-anion balanced diet (TRT-C) not only showed the lowest Ca retention (3.29 g $7d^{-1}$) but also showed net loss of phosphorus (P) (-0.17 g $7d^{-1}$) and the lowest Na digestibility (42.1%) with no changes in K metabolism compared to others. Results showed that reduced cation-anion balance in diet resulted in reduction of Ca retention.

INTRODUCTION

An increased need for calcium (Ca) to satisfy demands created by physiological changes such as pregnancy and lactation and to maintain the blood Ca concentration at an adequate level creates dramatic changes in Ca metabolism resulting in disturbances of the homeostatic mechanism for Ca. The initiation of lactation places one of the greatest stresses on Ca homeostasis: Hypocalcemic parturient paresis (milk fever) is a manifestation of this, although the cause of this disease has not been well established (Horst and Reinhardt, 1983).

There have been various measures for the prevention of milk fever: Administration of vitamin D_3 and its metabolites (McMurrar *et al.*, 1980; Hove and Kristiansen, 1982; Bar *et al.*, 1985; Goff *et al.*, 1985a; Sacks *et al.*, 1987) nutritional manipulation including feeding diets to prepartum cows containing low Ca (Beitz *et al.*, 1974; Going *et al.*, 1974), manipulation of amount of dietary Ca and phosphorus (P) and their ratio (Boda and Cole, 1954) and feeding diets containing acid-forming mineral salts (Ender *et al.*, 1971; Dishington, 1975; Dishington and Bjornstad, 1982).

The last preventative method involves the concept that feeding diets that have a negative dietary cation-anion balance, defined and calculated as a summation of milliequivalent of cation-forming mineral elements (sodium [Na^+] and potassium [K^+]) minus a summation of milliequivalent of anion-forming mineral elements (chloride [Cl^-] and sulfur

[S⁼]), has not received great interest in North America due to fact that ingredients used by Ender *et al* (1971) and Dishington (1975) were atypical of North American rations, such as beets, formic acid treated silage and herring meal. Recently Block, (1984) utilized various mineral salts to alter dietary cation-anion balance and demonstrated complete prevention of milk fever by feeding diets containing extremely negative dietary cation-anion balance (-128.7 meq kg^{-1} dry matter (DM)) compared to an incidence rate of 47.4% among cows fed a diet that had a cation-anion balance of +330.4 meq kg^{-1} DM. Diets had relatively high levels of dietary Ca (0.63% DM) and P (0.25% DM); mineral salts used to alter dietary cation-anion balance were $CaCl_2$, $MgSO_4$ and $Al_2(SO_4)_3$.

There are a few proposed mechanisms to how this preventative method works. A negative cation-anion balanced diet may increase the intestinal absorption of Ca by creating a reduction of pH in the gastrointestinal-tract causing an increase in the more soluble forms of Ca (ionized form) present in digesta; cause an alteration in acid-base balance of animals resulting in an increase of Ca availability from exchangeable Ca pool(s); cause a reduction in the intestinal Ca absorption by interference created by the presence of excess cation mineral elements such as aluminum (Al) and magnesium (Mg) in the diet. Studies that explored these possible mechanism(s) do not exist.

Objectives of this study were: to investigate the effects of feeding a reduced or negative cation-anion balanced

diets on absorption and retention of major minerals (Ca, P, Mg, K, Na, Cl, and S); to test differences in the effects caused by varying mineral salts used to reduce dietary cation-anion balance; to compare the above effects to that of injecting therapeutic doses of vitamin D_3.

MATERIALS AND METHODS

Animals

The experiment was conducted with ten wether lambs (suffolk crossbred) at an average initial body weight of 51.6±3.06 kg. Animals were placed in individual metabolic cages in a room where temperature was maintained at 20°C and had a photoperiod of 14 h light: 10 h dark. Prior to commencing the experiment animals were treated for internal parasites with Baymix (Farbenfabriken Bayer, Leverkusen). Animals had free access to distilled water at all times. Experimental diets as total mixed rations were offered *ad libitum* in two equal portions at 0830 h and at 1600 h.

Diets

Dietary treatments were grouped into two basal diets that contained high and low concentrations of Ca (high [HC] at 0.82% of total DM and low [LC] at 0.45% of total DM). Basal diets were composed of corn silage and either chopped alfalfa hay (HC) or timothy hay (LC). Within each calcium

levels, there were four dietary treatments differing in their dietary cation-anion balance. Composition of diets are presented in Table 1.1. The descriptions of dietary treatments within each level of dietary Ca (HC and LC) are as follows:

Control (CTR) -- composed of basal diets and 1.4% of total DM as mineral premix-A formulated to provide an excess amount of Na and K relative to Cl and S, which resulted in obtaining a positive cation-anion balanced diet;

Treatment A (TRT-A) -- same as CTR with addition of NH_4Cl at the level of 0.87% of total DM to create a metabolic acidosis;

Treatment B (TRT-B) -- composed of basal diets plus 0.5% of total DM of mineral premix-B, formulated to provide an excess amounts of Cl and S relative to Na and K and 0.2% $CaCl_2.2H_2O$, 0.86% $Al_2(SO_4)_3.18H_2O$ and 0.74% $MgSO_4.7H_2O$ to obtain a negative cation-anion balanced diet;

Treatment C (TRT-C) -- same as TRT-B except that 0.83% $FeSO_4$ was substituted for $Al_2(SO_4)_3.18H_2O$ to obtain a negative cation-anion balanced diet. Quantities of $FeSO_4$ supplemented was calculated to have an equivalent amount of S compared to that of $Al_2(SO_4)_3$ in TRT-B;

Treatment D (TRT-D) -- vitamin D (Poten D, Pfizer, Montreal) injection at a dose of 16670 IU kg^{-1} BW was administered intramuscularly (i.m.) to animals fed CTR. Injections were administered 2 days prior to the initiation of the collection period.

CTR, TRT-A, TRT-B, TRT-C, and TRT-D were fed at both HC

Table 1.1. Composition of diets differing in the dietary cation-anion balance with high (HC) and low (LC) calcium levels fed to sheep.

DIET	CTR[1] & TRT-D		TRT-A		TRT-B		TRT-C	
Ingredients	HC	LC	HC	LC	HC	LC	HC	LC
	%							
Alfalfa hay	38.5	-	38.5	-	38.5	-	38.5	-
Timothy hay	-	38.5	-	38.5	-	38.5	-	38.5
Corn silage	59.82	50.74	58.95	49.87	58.85	49.97	58.63	49.75
Soy bean meal	-	9.0	-	9.0	-	9.0	-	9.0
Mineral mix-A[2]	1.4	1.4	1.4	1.4	-	-	-	-
Mineral mix-B[3]	-	-	-	-	0.5	0.5	0.5	0.5
NH_4Cl	-	-	0.87	0.87	-	-	-	-
$CaCl_2.2H_2O$	-	-	-	-	0.20	0.20	0.20	0.20
$Al_2(SO_4)_3.18H_2O$	-	-	-	-	0.86	0.86	-	-
$MgSO_4.7H_2O$	-	-	-	-	0.74	0.74	0.74	0.74
$FeSO_4$	-	-	-	-	-	-	1.08	1.08
$NH_4H_2PO_4$	0.11	0.06	0.11	0.06	0.25	0.06	0.25	0.06
$CaHPO_4$	0.17	0.17	0.17	0.17	-	0.17	-	0.17
$CaCO_3$	-	0.13	-	-	-	-	-	-

[1] CTR=Control; TRT=Treatment.

[2] Mineral mix-A contains NaCl, $NaHCO_3$, Na_2CO_3, $CaCO_3$, $CaCl_2.2H_2O$, $MnSO_4.7H_2O$, ZnO, $FeSO_4.7H_2O$, $CuSO_4$, and KI.

[3] Mineral mix-B contains NaCl, $CaCl_2.2H_2O$, $ZnCl_2$, $MnSO_4.4H_2O$, $CuSO_4$, $CoCl_2.6H_2O$, $CoSO_4.7H_2O$, and HIO_3.

and LC resulting in ten dietary treatments.

Both mineral premix-A and -B were formulated to meet nutrient requirements (NRC, 1985) and to deliver excess cations (Na and K) or anions (Cl and S). Vitamin A and D 88,000 IU and 33,000 IU kg^{-1} DM, respectively were also supplemented in both mineral premixes. Ingredients of mineral premixes were NaCl, $CoCl_2$, $MnSO_4$, ZnO, $FeSO_4$, KI, $CaCO_3$, $NaHCO_3$, Na_2CO_3, $CaCl_2$, $CuSO_4$, $CoSO_4$, $ZnSO_4$ and HIO_3. Detailed descriptions of both mineral premixes are presented in Table 1.2. The cation-anion balance of diets was calculated by the quantities of Na, K, Cl, and S in diets. The equation for calculating dietary cation-anion balance was milliequivalents of $[(Na^+ + K^+) - (Cl^- + S^=)]$. Thus, manipulation through an increase in the quantities of anions (Cl and S) compared to that of cations (Na and K) in diets resulted in obtaining negative cation-anion balanced diets. All diets were formulated to meet requirements of mature sheep (NRC, 1986) except for Na, K, Cl, S, Ca, P and Mg where these mineral elements met or exceeded requirements. A quality of mineral salts used were at technical grade. Mineral premixes were mechanically mixed with other supplemental mineral salts and stored. At each feeding they were combined with other ingredients, hand mixed and offered to animals.

Experimental design

Animals were assigned randomly to one of the ten dietary treatments according to a split-plot design with a 2x5 fac-

Table 1.2. Composition of mineral premixes[1].

Ingredients	mix-A Diet CTR[2], TRT-A & -D	mix-B TRT-B & -C
	%	
NaCl	35.000	95.000
$NaHCO_3$	31.531	-
Na_2CO_3	30.000	-
$CaCO_3$	2.000	-
$CaCl_2.2H_2O$	0.805	2.805
$MnSO_4.4H_2O$	0.261	0.731
ZnO	0.181	-
$ZnCl_2$	-	0.834
$FeSO_4.7H_2O$	0.179	0.501
$CuSO_4$	0.018	0.050
$CoCl_2.4H_2O$	0.015	0.024
$CoSO_4.7H_2O$	-	0.020
KI	0.009	-
HIO_3	-	0.027

[1] Both minerals provided following trace elements per kg of diet; Mn=90mg, Fe=25mg, Cu=10mg, Co=5mg, I=10mg, and Zn=20mg.

[2] CTR=Control; TRT=Treatment.

torial arrangement (2 levels of calcium x 5 dietary treatments). There were five 21-day experimental periods consisting of a 14-day adaptation and a 7-day collection period.

Sample collections

Feces

A plastic bag was held with rubber cement around the rectum for collection of total feces during the collection period. Feces were collected twice daily, weighed and subsamples were collected. Dry matter (DM) of the daily samples was determined then subsamples were combined to obtain one fecal sample per animal per experimental period.

Urine

Urine was collected in polyethylene bottles and the pH was measured immediately by pH meter (Model 119 Fisher Scientific Ltd., Montreal, Quebec) then two proportional (10% of total excretion) samples were obtained. One daily sample was acidified with 1% volume of 10N H_2SO_4 for determination of N and the other sample was kept without acidification for determination of major mineral concentrations. Both urine samples were stored at 5°C. At the end of the 7-day collection period, daily urine samples from each animal were pooled and one sample per animal per period was

stored at -10°C for subsequent laboratory analysis.

Feed and orts

Daily samples of feed and orts were collected and stored at 5°C. At the end of each 7-day collection period feed and orts samples were composited and stored at -10°C for subsequent analysis of dry matter (DM) with a subsample dried in a forced-air oven at 65° for 48 h for nutrient analyses.

Blood collections

Three hours after the morning feeding on day-7 of the collection period, blood samples were obtained from the jugular vein. Blood samples were collected into two test tubes; one test tube containing dextrose citrate as anticoagulant and preservative for determination of hydroxyproline (OHPro) and the second test tube containing lithium heparin as anticoagulant for determination of plasma major minerals. Additional blood samples from animals injected with vitamin D_3 (TRT-D) were collected on day 1, 3, 5 and 7 after the administration of vitamin D_3 (day 0). Blood samples were centrifuged at 750 x G for 15 min (International centrifuge, Universal Model U.V, International Equipment Co., Boston, Massachusetts) immediately after the collection and plasma was recovered using a pasteur pipette and transferred to a small plastic scintillation counter vial for storage at -10°C until subsequent analysis.

Analytical procedure

Fecal pH was measured by mixing 20 g of fresh feces with 80ml of glass distilled water.

Dry matter (DM) of feed and orts were determined by toluene distillation (Dewar and McDonald, 1961) and DM of daily feces were determined by placing samples in a forced-air oven at 65°C for 48 h. Dried feed, orts and feces samples were ground through a hammer mill (2 mm screen) before laboratory analyses.

Composited feed, orts, feed and urine samples were analyzed for N with Kjel-Foss macroautomatic analyzer (Foss Electric, Hillerod, Denmark). After wet digestion with HNO_3 and $HClO_4$, samples were analyzed for P by the alkalimeter ammonium molybdate method (AOAC, 1984) and for Ca, Mg, Na, K, and iron (Fe) with atomic absorption spectrophotometer (Perkin Elmer 360, Norwalk, Connecticut). Contents of S were measured with the turbidimetric method of Berblung and Sorbo (1960).

For the determination of Cl in feed, orts and feces, 20ml of 1N HNO_3 was added to 1g of sample and mixed for 15 min by a shaker (model 75 Burrell Corporation, Pittsburg, PA) for extraction then centrifuged at 4,400 x G for 15min to obtain a clear supernatant. Concentration of Cl in the supernatant was analyzed by an indirect method in which excess silver, as $AgNO_3$, was added to the sample solution then free silver was measured by atomic absorption spectrophotometer (Anonymous, 1982).

Contents of Al in feed and feces were measured by the method of Hendershot (1985).

For determination of major minerals in plasma, 2 ml of plasma was added to an equal volume of 20% (v/w) trichloroacetic acid, vortexed and kept for 15 min at room temperature then centrifuged at 750 x G for 20 min. The clear supernatant that was recovered was used for mineral analyses by the same procedures as described for feed mineral analyses except for inorganic S determination.

The concentration of inorganic S in plasma was determined with the turbimetric method of Berblung and Sorbo (1960) modified by Krijgsheld *et al.* (1979). To 0.5 ml of plasma, 2 ml of trichloroacetic acid (5% v/w) were added, vortexed and the mixture was allowed to stand for 10min at room temperature. After centrifugation at 4,400 x G for 15 min, one ml of clear supernatant was mixed with 0.25 ml $BaCl_2$ reagent (10 g $BaCl_2$ and 100 g dextran per one liter of glass distilled water) and the absorbance was read after precisely 35 min at 360 nm against a sample background (1 ml supernatant and 0.25 ml reagent containing 100 g dextran in one liter of glass distilled water).

Free hydoxyproline (OHPro)in plasma was determined by the method of Plamerini *et al.* (1985) using an HPLC (Vista 5500, Varian. Palo Alro, California)

Statistical analysis

Statistical analyses were performed at the McGill Comput-

ing Center by the Statistical Analysis System (SAS Institute Inc., Box 8000, Cary, North Carolina). Differences due to period, the levels of dietary Ca and treatments were evaluated by analysis of variance for a split-plot design (Steel and Torrie, 1980) in which dietary treatments acted as subunits. Linear model used was following;

$$Y_{ijk} = u + A_i + B_j + C_{ij} + D_k + (BD)_{jk} + E_{ijk}$$

where

u=common mean,

A_i =effect of ith block,

B_j =effect of jth dietary Ca level,

C_{ij} =error term of whole unit=interaction effect of ith block and jth dietary Ca level,

D_k =effect of kth treatment,

$(BD)_{jk}$ =interaction effect of jth dietary Ca level and kth treatment,

E_{ijk} =random error=error term of subunit.

A least-squares analysis was also used to obtain least-squares estimates of differences between subunits for comparison purposes. In order to evaluate the existance of first order carry-over effect of treatments, the method of Lucas (Lucas, 1983) was used, where the same linear model described above except that effect of the kth treatment was divided into true effect of the kth treatment and true carry-over effect of the kth treatment from the proceeding treatment.

RESULTS

Chemical analyses of diets

Chemical analyses of diets are presented in Table 1.3. Dietary levels of Ca and P and their ratio were 0.82%, 0.30%, and 2.73 for the HC diets and 0.48%, 0.32%, and 1.50 for the LC diets, respectively. Major differences between dietary treatments for both HC and LC groups were levels of cation-forming mineral elements (Na and K) and anion-forming mineral elements (Cl and S). TRT-B and TRT-C had low Na and high Cl and S concentrations compared to CTR to obtain a reduced dietary cation-anion balance whereas reduced dietary cation-anion balance value of TRT-A was obtained by increasing a concentration of Cl alone compared to that of CTR.

Dietary cation-anion balance values (meq kg^{-1} DM) were calculated from the mineral analyses and presented in Table 1.3. For the HC group, dietary cation-anion balances were +284.2, +61.67, -27.3 and -32.17 for CTR (and TRT-D), TRT-A, TRT-B and TRT-C , respectively, whereas values were +343.2, +218.3, +62.6 and - 13.0, respectively in LC group. Table 1.4 shows chemical analyses and dietary cation-anion balance with HC and LC groups combined. Dietary cation-anion balance were +314, +139, +18, and -22 for CTR (and TRT-D), TRT-A, TRT-B, and TRT-C, respectively, for HC and LC combined.

Table 1.3. Mineral composition of diets fed to sheep with two levels of dietary calcium[1,2].

Nutrient	High calcium				Low calcium			
	CTR	TRT-A	TRT-B	TRT-C	CTR	TRT-A	TRT-B	TRT-C
Crude protein (%)	12.09±0.28	13.20±0.48	12.77±0.59	12.00±0.3	11.30±0.54	12.8±0.51	11.4±0.54	11.42±0.48
Phosphorus(%)	0.31±0.007	0.31±0.006	0.31±0.006	0.30±0.016	0.32±0.01	0.32±0.01	0.31±0.01	0.32±0.02
Calcium(%)	0.85±0.054	0.79±0.047	0.84±0.065	0.82±0.051	0.48±0.1	0.47±0.02	0.46±0.04	0.48±0.04
Magnesium(%)	0.24±0.018	0.24±0.019	0.31±0.023	0.31±0.022	0.21±0.006	0.21±0.01	0.26±0.008	0.28±0.005
Sodium(%)	0.53±0.032	0.53±0.025	0.30±0.034	0.28±0.029	0.54±0.06	0.56±0.01	0.31±0.03	0.27±0.01
Potassium(%)	1.35±0.161	1.25±0.191	1.32±0.145	1.34±0.178	1.37±0.10	1.38±0.09	1.34±0.12	1.41±0.12
Chloride(%)	0.59±0.038	1.20±0.055	0.69±0.039	0.65±0.033	0.43±0.01	0.86±0.05	0.56±0.04	0.49±0.03
Sulfur(%)	0.20±0.017	0.24±0.027	0.48±0.025	0.50±0.040	0.20±0.02	0.19±0.09	0.41±0.02	0.56±0.03
Cation-anion balance[3] (meq kg^{-1} DM)	284±25.9	61±46.4	-27±46.7	-32±52.7	343±34.9	218±43.1	63±18.9	-13±16.5

[1] Values are presented as mean±standard error.
[2] CTR=Control; TRT=Treatment.
[3] Milliequvalent of $[(Na^{+}+K^{+})-(Cl^{-}+S^{=})]$.

Table 1.4. Mineral composition of diets differing in the dietary cation -anion balance (high and low calcium groups combined)[1,2].

Nutrient	CTR & TRT-D	TRT-A	TRT-B	TRT-C
Crude protein (%)	11.7±0.31	13.0±0.33	11.8±0.41	11.7±0.29
Phosphrus (%)	0.32±0.006	0.32±0.022	0.31±0.008	0.31±0.011
Calcium (%)	0.67±0.07	0.63±0.06	0.65±0.07	0.65±0.06
Magnesium (%)	0.22±0.01	0.23±0.01	0.28±0.01	0.29±0.01
Sodium (%)	0.54±0.03	0.55±0.01	0.30±0.08	0.27±0.01
Potassium (%)	1.36±0.09	1.32±0.10	1.33±0.04	1.37±0.10
Chloride (%)	0.51±0.03	1.03±0.06	0.63±0.04	0.57±0.03
Sulfur (%)	0.20±0.01	0.22±0.02	0.44±0.02	0.53±0.03
Cation-anion[3] balance (meq kg^{-1})	314±22.7	139±39.7	18±28.2	-22±26.3

[1] Values are presented as mean±standard error.
[2] CTR=Control; TRT=Treatment.
[3] milliequvalent of [(Na+K)-(Cl+S)].

Feed consumption and body weight (BW) changes

Least-squares means of DM intake, DM digestibility, body weight (BW) change, water intake, fecal and urine output and their pH are presented in Tables 1.5 and 1.6. Intake of DM was affected by both the level of dietary Ca and dietary treatment. Animals in HC had a tendency ($P<.1$) for higher intake of DM (1949 g d^{-1}) than that for LC, although, when expressed per unit metabolic BW (g d^{-1} $kg^{-1}BW^{.75}$) the values did not differ ($P>.1$) between the two groups.

A comparison between dietary treatments revealed that animals fed TRT-C (supplemented with $FeSO_4$ as the anion-supplement mineral salt) showed a reduction ($P<.05$) in daily DM intake (1687 g and 1742 g d^{-1} for both HC and LC groups, respectively) compared to those fed TRT-A (2130 and 1920 g d^{-1} for HC and LC groups, respectively) and those fed TRT-D (2040 and 1920 g d^{-1} for HC and LC groups, respectively). Intake of DM was not different ($P>.1$) from those of CTR, however, feed intake expressed per unit metabolic BW in TRT-C was lower ($P<.05$) than CTR. Concurrent with a reduction in DM intake observed, reductions ($P<.05$) in water intake and urine output were also observed in animals fed TRT-C compared to those of CTR. In spite of differences observed in intake of DM, there were no differences ($P>.1$) in DM digestibility or daily BW gain between dietary treatments (Table 1.5).

Urine pH of animals fed TRT-B and TRT-C (reduced or negative dietary cation-anion balance) were lower ($P<.01$) than

Table 1.5. Body weight (BW) change, dry matter (DM) intake, DM digestibility and urine and fecal pH of sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium [1,2].

	High calcium					Low calcium						main effects and interaction[3]		
Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM[4]	Ca	Anion	Ca * Anion
Body weight change ($g\ d^{-1}$)	206	159	92	135	191	159	180	179	185	138	42.2	NS	NS	NS
DM intake ($g\ d^{-1}$)	1890	2130	1998	1687	2040	1834	1866	1759	1742	1920	106.8	*	**	NS
DM intake ($g\ d^{-1}\ kg^{-1}BW^{.75}$)	83.9	92.9	88.7	74.6	90.5	87.5	85.0	84.0	77.0	87.7	4.55	NS	**	NS
DM digestibility (%)	66.1	65.8	63.1	65.3	65.1	66.5	61.9	64.0	65.8	65.7	1.76	NS	NS	NS
Water intake ($L\ d^{-1}$)	4.2	5.0	5.2	3.3	4.8	4.6	4.7	4.1	3.7	4.2	0.45	*	**	NS
Fecal out put ($g\ DM\ d^{-1}$)	640	729	737	689	707	612	687	634	594	659	40.76	*	**	NS
Urine ($L\ d^{-1}$)	2.19	2.36	2.62	1.32	1.93	2.19	1.88	1.58	1.22	1.61	0.432	NS	**	NS
Urine pH	8.85	8.57	7.69	7.91	8.65	8.75	8.57	8.35	8.12	8.66	0.266	NS	**	NS
Fecal pH	8.21	8.14	8.43	8.29	8.30	8.23	7.99	8.13	8.23	7.97	0.108	**	*	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); * P<.1; ** P<.05; *** P<.01.
[4] SEM=Standard error of mean.

Table 1.6. Body weight (BW) change, dry matter (DM) intake, DM digestibility, urine and fecal pH of sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM[3]	HC	LC	SEM
Body weight change ($g\ d^{-1}$)	183	170	136	160	165	29.8	157	168	23.5
Initial BW (kg)	56.9	60.1	58.9	60.9	59.5	2.41	61.1	57.4	1.90
DM intake ($g\ d^{-1}$)	1862^{ab}	1998^{a}	1878^{ab}	1714^{b}	1980^{a}	75.51	1949^{A}	1824^{B}	52.0
DM intake ($g\ d^{-1}\ kg^{-1}BW^{.75}$)	85.7^{a}	88.9^{a}	86.4^{a}	75.8^{b}	89.1^{a}	3.22	86.1	84.3	2.63
DM digestibility (%)	66.3	63.9	63.5	65.5	65.4	1.24	65.1	64.8	0.68
Water intake ($L\ d^{-1}$)	4.4^{a}	4.9^{a}	4.7^{a}	3.6^{b}	4.5^{a}	0.32	4.5^{A}	4.3^{B}	0.12
Fecal out put (DM $g\ d^{-1}$)	626	708^{a}	685^{a}	591^{b}	683^{a}	28.8	681^{A}	637^{B}	20.9
Urine ($L\ d^{-1}$)	2.19^{a}	2.12^{ab}	2.10^{ab}	1.27^{b}	1.77^{ab}	0.306	2.09	1.70	0.252
Urine pH	8.80^{a}	8.57^{a}	8.02^{b}	8.02^{b}	8.66^{a}	0.188	8.34	8.49	0.143
Fecal pH	8.22^{A}	8.06^{B}	8.28^{A}	8.26^{A}	8.14^{AB}	0.080	8.27^{a}	8.11^{b}	0.050

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standard error of mean; HC=High Ca; LC=Low Ca.
[ab] Means in the same row with different superscripts are different ($P<.05$).
[AB] Means in the same row with different superscripts are different ($P<.1$).

CTR. There was no reduction ($P>.1$) in urine pH of animals fed TRT-A (supplemented with NH_4Cl to create metabolic acidosis) compared to that of CTR, however, fecal pH of animals fed TRT-A showed a tendency ($P<.1$) to be lower than CTR.

An interaction between levels of dietary Ca and dietary cation-anion balance was not detected for any of the above parameters ($P>.1$).

Phosphorus (P) absorption, excretion and retention

Absorption of P was different ($P<.05$) between HC and LC groups as well as between dietary treatments (Tables 1.7 and 1.8). Animals fed HC diets absorbed greater ($P<.01$) amount of P than those fed LC diets (4.3 vs 1.2 g $7d^{-1}$). Apparent absorption rate (apparent digestibility) also showed the same differences between the two groups (9.1 and 2.0% for HC and LC group, respectively) during the 7-day collection period. Among dietary treatments, it was demonstrated that animals fed TRT-C excreted P via feces in excess (37.2 g 7 d^{-1}) of that ingested (37.0 g $7d^{-1}$). By contrast, fecal P excretion of animals fed other diets were below the intake and apparent digestibility ranged from 3.5% for TRT-A to 12.5% for TRT-D (Table 1.8) with no differences ($P>.1$) observed between them. A reduction in apparent digestibility for animals fed TRT-B was observed compared to only that of CTR ($P<.01$) and TRT-D ($P<.05$) owning to a high variability among animals fed the same diet.

Table 1.7. Phosphorus absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium[1,2].

Variable	High Ca					Low Ca					SEM[4]	main effects and interaction[3]		
	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D		Ca	Anion	Ca * Anion
Intake (g $7d^{-1}$)	40.95	46.76	42.86	34.68	44.00	41.64	42.34	37.72	39.35	41.37	2.518	NS	**	NS
Feces excretion (g $7d^{-1}$)	26.34	41.02	38.79	36.00	35.73	38.18	42.99	37.91	38.38	38.75	2.213	NS	NS	NS
Urine excretion (g $7d^{-1}$)	0.09	0.10	0.11	0.08	0.11	0.11	0.08	0.09	0.09	0.10	0.011	NS	**	NS
Absorption (g $7d^{-1}$)	4.61	5.74	4.07	-1.32	8.27	3.46	-0.66	-0.19	0.97	2.61	2.454	***	**	NS
Digestibility (%)	11.18	12.11	8.30	-4.91	18.72	7.89	-5.16	-0.46	1.71	6.21	6.338	***	NS	NS
Retention (g $7d^{-1}$)	4.51	5.64	3.96	-1.39	8.17	3.35	-0.74	-0.28	0.88	2.51	2.452	***	**	NS
Urine/Abs (%)	2.15	1.65	2.63	-	1.28	3.21	-	-	9.44	3.90	4.552	NS	NS	NS
Retention/Intake (%)	10.94	11.91	8.06	-	18.37	7.62	-	-	1.48	5.97	6.244	*	NS	NS
Retention/Abs (%)	97.85	98.34	97.37	-	98.72	96.79	-	-	98.55	96.10	4.552	NS	NS	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment;
[3] NS=Not significant (P>.1); * P<.1; ** P<.05; *** P<.01.
[4] SEM=Standard error of mean.

Table 1.8. Phosphorus absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Intake (g 7d^{-1})	41.30^{ab}	44.55^{a}	40.29^{ab}	37.02^{b}	42.68^{a}	1.780	41.85	40.48	1.16
Feces excretion (g 7d^{-1})	37.26	42.01	38.35	37.19	37.24	1.565	37.58	39.24	0.99
Urine excretion (g 7d^{-1})	0.11^{a}	0.09^{ab}	0.10^{ab}	0.08^{b}	0.11^{a}	0.008	0.09	0.09	0.005
Absorption (g 7d^{-1})	4.04^{ab}	2.54^{ab}	1.94^{ab}	-0.17^{b}	5.44^{a}	1.735	4.28^{a}	1.24^{b}	1.10
Digestibility (%)	9.53^{a}	3.47^{ab}	3.92^{ab}	-1.60^{b}	12.47^{a}	4.48	9.08^{a}	2.04^{b}	2.84
Retention (g 7d^{-1})	3.93^{ab}	2.45^{ab}	1.84^{ab}	-0.26^{b}	5.34^{a}	1.735	4.18^{a}	1.15^{b}	1.10
Urine/Abs (%)	2.60	3.54	5.00	-	1.91	6.93	2.31	7.69	4.52
Retention/Intake (%)	9.28	3.27	3.68	-	12.22	4.486	8.85^{A}	1.80^{B}	2.83
Retention/Abs (%)	97.40	96.49	94.99	-	98.09	6.93	97.73	92.34	4.52

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standar error of mean; HC=High Ca; LC=Low Ca.
[ab] Means in the same row with different superscripts are different (P<.05).
[AB] Means in the same row with different superscripts are different (P<.1).

Excretion of P via urine were small regardless of levels of dietary Ca or dietary treatments. Animals fed TRT-C excreted less ($P<.05$) P (83 mg $7d^{-1}$) than CTR (105 mg $7d^{-1}$) and TRT-D (104 mg $7d^{-1}$). When urinary P excretion was expressed as a percentage of that absorbed, no differences ($P>.1$) were observed between dietary treatments.

Final P balance for sheep fed HC diets (4.18 g $7d^{-1}$ and 8.85% of P ingested, respectively) were higher ($P<.01$) than for sheep fed LC diets (1.15 g $7d^{-1}$ and 1.8% of P ingested, respectively) due to differences observed in apparent digestibility of P. None of the dietary treatments differed ($P>.1$) in P retention compared to that of CTR; however, animals fed TRT-C showed an apparent loss of P (-0.26 g $7d^{-1}$).

Figures 1.1 and 1.2 show the relationship between dietary cation-anion balance (meq d^{-1}) and apparent absorption and retention of P, respectively. Positive linear relationships were obtained with correlation coefficient values (r) of 0.248 ($P=0.085$) and 0.247 ($P=0.087$), respectively. There was a positive correlation between dietary cation-anion balance intake (meq d^{-1}) and urinary excretion (g $7d^{-1}$)($r=0.311$, $P=0.029$).

Calcium (Ca) absorption, excretion and retention

Calcium metabolism is presented in Table 1.9 and 1.10. As expected, there was approximately a two-fold difference ($P<.01$) in intake of Ca between HC and LC diets (110.7 g vs

Figure 1.1. Relationship between the intake of dietary cation-anion balance and the apparent absorption of phosphorus (P) (O = high calcium, ● = low calcium groups) (r=0.248, P=0.085).

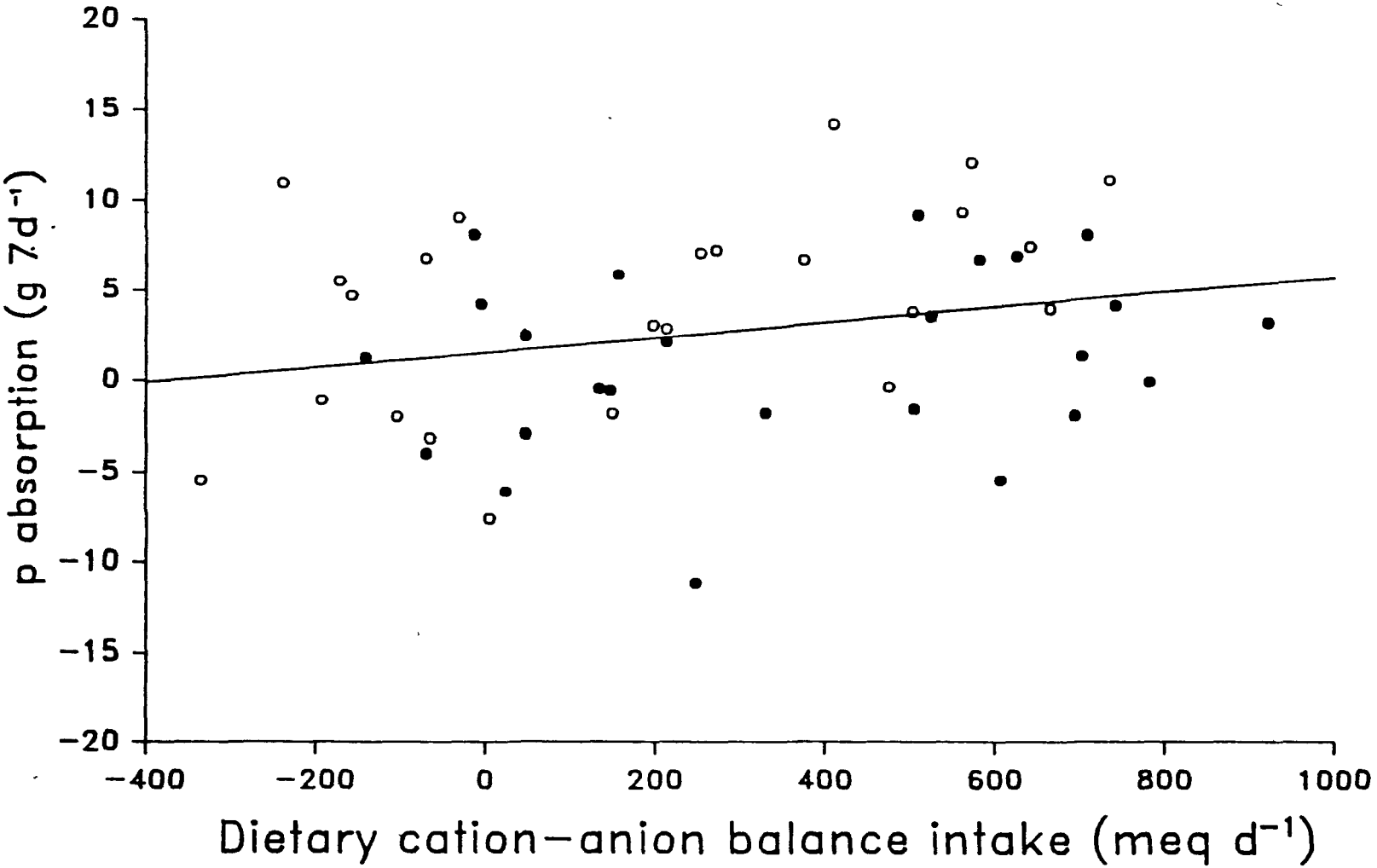

20
15
10
5
0
−5
−10
−15
−20
p absorption (g 7d⁻¹)
−400
−200
0
200
400
600
800
1000
Dietary cation−anion balance intake (meq d⁻¹)

Figure 1.2. Relationship between the intake of dietary cation-anion balance and the retention of phosphorus (P) (o = high calcium, ● = low calcium groups) (r= 0.247, P=0.087).

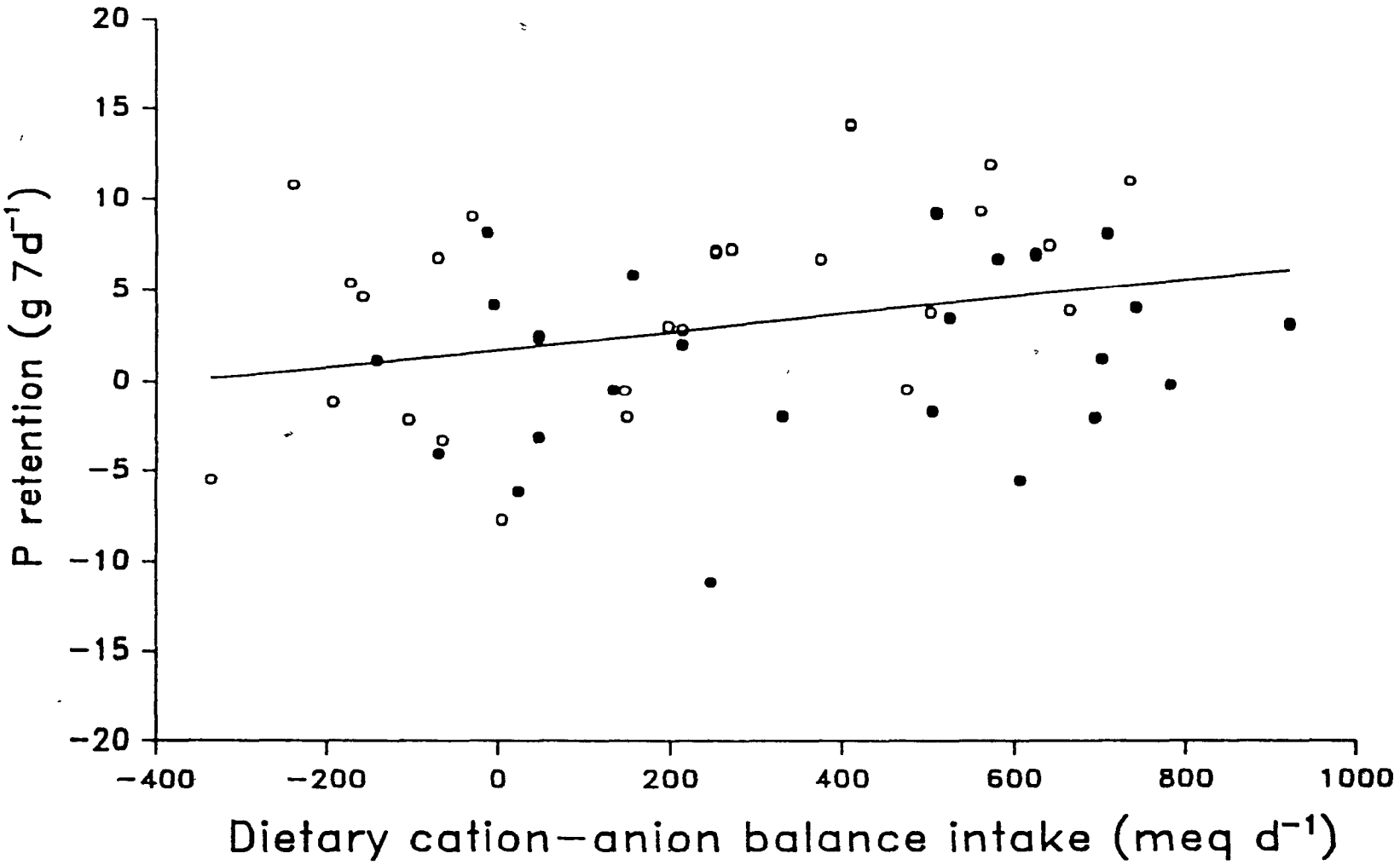

20
15
10
5
0
−5
−10
−15
−20
P retention (g 7d^{-1})
−400
−200
0
200
400
600
800
1000
Dietary cation−anion balance intake (meq d^{-1})

Table 1.9. Calcium absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium[1,2].

Variable	High Ca					Low Ca					SEM[4]	main effects and interaction[3]		
	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D		Ca	Anion	Ca * Anion
Intake (g $7d^{-1}$)	111.02	111.49	117.37	94.17	111.52	62.46	62.15	56.41	58.62	67.02	5.303	***	**	NS
Fecal excretion (g $7d^{-1}$)	91.63	100.96	93.14	83.90	99.97	50.70	54.89	47.85	53.93	52.55	5.175	***	NS	NS
urine excretion (g $7d^{-1}$)	0.15	2.30	5.68	5.63	0.89	0.63	3.89	2.38	2.74	0.41	1.143	NS	***	NS
Absorption (g $7d^{-1}$)	19.38	18.53	24.23	10.27	11.56	11.76	7.25	8.56	4.69	14.48	5.53	**	NS	NS
Digestibility (%)	18.19	15.99	18.58	10.25	12.39	19.18	10.18	14.65	8.85	21.91	5.411	NS	NS	NS
Retention (g $7d^{-1}$)	19.23	16.23	18.55	4.64	10.66	11.12	3.37	6.17	1.95	14.07	5.151	**	**	NS
Urine/Abs (%)	0.77	12.42	23.43	54.78	7.73	5.39	53.57	27.86	58.44	2.79	20.750	NS	NS	NS
Retention/Intake (%)	18.08	14.13	14.04	4.43	11.63	18.26	3.76	9.74	4.04	21.30	5.312	NS	**	NS
Retention/Abs (%)	99.23	87.58	76.56	45.22	92.27	94.60	46.44	72.14	41.56	97.19	20.750	NS	NS	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); ** P<.05; *** P<.01.
[4] SEM=Standard error of mean.

Table 1.10. Calcium absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Treatment Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Intake (g $7d^{-1}$)	86.74^{ab}	90.82^{a}	86.89^{ab}	76.39^{b}	89.27^{a}	3.750	110.71^{a}	61.33^{b}	2.44
Feces excretion (g $7d^{-1}$)	71.17^{ab}	77.93^{a}	70.49^{ab}	68.91^{b}	76.26^{ab}	3.659	93.92^{a}	51.98^{b}	2.31
Urine excretion (g $7d^{-1}$)	0.39^{a}	3.09^{b}	4.03^{b}	4.18^{b}	0.65^{a}	0.495	2.93	2.01	0.51
Absorption (g $7d^{-1}$)	15.57	12.89	16.39	7.48	13.02	3.913	16.79^{a}	9.35^{b}	2.47
Digestibility (%)	18.69	13.09	16.61	9.55	17.15	3.83	15.08	14.96	2.42
Retention (g $7d^{-1}$)	15.18^{a}	9.80^{ab}	12.36^{ab}	3.30^{b}	12.37^{ab}	3.64	13.86^{a}	7.34^{b}	2.30
Urine/Abs (%)	2.52	23.99	24.59	55.93	4.99	20.75	17.44	21.50	17.89
Retention/Intake (%)	18.17^{a}	8.94^{ab}	11.89^{ab}	4.23^{b}	16.47^{a}	3.756	12.46	11.42	2.38
Retention/Abs (%)	97.48	76.01	75.41	44.07	95.01	20.75	82.56	78.49	17.89

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standar error of mean; HC=High Ca; LC=Low Ca.
[ab] Means in the same row with different superscripts are different ($P<.05$).

61.3 g $7d^{-1}$)(Table 1.10). For the HC diets, animals fed TRT-C had a reduced intake of Ca (94.17 g $7d^{-1}$) than others because of a reduction of DM intake observed for this dietary treatment. A major excretion route of the ingested Ca was via feces and amounts excreted were a reflection of Ca ingested. Therefore, the proportion of Ca ingested that was excreted via feces was similar between the HC and LC diets at 84.9% and 85.0%, respectively . Apparent digestibility of Ca was similar between HC (15.1%) and LC groups (15.0%) resulting in higher ($P<.05$) Ca absorption by animals fed HC diets (16.79 g $7d^{-1}$) than that of animals fed LC diets (9.35 g $7d^{-1}$), and there was no difference ($P>.1$) observed between dietary treatments and animals fed TRT-C showed the lowest values in both HC and LC groups (10.25% and 8.85% for TRT-C in HC and LC groups, respectively).

Excretion of Ca via urine showed major differences between dietary treatments. The combined data for HC and LC diets (Table 1.10) showed that animals fed reduced or negative cation-anion balanced diets (TRT-A,TRT-B and TRT-C), regardless of the magnitude of negativity of their dietary cation-anion balance, excreted more ($P<.01$) Ca in urine than animals fed CTR or TRT-D. When the urinary Ca excretion was expressed as a proportion of Ca absorbed, similar patterns of differences between dietary treatments were observed.

Final Ca balance revealed that all animals were in a positive balance, and sheep fed HC diets retained greater ($P<.05$) quantities of Ca than those fed LC diets (13.86 vs

7.34 g $7d^{-1}$, respectively). Animals fed TRT-C showed a reduced ($P<.05$) Ca retention than CTR. When Ca balance was expressed as a proportion of Ca ingested, animals fed TRT-C had lower ($P<.05$) and animals fed TRT-A tended to have a lower ($P<.1$) retention than animals fed CTR (Table 1.10). No interactions were observed in Ca metabolism.

Figures 1.3 and 1.4 show the relationships between dietary cation-anion balance intake (meq d^{-1}) and apparent absorption and retention of Ca, respectively. There was no significant ($P=0.245$) correlation observed between intake of dietary cation-anion balance (meq d^{-1}) and apparent Ca absorption (g $7d^{-1}$). However, a positive linear correlation ($r=0.305$, $P=0.032$) between intake of dietary cation-anion balance (meq $7d^{-1}$) and Ca retention (g $7d^{-1}$) was obtained (Figure 1.4) mainly due to a strong negative linear correlation ($r=0.328$ and $P=0.0001$) observed between intake of dietary cation-anion balance (meq d^{-1}) and urinary Ca excretion (g $7d^{-1}$)(Figure 1.5).

The relationships between apparent absorption and retention of Ca and P are presented in Figures 1.6 and 1.7. Correlation coefficient values of 0.65 ($P=.0001$) was observed in both relationship and the correlation equations calculated were as follows:

$$P_{abs} = -0.758 + 0.267 * Ca_{abs}$$

$$P_{ret} = -0.201 + 0.270 * Ca_{ret}$$

where;

P_{abs} = P absorption (g $7d^{-1}$),

Ca_{abs} = Ca absorption (g $7d^{-1}$),

Figure 1.3. Relationship between the intake of dietary cation-anion balance and the apparent absorption of calcium (Ca) (○= high calcium, ● = low calcium groups) (r=0.167, P=0.245).

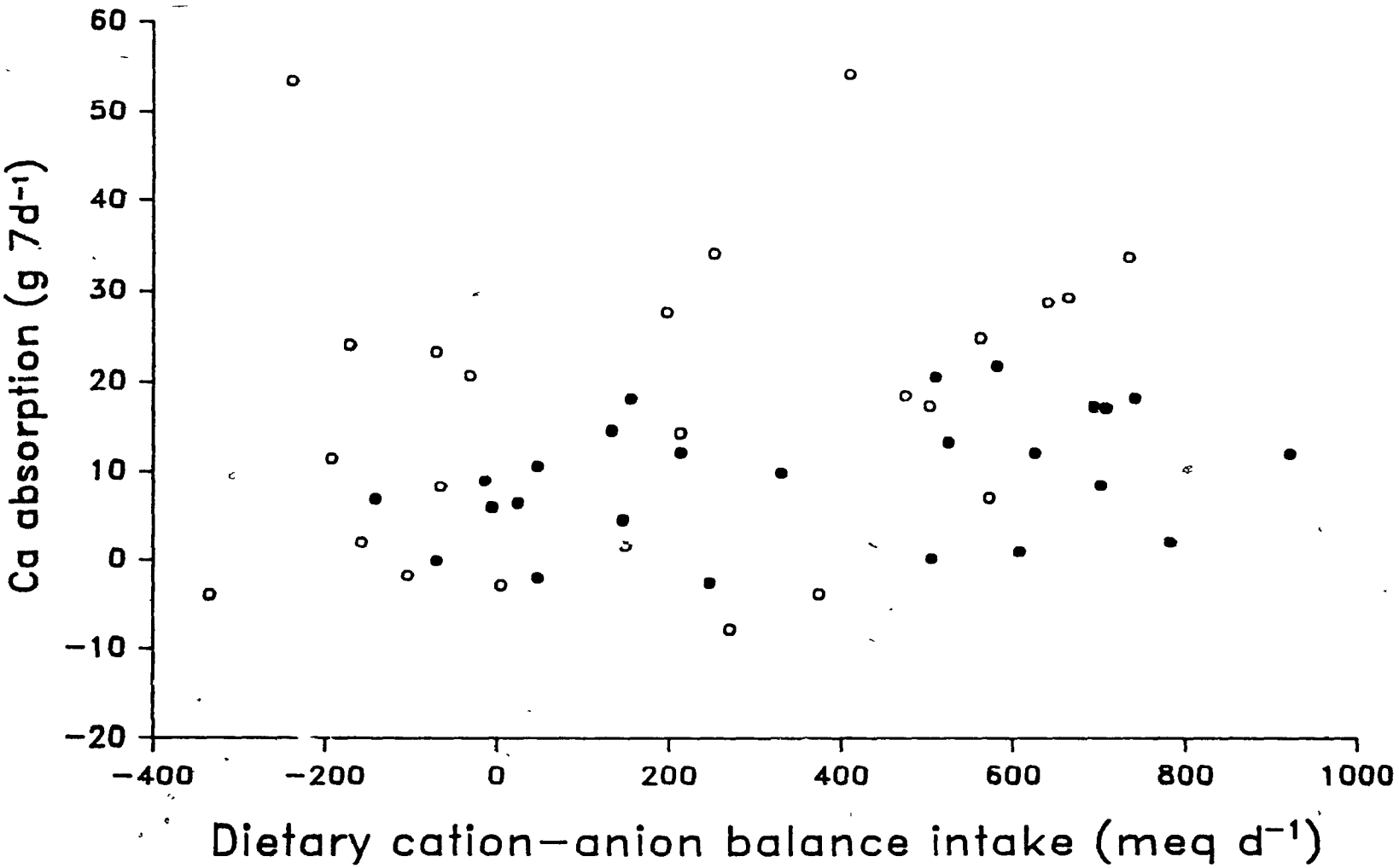

Ca absorption (g 7d-1)
60
50
40
30
20
10
0
-10
-20
-400
-200
0
200
400
600
800
1000
Dietary cation–anion balance intake (meq d-1)

Figure 1.4. Relationship between the intake of dietary cation-anion balance and the retntion of calcium (Ca) (o = high calcium, • = low calcium groups) ($r=0.0305$, $P=0.032$).

