Effects of manipulating the anion-cation balance in rations for prepartum dairy cows on hypocalcemic parturient paresis

by

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ABSTRACT

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A trial was conducted to investigate the response of prepartum dairy cows to reducing the level of dietary anion-cation balance by measuring the concentration of plasma macrominerals, the apparent absorption of macrominerals and the incidence of milk fever. Twenty prepartum Holstein cows were randomly allocated to 4 dietary treatments designated control, diets 1, 2 and 3, with a dietary anion-cation balance , measured as meq [(Na + K)-(Cl + S)] per kg of ration dry matter (DM) of +394.31, +120.65, +104.89 and +62.20 , respectively. The total ration DM contained 1.38% calcium (Ca), and 0.76% phosphorus (P). Incidence of milk fever was lower for cows fed diets 1, 2 and 3 (60% incidence) than for cows fed the control diet (80% incidence). Reducing the level of dietary anion-cation balance decreased the severity of the decline of plasma Ca during the periparturient period and delayed the time of observing the lowest concentration of plasma Ca. Strong negative correlations were observed during the periparturient period,

particularly at parturition, between the level of dietary anion-cation balance and the concentration of plasma Ca and plasma magnesium (Mg). Apparent absorption of Ca and Mg were not influenced by dietary treatment during period 1 (from day-24 to day-21 prepartum), however, apparent absorption was reduced in cows fed diets 2 and 3 compared to cows fed the control diet and apparent absorption of Mg was reduced in cows fed diets 1, 2 and 3 compared to cows fed the control diet during period 2 (from day-7 prepartum until day-1 postpartum).

Results showed that reducing the level of dietary anion-cation balance influenced the concentration of plasma Ca and Mg as well as their apparent absorption. This effect was more accentuated during the periparturient period and may be beneficial in the prevention of milk fever.

L'influence de la manipulation de la balance des anions et des cations dans les rations pré-vêlages pour vaches laitières sur la parésie hypocalcémique de parturition

RESUME

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Une expérience fut menée afin d'analyser la réaction des vaches laitières à une diminution de la balance alimentaire entre les anions et les cations dans les rations pré-vêlages, en mesurant la concentration des macrominéraux dans le plasma, l'absorption apparente de ces macrominéraux et l'incidence de la fièvre vitulaire. Pour cela, vingt vaches taries Holstein furent assignées aléatoirement à 4 différents traitements alimentaires désignés comme témoin, rations 1, 2 et 3, avec une balance alimentaire entre les anions et les cations mesurée comme milliéquivalents [(Na + K)-(Cl + S)] par kg de matière sèche (MS) de la ration, avec une valeur de +394.31, +120.65, +104.89 et +62.20, respectivement. La ration totale mélangée (base MS) contenait 1.38% de calcium (Ca) et 0.76% de phosphore (P). L'incidence de la fièvre vitulaire fut moins élevée pour les vaches consommant les rations 1, 2 et 3 (incidence de 60%) que pour les vaches consommant la ration témoin (incidence de 80%). Une réduction de la balance alimentaire entre les anions et les cations a atténué la diminution de la concentration du calcium (Ca)

dans le plasma durant la période péripartale et a retardé le moment où la plus faible concentration du Ca dans le plasma est observée. Des corrélations négatives furent observées durant la période péripartale, particulièrement à la parturition, entre la balance alimentaire d'anions et de cations et la concentration du Ca et du magnésium (Mg) dans le plasma. L'absorption apparente du Ca et du Mg ne furent pas influencées par les traitements alimentaires durant la période 1 (à partir du jour-24 jusqu'au jour-21 prépartum), cependant, durant la période 2 (à partir du jour-7 prépartum jusqu'au jour 1 postpartum), l'absorption apparente du Ca fut diminuée pour les vaches consommant les rations 2 et 3 comparée aux vaches consommant la ration témoin, et l'absorption apparente du Mg fut diminuée pour les vaches consommant les rations 1, 2 et 3 comparée aux vaches consommant la ration témoin.

Les résultats ont démontré qu'une réduction de la balance alimentaire entre les anions et les cations influence la concentration du Ca et du Mg dans le plasma ainsi que leur absorption apparente. Cet effet fut plus accentué durant la période péripartale et peut être avantageux dans la prévention de la fièvre vitulaire.

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I. INTRODUCTION

Milk fever (hypocalcemic parturient paresis) is an afebrile metabolic disorder that is typically associated with parturition and the onset of lactation. Animals that develop milk fever are unable to meet the sudden demand for calcium (Ca) that is lost from blood for synthesis of colostrum, which occurs at a faster rate than it is replaced (Braithwaite, 1976). In fact, approximately 2.5 g of Ca is extracted from blood for each kg of colostrum produced. This is approximately equal to the total amount of Ca present in the blood at any given time. Therefore, a dairy cow producing 25 kg colostrum will have to replace her total blood Ca every hour (Horst, 1986). Although a decline in concentration of plasma Ca is a normal event in cows at parturition, the decline is significantly greater in cows with milk fever (Braithwaite, 1976). Moreover, milk fever, which is characterized by hypocalcemia, is generally associated with hypophosphatemia. Typical symptoms of the disease include the inability to rise, a typical recumbant position, lowered temperature of extremities, grinding of teeth and inappetance (Block, 1984).

Milk fever is a " human-induced " disease because it is assumed that no such disease existed two or three centuries ago (Fish, 1927), but began to be known when human started to feed and select cows for higher milk producing capacity. Milk fever was first mentioned in the literature in Germany by Eberhardt in 1793 as reported by Hutyra _et al_.(1938). Since that time, there have been numerous theories as to the cause of milk fever. Hibbs (1950) listed some thirty theories in his review article on milk fever , most of them being disproved along the way. An important finding was that blood Ca declined by as much as 60% in animals with milk fever (Littledike and Wright, 1925). This observation led to a new form of treatment for milk fever, where calcium salts were injected intravenously to combat the effects of hypocalcemia. Although improved techniques for treating the disease have decreased the mortality of cows, the cause and prevention of the disease still remain obscure. Today, the incidence of milk fever varies greatly on an individual herd basis but represented 5% of the dairy cows in United States (Horst, 1986) and 8% of Swedish dairy cattle (Jonsson, 1978). It is associated with an important economic loss due to the reported 14% loss of the subsequent milk production (Block, 1984), the reduced productive life of a dairy cow of 3 to 4 years (Curtis _et al_., 1983) and the increased veterinary and labor costs. Moreover, it was reported (Horst, 1986) that cows with milk fever were three to nine times more likely to contract other postpartum disorders such as dystocia, displaced abomasum, retained placenta, ketosis and mastitis.