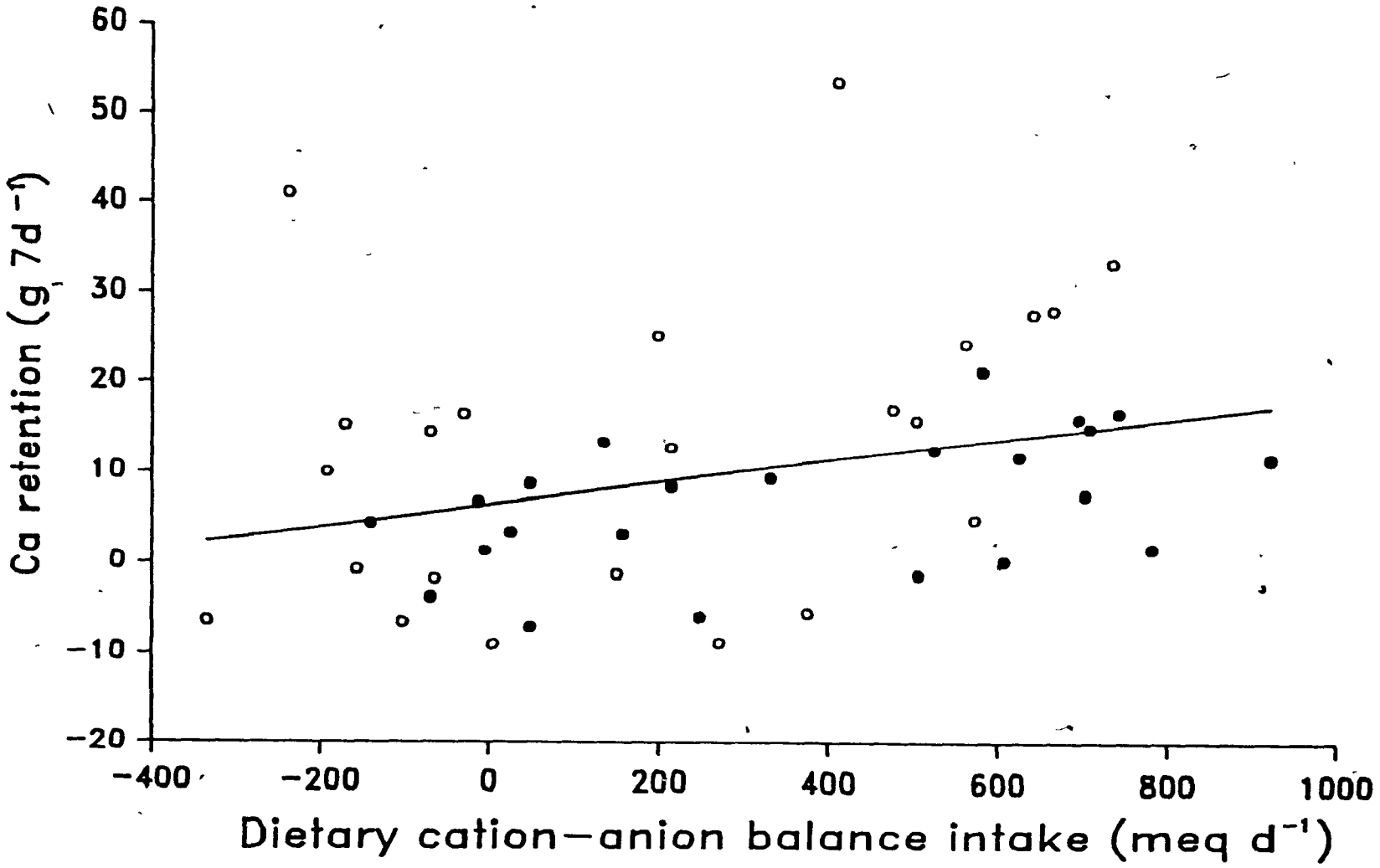

60
50
40
30
20
10
0
−10
−20
Ca retention (g 7d⁻¹)
−400
−200
0
200
400
600
800
1000
Dietary cation−anion balance intake (meq d⁻¹)

Figure 1.5. Relationship between the intake of dietary cation-anion balance and the urinary calcium (Ca) excretion (o = high calcium, • = low calcium groups).

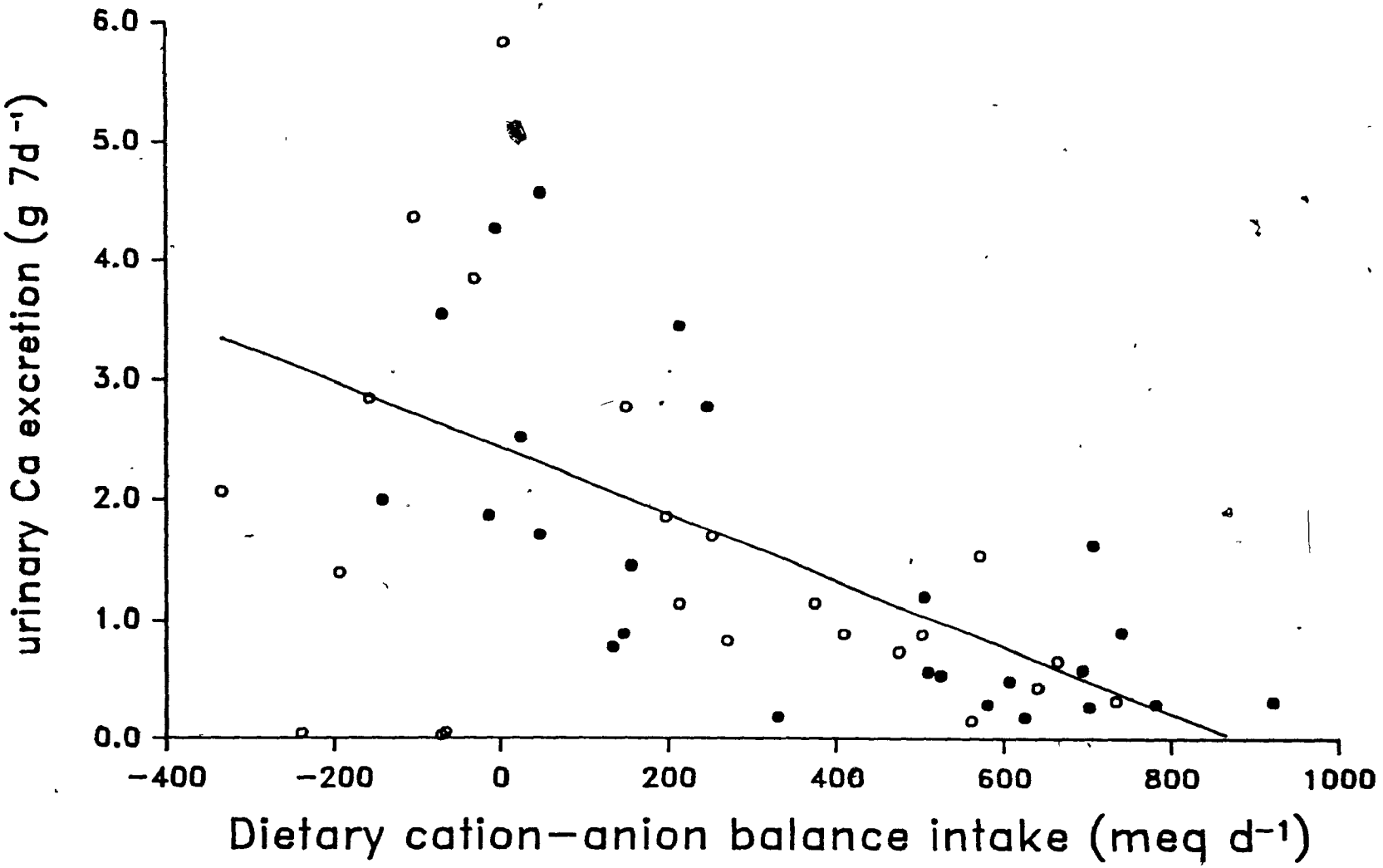

urinary Ca excretion (g 7d⁻¹)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
−400
−200
0
200
400
600
800
1000
Dietary cation−anion balance intake (meq d⁻¹)

Figure 1.6. Relationship between the apparent absoprtion of calcium (Ca) and phosphorus (P).

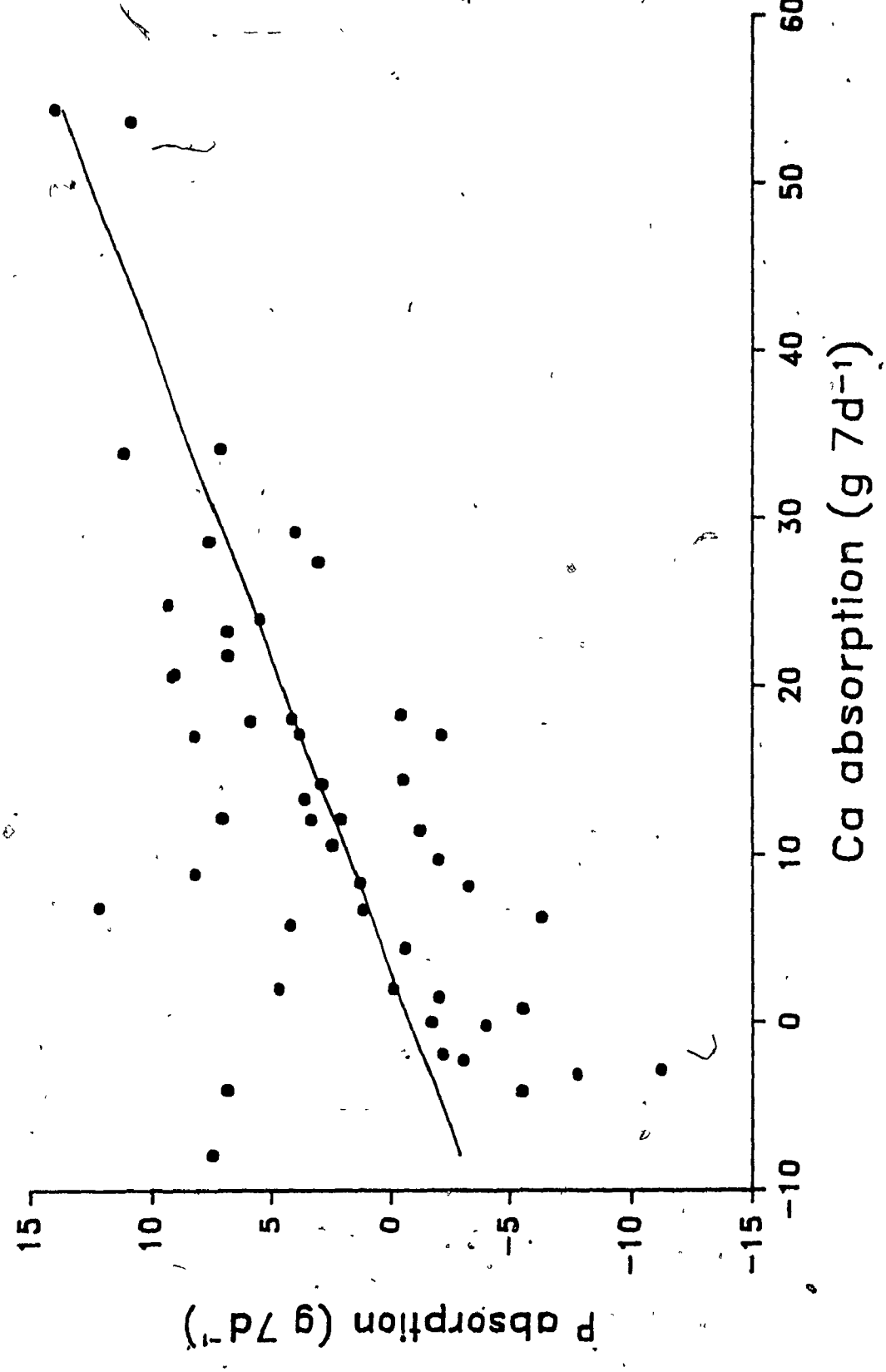
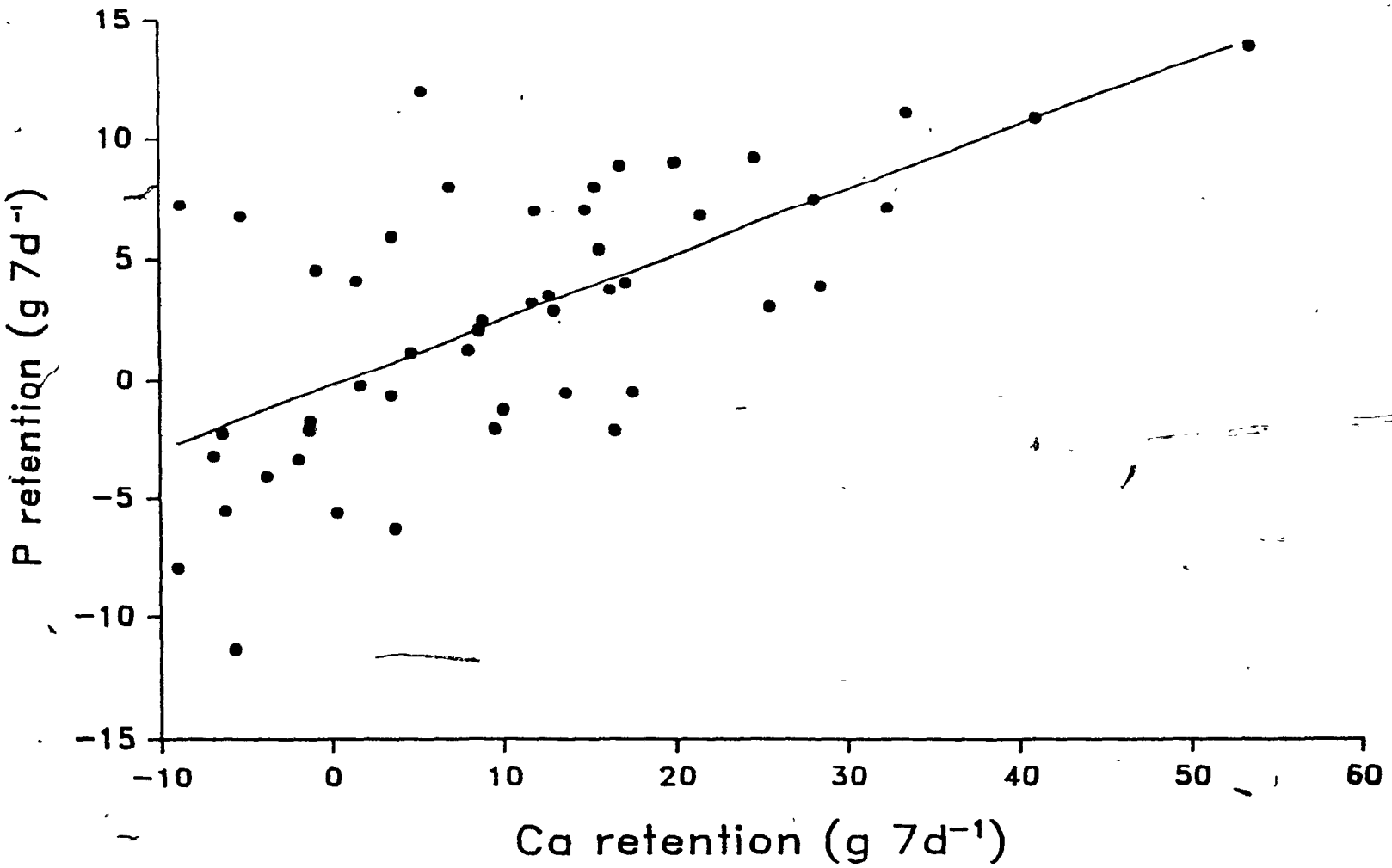

P absorption (g 7d⁻¹)
15
10
5
0
−5
−10
−15
Ca absorption (g 7d⁻¹)
−10
0
10
20
30
40
50
60

Figure 1.7. Relationship between the retention of calcium (Ca) and phosphorus (P).


15
10
5
0
−5
−10
−15
P retention (g 7d⁻¹)
−10
0
10
20
30
40
50
60
Ca retention (g 7d⁻¹)

P_{ret} = P retention (g $7d^{-1}$),

Ca_{ret} = Ca retention (g $7d^{-1}$).

Magnesium (Mg) metabolism

As expected, animals fed TRT-B and TRT-C, where $MgSO_4$ was supplemented as anion-supplemental mineral salts, had higher ($P<.05$) Mg intake than those fed other dietary treatments (Tables 1.11 and 1.12). Feces accounted for most of the Mg excretion, as was observed in P and Ca metabolism, and was reflected by the amount of Mg ingested. Thus, no differences were observed in apparent digestibility (%) or absorption (g $7d^{-1}$) between dietary treatments.

Total urinary Mg excretion was a reflection of the amount of Mg ingested and animals fed TRT-B and TRT-C excreted more ($P<.01$) than CTR but no differences were observed when the values were expressed as a proportion of that absorbed.

None of dietary treatments showed differences in Mg retention (g $7d^{-1}$)($P>.1$) from CTR, however, there was a difference ($P<.05$) between the group with the lowest retention (TRT-B) and the group with the highest retention (TRT-A).

Sodium (Na) metabolism

As expected, intake of Na was reduced ($P<.01$) for animals fed the reduced or negative cation-anion balanced

Table 1.11. Magnesium absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium[1,2].

	High Ca					Low Ca						main effects and interaction[3]		
Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM[4]	Ca	Anion	Ca * Anion
Intake (g $7d^{-1}$)	31.68	35.53	42.98	35.99	33.01	26.59	27.75	32.06	33.74	28.53	2.129	***	***	NS
Fecal excretion (g $7d^{-1}$)	22.61	24.97	32.64	25.11	24.81	19.09	20.98	26.86	24.70	20.67	1.381	**	***	NS
Urine excretion (g $7d^{-1}$)	4.69	4.54	7.98	8.77	5.51	5.90	5.18	5.94	6.66	4.10	0.700	*	***	NS
Absorption (g $7d^{-1}$)	9.07	10.56	10.34	10.88	8.20	7.50	6.77	5.20	9.05	7.86	1.759	*	NS	NS
Digestibility (%)	28.71	30.02	22.42	30.01	24.56	27.51	19.65	15.44	26.30	27.33	4.754	NS	NS	NS
Retention (g $7d^{-1}$)	4.37	6.02	2.35	2.11	2.69	1.61	1.59	-0.70	2.39	3.76	1.420	**	**	NS
Urine/Abs (%)	52.55	43.02	77.23	80.58	67.16	87.90	76.48	114.06	73.58	52.15	10.890	NS	**	NS
Retention/Intake (%)	13.17	17.15	4.03	5.55	7.69	5.16	0.47	-	6.66	12.50	4.720	*	NS	NS
Retetion/Abs (%)	47.44	56.98	22.77	19.42	32.83	21.14	23.52	-	26.42	47.85	10.890	NS	**	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); * P<.1; ** P<.05; *** P<.01.
[4] SEM=Standard error of mean.

Table 1.12. Magnesium absorption and retention by sheep fed different cation-anion balanced diet or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Treatment Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Intake (g $7d^{-1}$)	29.13^{a}	31.64^{a}	37.52^{b}	34.87^{b}	30.77^{a}	1.506	35.84^{a}	29.74^{b}	0.981
Feces excretion (g $7d^{-1}$)	20.85^{a}	22.97^{a,b}	29.75^{c}	24.90^{b}	22.74^{a,b}	0.495	26.03^{a}	22.46^{b}	0.62
Urine excretion (g $7d^{-1}$)	5.29^{a}	4.86^{a}	6.96^{b}	7.71^{b}	4.80^{a}	0.495	6.30^{A}	5.55^{B}	0.31
Absorption (g $7d^{-1}$)	8.28	8.67	7.77	9.96	8.03	1.243	9.81^{A}	7.28^{B}	0.79
Digestibility (%)	28.11	24.84	18.93	28.16	25.09	3.362	27.15	23.25	2.27
Retention (g $7d^{-1}$)	2.99^{a,b}	3.81^{a}	0.81^{b}	2.55^{a,b}	3.23^{a,b}	1.004	3.51^{a}	1.72^{b}	0.64
Urine/Abs (%)	63.91^{a,b}	56.09^{a}	89.56^{b}	77.40^{a,b}	59.82^{a}	10.89	64.21	76.32	12.31
Retention/Intake (%)	9.16	8.81	0.24	6.11	10.10	3.338	9.52^{A}	4.25^{B}	2.11
Retention/Abs (%)	36.09^{a,b}	43.91^{a}	10.44^{b}	25.61^{a,b}	40.19^{a}	10.89	35.79	23.69	12.31

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standar error of mean; HC=High Ca; LC=Low Ca.
^{abc} Means in the same row with different superscripts are different ($P<.05$).
^{AB} Means in the same row with different superscripts are different ($P<.1$).

diets (TRT-B and TRT-C) compared to those fed higher dietary cation-anion balanced diets (CTR, TRT-A and TRT-D) because of the high Na content in premix-A. Apparent digestibility (%) and absorption (g $7d^{-1}$) for Na were also reduced ($P<.01$) for animals fed TRT-B and TRT-C than those fed higher dietary cation-anion balanced diets (Table 1.13). Apparent digestibility of Na was considerably higher than for the divalent minerals such as Ca and Mg and ranged from 40.67 to 75.58% (Table 1.13).

Animals fed TRT-B and TRT-C were in a negative balance (-2.72 and -11.78 g $7d^{-1}$, respectively) because of the lower ($P<.05$) apparent digestibility and a higher urinary excretion than those fed CTR who showed a positive Na balance (9.29 g $7d^{-1}$)(Table 1.14).

Potassium (K) metabolism

None of dietary treatments showed differences ($P>.1$) in K metabolism parameters compared to CTR (Table 1.15). However, animals fed TRT-C showed a tendency ($P<.1$) for reduced absorption (g $7d^{-1}$) than TRT-D. This was a reflection of differences ($P<.05$) observed in the intake of DM between the two dietary treatments (Table 1.16). An increased apparent digestibility and an increased rate of urinary excretion expressed as a proportion of that absorbed confirmed that the metabolism of K was controlled mainly through the kidney rather than the gastrointestinal tract (Table 1.16).

Table 1.13. Sodium absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of calcium[1,2].

	High Ca					Low Ca						main effects and interaction[3]		
Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM[4]	Ca	Anion	Ca * Anion
Intake (g $7d^{-1}$)	66.80	78.92	41.08	31.58	73.10	69.97	73.58	37.25	31.90	64.45	4.965	NS	***	NS
Feces excretion (g $7d^{-1}$)	17.05	26.34	17.29	16.87	26.99	21.48	30.24	16.13	18.82	26.19	2.720	NS	**	NS
Urine excretion (g $7d^{-1}$)	40.21	44.15	25.69	40.12	37.57	39.44	29.80	24.66	11.23	33.28	7.476	*	**	NS
Absorption (g $7d^{-1}$)	49.75	52.58	23.79	14.71	46.11	48.48	43.35	21.12	13.08	38.26	5.800	NS	***	NS
Digestibility (%)	75.58	66.87	56.47	43.56	62.33	66.82	59.93	56.06	40.67	58.85	6.211	NS	***	NS
Retention (g $7d^{-1}$)	9.54	8.43	-1.90	-25.41	8.54	9.05	13.55	-3.55	1.85	4.99	8.295	NS	**	NS
Urine/Abs (%)	80.83	83.96	107.98	272.70	81.47	81.34	68.74	125.48	107.10	86.96	20.250	NS	**	NS
Retention/Intake (%)	17.60	9.50	-23.74	-108.83	10.51	11.03	16.37	-5.44	3.50	7.22	26.689	NS	*	NS
Retention/Abs (%)	19.17	16.04	-7.98	-172.74	18.52	18.66	31.26	-25.48	-7.09	13.04	20.250	NS	NS	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); * P<.1; ** P<.05; *** P<.01.
[4] SEM=Standard error of mean.

Table 1.14. Sodium absorption and retention by sheep fed different cation-anion balanced diet or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Treatment Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Intake (g $7d^{-1}$)	68.38^{a}	76.25^{a}	39.16^{b}	31.74^{b}	68.78^{a}	3.511	58.29	55.43	2.23
Feces excretion (g $7d^{-1}$)	19.27^{a}	28.29^{b}	16.71^{a}	17.85^{a}	26.59^{b}	1.924	20.91	22.57	1.22
Urine excretion (g $7d^{-1}$)	39.82^{a}	$36.97^{a\,b}$	25.17^{b}	$25.68^{a\,b}$	$35.42^{a\,b}$	5.286	37.55^{A}	27.68^{B}	3.34
Absorption (g $7d^{-1}$)	49.11^{a}	47.96^{a}	22.45^{b}	13.89^{b}	42.19^{a}	4.101	37.38	32.86	2.59
Digestibility (%)	71.20^{a}	$61.90^{a\,b}$	56.27^{b}	42.11^{c}	$60.59^{a\,b}$	4.392	60.96	55.87	2.78
Retention (g $7d^{-1}$)	$9.29^{a\,b}$	10.99^{a}	$-2.72^{a\,b}$	-11.78^{b}	6.76^{a}	5.866	-0.16	5.18	3.71
Urine/Abs (%)	81.10^{a}	77.08^{a}	$112.2^{a\,b}$	184.81^{b}	$83.96^{a\,b}$	20.25	100.43	84.24	20.25
Retention/Intake (%)	14.31^{A}	12.94^{A}	$-14.59^{A\,B}$	-52.41^{B}	8.87^{A}	18.873	-18.89	6.54	11.94
Retention/Abs (%)	18.92	22.92	-	-	16.04	20.25	-	15.76	20.25

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standar error of mean; HC=High Ca; LC=Low Ca.
$^{a\,b\,c}$ Means in the same row with different superscripts are different ($P<.05$).
$^{A\,B}$ Means in the same row with different superscripts are different ($P<.1$).

Table 1.15. Potassium absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium[1,2].

Variable	High Ca					Low Ca					SEM[4]	main effects and interaction[3]		
	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D		Ca	Anion	Ca * Anion
Intake (g $7d^{-1}$)	173.5	182.7	184.8	158.1	180.9	178.3	180.9	163.1	173.9	193.1	10.05	NS	**	NS
Fecal excretion (g $7d^{-1}$)	9.45	13.13	13.90	10.92	13.83	16.13	13.97	14.26	14.92	14.07	2.287	NS	NS	NS
Urine excretion (g $7d^{-1}$)	121.9	121.2	125.0	95.5	127.6	123.7	113.8	108.9	110.3	117.8	9.17	NS	NS	NS
Absorption (g $7d^{-1}$)	164.1	169.59	170.9	147.3	167.1	162.2	168.0	148.9	159.0	179.1	9.39	NS	*	NS
Digestibility (%)	94.16	91.71	92.20	92.95	92.05	91.13	91.81	91.24	92.16	92.80	1.17	NS	NS	NS
Retention (g $7d^{-1}$)	42.22	48.39	45.91	51.71	39.49	38.51	54.15	40.01	48.71	61.24	9.867	NS	NS	NS
Urine/Abs (%)	72.86	75.19	73.38	64.67	77.40	76.39	70.36	74.33	71.38	67.44	3.910	NS	NS	NS
Retention/Intake (%)	23.05	23.29	24.47	32.93	20.99	21.55	27.44	23.52	25.95	30.18	4.919	NS	NS	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); * P<.1; ** P<.05.
[4] SEM=Standard error of mean.

Table 1.16. Potassium absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Treatment Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Intake (g $7d^{-1}$)	175.96^{ab}	182.34^{ab}	174.00^{ab}	166.08^{a}	187.04^{b}	7.106	176.05	178.12	4.63
Feces excretion (g $7d^{-1}$)	12.79	13.55	14.08	12.92	13.95	1.617	12.25	14.67	1.02
Urine excretion (g $7d^{-1}$)	122.81	117.52	116.96	102.95	122.73	6.487	118.26	114.92	4.10
Absorption (g $7d^{-1}$)	163.17^{AB}	168.79^{AB}	159.92^{AB}	153.16^{A}	173.09^{B}	6.645	163.81	163.45	4.20
Digestibility (%)	92.64	91.76	91.72	92.55	92.43	0.826	92.62	91.83	0.52
Retention (g $7d^{-1}$)	40.37	51.27	42.96	50.21	50.36	6.977	45.54	48.52	4.41
Urine/Abs (%)	74.83	72.78	73.83	68.03	72.42	3.91	72.70	71.98	2.37
Retention/Intake (%)	22.30	25.37	23.99	29.44	25.59	3.478	24.95	25.73	2.20
Retention/Abs (%)	25.17	27.21	26.15	31.97	27.58	3.91	27.30	28.02	2.37

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standar error of mean; HC=High Ca; LC=Low Ca.
[ab] Means in the same row with different superscripts are different ($P<.05$).
[AB] Means in the same row with different superscripts are different ($P<.1$).

Chloride (Cl) metabolism

As expected, animals fed TRT-A had an increased ($P<.01$) Cl intake (Table 1.17, 1.18). There were no differences ($P>.1$) in apparent digestibility between dietary treatments. Absorption of Cl from the GI-tract was very high (above 90%) and confirms that the kidney is the major homeostatic organ. A intake of Cl by sheep fed HC diets was greater ($P<.01$) than that for sheep fed LC diets. This was caused by the difference observed in Cl concentration between alfalfa hay (0.52%) and timothy hay (0.35%). This difference also caused differences ($P<.05$) in Cl retention.

Sulfur (S) metabolism

Compared to Cl, sulfur metabolism showed a different homeostatic metabolism (Table 1.19). Apparent digestibility increased ($P<.01$) as intake of S increased. This resulted in greater ($P<.05$) retention by animals fed TRT-B and TRT-C than QTR (Table 1.20). However, since animals fed TRT-B and TRT-C excreted excess S in urine , there was no difference ($P>.1$) observed in S retention by sheep fed different dietary treatments. Retention of S expressed as a proportion of that ingested differed more than 10-fold (range from 2.71 to 60.41%) but no difference ($P>.1$) was observed between dietary treatments because of large variations between animals.

Table 1.17. Chloride absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium[1,2].

	High Ca					Low Ca						main effects and interaction[3]		
Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM[4]	Ca	Anion	Ca * Anion
Intake (g $7d^{-1}$)	78.8	179.6	99.7	76.3	85.8	54.7	116.3	69.5	55.6	53.7	7.29	***	***	NS
Fecal excretion (g $7d^{-1}$)	3.12	4.97	5.79	4.04	3.81	3.56	3.44	3.35	3.12	4.30	0.81	NS	NS	NS
Urine excretion (g $7d^{-1}$)	46.3	108.0	55.4	48.1	64.8	39.5	102.6	57.3	28.2	43.4	8.09	***	***	NS
Absorption (g $7d^{-1}$)	75.7	174.6	93.9	72.3	81.9	51.1	102.6	66.1	52.5	49.4	7.32	***	***	NS
Digestibility (%)	95.8	97.2	94.3	94.7	95.7	93.4	96.6	95.1	94.4	92.1	1.00	NS	NS	NS
Retention (g $7d^{-1}$)	29.4	66.6	38.5	24.2	17.2	11.6	10.3	8.81	24.3	6.03	7.59	***	**	*
Urine/Abs (%)	61.6	62.8	58.9	65.7	78.9	77.4	91.5	84.0	55.4	88.2	9.42	*	NS	NS
Retention/Intake (%)	36.9	36.3	38.7	32.7	20.3	21.3	8.25	15.2	42.4	10.7	9.01	*	NS	NS
Retention/Abs (%)	38.4	37.2	41.1	34.3	21.1	22.6	8.48	15.9	44.6	11.8	9.42	*	NS	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); * P<.1; ** P<.05.
[4] SEM=Standard error of mean.

Table 1.18. Chloride absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Treatment Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Intake (g $7d^{-1}$)	66.7^{a}	147.9^{b}	84.6^{c}	65.9^{a}	69.7^{a}	5.15	104.0^{a}	69.92^{b}	3.67
Feces excretion (g $7d^{-1}$)	3.34	4.21	4.57	3.58	4.06	0.57	4.35	3.56	0.41
Urine excretion (g $7d^{-1}$)	$42.9^{a,b}$	105.3^{c}	56.3^{a}	38.1^{a}	$54.1^{a,b}$	5.72	64.5^{a}	54.2^{b}	4.08
Absorption (g $7d^{-1}$)	63.4^{a}	143.7^{b}	80.0^{c}	62.4^{a}	$65.7^{a,c}$	5.18	99.7^{a}	66.4^{b}	3.69
Digestibility (%)	94.6	96.9	94.7	94.6	93.9	0.71	95.6	94.3	0.50
Retention (g $7d^{-1}$)	20.50^{a}	38.43^{b}	23.68^{a}	24.23^{a}	11.59^{a}	5.37	35.17^{a}	12.20^{b}	3.83
Urine/Abs (%)	69.49	77.14	71.45	60.54	83.52	7.20	65.58^{A}	79.29^{B}	4.75
Retention/Intake (%)	29.07	22.28	26.97	37.51	15.51	6.38	32.97^{A}	19.56^{B}	4.56
Retention/Abs (%)	30.51	22.86	28.54	39.45	16.48	6.67	34.42^{A}	20.71^{B}	4.75

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standar error of mean; HC=High Ca; LC=Low Ca.
[abc] Means in the same row with different superscripts are different ($P<.05$).
[AB] Means in the same row with different superscripts are different ($P<.1$).

Table 1.19. Sulfur absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium[1,2].

	High Ca					Low Ca						main effects and interaction[3]		
Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM[4]	Ca	Anion	Ca * Anion
Intake (g $7d^{-1}$)	26.5	35.7	69.9	58.5	27.7	25.7	21.2	50.0	65.4	23.1	4.06	*	***	**
Fecal excretion (g $7d^{-1}$)	11.1	12.8	14.8	17.9	9.67	9.8	11.7	11.8	16.5	10.2	1.20	NS	***	NS
Urine excretion (g $7d^{-1}$)	0.09	1.92	25.71	34.49	1.15	0.14	1.11	37.96	31.69	11.98	6.13	NS	***	NS
Absorption (g $7d^{-1}$)	15.4	22.9	54.8	40.9	10.0	15.9	9.55	38.2	49.5	12.9	3.82	NS	***	**
Digestibility (%)	57.04	62.87	78.56	69.05	63.25	60.99	37.73	76.20	73.35	55.72	4.87	NS	***	*
Retention (g $7d^{-1}$)	15.6	20.9	29.1	6.1	16.9	15.8	10.9	2.7	17.2	0.92	7.12	NS	NS	NS
Urine/Abs (%)	0.5	61.5	50.6	83.7	8.2	0.9	11.6	102.6	59.9	110.2	36.42	NS	NS	NS
Retention/Intake (%)	58.9	58.5	39.3	11.5	58.7	60.4	51.0	5.3	27.33	2.7	20.26	NS	NS	NS
Retention/Abs	100.5	93.9	49.4	16.3	91.8	99.1	118.0	6.9	40.1	7.1	36.42	NS	NS	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); * P<.1; ** P<.05; *** P<.01.
[4] SEM=Standard error of mean.

Table 1.20. Sulfur absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Treatment Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Intake (g $7d^{-1}$)	26.1[a]	28.5[a]	59.8[b]	61.9[b]	25.4[a]	2.86	43.6[A]	37.1[B]	2.04
Feces excretion (g $7d^{-1}$)	10.5[a]	12.3[a b]	13.3[b]	17.2[c]	9.9[a]	0.84	13.3	11.9	0.60
Urine excretion (g $7d^{-1}$)	0.12[a]	1.52[a]	31.8[b]	33.1[b]	6.6[a]	4.35	12.6	16.1	3.09
Absorption (g $7d^{-1}$)	15.6[a]	16.2[a]	46.5[b]	44.7[b]	15.5[a]	2.70	30.4	25.1	1.92
Digestibility (%)	59.0[a]	50.0[a]	77.4[b]	71.2[b]	59.5[a]	3.45	66.2	60.1	2.45
Retention (g $7d^{-1}$)	15.5	14.9	14.7	11.7	8.9	5.03	17.7	9.0	3.59
Urine/Abs (%)	2.5	9.4	76.4	71.8	59.2	25.70	29.6	51.1	18.36
Retention/Intake (%)	58.9	52.5	19.4	19.4	30.7	14.32	45.1	27.2	10.21
Retention/Abs (%)	99.7	91.9	23.6	28.2	40.8	25.76	70.4	48.19	18.36

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standar error of mean; HC=High Ca; LC=Low Ca.
[abc] Means in the same row with different superscripts are different ($P<.05$).
[AB] Means in the same row with different superscripts are different ($P<.1$).

Nitrogen (N) metabolism

Tables 1.21 and 1.22 show N metabolism of animals. In spite of higher intake of N by animals fed TRT-A, no differences ($P>.1$) were observed in the apparent digestibility but resulted in obtaining a greater ($P<.01$) N absorption (g $7d^{-1}$) by animals fed TRT-A than those fed the other dietary treatments (Table 1.22). Animals fed TRT-C showed a reduced ($P<.01$) fecal N excretion than those fed TRT-B, even though their N intake was similar. Thus, final retention expressed as a proportion of that ingested by animals fed TRT-C (23.85%) was greater ($P<.1$) than for TRT-B (11.07%).

Plasma major minerals profiles

Concentration of inorganic P (Pi) in plasma was affected ($P<.05$) by dietary treatments but not by levels of dietary Ca ($P>.1$)(Table 1.23). Animals fed TRT-B had greater ($P<.05$) concentrations (5.26 mg dL^{-1}) than that for TRT-C (4.40 mg dL^{-1}), although neither were different ($P>.1$) from CTR (4.98 mg dL^{-1})(Table 1.24).

Plasma Ca concentration was relatively low and ranged from 8.72 to 9.46 mg dL^{-1}. Animals fed TRT-A and TRT-D showed higher ($P<.05$) plasma Ca concentrations compared to those fed CTR (Table 1.24). Interaction existed between the level of dietary Ca and dietary cation-anion balance ($P<.05$) suggesting that the response of changing dietary cation-anion balance altered Ca concentrations in plasma and was dif-

Table 1.21. Nitrogen absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium[1,2].

Variable	High Ca					Low Ca					SEM[4]	main effects and interaction[3]		
	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D		Ca	Anion	Ca * Anion
Intake (kg $7d^{-1}$)	0.996	1.241	1.070	0.875	1.071	0.929	1.059	0.883	0.874	0.956	0.054	***	***	NS
Feces excretion (kg $7d^{-1}$)	0.353	0.388	0.392	0.301	0.398	0.314	0.359	0.344	0.315	0.475	0.025	**	*	NS
Urine excretion (kg $7d^{-1}$)	0.419	0.554	0.474	0.363	0.429	0.444	0.499	0.537	0.359	0.475	0.063	NS	NS	NS
Absorption (kg $7d^{-1}$)	0.642	0.853	0.678	0.565	0.674	0.615	0.701	0.539	0.559	0.627	0.044	*	***	NS
Digestibility (%)	64.33	68.95	62.85	64.73	63.12	65.45	64.78	60.62	63.70	65.44	2.279	NS	NS	NS
Retention (kg $7d^{-1}$)	0.223	0.299	0.231	0.202	0.244	0.171	0.246	0.001	0.200	0.152	0.651	**	*	NS
Urine/Abs (%)	34.99	35.11	32.99	36.15	36.36	27.62	21.53	0.55	38.89	23.84	8.740	NS	NS	NS
Retention/Intake (%)	22.25	24.36	20.97	23.38	23.16	18.10	16.33	1.17	24.31	15.63	7.028	*	*	NS
Retention/Abs (%)	65.00	64.89	67.01	63.85	63.69	72.38	78.69	99.44	61.11	76.16	8.740	NS	NS	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); * P<.1; ** P<.05; *** P<.01.
[4] SEM=Standard error of mean.

Table 1.22. Nitrogen absorption and retention by sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Treatment Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Intake (kg 7d^{-1})	0.962^{a}	1.151^{b}	0.976^{a}	0.874^{a}	1.014$^{a b}$	0.038	1.051^{a}	0.940^{b}	0.0249
Feces excretion (kg 7d^{-1})	0.334$^{A B}$	0.373^{A}	0.368^{A}	0.312^{B}	0.364^{A}	0.0176	0.368^{a}	0.332^{b}	0.011
Urine excretion (kg 7d^{-1})	0.432	0.526	0.492	0.361	0.452	0.0450	0.443	0.463	0.028
Absorption (kg 7d^{-1})	0.629$^{a b}$	0.777^{c}	0.608$^{a b}$	0.562^{a}	0.650^{b}	0.0314	0.682^{A}	0.608^{B}	0.020
Digestibility (%)	64.89	66.86	61.73	64.21	64.28	1.612	64.79	63.99	1.020
Retention (kg 7d^{-1})	0.197$^{A B}$	0.251^{A}	0.116^{B}	0.201$^{A B}$	0.198$^{A B}$	0.0460	0.240^{a}	0.145^{b}	0.029
Urine/Abs (%)	69.10	71.68	83.22	62.48	69.93	8.74	64.88	77.51	5.29
Retention/Intake (%)	20.18$^{A B}$	20.35$^{A B}$	11.07^{A}	23.85^{B}	19.39$^{A B}$	4.969	22.83^{A}	15.11^{B}	3.14
Retention/Abs (%)	30.89	28.32	16.77	37.52	30.07	8.74	35.11	22.49	5.29

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standar error of mean; HC=High Ca; LC=Low Ca.
$^{a b c}$ Means in the same row with different superscripts are different ($P<.05$).
$^{A B}$ Means in the same row with different superscripts are different ($P<.1$).

Table 1.23. Blood parameters of sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium[1,2].

Parameter	High Ca					Low Ca					SEM[4]	main effects and interaction[3]		
	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D		Ca	Anion	Ca * Anion
Phosphorus (mg dL^{-1})	4.81	4.90	5.43	4.36	4.85	5.14	4.87	5.08	4.44	4.97	0.30	NS	**	NS
Calcium (mg dL^{-1})	8.96	9.18	8.94	8.72	9.46	8.84	9.20	9.15	8.86	9.01	0.11	NS	***	**
Magnesium (mg dL^{-1})	2.42	2.33	2.54	2.67	2.34	2.25	2.24	2.55	2.65	2.24	0.08	NS	***	NS
Sodium (meq L^{-1})	147.4	144.4	148.2	142.7	148.5	144.4	144.4	147.5	144.8	143.7	3.19	NS	NS	NS
Potassium (meq L^{-1})	4.46	4.32	4.38	4.28	4.31	4.19	4.49	4.40	4.31	4.27	0.11	NS	NS	NS
Sulfate (mmol L^{-1})	0.89	1.06	1.25	1.45	1.08	1.06	1.16	1.32	1.05	0.83	0.13	NS	**	NS
Chloride (meq L^{-1})	104.22	100.47	103.94	103.48	104.10	103.27	103.29	100.94	102.98	104.29	1.708	NS	NS	NS
OHPro[5] (nmol L^{-1})	6.13	5.65	6.11	5.87	5.75	7.23	5.75	6.81	5.94	6.43	0.375	**	**	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); ** P<.05; *** P<.01.
[4] SEM=Standard error of mean.
[5] Hydroxyproline.

Table 1.24. Blood parameters of sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Parameter	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Phosphorus (mg dL^{-1})	4.98^{ab}	4.89^{ab}	5.26^{a}	4.40^{b}	4.90^{ab}	0.21	4.87	4.90	0.13
Calcium (mg dL^{-1})	8.89^{a}	9.19^{b}	9.04^{ab}	8.79^{a}	9.27^{b}	0.08	9.06	9.01	0.04
Magnesium (mg dL^{-1})	2.33^{a}	2.28^{a}	2.54^{b}	2.66^{b}	2.29^{a}	0.05	2.46	2.39	0.04
Sodium (meq L^{-1})	145.9	144.4	147.9	143.8	146.1	2.26	146.2	144.9	1.42
Potassium (meq L^{-1})	4.32	4.40	4.39	4.30	4.29	0.07	4.35	4.33	0.05
Sulfate (mmol L^{-1})	0.97^{a}	1.11^{ab}	1.28^{b}	1.25^{b}	0.95^{a}	0.09	1.15	1.08	0.06
Chloride (meq L^{-1})	103.73	103.97	102.44	103.23	104.05	1.207	104.80	102.90	0.98
OHPro[3] (nmol L^{-1})	6.68^{a}	5.70^{b}	6.46^{ab}	5.91^{ab}	6.09^{ab}	0.300	5.90^{a}	6.43^{b}	0.12

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standar error of mean; HC=High Ca; LC=Low Ca.
[3] Hydroxyproline.
^{ab} Means in the same row with different superscripts are different ($P<.05$).

ferent between sheep fed HC and LC diets. In sheep fed HC diets, animals fed TRT-A and TRT-D was higher ($P<.01$) plasma Ca concentrations than those fed CTR, whereas differences ($P<.05$) were only apparent between TRT-A and CTR for the LC diets and failed to demonstrate the effect of vitamin D_3 injection on plasma Ca concentrations.

Concentration of Mg in plasma reflected the amounts of dietary Mg ingested where animals fed TRT-B and TRT-C had higher concentrations ($P<.01$) than those fed other dietary treatments. No difference ($P>.1$) was observed between HC and LC diets.

No differences ($P>.1$) were observed in plasma Na concentrations between dietary treatments and ranged from 143.7 meq L^{-1} for TRT-C to 147.8 meq L^{-1} for TRT-B.

Plasma K concentrations ranged from 4.29 meq L^{-1} for TRT-D to 4.40 meq L^{-1} for TRT-A and no differences ($P>.1$) were found between dietary treatments.

Plasma inorganic sulfate (SO_4) concentrations reflected the amounts of dietary SO_4 that animals consumed where the reduced or negative cation-anion balanced diets (TRT-B and TRT-C) showed greater ($P<.05$) plasma concentration than those fed CTR and TRT-D.

No differences ($P>.1$) were observed in plasma chloride concentration among dietary treatments and ranged from 102.44 meq L^{-1} for CTR to 104.05 meq L^{-1} for TRT-D.

Plasma free hydoxyproline (OHPro) concentrations are shown in Tables 1.23 and 1.24. Animals fed TRT-A had reduced ($P<.05$) concentrations than those fed CTR. There were no dif-

ferences ($P>.1$) between animals fed reduced or negative cation-anion balanced diets (TRT-B and TRT-C) and higher cation-anion balanced diets (CTR). The LC diets demonstrated a greater ($P<.05$) concentration than those fed HC diets indicating that feeding low dietary Ca increased the bone mobilization processes regardless of dietary treatments.

Table 1.25 shows a regression coefficient analysis between intake of dietary cation-anion balance (meq d^{-1}) and plasma major mineral concentrations. Only the concentration of Mg in plasma was significantly correlated ($P=0.0001$) with intake of dietary cation-anion balance and an equation describing this relationship was;

$Mg(mg\ dL^{-1}) = 2.53 - 3.815 * AC\text{-}balance(kg\ d^{-1})$ (Figure 1.8)

DISCUSSION

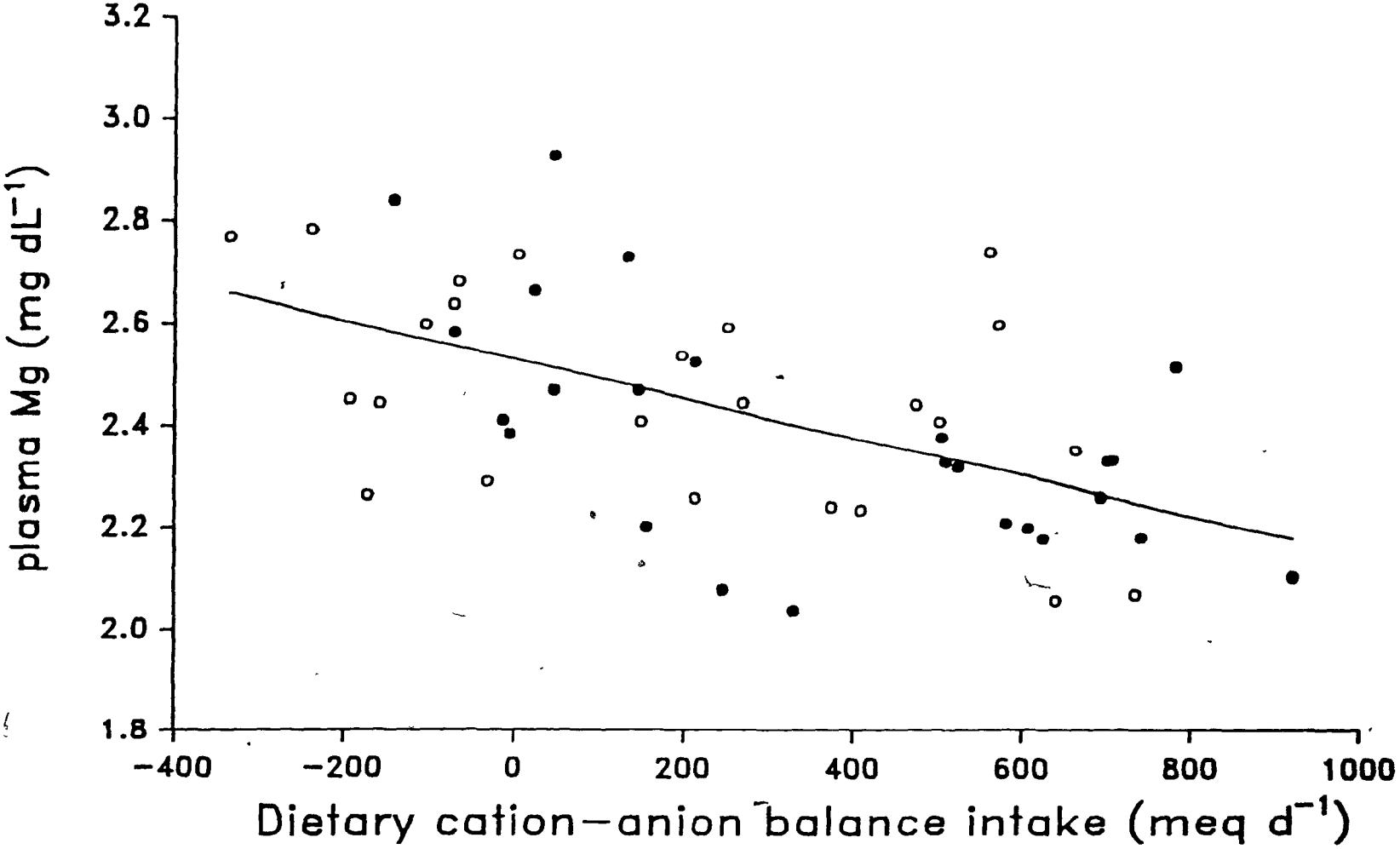
Our data suggest that feeding reduced or negative cation-anion balanced diets obtained by supplementing anion-forming mineral salts exerted some effects on Ca metabolism as indicated by an increase in urinary Ca excretion rate without corresponding changes in their intestinal Ca absorption rate. A 10.7 and 7.9-fold increase in absolute amount of Ca excreted in urine by animals fed TRT-B and TRT-C, respectively, were observed compared to that of CTR. The carry-over effect from the proceeding treatment was not detected in any parameters measured using the method of Lucas (Lucas, 1983), therefore, carry-over effects including that

Table 1.25. Regression coefficiet analyses of blood paramters and dietary cation-anion balance.

Variable	r	n	Pr
Phosphorus	0.169	50	0.249
Calcium	0.170	50	0.242
Magnesium	-0.562	50	0.0001
Sodium	-0.061	50	0.679
Potassium	0.081	50	0.580
Chloride	-0.102	50	0.491

r Correlation coefficient.
n Number of observations.
Pr Probability.

Figure 1.8. Relationship between the intake of dietary cation-anion balance and the concentration of magnesium (Mg) in plasma (o = high calcium, ● = low calcium groups)(r=0.562, P=0.0001).


3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
plasma Mg (mg dL^{-1})
−400
−200
0
200
400
600
800
1000
Dietary cation−anion balance intake (meq d^{-1})

from the injection of vitamin D_3 will not be discussed within this study.

A major site of Ca absorption in GI-tract is postulated to be the upper small intestine in ruminants (Ben-Ghedalia *et al.*, 1975; Yano *et al.*, 1979; Ivan *et al.*, 1983) as evidenced by a decreasing in Ca solubility of digesta with increasing distance from the pylorus. Although, the Ca absorption process has been shown to involve both the diffusive and active transport mechanisms in chicken (Wasserman and Taylor, 1969) and in rats (Pansu *et al.*, 1981), no clear evidence for the existence of an active component for Ca absorption in small intestinal in adult sheep has been found (Phillipson and Storry, 1965). The active component of Ca absorption is, relatively, more important at low concentrations of Ca within the small intestinal lumen and is also expected to be more evident in the young animals (Pansu *et al.*, 1983a). Furthermore, efficiency of absorption of dietary Ca from the small intestine of the ruminants was observed to increase in response to a reduced intake of the dietary Ca (Abdel-Hafeez *et al.*, 1982). Considering that the Ca level in LC diets (0.48% DM) was relatively higher than requirements (0.35% DM)(NRC, 1985) and no differences were observed in apparent digestibility of Ca between sheep fed HC and LC diets in our study, the non-saturable passive diffusion absorption process was likely responsible for most of Ca absorption (with a depression in the active absorption process). This may also explained the lack of change in apparent absorption rate of Ca by animals injected with vitamin

D_3 (TRT-D) compared to that of CTR, since vitamin D_3 is a potent stimulator of the active absorption process (Pansu *et al.*, 1983b) through the action of Ca-binding protein (CaBP) but has little effects on the non-saturable passive absorption process (Pansu *et al.*, 1981).

Ca absorption rates of 15 to 20% observed in this study by feeding either chopped alfalfa (HC) or timothy hay (LC) confirmed the values calculated from the data of Braithwaite (1975) for sheep with a similar intake of Ca.

A reduction in intake of DM observed by sheep fed TRT-C may be a direct result from supplementation of excess Fe as $FeSO_4$ in this diet. Iron contents of TRT-C for HC and LC groups were 1134 ± 373 and 1161 ± 243 ppm, respectively, whereas CTR contained 152 and 166 ppm for the HC and LC diets, respectively. Supplementation of Fe as $FeSO_4$ at levels of >1000ppm were demonstrated to have an adverse effects on feed intake and body weight gain in steers (Standish *et al.*, 1969; 1971) as well as in calves (Koong *et al.*, 1970). Excess Fe in TRT-C may have combined with dietary phosphate to form unabsorbable iron phosphate, rendering it unavailable to animals, as evidenced by the negative P balance by animals fed TRT-C as well as the reduction in plasma inorganic P concentrations compared to that of CTR. Standish *et al.* (1971) reported a decrease in P absorption with an increase in dietary Fe from 100 to 1000 ppm. Even though availability of Fe is generally low, apparent absorption values of 3 to 10% has been reported (Underwood, 1977), and $FeSO_4$ has been shown to be a readily available source of Fe

for ruminants (Ammerman *et al.*, 1967). Another possibility exists explaining the reduction in intake of DM observed by animals fed TRT-C, i.e. the excess amount of S (0.53% DM) in the diet. Johnson *et al.* (1968) demonstrated that feeding 0.5% of total DM as S in the form of Na_2SO_4 or $CaSO_4$ resulted in a reduced intake of DM. However, other studies have observed no harmful effects from feeding relatively high levels (up to 1.72% of total DM) of S (Chalupa *et al.*, 1971; Gawthorne and Nader, 1976).

In spite of the potential adverse relationship existing between dietary Fe and P, the effects of excess dietary Fe on Ca metabolism has not been established. Standish *et al.* (1971) and Koong *et al.* (1970) reported no differences in plasma Ca concentrations of ruminants supplemented up to 2,000 ppm of Fe, whereas others reported a reduction in plasma Ca concentrations with an increase in dietary Fe contents in rats (Harmon *et al.*, 1968) and in ruminants up to 400 ppm Fe (Standish *et al.*, 1969).

Irrespective of the anion-forming mineral salts used to reduce dietary cation-anion balance, a correlation between the digestibility of dietary Ca and daily ingested dietary cation-anion balance values seemed to have two phases (Lomba *et al.*, 1978); a positive linear relationship with positive cation-anion balanced diets and a negative linear relationship obtained with negative cation-anion balanced diets. There was no significant correlation obtained in this study between intake of dietary cation-anion balance (meq d^{-1}) and Ca absorption (g $7d^{-1}$), although a positive linear

relationship (r=0.305 and P=0.032) was observed between the dietary cation-anion balance and Ca retention (g $7d^{-1}$). A correlation using only negative cation-anion balanced diets and Ca retention in this study was limited by a small number of observations with a negative cation-anion balanced diets (n=14), thus, no significant correlation was found between them (P=0.64). Ender *et al.* (1971) reported that feeding acidic diets to dairy cows prepartum improved the Ca balance during first four days postpartum and produced fewer incidences of milk fever compared to cows fed non-acidic control diet. However, no differences were observed in Ca balance prepartum: The acidic diets were obtained by supplementing H_2SO_4 and HCl to the control diets.

A high amount of Al (>2000ppm) in diets is known to cause a reduction in feed intake and absorption of P from the small intestine (Valdivia *et al.*, 1982; Allen, 1984). Al contents of TRT-B (supplemented with $Al_2(SO_4)_3$) was much lower than 2000 ppm (719±98 ppm) and animals fed this diet did not show any differences in intake of DM or P metabolism compared to CTR. Thus it is unlikely that this level of Al in diets, *per se*, caused a direct effects on major mineral metabolism.

An increased urinary Ca excretion was observed by animals fed reduced cation-anion balanced diets (TRT-A, TRT-B and TPT-C) and differences observed in total urinary Ca excretion (g $7d^{-1}$) for animals fed TRT-A, TRT-B, and TRT-C were 10.28, 10.67 and 7.89 times higher than that of CTR, respectively. The urinary Ca excretion expressed as a

proportion of Ca absorbed (%) indicated that animals fed the most negative cation-anion balanced diet (TRT-C) excreted 56% of the Ca absorbed whereas only a 2.5% of Ca absorbed was excreted in the urine of animals fed CTR. A strong negative linear relationship was observed between the dietary cation-anion balance and urinary Ca excretion (Figure 1.5) in this study which suggests the existence of a mechanism(s) where dietary cation-anion balance affects kidney function in mineral reabsorption processes (Stacey and Wilson, 1970). Furthermore, animals fed TRT-C had lower concentration of Ca in plasma than that of CTR suggesting that the filtered load of Ca at the kidney tubule should be smaller for sheep fed TRT-C than CTR, yet the amount of urinary Ca excretion was the highest. Thus, the existence of inhibitory effects of renal Ca reabsorption by feeding negative cation-anion balanced diets is suggested.

Braithwaite (1972) hypothesized that urinary Ca excretion was under control of some renal mechanisms, which is affected by pH. Sutton *et al.* (1979) also demonstrated, in dogs the presence of a component of tubular Ca resorption situated beyond the proximal tubule that was inhibited by chronic metabolic acidosis and enhanced by metabolic alkalosis. In our study, a reduction in urinary pH observed in animals fed TRT-B and TRT-C compared to that of CTR indicates an alternation of acid-base status caused by feeding reduced cation-anion balanced diets. This confirmed the result of Fredeen *et al.* (1988a) who reported a reduction in urine pH and bicarbonate (HCO_3^-) excretion in urine by goats

fed a diet with Na+K-Cl value of -84 (meq kg^{-1} DM) compared to that of fed a diet with Na+K-Cl value of 467 (meq kg^{-1} DM). However, no reduction in urinary pH of animals fed TRT-A which included NH_4Cl at the level of 0.87% of total DM was observed. Braithwaite (1972) and Bushman *et al.* (1968) observed a significant reduction in urinary pH by feeding 1% of total DM NH_4Cl in sheep but a level of 0.5% of total DM NH_4Cl did not show the effect on urine pH.

Providing exogenous sources of anions (SO_4 and Cl) were shown to cause increased urinary Ca excretion (Whiting and Draper, 1980; Whiting and Cole, 1986). With observations that SO_4 is reabsorbed by the kidney at minimum rate and also selectively complexes with free Ca in the renal tubular ultrafiltrate leading to a decrease in the Ca reabsorption (Whiting and Draper, 1981b; Bushinsky *et al*, 1982; Jacob *et al.*, 1983), it was hypothesized that SO_4 should produce a greater calciuric effect than Cl when anions were given in equivalent amounts. However, Whiting and Cole (1986) using rats concluded that there was no difference in the calciuric effect between dietary SO_4 and Cl but intestinal absorption of dietary Cl was greater than that of dietary SO_4. In ruminants, the amount of recycling of endogenous SO_4 either through salivary secretion and/or directly through the rumen wall would vary considerably among various diets (Kandylis, 1983). Therefore, the available SO_4 to ruminants might not directly reflect the amount of dietary SO_4.

No measurements were made of the calcitropic hormones in this study. However, the hypercalciuric effect of acidic-

induced diets was shown to be independent of PTH as well as $1,25(OH)_2D_3$ (Bushinsky *et al.*, 1982; Marone *et al.*, 1983).

The existence of direct linear relationship in apparent absorption as well as retention between Ca and P observed in this study confirmed the results of Braithwaite (1975; 1979) and Harrison and Harrison (1961) using Ca-deficient animals. Results of our study indicated that even in Ca-replete animals, P retention was controlled by the rate of Ca retention. Apparent absorption rate (%) of P obtained in our study ranged between -1.6% to 12.5% and were similar to that of Braithwaite (1984) who reported 4.5%. However, the apparent absorption may not reflect the amount of available dietary P since it is assumed that higher endogenous fecal losses occurs with higher dietary P intake than requirements and represents a resecretion mainly in the saliva. (Field *et al.*, 1982).

In non-ruminants as well as in ruminants, it is well established that diet low in P tend to be associated with hypercalcemia and, consequently, a depressed secretion of PTH (Fox and Care, 1978). In non-ruminants, despite the concomitant hypercalcemia observed with feeding low P diets, there is usually an increase in the plasma concentration of $1,25(OH)_2D_3$ and a consequent increase in the efficiency of absorption of Ca from the small intestine (Kenny, 1981b). It is apparent from our results that animals with P depletion resulted from a reduction in available dietary P caused by excess Fe supplementation (TRT-C) and lead to a reduction in the efficiency of absorption of Ca. This anomaly presumably

resulting from a depressed plasma concentration of $1,25(OH)_2D_3$, the production of which is not enhanced by hypophosphotaemia resulted from P depletion, although the reduction in plasma P concentrations of animals fed TRT-C was found only to be a trend ($P<.1$) compared to that of CTR.

Abdel-Hafeez *et al.* (1982) suggested that a reduction in plasma $1,25(OH)_2D_3$ concentrations was a direct result of altered activity of the enzyme involved, $25(OH)D_3$-1-hydroxylase, and neither PTH or calcitonin could be involved in this change since dietary P deficiency was shown to cause similar changes in plasma concentrations of Ca, P and $1,25(OH)_2D_3$ between thyroparathyroidectomized and intact ruminants. This enzyme, $25(OH)D_3$-1-hydroxylase perhaps has a higher requirement for P than in non-ruminants so that it is maximally stimulated only when sheep were fed a relatively high P diets and significant amounts of P were excreted in urine.

The effect of the administration of a single massive dose (16670 IU $Kg^{-1}BW$) of vitamin D_3 was to cause a small but significant increase in plasma Ca concentrations compared to CTR. This result is in agreement with others (Thomas *et al.*, 1981; Hidiroglou and Hidiroglou, 1982; Hidiroglu *et al.*, 1984). An increase in the concentration of Ca in plasma with the injection of vitamin D_3 was a result of the action of $1,25(OH)_2D_3$ acting in the small intestine to increase Ca absorption particularly by the active absorption process and in bone to increase bone Ca resorption (Putkey and Norman, 1982). The magnitude of increase in concentrations of plasma

Ca observed in this study, however, was not as great as observed with cows (Horst and Reinhardt, 1983). This may have resulted from a long time interval between the injection of vitamin D_3 and the sampling of blood (10 days). Thomas (1981) reported that the effect of administeration (i.m.) with various doses of vitamin D_3 on plasma concentrations of Ca in lambs was a quadratic with respect to time. There also seemed to be differences between sheep and cattle for the hydroxylation process of vitamin D_3 required to synthesize the active metabolite $1,25(OH)_2D_3$ after the administration of large doses of vitamin D_3. In cattle, an increase in $25(OH)D_3$ was observed 7 days after administration (i.m.) of a large dose of vitamin D_3 (Hollis *et al.*, 1977), whereas an increase was observed within 1 day following the injection in sheep (Hidiroglou and Knipfel, 1984).

Results indicated that feeding a reduced cation-anion balanced diet to sheep regardless of the type mineral salts used caused an elevation in urinary Ca excretion without affecting intestinal apparent Ca absorption or plasma Ca concentrations. A positive correlation ($r=0.305$) was obtained between dietary cation-anion balance intake (meq g^{-1}) and Ca retention (g $7d^{-1}$). The lowest cation-anion balanced diet in our study (-32 meq kg^{-1} DM), however, may not be low enough to observe the positive effect of extremely reduced cation-anion balanced diets (<-100 meq kg^{-1}DM) on Ca balance (Lomba *et al.*, 1978).

Minerals (Mg, Al, and Fe) that accompanied the anions (Cl and S) with the form of salts used to reduced the dietary

cation-anion balance showed little or no direct effect on Ca metabolism, although some indirect effects of excess minerals were apparent (reduction in feed intake). Because of the importance of balancing dietary fixed ions in acid-base control of animals (Stewart, 1978), mode of action of feeding reduced cation-anion balanced diet must involve an alteration of the acid-base status of animals toward a metabolic acidosis resulting in pertubations of Ca metabolism.

IV. EFFECTS OF MANIPULATING DIETARY CATION-ANION BALANCE ON THE RESPONSE TO EXPERIMENTALLY INDUCED HYPOCALCEMIA IN SHEEP.

Abstract

A study was conducted to examine the effect of dietary cation-anion balance on bone calcium (Ca) mobilization, measured by challenging animals with 5.6% EDTA infusion at a dose rate of 1.4 ml kg^{-1} BW. Dietary cation-anion balance was calculated as meq [(Sodium (Na) + Potassium (K))-(Chloride (Cl)+ Sulfur (S)]. Ten crossbred wether lambs (average body weight 67.8 kg) were fed rations with high Ca (HC) or low Ca (LC)(0.75% [HC] and 0.45% [LC]) and five different treatments, four of which differed in their mineral supplementation. cation-anion balance (meq kg^{-1} DM) were +354, +200, +79, and +37 for control (CTR), treatments (TRT) -A, -B, and -C, respectively. The fifth TRT (TRT-D) was injection of vitamin D_3 (16670 IU kg^{-1} BW) to sheep fed CTR. A 2x5 factorial split-plot design with five 15-day periods of 14 days adaptation followed by EDTA infusion was used. Total and EDTA titratable plasma Ca concentrations (mg dL^{-1}) were; 8.62, 7.99; 8.43, 8.17; 8.21, 7.90; 8.42, 7.40; 8.06, 7.99 for CTR, TRT-A, -B, -C and -D, respectively. For CTR, TRT-A, -B, -C and -D, respectively, the decreases in plasma titratable Ca (mg dL^{-1}) by the end of EDTA infusion (120 min) were 4.56, 4.20, 3.56, 4.02, and 3.67; the recoveries of plasma titratable Ca during 240 min post-infusion recovery period were 3.15, 3.10, 3.21, 3.25, and 2.47; the percentage changes in plasma titratable Ca from pre-infusion at the end of the recovery period were -14.15, -11.03, -3.46, -7.69, and -12.09. Amount of Ca mobilized (mmol) and Ca mobilization rate (mmol min^{-1}) during

EDTA infusion period were; 10.87, 0.094; 14.56, 0.124; 12.96, 0.109; 14.74, 0.125 and 19.83, 0.158 for CTR, TRT-A, -B, -C, and -D, respectively. Thus, reducing dietary cation-anion balance in diet increased ability to mobilize Ca during hypocalcemia and the magnetude of increase was similar to that of vitamin D_3 injection.

INTRODUCTION

Calcium (Ca) concentrations in plasma are tightly regulated by calcitropic hormones and is generally maintained within a narrow range despite a large fluctuation exists in Ca demand created by various physiological states such as gestation and lactation (Toverud and Boass, 1979). Hypocalcemic parturient paresis (milk fever) that occurs in dairy cows is a metabolic disease where the Ca homeostatic mechanism is disturbed by a sudden increase in Ca demand at the initiation of lactation (Littledike *et al.*, 1981).

There have been numerous measures proposed for the prevention of milk fever (Littledike *et al.*, 1981). These measures are designed to create an increase in the sensitivity of regulatory mechanisms for blood Ca by increasing the availability of Ca from both exogenous (dietary) and endogenous sources at parturition when milk fever is likely to occur.

Boda and Cole (1954) were first to demonstrate that feeding diets high in Ca to prepartum cows resulted in an increased incidence of milk fever. Ender *et al.* (1962a) explored the etiology of milk fever by feeding different amounts of Ca and phosphorus (P) to cows and concluded that the effect of dietary Ca and P on the induction or prevention of milk fever did not depend upon the absolute amounts of each mineral but was more related to the alkaline and acid components of the diet. Cation-anion balance is defined and calculated as the summation of the milliequivalents of

anion-forming mineral elements (sodium (Na) and potassium (K)) minus the summation of the milliequivalent of cation-forming mineral elements (chloride (Cl) and sulfur (S)). Diets composed of beets, that is alkalogenic and has a positive cation-anion balance diets due to their high contents of Na and K and low contents of Cl and S were found to induce milk fever. Diets consisting of A.I.V. (formic acid treated) silage that had high levels of Cl and S in relation to Na and K contents resulted in negative cation-anion balanced diets and low pH values had pronounced preventive effect.

Dishington (1975) prevented milk fever by feeding diets that had negative cation-anion balances obtained by using combinations of commercially available mineral ingredients, namely $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, and $Al_2(SO_4)_3.18H_2O$: To prevent milk fever a minimum dietary cation-anion balance intake of -255 meq d^{-1} animal was needed. However, Ca and P contents of the experimental diets were not reported. Recently, Block (1984) used the same supplemental mineral salts as Dishington (1975) to obtain a dietary cation-anion balance of -127.8 meq kg^{-1} dry matter (DM) and a control diet with a positive cation-anion balance value of +330.4 meq kg^{-1} DM. Both diets contained high Ca (0.65% DM) and P (0.24% DM). These diets were fed to prepartum cows from 45 days prepartum to parturition. Cows fed the negative cation-anion balanced diet did not develop milk fever, whereas a 47.4% incidence was observed in cows fed the control diet. He observed that animals fed the negative cation-anion

balanced diet had a higher concentration of plasma hydroxyproline (OHPro), indicating an increased bone mobilization, from day-4 prepartum to day-2 postpartum when Ca-stress was the greatest. Leclerc (1986) also observed that reducing cation-anion balance of rations from +394 to +62 (meq kg^{-1} dry matter (DM)) decreased the incidence of milk fever when rations were fed to prepartum cows. Thus, Block (1984) hypothesized that feeding negative cation-anion balanced diets causes an increase in availability of Ca from bone either directly or indirectly through the action of calcitropic hormones, namely parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D3$).

Objectives of this experiment were: to investigate the effect of feeding reduced or negative cation-anion balanced diets to sheep on the response to acute hypocalcemia experimentally induced by continuous infusion of disodium ethylenediamine tetraacetic acid (Na_2EDTA); to test if there is a difference in response caused by varying the mineral salts supplementation used to reduce the cation-anion balance of diets; to compare the above responses to sheep injected with theraputic doses of vitamin D_3.

MATERIALS AND METHODS

Animals

The experiment was conducted with ten wether lambs (suffolk crossbred) with an initial average weight of

66.5±8.65 kg. Animals were placed in individual metabolic cages in a room where temperature was maintained at 20°C and had a photoperiod of 14 h light: 10h dark. Animals had free access to distilled water at all time and were offered the experimental diets as total mixed rations *ad libitum* in two equal portions at 0830 h and 1600 h.

Diets

The ingredients of the experimental diets were the same in Exp. 1 (Table 1.1). Basal diets were grouped into the two levels of Ca , high Ca [HC] (0.74% of total DM) and low Ca [LC] (0.45% of total DM) group and in each group there were four dietary treatments as in Exp.1.

A fifth treatment (TRT-D) in which an administration of vitamin D_3 at a dose of 16670 IU kg^{-1}BW (Potent D, Pfizer, Montreal, Quebec) to animals fed CTR was also included.

Experimental design

Animals were randomly assigned to one of the 10 dietary treatments according to a 2x5 factorial split-plot design with 2 levels of dietary Ca and 5 different treatments with five 15-day experimental periods consisting of a 14-day adaptation period followed by the EDTA-infusion study. Animals were assigned to TRT-D during the last period of the experiment in order to minimize the carry-over effects of injecting vitamin D_3.

Jugular vein catheterization

A polyethylene cannula (I.D. .086 cm O.D. .127 cm Clay Adams Inc., Parsippany, NJ.) was inserted into both left and right jugular veins the day before the infusion study. The placement of the catheters into the veins was performed by inserting a disposable needle (18 Ga) into the vein, through which the catheter was introduced into the vein. Once 10 to 15 cm of the catheter was inside the jugular vein toward the superior vena cava the needle was removed and the catheter was fixed to the skin on the neck with several stitches and covered with adhesive tape to prevent creating kinks and rubbing. In order to facilitate frequent blood samplings, the external end of the catheter was extended from the neck to the middle of the back of animals where the catheter was stabilized by adhesive tape. A blunt needle (21 Ga) covered with its injection cap was placed at the external end of the catheter, thus allowing the connection of the catheter with a disposable syringe for blood sampling. The catheter was kept filled with heparinized physiological saline (50 IU heparin ml^{-1}) solution to prevent the formation of blood clots inside the catheter.

Induction of hypocalcemia

One of the jugular vein cannulae was used for the infusion of Na_2EDTA (Anachemia, Lachine, Quebec) solution. The solution was made by dissolving 56 g of $Na_2EDTA.2H_2O$ in ap-

proximately 80 ml of physiological saline (Abbot Laboratories Co., Montreal, Quebec), then adjusting pH to 6.8 to 7.0 with 5N sodium hydroxide (NaOH) and made up to 1 liter with more physiological saline. This 5.6% Na_2EDTA solution would effectively remove 0.15 mmol Ca per ml assuming one mole of EDTA will chelate one mole of Ca. The EDTA solution was infused at the constant rate of 1.4 ml min^{-1} by means of a peristaltic pump.

A total dose of Na_2EDTA given to animals was calculated from body weight (BW) and an infusion rate of 1.7 min kg^{-1} BW was used. This dose was previously found suitable for rapidly reducing plasma Ca level approximately by 50% in a preliminary trial as shown in Figure 2.1. The duration of infusion of the solution of 5.6% EDTA was an average of 122 min per animal and an average amount of EDTA infused was 9.56 g Na_2EDTA per animal.

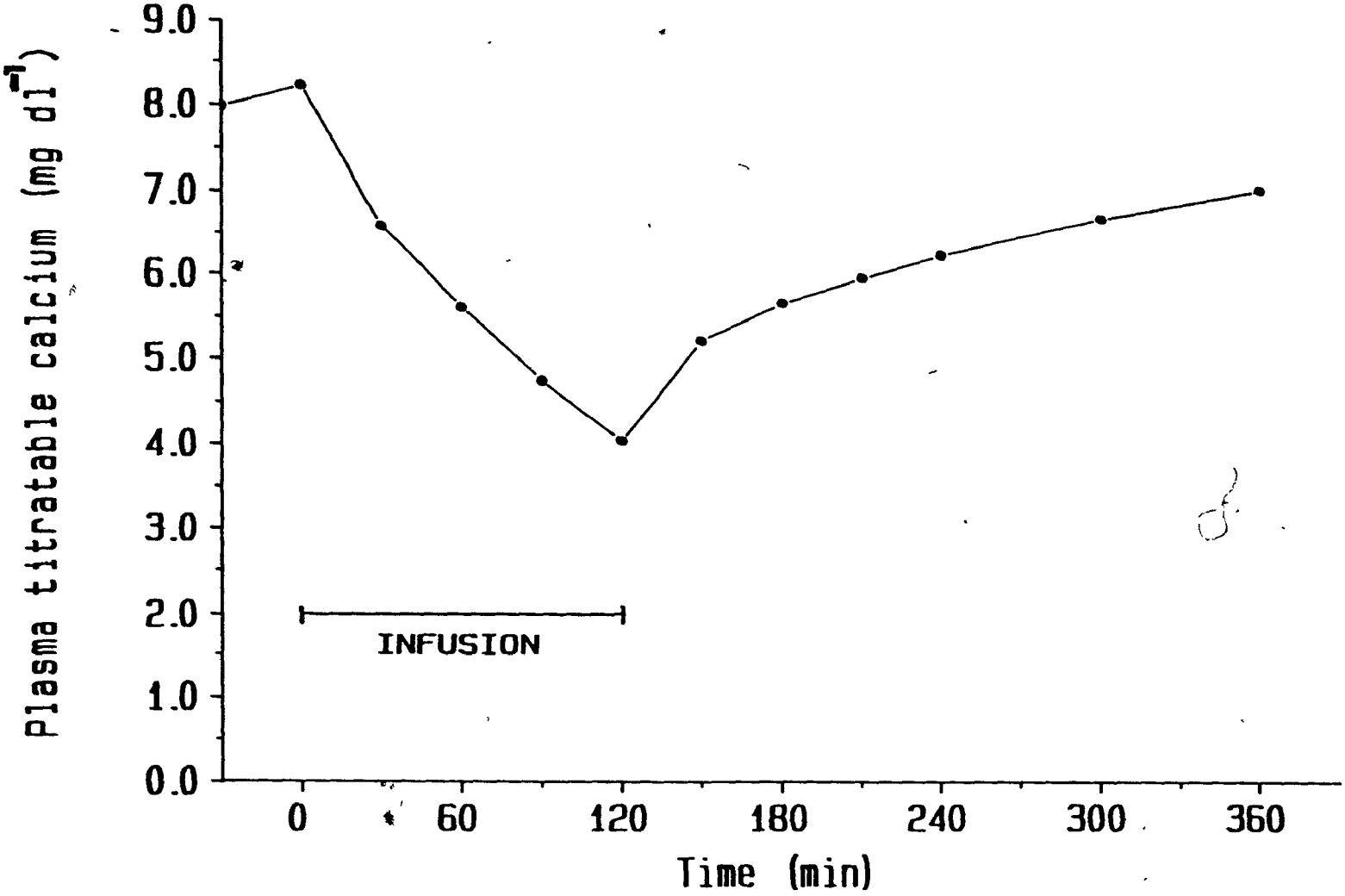
At the end of the experiments, five animals were infused with physiological saline solution alone at rate of 1.7 min kg^{-1} BW to examine the differences in plasma concentration of Ca, P, and Mg with infusing either EDTA or physiological saline.

Animals were prevented from becoming recumbent by supporting them with a pair of steel bars under their abdomen while animals stood in a cage during the period of infusion.

Blood collection

Two blood samples were collected prior to the start of

Figure 2.1. Changes in plasma titratable calcium concentration with the EDTA infusion.


Plasma titratable calcium (mg dl^{-1})
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
INFUSION
0
60
120
180
240
300
360
Time (min)

infusion of the EDTA solution. Samples were taken every 30 min during the infusion period and at the termination of infusion. Samples were taken every 10min during first 30-min post-infusion, then every 30 min until 120 min post-infusion. Thereafter, additional samples were taken at 180 and 240 min post-infusion.

Approximately 12 ml of blood was collected into heparinized 25 ml disposable syringes, immediately transferred into heparinized 15 ml test tubes and thoroughly mixed. Samples were centrifuged at 750 x G (International centrifuge, Universal model U.V. International Equipment Co., Boston, Massachusetts.) for 15 min, and plasma was separated immediately and stored at -10°C for subsequent analyses.

The cannulae were kept patent by filling them with heparinized physiological saline (50 IU heparin ml^{-1}) between samplings. Immediately after the last blood sample had been taken the cannula were removed.

Chemical analyses

Dry matter (DM) of feed and orts were determined by the toluene distillation (Dewar and McDonald, 1961) and DM of daily feces were determined by placing samples in a forced-air oven at 65°C for 48 hours. Dried feed, orts and feces samples were ground through a hammer mill (2 mm screen) before laboratory analyses.

Composited feed, orts, feed and urine samples were

analyzed for nitrogen with Kjel-Foss macroautomatic analyzer (Foss Electric, Hillerod, Denmark). After wet digestion with HNO_3 and $HClO_4$, samples were analyzed for P by the alkalimeter ammonium molybdate method (AOAC, 1984) and for Ca, magnesium (Mg), Na, K, and iron (Fe) with atomic absorption spectrophotometer (Perkin Elmer 360, Norwalk, Connecticut). One percent (w/v) lanthanum oxide was added prior to the determination of Ca and Mg in order to minimize interference from the presence of phosphate (PO_4) as well as sulfate (SO_4). Sulfur contents were measured by the turbidimetric method of Berblung and Sorbo (1960).

For the determination of chloride in feed, orts and feces, 20 ml of 1 N HNO_3 was added to 1g of sample and mixed for 15 min by a shaker (model 75 Burrell Corporation, Pittsburgh, PA) for extraction then centrifuged at 4,400 x G for 15 min to obtain a clear supernatant. Chloride concentrations of the supernatants were analyzed by an indirect method in which a known amounts of excess silver, as $AgNO_3$, was added to the sample solution then free silver was measured by atomic absorption spectrophotometer (Anonymous, 1982).

Aluminum (Al) contents of feed and feces were measured by the method of Hendershot (1985).

For determination of minerals in plasma, two ml of plasma were added to an equal volume of 20% (v/w) trichloroacetic acid, vortexed and kept for 15 min at room temperature then centrifuged at 750 x G for 20 min. The clear supernatants recovered were used for mineral analyses above.

Plasma EDTA titratable free Ca ($_f$Ca) was determined by a direct titration with Na_2EDTA in the presence of NaOH by the method of Ward *et al.* (1960) using cal-red (Calcon-carboxylic acid. Sigma Chemical Co.Ltd., St.Louis, MO) as an indicator. Plasma inorganic P (Pi) was determined by the method of Fiske and Subbarow (1925).

Calculation of the Ca mobilization rate during EDTA infusion (method was taken from Contreras *et al.*(1982)).

Assuming that one molecule of EDTA binds to one free Ca ion ($_f$Ca) in blood and that no Ca were mobilized, the Ca equivalent of the EDTA infused (I), would be V*@($_f$Ca) where @($_f$Ca) is the decrease in the concentration of $_f$Ca during the infusion period and V is the volume of the compartment within which there is rapid equilibration of $_f$Ca.

When Ca is mobilized into this compartment (V) from other available Ca sources (i.e. extracellular fluid, skeleton, etc.) then @($_f$Ca) will be smaller. If Q were the amount of Ca mobilized, then

$$I-Q = V*@(_fCa)$$

and $$Q = I-V*@(_fCa) \quad (1)$$

If the infusion of EDTA lasts T time units (min) then the rate of Ca mobilization R is given

$$R = Q/T = (I-V*@(_fCa))/\ T \quad (2)$$

An independent measurement of V can be obtained from the changes in the plasma concentration of Ca bound to EDTA (CaEDTA) during the infusion. The concentration of CaEDTA

increases during the infusion of EDTA and is measured as the difference between total Ca concentrations (CaEDTA + ₜCa) and the concentration of ₜCa in plasma. An increase in concentration of CaEDTA is (and was found to be) approximately linear during the EDTA infusion. If none of the CaEDTA were excreted, then

$$I = V*\Delta(CaEDTA)$$

where Δ(CaEDTA) represents the increase in concentration during the infusion. However, CaEDTA is excreted through the kidney (Foreman *et al.*, 1953; Payne *et al.*,1963) at a rate which can be determined from the rate of decline in its concentration after the infusion of EDTA has stopped. This rate of decline is (and was found to be) mono-exponential and the rate constant (K) that can be derived from the slope of the regression line obtained by plotting the natural logarithm of the plasma CaEDTA concentration against time (min) after the end of the infusion. If it is assumed that CaEDTA is excreted through the kidney at the same rate during the infusion of EDTA solution, then the total amount of CaEDTA excreted during the infusion would be

$$K*(\underline{CaEDTA})*V*T$$

where (CaEDTA) is the mean concentration of CaEDTA during the infusion. Because the increase in concentration of CaEDTA is (and found to be) approximately linear, its mean concentration can be obtained by

$$(\underline{CaEDTA})=\Delta(CaEDTA)/2$$

and the total amount of CaEDTA excreted during the infusion

$$=K*V*\Delta(CaEDTA)/2*T \qquad (3)$$

and

$$I = V*@(CaEDTA) + K*V*@(CaEDTA)/2*T$$

$$= (V*(2*@(CaEDTA)) + (K*@(CaEDTA)*T))/2 \quad (4)$$

and $$V = 2*I/(@(CaEDTA)*(K*T+2)) \quad (5)$$

By substituting this expression for V in equations (1) and (2), the amount of Ca mobilized (Q) and the Ca mobilization rate (R), respectively, were calculated.

The Ca mobilization rate, R, is the average rate at which ^{45}Ca was mobilized into the compartment whose volume is V, during the infusion of EDTA and it is independent of measurements of the rate of recovery of ^{45}Ca concentration after the infusion had stopped.

Statistical analysis

Statistical analyses were performed at McGill computing center by the statistical analysis system (SAS Institute Inc., Box 8000, Cary, N.C.). Differences due to animals and dietary treatments were evaluated by analysis of variance for a split-plot design (Steel and Torrie, 1980) with dietary treatments within each level of Ca was taken as subunits. Linear model used was following;

$$Y_{ijk} = u + A_i + B_j + C_{ij} + D_k + (BD)_{jk} + E_{ijk}$$

where

u=common mean,

A_i =effect of ith block,

B_j =effect of jth dietary Ca level,

$C_{i,j}$ =error term of whole unit (dietary Ca level),

=interaction effect of ith block and jth level of dietary Ca,

D_k =effect of kth treatment,

$(BD)_{j,k}$ =interaction effect of jth level of dietary Ca and kth treatment,

$E_{i,j,k}$ =error term of subunit (random error).

A least-squares analysis was also used to obtain the least-squares estimates of differences between subunits for comparison purposes.

Differences in plasma mineral concentration between the infusion of EDTA or physiological saline were evaluated with a nested design where blood samplings were nested within a sheep and linear model used was following;

$$Y_{lmn} = u + F_l + J_m + (FJ)_{lm} + K_{ln} + E_{lmn}$$

where

u=common mean,

F_l =effect of lth sheep,

J_m =effect of mth infusion,

$(FJ)_{lm}$ =interaction effect of lth sheep and mth infusion,

K_{ln} =effect of nth blood sampling within lth sheep,

E_{lmn} =random residual.

RESULTS

Experimental diets

Chemical analyses of the experimental diets are presented in Tables 2.1 and 2.2. As intended, dietary Ca level was different ($P<.01$) between HC (0.75%) and LC (0.45%) diets (Table 2.2). A dietary cation-anion balance (meq kg^{-1} DM) for CTR (and TRT-D), TRT-A, TRT-B, and TRT-C were +358.2, +121.6, +41.0 and +51.9 for HC diets ; +350.3, +262.7, +127.8 and +23.19 for LC diets, respectively.

Intake of DM and body weight changes

There was no difference ($P>.1$) in intake of DM between sheep fed HC and LC diets. In both groups, animals fed TRT-C showed an reduction ($P<.05$) of DM intake compared to those of other dietary treatments (Table 2.3). Animals fed reduced cation-anion balanced diets (TRT-B and TRT-C) lost their BW whereas animals fed diets higher in cation-anion balance (CTR, TRT-A and TRT-D) gained weight. Intake of dietary cation-anion balance were +570.0, +333.8, +131.1, +49.6, and 573.1 meq d^{-1} for CTR, TRT-A, TRT-B, TRT-C, and TRT-D, respectively (Table 2.3).

Clinical effects of intravenous infusion of EDTA

The administration of 5.6% EDTA at the rate of 1.4

Table 2.1. Mineral composition of diets differing in their dietary cation-anion balance with 2 levels of dietary calcium [1,2].

Nutrient	High calcium				Low calcium			
	CTR & TRT-D	TRT-A	TRT-B	TRT-C	CTR & TRT-D	TRT-A	TRT-B	TRT-C
Crude protein (%)	10.95±0.15	12.90±0.06	11.49±0.16	11.20±0.15	10.75±0.28	12.34±0.31	11.03±0.32	11.87±0.59
Phosphorus (%)	0.29±0.01	0.34±0.01	0.29±0.02	0.30±0.01	0.34±0.003	0.32±0.01	0.34±0.01	0.31±0.01
Calcium (%)	0.69±0.03	0.76±0.05	0.06±0.02	0.75±0.04	0.45±0.02	0.44±0.01	0.48±0.004	0.42±0.01
Magnesium (%)	0.26±0.03	0.26±0.02	0.30±0.01	0.33±0.01	0.28±0.01	0.24±0.01	0.34±0.01	0.32±0.02
Sodium (%)	0.54±0.07	0.56±0.06	0.24±0.03	0.22±0.02	0.40±0.05	0.50±0.06	0.22±0.07	0.22±0.02
Potassium (%)	1.55±0.08	1.33±0.02	1.54±0.08	1.59±0.02	1.46±0.07	1.38±0.01	1.55±0.03	1.31±0.08
Chloride (%)	0.53±0.05	1.18±0.05	0.59±0.03	0.58±0.05	0.25±0.01	0.71±0.06	0.32±0.02	0.33±0.03
Sulfur (%)	0.20±0.06	0.24±0.01	0.47±0.04	0.46±0.03	0.21±0.03	0.17±0.01	0.44±0.02	0.50±0.03
Cation-anion balance[3] (meq kg^{-1})	358±6.2	121±7.4	41±18.6	52±43.8	350±11.6	263±7.2	128±23.1	23±4.0

[1] Values are presented as mean±standard error.
[2] CTR=Control; TRT=Treatment.
[3] Calculated as milliequivalents of [Na+K]-[Cl+S].

Table 2.2 Mineral composition of diets differing in their dietary cation-anion balance fed to sheep (High [HC] and low [LC] calcium groups combined) [1,2].

Nutrient	CTR	TRT-A	TRT-B	TRT-C	HC	LC
	(%)					
Crude protein	10.85±0.15	12.59±0.32	11.28±0.18	11.53±0.31	11.81±0.21	11.46±0.20
Phosphorus	0.32±0.01	0.32±0.01	0.31±0.01	0.30±0.01	0.32±0.01	0.33±0.01
Calcium	0.57±0.06	0.58±0.06	0.64±0.05	0.52±0.08	0.75±0.01	0.45±0.01
Magnesium	0.27±0.01	0.25±0.01	0.32±0.01	0.32±0.01	0.28±0.01	0.28±0.01
Potassium	1.51±0.05	1.39±0.04	1.54±0.04	1.45±0.12	1.47±0.04	1.48±0.04
Chloride	0.39±0.07	0.92±0.09	0.46±0.02	0.48±0.02	0.67±0.05	0.38±0.03
Sulfur	0.20±0.06	0.20±0.01	0.46±0.02	0.48±0.02	0.32±0.02	0.30±0.02
Cation-anion balance[3] (meq kg^{-1})	354±6.1	200±25.3	79±20.4	37±20.7		

[1] Values are presented as mean±standard error.
[2] CTR=Control; TRT=Treatment; HC=High Ca; LC=Low Ca.
[3] Calculated as milliequivalents of [Na+K]-[Cl+S].

Table 2.3. Body weight (BW), dry matter (DM) and dietary cation-anion balance intake (meq d^{-1}) by sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Initail BW (kg)	65.25[a]	67.09[ab]	70.91[b]	68.65[b]	69.94[b]	0.59	68.73	68.29	0.42
Final BW (kg)	68.82	69.25	69.48	68.14	72.37	0.34	69.38	69.83	0.31
BW changes (kg)	3.57[a]	2.16[ab]	-1.43[b]	-0.51[b]	2.43[a]	0.72	0.65[a]	1.54[b]	0.27
DM intake ($g\ d^{-1}$)	1610[a]	1669[a]	1648[a]	1321[b]	1661[a]	38.4	1593	1570	29.7
DM intake ($g\ kg^{-1}\ BW^{0.75}\ d^{-1}$)	68.04[a]	69.72[a]	68.80[a]	56.05[b]	69.72[a]	1.340	66.60	65.32	1.07
Cation-anion balance[3] (meq d^{-1})	+570.0[a]	+333.8[b]	+131.1[c]	+49.6[c]	+573.1[a]	40.60			

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standard error of mean; HC=High Ca; LC=Low Ca.
[3] Calculated as milliequivalent of [Na+K]-[Cl+S].
[abc] Means in the same row with different superscripts are different ($P<.05$).

ml kg^{-1} BW for the duration of 1.7 min kg^{-1} BW rarely gave rise to the visual symptoms of hypocalcemia. Increased respiration rates and muscular tremors occurred occasionally, but none of the animals experienced recumbency or comatose during the infusion period.

Plasma parameters

Plasma parameters for animals prior to the infusion of EDTA solution are presented in Tables 2.4 and 2.5. There was no difference ($P>.1$) in total plasma Ca (tCa) concentrations between sheep fed the HC (8.34 mg dL^{-1}) and LC (8.36 mg dL^{-1}) diets. Also no differences ($P>.1$) were observed between dietary treatments (8.62, 8.43, 8.21, 8.42 and 8.06 mg dL^{-1} for CTR, TRT-A, TRT-B, TRT-C and TRT-D, respectively). This indicated that reducing cation-anion balance of diets did not alter the plasma tCa concentrations. However plasma Pi and Mg concentrations were affected by dietary treatments. Animals fed TRT-C had a reduced ($P<.05$) plasma Pi and an increased ($P<.05$) plasma Mg concentration compared to TRT-D, although they were not different ($P<.1$) from that of CTR because of the high variability for sheep within the same dietary treatment.

Changes in plasma Ca concentration with EDTA infusion

During the infusion of EDTA, the rate of decrease in tCa concentrations (mg dL^{-1}) observed with all animals was al-

Table 2.4. Blood parameters of sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium levels[1,2].

Parameters	High calcium					Low calcium					SEM[4]	main effects and interaction[3]		
	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D		Ca	Anion	Ca * Anion
Calcium (mg dL^{-1})	8.68	8.53	8.31	8.36	7.81	8.56	8.34	8.11	8.48	8.32	0.36	NS	NS	NS
Phosphorus (mg dL^{-1})	4.59	4.54	4.55	4.40	5.33	4.91	4.40	4.84	3.79	5.09	0.85	NS	**	NS
Magnesium (mg dL^{-1})	2.01	1.93	2.10	2.19	1.97	2.01	1.92	2.06	2.23	1.67	0.09	NS	**	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); ** P<.05.
[4] SEM=Standard error of mean.

Table 2.5. Blood parameters of sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Plasma parameter	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Total calcium (mg dL^{-1})	8.62	8.43	8.21	8.42	8.06	0.360	8.34	8.36	0.290
Phosphorus (mg dL^{-1})	4.75[ab]	4.46[ab]	4.69[ab]	4.09[a]	5.21[b]	0.851	4.68	4.60	0.690
Magnesium (mg dL^{-1})	2.01[ab]	1.93[ab]	2.08[ab]	2.21[a]	1.82[b]	0.086	2.04	1.98	0.072

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standard error of mean; HC=High Ca; LC=Low Ca.
[ab] Means in the same row with different superscripts are different ($P<.05$).

most linear as evidenced by an average regression coefficient value ($r=0.935\pm0.052$). Table 2.6 shows regression equations calculated from timed samples (n=5) of individual animals which describes the mean decrease of $_f$Ca during the infusion period. No difference ($P>.1$) was observed between sheep fed HC and LC diets or between dietary treatments. Figure 2.2 shows a difference in response to the infusion of either EDTA solution or physiological saline. Animals showed no differences in plasma $_f$Ca concentrations during the infusion with physiological saline or in the recovery period thereafter. However, there was a 50 % reduction (8 to 4 mg dL^{-1}) in plasma $_f$Ca created by the infusion of EDTA solution.