II. LITERATURE REVIEW

A. Factors contributing to milk fever

1. Age

Milk fever is uncommon before the third lactation and its incidence is highest from the fifth to the eighth lactation (Curtis _et al._, 1984). Dishington (1974) had reported a decreasing tendancy to contract milk fever in the oldest group as was also found by Jonnson (1960) with cows at their tenth and later lactations. Possible explanations concerning the increased tendancy to contract milk fever for cows at their third or greater lactation, may include a reduced efficiency of Ca utilization (Bush _et al._, 1970), a reduced rate of bone turnover and a reduced size of the exchangeable Ca pool in older animals (Braithwaite, 1976). Hansard _et al._ (1954) had reported a reduction in the true digestibility of Ca as animals aged, combined with an increased amount of endogenous fecal Ca, indicating that the older animals not only absorbed less but also retained less Ca than the younger animals.

2. Breed

The susceptibility to milk fever appears to differ with breed. Numerous researchers have found the Jersey breed to have the highest incidence (Bush et al., 1970; Littledike et al., 1981) with the Guernsey and the Holstein breeds being reported to be at high risk (Curtis et al., 1984; Block, 1984).

3. Genetic

According to Jonnson (1978), there is a clear-cut hereditary predisposition to milk fever. Based on analysis of bull-daughter groups, heritability was estimated at 0.13; presumably, the figure would be even higher if analysis were based on cow:daughter analysis and consequently, in Sweden, a cow is disqualified as a dam of an AI bull if she has contracted milk fever.

4. Hormonal status

Plasma levels of estrogen have been reported to be significantly higher for animals that develop milk fever than control animals in the prepartum period (Hollis et al., 1981). Bone resorption, measured by plasma levels of hydroxyproline, started to increase for the paretic animals when plasma levels of estrogen started to decrease. Estrogen has been shown to inhibit bone resorption in organ culture (Stern, 1969). Therefore, Hollis et al. (1981) suggested that the lag period for paretic cows in their skeletal response may be due to high concentrations of estrogen in the blood supply to the skeleton. However, Muir et al (1972) did not find any effect of estrogen or progesterone on bone resorption when they measured urinary excretion of hydroxyproline and, neither estrogen or progesterone were found to influence the incidence of milk fever when administered to cows for several days before parturition (Bargeloh et al., 1975).

Concentrations of plasma cortisol were observed to be higher in cows that contract milk fever than in healthy cows (Waage et al., 1984). Although the mechanism is unknown, the authors speculated that increased plasma cortisol concentration may inhibit intestinal absorption of Ca. The exact role of this hormone as a precipating agent of milk fever is still in question. It may be that cortisol allows the animal to respond better to a Ca stress (Horst and Reinhardt, 1983), as recent data suggested that cortisol may up-regulate $1,25(OH)_2$ D receptor in bone (Manolagas et al., 1979). This response may explain the protective effect of cortisol against induced hypocalcemia goats (Horst and Jorgensen, 1982).

5. Nutritional implications

5.1. Ca:P ratio and absolute quantity

Primary work on the influence of various amounts and ratios of Ca and P on the incidence of milk fever was done by Boda and Cole (1954). They suggested that the Ca to P ratio (Ca:P) of the prepartum diet had a direct effect on the incidence of milk fever. Jonnson (1978) showed numerous experiments that have been carried out with different Ca:P and with normal and high intake of Ca. Although results were conflicting and inconclusive, they suggested a higher incidence of milk fever when feeding high levels of Ca in prepartum diets with no, or only minor importance of the Ca:P. Braithwaite (1976) stipulated that there was little agreement on an optimum dietary Ca:P, probably because the ratio of available Ca:P is more important than the total amount of these elements in the diet. Some studies emphasized the importance of the Ca:P ratio rather than the absolute amount of both elements. In fact, Kendall (1968) reported an incidence of milk fever of 45% when cows were fed a ratio of Ca:P of 4.3:1 or 0.9:1, but the incidence was reduced to 5% when the ratio was 2.3:1. Gardner (1970) also speculated that the optimum ratio should be 2.3:1 since it is approximately the ratio of Ca:P in bone. However, some recent studies have demonstrated the importance of the absolute quantity of Ca and P. Schroeder (1973) concluded that there was no indication that a narrow ratio (1:1) will prevent or reduce the incidence of milk fever compared to a wide ratio (6.8:1), but they observed some beneficial effects on plasma Ca when cows were fed a prepartum diet low in Ca. Work done by Kichura et al. (1982) supports the previous finding since they found that a prepartum diet low in Ca (9.5 g/day), regardless of dietary intake of P, will decrease the incidence of milk fever. When cows received a prepartum diet high in Ca (150 g/day), they had a higher blood Ca level prepartum but were less able to maintain serum Ca levels through parturition. Cows did not have an increase in urinary hydroxyproline excretion near parturition as did cows fed the prepartum diet low in Ca (Black et al. 1973). A prepartum diet low in P (10 g/day) in the presence of high levels of Ca will help to prevent milk fever compared to a prepartum diet high in Ca and P (Kichura et al., 1982). In this latter case, the importance of the absolute quantity of Ca and P rather than the Ca:P ratio is stressed since the prepartum diet high in Ca and low in P had a wide Ca:P ratio (8.6:1) and the prepartum diet high in Ca and P had a narrow Ca:P ratio (1.05:1). High intake of P is known to suppress kidney formation of the 1,25(OH)$_2$D$_3$ needed for the production of calcium-binding protein in the intestine (Reinheardt and Conrad, 1980).