Mean decreases in plasma $_f$Ca concentrations during the EDTA infusion are presented in Figure 2.3 and Tables 2.7 and 2.8. The decrease in plasma $_f$Ca concentration was expressed as actual values (mg dL^{-1}) and as a percentage of change from the of pre-infusion value. The latter expression was employed to overcome differences in the initial (pre-infusion) plasma $_f$Ca concentrations between animals.

Decreases in concentrations of plasma $_f$Ca during the EDTA infusion were not different ($P>.1$) between sheep fed the HC and LC diets (4.11 vs 3.90 mg dL^{-1} or 49.98 vs 48.06%, respectively). Animals fed TRT-B and TRT-D demonstrated a smaller ($P<.05$) reduction in plasma $_f$Ca concentrations than CTR during the EDTA infusion (Table 2.8). The tables also show the recovered plasma $_f$Ca concentrations during the 240 min post-infusion (recovery period). Animals fed TRT-D

Table 2.6. Regression equation and correlation coefficient of plasma free calcium ($_f$Ca) concentration during the EDTA infusion in sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1].

Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC
Slope	-0.034	-0.033	-0.036	-0.034	-0.029	0.003	-0.033	-0.032
Intercept	8.03	7.58	7.91	7.83	8.14	0.175	7.96	7.96
r^2	0.953	0.926	0.941	0.932	0.920		0.938	0.930

[1] CTR=Control; TRT=Treatment; SEM=Standard error of mean; HC=High Ca; LC=Low Ca.
[2] Correlation coefficient.

Figure 2.2. Changes in plasma titratable calcium concentration with the infusion of physiological saline (x) or 5.6% EDTA solution in sheep fed high (O) or low (●) dietary calcium levels.

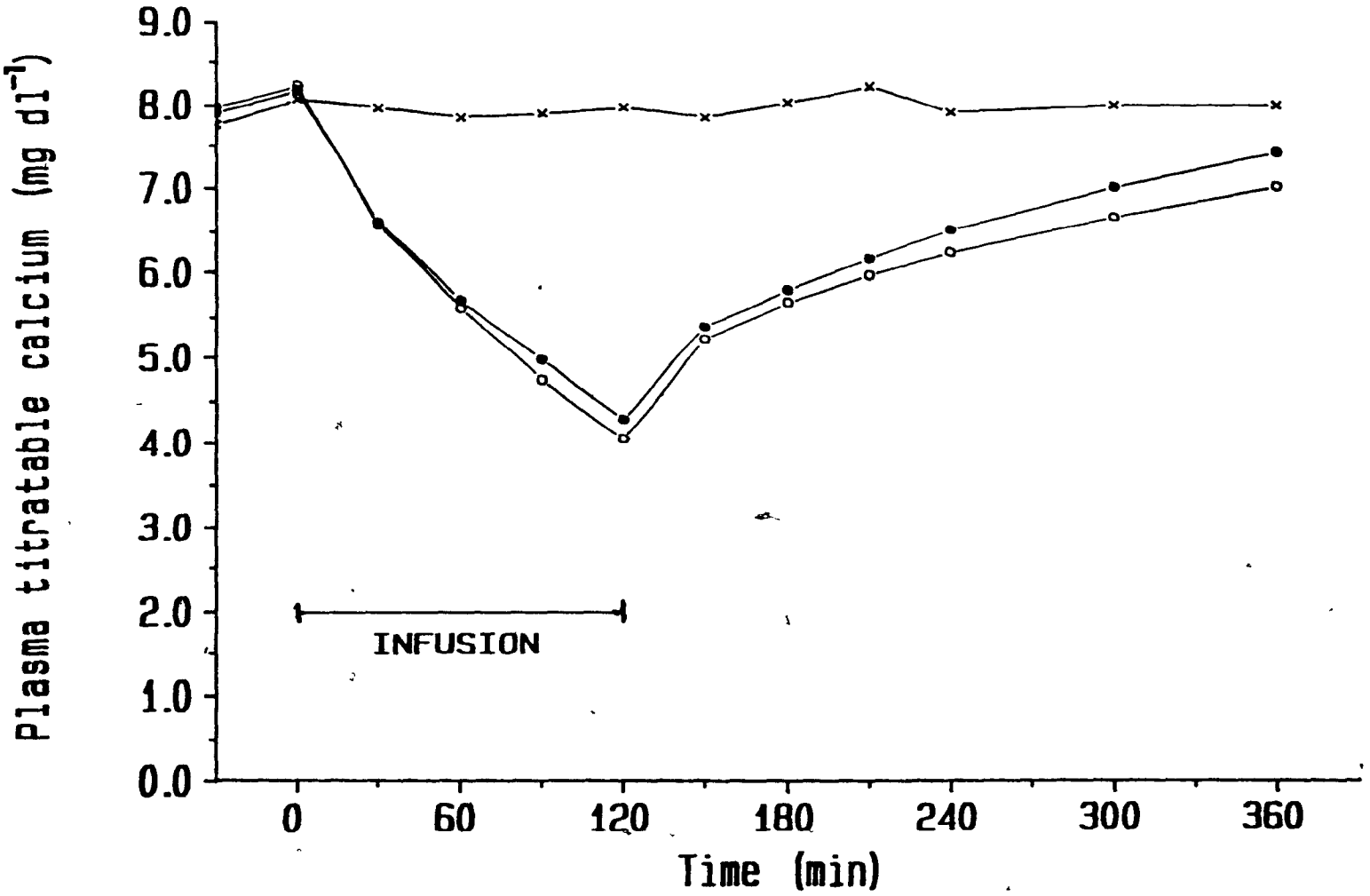

Plasma titratable calcium (mg dl⁻¹)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
INFUSION
0
60
120
180
240
300
360
Time (min)

Figure 2.3. Changes in plasma titratable calcium concentration with the EDTA infusion in sheep fed different anion-anion balanced diets (× =CTR, ○ =TRT-A, ▽ =TRT-B, □ =TRT-C) or vitamin D_3 injected (● =TRT-D) (high and low calcium groups combined).

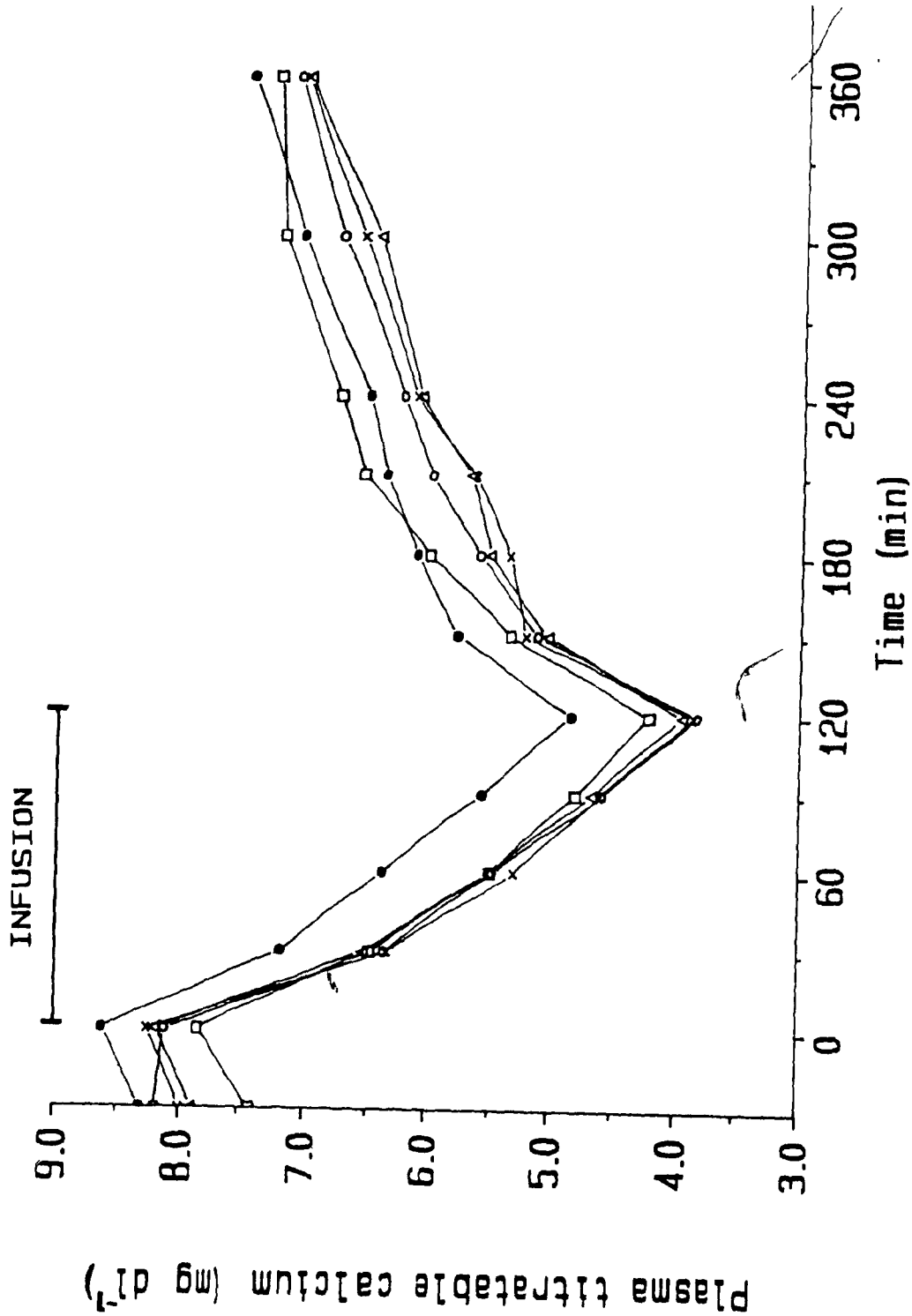

Plasma titratable calcium (mg dl⁻¹)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
INFUSION
0
60
120
180
240
300
360
Time (min)

Table 2.7. Response of plasma free calcium ($_f$Ca) concentration with the infusion of 5.6% EDTA solution by sheep fed different cation-anion balanced diets or injected with vitamin D_3 with two levels of dietary calcium[1,2].

Variable	High calcium					Low calcium					SEM[4]	main effects and interaction[3]		
	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D		Ca	Anion	Ca * Anion
Decreased Ca during infusion (mg dL^{-1})	5.05	4.26	3.69	4.04	3.50	4.08	4.15	3.42	4.00	3.85	0.350	NS	**	NS
(% from baseline)	56.64	51.95	47.15	52.21	41.93	53.10	49.65	44.10	50.28	43.15	3.375	NS	**	NS
Recovered Ca after infusion (mg dL^{-1})	3.43	3.24	3.09	2.82	2.26	2.87	2.96	3.33	3.68	2.67	0.370	NS	*	NS
(% from baseline)	38.05	40.36	39.63	37.17	27.63	38.33	35.02	44.32	46.43	29.97	5.440	NS	**	NS
Total Ca change (mg dL^{-1})	-1.62	-1.21	-1.02	-1.19	-0.60	-0.09	-1.22	-0.31	-1.24	-1.18	0.459	NS	**	NS
(% from baseline)	-18.59	-15.05	-11.59	-7.52	-14.30	-14.77	-3.86	-14.64	+0.22	-13.18	5.727	NS	**	NS
Half recovery time $T_{1/2}$ (min)	167.6	131.5	130.6	103.7	155.55◇	157.3	104.5	122.8	115.9	158.0	15.08	NS	**	NS

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment.
[3] NS=Not significant (P>.1); * P<.1; ** P<.05.
[4] SEM=Standard error of mean.

Table 2.8. Responses of plasma free calcium ($_f$Ca) concentration with the infusion of 5.6% EDTA solution by sheep fed different cation-anion balanced diets or injected with vitamin D_3 (high [HC] and low [LC] calcium groups combined)[1,2].

Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
Decreased Ca during infusion (mg dL^{-1})	4.56^{a}	4.20^{ab}	3.56^{b}	4.02^{ab}	3.67^{b}	0.245	4.11	3.89	0.144
(% from base line)	54.87^{a}	50.80^{ab}	45.62^{b}	51.25^{ab}	42.54^{b}	2.643	49.98	48.06	1.552
Recovered Ca after infusion (mg dL^{-1})	3.15^{A}	3.10^{AB}	3.21^{A}	3.25^{A}	2.47^{B}	0.261	2.97	3.10	0.153
(% from base line)	39.19^{ab}	37.69^{ab}	41.98^{a}	41.80^{a}	28.80^{b}	3.808	36.57	38.81	2.236
Total Ca change (mg dL^{-1})	-1.67^{a}	-1.31^{ab}	-0.37^{b}	-0.95^{ab}	-1.37^{ab}	0.403	-1.34	-0.92	0.237
(% from base line)	-14.15^{a}	-11.03^{ab}	-3.46^{b}	-7.69^{ab}	-12.09^{ab}	3.040	-11.41	-7.96	1.900
Half recovery time $T_{1/2}$ (min)	162.5^{a}	118.0^{ab}	126.7^{ab}	109.8^{b}	156.8^{ab}	13.35	137.8	131.7	7.70

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standard error of mean; HC=High Ca; LC=Low Ca.
^{ab} Means in the same row with different superscripts are different ($P<.05$).
^{AB} Means in the same row with different superscripts are different ($P<.1$).

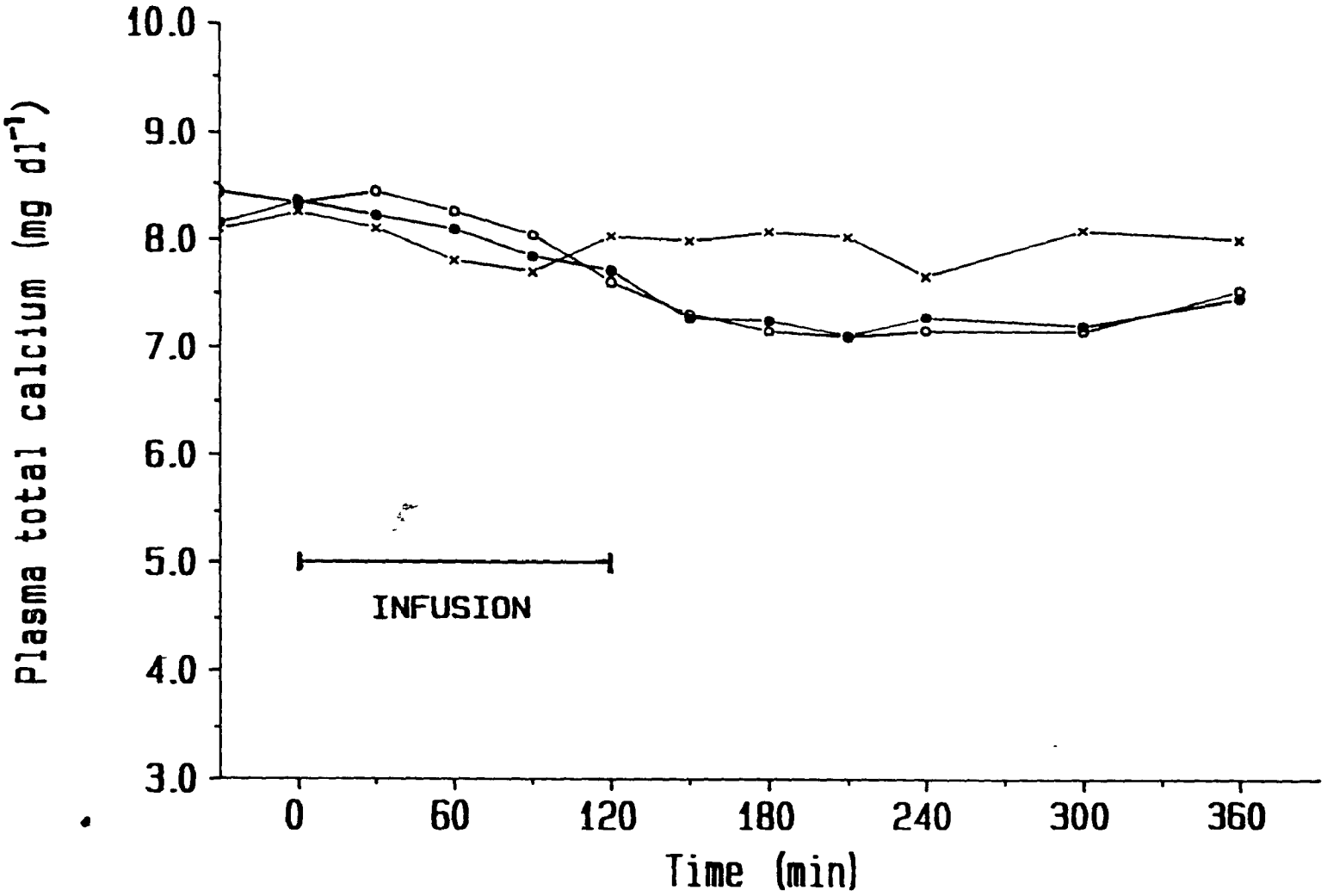
recovered (2.47 mg dL^{-1}) slightly ($P<.1$) smaller concentrations of $_{f}Ca$ compared to that of CTR (3.15 mg dL^{-1}). This trend, however, was not observed when values were expressed as % of the pre-infusion concentrations. When the changes in plasma $_{f}Ca$ concentrations during the entire collection periods are compared no difference ($P>.1$) was found between sheep fed the HC and LC diets. Only animals fed TRT-C demonstrated changes ($P<.05$) from CTR in both absolute levels of $_{f}Ca$ (-0.37 vs -1.67 mg dL^{-1}) and expressed as % from the base line (-3.46 vs -14.15%, respectively).

During the recovery period (240 min), none of animals had recovered plasma $_{f}Ca$ concentrations to the pre-infusion concentrations (Figure 2.3). Thus recovery rates during the recovery period were calculated as the time by which concentrations of $_{f}Ca$ in plasma regained one-half of that which it declined to during the infusion period ($T_{1/2}$), as expressed by Payne (1964). Animals fed TRT-C demonstrated faster ($P<.05$) $T_{1/2}$ than CTR and animals fed reduced cation-anion balanced diets (TRT-A, TRT-B and TRT-C) showed a trend ($P<.1$) of faster $T_{1/2}$ than CTR.

Changes in plasma total Ca ($_{t}Ca$) concentration

Figure 2.4 illustrates the changes in $_{t}Ca$ concentrations of animals infused with either physiological saline or EDTA solution. Animals infused with EDTA showed a sigmoid shape curve where there was an initial increase followed by a gradual decrease of $_{t}Ca$ during the infusion and early

Figure 2.4. Changes in plasma total calcium concentration with the infusion of physiological saline (×) or 5.6% EDTA solution in sheep fed high (o) or low (●) dietary calcium levels.


10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
Plasma total calcium (mg dl^{-1})
INFUSION
0
60
120
180
240
300
360
Time (min)

recovery periods, which then increased at the late stages of recovery period. Analysis of variance (Table 2.9) shows that the sheep*infusion interaction was not significant (P=0.929) indicating that the responses of plasma ₁Ca concentration was not different between animals infused with either EDTA or physiological saline.

Plasma inorganic phosphorus (Pi)

Changes in plasma Pi concentrations with the infusion are presented in Figure 2.5. Plasma Pi concentrations of animals infused with EDTA demonstrated a reduction during the infusion compared to the pre-infusion concentrations and remained at lower concentration throughout the recovery period. There was no difference (P>.1) observed in plasma Pi between sheep fed HC and LC diets. In order to study the effects of EDTA infusion on plasma Pi concentrations, a comparison was made between the infusion of EDTA and physiological saline. Analysis of variance (Table 2.10) shows that the sheep*infusion interaction was significant (P=.001) indicating that the response of plasma Pi to the induction of hypocalcemia created by the infusion of EDTA was significantly different from that of the infusion of physiological saline solution. Figure 2.5 shows that animals infused with physiological saline maintained higher plasma Pi concentrations than those infused with EDTA during the infusion and recovery periods. Differences in plasma Pi concentrations between dietary treatments are presented in

Table 2.9. Analysis of variance of total calcium (tCa) concentration in plasma of sheep fed different cation-anion balanced diets.

Variable	DF	MS	F-ratio	Pr
Sheep	4	1.695	0.16	0.958
Infusion	1	10.943	4.15	0.044
Sheep*infusion	4	2.320	0.22	0.927
Sheep within infusion	11	263.777	9.10	0.001
Error	108	2.635	-	

DF Degrees of freedom.
MS Mean square.
Pr Probability.

Figure 2.5. Changes in plasma phosphorus concentrations with the infusion of physiological saline (×) or 5.6 % EDTA solution in sheep fed high (○) or low (●) dietary calcium levels.

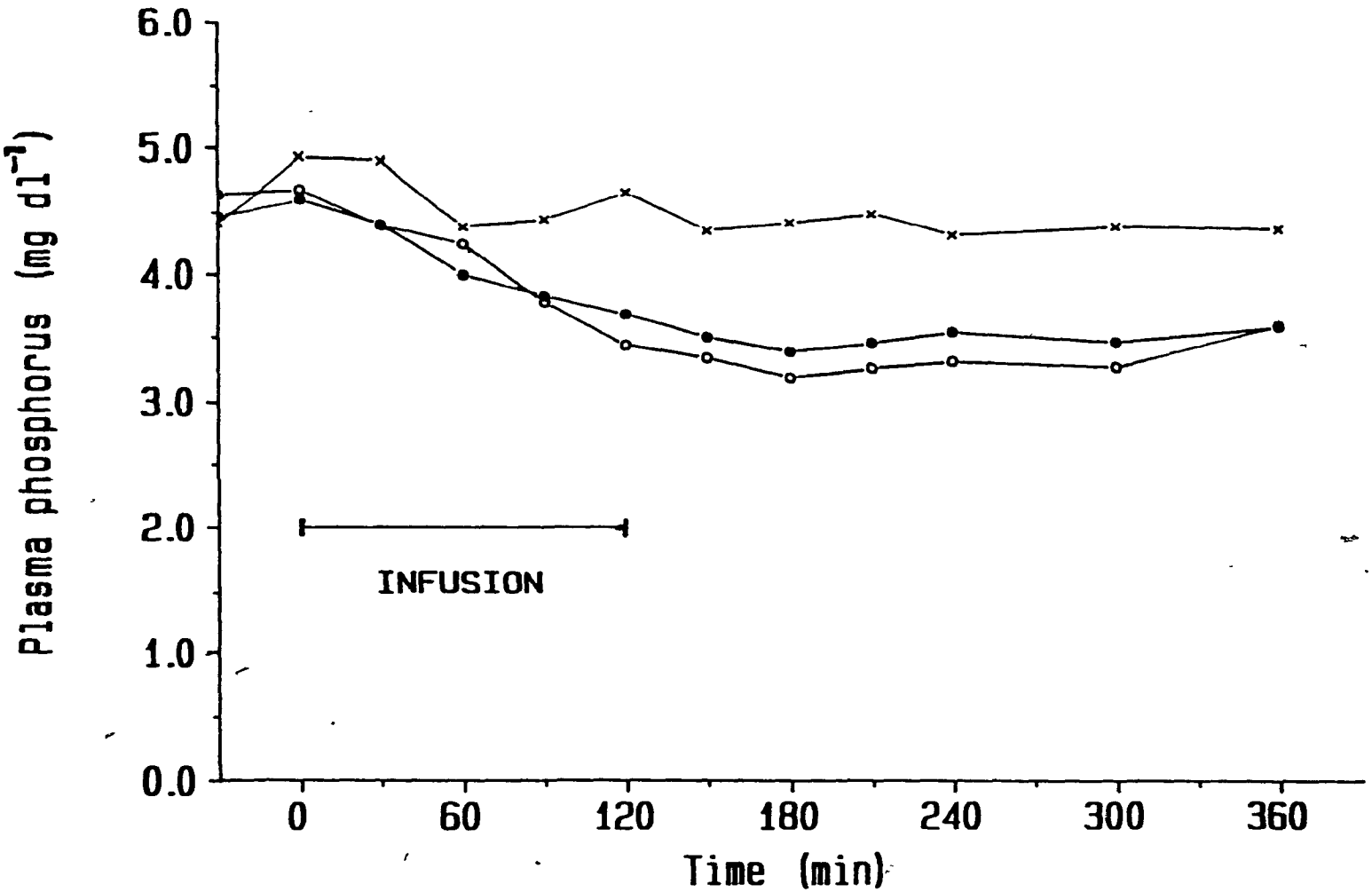

Plasma phosphorus (mg dl^{-1})
6.0
5.0
4.0
3.0
2.0
1.0
0.0
INFUSION
0
60
120
180
240
300
360
Time (min)

Table 2.10. Analysis of variance of phosphorus (Pi) concentration in plasma of sheep fed different cation-anion balanced diets.

Variable	DF	MS	F-ratio	Pr
Sheep	4	28.643	42.73	0.0001
Infusion	1	0.012	0.07	0.787
Sheep*infusion	4	7.200	10.75	0.001
Sheep within infusion	12	10.480	5.21	0.001
Error	107	0.107	-	

DF Degrees of freedom.
MS Mean square.
Pr Probability.

Figure 2.6. Animals fed TRT-C showed a trend (P<.1) for lower plasma Pi concentrations during the infusion period than CTR and animals fed TRT-A and TRT-D had higher (P<.05) plasma Pi than CTR during the recovery period.

Plasma Magnesium (Mg)

Changes in plasma Mg concentration with the 5.6% EDTA infusion are presented in Figure 2.7. There was no difference (P>.1) in plasma Mg concentrations throughout the sampling period between sheep fed HC and LC diets. Analysis of variance (Table 2.11) revealed that the sheep*infusion interaction was not significant (P=.750) indicating that the response of plasma Mg was not significantly different between the infusion of 5.6% EDTA solution and physiological saline. However, there were differences (P<.001) in plasma Mg concentrations with the time of blood sampling. A comparison of plasma Mg concentrations between dietary treatments (Figure 2.8) shows that animals fed TRT-D showed lower (P<.05) plasma Mg concentrations particularly during the recovery period, compared to CTR. This was opposite relationship to the responses in plasma Pi concentrations (Figure 2.5). Plasma Mg concentration of animals fed TRT-C was higher (P<.05) than CTR during the sampling period.

Relation between plasma Ca, Pi and Mg

Table 2.12 shows a within-animal regression-correlation

Figure 2.6. Changes in plasma phosphorus concentrations with the EDTA infusion in sheep fed different cation-anion balanced diets (× =CTR, ○ =TRT-A, △ =TRT-B, □ =TRT-C) or vitamin D_3 injected (● =TRT-D) (high and low calcium groups combined). Values listed the bottom of the figure are standard error of mean.

* Values differ significantly ($P<.05$).

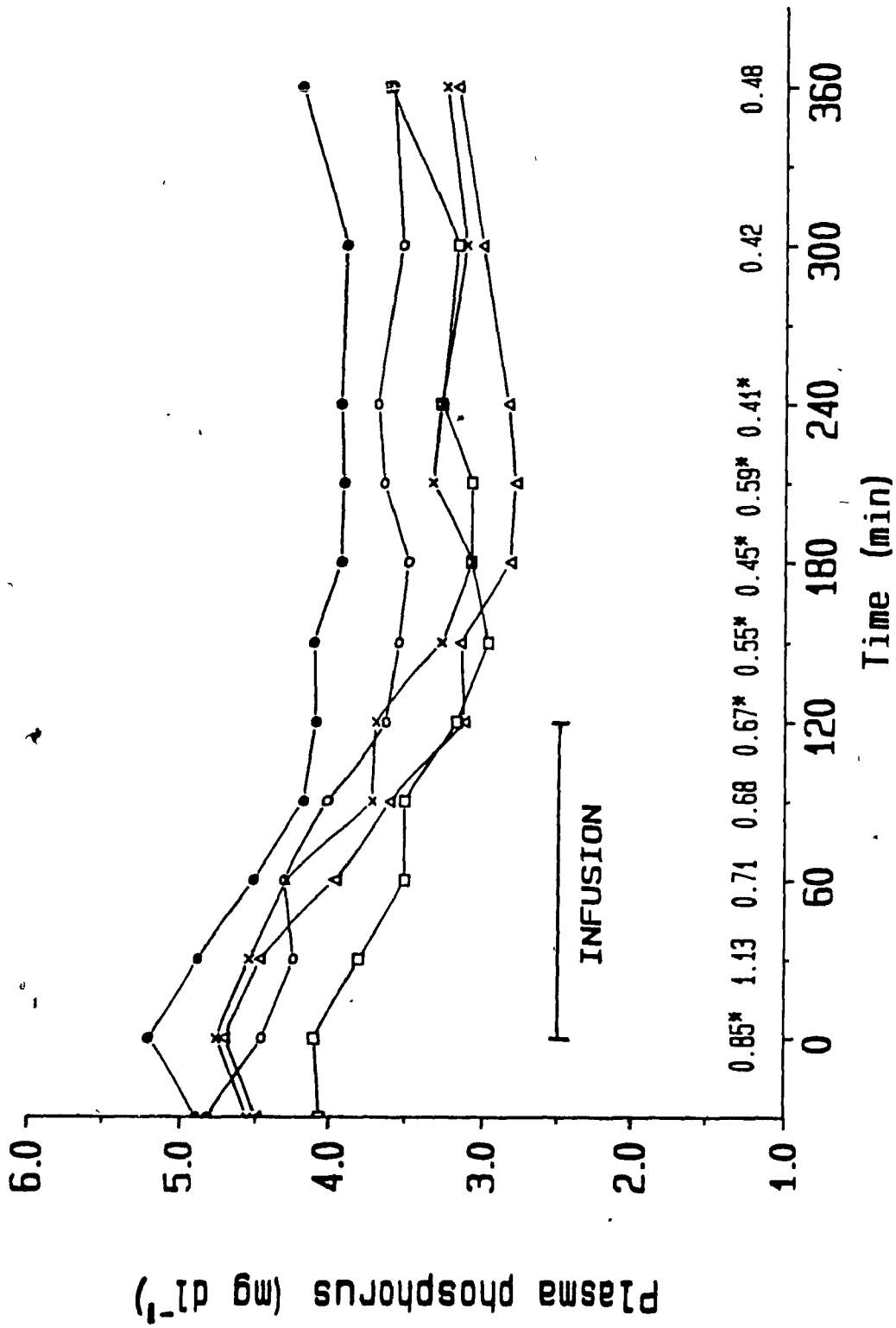

Plasma phosphorus (mg dl⁻¹)
6.0
5.0
4.0
3.0
2.0
1.0
INFUSION
0.85* 1.13 0.71 0.68 0.67* 0.55* 0.45* 0.59* 0.41* 0.42 0.48
0
60
120
180
240
300
360
Time (min)

Figure 2.7. Changes in plasma magnesium concentrations with the infusion of physiological saline (x) or 5.6% EDTA solution in sheep fed high (o) or low (•) dietary calcium levels.

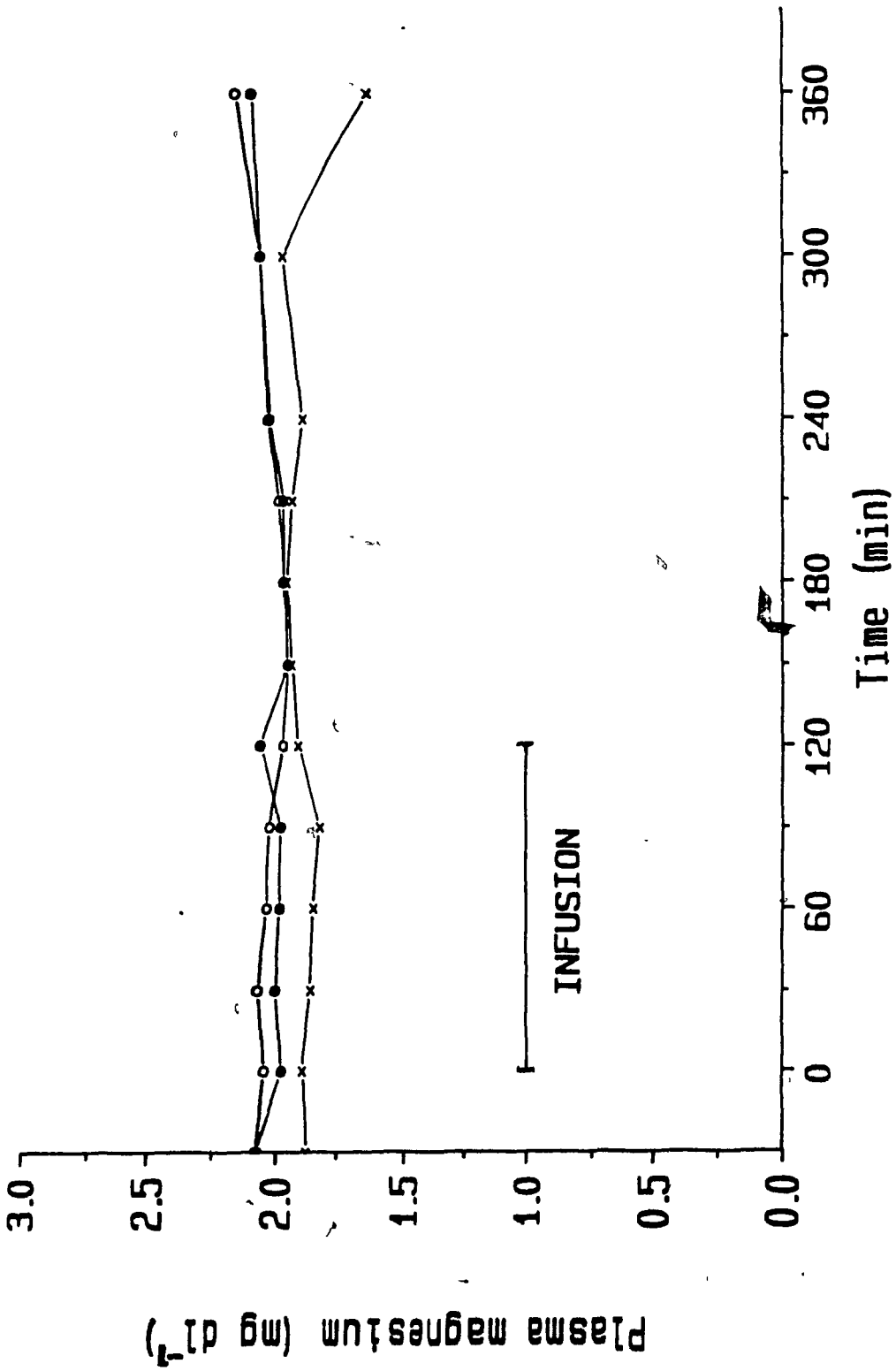

Plasma magnesium (mg dl⁻¹)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
INFUSION
0
60
120
180
240
300
360
Time (min)

Table 2.11. Analysis of variance of magnesium (Mg) concentration in plasma of sheep fed different cation-anion balanced diets.

Variable	DF	MS	F-ratio	Pr
Sheep	4	0.997	1.33	0.264
Infusion	1	0.038	0.20	0.652
Sheep*infusion	4	0.361	0.48	0.750
Sheep within infusion	11	16.787	8.14	0.001
Error	108	0.188	-	

DF Degrees of freedom.
MS Mean square.
Pr Probability.

Figure 2.8 Changes in plasma magnesium concentrations with the EDTA infusion in sheep fed different cation-anion balanced diets (× =CTR, ○ =TRT-A, △=TRT-B, □ =TRT-C) or vitamin D_3 injected (● =TRT-D) (high and low calcium groups combined).

Values listed the bottom of the figure are standard error of menas.

* Values differ significantly ($P<.05$).

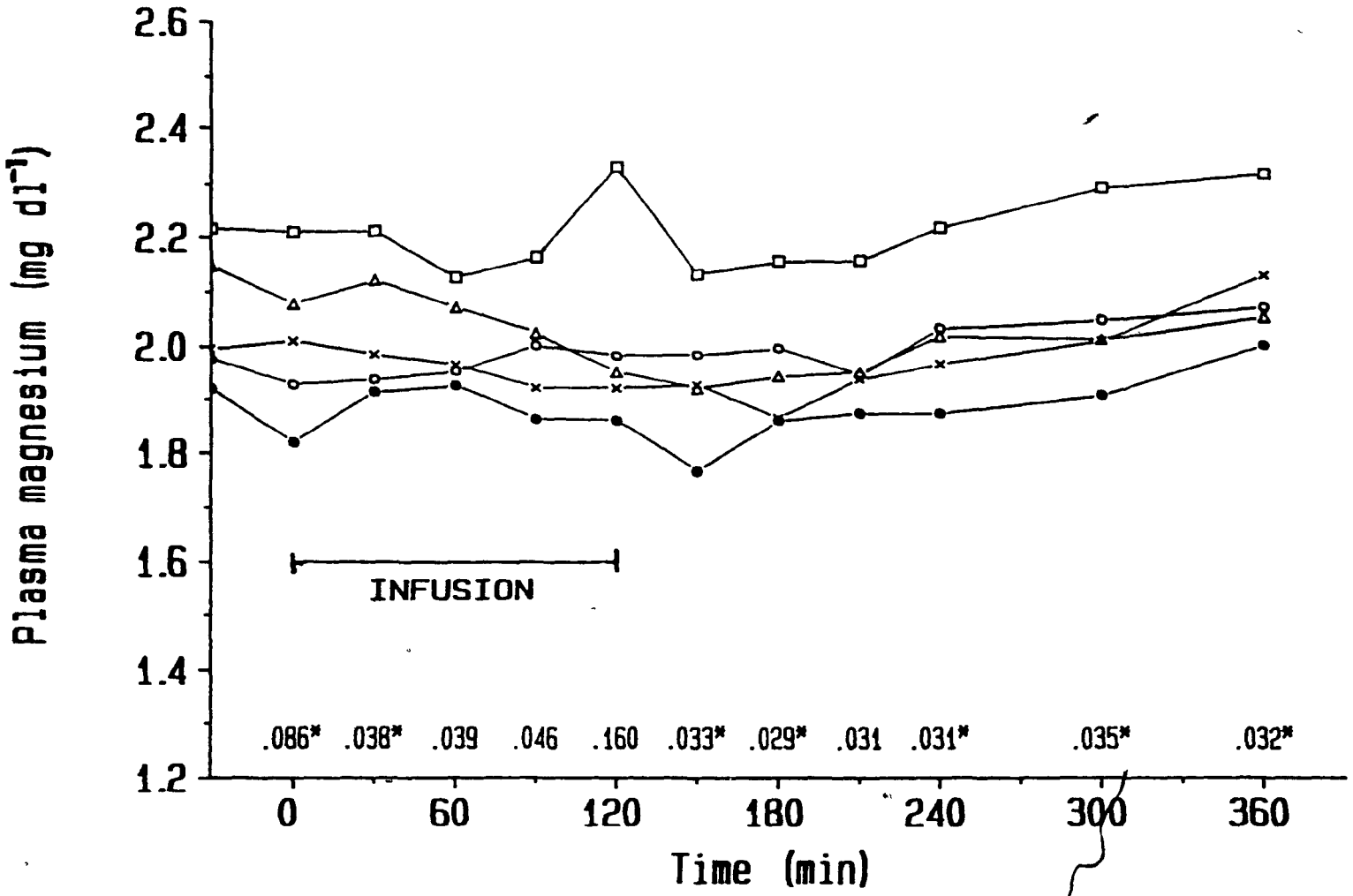

Plasma magnesium (mg dl⁻¹)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
INFUSION
.086* .038* .039 .046 .160 .033* .029* .031 .031* .035* .032*
0
60
120
180
240
300
360
Time (min)

Table 2.12. Within-animal regression-correlation and slope analyses, dependent variable is plasma phosphorus (Pi) (mmol L^{-1}) and independent variable is plasma free calcium ($_f$Ca) (mmol L^{-1}).

Pen	b±Sb[1]	r^2	n	Pr
1	0.520±0.085	0.391	61	0.0001
2	0.146±0.109	0.029	63	0.1860
3	0.528±0.073	0.461	64	0.0001
4	0.217±0.107	0.066	60	0.0467
5	0.209±0.085	0.098	58	0.0168
6	0.353±0.145	0.089	63	0.0175
7	0.349±0.140	0.094	62	0.0153
8	0.236±0.085	0.109	62	0.0071
9	0.309±0.120	0.096	64	0.0129
10	0.302±0.108	0.112	64	0.0069

[1] Slope±Standard error of slope.
r^2 Coefficient of determination.
n Number of observations.
Pr Probability.

analysis using plasma Pi (mmol L^{-1}) as a dependent variable and plasma $_{f}Ca$ (mmol L^{-1}) as an independent variable. Except for one animal (pen 2), a significant correlation was obtained in all animals regardless of dietary treatments. A significant correlation was also obtained for the within-diet regression correlation analysis (Table 2.13), although animals fed TRT-A failed to show a significant correlation (P=0.49).

Tables 2.14 and 2.15 show a regression-correlation analysis between plasma Mg (mmol L^{-1}) and plasma $_{f}Ca$ (mmol L^{-1}) as a dependent and independent variable, respectively. A significant correlation was obtained for all animals and ranged from r=0.33 for pen 3 to r=0.49 for pen 5, and for all dietary treatments it ranged from r=0.37 for TRT-D to r=0.51 for TRT-C.

Calcium mobilization rate

Ca mobilization capacities for animals calculated by the equation of Contreras *et al.* (1982) are presented in Tables 2.16 and 2.17. The volume of distribution of CaEDTA (V), which represented the volume of the compartment within which there is rapid equlibration of $_{f}Ca$, did not differ (P>.1) between sheep fed the HC and LC diets or between dietary treatments (range from 7.83 L for TRT-D to 12.57 L for CTR). A mean of V/BW, which is expressed as a percentage of BW, ranged from 11.22% for TRT-D to 17.80% for CTR. There were no differences (P>.1) observed between dietary treat-

Table 2.13. Within-diet regression correlation and slope analyses, dependent variable is plasma phosphorus (Pi) (mmol L^{-1}) and independent variable is plasma free calcium ($_f$Ca) (mmol $_fL^{-1}$) concentration (high and low calcium groups combined)[1].

Treatments	b±Sb[2]	r^3	n	Pr
CTR	0.334±0.104	0.078	123	0.0017
TRT-A	0.063±0.092	0.004	123	0.4935
TRT-B	0.420±0.077	0.190	129	0.0001
TRT-C	0.353±0.072	0.165	122	0.0001
TRT-D	0.145±0.078	0.027	127	0.0671
HC	0.376±0.063	0.102	318	0.0001
LC	0.224±0.049	0.063	306	0.0001

[1] CTR=Control; TRT=Treatment; HC=High Ca; LC=Low Ca.
[2] Slope±Standard error of slope.
[3] Coefficient of determination.
n Number of observations.
Pr Probability.

Table 2.14. Within-animal regression correlation and slope analyses. dependent variable is plasma magnesium (Mg) (mmol L^{-1}) and independent variable is plasma free calcium ($_{f}Ca$) (mmol L^{-1}) concentrations.

Pen	b±Sb[1]	r^2	n	Pr
1	0.235±0.084	0.119	60	0.0069
2	0.305±0.074	0.217	63	0.0001
3	0.253±0.091	0.111	64	0.0072
4	0.342±0.085	0.219	59	0.0002
5	0.394±0.094	0.239	58	0.0001
6	0.469±0.109	0.231	64	0.0001
7	0.388±0.090	0.225	64	0.0010
8	0.265±0.077	0.225	64	0.0001
9	0.381±0.101	0.188	63	0.0004
10	0.327±0.074	0.238	64	0.0001

[1] Slope±Standard error of slope.
r^2 Coefficient of determination.
n Number of observations.
Pr Probability.

Table 2.15. Within-diet regression correlation and slope analyses. dependent variable is plasma magnesium (Mg) (mmol L^{-1}) and independent variable is plasma free calcium ($_f$Ca) (mmol L^{-1}) (high and low calcium groups combined)[1].

Treaments	b±Sb[2]	r^2[3]	n	Pr
CTR	0.363±0.057	0.251	121	0.0001
TRT-A	0.311±0.058	0.190	126	0.0001
TRT-B	0.279±0.057	0.157	129	0.0001
TRT-C	0.433±0.066	0.262	123	0.0001
TRT-D	0.279±0.062	0.139	126	0.0001
HC	0.360±0.037	0.227	318	0.0001
LC	0.269±0.039	0.137	307	0.0001

[1] CTR=Control; TRT=Treatment; HC=High Ca; LC=Low Ca.
[2] Slope±Standard error of slope.
[3] Coefficient of determination.
n Number of observations.
Pr Probability.

Table 2.16. Calcium mobilization rate by sheeps fed different cation-anion balnced diets or injected with vitamin D_3 with two levels of dietary calcium measured by the infusion of 5.6% EDTA solution[1,2].

Variable	High calcuim					Low calcium					SEM[4]	main effects and interaction[3]		
	CTR	TRT-A	TRT-B	TRT-C	TRT-D	CTR	TRT-A	TRT-B	TRT-C	TRT-D		Ca	Anion	Ca * Anion
V^5 (L)	9.99	10.89	13.01	12.61	7.77	15.14	10.26	9.14	9.49	7.89	2.92	NS	NS	NS
V/BW (%)	14.02	15.95	18.76	19.28	11.04	21.58	14.38	12.93	13.69	11.40	4.94	NS	NS	NS
Q^6 (mmol)	11.51	13.92	10.87	12.48	20.73	10.23	15.09	15.06	17.03	18.93	2.848	NS	**	NS
R^7 (mmol min^{-1})	0.102	0.115	0.093	0.104	0.166	0.087	0.133	0.126	0.145	0.151	0.025	NS	**	NS

1 Least-squares means.
2 CTR=Control; TRT=Treatment.
3 NS=Not significant (P>.1); ** P<.05.
4 SEM=Standard error of mean.
5 The volume of the compartment within which there is rapid equilibration of free calcium.
6 The amount of calcium mobilized from V during EDTA infusion period.
7 The rate of calcium mobilization from V.

Table 2.17. Calcium mobilization rate by sheep fed different cation-anion balanced diets or injected with vitamin D_3 measured by the infusion of 5.6% EDTA solution (high and low calcium groups combined)[1,2].

Variable	CTR	TRT-A	TRT-B	TRT-C	TRT-D	SEM	HC	LC	SEM
V[3] (L)	12.57	10.58	11.08	11.05	7.83	2.251	10.86	10.38	1.252
V/BW (%)	17.80	15.16	15.84	16.48	11.22	3.230	15.81	14.80	1.680
Q[4] (mmol)	10.87[a]	14.50[a b]	12.96[a]	14.75[a b]	19.83[b]	2.218	13.90	15.27	1.152
R[5] (mmol min^{-1})	0.094[a A]	0.124[B]	0.109[a A]	0.125[B]	0.158[b]	0.017	0.116	0.128	0.10

[1] Least-squares means.
[2] CTR=Control; TRT=Treatment; SEM=Standard error of mean; HC=High Ca; LC=Low Ca.
[3] The volume of the compartment within which there is rapid equilibration of free calcium.
[4] The amount of calcium mobilized from V during EDTA infusion period.
[5] The rate of calcium mobilization from V.
[a b] Means in the same row with different superscripts are different ($P<.05$).
[A B] Means in the same row with different superscripts are different ($P<.1$).

ments because of a high variation evidenced by a high standard error ($\pm$2.251 L for V and $\pm$3.23% for V/BW).

Dietary cation-anion balance influenced the total amounts of Ca mobilized (Q) and Ca mobilization rates (R). The amount of Ca mobilized during the 5.6% EDTA infusion by animal in TRT-D was greater ($P<.05$) than that of CTR (19.83 vs 10.87 mmol) and animal fed TRT-A and TRT-C showed slight but significant increase ($P<.1$) in calcium mobilization rate (R) than that of CTR.

DISCUSSION

With creating hypocalcemia experimentally by the infusion of a 5.6% solution of EDTA solution, animals fed the lowest cation-anion balanced diet (TRT-C) demonstrated a resistance to a reduction in plasma ${}_f$Ca concentrations almost to the same magnitude as that of animals injected with vitamin D_3 (TRT-D). This was also reflected in a greater Ca mobilization capacity measured by the method of Contreras *et al* (1982).

There have been other studies using the infusion of EDTA to determine the rate of Ca mobilization (Payne, 1964b; Ramberg *et al.*, 1967; Horst and Jorgensen, 1982; Waage *et al.*, 1984). The responses to the induced hypocalcemia should reflect both a size and a responsiveness of immediately mobilizable Ca pools (blood Ca, interstitial Ca and exchangeable bone Ca). A reduction in plasma Ca concentration, particularly in the ionized form of Ca, created by the infusion

of chelating agents such as EDTA or phosphate (PO_4) is encountered by the secretion of PTH within minutes of infusion (Fisher *et al.*, 1973; Blum *et al.*, 1974a; 1981): This is mediated by plasma-membrane-bound adenylate cyclase (Abe and Sherwood, 1972). Blum *et al.* (1974b) reported that a small change in concentration of plasma ionized Ca (0.1 mg dL^{-1}) triggered a significant response to the secretion of PTH. This PTH secretion will increase plasma Ca concentration through an enhancement of the bone resorption process.(Mayer *et al.*, 1967; Vaes, 1968; Silve *et al.*, 1981). It is believed that 1 to 1.5 h is required for PTH to increase concentrations of Ca in plasma (Saeki and Ashi, 1981).