5.2. Magnesium

Evidence of an existing link between magnesium status of the cow and milk fever has been emphasized by various workers. In a review article, Sansom *et al*. (1983) reported facts that support the existence of clinical, experimental and theoretical evidence for a connection between the occurrence of subclinical hypomagnesaemia and milk fever. Vitamin D_3 (Payne *et al*., 1970) and 1 -hydroxyvitamin D_3 have both been found to be ineffective when administered to cows that were midly hypomagnesaemic. When cattle were hypomagnesaemic, they were less able to mobilise Ca than normo-magnesaemic animals in response to an artificially induced hypocalcemia (Sansom *et al*., 1982; Contreras *et al*., 1982). The effect of the magnesium status of the animal on the incidence of milk fever may also be related to the age of the animal because workers (Sansom *et al*., 1982; Contreras *et al*., 1982) showed that hypomagnesaemia reduced the Ca mobilisation rate in older animals by a greater proportion than it reduced it for younger animals. Magnesium has been shown to play an important role in the synthesis and/or the release of parathyroid hormone (PTH), and in the action of PTH upon the gastrointestinal tract or bone in rats (Anast *et al*., 1972; Rude *et al*., 1976).

5.3. High-grain and high- protein diet

An increase in the content of grain in prepartum ration from 0.5 to 1.0 percent of body weight has been reported to be effective in the prevention of milk fever (Kendall _et al._, 1966). Other researchers (Curtis _et al._, 1984) found an inverse relationship between feeding high levels of grain in the prepartum diet and incidence of milk fever. However, results were not confirmed in other studies where a higher incidence of milk fever was noted when concentrates were added to a roughage diet during the dry period (Emery _et al._, 1969; Gardner, 1969) Beneficial effects of high grain diets fed prepartum on the incidence of milk fever may be due to an acidifying effect of the diet. This was supported by a study by Harmon and Britton (1983) who observed a disturbance in the acid-base balance (decreased blood pH and bicarbonate) in lambs fed a high grain diet, an increase in the excretion of urinary Ca and an increase in the excretion of urinary hydroxyproline.

Intake of high levels of protein during the prepartum period was also reported to decrease the incidence of milk fever (Curtis _et al._, 1984). In the rat, a high protein diet will increase excretion of urinary Ca by increasing the intestinal absorption of dietary Ca and by shifting the route of excretion of endogenous Ca from the feces to the urine. No effect were observed on bone resorption (Bell *et al.*, 1975; Whiting and Draper, 1981).

B. The calcium homeostatic mechanism with special reference to milk fever

Calcium homeostasis is regulated through three major mechanisms: absorption by the intestinal mucosa cells; urinary Ca reabsorption; and bone resorption. In the transition from pregnancy to lactation, the maternal drain of Ca to the fetus ceases but it increases to a greater extent within a few hours for the formation of colostrum. Because of delays inherent in the feedback pathways, hypocalcemia develops in most cows at the onset of lactation but more severely for cows that contract milk fever (Ramberg, 1984).

1. Intestinal absorption of calcium

The physiological need for Ca by the animal and the content of Ca in the diet will determine the efficiency of Ca absorption (Horst, 1986). Furthermore, the age of the animal will have a significant influence on the ability of the intestine to adapt to Ca stress. As an animal ages, the efficiency of intestinal absorption of Ca in response to Ca stress will be less than for a younger animal (Horst *et al*, 1978). The absorption of Ca from the gastrointestinal tract mainly occurs in the upper intestine (Horst, 1986). Calcium is absorbed by two processes; active transport and passive diffusion where 1,25$(OH)_2$ D_3 appears to influence both processes (Wasserman, 1981). The 1,25$(OH)_2D_3$ will stimulate the synthesis of calcium-binding protein, which controls active Ca transport into the mucosa cell of the intestine. Transport of Ca out of the cell is against a concentration gradient and is accomplished via a high affinity Ca-activated adenosine triphosphatase or Na^+/Ca^+ exchange (Wasserman, 1981). Dependence on intestinal absorption of Ca to recover from hypocalcemia during the periparturient represents a precarious situation that may lead to an unstable response (Ramberg *et al*, 1984). In fact, hypocalcemia may decrease gut motility as well as the movement of Ca from the rumen to absorption sites in the small intestine (Moodie, 1960) because smooth muscle is dependent on extracellular Ca for its contractile activity (Ramberg *et al*, 1984). Anorexia has also been reported to occur at the onset of lactation (Marquardt *et al*, 1977), which will decrease the supply of Ca for intestinal absorption. Development of milk fever, if related to an impairment of the intestinal absorption of Ca, is not due to a lack of plasma 1,25$(OH)_2D_3$ concentration since it was shown that it responds appropriately and rapidly in paretic cows (Horst *et al*., 1978; Horst and Reinhardt, 1983). The delay in response, if it exists, should be related to an intestinal target organ responsiveness to blood-borne feedback signals (Ramberg et al., 1984).

2. Bone resorption

Calcium mobilization from bone to support the concentration of plasma Ca is under the influence of the hormones, $1,25(OH)_2 D_3$ and PTH (Horst, 1986). At the onset of lactation, the ability of bone to respond by resorptive processes is somewhat refractory to the stimulus of PTH and $1,25(OH)_2D_3$ thus, resorption of bone may play a minor role for the maintenance of plasma Ca until 1 or 2 weeks postpartum (Ramberg et al., 1984). However, if cows are fed a prepartum diet low in Ca, the bone will contribute to the Ca pool. There is only a small fraction of the Ca of bone that is rapidly exchangeable with the ionic Ca in blood and soft tissue, and the size of the exchancheable Ca pool has been shown to be reduced with age (Braithwaite, 1976). It has been demonstrated that paretic cows will have increased concentration of plasma PTH and $1,25(OH)_2 D_3$ (Horst et al., 1978) and lower concentration of plasma calcitonin than non-paretic animals (Mayer et al., 1975). However, these cows will have an acute inability to resorb Ca from bone (Horst, 1986). Microradiographic and histological evaluation of cortical and trabecular bone taken from milk fever-prone cows suggested an impairment of osteoclast function possibly resulting from either a lack of or a malfunction in monocytes (Horst, 1986).

Recently, some researchers have suggested a possible role for the metabolite 24,25$(OH)_2D_3$ with the onset of milk fever. This metabolite appears to be involved in the process of bone formation (Norman, 1980), A negative correlation has been established between plasma 24,25$(OH)_2D_3$ and concentration of plasma Ca (Smith et al., 1982). Also, Barton et al. (1984) have shown that injecting pharmacological dose of 24,25$(OH)_2D_3$ to dairy cows around parturition resulted in an increased incidence of milk fever. However, Horst et al. (1979) did not observe significant differences in plasma 24,25$(OH)_2D_3$ between paretic or non-paretic cows. Therefore, Horst (1986) stipulated that under physiological conditions, 24,25$(OH)_2D_3$ is unlikely to play any major role in the incidence of milk fever.

3. Calcium excretion

3.1. Urinary calcium

The bovine kidney is highly efficient in conserving calcium since 99% of the glomerular filtered Ca is reabsorbed (Kronfeld et al, 1976). In cattle, urinary excretion of Ca does not vary with dietary intake of Ca (Ramberg et al, 1975), however, it is substantially increased with P deficiency (Nel et al, 1974) or after the ingestion of acidic substances (Vagg et al, 1970). Therefore, it is an important route of excretion in times of stress (Braithwaite, 1976). However, urinary excretion of Ca plays only a minor role in combating hypocalcemia (Ramberg et al, 1975); its role may be more important in alleviating hypercalcemia (Ramberg et al, 1975).

3.2. Endogenous fecal calcium

Endogenous fecal excretion of Ca has been reported to increase linearly with feed intake but it is independant of intake of dietary Ca (Braithwaite, 1982). The physiological states of pregnancy or lactation did not alter the endogenous loss of fecal Ca (Braithwaite, 1982), however, this loss increased slightly in cows made hypercalcemic by the administration of parathyroid extract (Mayer et al, 1967).

3.3. Calcium loss in colostrum

Colostrum contains twice as much Ca as milk, thus the secretion of one litre of colostrum would remove all the Ca contained in 24 litres of plasma, equivalent to the plasma in a 500 kg cow (Ramberg et al, 1975). Studies of the movement of radioactive Ca indicated a delay of 6 hours between plasma and milk, which may represent a Ca compartment in the udder

(Kronfeld *et al*, 1971). Secretion of Ca into colostrum is not greater for paretic cows (Hibbs, 1950) and may actually be reduced (Nurmio, 1968). Calcium removal from the blood to colostrum begins during the prepartum period, and the rate of this extraction of Ca may be important in contributing to the development of hypocalcemia (Ramberg *et al*, 1975).

C. Prevention of milk fever

Prevention is the most desirable means of reducing the economic losses occurring from milk fever. Because of the importance of calcium metabolism in the etiology of milk fever, preventative approaches have been focused in this direction. Dietary manipulations as well as injections of vitamin D_3 and its metabolites have been reported in the literature as possible methods for reducing the incidence of milk fever.

1. Vitamin D_3 and its metabolites

Large oral doses of vitamin D_3 (20-30 million units) given at least 3 days prior to parturition and for no more than 7 days, have successfully prevented milk fever (Hibbs and

Conrad, 1960). Also, a single intramuscular dose of 10 million units of vitamin D_3 given within 10 days prepartum afforded reasonable protection against milk fever (Payne and Manston, 1967). However, accurate prediction of the date of parturition is needed, otherwise, repeated treatments may lead to toxicity problems (Littledike and Horst, 1982). Moreover, such treatments will significantly reduce the incidence of milk fever for cows prone to the disorder but may increase the incidence in normally healthy cows and therefore, has the disadvantage that it cannot be used for all animals in a herd (Braithwaite, 1976). This latter statement places the oral treatments at a particular disadvantage. The recent discovery of active metabolites of vitamin D_3 has led to renewed interest in the prevention of milk fever since these metabolites have advantages over vitamin D_3 itself. They act more quickly, are required in smaller amounts and are metabolized faster. Therefore, they can be applied at more frequent intervals (Braithwaite, 1976). Hove et al. (1983) compared intramuscular injection of $1,25(OH)_2D_3$ and of $1-(OH)D_3$ and found that, for the same dose, plasma $1,25(OH)_2D_3$ will peak higher at a faster rate when injected the $1,25(OH)_2D_3$ than when injected with $1-(OH)D_3$. They also observed that the decline from peak concentration will occur more slowly after an oral treatment (of either one of the metabolites) than after an intramuscular treatment. This latter finding may have some importance since Horst (1986) observed an inhibitory effect of these compounds on kidney 1, hydroxylase and a dependance of the animals on exogenous 1,25$(OH)_2D_3$ to reverse their hypocalcemia. It seems that a possible reason for deleterious effects of parenteral injections of pharmalocogical doses of 1,25$(OH)_2D_3$ will be caused by the acute removal of the drug from the injection site, therefore, an oral dose may be a useful alternative to the parenteral treatment (Hove et al., 1983).

2. Dietary manipulations

2.1. Ca:P ratio and absolute quantity

Whenever it is possible, feeding a prepartum diet low in Ca is the desirable choice for preventing the incidence of milk fever (Block, 1984). In fact, it has been shown (Goings et al., 1974) and accepted by the National Research Council (NRC, 1978) that this method will effectively prevent milk fever. Recommended levels of Ca and P in the prepartum diet are 0.37% and 0.26%, respectively (NRC, 1978). More Ca may be included in the diet of dry cows if the amount of P is controlled strictly at the level recommended by NRC (1978) (Julien et al., 1976) and the total amount of Ca consumed by cows does not exceed 100 g per day for Holsteins and 60 g daily for Jerseys (Jorgensen, 1973).