A continuous flow of Ca to the intestine is available in ruminant (Hove and Hilde, 1984), which leads to the hypothesis that an even rate of intestinal Ca absorption might cause fluctuations of plasma Ca to be so small that the parathyroid gland lacks excercise over plasma Ca. Thus, the importance of PTH and its involvement in Ca metabolism in ruminants is questioned. However, studies showed that there was no delay or deficiency of PTH secretion at parturition in cows. (Horst *et al.*, 1978a; Blum *et al.*, 1981).

Black *et al.* (1973b) and Belyea *et al.* (1975) reported an inverse relationship between plasma Ca and dietary Ca concentrations during the period of EDTA infusion in Ca-deficient cows created by feeding a diets with low dietary Ca (0.25% DM) . Requirements of Ca for their experimental animals were 0.54 to 0.60% DM. However, in our study, there were no differences in either plasma iCa or tCa concentra-

tions between the sheep fed HC and LC diets regardless of dietary treatment. Dietary Ca concentration of LC diets (0.45%), which was above the requirements of 0.35% DM (NRC 1985), was probably too high to cause any change in Ca metabolism compared to that of Ca-deficient animals. A Ca balance study using same dietary treatments (Exp. 1) showed that animals fed LC diets were in a positive Ca balance, whereas Ca-deficient animals should show a negative Ca balance (NRC 1985). This should lead to an increase in sensitivity and induce greater responses to the PTH secretion than that of Ca-replete animals (Blum *et al.*, 1974b). This resulted in a increase in the resorption of bone Ca (Black *et al.*, 1973b). Braithwaite (1979), however, reported in the kinetic study that Ca-replete wethers fed dietary Ca of either 40 or 100 mg d^{-1} kg^{-1} BW showed a similar size of the rapidly exchangeable Ca pool (34.7 vs 33.8 mg kg^{-1} BW), slowly exchangeable Ca pool in bone (30.8 vs 33.2 mg kg^{-1} BW) and the rate of resorption from bone (17.9 vs 17.6 mg d^{-1} kg^{-1} BW), respectively. Our study also showed no differences in volume of compartment (V)(11.94 and 12.17 L) or Ca mobilization (Q)(12.97 and 13.47 mmol) between animals fed high (HC) and low (LC) dietary Ca concentrations, respectively.

PTH increases the conversion of 25-hydroxyvitamin D_3 ($25(OH)D_3$) to $1,25(OH)_2D_3$ (Kumar, 1984), which enhances dietary Ca absorption from the small intestine (Pansu *et al.*, 1983b). However, altered gastrointestinal absorption of dietary Ca probably did not account for the change observed in plasma †Ca concentration in responses to the EDTA infusion

in this study. Bishop *et al.* (1983) reported that a minimum of 6 h was required to increase Ca-binding protein (CaBP) contents thus increasing Ca absorption from the small intestine. Therefore, Ca absorption from the gastrointestine probably had similar and small effects in responding to the Ca outflow regardless of dietary treatments during the EDTA infusion.

Animals fed TRT-C demonstrated more resistance to the hypocalcemic situation than that of CTR evidenced by a slower decrease in plasma Ca concentrations during the infusion period and a faster recovery ($T_{1/2}$) during the recovery period. There is no data available for $T_{1/2}$ values using sheep as a experimental model. However, it seems that species and physiological status of animals would create a variation in $T_{1/2}$ values. Belyea *et al.* (1975) reported with EDTA induced hypocalcemia in lactating cows (43 mgEDTA kg^{-1} BW h^{-1} for 4 h) that control cows showed $T_{1/2}$ values of 240 to 320 min whereas Ca-deficient cows showed values of 30 to 60 min. Black *et al.* (1973b) measured $T_{1/2}$ values of Jersey cows fed either normal (25g d^{-1}) or high Ca diets (150g d^{-1}) using the same EDTA dose as Belyea *et al.* (1975) and found that a $T_{1/2}$ value for control cows was about 300 min compared to cows fed high Ca diet of 600 min.

The data obtained in our study does not allow us to provide a possible mechanism(s) whereby dietary cation-anion balance would influence the responsiveness to the induced hypocalcemia. However, lowering the cation-anion balance in diets seems to have same effects on Ca mobi-

lization regardless of the choice of mineral salts used to supply anion-forming mineral elements (Cl and S), namely NH_4Cl alone (TRT-A), a combination of $Al_2(SO_4)_3$ and $MgSO_4$ (TRT-B) or $FeSO_4$ and $MgSO_4$ (TRT-C). Although, comparison between animals fed TRT-A, TRT-B and TRT-C could not confirm this effect because there were differences in their dietary cation-anion balance (+199, +79, and +37 meq kg^{-1} DM, respectively).

No differences observed in response to the EDTA infusion between animals fed TRT-A and TRT-B compared to that of CTR may implied that differences in their dietary cation-anion balance from that of CTR (155 and 275 meq kg^{-1} for TRT-A and TRT-B, respectively) was not large enough and also that dietary cation-anion balance of TRT-A (+199 meq kg^{-1}) and TRT-B (+79 meq kg^{-1}) themselves were not low enough to observe the effect of a reduced dietary cation-anion balance on Ca metabolism compared to that of higher dietary cation-anion balance. Block (1984) fed a positive cation-anion balanced diet (+330.4 meq kg^{-1}DM) and a negative diet (-127.8 meq kg^{-1}DM) to cows from 45 days prepartum to parturition and found that cows fed a negative diet showed higher concentrations of free hydroxyproline (OHPro) in plasma than those fed the positive diet for 4 days prepartum to day-2 postpartum. Fredeen *et al.* (1988b) also found in a kinetic study that sheep fed a reduced cation-anion balanced diet (-21.4 meq kg^{-1}DM) showed a greater rate of Ca mobilization from bone than that of fed a diet with very positive cation-anion balance (+713 meq kg^{-1}DM),

although their dietary cation-anion balance was defined and calculated without S contents ($Na^+ + K^+ - Cl^-$).

Feeding reduced cation-anion balanced diets to ruminants resulted in induction of a subclinical mild acidosis (Fredeen, 1984). Acid-base status of animals was not determined in our study, however, animals with a same dietary treatments as TRT-B and TRT-C showed a reduced pH of urine compared with CTR (Exp. 1). Payne *et al.* (1970) and Vagg and Payne (1970) have shown that feeding NH_4Cl-supplemented diets to induce acidosis in goats and cows increased the size of the exchangeable Ca pool due to a alterations in bone metabolism. Systemic metabolic acidosis might stimulates a slow dissociation of alkaline bone salts in order to increase the buffering capacity of the extracellular fluid, thus making bone more sensitive to endocrinological signals to recover from a hypocalcemic situation (Barzel and Jowsey, 1969). However, Braithwaite (1972) with sheep and Bell *et al.*(1977) with rats found that metabolic acidosis did not alter the bone resorption rate but increased urinary Ca excretion. The reduced Ca reabsorption from the renal tubules of metabolically acidotic animals was probably caused by a blunt response of adenyl cyclase to PTH in the renal cortex (Beck *et al.*, 1975) and/or an impaired conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ by renal cortical cells (Lee *et al.*, 1977).

High amounts of dietary aluminum (Al) (>2000 ppm) are known to cause a reduction in intestinal absorption of P (Valdivia *et al.*, 1982) and to stimulate intestinal Ca ab-

sorption through an increase in 25(OH)D_3-1-hydroxylase activity as well as an increase in response of intestinal mucosa cells to 1,25(OH)$_2D_3$ (Horst and Reinhardt, 1983). Miller and Levine, (1974) also demonstrated a negative correlation between Al concentrations in plasma and circulating PTH levels. However, Al contents of TRT-B were much lower (717±98 ppm) than in the above studies and feed intake as well as plasma Pi concentrations by animals fed TRT-B were not different from those of CTR. Therefore, it is unlikely that this level of dietary Al causes a direct effect upon Ca metabolism.

The lack of effects of vitamin D_3 injection on total changes of plasma ^{45}Ca concentration and $T_{1/2}$ recovery time observed with EDTA infusion compared to those of CTR was unexpected, although a reduction in plasma ^{45}Ca during the infusion was 12% smaller than that for CTR (42.54 and 54.87%, respectively). Vitamin D_3, particularly the active metabolite 1,25(OH)$_2D_3$, is a potent stimulator of bone resorption (Holick *et al.*, 1976). It was demonstrated that 1,25(OH)$_2D_3$ is capable of increasing the number and activity of osteoclast, which are localized to regions of the bone resorption process and this action was observed in the absence of PTH (Holtrop *et al.*, 1981). Koeffler *et al.* (1984) also demonstrated that 1,25(OH)$_2D_3$ can stimulate the differentiation of promyelocytes into monocytes, which are precursors of the osteoclast. However, Braithwaite (1981) with sheep, demonstrated that the injection of 1-alpha-(OH)D_3 did not change either the rapidly exchangeable Ca pool of

soft tissue or the slowly exchangeable Ca pool of bone. Our study confirmed that there were no differences in V or V/BW (%), represented as a volume of the compartment to which Ca moves and expressed as a proportion of BW, respectively, between animals fed CTR and TRT-D.

Muir *et al.* (1968) conducted a similar study to ours and found that there was no difference in $T_{1/2}$ between control and vitamin D_3 supplemented cows but the amount of plasma Ca reduced during the EDTA infusion was smaller than that for control cows. Thus, they concluded that animals fed with vitamin D_3 supplementation could withstand a similar drain of blood Ca at least one and a half times longer than that of control cows. The amounts of Ca mobilized with the infusion of EDTA by animals in TRT-D was 30.2% greater than that of CTR in our study measured by the method of Contreras *et al.* (1982). Animals fed TRT-A and TRT-B also showed 10.0 and 9.8% improvements, respectively, in Ca mobilization rate compared to that of CTR.

A direct relationship observed in our study between the concentrations of plasma Pi and $_f$Ca during the induction and spontaneous recovery periods from hypocalcemia confirmed the result of Daniel and Moodie (1979). A significant regression of plasma Pi on plasma $_f$Ca concentrations with the EDTA infusion was observed in nine out of ten animals used and a mean regression coefficient using the pooled data of 0.287 ($P<.001$) was observed. The "b" value using the pooled data was 0.297 which was lower than findings of others (Belyea *et al.*, 1975; Daniel and Moodie, 1979) who

reported, in cows, that plasma Pi fell by approximately 0.4 mmol per fall of 1 mmol in plasma ₁Ca concentration created by the infusion of EDTA or oxalate. This direct relationship observed in equal molar proportions between plasma ₁Ca and Pi during the induction and recovery periods of EDTA infusion can lead to the postulation that the changes in Pi observed may be due to the action of PTH, the secretion of which was stimulated by falling plasma Ca concentrations (Blum *et al.*, 1981). PTH lowers the concentration of plasma Pi either by increasing PO_4 excretion via kidney (Puschett, 1978) or via salivary glands (Ramp and Waite, 1982).

Lack of a significant relationship between plasma Pi and ₁Ca observed in animals fed TRT-A ($P=0.493$) reasonably suggests that NH_4Cl supplementation created alterations either in the amount of PTH secreted in response to the induced hypocalcemia due to changes in parathyroid gland responsiveness or in the responses of target organs (i.e., bone and kidney) to PTH. However, a balance study using the same diet (0.87% NH_4Cl of total DM) showed that there was no change in urinary P excretion compared to CTR (Exp. 1).

A direct correlation between the concentrations of plasma ₁Ca and Mg with the EDTA infusion was also obtained in this study. The regression coefficient and "b" values of 0.418 and 0.310, respectively, using the pooled data were highly significant ($P<.0001$). Those values were different from those of Daniel (1980) who reported 0.34 and 0.054 for regression coefficient and "b" value, respectively. He, however, found no correlation between plasma ₁Ca and Mg

concentrations.

Animals fed TRT-C showed an increased concentration of plasma Mg than TRT-D with EDTA infusion, particularly in the recovery period and both treatments (TRT-C and TRT-D) showed higher bone mobilization rates than CTR. It was shown that animals with hypomagnesemia developed an impaired release of PTH (Anaat *et al.*, 1976), a decrease in end-organ responses to PTH (McManus *et al.*, 1971) and a reduction in Ca mobilization rate (Contreras *et al.*, 1982), whereas hypermagnesemia created from the intravenous infusion of Mg produced a marked increase in circulating PTH levels within in one minute (Rude *et al.*, 1978). The nature of the mechanisms by which Mg deficiency impairs PTH secretion as well as its action on target organs has been thought to involve the adenylase-cyclic-AMP system (Rude and Singer, 1981), which is Mg dependent (Radriquez *et al.*, 1978).

Samson *et al.* (1983) reported with cows, that plasma Mg concentrations of less than 2.07 mg dL^{-1} (0.85 mmol L^{-1}) was related to a high incidence of milk fever and cows suffered from a reduced ability to mobilized Ca from the exchangeable Ca pool in response to the induced hypocalcemia (Contreras *et al.*, 1982). In our study, animals injected with vitamin D_3 (TRT-D) and fed TRT-C had relatively high plasma Mg concentration of 1.82 and 2.21 mg dL^{-1}, respectively, pre-infusion. It is, therefore, conceivable that a resistence to experimentally induced hypocalcemia observed in animals fed TRT-C or with TRT-D may be activated through two different mechanisms. Animals fed TRT-C could mediate the resistance

through high concentrations of plasma Mg and reduced dietary cation-anion balance resulted in not only an increase in circulating PTH level but also an increase in a sensitivity of target organ (i.e., bone) for Ca mobilization with a possible elevation of vitamin D_3 metabolites. Conversly, an increase in circulating vitamin D_3 metabolites, particularly $1,25(OH)_2D_3$, may be the sole mechanism operating in animals on TRT-D to increase bone mobilization with little increase in the action of PTH due to low concentrations of plasma Mg.

The results suggested that action of feeding reduced cation-anion balanced diets used as a preventative method for milk fever (Block, 1984; Leclerc, 1986) was through changes in Ca homeostatic mechanism to increase capacity to mobilize Ca from immediately mobilizable Ca pool (blood Ca., intestinal Ca, and exchangeable bone Ca). The magnitude of this change observed associated with feeding reduced cation-anion balanced diet was comparable to that of vitamin D_3 injection, which is frequently used as a prophylactic method of milk fever (Goff *et al.*, 1987) but may present problems due to resistance to vitamin D_3 (Horst and Reinhardt, 1983) or toxicity (Littledike and Horst, 1982).

V. EFFECTS OF REDUCING DIETARY CATION-ANION BALANCE ON CALCIUM KINETICS IN SHEEP

Abstract

A calcium (Ca) kinetic study with a four-compartment model being fitted to radioisotope and balance data using CONSAM (Conversational, Simulation, Analysis, and Modeling) computer program was conducted to examine effects of dietary cation-anion balance, calculated as meq [(Sodium (Na^+) + Potassium (K^+)) - (Chloride (Cl^-) + Sulfur ($S^=$))]. Twelve crossbred wether lambs were used as eucalcemic (period 1) and simulated lactational Ca loss induced by continuous infusion of EGTA (period 2). Dietary cation-anion balance (meq kg^{-1} DM) was manipulated by supplementation of various mineral salts and were +339, +35, and -127 during period 1 and +429, +68, and -147 during period 2 for control (CTR), treatment (TRT) -A, and -B, respectively. Additional mineral salts used in TRT-A and TRT-B were $CaSO_4$, $Al_2(SO_4)_3$, $MgSO_4$, $CaCl_2$, and $FeSO_4$. Animals responded to simulated lactational Ca loss (period 2) by increasing true intestinal Ca absorption and bone resorption and by reducing bone accretion. No difference was observed in plasma total Ca concentration but TRT-A and -B had increased plasma ionized Ca concentration during both periods. TRT-A and -B showed hypercalciuria during both periods and TRT-B increased true intestinal Ca absorption and reduced bone accretion during period 2. The size of total exchangeable Ca pool (g) and amount of Ca movement between them (g d^{-1}) for CTR, TRT-A, and -B were; 11.3, 95.4; 14.1, 110.5; 12.0, 138.0 during period 1 and 10,8, 117.1; 14.4, 149.9; 11.0, 129.3 during period 2, respectively. Feeding reduced

cation-anion balanced diet increased Ca flux through exchangeable Ca pool with no changes in the size of pool particularly when Ca demand was increased.

INTRODUCTION

The calcium (Ca) homeostatic mechanism operates very tightly to maintain extracellular Ca^{++} within physiological ranges (8-11 mg dL^{-1}). A change in physiological status such as the initiation of lactation creates a rapid disturbance in this mechanism. Plasma Ca exchanges with a larger mass of Ca in soft tissues and bone surfaces, which may function to buffer the effect of such rapid changes in these pools. The size of the exchangeable Ca pool can be determined from kinetic analysis of plasma Ca specific activity after intravenous radioactive-tracer administration (Aubert and Milhaud, 1960).

Dietary cation-anion balance, defined as milliequivalents of [(Sodium (Na^+) + Potassim (K^+))-(Chloride (Cl^-) + Sulfur ($S^=$))], causes changes in Ca mechanism as evidenced in the literature. A negative cation-anion balanced diet has excessive amounts of anions in relation to cations in the diet, thus is considered acidogenic, whereas a positive cation-anion balanced diet is considered alkalogenic in nature. It was suggested that acid-base balance affected by feeding acidogenic diets alters Ca metabolism (Gupta *et al.*, 1970; Girndt *et al.*, 1979; Fredeen, 1984; James and Wohlt, 1985), via bone resorption (Horst and Jorgenson, 1973; Newell and Beaucheme, 1975; Kunkel *et al.*, 1986), intestinal Ca absorption (Braithwaite, 1972; Verdaris and Evans, 1975), and renal handling of Ca (Stacey and Wilson, 1970; Sutton *et al.*, 1979; Bichara *et al.*, 1986). Bacause of its effects on Ca metabo-

lism several studies have conducted and demonstrated that feeding reduced or negative cation-anion balanced diets prepartum to dairy cows decreases the incidence of prepartal hypocalcemia (milk fever) whereas alkalogenic diet increases its incidence (Ender *et al.*, 1971; Dishington, 1975; Dishington and Bjornstad, 1982; Block, 1984). However, effects of manipulating dietary cation-anion balance on Ca kinetics has been studied only in one study (Fredeen *et al.*, 1988b) with goats.

The objective of this experiment was to determine the effect of changing dietary cation-anion balance (achieved by supplementation of various mineral salts) on Ca kinetics using a four-compartment model in sheep during normal and during simulated lactational Ca losses caused by continuous infusion of a 4.5% solution of EGTA.

MATERIALS AND METHODS

Animals

The experiment was conducted with 12 wether lambs (suffolk crossbred) at an average age of 2 years. Animals were placed in individual metabolic cages in a room where temperature was maintained at 20°C and had a photoperiod of 14 h light:10 h dark. Prior to start of the experiment animals were treated for gastrointestinal parasites with Tramisol (Cyanamide, Montreal, Quebec).

Diets

Three dietary treatments; positive (CTR), near zero (TRT-A), and negative (TRT-B) cation-anion balanced diets were formulated by adding various mineral salts to a basal diet composed of corn silage and chopped alfalfa hay. Composition of diets and mineral premixes are presented in Tables 3.1 and 3.2, respectively. Mineral premixes were formulated to meet trace mineral requirements of animals (NRC, 1985) and to deliver excess anions or cations, depending on dietary treatments. All the mineral salts utilized were lab grade. Rations were offered as total mixed rations twice daily at 0800h and 1600h with refusals weighed and discarded before the feeding at 0800h.

Experimental Design

Twelve lambs were assigned to four blocks according their body weight (BW) and within each block they were randomly assigned to one of three diets in a random block design. Each 35-day study consisted of a 14-day initial adjustment period and two 7-day collection period with a 7-day interval between them. From day 8 of the adjustment period and thereafter animals in each block were pair-fed to the level of lowest consumption within the block in order to equalize feed intake between dietary treatments. On day-14 of the adjustment period animals were restrained and both jugular veins were catheterized as described in Exp.2. The initial 7-day collec-

Table 3.1. Feed composition for sheep fed different cation-anion balanced diets[1].

Ingredient	CTR	TRT-A	TRT-B
Alfalfa hay	38.5	38.5	38.5
Corn silage	58.95	57.24	56.5
Mineral mix[2]	2.0	2.0	2.0
$CaCO_3$	0.55	0.27	-
$CaSO_4$	-	0.19	0.50
$Al_2(SO_4)_3 . 18H_2O$	-	0.86	0.86
$MgSO_4 . 7H_2O$	-	0.74	0.74
$CaCl_2 . 2H_2O$	-	0.20	0.30
$FeSO_4 . 7H_2O$	-	-	0.60

[1] CTR=Control; TRT=Treatment.
[2] See table 3.2 for composition.

Table 3.2. Composition of mineral mixtures[1,2,3].

Mineral salt	CTR	TRT-A	TRT-B
	---------- % ----------		
NaCl	53.902	68.037	70.277
$CoCl_2$	0.015	-	-
$CoSO_4$	-	0.018	0.018
$MnCl_2$	0.351	-	-
$MnSO_4$	-	0.3	0.3
ZnO	0.467	-	-
$ZnSO_4$	-	0.926	0.926
KI	0.004	-	-
$HICO_3$	-	0.005	0.005
$CuCl_2$	0.063	-	-
$CuSO_4$	-	0.076	0.076
$CaCO_3$	20.0	-	-
$CaCl_2$	-	29.44	-
$CaSO_4$	-	-	27.20
$FeSO_4.7H_2O$	1.198	1.198	1.198
Na_2CO_3	24.0	-	-

[1] CTR=Control; TRT=Treatment.
[2] Mineral mixtures provide following (ppm) 20 Mn, 50 Fe, 6 Cu, 0.8 Co, 0.6 I, 75 Zn.
[3] Vit A (71250 IU kg^{-1} mix) and vit D (26250 IU kg^{-1} mix) were added.

tion period was designed to establish effects of experimental diets on Ca kinetics during the eucalcemic state. Ca-45 as $^{45}CaCl_2$ in aqueous solution with a specific activity of 10.3 to 12.3 Ci g^{-1} was adjusted so as to contain 80 μCi ml^{-1}. An injection of 5 μCi kg^{-1}BW was administered via one of the jugular vein catheters at 0900h on day 1 of the collection period. The second collection period was identical to the first except that at 24h prior to Ca^{45} injection and thereafter, Ethylene glycol-bis (*B*-amino-ethyl ether) N,N,N',N'-tetra acetic acid (EGTA, Sigma, St-Louis,MO) was infused intravenously at a dose rate of 55 mmol d^{-1}, thereby producing a standardized rate of Ca loss. A 1:1 chelation ratio of EGTA to Ca was assumed (Fox and Heath, 1982) and an amount of EGTA infused was calculated to simulate a lactational Ca loss with a milk production level of 1.3 kg milk d^{-1} and assuming Ca content in milk of 0.17%.

EGTA solution

The jugular vein catheter on one side was used for the infusion of EGTA solution. This solution was made by dissolving 650 g of EGTA initially with 500 ml of NaOH(5N) and 6 L of physiological saline, vortexed over-night then brought up to a 14 L solution after adjusting the pH to 7.4 with 5N NaOH. The final volume was 14.375 L by adding sterilized glass distilled water. Antibacterial agent Trivetrin (Coopers, Willowdale, Ontario) was also added (5 ml L^{-1}). This solution was delivered by a peristaltic pump (Minipuls

2, Level, France) with approximate infusion rate of 19.5 ml h^{-1}. To assure sterility of solution the infusa was passed through a syringe filter (.45 μm Nalge Co., Rochester, NY).

Sample collection

Details of feed, orts, urine, and feces collection have been described in Exp. 1. During the collection period, additional daily samples of urine and feces were obtained for radioactivity measurements prior to the morning feeding.

Blood sampling

Immediately after isotope injection, blood samples (5 to 7 ml) were collected into a heparinized 10 ml syringe according to the following 7-days schedule:

Post-injection time	Frequency of sampling
1-5 min	0.5 min
5-10 min	1 min
10-20 min	2 min
20-30 min	5 min
30-150 min	15 min
3-4 h	30 min
4-11 h	1 h
24h to 7th day	each 3h from 0900h to 2100h

Blood samples were immediately transferred to 10 ml test tube kept on ice bucket and centrifuged at 750 x G for 15

min to collect plasma within 2 h of sampling.

Analyses

Diets

Feed, orts, and feces samples were placed in a forced-air oven for 72 h for dry matter (DM) determination and were ground through a 2 mm screen in a hammer mill. For mineral determination, feed, orts, and feces were first wet-ashed with a mixture of concentrated nitric acids and perchloric acid (10:1). The digested solution were analyzed for Ca, Magnesium (Mg), Na, K, Iron (Fe), and aluminum (Al) by Perkin-Elmer 2830 atomic absorption spectrophotometer (Perkin-Elmer. Norwall, Conneticut) at appropriate dilution rates.

The determination of inorganic S content in feed, orts, feces, urine, and plasma were as decribed in Exp. 1. For Cl measurements, a modified method of potentiometric titration procedure (La Croix *et al.*, 1970) was employed using a Cl ion specific electrode (Orion Model 94-17A). Feed and orts (0.5 g) and feces (2 g) were suspended in 20 ml of 0.1N Nitric acid and vigorously mixed at 10-15 min intervals for at least 1 h; Cl concentration of the suspended solution was determined by the double end point titration method with 0.0282N $AgNO_3$ as the titrant.

Plasma mineral determinations have been described in Exp.1. Ionic Ca was determined in plasma samples by Nova 7 (Nova Biomedical. Waltham, MA) and values were normalized to plasma pH of 7.40.

Radioactivity Measurements

Ground feces samples (1 g) were ashed overnight at 600°C in a furnace (Thermolyne Sybron, Dubuque, IO), and the ash was dissolved in 20% V/V HCl, evaporated to dryness on a hot plate at 100°C then redissolved in 20% V/V HCl, and brought up to a volume of 10 ml with 20% V/V HCl. After being centrifuged at 500 x G for 15 min, the clear supernatent was collected. One ml of the supernatants as well as daily samples of unacidified urine (1 ml) and plasma (1 ml) were dissolved with 9ml of scintillation cocktail (Universal cocktail. ICN Biomedical Inc., Montreal, Quebec) in a 20 ml scintillation vial and radioactivity was measured by liquid scintillation spectrometry (1209 Rackbeta, LKB, Bromma, Sweden) with following specifications;

Isotope	Ca-45
Time	600sec
External standard time	15 sec
External standard counts	900000
Window	5-725
Count1	20000

Quenching curves for plasma, urine, and feces were constructed separately by mixing varied amounts of normal non-radioactive samples with known amounts of Ca^{45} utilized to obtain disintegration per minute (DPM) of samples. Specific activity (SA) of the plasma was then expressed as a fraction of the injected dose per gram of Ca, while excretion of radioactivity in urine and feces were expressed as a cumula-

tive fraction of injected dose.

Kinetic analysis

The four compartment series model of Ca kinetics of Ramberg *et al.* (1970) was adapted for ruminants from a model of normal humans (Neer *et al.*, 1967). The schematic presentation of this model is presented in Figure 3.1. The model consists of a collection of interconnected compartments in a steady state (equilibrium). Four compartments represent the number of exponents required to best describe the plasma SA decay curve over a period of 7 days (Aubert *et al.*, 1963) and is described by the equation:

$$R_{s(t)} = A_1 e^{-a_1 t} + A_2 e^{-a_2 t} + A_3 e^{-a_3 t} + A_4 e^{-a_4 t}$$

where:

$R_{s(t)}$ = Plasma SA at time t,

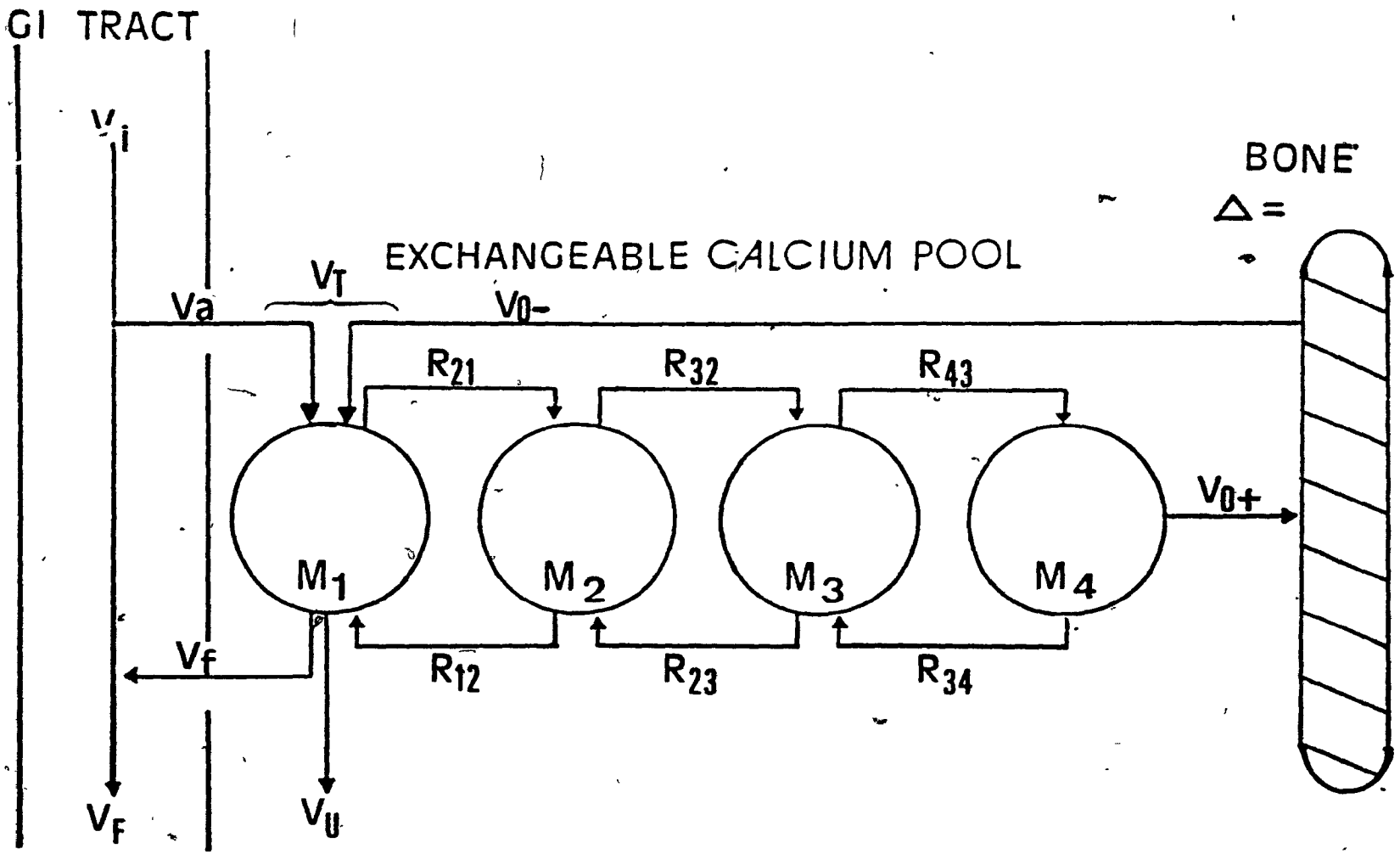
A_1 = "intercept" terms with dimension of concentration of rate constants, and

a_1 to a_4 = the overall distribution and elimination rate constants.

Four individual compartments represent a region or homogenous chemical spaces that have identical transition probabilities (turn-over rate) of exchangeable Ca (Aubert *et al.*, 1963) rather than to represent separate physical entities (i.e. organ). The notation described in Figure 3.1 follows those of Aubert and Milhaud (1960) and Aubert *et al.* (1963);

M = Unit of mass between which various Ca exchanges occur.

Figure 3.1. Scheme of calcium metabolism with the four compartment model. Total exchangeable calcium pool consists of four compartments (M_1, M_2, M_3, and M_4). Inflows of calcium into the system are from the GI tract (Va) and bone (Vo-), and excretory losses go to feces (Vf), urine (Vu), and bone (Vo+). Calcium retention is Δ. Rij represents rate of calium transport Mj to Mi.


GI TRACT
V_i
BONE
$\Delta =$
EXCHANGEABLE CALCIUM POOL
V_T
Va
V_{0-}
R_{21}
R_{32}
R_{43}
M_1
M_2
M_3
M_4
V_{0+}
V_F
R_{12}
R_{23}
R_{34}
V_F
V_U

V = The rate in units of mass per unit time of an exchange process.

Notation for unidirectional processes and for exchange (in g d^{-1}) are as follows:

V_I = Dietary Ca intake,

V_F = Total fecal Ca excretion,

Δ = Ca balance of system,

V_f = Endogenous fecal Ca excretion,

V_u = Urinary Ca excretion,

$V_a = V_I + V_f - V_F$ = The amount of dietary Ca that was absorbed,

V_{0+} = Unidirectional movement of Ca from the system into bone (accretion),

V_{0-} = Movement of unlabelled Ca from bone into the system (resorption),

M_i = The mass of Ca in compartment i,

$M_T = M_1 + M_2 + M_3 + M_4$,

R_{ij} = Rate of stable Ca transport from j to i.

Compartments 1 through 4 represent dilution of isotope in progressively large masses of Ca that together comprise the total exchangeable Ca pool (M_T). Compartment 1 is the site of tracer injection and is sampled via the blood. It was assumed that inflow of Ca from the intestine (V_a) and from bone (V_{0-}) enter compartment 1 and excretory losses of Ca to feces (V_f) and urine (V_u) leave only from compartment 1, whereas compartment 4 is the site of loss of Ca to non-exchanging bone (V_{0+}). Because the system is assumed to be in the steady state, the mass of Ca in any compartment is to remain con-

stant for the duration of the study. Net inflow into the system, therefore, equals net outflow from M_T (Aubert *et al.*, 1963). If M_T is in dynamic equilibrium, then

$$V_{out\,MT} = V_{in\,MT};$$

In other words, changes in M_T must be equal to 0 and since the intestine does not store significant amount of Ca (Aubert *et al.*, 1963) then changes in Ca balance of animals represents the difference between the rates of Ca deposition into and removal from bone rather than alteration in the mass of the labile Ca pool. Net transfer through V_T is equivalent to the reciprocal of the time integral from zero to infinity of the plasma SA decay curve (Ramberg *et al.*, 1976). Thus,

$$V_T = V_{0-} + V_a = V_f + V_u + V_{0+}$$

$$\text{Ca balance} = V_{0+} - V_{0-}$$

The computer program, CONSAM (Conversational, Simulation, Analysis, and Modeling), was used to fit the model to each data set. All sample radioactivities (CPM) were adjusted for decay , background, and quenching and plasma samples were expressed as SA (DPM g^{-1} Ca) as a proportion of injected dose. Cumulative urine and feces radioactivity (DPM) were also expressed as a proportion of injected dose.

Steady state parameters were determined after estimates were entered for proportionality (K_i) and rate ($L_{i,j}$) constants. Then, the program adjusted those initial estimates of the parameters by an iterative process until a least-squares fit of the data was obtained. In other words, a best fit solution was obtained when its residual sums of squares reached a minimum value. The model type used was linear dif-

ferential equations with constant efficiencies (Chu and Berman method)(Chu and Berman, 1974).

The final solution printout included the parameter values ($L_{i,j}$), estimate of their uncertainty and the steady-state solution (M_i and $R_{i,j}$).

Statistical analysis

Dietary treatment of differences in mineral balance, blood parameters, and Ca^{45} kinetic parameters were evaluated by analysis of variance for a random block design (Steer and Torrie , 1980) and Scheffe's Test was utilized for comparison purpose between the treatments. Comparison between eucalcemic and EGTA infused sheep was evaluated by "t" test for a paired observation (Steer and Torrie, 1980). All statistical analysis were carried out on the Statistical Analysis System (SAS institute Inc., Cary, NC).

RESULTS

Diets and intake

Mineral composition of diets is shown in Table 3.3. Dietary Ca level (0.88 to 1.05% DM) and Ca to phosphorus (P) ratio (2.93 to 3.39) were above requirements (0.42% and 2.0, respectively)(NRC 1985). Cation-anion balance of diets for CTR, TAT-A, and TRT-B, respectively, were +339, +35, and -127 during the eucalcemic period (period 1) and +429, +68,

Table 3.3. Mineral composition of diets[1,2].

Mineral	Eucalcemic period			EGTA-infusion period[3]		
	CTR	TRT-A	TRT-B	CTR	TRT-A	TRT-B
Calcium (%)	0.99±.08	0.95±.07	0.88±.10	1.05±.07	0.95±.10	0.94±.10
Magnesium (%)	0.21±.01	0.26±.03	0.25±.02	0.19±.01	0.24±.03	0.26±.01
Phosphorus (%)	0.30±.01	0.30±.01	0.30±.01	0.31±.01	0.29±.01	0.30±.01
Sodium (%)	0.66±.08	0.63±.14	0.56±.13	0.78±.15	0.62±.12	0.61±.03
Potassium (%)	1.62±.05	1.58±.04	1.63±.09	1.70±.08	1.65±.07	1.68±.08
Chloride (%)	0.85±.03	1.31±.18	1.44±.16	0.88±.08	1.24±.14	1.41±.09
Sulfur (%)	0.19±.05	0.43±.06	0.61±.09	0.15±.01	0.44±.07	0.71±.07
Iron (ppm)	411±127	405±108	1278±172	371±203	257±32	1454±143
Aluminum (ppm)	359±108	1164±284	856±130	114±59	1075±296	1128±61
Cation-anion balance[4] (meq kg^{-1} DM)	338±19	35±17	-127±20	429±23	68±18	-147±29

[1] Values are presented as mean±standard error.
[2] CTR=Control; TRT=Treatment.
[3] EGTA-infusion used to simulate lactational calcium loss in blood.
[4] Calculated as meq $[Na^+ + K^+] - [Cl^- + S^=]$.

and -147 during the EGTA-infusion period (period 2), respectively.

Body weight (BW), feed intake, feces and urine volume, and daily cation-anion balance intake are presented in Table 3.4. No differences ($P>.1$) were observed in feed intake due to the pair-feeding regimen, however, differences ($P<.01$) were observed between blocks. Feed intake (g d^{-1} kg^{-1} $BW^{.75}$) of block 1 to 4 were 66.1, 53.7, 67.9, and 82.5 in period 1 and 60.2, 61.7, 78.8, and 84.3 in period 2, respectively. Urine volume was increased ($P<.05$) by EGTA-infusion. Dietary cation-anion balance intake (meq d^{-1}) were +375, +37, and -136 in period 1 and +493, +79, and -210 in period 2 for CTR, TRT-A, and TRT-B, respectively.

Plasma minerals

Infusion rate of EGTA and calculated Ca chelation rates are shown in Table 3.5. Rates of infusion were measured prior to infusion into animals. Loss of Ca from the exchangeable Ca pool was calculated to be approximately 55 mmol d^{-1}, which was equivalent to a milk production of 1.3 kg d^{-1} (assuming a milk Ca concentration of 0.17%).

Blood mineral concentrations are shown in Table 3.6. A mean plasma total Ca level was calculated using approximately 70 samples collected over the 7-day collection period. Plasma Ca concentration did not differ ($P>.1$) between treatments, however, EGTA infusion increased ($P<.01$) concentration of plasma Ca. The ionic form of plasma Ca (Ca^{++}) differed be-

Table 3.4. Body weight, feed intake, excreta volume, and daily dietary cation-anion balance intake by sheep fed different cation-anion balanced diets during the eucalcemic and EGTA-infusion periods[1].

	Eucalcemic period				EGTA infusion period				Significance[2]
	CTR	TRT-A	TRT-B	SEM	CTR	TRT-A	TRT-B	SEM	Period
Initial body weight (kg)	38.3	41.5	41.6	1.43	38.9	42.6	42.9	1.17	NS
Feed intake (kg d^{-1})	1.11	1.10	1.09	0.06	1.13	1.16	1.20	0.09	NS
(g d^{-1} $kg^{-1}BW^{.75}$)	71.9	67.2	65.6	3.2	72.4	69.1	70.0	4.6	NS
Feces excretion (kg d^{-1})	0.42	0.39	0.40	0.02	0.42	0.43	0.44	0.03	NS
Urine excretion (L d^{-1})	1.17	1.10	1.35	0.13	1.46	1.54	1.75	0.13	**
Anion-cation balance (meq d^{-1})	375.7[a]	37.2[b]	-136.2[c]	40.7	493.5[a]	78.6[b]	-209.7[c]	13.2	NS

[1] CTR=Control; TRT=Treatment; SEM=Standard error of mean.
[2] NS=Not significant (P>.05); ** Significant (P<.05).
[abc] Means with different superscripts are different (P<.05) within each periods.

Table 3.5. Rate and volume of EGTA solution infused into sheep fed different cation-anion balanced diets[1,2].

Sheep #	Diet	Infused volume (ml h^{-1})	Duration (h)	Chelation rate[3] (mmol Ca d^{-1})	(mmol d^{-1} kg^{-1} BW)	Simulated milk production (ml d^{-1})[4]
1	CTR	19.45	192	55.55	1.34	1307
2	TRT-A	19.54	192	55.81	1.34	1313
3	TRT-B	19.84	192	56.66	1.66	1333
4	CTR	19.45	193	55.55	1.76	1307
5	TRT-B	19.84	193	56.66	1.25	1333
6	TRT-A	19.54	193	55.81	1.34	1313
7	CTR	19.00	192	54.26	1.33	1277
8	TRT-B	19.36	192	55.29	1.38	1301
9	TRT-A	19.74	192	56.38	1.43	1327
10	CTR	19.00	192	54.26	1.29	1277
11	TRT-B	19.33	192	55.21	1.27	1299
12	TRT-A	19.03	192	54.35	0.96	1279

[1] EGTA concentration was .119mmol (4.5% EGTA).
[2] CTR=Control; TRT=Treatment.
[3] Assuming a 1:1 chelation ratio (EGTA:calcium).
[4] Milk production was calculated based on milk calcium level of .17%.

Table 3.6. Blood parameters of sheep fed different cation-anion balanced diets during the eucalcemic and EGTA-infusion periods[1].

Parameter	Eucalcemic period				EGTA infusion period				Significance[2]
	CTR	TRT-A	TRT-B	SEM	CTR	TRT-A	TRT-B	SEM	period
Total Calcium (mg dL^{-1})	8.81	8.73	8.52	0.15	9.64	9.77	9.36	0.20	***
Ionic Calcium (mg dL^{-1})	3.3[a]	4.08[b]	3.84[b]	0.16	3.32[a]	3.96[b]	4.20[b]	0.11	NS
Total/Ionic Ca (%)	38.8[a]	46.6[b]	44.6[b]	1.61	35.5[a]	40.8[a b]	44.8[b]	1.87	*
Magnesium (mg dL^{-1})	3.9	3.6	3.4	0.12	3.7	3.7	3.7	0.07	NS
Phosphorus (mg dL^{-1})	4.7[a]	4.2[a b]	3.9[b]	0.19	5.2[a]	3.9[b]	4.3[b]	0.24	NS
Sodium (meq L^{-1})	150.4	138.9	144.2	2.72	143.3	143.1	150.1	1.74	NS
Potassium (meq L^{-1})	4.9	5.2	4.6	0.12	5.0	5.5	4.9	0.12	NS
Chloride (meq L^{-1})	109.5	108.7	106.7	2.08	108.6	106.8	107.8	0.83	NS
Sulfate (mmol L^{-1})	-2.1	2.7	3.0	0.49	2.9	3.0	3.2	0.26	NS

[1] CTR=Control; TRT=Treatment.
[2] NS=Not significant (P>.1); * Significant (P<.1); *** Significant (P<.01).
[a b] Means with different superscripts are different (P<.05) within each periods.

tween treatments; sheep fed reduced or negative cation-anion balanced diets (TRT-A and TRT-B) had increased Ca^{++} ($P<.01$) than CTR during both periods and this is reflected in the ratio of Ca^{++} to total Ca. Figures 3.2 and 3.3 show plasma Ca concentration during the 7-day collection periods of the eucalcemic (period 1) and EGTA infused period (period 2), respectively. Plasma Ca concentration of individual animals (block 4) rather than a mean value was chosen to create the figures because of differences ($P<.01$) in feed intake between blocks. A large fluctuation was observed during the early part of the collection phase (first 2 h), which may reflect the consequences of the frequent blood sampling regimen. The figures also illustrate that there was fluctuation in plasma Ca level during the entire collection period.

Plasma inorganic P concentration was the only other mineral that was affected by dietary treatments with that of CTR being greater ($P<.01$) than TRT-B during the eucalcemic period and greater than both TRT-A and TRT-B during the EGTA-infusion period. In addition, plasma P level was positively correlated with daily cation-anion balance intake ($r=0.52$, $P=0.009$).

Radiotracer analysis

Data on plasma SA and cumulative excretion of isotope in urine and feces of sheep 19 (CTR), 21 (TRT-A), and 20 (TRT-B) during the eucalcemic and EGTA-infusion periods are shown in Figures 3.4, 3.5, 3.6, and 3.7. As described in the materials

Figure 3.2. Changes in concentrations of calcium in plasma of eucalcemic sheep fed CTR (C), TRT-A (A), and TRT-B (B) during the 7-day collection period.

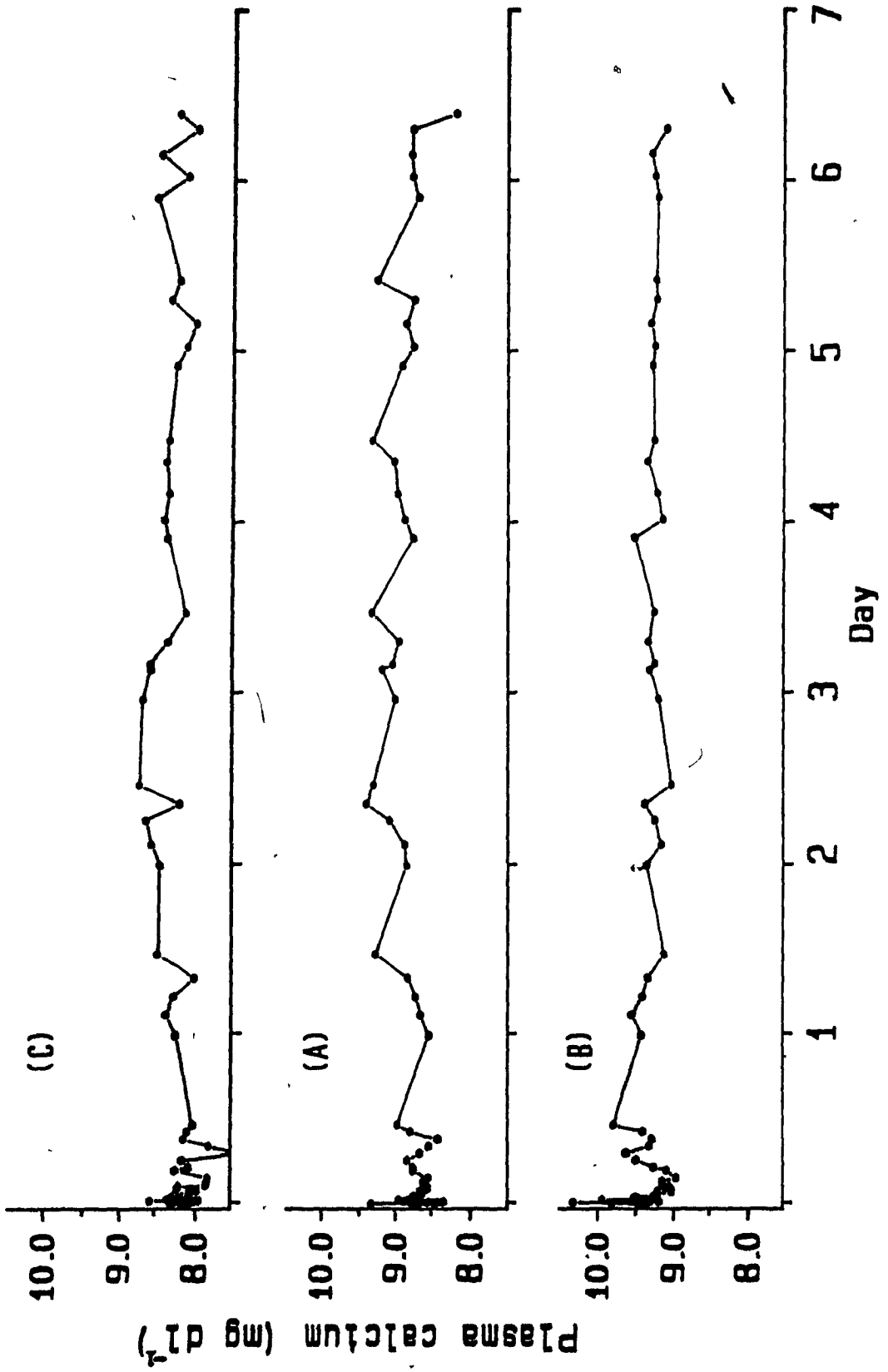

Plasma calcium (mg dl⁻¹)
10.0
9.0
8.0
(C)
(A)
(B)
1
2
3
4
5
6
7
Day

Figure 3.3. Changes in concentrations of calcium in plasma of EGTA-infusion sheep fed CTR (C), TRT-A (A), and TRT-B (B) during the 7-day collection period.