2.2. Addition of acid

For more than fifty years attention had been given to the addition of acid to diets of dairy cow in order to influence the homeostatic mechanism of calcium. In fact, Hart et al. (1931) fed 115 or 230 ml of concentrated hydrochloric acid (HCl) to lactating dairy cows and observed an increased urinary excretion of Ca as well as an increased intestinal absorption of Ca. The same trends were observed by Freeden et al. (1984) when they offered a ration containing 1.2 per cent HCl to non-lactating goats. Treated goats also developed a metabolic acidosis indicated by a lower blood pH, pCO_2 and HCO_3^- than control animals. Addition of ammonium chloride (NH_4Cl) to diets of non-lactating goats (Horst and Jorgensen, 1971) decreased urinary pH and increased urinary excretion of Ca and Cl. Bushinsky et al. (1982) observed a suppression of the increase in $1,25(OH)_2D_3$ levels for rats fed a diet low in Ca when they created a chronic metabolic acidosis by adding 1.5 per cent NH_4Cl to the diet. The PTH response to the diet low in Ca was not suppressed. Although total serum Ca and P levels were not affected by acidosis, blood ionized Ca levels were increased. Few studies have been done on the acidification of the diet in order to prevent the incidence of milk fever. Kendall et al. (1969) observed a lower incidence of milk fever and a higher concentration of plasma Ca and P on day-1 postpartum when they added 150 g of NH_4Cl to prepartum diets of dairy cows. However, in this particular study, the number of cows was too small to allow definite conclusions concerning the preventative effect of the diet (Jonsson, 1978). Reduction of the pH of the prepartum diet, using 1.65 per cent phosphoric acid in the grain mixture, increased calcium absorption in the early postparturient period (Verdaris and Evans, 1975). The highest calcium absorption was observed in the high calcium-low pH group. In the experiment of Dishington (1975), lowering the pH of the prepartal diet using an AIV-silage, which is a mineral acid composed of hydrochloric acid and sulfuric acid, did not appear to reduce the incidence of milk fever. They stipulated that the dietary anion-cation balance had more importance than the pH of the diet in the prevention of milk fever.

2.3. Manipulating the dietary anion-cation balance

Anion-cation balance refers to a balance between the total anions and the total cations (Neathery, 1981). It is expressed as milliequivalents (meq) per g or per kg ration dry matter (DM). This concept has been used in research in poultry nutrition with the calculation including the cations, sodium and potassium and the anion, chloride (Sauveur and Mongin, 1978). The importance of this concept in poultry is emphasized by the effect of anion-cation balance on the acid-base status of the animal (Cohen and Hurwitz, 1974) and on the egg shell strength of the laying hen (Hamilton, 1981). In ruminant nutrition, the importance of the anion-cation balance, as described by the poultry researchers, has been pointed out by Wheeler (1981). He observed a consistent improvement either in weight gain for beef steers or in milk production for dairy cows when the anion-cation balance of the control diet had been near 10 meq per 100 g of ration DM. Manipulating the dietary anion-cation balance has also been used to prevent milk fever in dairy cows. Norwegian workers defined the anion-cation balance (alkali-alkalinity) as the summation in milliequivalents, of the cations, sodium and potassium minus the summation of the anions, chloride and sulfur. Milk fever was successfully prevented in 92 per cent of the cases when prepartum dairy cows were fed rations with a negative anion-cation balance, composed of a mixture of mineral salts with a high content of Ca (Dishington, 1975). A better response to the diet was observed when dietary concentration of Ca was high compared to low (Ender _et al_., 1971), which may be explained by the fact that negative dietary anion-cation balance increased absorption of Ca when cows are kept in positive balance of Ca but not in negative balance (Lomba _et al_., 1978). In most of the cases, concentration of plasma Ca and P were maintained higher in cows fed the negative anion-cation balance (Dishington, 1975). Beneficial effects of a negative dietary anion-cation balance, using the same mineral salts as Dishington (1975), was observed by Block (1984). Milk fever was prevented in 100 per cent of the cases when prepartum dairy cows were fed a diet of -128.5 meq per kg of ration DM but, 47 per cent of the cows did get milk fever when they received a diet of +330.5 meq per kg of ration DM. Concentration of plasma Ca and P were maintained higher during the periparturient period in cows fed the negative anion-cation balance. Plasma hydroxyproline levels started to increase during the prepartal period and remained higher until day-2 postpartum in cows fed the negative diet.

The promising results obtained by the Norwegian workers (Ender et al., 1971; Dishington, 1975) and by Block (1984) led to the design of the trial that follows with the following hypothesis: Reducing the dietary anion-cation balance of rations that are high in Ca and P for prepartum cows, will decrease the severity of the decline of plasma Ca during the periparturient period, which will reduce the incidence of milk fever. The objectives of the trial were to determine the response of prepartum dairy cows to a reduced dietary anion-cation balance by investigating the concentration of plasma macrominerals, the apparent absorption of macrominerals and the incidence of milk fever.

III. MATERIALS AND METHODS

1. Animals and management

Twenty Holstein dairy cows from the Macdonald College Farm herd were used that ranged in age from 4 years 6 months to 10 years, and ~~had completed~~ 3 to 7 lactations. Body weight at 45 days prepartum ranged from 745 to 993 kg. Milk produced in the previous lactation (calculated for 305 days) ranged from 6,840 kg to 11,140 kg of milk. At 45 days before the expected calving date, cows were transferred to individual box stalls with sawdust used as bedding. At that time, cows received a 5 ml injection of vitamin A-D composed of 2.5×10^6 IU of vitamin A and 3.75×10^5 IU of vitamin D (MTC Pharmaceutical, Missisauga, Ontario.) and a 14 ml injection of vitamin E-Selenium composed of 42 mg of sodium selenite , 1904 IU of d-tocopheryl acetate and 21% benzyl alcohol used as a preservative (Dystocel,Rogar/STB. Division BTI product). A second dose of vitamin E-Selenium was given 3 weeks before the expected calving date.

Access to automatic waterers was unrestricted. Water was analysed for mineral composition (see Table 1). Because of the impossibility to control the intake of water, it was decided to disregarde this possible source of mineral.

Table 1. Mineral composition of the Macdonald Farm water.