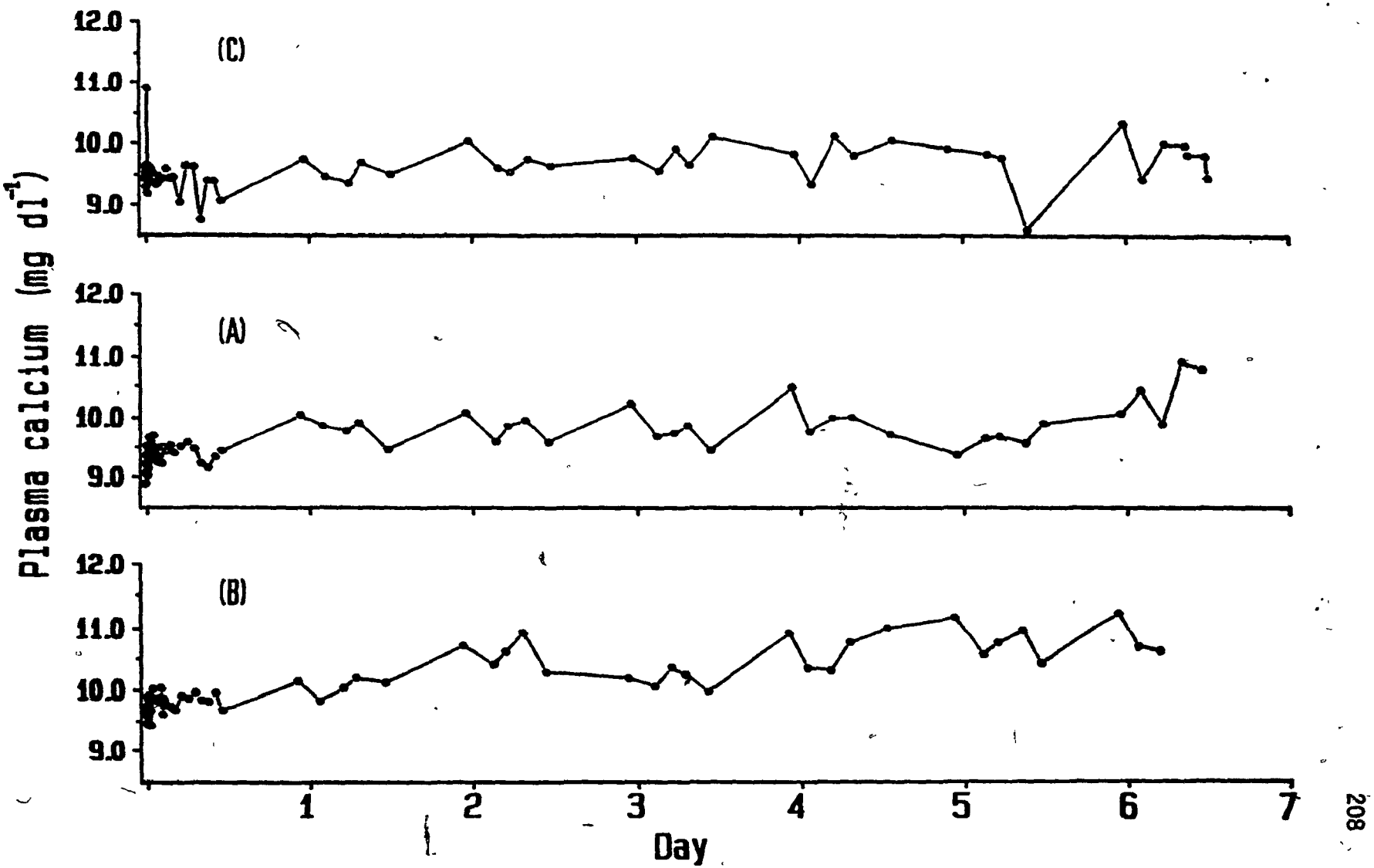

(C)
(A)
(B)
12.0
11.0
10.0
9.0
Plasma calcium (mg dl^{-1})
1
2
3
4
5
6
7
Day

Figure 3.4. Plasma specific activity (SA) of eucalcemic sheep fed different cation-anion balanced diets (○ =CTR, △ =TRT-A, □ =TRT-B).

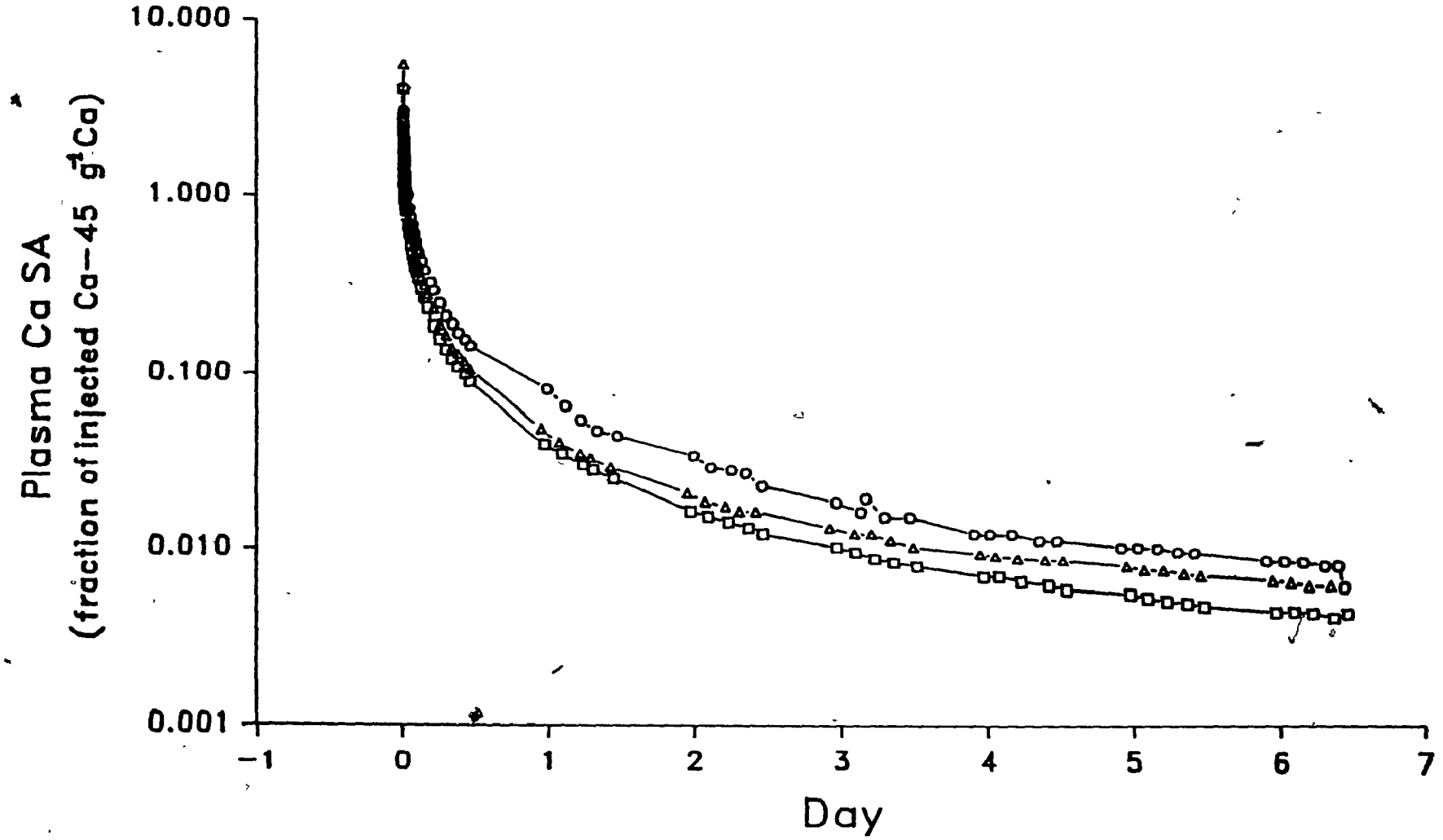

10.000
1.000
0.100
0.010
0.001
Plasma Ca SA
(fraction of injected Ca—45 g⁻¹Ca)
−1
0
1
2
3
4
5
6
7
Day

Figure 3.5. Plasma specific activity (SA) of EGTA-infused sheep fed different cation-anion balanced diets (O =CTR, Δ=TRT-A, □=TRT-B).

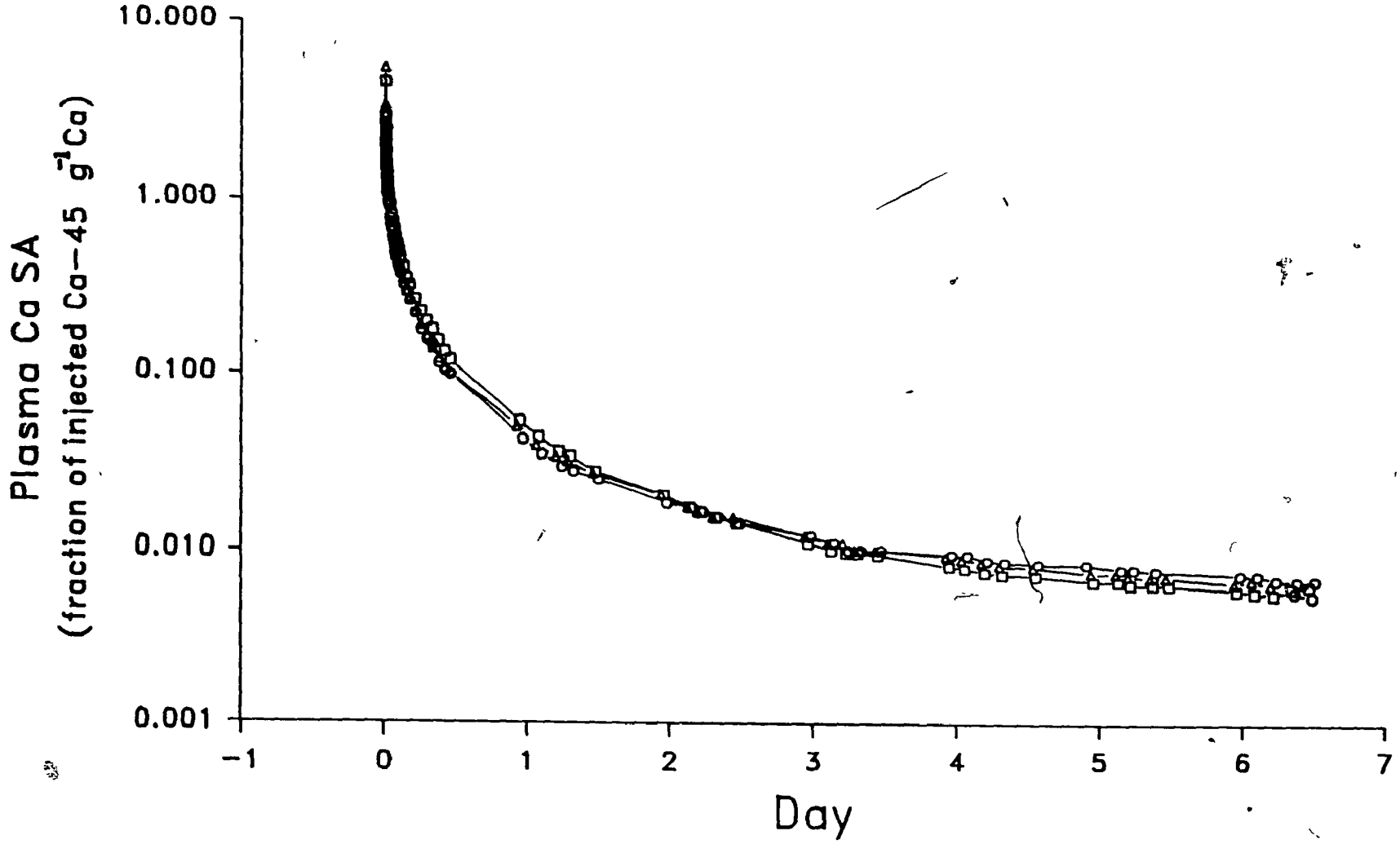

Plasma Ca SA
(fraction of injected Ca-45 g^{-1}Ca)
10.000
1.000
0.100
0.010
0.001
−1
0
1
2
3
4
5
6
7
Day

Figure 3.6. Cumulative isotope excretion in urine of eucalcemic sheep (○ =CTR, △ =TRT-A, □ =TRT-B) or EGTA-infused sheep (● =CTR, ▲ =TRT-A, ■ =TRT-B) fed different cation-anion balanced diets.

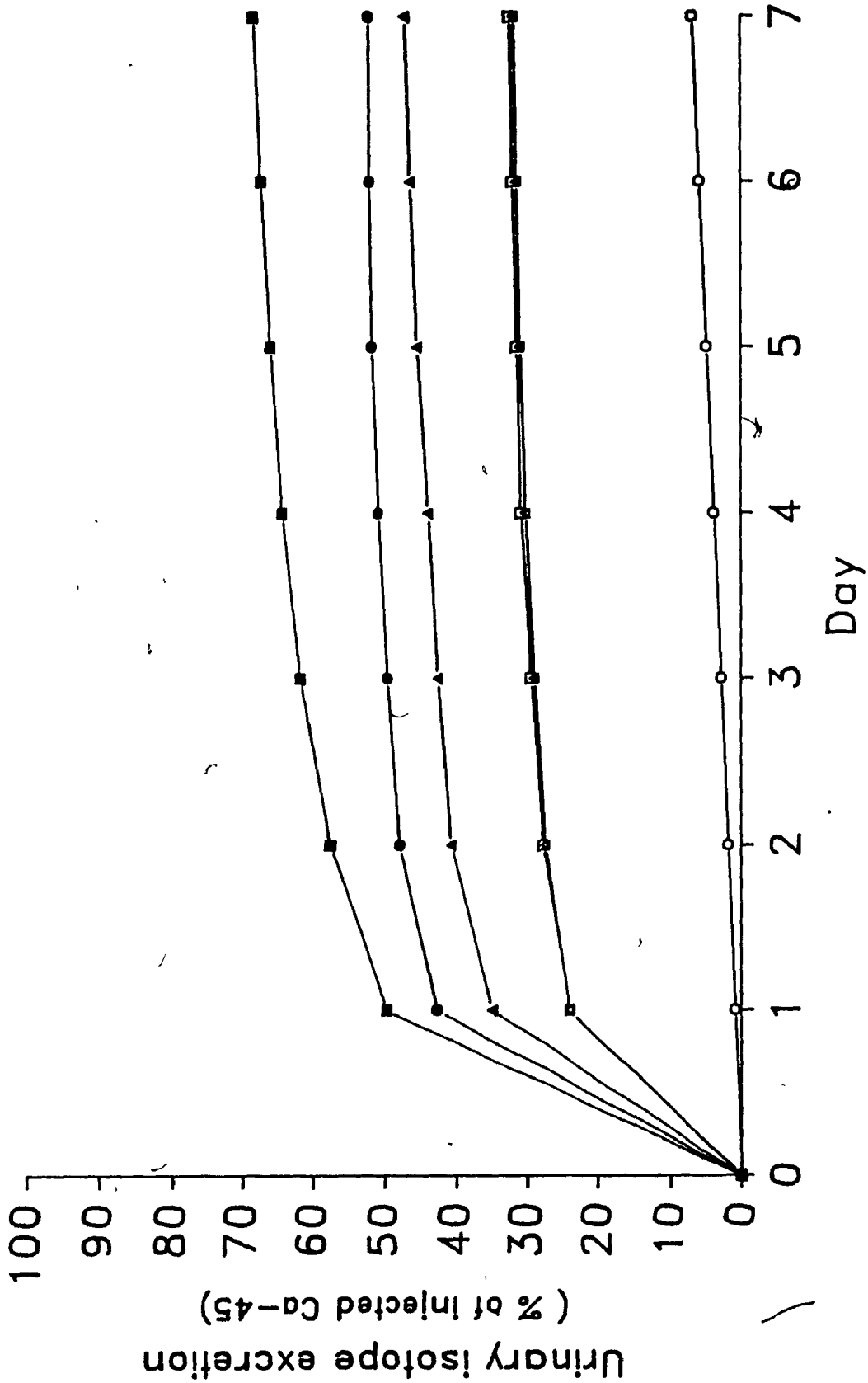
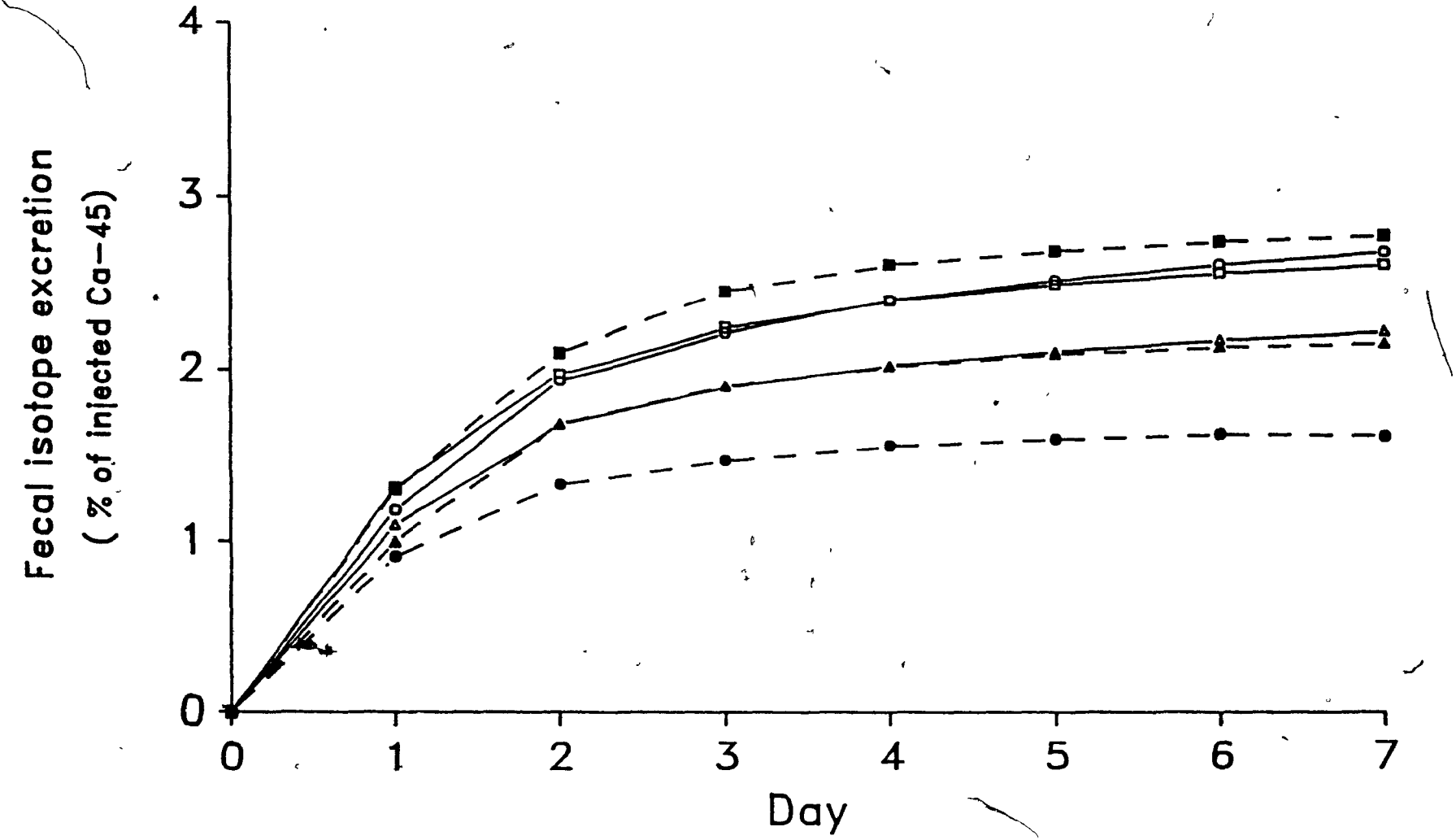

Urinary isotope excretion
(% of injected Ca-45)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Day

Figure 3.7. Cumulative isotope excretion in feces of eucalcemic sheep (○=CTR, △=TRT-A, □=TRT-B) or EGTA-infused sheep (●=CTR, ▲=TRT-A, ■=TRT-B) fed different cation-anion balanced diets.


Fecal isotope excretion
(% of injected Ca-45)
4
3
2
1
0
0
1
2
3
4
5
6
7
Day

and methods section, four exponential terms were used to fit the entire curve of plasma SA (expressed as a fraction of injected dose). Plasma SA declined rapidly after the injection of Ca^{45}, then plateaued.

Both TRT-A and TRT-B increased urinary excretion of isotope greatly compared to that of CTR during the eucalcemic period. However, this difference became smaller during the EGTA-infusion period because urinary isotope excretion during this period represented a combination of endogenous urinary Ca and EGTA- chelated Ca which represents the simulated lactational Ca loss. Total isotope excretion (urine and feces) of CTR during the eucalcemic and EGTA-infusion periods were comparable to those of non-lactating and lactating cows, respectively (Ramberg *et al.*, 1970).

The early portions of the cumulative fecal excretion curves (first 2 d) appeared to be affected by a delay in the appearance of isotope excreted, therefore, only the latter portion (day 3 to 7) of data points were utilized in the fitting process since this would minimized the effect of the lag time without resorting to a correction in the computer model for intestinal transit time.

Rate and proportionality constants for CTR, TRT-A, and TRT-B are presented in Tables 3.7, 3.8, and 3.9 for the eucalcemic period and in Tables 3.10, 3.11, and 3.12 for the EGTA-infusion period, respectively. Because the physiological state of animals was changed experimentally (i.e. normal or simulated lactation), it was necessary to change the parameters representing exchanges between the compartment

Table 3.7. Rate constant ($L_{4\,3}$) and proportionality constant (K_1) of calcium metabolism in eucalcemic sheep fed CTR.

Constant		Sheep # 1	4	7	10	Mean	SEM
$L_{2\,1}$	d^{-1}	62.2	210.6	186.0	136.1	148.7	32.74
$L_{1\,2}$	d^{-1}	38.7	92.1	86.1	85.8	75.6	12.40
$L_{3\,2}$	d^{-1}	10.1	14.8	16.8	17.2	14.8	1.63
$L_{2\,3}$	d^{-1}	2.96	2.35	3.77	2.46	2.89	0.32
$L_{4\,3}$	d^{-1}	2.60	1.49	1.89	1.72	1.93	0.24
$L_{3\,4}$	d^{-1}	0.248	0.120	0.201	0.148	0.179	0.028
$L_{0\,4}$	d^{-1}	0.346	0.210	0.273	0.219	0.262	0.031
L_{u}	d^{-1}	0.200	0.098	0.159	0.046	0.126	0.034
L_{f}	d^{-1}	2.64	3.67	2.44	2.29	2.76	0.31
K_1	d^{-1}	1.95	5.79	3.99	3.01	3.69	0.81

SEM Standard error of mean.

Table 3.8. Rate constant (L_{ij}) and proportionality constant (K_1) of calcium metabolism in eucalcemic sheep fed TRT-A.

Constant		Sheep # 2	6	9	12	Mean	SEM
L_{21}	d^{-1}	100.3	119.0	125.8	256.1	150.3	35.67
L_{12}	d^{-1}	64.1	53.9	73.8	149.5	85.3	21.76
L_{32}	d^{-1}	13.7	10.5	14.2	19.0	14.3	1.75
L_{23}	d^{-1}	3.03	1.50	2.53	3.10	2.54	0.37
L_{43}	d^{-1}	1.73	1.02	1.52	2.00	1.57	0.21
L_{34}	d^{-1}	0.227	0.094	0.186	0.229	0.184	0.032
L_{0+}	d^{-1}	0.322	0.122	0.213	0.201	0.214	0.041
L_{u}	d^{-1}	0.392	0.846	2.29	4.29	1.95	0.88
L_{f}	d^{-1}	2.13	2.23	2.61	2.87	2.45	0.17
K_1	d^{-1}	3.58	2.69	3.15	3.42	3.21	0.19

SEM Standard error of mean.

Table 3.9. Rate constant ($L_{i,j}$) and proportionality constant (K_1) of calcium metabolism in eucalcemic sheep fed TRT-B.

Constant		Sheep # 3	5	8	11	Mean[a]	SEM
$L_{2,1}$	d^{-1}	184.2	163.3	173.3	276.7	208.0	34.9
$L_{1,2}$	d^{-1}	118.3	57.5	115.7	128.9	101.6	22.2
$L_{3,2}$	d^{-1}	18.5	12.5	18.5	16.0	15.7	1.75
$L_{2,3}$	d^{-1}	3.53	2.56	3.20	2.78	2.96	0.29
$L_{4,3}$	d^{-1}	1.88	1.77	1.91	1.77	1.81	0.04
$L_{3,4}$	d^{-1}	0.215	0.210	0.201	0.199	0.208	0.005
$L_{0,+}$	d^{-1}	0.234	0.302	0.275	0.221	0.253	0.025
L_u	d^{-1}	2.12	0.588	0.021	4.85	2.52	1.24
L_f	d^{-1}	0.305	2.03	1.88	3.77	2.03	0.99
K_1	d^{-1}	4.09	3.68	3.42	3.15	3.64	0.27

[a] Sheep 8 excluded before calculation of means because of illness.
SEM Standard error of mean.

Table 3.10. Rate constant (L_{ij}) and proportionality constant (K_1) of calcium metabolism in EGTA infused sheep fed CTR.

Constant		Sheep # 1	4	7	10	Mean	SEM
L_{21}	d^{-1}	210.5	206.9	255.9	92.6	191.5	34.80
L_{12}	d^{-1}	118.8	108.3	103.8	71.9	100.7	10.08
L_{32}	d^{-1}	14.7	16.9	15.2	15.2	15.49	0.47
L_{23}	d^{-1}	2.93	4.32	3.69	2.99	3.49	0.33
L_{43}	d^{-1}	1.44	2.08	1.51	1.63	1.67	0.14
L_{34}	d^{-1}	0.216	0.292	0.319	0.253	0.270	0.022
L_{0+}	d^{-1}	0.122	0.165	0.145	0.104	0.134	0.013
L_u	d^{-1}	5.46	6.54	8.30	5.09	6.35	0.72
L_f	d^{-1}	1.53	2.90	2.68	1.51	2.16	0.37
K_1	d^{-1}	3.84	4.70	4.36	2.22	3.78	0.55

SEM Standard error of mean.

Table 3.11. Rate constant (L_{ij}) and proportionality constant (K_1) of calcium metabolism in EGTA infused sheep fed TRT-A.

Constant		Sheep # 2	6	9	12	Mean	SEM
L_{21}	d^{-1}	262.0	221.6	125.6	313.9	230.8	39.8
L_{12}	d^{-1}	124.2	122.0	70.4	129.8	111.6	13.8
L_{32}	d^{-1}	20.55	19.42	13.47	16.44	17.47	1.59
L_{23}	d^{-1}	4.24	3.69	2.73	3.44	3.53	0.31
L_{43}	d^{-1}	1.79	1.98	1.43	1.76	1.74	0.11
L_{34}	d^{-1}	0.231	0.258	0.208	0.235	0.233	0.010
L_{04}	d^{-1}	0.096	0.177	0.106	0.163	0.136	0.020
L_{u}	d^{-1}	4.48	5.27	5.57	8.00	5.83	0.76
L_{f}	d^{-1}	1.52	2.24	4.63	3.53	2.98	0.69
K_1	d^{-1}	4.48	2.95	2.76	4.17	3.59	0.43

SEM Standard error of mean.

Table 3.12. Rate constant (L_{ij}) and proportionality constant (K_1) of calcium metabolism in EGTA infused sheep fed TRT-B.

Constant		Sheep # 3	5	8	11	Mean	SEM
L_{21}	d^{-1}	351.7	162.4	220.3	119.1	213.4	50.5
L_{12}	d^{-1}	153.7	70.1	122.1	89.7	108.9	18.4
L_{32}	d^{-1}	16.5	13.3	12.5	12.1	13.6	1.01
L_{23}	d^{-1}	4.17	2.57	2.62	2.86	3.06	0.38
L_{43}	d^{-1}	1.62	1.69	1.56	1.21	1.52	0.11
L_{34}	d^{-1}	0.302	0.234	0.225	0.214	0.244	0.020
L_{04}	d^{-1}	0.154	0.167	0.192	0.099	0.153	0.019
L_{u}	d^{-1}	5.51	4.31	4.99	6.74	5.39	0.51
L_{f}	d^{-1}	2.16	1.95	2.27	2.71	2.27	0.16
K_1	d^{-1}	4.84	2.98	3.72	2.74	3.57	0.47

SEM Standard error of mean.

($L_{i,j}$) from one study to the next, even though two isotope studies were carried out in the same animals within a short period of time (2 weeks). In addition, the parameters representing loss of isotope from the system to the excreta (L_i =intestine and L_u =urine) and to bone ($L_{0,*}$) were changed in order to avoid inconsistencies in fitting calculated model to actual data. Proportionality constant is the equivalent to the calculated SA of plasma Ca at zero time, in other word, it is the reciprocal of distribution in compartment 1.

Data from sheep 8 during the eucalcemic period was excluded before calculating a mean value because coprostasis developed during the last three days of the collection period.

Probability of differences between means is presented in Table 3.13. During the eucalcemic period, L_u (urinary Ca excretion) was the only parameter that showed difference ($P<.1$) between dietary treatments; TRT-B (2.52 d^{-1}) was greater than CTR (0.13 d^{-1}). Similarly, $L_{3,2}$ (rate of Ca transport from compartment 2 to 3) was the only difference ($P<.1$) found during the EGTA-infusion period; TRT-A (17.5 d^{-1}) was greater than TRT-B (13.6 d^{-1}). Table 3.14 shows the mean and probability of differences observed between the eucalcemic and EGTA-infusion periods. EGTA-infused lambs had greater $L_{3,4}$ (rate of Ca transport from compartment 4 to 3)(0.249 d^{-1}) and L_u (5.85 d^{-1}) values than those of eucalcemic lambs (0.189 d^{-1} and 1.44 d^{-1}, respectively). In general, rate and proportionality constants were greater during the EGTA-infusion than the eucalcemic period.

Table 3.13. Probability of dietary treatment and EGTA infusion differences in mean rate constant and proportionality constants of sheep.

Constnant		Dietary treatment		
		Eucalcemic	EGTA infusion	Infusion
$L_{2\,1}$	d^{-1}	.423	.826	.137
$L_{1\,2}$	d^{-1}	.634	.862	.122
$L_{3\,2}$	d^{-1}	.802	.067	.590
$L_{2\,3}$	d^{-1}	.403	.607	.590
$L_{4\,3}$	d^{-1}	.393	.321	.378
$L_{3\,4}$	d^{-1}	.644	.494	.004
L_{0+}	d^{-1}	.567	.706	.0003
L_u	d^{-1}	.092	.646	.0001
L_f	d^{-1}	.649	.395	.962
K_1	d^{-1}	.833	.946	.710

Steady state parameter size and exchange rates between compartments for the eucalcemic period are shown in Tables 3.14, 3.15, and 3.16 and for the EGTA-infusion period in Tables 3.17, 3.18, and 3.19 for CTR, TRT-A, and TRT-B, respectively. Compartments (M_i) collectively comprise the total exchangeable Ca pool (M_T). Exchange rates and mass Ca movements (g d^{-1}) are between compartments M_j from M_i ($R_{i,j}$), fluxes from M_T to intestine (V_ℓ), urine (V_u), and bone (V_{0+}) or influxes from intestine (V_a) and bone (V_{0-}). The latter two influxes collectively comprise total entry rate (V_T) into M_1.

Figures 3.8, 3.9, and 3.10 represent mean compartment sizes and exchange rates between compartments for sheep fed CTR, TRT-A, and TRT-B, respectively, during the eucalcemic period and Figures 3.11, 3.12, and 3.13 show the same parameters during the EGTA-infusion period. Probability of differences between means are presented in Table 3.20. Intake of Ca, ranged from 7.6 to 16.7 g d^{-1} and varied between blocks since the paired feeding regimen was carried out only within each block. There was, however, no difference ($P>.1$) in mean Ca intake between the dietary treatments. Regardless of dietary treatment all sheep were in positive Ca balance (Δ) in both period except sheep 2 fed CTR during the EGTA-infusion period (-0.37 g d^{-1}). The total Ca inflow into the exchangeable pool (V_T) was comprised entirely of intestinal absorption (V_a) during both periods.

Only urinary Ca excretion (V_u, % of intake) was affected by dietary treatments during both periods in that both TRT-A

Table 3.14. Compartment masses and rates of calcium transport in eucalcemic sheep fed CTR.

Parameter	Sheep # 1	4	7	10	Mean	SEM
Calcium intake (g d^{-1})	12.0	9.1	10.6	15.9	11.9	1.45
V_a, (g d^{-1})	2.96	2.73	3.37	7.36	4.10	1.09
(% intake)	24.6	30.1	31.7	42.4	33.2	4.66
V_{0+}, (g d^{-1})	2.58	1.59	1.99	2.54	2.18	0.24
(% intake)	21.4	17.6	18.8	16.0	18.4	1.14
V_{0-}, (g d^{-1})	1.01	-0.50	-0.74	-4.04	-1.07	1.06
(% intake)	8.40	-5.55	-6.97	-25.49	-7.41	6.96
Balance (g d^{-1})	1.56	2.10	2.73	6.58	3.25	1.14
V_T, (g d^{-1})	3.97	2.23	2.63	3.32	3.04	0.17
V_F, (g d^{-1})	10.4	6.97	7.87	9.26	8.63	0.76
V_f, (g d^{-1})	1.35	0.62	0.61	0.76	0.84	0.17
(% intake)	11.23	6.88	5.75	4.80	7.39	5.85
a, (%)	24.6	30.1	31.7	46.4	33.2	4.66
V_u, (g d^{-1})	0.04	0.009	0.021	0.011	0.021	0.007
(% intake)	1.16	0.08	0.19	0.07	0.18	0.06
M_1, (g)	0.51	0.17	0.25	0.33	0.32	0.072
M_2, (g)	0.75	0.37	0.52	0.49	0.53	0.078
M_3, (g)	1.70	1.71	1.80	2.43	1.91	0.176
M_4, (g)	7.44	8.26	7.16	11.16	8.50	0.92
M_T, (g)	10.4	10.5	9.73	14.4	11.27	1.07
R_{21}, (g $^{-1}$d)	31.6	35.9	46.6	44.6	39.7	3.55
R_{12}, (g d^{-1})	29.0	34.4	44.6	42.0	37.5	3.58
R_{32}, (g d^{-1})	7.60	5.63	8.74	8.60	7.64	0.72
R_{23}, (g d^{-1})	5.02	4.04	6.78	6.06	5.48	0.60
R_{43}, (g d^{-1})	4.43	2.51	3.40	4.25	3.65	0.44
R_{34}, (g d^{-1})	1.85	0.92	1.44	1.71	1.48	0.21

SEM Standard error of mean.

Table 3.15. Compartment masses and rates of calcium transport in eucalcemic sheep fed TRT-A.

Parameter	Sheep # 2	6	9	12	Mean	SEM
Calcium intake ($g\ d^{-1}$)	10.7	11.4	9.23	14.0	11.4	1.00
V_a, ($g\ d^{-1}$)	4.48	3.02	2.58	5.13	3.86	0.60
(% intake)	41.8	26.3	27.9	36.6	33.16	3.65
V_{o+}, ($g\ d^{-1}$)	1.43	2.23	1.78	2.14	1.90	0.18
(% intake)	13.3	19.4	19.3	15.3	16.8	1.51
V_{o-}, ($g\ d^{-1}$)	-2.41	0.348	0.577	-1.03	-0.63	0.693
(% intake)	-22.5	3.03	6.24	-7.38	-5.16	6.48
Balance ($g\ d^{-1}$)	3.84	1.88	1.21	3.18	2.53	0.60
V_T, ($g\ d^{-1}$)	2.06	3.36	3.16	4.09	3.17	0.42
V_F, ($g\ d^{-1}$)	6.83	9.27	7.48	9.74	8.33	0.70
V_f, ($g\ d^{-1}$)	0.59	0.83	0.93	0.84	0.77	0.06
(% intake)	5.54	7.24	8.96	5.99	6.93	0.77
a_1 (%)	41.8	26.32	27.9	36.5	33.16	3.65
V_u, ($g\ d^{-1}$)	0.043	0.306	0.550	1.11	0.502	0.227
(% intake)	0.40	2.67	5.95	7.90	4.23	1.67
M_1, (g)	0.28	0.37	0.32	0.29	0.31	0.02
M_2, (g)	0.44	0.75	0.52	0.49	0.55	0.07
M_3, (g)	1.47	3.81	2.19	2.30	2.44	0.49
M_4, (g)	4.62	19.7	8.37	10.7	10.84	3.2
M_T, (g)	6.81	24.6	11.4	13.8	14.14	3.78
R_{21}, ($g\ d^{-1}$)	26.6	46.1	39.9	75.1	46.9	10.2
R_{12}, ($g\ d^{-1}$)	25.2	43.9	38.1	72.9	45.0	10.1
R_{32}, ($g\ d^{-1}$)	5.49	8.35	7.32	9.24	7.60	0.80
R_{23}, ($g\ d^{-1}$)	4.07	6.12	5.54	7.10	5.70	0.63
R_{43}, ($g\ d^{-1}$)	2.42	4.01	3.34	4.59	3.59	0.47
R_{34}, ($g\ d^{-1}$)	0.99	1.78	1.56	2.45	1.69	0.30

SEM Standard error of mean.

Table 3.16. Compartment masses and rates of calcium transport in eucalcemic sheep fed TRT-B.

Parameter	Sheep # 3	5	8	11	Mean[a]	SEM
Calcium intake ($g\ d^{-1}$)	8.27	12.5	11.8	13.6	11.5	1.64
V_a, ($g\ d^{-1}$)	1.51	6.14	8.68	2.90	3.52	1.37
(% intake)	18.3	49.1	73.4	21.2	29.6	9.83
V_{0+}, ($g\ d^{-1}$)	1.49	2.63	2.00	2.65	2.26	0.38
(% intake)	18.0	21.1	16.9	19.44	19.52	0.87
V_{0-}, ($g\ d^{-1}$)	1.16	-2.93	-6.08	2.33	0.19	1.59
(% intake)	14.0	-23.45	-51.5	17.06	2.54	13.02
Balance ($g\ d^{-1}$)	0.33	5.56	8.09	0.33	2.07	1.75
V_T, ($g\ d^{-1}$)	2.67	3.21	2.59	5.23	3.70	0.78
V_F, ($g\ d^{-1}$)	7.51	6.91	3.70	11.9	8.79	1.59
V_f, ($g\ d^{-1}$)	0.751	0.554	0.550	1.19	0.833	0.189
(% intake)	9.08	4.43	4.65	8.74	7.42	1.50
a, (%)	18.35	49.14	73.39	21.24	29.56	9.83
V_u, ($g\ d^{-1}$)	0.428	0.026	0.040	1.379	0.611	0.401
(% intake)	5.17	0.208	0.338	10.11	5.16	2.86
M_1, (g)	0.23	0.27	0.29	0.32	0.27	0.023
M_2, (g)	0.45	0.73	0.42	0.66	0.58	0.117
M_3, (g)	1.43	2.43	1.81	2.84	2.23	0.42
M_4, (g)	6.11	8.70	7.28	11.98	8.93	1.70
M_T, (g)	8.13	12.1	9.81	15.8	12.02	2.21
R_{21}, ($g\ d^{-1}$)	49.3	71.5	50.7	87.7	60.5	13.67
R_{12}, ($g\ d^{-1}$)	47.9	41.9	48.7	85.1	58.3	13.51
R_{32}, ($g\ d^{-1}$)	7.14	9.12	7.80	10.6	8.94	0.99
R_{23}, ($g\ d^{-1}$)	5.65	6.49	5.79	7.91	4.31	1.78
R_{43}, ($g\ d^{-1}$)	2.86	4.46	3.47	5.04	4.12	0.65
R_{34}, ($g\ d^{-1}$)	1.37	1.83	1.46	2.38	1.86	0.29

SEM Standard error of mean.

[a] Sheep 8 excluded before calculation of means because of illness.

Table 3.17. Compartment masses and rates of calcium transport in EGTA infused sheep fed CTR.

Parameter	Sheep # 1	4	7	10	Mean	SEM
Calcium intake ($g\ d^{-1}$)	10.7	12.3	10.6	16.1	12.4	1.29
V_a, ($g\ d^{-1}$)	2.33	5.31	4.04	7.32	4.75	1.05
(% intake)	21.7	43.2	38.1	45.4	37.1	5.37
V_{0+}, ($g\ d^{-1}$)	0.955	0.993	0.958	1.169	1.02	0.05
(% intake)	8.89	8.08	9.04	7.25	8.31	0.41
V_{0-}, ($g\ d^{-1}$)	0.642	-2.44	-0.856	-3.51	-1.54	0.90
(% intake)	5.97	-19.9	-8.08	-21.7	-10.9	6.40
Balance ($g\ d^{-1}$)	0.313	3.43	1.81	4.68	2.56	0.95
V_T, ($g\ d^{-1}$)	2.97	2.87	3.19	3.82	3.21	0.21
V_F, ($g\ d^{-1}$)	8.82	7.59	7.17	9.49	8.27	0.54
V_f, ($g\ d^{-1}$)	0.401	0.618	0.615	0.681	0.579	0.061
(% intake)	3.73	5.03	5.80	4.22	4.70	0.46
a, (%)	21.7	43.25	38.14	45.4	37.1	5.37
V_u, ($g\ d^{-1}$)	1.62	1.26	1.61	2.00	1.62	0.14
(% intake)	15.0	10.3	15.2	12.2	13.2	1.20
M_1, (g)	0.262	0.213	0.231	0.451	0.289	0.055
M_2, (g)	0.459	0.397	0.557	0.564	0.494	0.040
M_3, (g)	1.97	1.32	2.01	2.46	1.94	0.23
M_4, (g)	8.55	6.02	6.52	11.2	8.08	1.18
M_T, (g)	11.2	7.95	9.32	14.7	10.8	1.46
R_{21}, ($g\ d^{-1}$)	53.4	44.0	58.1	41.7	49.3	3.87
R_{12}, ($g\ d^{-1}$)	52.4	43.0	57.2	40.6	48.3	3.91
R_{32}, ($g\ d^{-1}$)	6.63	6.70	8.38	8.54	7.56	0.52
R_{23}, ($g\ d^{-1}$)	5.68	5.71	7.42	7.37	6.55	0.49
R_{43}, ($g\ d^{-1}$)	2.80	2.75	3.06	4.01	3.16	0.29
R_{34}, ($g\ d^{-1}$)	1.85	1.76	2.10	2.84	2.14	0.25

SEM Standard error of mean.

Table 3.18. Compartment masses and rates of calcium transport in EGTA infused sheep fed TRT-A.

Parameter	Sheep # 5	6	9	12	Mean	SEM
Calcium intake (g d^{-1})	7.6	14.7	8.83	15.5	11.6	2.01
V_a, (g d^{-1})	1.45	4.99	3.75	6.43	4.16	1.06
(% intake)	19.2	34.1	42.4	41.5	34.3	5.38
V_{0+}, (g d^{-1})	1.04	2.08	1.33	1.61	1.52	0.22
(% intake)	13.7	14.2	15.0	10.4	13.3	1.02
V_{0-}, (g d^{-1})	1.41	0.158	0.071	-1.49	0.04	0.594
(% intake)	18.6	1.08	0.803	-9.58	2.73	5.85
Balance (g d^{-1})	-0.37	1.93	1.26	3.10	1.48	0.72
V_T, (g d^{-1})	2.87	5.15	3.82	4.95	4.19	0.53
V_F, (g d^{-1})	6.52	10.4	5.99	9.93	8.22	1.14
V_f, (g d^{-1})	0.394	0.760	0.908	0.846	0.727	0.115
(% intake)	5.19	5.19	10.29	5.45	6.53	1.25
a, (%)	19.2	34.1	42.4	41.5	34.3	5.38
V_u, (g d^{-1})	1.43	2.31	1.58	2.49	1.95	0.26
(% intake)	18.9	15.7	17.9	16.03	17.2	0.76
M_1, (g)	0.223	0.339	0.362	0.239	0.291	0.035
M_2, (g)	0.457	0.598	0.628	0.567	0.563	0.037
M_3, (g)	1.97	2.57	2.67	2.24	2.37	0.16
M_4, (g)	10.8	11.7	12.4	9.88	11.2	0.55
M_T, (g)	13.4	15.2	16.0	12.9	14.4	0.74
R_{21}, (g d^{-1})	57.8	75.0	45.5	75.2	63.4	7.22
R_{12}, (g d^{-1})	56.8	72.9	44.2	73.6	61.9	7.05
R_{32}, (g d^{-1})	9.40	11.6	8.46	9.32	9.70	0.67
R_{23}, (g d^{-1})	8.36	9.52	7.17	7.71	8.19	0.51
R_{43}, (g d^{-1})	3.52	5.11	3.83	3.94	4.10	0.35
R_{34}, (g d^{-1})	2.49	3.03	2.53	2.32	2.59	0.15

SEM Standard error of mean.

Table 3.19. Compartment masses and rates of calcium transport in EGTA infused sheep fed TRT-B.

Parameter	Sheep # 3	5	8	11	Mean	SEM
Calcium intake ($g\ d^{-1}$)	8.7	17.3	10.5	16.7	13.3	2.18
V_a, ($g\ d^{-1}$)	4.55	8.20	5.56	6.38	6.17	0.77
(% intake)	52.5	47.3	53.8	38.1	47.9	3.56
V_{0+}, ($g\ d^{-1}$)	0.883	0.167	1.27	0.681	0.752	0.232
(% intake)	10.2	0.961	12.16	4.06	6.84	2.61
V_{0-}, ($g\ d^{-1}$)	-1.98	-5.33	-1.89	-1.62	-2.71	0.87
(% intake)	-22.8	-30.7	-18.0	-9.69	-20.3	4.41
Balance ($g\ d^{-1}$)	2.86	5.49	3.17	2.30	3.46	0.70
V_T, ($g\ d^{-1}$)	2.57	2.87	3.67	4.76	3.47	0.49
V_F, ($g\ d^{-1}$)	4.56	9.79	5.48	11.36	7.80	1.64
V_f, ($g\ d^{-1}$)	0.447	0.654	0.611	0.991	0.676	0.114
(% intake)	5.16	3.77	5.80	5.91	5.16	0.49
a, (%)	52.5	47.3	53.8	38.1	47.9	3.56
V_u, ($g\ d^{-1}$)	1.24	2.05	1.88	3.09	2.06	0.38
(% intake)	14.3	11.8	17.8	18.4	15.6	1.55
M_1, (g)	0.214	0.336	0.276	0.365	0.298	0.034
M_2, (g)	0.470	0.747	0.475	0.477	0.543	0.068
M_3, (g)	1.66	3.04	1.81	1.78	2.07	0.32
M_4, (g)	6.01	12.8	6.86	6.84	8.13	1.58
M_T, (g)	8.36	16.9	9.42	9.46	11.0	1.98
R_{21}, ($g\ d^{-1}$)	69.4	54.5	56.7	43.5	56.0	5.32
R_{12}, ($g\ d^{-1}$)	68.6	52.4	55.4	42.8	54.8	5.32
R_{32}, ($g\ d^{-1}$)	7.72	9.92	5.92	5.77	7.33	0.97
R_{23}, ($g\ d^{-1}$)	6.84	7.79	4.64	5.09	6.09	0.74
R_{43}, ($g\ d^{-1}$)	2.63	5.13	2.77	2.15	3.17	0.67
R_{34}, ($g\ d^{-1}$)	1.75	3.00	1.49	1.47	1.92	0.36

SEM Standard error of mean.

Figure 3.8. Mean compartment masses and rate of calcium transport in eucalcemic sheep fed CTR. Values are means ± SEM.