Mineral	Concentration (ppm)
Calcium	17.10
Phosphorus	3.38
Magnesium	2.75
Iron	0.00

Cows were weighed weekly at 0900 h. Cows were fed their experimental diets twice daily at 0500 h and 1700 h from day 45 before expected calving date until the day of parturition when they were switched to the normal feeding program of the Macdonald Farm. Daily rations were prepared once daily at 1700 h and divided into 2 separate buckets, one for the feeding of the next morning and one for the feeding of the same afternoon as mixing. Ingredients were weighed and mixed manually to obtain a homogeneous ration. Ration refusals were weighed once daily immediately prior to the afternoon feeding and discarded. Refusal samples were collected weekly for analysis of dry matter (DM). Two representative samples were collected weekly,one for the analysis of dry matter and the other was frozen (-20° C) for further analysis. Ration mixing and amounts offered on a percentage of body weight basis were corrected weekly for DM. Intake of DM was restricted to 2% of body weight to overcome possible problems of fat cows.

Cows were diagnosed as having milk fever only if they exhibited two or more of the typical symptoms of the disease (ie. loss of appetite, inability to rise, cold extremities, typical recumbant position, grinding of teeth). Cows diagnosed with milk fever were treated intravenously with 500 ml of a 1.7% calcium gluconate solution (Cal-dextrose). If cows did not respond to treatment or relapsed, they received a 500 ml intravenous injection of 23% calcium borogluconate

(Cal-Mag-K).Dose number varied according to the severity of the disease. Blood samples were taken immediately prior to the solution infusion.

2. Preparation and Composition of the Diet

Composition of experimental diets is shown in Table 2. Ration were composed (DM basis) of 57% alfalfa haylage and approximatively 38% corn silage and 5% mineral mix. Four different rations were offered to cows according to their respective treatments that varied depending on the anion-cation balance of the treatment. Anion-cation balance was calculated using the equation: milliequivalents [(Na^+ + K^+) - (Cl^- + $S^=$)] and expressed per kg of ration DM (Block,1984). Alfalfa haylage and corn silage were harvested at the Macdonald College Farm. Mineral salts used to alter the anion-cation balance such as calcium phosphate ($CaHPO_4$), calcium sulfate ($CaSO_4$), sodium phosphate monobasic ($NaH_2PO_4.H_2O$), calcium carbonate ($CaCO_3$), magnesium sulfate ($MgSO_4.7H_2O$), iron sulfate ($FeSO_4.H_2O$) and potassium iodide (KI) were purchased through Fortamix (division of BASF CANADA Inc.) and anhydrous cupric sulfate ($CuSO_4$), crystal cupric chloride ($CuCl_2.2H_2O$), manganous sulfate monohydrate ($MnSO_4.H_2O$), crystal manganous chloride ($MnCl_2.4H_2O$) and granular zinc chloride ($ZnCl_2$) were purchased through Anachemia Ltd. Montreal.

Table 2. Composition of rations offered to cows beginning at day-45 before expecting calving date until parturition.

Ingredients	Control	Trt 1	Trt 2	Trt 3
	--------% of total dry matter---------------			
Alfalfa haylage	57	57	57	57
Corn silage	38.5	38.7	38.3	38.07
Mineral mix	4.48	4.30	4.52	4.93
$NaH_2PO_4.H_2O$	1.9	0.65	0.65	0.65
$CaHPO_4$	0.68	1.91	1.91	1.91
$CaSO_4$	—	1.48	1.48	1.48
$CaCO_3$	1.88	—	—	—
$MgSO_4.7H_2O$	—	0.22	0.22	0.44
$FeSO_4.H_2O$	—	0.01	0.22	0.40
$CuSO_4$ (ppm)	—	105	205	400
$CuCl_2.2H_2O$ (ppm)	56	—	—	—
$MnSO_4.H_2O$ (ppm)	—	70	70	70
$MnCl_2.4H_2O$ (ppm)	86	—	—	—
$ZnCl_2$ (ppm)	56	56	56	56
KI (ppm)	0.65	0.65	0.65	0.65
Calculated anion-cation balance (meq/kg DM)	+400	+200	+100	+50

Mineral salts were mixed for 15 minutes in a stainless steel mixing bowl with a 50 kg capacity using stainless blades. Batches of 15 kg mineral mix were prepared and stored in covered plastic containers.

3. Blood sampling

Blood samples were taken once a day at 0800 h on days 45,35,25,20,15,14,13,12,11,10,9 and 8 prepartum, twice daily at 0800 h and 1800 h from day-7 prepartum to day-3 postpartum, followed by sampling once daily at 0800 h on days 5 and 10 postpartum. Blood samples were taken from the tail vein to avoid possible stress due to frequent blood sampling. Vacutainers with 7 ml capacity containing 100 usp units of lithium heparin as an anticoagulant were used with a 20 gauge 1/2 in needle (Becton Dickinson & Company). Blood samples were then centrifuged at 1100 x G for 10 minutes (Sorvall RC2-B automatic refrigerated centrifuge). Plasma was recovered using a Pasteur pipette and transferred into a 7 ml plastic vial and frozen (-20° C) for further analysis.

4. Digestibility study

Two digestibility studies were conducted, one from day-24 to day-21 prepartum and the second one from day-7 prepartum to day-1 postpartum. Feces samples were not collected at day-1 postpartum when cows tend to become anorexic . Chromic sesquioxide (Cr_2O_3) was used as a marker and mixed with the mineral premix to obtain 0.5% Cr_2O_3 in the total ration DM. An adaptation period of 7 days was allowed before each collection period to obtain complete recovery of the Cr_2O_3 in the feces. The equation used to calculate the percentage of DM digestibility was:

$$\% \text{ DM. digestibility} = 100 - \left(\frac{\% \, Cr_2O_3 \text{ feed} \times 100}{\% \, Cr_2O_3 \text{ feces}} \right)$$

Calculation of the apparent absorption of the minerals was:

% apparent absorption of mineral=

$$100 - \left(\frac{\% \, Cr_2O_3 \text{ feed} \times \% \text{ mineral feces} \times 100}{\% \, Cr_2O_3 \text{ feces} \;\; \% \text{ mineral feed}} \right)$$

Fecal grab samples were collected three times a day, at 0800h 1200 h and 1600 h and immediately stored in a refrigerator (8° C) until the end of the collection period, when they were dried in a forced-air oven (Despatch

Industries Inc.) at 50° C for 48 h. Dry feces were ground using a Thomas-Wiley Laboratory Mill (model 4) with a 1 mm screen and mixed thoroughly for 5 minutes. Subsamples of 200 grams were kept for further analysis.