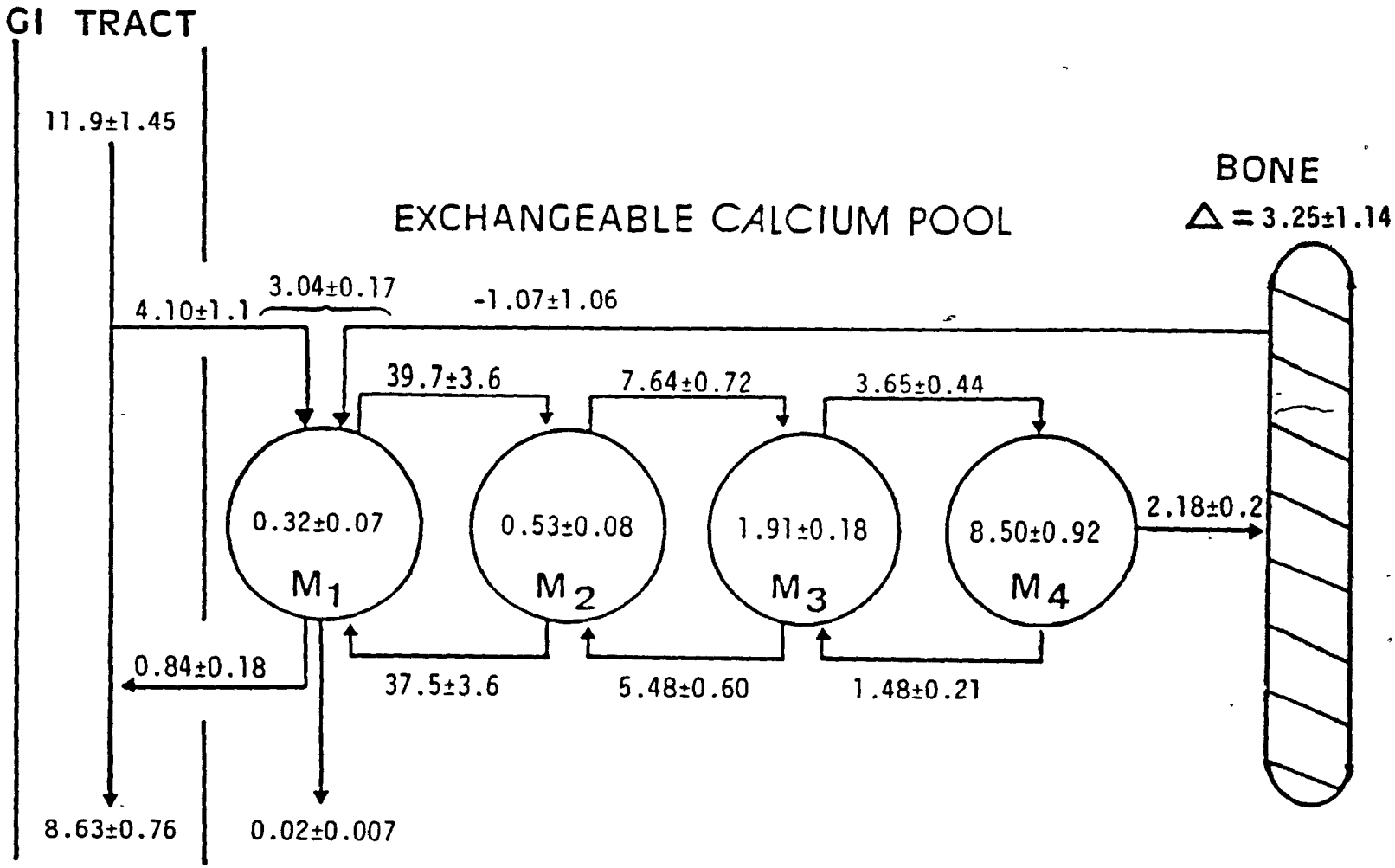

GI TRACT
11.9±1.45
EXCHANGEABLE CALCIUM POOL
BONE
Δ = 3.25±1.14
4.10±1.1
3.04±0.17
-1.07±1.06
39.7±3.6
7.64±0.72
3.65±0.44
0.32±0.07
M1
0.53±0.08
M2
1.91±0.18
M3
8.50±0.92
M4
2.18±0.2
0.84±0.18
37.5±3.6
5.48±0.60
1.48±0.21
8.63±0.76
0.02±0.007

Figure 3.9. Mean compartment masses and rate of calcium transport in eucalcemic sheep fed TRT-A. Values are means ± SEM.

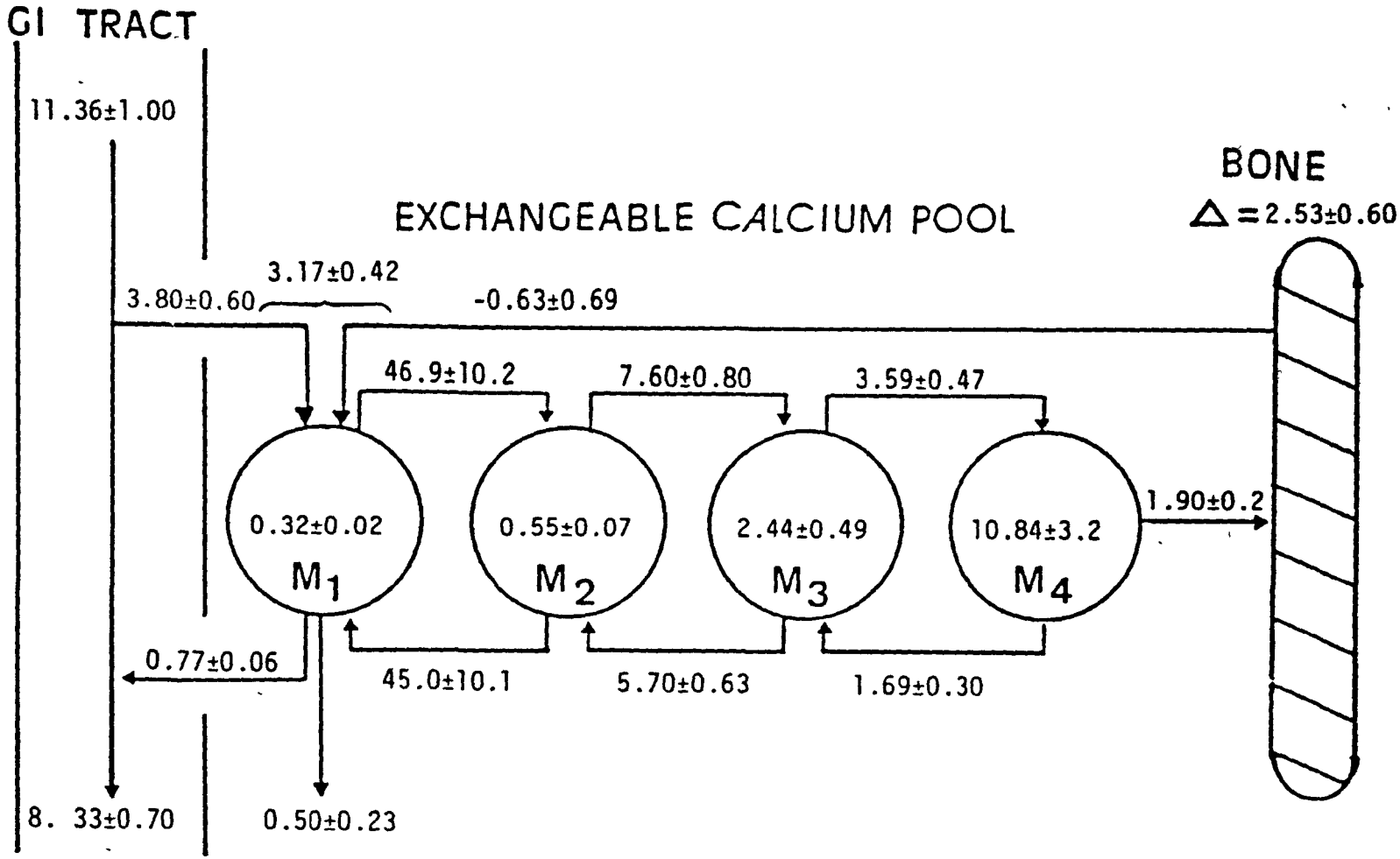

GI TRACT
11.36±1.00
EXCHANGEABLE CALCIUM POOL
BONE
Δ = 2.53±0.60
3.17±0.42
3.80±0.60
-0.63±0.69
46.9±10.2
7.60±0.80
3.59±0.47
0.32±0.02
M1
0.55±0.07
M2
2.44±0.49
M3
10.84±3.2
M4
1.90±0.2
0.77±0.06
45.0±10.1
5.70±0.63
1.69±0.30
8. 33±0.70
0.50±0.23

Figure 3.10. Mean compartment masses and rate of calcium transport in eucalcemic sheep fed TRT-B. Values are means ± SEM.

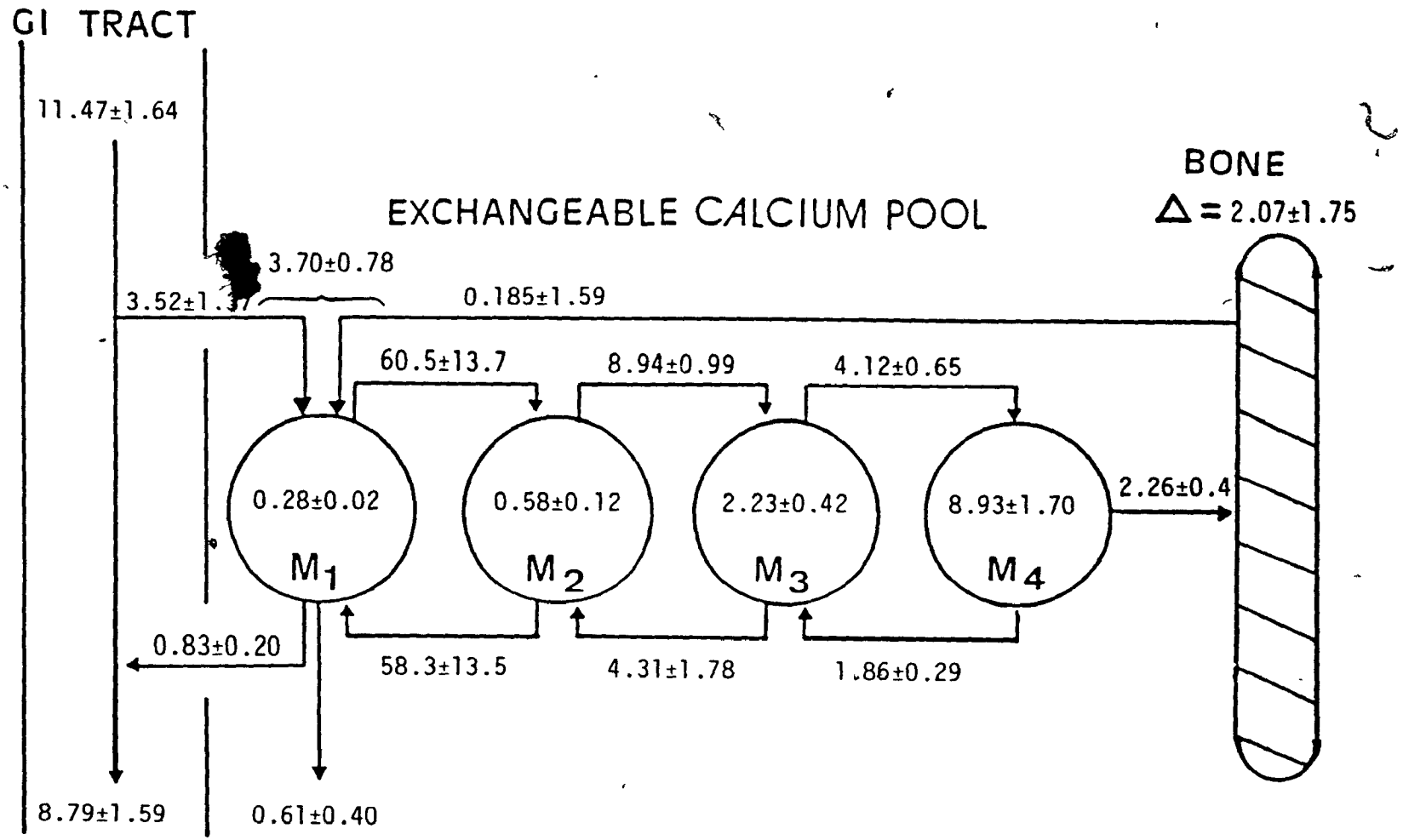

GI TRACT
11.47±1.64
EXCHANGEABLE CALCIUM POOL
BONE
Δ = 2.07±1.75
3.70±0.78
3.52±1.37
0.185±1.59
60.5±13.7
8.94±0.99
4.12±0.65
0.28±0.02
M1
0.58±0.12
M2
2.23±0.42
M3
8.93±1.70
M4
2.26±0.4
0.83±0.20
58.3±13.5
4.31±1.78
1.86±0.29
8.79±1.59
0.61±0.40

Figure 3.11. Mean compartment masses and rate of calcium transport in EGTA-infused sheep fed CTR. Values are means ± SEM.

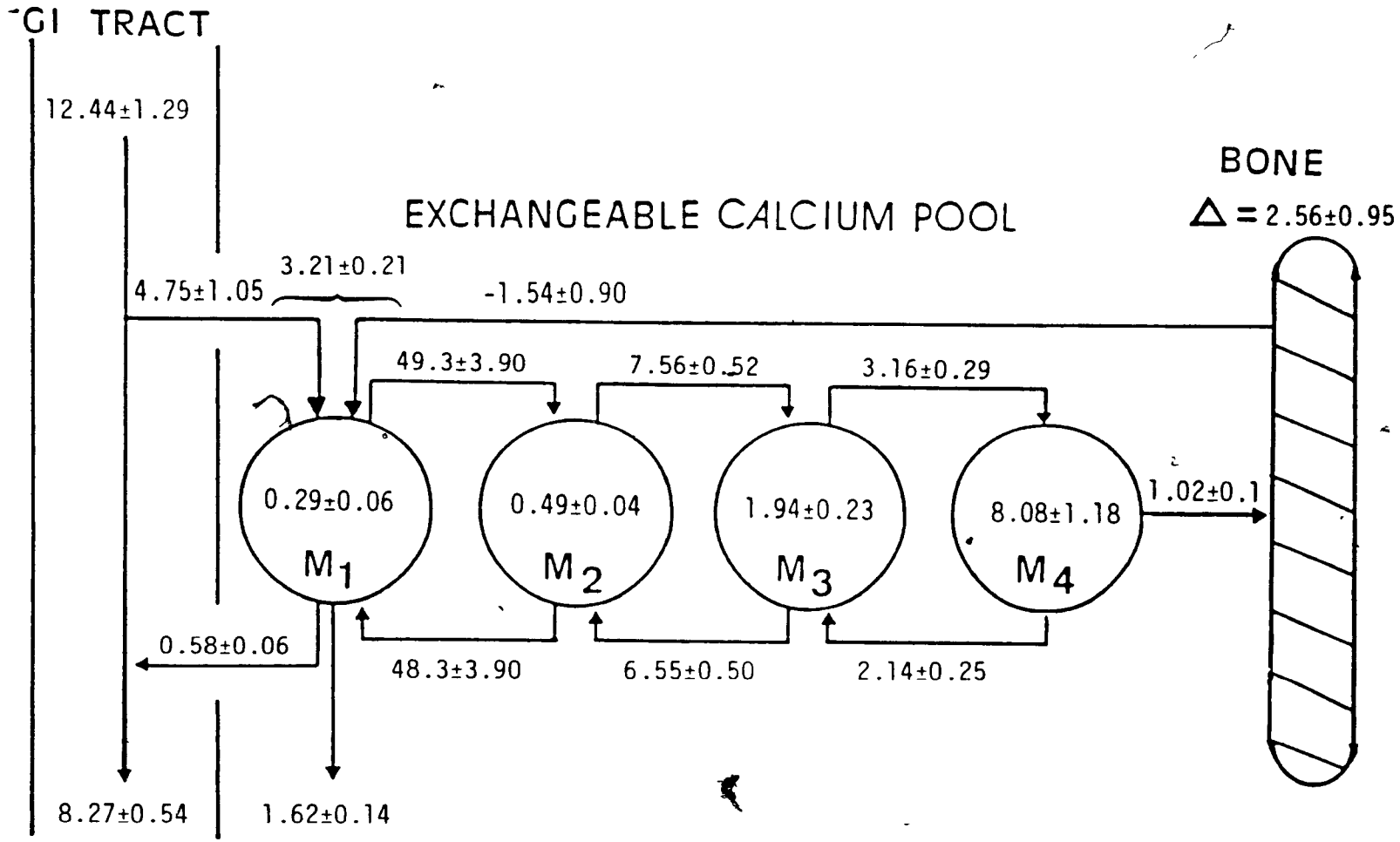

GI TRACT
12.44±1.29
EXCHANGEABLE CALCIUM POOL
BONE
Δ = 2.56±0.95
4.75±1.05
3.21±0.21
-1.54±0.90
49.3±3.90
7.56±0.52
3.16±0.29
0.29±0.06
M1
0.49±0.04
M2
1.94±0.23
M3
8.08±1.18
M4
1.02±0.1
0.58±0.06
48.3±3.90
6.55±0.50
2.14±0.25
8.27±0.54
1.62±0.14

Figure 3.12. Mean compartment masses and rate of calcium transport in EGTA-infused sheep fed TRT-A. Values are means ± SEM.

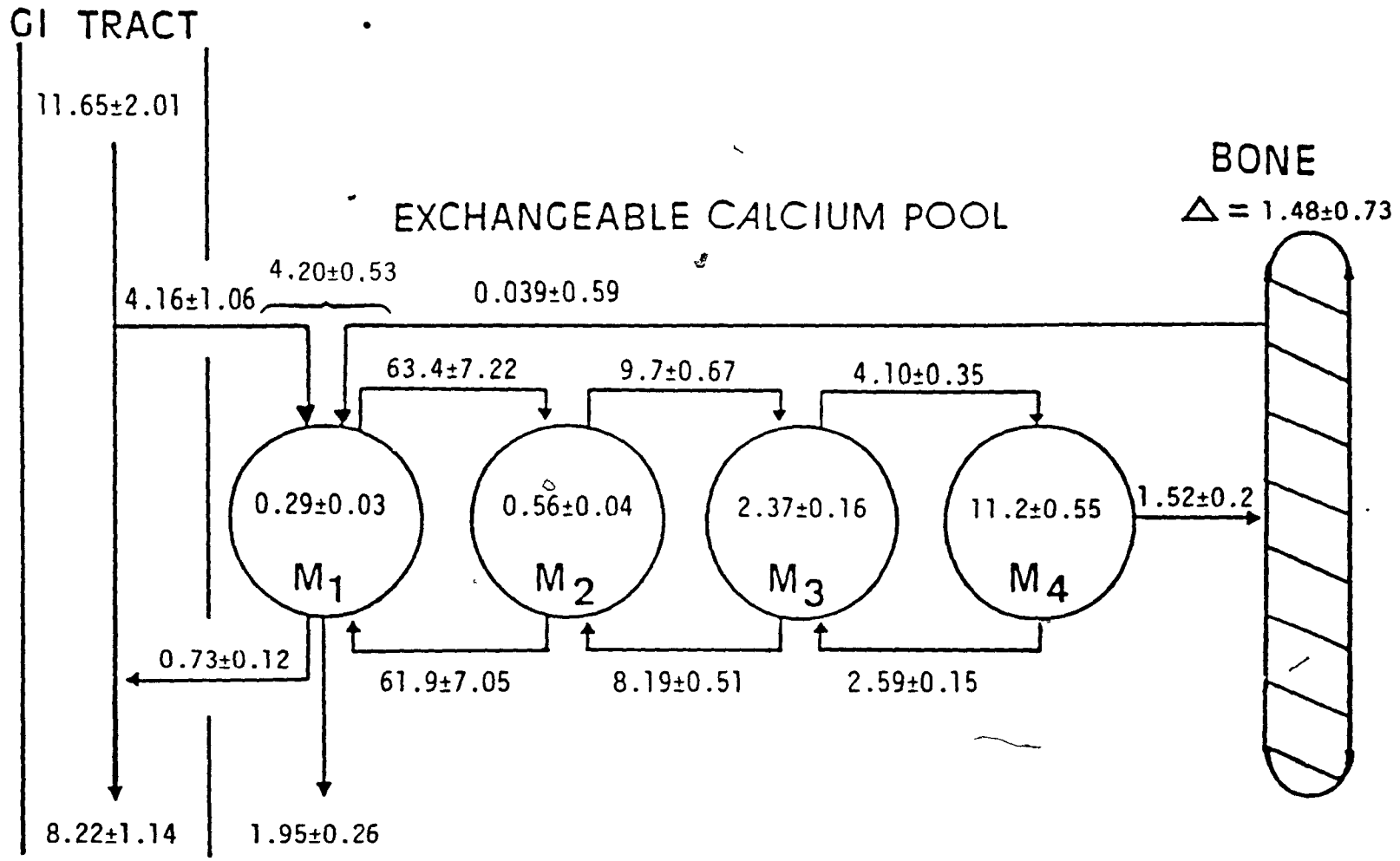

GI TRACT
11.65±2.01
EXCHANGEABLE CALCIUM POOL
BONE
Δ = 1.48±0.73
4.16±1.06
4.20±0.53
0.039±0.59
63.4±7.22
9.7±0.67
4.10±0.35
0.29±0.03
M1
0.56±0.04
M2
2.37±0.16
M3
11.2±0.55
M4
1.52±0.2
0.73±0.12
61.9±7.05
8.19±0.51
2.59±0.15
8.22±1.14
1.95±0.26

Figure 3.13. Mean compartment masses and rate of calcium transport in EGTA-infused sheep fed TRT-B. Values are means ± SEM.

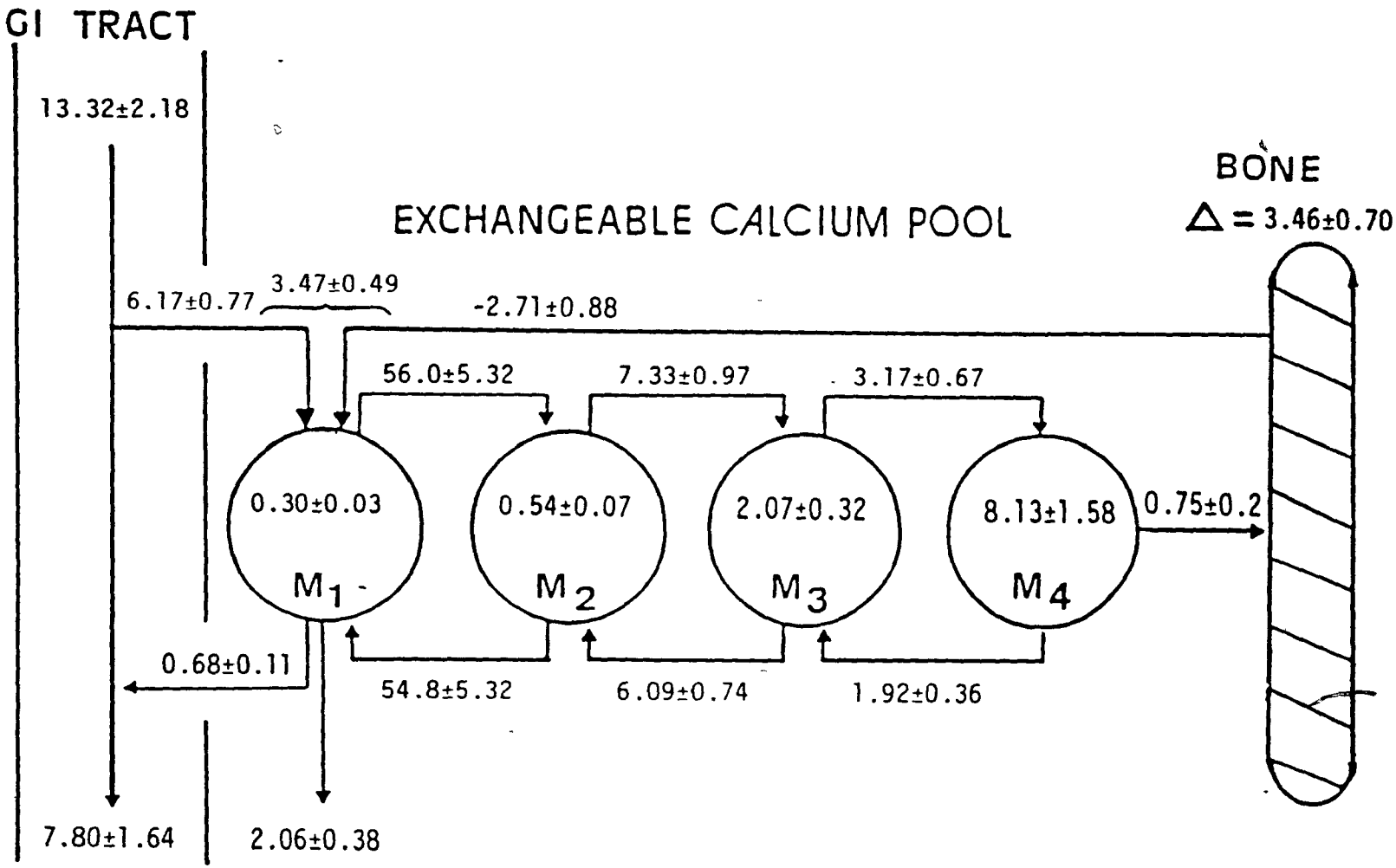

GI TRACT
13.32±2.18
EXCHANGEABLE CALCIUM POOL
BONE
Δ = 3.46±0.70
6.17±0.77
3.47±0.49
-2.71±0.88
56.0±5.32
7.33±0.97
3.17±0.67
0.30±0.03
M1
0.54±0.07
M2
2.07±0.32
M3
8.13±1.58
M4
0.75±0.2
0.68±0.11
54.8±5.32
6.09±0.74
1.92±0.36
7.80±1.64
2.06±0.38

Table 3.20. Probability of dietary treatments and EGTA infusion differences in mean compartment sizes and transfer rates of sheep.

Constant	Dietary treatment	
	Eucalcemic	EGTA infusion
Calcium intake ($g\ d^{-1}$)	.780	.388
V_a, ($g\ d^{-1}$)	.854	.051
(% intake)	.883	.174
V_{0+}, ($g\ d^{-1}$)	.684	.121
(% intake)	.369	.036
V_{0-}, ($g\ d^{-1}$)	.756	.070
(% intake)	.739	.050
Balance ($g\ d^{-1}$)	.771	.157
V_T, ($g\ d^{-1}$)	.744	.163
V_F, ($g\ d^{-1}$)	.970	.909
V_f, ($g\ d^{-1}$)	.958	.237
(% intake)	.961	.201
a, (%)	.883	.174
V_u, ($g\ d^{-1}$)	.147	.304
(% intake)	.099	.058
M_1, (g)	.803	.989
M_2, (g)	.971	.666
M_3, (g)	.535	.583
M_4, (g)	.639	.258
M_T, (g)	.650	.318
R_{21}, ($g\ d^{-1}$)	.163	.380
R_{12}, ($g\ d^{-1}$)	.165	.394
R_{22}, ($g\ d^{-1}$)	.344	.129
R_{23}, ($g\ d^{-1}$)	.795	.128
R_{43}, ($g\ d^{-1}$)	.818	.296
R_{34}, ($g\ d^{-1}$)	.626	.309

and TRT-B (4.23, 5.16% during the eucalcemic period and 17.2, 15.6% during the EGTA-infusion period, respectively) were higher ($P<.1$) than CTR (0.18 and 13.2% during the eucalcemic and EGTA-infusion periods, respectively). No other constants were different ($P>.1$) during the eucalcemic period.

During the EGTA-infusion period, V_a (g d^{-1}), V_{0+} (% of intake), and V_{0-} (both as g d^{-1} and % of intake) were affected by the dietary treatments. Ca absorption (V_a) by sheep fed TRT-B (6.2 g d^{-1}) was higher ($P=0.05$) than that of TRT-A (4.16 g d^{-1}), however, no difference ($P>.1$) was observed when expressed as % of intake (true Ca absorption rate). Bone accretion V_{0+} (loss of Ca to non-exchanging bone) expressed as % of intake was higher ($P=0.04$) in TRT-A (13.3%) than TRT-B (6.84%) but neither were different ($P>.1$) from CTR (8.31%). Bone resorption rate V_{0-} showed the same pattern of difference that TRT-A (2.73%) was higher ($P=0.05$) than TRT-B (-20.3%).

Table 3.21 shows mean values of the eucalcemic and EGTA-infusion periods and their probability of differences. Simulated lactation by means of continuous EGTA infusion caused changes in Ca metabolism with a 48% reduction in bone accretion V_{0+} (18.15 vs 9.49%), and slight but significant ($P=0.09$) increase in true absorption rate (V_{0-} % of intake)(39.8 vs 32.2%) compared to that of the eucalcemic period. However, no difference ($P>.1$) was found in total Ca inflow into the exchangeable Ca pool (V_T) due mainly to the lack of difference ($P>.1$) found in the other components of V_T, V_{0-}. A difference found in true absorption rate was

Table 3.21. Mean compartment sizes and transfer rates of sheep during the eucalcemic and EGTA-infusion periods and their probability of differences.

Constant	Eucalcemic	EGTA-infused	Probability
Calcium intake ($g\ d^{-1}$)	11.6	12.5	.478
V_a, ($g\ d^{-1}$)	3.83	5.04	.136
(% intake)	32.2	39.8	.096
V_{0+}, ($g\ d^{-1}$)	2.10	1.09	.001
(% intake)	18.1	9.49	.001
V_{0-}, ($g\ d^{-1}$)	-0.57	-1.40	.306
(% intake)	-3.88	-9.50	.369
Balance ($g\ d^{-1}$)	2.66	2.50	.833
V_T, ($g\ d^{-1}$)	3.41	3.63	.553
V_F, ($g\ d^{-1}$)	8.57	8.09	.566
V_f, ($g\ d^{-1}$)	0.813	0.660	.123
(% intake)	7.15	5.46	.050
a, (%)	32.2	39.8	.096
V_u, ($g\ d^{-1}$)	0.357	1.88	.001
(% intake)	3.01	15.3	.001
M_1, (g)	0.305	0.293	.710
M_2, (g)	0.551	0.533	.739
M_3, (g)	2.19	2.12	.796
M_4, (g)	9.47	9.13	.817
M_T, (g)	12.52	12.09	.804
R_{21}, ($g\ d^{-1}$)	47.99	56.25	.217
R_{12}, ($g\ d^{-1}$)	45.90	54.99	.171
R_{32}, ($g\ d^{-1}$)	7.98	8.20	.756
R_{23}, ($g\ d^{-1}$)	5.24	6.94	.021
R_{43}, ($g\ d^{-1}$)	3.75	3.48	.479
R_{34}, ($g\ d^{-1}$)	1.66	2.22	.019

reflected in a 24% reduction in endogenous fecal excretion (V_f). In general, EGTA infusion increased compartment mass and transfer rates between them.

DISCUSSION

In previous experiments, it was shown that manipulating dietary cation-anion balance, defined as meq [($Na^+ + K^+$)-($Cl^- + S^=$)], altered Ca metabolism (Exp.1 and Exp.2). Negative cation-anion balanced diets that contained excess of anions in relation to cations greatly increased urinary Ca excretion while not affecting blood Ca concentration. This led to the assumption that bone resorption must be increased in order to assure normal extracellular Ca level. In this experiment, the kinetics of Ca metabolism in sheep fed different cation-anion balanced diets were determined using the model of Ramberg *et al.* (1970) and was fit to a combination of conventional Ca balance and radioisotope data using the computer program CONSAM (Berman and Weiss, 1978). Studies were carried out using normal (eucalcemic period) and EGTA-infused sheep (EGTA-infused period). The latter was used as a model of simulated lactational Ca loss, thus allowing the comparison of Ca kinetic changes that occur caused by the dietary treatments in a situation where the Ca homeostatic mechanism is greatly and abruptly disturbed (i.e. at the onset of lactation).

Similar to the findings in Exp.1, sheep fed reduced and negative cation-anion balanced diets (TRT-A and TRT-B,

respectively), increased urinary Ca excretion (V_u) compared to CTR, however, no changes were observed in Ca balance. Others (Vagg and Payne, 1970; Braithwaite, 1972) also have observed hypercalciuria by feeding NH_4Cl to ruminants and showed an increase in exchangeable Ca pool size (M_T) which was associated with enhanced absorption from intestine, depressed bone accretion (Vagg and Payne, 1970) and enhanced bone resorption (Braithwaite, 1972). Fredeen *et al.* (1984) compared Ca kinetics of does (goats) fed an acidogenic diet supplemented with HCl to does fed an alkalogenic diet supplemented with NaOH. Hypercalciuria and a reduction of bone accretion rate were found in acidogenic diet. Oral administration of HCl to uremic rats also resulted in an increase of osteoclastic density and a reduction in bone mineralization rate (Chan *et al.*, 1985). Hypercalciuria observed in the above studies and others may suggest that the effect is associated with a mild metabolic acidosis created by feeding acidogenic diets rather than direct effect of the diet. However, some studies could not confirm the effect on bone metabolism (Bell *et al.*, 1977). Thus, it was suggested that the effects of metabolic acidosis on bone metabolism become apparent after a long adaptation period or with rapidly growing young animals (Whiting and Draper, 1981b). Dietary acid stress provided by sulfate (SO_4) and/or NH_4Cl in rats showed an accelerated bone resorption only after 2 months of adaptation (Whiting and Draper, 1980) or young animals with rapid bone turnover (Kunkel *et al.*, 1986).

Previous kinetic studies demonstrated that an increased

Ca retention by feeding increasing dietary Ca resulted in an increase in bone resorption (V_{0-}) but minimum to no changes in bone accretion (V_{0+}) in rats (Bronner and Aubert, 1965; Cohn *et al.*, 1968) and in ruminants (Braithwaite and Riazuddin, 1971). Furthermore, cows that suffered from parturient hypocalcemia at the onset of lactation showed a temporally reduction in outflow of Ca from bone (Ramberg *et al.*, 1970). This suggested that bone resorption process plays a important feedback mechanism for Ca homeostasis (Braithwaite, 1983a). In this experiment, no correlation ($P>.1$) was observed between Ca intake or retention and bone accretion rate ($r=0.03$ and 0.10, respectively) but a strong negative correlation was found between bone mobilization rate and Ca intake ($r=-0.37$, $P=0.07$) or Ca balance ($r=-0.53$, $P=0.008$).

In some studies, the total inflow into compartment 1 (V_T) was exceeded by the calculated value for Ca absorbed from the intestine (V_a). Consequently, some negative values for Ca mobilization from bone (V_{0-}) were obtained by calculating $V_{0-} = V_T - V_a$. Negative values of V_{0-} were also reported in cows and rats fed relatively high dietary Ca (1.4% DM)(Ramberg *et al.*, 1976; Bronner and Aubert, 1965), and in cows prior to the onset of lactation (Ramberg *et al.*, 1970). The inherent errors of balance techniques would increase as dietary Ca intake increases and most of the error involve small cumulative losses in feces and urine and incomplete measurements of intake, both of which favor a positive balance and might lead to overestimation of V_T and/or underestimation of V_a. However, these possible errors could not account for the mag-

nitude of the negative V_{0-} values that were observed in our trial (i.e. TRT-B during the EGTA-infusion period). Some fluctuation in fecal output ($g\ d^{-1}$) was observed with a minimum changes in feed intake during the 7-day collection period. Even though, Ca retention within the gastrointestinal tract is known to be a minimum (Aubert *et al.*, 1963), a long digesta transit time in ruminants compared to that of non-ruminant with uneven excretion rates might lead to overestimation of Ca balance which defined in this model to be a consequence of the process involves only in bone. Some implications of negative values of V_{0-} have been discussed by Bronner (1967). One of the possible explanations is that a fraction of the absorbed Ca may be deposited into bone without equilibrating with all the Ca in compartment 1, which implies the existence of non-exchanging or slowly exchanging fraction of the blood Ca that can not be traced by intravenous injection of isotope (Ca^{45})(Visek *et al.*, 1953). Existence of a slowly exchanging fractions of blood Ca has been reported in some species but not successfully in ruminants (Ramberg *et al.*, 1970). However even in ruminants, some indirect evidence based on the dissimilarity of the specific activity of urine or milk and plasma Ca (Visek *et al.*, 1953; Giese and Comar, 1964) suggests that existence of such a fraction remains a possibility. Unphysiological negative values of bone mobilization rate (V_{0-}) observed in some of animals in this study suggest that this model might not be applicable for Ca kinetics study where a large difference in the level of dietary Ca exists. In this study, however,

variation of the level of Ca in experiment diets was relatively small (0.88 to 1.05% DM). Therefore, the utilization of a four-compartment model to investigate the effect of manipulating dietary cation-anion balance on Ca kinetics is justified in this study.

In this experiment, the total exchangeable Ca pool sizes (M_T) in the eucalcemic period were approximately 3 to 4 times larger than those in sheep measured by kinetic studies using a two compartment model (Hidiroglou and Hidiroglou, 1982; Braithwaite *et al.*, 1969; Braithwaite and Riazuddin, 1971), while M_T were 50% smaller than those of mature non-lactating cows determined by the four-compartment model as used in this experiment (Ramberg *et al.*, 1970). Expressed as g kg^{-1}BW, a mean exchangeable Ca compartments M_1 to M_4, respectively, were 0.007, 0.013, 0.053, and 0.23 during the eucalcemic period and 0.007, 0.013, 0.051, and 0.22 during the EGTA-infusion period in this experiment compared to 0.012, 0.020, 0.045, and 0.10 for non-lactating cows (Ramberg *et al.*, 1970). Values were higher because of relatively higher V_T and V_{0+}, which reflect high dietary Ca intake in this experiment compared to that of Ramberg *et al.* (1970) (0.30 vs 0.175 g Ca kg^{-1}BW). This relatively high dietary Ca intake ranged from 11 to 13 gd^{-1} compared to that of requirements (7.7 g d^{-1})(NRC 1985) is probably attributed to a small changes in M_T between the eucalcemic and EGTA-infusion period.

There was no difference ($P>.1$) in the size of total exchangeable Ca pool among dietary treatments during both periods. This confirmed the results of Fredeen *et al.*

(1988b). They also hypothesized that size of the exchangeable Ca pool is influenced only by the action of parathyroid hormone (PTH), secretion of which is triggered by a reduction of blood ionized form of Ca (Ca^{++}) (Fisher *et al.*, 1973). A positive correlation between plasma PTH and the size of exchangeable Ca pool was observed in nonpregnant nonlactating cows (Ramberg *et al.*, 1976) and in EDTA-infused hypocalcemia cows (Ramberg *et al.*, 1967). Increased plasma Ca^{++} concentrations were observed in animals fed reduced cation-anion balanced diets (TRT-A and TRT-B) in this experiment. This should result in a corresponding suppression of PTH secretion leading to a reduction in the size of exchangeable Ca pool. The discrepancy that exists between the two findings may be caused by alteration in acid-base status of animals. Bushinsky *et al.* (1982) created metabolic acidosis in rats by feeding NH_4Cl and found an increased serum Ca^{++} concentrations but could not detect a reduction in plasma PTH concentrations. Therefore, direct effects or consequences of of disturbing acid-base balance of animals toward acidosis by feeding reduced cation-anion balanced diets may be an altered relationship between PTH secretion and plasma Ca^{++} concentrations.

It is well established that endogenous fecal Ca (V_f) is affected by DM intake and not affected by dietary Ca intake (Braithwaite, 1983a). Small variations observed in this experiment also agrees with previous findings (Ramberg *et al.*, 1970). V_f represents the amount of Ca that was secreted into the intestine and not reabsorbed. A 24% reduction in V_f ob-

served in EGTA-infused lambs may reflect either a decrease in secretion of Ca into the intestine or a more efficient reabsorption of the endogenously secreted gastrointestinal Ca. The latter is more consistent with the change observed in true Ca absorption rate (V_a % of intake). A positive correlation ($r=0.64$, $P=0.007$) was observed between V_f and V_F (fecal Ca excretion) indicating that the endogenously secreted and dietary Ca were mixed within the intestine and no discrimination in the reabsorption process occurred.

Simulated lactational Ca loss created by continuous EGTA-infusion resulted in transient responses in the Ca homeostatic mechanism which reflected the kinetics of the feedback signals involved in the control of plasma and bone Ca. Included in these pathways are the kinetic parameters associated with secretion, metabolism and action of feedback modulators such as PTH, calcitonin (CT), and 1,25-dihydroxyvitaminD_3 ($1,25(OH)_2D_3$) and those on their cellular targets in intestine, kidney, and bone. During this period, effects of reducing dietary cation-anion balance on Ca kinetics became significant. Elevated intestinal absorption of Ca (V_a) with concomitant decrease in bone accretion (V_{0+}) to accommodate an increase in Ca demand was observed in cows (Ramberg *et al.*, 1984) and in ewes (Braithwaite, 1983a). However, there is no hormonal study available in the literature investigating effects of manipulating dietary cation-anion balance.

Several studies indicated that prepartum intake of acidogenic and alkalogenic components of diet is more impor-

tant than the level of dietary Ca with regard to the incidence of hypocalcemic parturient paresis (milk fever)(Ender *et al.*, 1971; Dishington, 1975; Block, 1984). Conventionally, high dietary Ca is considered to be one of the most important predisposing factors of milk fever (Goff *et al.*, 1987). However, when an acidogenic diet was fed to cows predisposed to milk fever prepartum, it was observed that relatively high dietary Ca may be beneficial (Ender *et al.*, 1971; Dishington, 1975; Verdaris and Evans, 1975). Anderson *et al.* (1970) found the feeding of elevated dietary Ca enhanced absorption, retention and depressed bone resorption but had no effects on the exchangeable Ca pool size, which forced animals to become severely hypocalcemia at parturition (Ramberg *et al.*, 1976). Therefore, it was suggested that a size of the exchangeable Ca pool in preparturient cows may be of primary importance. Fredeen (1984) demonstrated a beneficial effect of feeding acidogenic diets on Ca homeostasis to recover from sudden loss of Ca via manipulation of acid-base status of animal. Mild metabolic acidosis imposed by acidogenic diets elevated exchangeable Ca pool (Vagg and Payne, 1970; Fredeen, 1984). Furthermore, incidence of milk fever is dramatically increased when diets are alkalogenic and reduced when diets are acidogenic (Ender *et al.*, 1971; Dishington, 1975; Block, 1984). In this study, hypercalciuria caused by feeding reduced and/or negative cation-anion balanced diet counteract and eliminate the excess Ca that was absorbed and resulted in no changes in Ca retention but increased or maintained a high flux through the exchangeable Ca pool. This was achieved

without the inhibitory effects on bone resorption observed when alkalogenic diet with relatively high dietary Ca were fed (Fredeen, 1984).

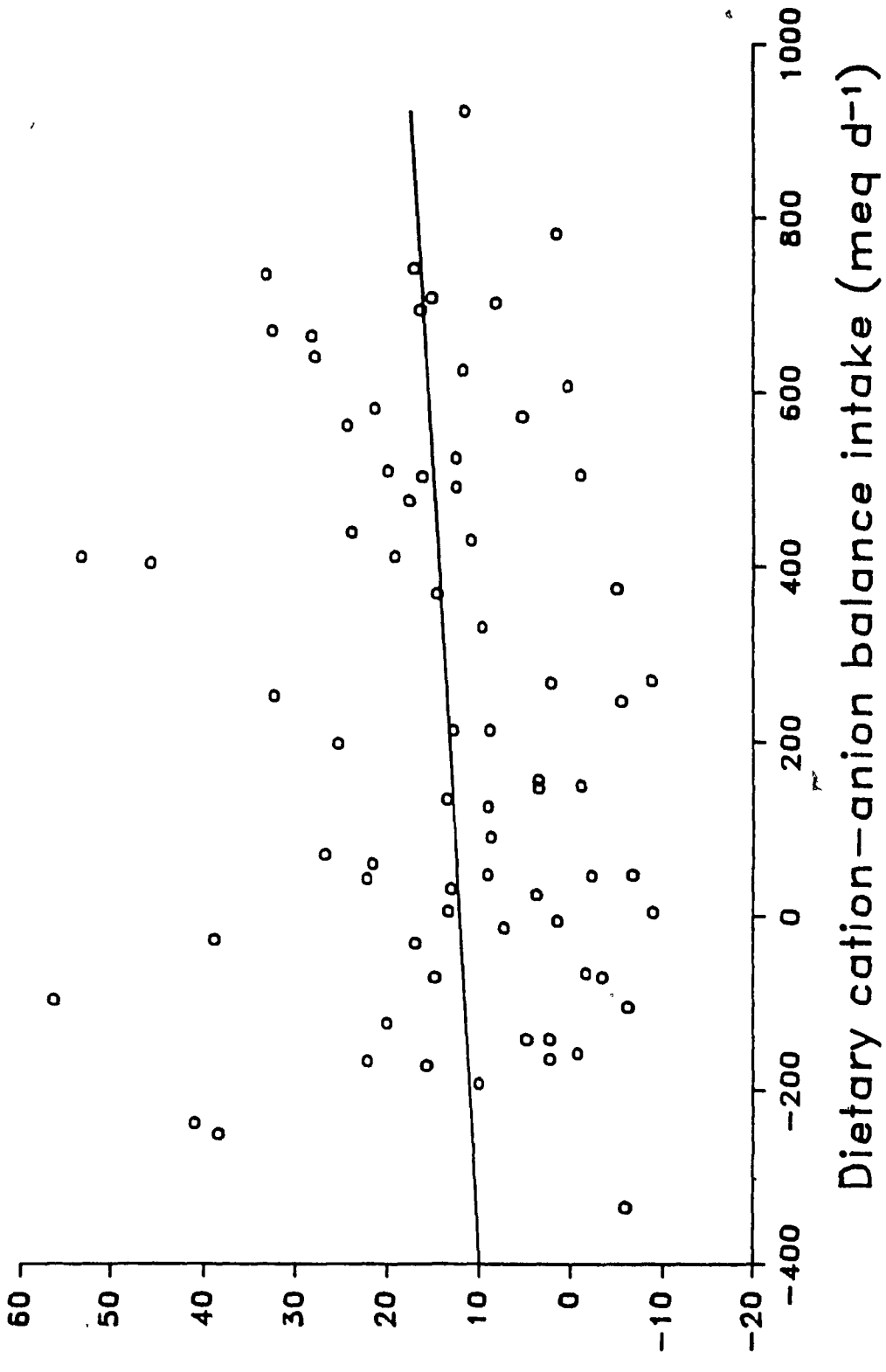
Results obtained from this experiment indicated that feeding reduced cation-anion balanced diets to sheep resulted in an increased Ca flux through the exchangeable Ca pool but had no effects on the size of Ca pool. This effect was maximized when Ca demand of animals was increased by simulated lactational Ca loss caused by continuous infusion of EGTA. Even though plasma total Ca concentration was not affected by dietary treatments, plasma ionized Ca (Ca^{++}) concentrations were elevated in reduced cation-anion balanced diets. This indicated that there was a disturbances in interrelationship between the Ca-regulating hormones (PTH, vitamin D, and CT) and/or in responsiveness to target organs (bone, kidney, and intestine). Hypercalciuria associated with reduced dietary cation-anion balance with corresponding increases in intestinal Ca absorption and in bone resorption may play an important role in order to sustain a high level of Ca flux through the exchangeable Ca pool, especially in situation of high Ca demands such as in postparturient hypocalcemia.

VI. GENERAL DISCUSSION

Manipulating dietary cation-anion balance, measured as meq [Sodium (Na^+) + Potassium (K^+)]-[Chloride (Cl^-) + Sulfur ($S^=$)], achieved by supplementing various mineral salts has been used as a preventative method of hypocalcemic parturient paresis (milk fever)(Dishington, 1975; Dishington and Bjórnstad, 1982; Block, 1984; Leclerc, 1986). The assumption was that the dietary cation-anion balance of rations fed to cows prepartum was an overriding importance in determining Ca availability (Ender and Dishington, 1970) and that a positive diet reduced Ca absorption whereas a negative diets increased it (Ender *et al.*, 1971). Using the combined data in this study (n=73), a positive correlation (r=0.28, P=0.04) between intake of dietary cation-anion balance (meq d^{-1}) and Ca retention (g $7d^{-1}$) confirmed that of Lomba *et al.* (1978)(Figure 4.1.), but we could not detect a negative correlation using negative cation-anion balanced diets only as shown by Lomba *et al.* (1978) mainly due to a small sample size (n=21) and a large variation within dietary treatments.