5. Analytical procedures

5.1. Feeds

Frozen samples of alfalfa haylage and corn silage were thawed, composited on a monthly basis, dried in a forced-air oven at 50° C for 48 hours and ground in a Thomas-Wiley Laboratory Mill (model 4) with a 1 mm screen. These samples were analysed for nitrogen by the Kjeldahl method (AOAC,1984) using a Kjel-Foss macroautomatic analyser (A/S N. Foss Electric, Hillerod, Denmark). Wet digestion was conducted (AOAC 1984) before analysing samples for Ca,Mg,Na,K and S. Digested samples were diluted 1:100 with deionized distilled water. For analysis of calcium, an aliquot of the digested alfalfa haylage was diluted 1:25.25; 25% of the total volume was a 1% lanthanum oxide and the remainder was deionized distilled water. For corn silage, a dilution of 1:5.05 was used with the same ratios of lanthanum oxide-to-water as the diluent. A standard curve was constructed using standards of

1, 4 and 7 ppm of calcium. For the analysis of magnesium, an aliquot of the digested alfalfa haylage was diluted 1:50.50 with deionized distilled water and the same proportion of lanthanum oxide than for the calcium analysis.The dilution used for corn silage was 1:49.49. A standard curve was constructed using standards of 0.1, 0.3 and 0.5 ppm of magnesium. For sodium, an aliquot of the digested alfalfa haylage was diluted 1:25.05; 0.001 % of the total volume was a 1000 ppm solution of potassium and the remainder was deionized distilled water. The dilution used for corn silage was 1:5.01. A standard curve was constructed using standards of 0.3, 0.8 and 1 ppm of sodium. For potassium, an aliquot of the digested alfalfa haylage was diluted 1:245.99; 0.002% of the total volume was a 1000 ppm solution of sodium and the remainder was deionized distilled water. The dilution used for corn silage was 1:96.43. A standard curve was constructed using standards of 1, 1.5 and 2 ppm of potassium. All the above mineral determinations were conducted on an Atomic Absorption Spectrophotometer (Perkin-Elmer model 360) using a direct method. For the determination of chloride, an indirect method was used by precipating a known excess of silver. The chloride extraction was a modified method of Cantliffe D.J. et al (1970). One half gram of alfalfa haylage sample was placed in a 40 ml plastic tube with 39 ml of 0.1 N HNO_3 (dilution of 1:39). This mixture was stoppered and shaken using a wrist-action shaker for 15 minutes. The tubes were placed in a centrifuge for 10 minutes at 11,100 x G. One ml of the extract was transferred into a 16x100 mm culture tube with 0.2 ml HNO_3 concentrated and 1 ml of a 500 ppm silver nitrate solution and mixed using a vortex; 7.8 ml of deionized distilled water was added prior to placing the samples in a centrifuge at 770 x G for 10 minutes. An aliquot of the supernatant was diluted 1:10 with deionized water for silver determination by Atomic Absorption Spectrophotometry (Perkin Elmer model 360). A standard curve was constructed using standards of 0.5, 2.5 and 5 ppm of silver. The following equation was used to calculate the chloride concentration (ppm):

((blank x 10) - (value x 10)) x 3.29 x dilution factor

For the corn silage sample, 1 g sample of feed was used with 20 ml of 0.1 N HNO_3 solution (dilution of 1:20). Phosphorus determination was conducted using the spectophotometric method (AOAC 1984) with molybdovanadate as the reagent. The digested alfalfa haylage was diluted 1:5.05 and the digested corn silage was diluted 1:2.50 with deionized distilled water. A standard curve was constructed using standards of 0, 1, 3 and 5 ppm of phosphorus.

5.2. Feces

Fecal samples were analysed for Ca, Mg, P, Na, K and Cl using the same methods as described in the feed section, however, different dilutions were used. Total dilution for analyses of calcium and phosphorus was 1:5150, 1:10,300 for magnesium, 1:20,000 for sodium, 1:2505 for potassium and 1:30 for chloride determination. Chromium (Cr) was also determined in fecal samples using a total dilution of 1:3980; 25% of the total volume was a 8% ammonium chloride solution and the remainder was deionized distilled water. A standard curve was constructed using standards of 1, 2, 4 and 5 ppm of chromium. The percentage of chromic sesquioxide (Cr_2O_3) was calculated as:

$$\frac{\text{ppm of Cr} \times \text{dilution factor}}{10{,}000 \times 0.34}$$

5.3. Blood and plasma

Plasma aliquots of 0.6 ml were taken after thawing frozen plasma. The samples were placed in 16 x 100 mm culture tubes with 2.4 ml of a 10% trichloroacetic acid solution and mixed with a vortex mixer. Ten minutes after mixing, the samples were centrifuged at 770 x G for 10 minutes. The supernatant was recovered with a Pasteur pipette and transferred to 16 x 100 mm culture tubes for analysis of Ca, Mg, P and K using the same methods described in the feed section, however,different dilutions were used. An aliquot of the supernatant was diluted 1:12 for analysis of calcium and magnesium, 1:5.05 for analysis of P and 1:20.24 for analysis of K. For analysis of Na and Cl, an aliquot of plasma sample was diluted 1:51 with deionized distilled water. For the Na determination, a second dilution of 1:99.1 was used; 0.001 % of the total volume was a 1000 ppm solution of potassium and the remainder was deionized distilled water. Chloride determination was conducted using the same indirect method as described in the feed section. For determination of sulfate, 0.6 ml of plasma sample was placed in a 10 ml plastic test tubes with a screw cap. A volume of 2.4 ml of 10% trichloroacetic acid solution was added and the solution was mixed with a vortex mixer. Ten minutes after mixing, samples were centrifuged at 11,000 x G for 10 minutes to obtain a clear supernatant. After centrifugation, sulfate (SO_4) concentration was determined using a modified method of Klaas et al. (1979). One ml of the clear supernatant was added to 0.25 ml of barium chloride ($BaCl_2$) reagent (20 g $BaCl_2$ and 100 g dextran per liter of deionized distilled water) and the absorbance was read after precisely 35 minutes at 360 nm (Beckman spectrophotometer model 35) against a sample background (1 ml supernatant and 0.25 ml reagent containing

100 g dextran in 1 liter of deionized distilled water). A standard curve was constructed using standards of 0, 10, 25, 50 and 75 ppm of sulfate.