Hypercalciuria observed in animals fed reduced or negative cation-anion balanced diets confirmed that of others (Braithwaite, 1972; Whiting and Cole, 1986; Fredeen *et al.*, 1988a). Furthermore, an alteration of acid-base status of animals suggested by changes in urine samples indicated that mild acidosis caused by feeding such diets may be responsible (Harmon and Britton, 1983) by decreasing tubular Ca reabsorption (Sutton and Dirks, 1978; Peraino and Suki, 1980) and/or

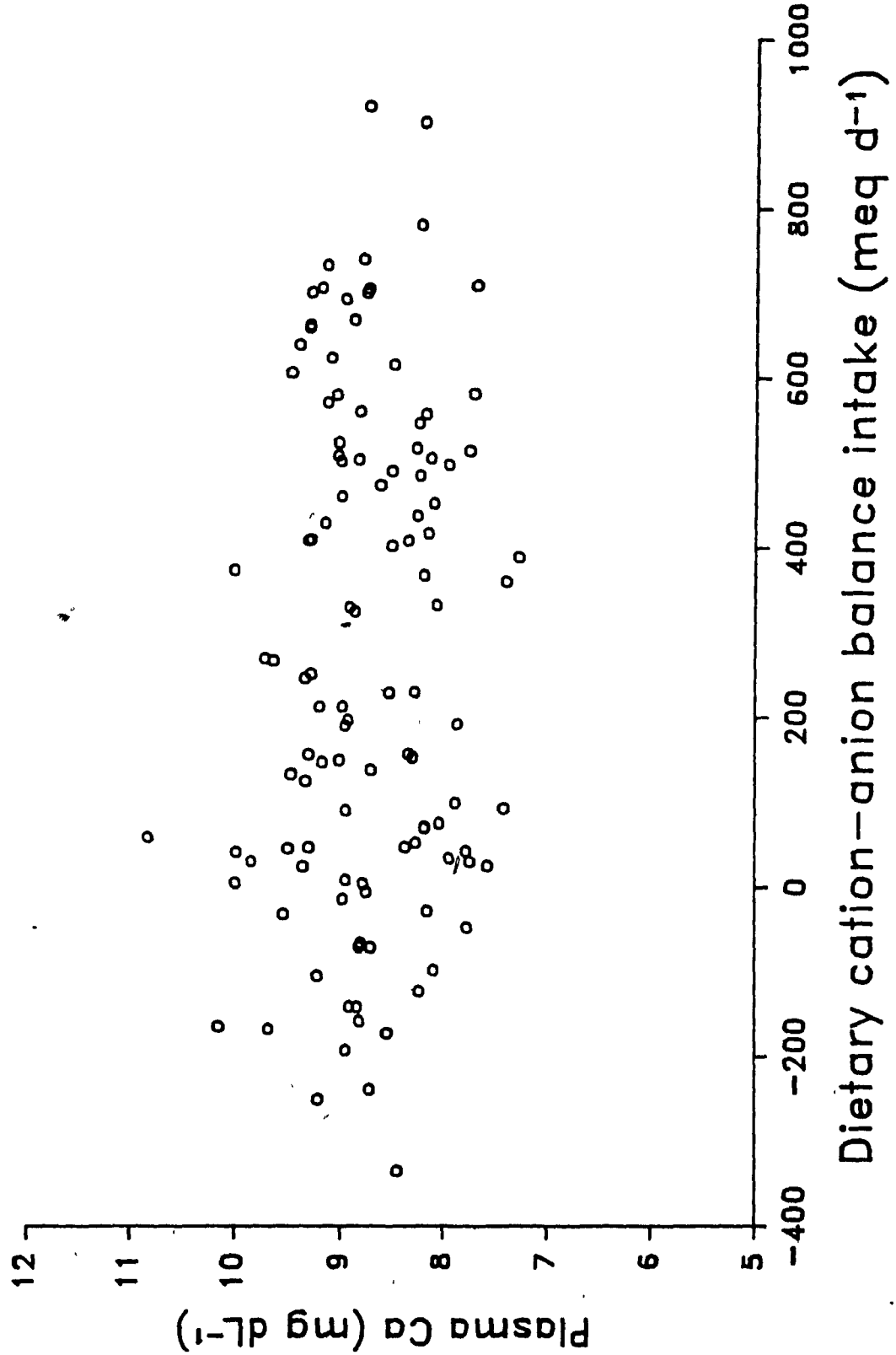
Figure 4.1. Relationship between the intake of dietary cation-anion balance and the retention of calcium (Ca) ($r=0.28$, $P=0.04$).


Ca retention (g 7d-1)
60
50
40
30
20
10
0
-10
-20
-400
-200
0
200
400
600
800
1000
Dietary cation-anion balance intake (meq d-1)

releasing Ca from bone (Braithwaite, 1972). However, there was no or little effects on Ca mobilization from bone associated with feeding reduced cation-anion balanced diets in this study as evidenced by lack of correlation observed between plasma free OHPro concentrations and intake of dietary cation-anion balance. Kunkel *et al.* (1986) also observed no changes in urinary excretion of OHPro by rats with dietary acid stress (supplementation of NH_4Cl or SO_4-containing mineral salts). There were also no changes observed in bone mobilization rate (V_{0-}) in the Ca kinetic study.

Effects of reducing cation-anion balance of diets on plasma total Ca concentration was minimum as shown in a combined data (Figure 4.2.) and as was in others (Braithwaite, 1972; Verdaris and Evans, 1975; Fredeen *et al.*, 1988a). Plasma ionized Ca (Ca^{++}) was elevated in animals fed reduced cation-anion balanced diets. This may have resulted from a direct effects of mild acidosis in animals. Chronic metabolic acidosis raised plasma Ca^{++} concentrations (Bushinsky *et al.*, 1982), resulting from a reduction of plasma protein bound Ca (Sutton and Dirks, 1978). An increased plasma Ca^{++} concentration is usually associated with a reduction in circulating PTH concentrations (Ramp and Waite, 1982). Metabolic acidosis, however, seems to disturb this relationship in that an increased plasma Ca^{++} concentration associated with acidosis did not depress PTH secretion (Bushinsky *et al.*, 1982) and may disturb a positive correlation that existed between the size of exchangeable Ca pool and plasma PTH concentrations (Ramberg *et al.*, 1967; 1976). No changes in the

Figure 4.2. Relationship between the intake of dietary cation-cation balance and the concentration of calcium (Ca) in plasma (r=0.059, P=0.79).


Plasma Ca (mg dL⁻¹)
12
11
10
9
8
7
6
5
−400
−200
0
200
400
600
800
1000
Dietary cation–anion balance intake (meq d⁻¹)

size of exchangeable Ca pool of animals fed reduced or negative cation-anion balanced diets support this theory.

An increased Ca mobilization rate during the induced acute hypocalcemia and an increase in the level of Ca flux through the exchangeable Ca pool associated with feeding reduced or negative cation-anion balanced diets through increases in Ca entry and clearance without affecting its size, particularly in animals with an increased Ca demand such as at the onset of lactation, may be the reason for beneficial effects of feeding such a diet to prepartum cows as a preventative method of milk fever.

VII. CONCLUSION

From the results obtained in three experiments presented the following conclusions can be drawn.

Reducing dietary cation-anion balance:

- did not affect dry matter(DM) intake and digestibility except when $FeSO_4$ (1.08% of total DM) was supplemented;
- disturbed acid-base balance of animals toward acidosis indicated by a reduced pH of urine;
- caused hypercalciuria;
- had a minimum effects on plasma total calcium (Ca) concentrations;
- caused a small reduction in plasma ionized Ca (Ca^{++}) concentrations;
- caused faster recovery time ($T_{1/2}$) after the infusion of EDTA;
- tended to increase the Ca mobilization rate from immediately mobilizable Ca pool in response to acute hypocalcemia caused by the infusion of EDTA and the magnitude of protective effect was comparable to that of vitamin D injection;
- had minimum effects on the size of exchangeable Ca pool;
- tended to increase the level of Ca flux through the exchangeable Ca pool.

Other notable observations were:

- Apparent absorption and retention of dietary Ca and phosphorus (P) was positively correlated;
- There was a positive linear correlation between intake of dietary cation-anion balance and Ca retention;
- Reducing dietary cation-anion balance was negatively correlated with plasma Mg concentrations;
- There was a direct correlation in equal molar proportions between plasma EDTA-titratable free Ca ($_f$Ca) and P concentrations with induced hypocalcemia;
- Reducing dietary cation-anion balance increased Ca absorption and urinary excretion and resulted in an increase in the level of Ca flux through the exchangeable Ca pool without affecting its size particularly when their Ca demand abruptly increased by simulated lactational Ca loss.

STATEMENTS OF ORIGINALITY

To the best of author's knowledge, the following imformation contained in this thesis constitutes an original contribution to the scientific literature.

1) The utilization of various mineral salts including trace minerals for reducing dietary cation-anion balance, defined as [(Sodium (Na^+) + Potassium (K^+))-(Chloride (Cl^-) + Sulfur ($S^=$))], from +350 to -150 (meq kg^{-1} dry matter) within the safety level of mineral elements in diet.

2) Reports on alteration of macromineral metabolism (calcium (Ca), phosphorus (P), magnesium (Mg), K, Na, Cl and S) and the concentration of plasma in relation with feeding reduced or negative cation-anion balanced diets.

3) Reports on an increasing Ca mobilization rate with a decreasing dietary cation-anion balance in the response to hypocalcemia created by continuous infusion of EDTA solution and the comparison between feeding reduced cation-anion balanced diets and vitamin D-injection on the capability of Ca mobilization rate from the immediately mobilizable Ca pool and the size of pool.

4) Reports on an increased concentration of plasma ionized form of Ca in relation with feeding reduced or negative cation-anion balanced diets.

5) Determination of Ca kinetics using a four-compartment model in sheep fed different cation-anion balanced diets causes by supplementation of various mineral salts during eucalcemic and simulated lactational Ca loss created by the continuous infusion of EGTA solution.

6) Demonstration of the sensitivity of changes observed in bone resorption rate with relatively high dietary Ca intake.

APPENDIX A.

SUPPLEMENTARY DATA - MAJOR MINERAL METABOLISM BY SHEEP FED DIFFERENT CATION-ANION BALANCED DIETS DURING THE EUCALCEMIC AND EGTA-INFUSION PERIOD

Table A.1. Magnesium absorption and retention by sheep fed different cation-anion balanced diets during the eucalcemic and EGTA-infusion periods[1].

Variable	Eucalcemic				EGTA infusion				Significance[2]
	CTR	TRT-A	TRT-B	SEM	CTR	TRT-A	TRT-B	SEM	Infusion
Intake ($g\ 7d^{-1}$)	16.1	19.8	18.2	0.74	15.3	19.3	21.5	1.86	NS
Feces ($g\ 7d^{-1}$)	9.91	14.2	12.6	0.82	9.43^{a}	14.1^{b}	12.8^{ab}	1.00	NS
Urine ($g\ 7d^{-1}$)	2.06	3.16	3.84	0.20	2.24^{a}	4.27^{ab}	5.28^{b}	0.58	**
Absorption ($g\ 7d^{-1}$)	6.24	5.55	5.65	0.27	5.85	5.26	8.77	1.25	NS
Digestibility (%)	39.5	24.9	31.9	3.00	38.8	25.6	39.6	5.00	NS
Balance ($g\ 7d^{-1}$)	4.18	2.39	1.81	0.45	3.60	0.98	3.48	0.86	NS
Urine/Absorption (%)	33.5	56.9	67.9	15.00	40.6	81.3	60.1	18.8	NS
Balance/Intake (%)	26.6^{A}	9.6^{B}	11.7^{AB}	4.0	23.9^{A}	4.1^{B}	15.5^{AB}	4.00	NS

[1] CTR=Control; TRT=Treatment; SEM=Standard error of mean.
[2] NS=Not significant (P>.1); ** Significant (P<.05).
[ab] Means with different superscripts were different (P<.05) within each period.
[AB] Means with different superscripts were different (P<.1) within each period.

Table A.2. Phosphorus absorption and retention by sheep fed different cation-anion balanced diets during the eucalcemic and EGTA-infusion periods[1].

Variable	Eucalcemic				EGTA infusion				Significance[2]
	CTR	TRT-A	TRT-B	SEM	CTR	TRT-A	TRT-B	SEM	Infusion
Intake ($g\ 7d^{-1}$)	23.6	23.0	22.8	1.25	24.2	23.8	25.4	1.81	NS
Feces ($g\ 7d^{-1}$)	18.2	18.9	19.1	1.10	20.4	20.5	21.6	1.09	*
Urine ($g\ 7d^{-1}$)	0.09	0.13	0.04	0.01	0.15	0.20	0.17	0.07	NS
Absorption ($g\ 7d^{-1}$)	5.38	4.16	3.61	0.97	3.83	3.32	3.86	0.93	NS
Digestibility (%)	22.9	17.9	15.9	3.00	15.0	11.3	11.5	4.00	*
Balance ($g\ 7d^{-1}$)	5.29	4.02	3.56	0.97	3.67	3.12	3.70	0.89	NS
Urine/Absorption (%)	1.94	3.11	1.43	1.00	6.32	1.52	1.88	1.54	NS
Balance/Intake (%)	22.5	17.3	15.8	3.05	14.3	10.5	10.6	3.09	NS

[1] CTR=Control; TRT=Treatment; SEM=Standard error of mean.
[2] NS=Not significant (P>.1); * Significant (P<.1).

Table A.3. Sodium absorption and retention by sheep fed different cation-anion balanced diets during the eucalcemic and EGTA infusion periods[1].

Variable	Eucalcemic				EGTA-infusion[2]				Significance[3]
	CTR	TRT-A	TRT-B	SEM	CTR	TRT-A	TRT-B	SEM	Infusion
Intake ($g\ 7d^{-1}$)	50.8	49.0	43.2	4.45	110.4[a]	98.9[b]	95.6[b]	11.2	***
Feces ($g\ 7d^{-1}$)	9.68	7.84	14.1	3.23	11.9	9.78	14.8	3.04	NS
Urine ($g\ 7d^{-1}$)	37.4	35.3	24.9	6.25	66.2	55.7	52.9	6.96	***
Absorption ($g\ 7d^{-1}$)	41.1	41.2	29.1	4.29	98.5[a]	89.5[b]	80.8[c]	2.87	***
Digestibility (%)	80.6[a]	83.3[a]	65.8[b]	2.03	89.4	90.1	86.2	2.45	***
Balance ($g\ 7d^{-1}$)	3.66	5.88	4.19	7.40	32.3	33.4	27.8	7.35	***
Urine/Absorption (%)	91.9	84.9	85.6	6.09	68.9	62.8	65.5	7.67	*
Balance/Intake (%)	6.65	12.5	9.70	6.08	27.7	32.5	30.0	6.56	*

[1] CTR=Control; TRT=Treatment; SEM=Standard error of mean.
[2] Intake include Na content of infused solution (NaCl).
[3] NS=Not significant (P>.1); * Significant (P<.1); *** Significant (P<.01).
[a b] Means with different superscripts were different (P<.05) within each periods.

Table A.4. Potassium absorption and retention by sheep fed different cation-anion balanced diets during the eucalcemic and EGTA infusion periods[1].

Variable	Eucalcemic				EGTA infusion				Significance[2]
	CTR	TRT-A	TRT-B	SEM	CTR	TRT-A	TRT-B	SEM	Infusion
Intake ($g\ 7d^{-1}$)	125.6	121.1	123.9	7.67	132.6	134.1	141.7	10.46	NS
Feces ($g\ 7d^{-1}$)	7.74[A]	5.38[B]	10.9[A]	2.07	6.36[A]	5.19[B]	7.38[A]	0.73	*
Urine ($g\ 7d^{-1}$)	97.2	94.8	99.3	3.28	101.7	111.6	114.6	4.36	*
Absorption ($g\ 7d^{-1}$)	117.8	115.7	113.0	7.66	126.2	128.9	134.3	10.13	*
Digestibility (%)	93.7	85.6	91.7	1.06	95.1	96.1	94.7	1.68	**
Balance ($g\ 7d^{-1}$)	20.6	20.9	13.6	5.01	24.5	17.3	19.7	6.48	NS
Urine/Absorption (%)	82.7	81.4	86.4	1.67	80.6	87.9	84.5	2.79	NS
Balance/Intake (%)	16.1	17.8	12.7	1.90	18.5	11.6	14.6	2.79	NS

[1] CTR=Control; TRT=Treatment; SEM=Standard error of mean.
[2] NS=Not significant (P>.1); * Significant (P<.1); ** Significant (P<.05).
[AB] Means with different superscripts were different (P<.05) within each periods.

Table A.5. Sulfur absorption and retention by sheep fed different cation-anion balanced diets during the eucalcemic and EGTA infusion periods[1].

Variable	Eucalcemic				EGTA infusion				Significance[2]
	CTR	TRT-A	TRT-B	SEM	CTR	TRT-A	TRT-B	SEM	Infusion
Intake (g 7d^{-1})	14.7^{a}	33.8^{b}	47.0^{c}	5.02	12.1^{a}	36.5^{b}	61.3^{c}	3.99	*
Feces (g 7d^{-1})	6.14^{A}	7.42^{AB}	9.83^{B}	1.17	6.54^{a}	10.3^{b}	12.7^{b}	1.09	**
Urine (g 7d^{-1})	0.17^{a}	20.9^{b}	32.5^{c}	3.48	0.29^{a}	25.6^{b}	33.1^{c}	4.12	NS
Absorption (g 7d^{-1})	8.54^{a}	26.4^{b}	37.2^{c}	4.14	5.57^{a}	26.2^{b}	48.5^{c}	3.08	NS
Digestibility (%)	57.1^{a}	77.5^{b}	78.9^{b}	4.08	46.2^{a}	71.4^{b}	78.9^{c}	2.45	**
Balance (g 7d^{-1})	8.37	5.42	4.71	2.84	5.28^{AB}	0.59^{A}	15.45^{B}	4.31	NS
Urine/Absorption (%)	2.50^{a}	83.1^{b}	88.1^{b}	7.52	5.10^{a}	97.8^{b}	70.6^{b}	11.56	NS
Balance/Intake (%)	55.9^{a}	13.5^{b}	9.83^{b}	7.94	43.9^{a}	1.62^{b}	23.7^{ab}	8.08	NS

[1] CTR=Control; TRT=Treatment; SEM=Standard error of mean.
[2] NS=Not significant (P>.1); ** Significant (P<.05).
^{abc} Means with different superscripts were different (P<.05) within each periods.
^{AB} Means with different superscripts were different (P<.1) within each periods.

APPENDIX B.

B. EFFECTS OF DIETARY CATION-ANION BALANCE ON INTESTINAL CALCIUM ABSORPTION IN RATS.

INTRODUCTION

It has been well established that intestinal calcium (Ca) absorption in rats eomprises of two processes, active and diffusive processes (Wasserman and Taylor, 1969; Walling, 1977; Pansu *et al.*, 1981). Former process is a saturable transmural movement in nature and is subject to physiological (Pansu and Bronner, 1981) and nutritional regulation (Armbrecht *et al.*, 1979). Whereas the latter is thought to be a linear function of Ca concentration in lumen and appears to be independent to the age or amount of Ca intake (Pansu *et al.*, 1983). One of the nutritional factors affecting Ca homeostasis is via alternation of acid-base status of animal such as metabolic acidosis. Gringt *et al.* (1979) demonstrated in rats that a proportion of ionic form of Ca to total Ca of plasma was elevated in a metabolic acidosis and was reduced in alkalosis. Bone formation and resorption would also be altered by acid-base balance. Deposiding alkaline bone minerals as storage during the intake of excess alkali and releasing bone minerals as bone resorption process during a period of acidosis (Barzel and Jowsey, 1969: Barton *et al.*, 1983).

Effects of metabolic acidosis on intestinal Ca absorption, however, is not well established. Lee *et al.* (1977)

showed that the intestinal content of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), a stimulator of active absorption process, decreases in metabolic acidosis indicating that Ca absorption would be decreased. However, other studies showed no changes or even an increased absorption rates (Lemann *et al.*, 1966; Litzow *et al.*, 1967; Weber *et al.*, 1971; Grindt and Delling, 1979; Gafter *et al.*, 1980). In this study, a diets differing in their dietary cation-anion balance, defined as meq [sodium (Na^+) + potassium (K^+)]-[chloride (Cl^-) + sulfur ($S^=$)], was used to demonstrate the effects of acid-base balance on the small intestinal Ca absorption measured using the *in situ* intestinal loop method and to investigate the effect of cation groups (aluminum (Al). magnesium (Mg), and iron (Fe)) of mineral salts used to alter cation-anion balance of diets.

MATERIALS AND METHODS

Animals and diets

A total of 80 Male rats, 36-42 days of age and 126-150 g body weight, obtained from Charles and River Canada Ltd were assigned randomly to 3 diets differing cation-anion balance. Composition of diets are presented in Table B.1. $Al_2(SO_4)_3 18H_2O$ and $MgSO_4$ were used to reduce dietary cation-anion balance in diet-2, whereas $MgSO_4$ and $FeSO_4$ were used in diet-3. Diets were formulated to meet the requirements of laboratory rats except for Na, K, Cl, S, Ca, and P, all of which were relatively exceeded the requirements. Animals were

Table B.1. Compositions of diets.

Indredients	Diet 1	Diet 2	Diet 3
	(%)		
Whole wheat flour	69.1	67.55	68.15
Vitamin-free casein	15.0	15.0	15.0
Cellulose	5.0	5.0	5.0
Corn oil	4.0	4.0	4.0
Mineral mixture[1]	5.0	5.0	5.0
Vitamin mixture[2]	1.0	1.0	1.0
$CaHPO_4$	0.8	0.8	0.8
$CaCl_2$	-	0.15	0.15
$CaCO_3$	0.1	-	-
$Al_2(SO_4)_3 18H_2O$	-	0.8	-
$MgSO_4$	-	0.7	0.7
$FeSO_4$	-	-	0.2

[1] Phillip and Hart mineral mixture. It contains followings (g/kg of diet) dicalcium phosphate 16.1; calcium carbonate 15.0; sodium chloride 8.35; magnesium sulfate 5.1; calcium phosphate 3.75; ferric citrate 1.375; magnesium sulfate 0.255; potassium iodine 0.04; copper sulfate 0.015; zinc sulfate 0.0125; cobalt chloride 0.0025.

[2] Composition of vitamin mixture are following (mg/kg of diet) vitamin A 19.8; vitamin D 1.38; vitamin E 110.0; Ascorbic acid 495; Inositol 55; Choline 2277; Menadione 24.75; D-aminobenzoic acid 55; Riboflabin 11; Thiamine 11; D-calcium pantothenete 33; Biotin 0.22; Folic acid 0.99; Vit B12 0.015.

housed in individual metabolic cages for balance study and diets were fed *ad libitum*. The experiment was consisted of 2-weeks diet adaptation and 7-day collection period followed by the intestinal Ca absorption study.

Calcium absorption study

Unfasted rats were anesthesized with sodium pentobarbital (30 mg kg^{-1} body weight (BW)) and a longitudinal abdominal incision was made to expose the whole length of the intestine. Duodenum, jejunum and ileum loops were made according the following procedure. The duodenum loop was made by ligating the small intestine at the pylorus and approximately 10 cm distally which was 2 cm beyond the ligament of Tritz. The jejunum loop was made by ligating at 10 and 20 cm distal from the ligament of Treitz. The ileum loop was made by ligating at the ileum-ceacum junction and 10 cm proximally. Before tightening the second ligation on the intestinal loops, 1.0 ml of test solution containing Ca^{45} was injected inside of the loops with the aid of a calibrated siringe then the ligation was tightened immediately after so that no leakage of the test solution from the loop would occur.

After injecting the test solution into three intestinal loops, the entire exposed small intestine was replaced into the abdominal cavity and the incision was clipped off then animals were put back into individual cages. Animals were usually recovered from anesthesia within 30 min and were kept at room temperature. A 120 min after the injection of the

test solution, animals were reanesthesized with chloroform, blood samples were taken by heart puncture, and the three intestinal loops were removed from rat. Then the loops were ashed for overnight and dissolved with a small amount of concentrated HCL (12N) and crucible was washed twice with a small volume of distilled water and transferred to 20 ml scintillation vials. Standard loops were also prepared in order to correct a loss of efficiency during the preparation procedure. They were the same intestinal loops as the experimental loops but excising immediately after the injection of the test solution. Radioactivity of the standard loops were used as amounts of instilled in the experimental loops. Radioactivity of solutions (plus 9ml PCS as a scintillator) was counted by liquid scintillation counter (Beckman LS-235) with following settings; preset error 0.3%, preset time 60 sec, lower discrimination window 0.5V and higher discriminator 9.9V.

Ca absorption was calculated as the difference between the total amount of instilled and recovered Ca^{45} assuming that a specific activity of Ca in the injected test solution did not change during the experimental period (120 min). In order to find Ca absorption, 6 test solutions different in their Ca concentration were used. The test solutions contained 1, 10, 25, 50, 100, and 150 mmol $CaCl_2$ and approximately 0.1 $\mu Ci\ ml^{-1}$ of $Ca^{45}Cl_2$ (800 $mCi\ nmol^{-1}$). NaCl was also added to the test solution containing 1-50 mmol $CaCl_2$ to render them isomolar with plasma (Table B.2).

Table B.2. Composition and characteristics of test solutions.

No.	(Ca^{2+}) mM	Radioactivity microCi/ml	NaCl[1] mM	Specific acitivity nanoCi/microMCa
1	1	0.1	135.0	100
2	10	0.1	121.5	10
3	25	0.1	99.0	4
4	50	0.1	61.5	2
5	100	0.1	0	1
6	150	0.1	0	0.67

[1] NaCl was added to the test solution to render them isomolar with plasma (271 mM).

Calculation of Ca absorption

The absorption curve was described by the following equation;

$J_{m-s} = J_{max} * (Ca_L^{2+}) / (K_t + (Ca_L^{2+})) + P * (Ca_L^{2+})$

where J_{m-s} --- total Ca flow from the lumen to the blood.

J_{max} --- the maximum saturable Ca flow.

(Ca_L^{2+}) - the luminal Ca concentration.

K_t --- the luminal Ca concentration at which $J_{max}/2$ is attained.

P --- diffusivity constant.

First part of the equation represents the active process which characterizes in saturable nature and second term represents the diffusive process. Experimentally J_{m-t} represents the difference between the amount of Ca injected and recovered from the loops at the end of the experimental period (120 min). (Ca_L^{2+}) represents Ca concentration of the injected test solution. The observed Ca absorption rate is a combination of both processes. Thus, in order to differentiate two components from a single observed curve, following procedure were exercised;

To arrive at the linear portion of the equation, a regression equation was derived by least-squares method (SAS) to estimate the best fit linear regression model. The procedure was based on individual loops at the upper Ca concentration ($50<(Ca_L^{2+})>150$ mmol).

To calculate K_t values, apparent half-saturation constant of the saturable process, a mean J_{m-s} value at the higher

concentration ($50<(Ca_L^{2+})>150$ mmol) with the predicted values for $P^*(Ca_L^{2+})$ was substracted from the observed values of J_{m-s}, then divided by two.

To obtain J_{max}, maximum saturable component of transepithelial Ca movements, observed J_{m-s}, predicted $P^*(Ca_L^{2+})$, and calculated K_t at lower concentration ($(Ca_L^{2+})>50$ mmol) was fit in the equation. This resulted in obtaining several values for J_{max}, thus , a mean and standard error was calculated.

Statistical analysis

Dietary treatment of differences in Ca absorption parameters were evaluated by analysis of variance and scheff's test was utilized for the comparison purpose between the treatments. All statistical analysis were carried out on the Statistical Analysis System (SAS institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Mineral composition and cation-anion balance of diets are presented in Table B.3. Ca content (1.03%) and Ca to phosphorus ratio (1.67) of experimental diets were higher than requirements (0.5% and 1.25, respectively). Cation-anion balance (meq kg^{-1} dry matter (DM)) of diet-1, diet-2, and diet-3 were +46.3, -52.9, and -53.3, respectively. Table B.4 shows body weight (BW), feed and water intake, and urine pH.

Table B.3. Nutrient composition of diets.

Nutrient	Diet-1	Diet-2	Diet-3
Calcium (%)	1.034	1.034	1.034
Phosphorus (%)	0.789	0.786	0.787
Ca:P	1.67	1.67	1.67
Sodium (%)	0.304	0.303	0.303
Potassium (%)	0.761	0.759	0.760
Chloride (%)	0.518	0.589	0.589
Sulfur (%)	0.226	0.351	0.352
Magnesium (%)	0.088	0.156	0.157
Cation-anion balance[1] (meq/kg)	+46.30	-52.86	-53.27

[1] Milliequvalent of [(Na+K)-(Cl+S)].

Table B.4. Body weight, feed and water intake and urine pH of rats fed diets differing in their dietary cation-anion balance.

Diet	No.observation	Initial BW (g)	Final BW (g)	BW change (g)	Feed intake (g/d)	Water intake (g/d)	Urine pH
1	30	242.9	281.0	5.44[a]	37.4[a]	37.4	7.22[a]
2	28	242.2	273.5	5.22[a]	24.3[b]	34.4	5.97[b]
3	29	237.3	277.0	6.74[b]	22.2[c]	37.2	6.79[c]
SEM		15.10	7.65	1.22	2.44	5.18	0.31

[abc] Means in the same colum with different superscripts are different (P<.05).

Rats fed diet-3 gained more ($P<.05$) weight during the 7-day collection period than those of diet-1 or diet-2, although feed intake of this group was the lowest ($P<.05$). Rats fed either diet-2 or diet-3 showed a reduced ($P<.05$) urine pH than that of diet-1 indicating that feeding negative cation-anion balanced diets created a mild acidosis in rats.

Figures B.1, B.2, and B.3 present Ca absorption by the duodenum, jejunum and ileum intestinal loops of rats, respectively. Regardless of diets offered, the duodenum and proximal jejunum loops showed existence of both the active and diffusive processes whereas the ileum loop showed only diffusive process. This confirmed that the active absorption process is operating only in upper part of the small intestine. This confirmed the result of Behar and Kerstein (1976) and Pansu *et al.* (1981; 1983a; 1983b). The saturable component of absorption was lower in the jejunum loops indicating that the duodenum absorbed more Ca than the jejunum by the active absorption process. Table B.5 represents detailed values of Ca absorption by the intestinal loops. A total amount of Ca absorbed by the duodenum loops was about one half of that of either the jejunum or ileum loops. Effect of feeding a reduced or negative cation-anion balanced diet on intestinal Ca absorption was not apparent from the figures. Table B.6 shows the active component of Ca absorption. There was no detectable active process in distal part of the small intestine in rats fed any of diets. Even though, there was no difference ($P>.05$) in the total amount of Ca absorbed among the experimental diets, some differences were found in both

Figure B.1. Duodenum calcium absorption (*in situ* loop method) of 55-day old male rats fed diet-1 (A), diet-2 (B), and diet-3 (C). Observed values (I) consist of diffusive (II) and active (III) absorption procceses.

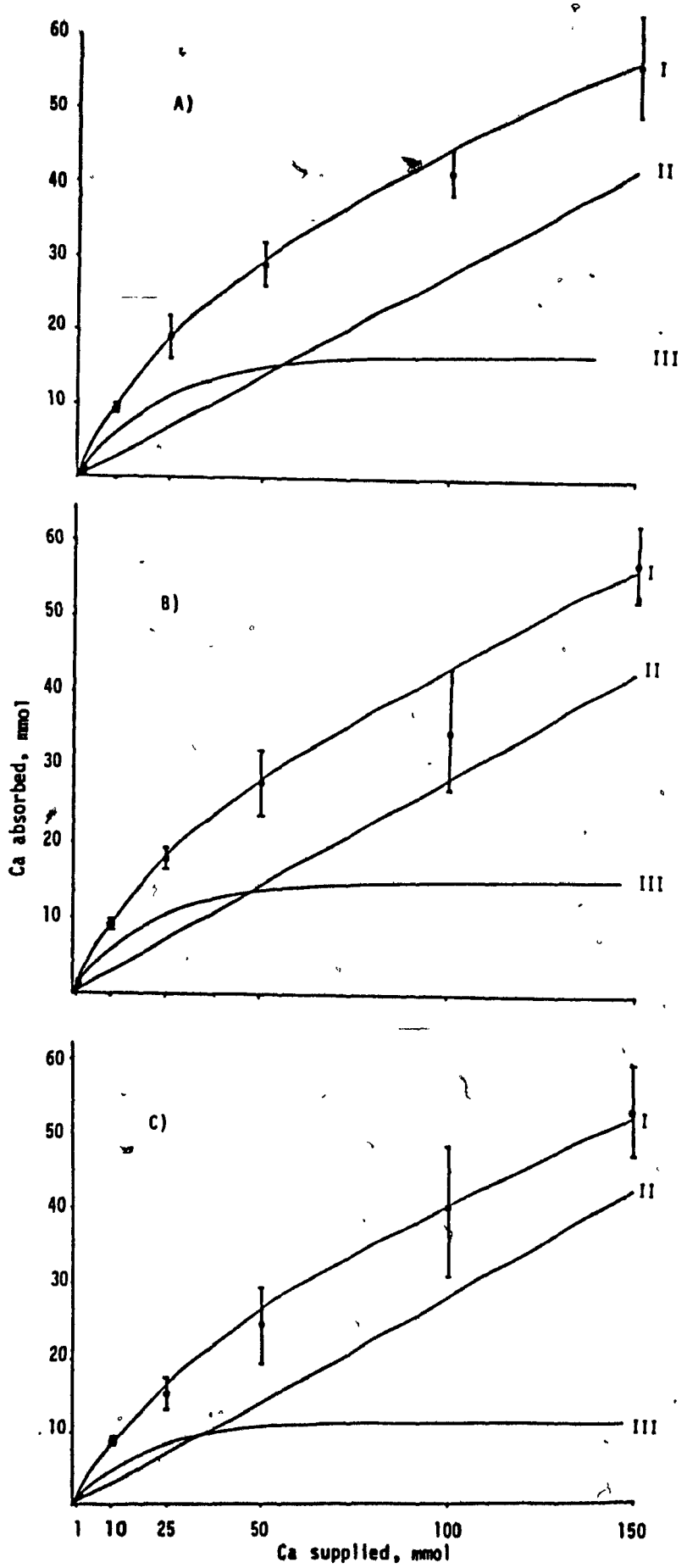

A)
B)
C)
I
II
III
60
50
40
30
20
10
Ca absorbed, mmol
1
10
25
50
100
150
Ca supplied, mmol

Figure B.2. Jejunum calcium absorption (*in situ* loop method) of 55-day old male rats fed diet-1 (A), diet-2 (B), and diet-3 (C). Observed values (I) consist of diffusive (II) and active (III) absorption proceses.

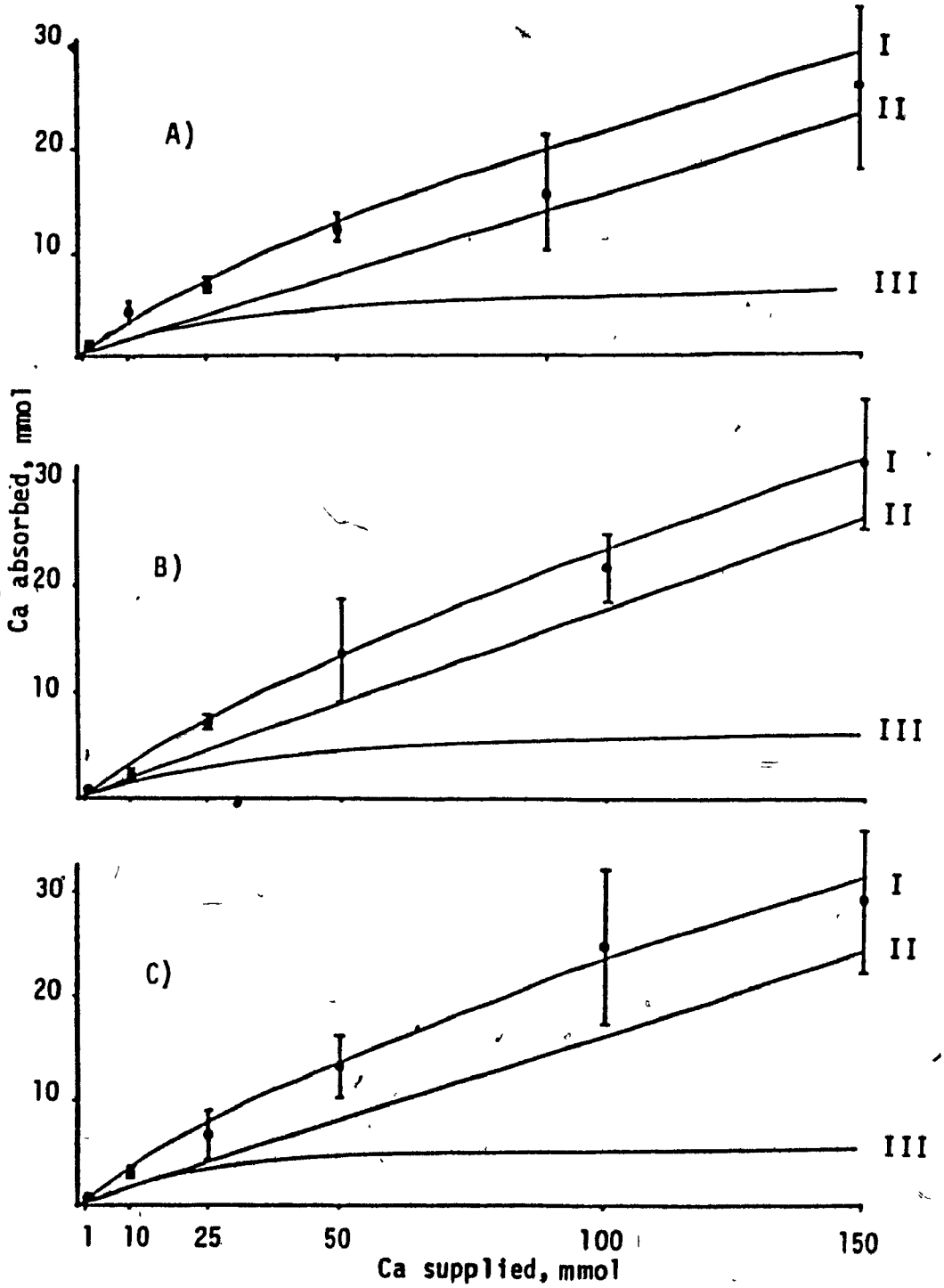

30
20
10
A)
I
II
III
Ca absorbed, mmol
30
20
10
B)
I
II
III
30
20
10
C)
I
II
III
1
10
25
50
100
150
Ca supplied, mmol

Figure B.3. Ileum calcium absorption (*in situ* loop method) of 55-day old male rats fed diet-1 (A), diet-2 (B), and diet-3 (C).

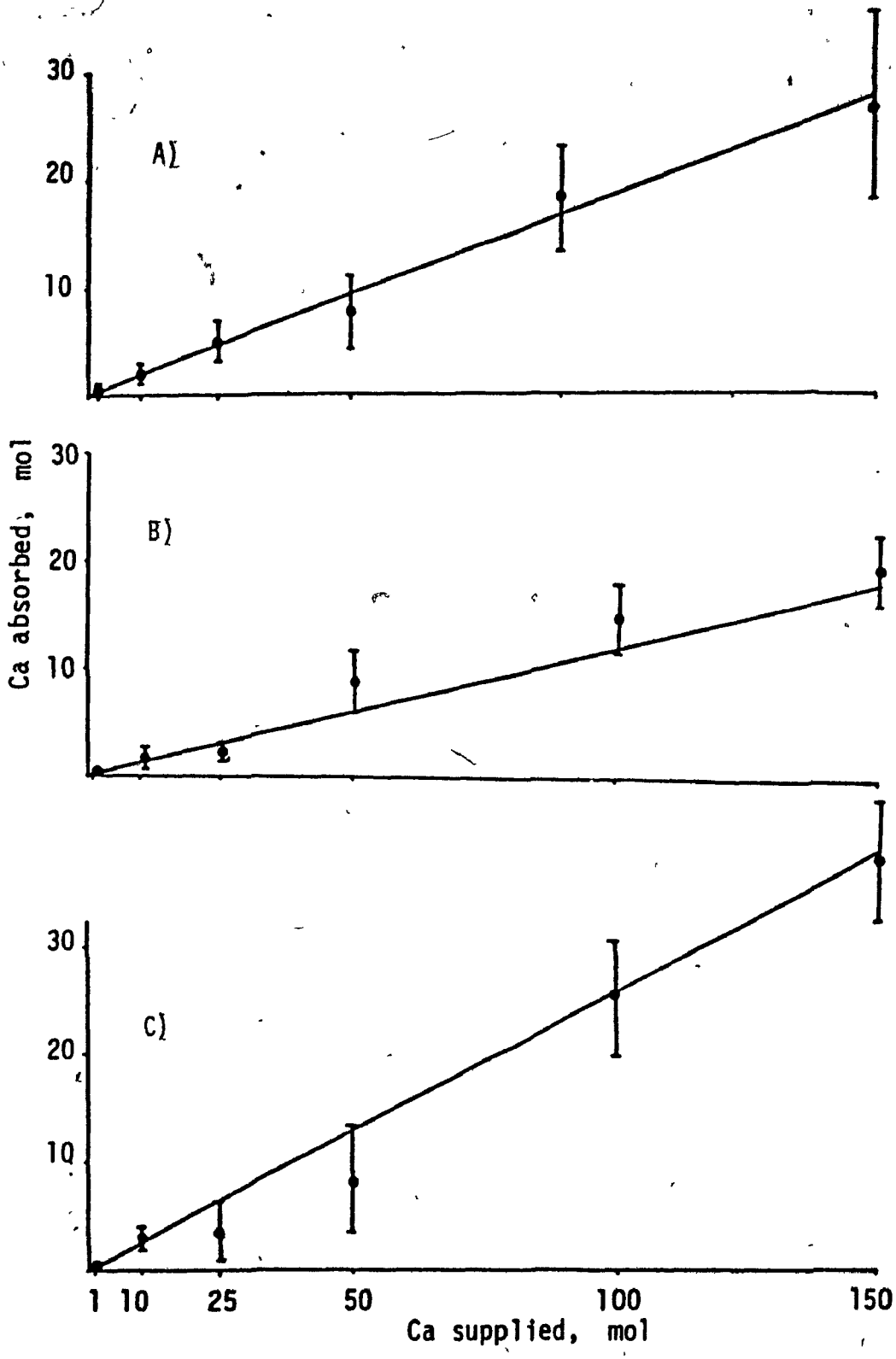

A)
B)
C)
30
20
10
Ca absorbed, mol
1 10 25 50 100 150
Ca supplied, mol

Table B.5. Calcium absorption by the intestinal loops of rats fed different cation-anion balanced diets[1].

Diet	(Ca^{2+}) of instilled solution (mM) 1	10	25	50	100	150
			(Duodenum loops)			
1	0.96±0.04 (4)	9.38±0.16 (2)	18.36±3.1 (5)	29.07±2.95 (3)	41.38±2.5 (3)	56.71±7.21 (3)
2	2.96±0.03 (4)	9.19±0.48 (4)	17.68±1.51 (4)	27.96±4.83 (5)	34.84±7.25 (4)	57.63±5.85 (3)
3	0.92±0.03 (4)	8.71±0.53 (3)	15.45±2.69 (4)	24.16±5.28 (4)	39.21±8.46 (4)	51.98±8.33 (5)
			(Jejunum loops)			
1	0.46±0.10[a] (4)	4.11±1.51 (4)	6.89±0.50 (4)	12.05±1.05 (3)	15.37±6.53 (3)	25.78±7.57 (3)
2	0.41±0.17 (3)	2.17±0.54[b] (3)	7.17±0.48 (3)	13.65±5.13 (4)	21.83±3.16 (4)	31.89±6.81 (4)
3	0.41±0.03 (4)	3.10±0.22[a b] (4)	6.72±2.62 (5)	13.04±3.59 (5)	24.85±7.64 (4)	29.30±7.46 (5)
			(Ileum loops)			
1	0.29±0.07[a b] (6)	2.23±0.90 (4)	5.07±1.87 (5)	7.82±3.30 (4)	18.65±4.74[a b] (3)	26.79±8.87[a] (3)
2	0.13±0.04[a] (3)	1.28±1.35 (3)	1.91±0.65 (3)	8.81±3.60 (5)	14.08±3.69[a] (4)	18.75±3.33[a] (4)
3	0.37±0.19[b] (3)	2.96±0.93 (4)	3.36±2.06 (4)	7.91±5.40 (5)	25.51±5.46[b] (4)	37.92±5.13[b] (4)

[1] Values are mean±standard deviation and the number of observations presented in parenthesis.
[a b] Means in the same column within each segment of intestinal loops with different superscripts are different ($P<.05$).

Table B.6. Active (saturable) component of calcium absorption by rats fed different cation-anion balanced diets[1].

Diet	J_{max}[2]	K_t[3]
	(Duodenum loops)	
1	16.29±1.96[a]	14.76±0.36[a]
2	15.19±1.62[a b]	14.52±0.35[a]
3	10.78±1.13[b]	10.63±0.27[b]
	(Ileum loops)	
1	3.75±0.69	4.99±0.13[a b]
2	2.41±0.99	4.22±0.27[a]
3	3.21±0.83	5.99±0.50[b]

[1] Values are mean±standard error. No detectable values are found in the ileum loops.
[2] J_{max} = the maximum saturable flux from lumen to the blood.
[3] K_t = luminal concentration at which J_{max} is attained.
[a b] Means in the same column and same intestinal loops with different superscripts are different ($P<.05$).

maximum saturable flux (J_{max}) and apparent half-saturation constant (K_t) of the duodenum and jejunum loops. In the duodenum loops of rats fed diet-3 had lower ($P>.05$) J_{max} than that of diet-1 and had lower ($P<.05$) K_t than those of diet-1 or diet-2. However, in the jejunum loops, rats fed diet-3 showed higher ($P>.05$) K_t than that of diet-1. Jmax values of duodenum loop observed in this experiment were similar to that of Pansu *et al.* (1983b) who reported J_{max} value of 18 umol in 60 day-old rats. However, J_{max} and K_t values of this experiment in general were lower than others (Pansu *et al.*, 1981; 1983a; 1983b). This difference may be caused by relatively high dietary Ca (1.03%) in this experiment compared to that of others (0.44%). Pansu *et al.* (1981) reported a reduction of J_{max} value from 28 to 18 umol with a n increase in dietary Ca level of 0.17 to 0.44%DM.

$1,25(OH)_2D_3$ is the most biologically active metabolites of vitamin D with respect to intestinal absorption of Ca, and alterations in intestinal absorption of Ca particularly in active absorption process appear to be mediated by changes in $1,25(OH)_2D_3$ (Dostal and Toverud, 1984: Yeh and Aloia, 1984). Lee *et al.* (1977) reported that a conversion of 25-hydroxyvitamin D_3 ($25(OH)D_3$) to $1,25(OH)_2D_3$ was impaired by systemic metabolic acidosis. However, metabolic acidosis failed to alter the concentration of $1,25(OH)_2D_3$ in plasma (Gafter *et al.*, 1980; Bushinsky *et al.*, 1982) and intestinal absorption of Ca (Gafter *et al.*, 1980). Reduction of J_{max} and K_t in duodenum loops of rats fed diet-3 in this experiment, therefore, might not be caused by the possible reduction in

$1,25(OH)_2D_3$ resulted from acidosis. This agrees with the finding that rats fed diet-2, which showed a similar reduction in urine pH compared to that of diet-3, did not showed a reduction in J_{max} or K_t values.

Table B.7 shows the diffusive component of the intestinal absorption. The slope of the non-saturable function (P) of duodenum observed in this experiment was similar to that of Zarnitzer and Bronner (1971) who reported an average value of 0.25 in animals on high- or low-Ca intake or of different ages. The slopes (P) of the duodenum loops were higher ($P<.05$) than those of the jejunum and ileum loops irrespective of diets offered. This indicated that not only the active component but also the diffusive component of Ca absorption was the most active in the duodenum segments of the small intestine. There was no difference ($P>.05$) in any parameters with dietary treatments in duodenum and jejunum intestinal loops. In ileum loop, however, proportionality constant (P) of diet-2 was lower ($P<.05$) and that of diet-3 was higher (P,.05) than CTR.

A possible adverse effect of cation group (Al, Fe, and Mg) accompanying anion group (Cl and S) on Ca absorption exist as evidenced by balanced studies (Alcock and MaCintyre, 1962: Barton *et al.*, 1983), however, there is no data available to indicate that these cations, in excess, affect a specific component of absorption processes.

Table B.7. Diffusive (unsaturable) component of calcium absorption by rats fed different cation-anion balanced diets[1].

Diet	No. observation	P[2]	r	Intercept
		(Duodenum loops)		
1	8	0.276±0.03	0.89	14.76±3.9
2	11	0.283±0.05	0.76	11.77±5.0
3	13	0.278±0.04	0.76	10.58±4.9
		(Jejunum loops)		
1	8	0.137±0.05	0.55	4.0±5.0
2	11	0.182±0.04	0.78	4.2±3.8
3	12	0.163±0.04	0.57	5.9±4.3
		(Ileum loops)		
1	9	0.179±0.02[a]	0.93	0.15±1.0
2	12	0.130±0.01[b]	0.92	0.30±0.9
3	13	0.262±0.02[c]	0.96	-1.80±1.3

[1] Values are mean±standard error.
[2] P=Proportionality constant (diffusivity constant) slope.
[abc] Means in the same column and same intestinal loops with different superscripts are different ($P<.05$).

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