IV. RESULTS

1. Feed intake and body weight changes

Cows in the control group had a lower (P<0.05) body weight than cows in treatment groups 1,2 and 3, with cows fed diet 3 having a higher (P<0.05) body weight than cows fed diets 1 and 2 (Table 3). Dry matter intake (Table 4), expressed as kg of dry matter (DM) per day, was higher (P<0.05) in treatment groups 1 and 3 than control and treatment group 2. However, when DM intake was expressed as a percentage (%) of body weight (B.W.), there were no differences (P>0.05) between control and treatments 1 and 3, but intake was lower (P<0.05) in cows fed diet 2. The same results were observed when DM intake was expressed as percentage of metabolic body weight. Feed intake preceding and at parturition was not different (P>0.05) between diets (Table 5) however, all cows exhibited their lowest feed intake (% of B.W.) on the day of parturition.

TABLE 3. Average body weight (BW) of cows fed diets with different anion-cation balances from day-45 prepartum to parturition.

TREATMENT	Body Weight (kg)	
	Mean[1]	S.E.[2]
Control	792.97[a]	3.48
Diet 1	837.63[b]	3.31
Diet 2	866.52[c]	3.29
Diet 3	828.48[b]	3.29

[1] Values expressed as LSMeans
[2] Standard error of LSMeans
[a,b,c] Values within column with different superscripts differ ($P<0.05$)

TABLE 4. Daily dry matter intake (DMI) as kg, as a percentage of body weight (% BW) and as a percentage of metabolic body weight (% MBW) for cows fed diets with different anion-cation balance from day-45 prepartum to parturition.[1]

Treatment	DMI (kg)		DMI (% BW)		DMI (%MBW)	
	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.
Control	12.03[a]	.19	1.52[a]	.02	8.07[a]	.12
Diet 1	12.72[b]	.18	1.54[a]	.02	8.24[a]	.12
Diet 2	11.72[a]	.18	1.36[b]	.02	7.36[b]	.12
Diet 3	13.03[b]	.18	1.58[a]	.02	8.45[a]	.12

[1] Values expressed as LSMeans ±standard error of LSMeans
[a,b] Values within columns with different superscripts differ ($P<0.05$)

TABLE 5. Daily dry matter intake (DMI) as a percentage of body weight (% BW) from day-5 prepartum to parturition for cows fed diets with different anion-cation balances.

Treatment	DMI (% BW)[1]					
	Days prepartum					
	0	1	2	3	4	5
Control	.78	1.09	1.34	1.43	1.40	1.38
S.E.	.29	.30	.24	.29	.26	.28
Diet 1	.60	0.99	1.10	1.05	1.08	1.25
S.E.	.29	.30	.24	.25	.23	.24
Diet 2	.92	1.05	1.25	1.28	1.15	1.20
S.E.	.25	.26	.21	.25	.23	.24
Diet 3	1.01	1.45	1.48	1.47	1.44	1.58
S.E.	.25	.26	.21	.25	.23	.24

[1] Values expressed as LSMeans ± standard error of LSMeans

2. Experimental diets

2.1. Dietary anion-cation balance

Dietary anion-cation balance of the control diet was the most positive ($P<0.05$) and diet 3 the least positive ($P<0.05$) while diets 1 and 2 were between those of the control and diet 3, with diet 1 being more positive ($P<0.05$) than diet 2. The difference between the dietary anion-cation balance of diet 3 and diet 1 was less than 60 meq/kg ration DM (Table 6).

2.2. Composition of diets

The composition of diets is shown in Table 7. The concentration of crude protein and net energy of lactation were not different ($P>0.05$) between diets. The concentration of calcium (Ca) was higher ($P<0.05$) in the control diet than in diets 1,2 and 3. The concentration of phosphorus (P) in diet 2 was lower ($p<0.05$) than in the control diet but similar to diets 1 and 3. The control diet had the highest ratio of Ca-to-P (2.02:1), with no difference between diets 1,2 and 3 (1.71:1, 1.73:1, 1.73:1, respectively). The magnesium (Mg), potassium (K) and iron (Fe) concentration were not different between diets. However, the content of

TABLE 6. Dietary anion-cation balance per kg ration dry matter (DM) for cows fed different diets from day-45 prepartum to parturition[2]

Treatment	Dietary anion-cation balance[1] (meq/kg ration DM)	
	X	S.E.
Control	+394.31[a]	2.80
Diet 1	+120.65[b]	2.46
Diet 2	+104.89[c]	2.41
Diet 3	+62.20[d]	2.40

[1] Values expressed as LSMeans ± standard error of LSMeans
[2] Calculated as milliequivalents °(Na+K)-(Cl+S)8 from nutrient analysis assuming the molecular weight of 23.01, 39.1, 35.45 and 32.0 for Na, K, Cl and S.
[a,b,c,d] Values within column with different superscripts differ (P<0.05)

TABLE 7. Nutrient composition of diets (dry basis) with different anion-catión balances fed to cows from day-45 prepartum to parturition[1]

Nutrient	Control	Diet 1	Diet 2	Diet 3	S.E.
Crude protein, %	13.66	13.57	13.04	13.65	.25
Net energy lactation mcal/kg[2]	1.38	1.38	1.38	1.38	
Calcium, %	1.58^{a}	1.32^{b}	1.28^{b}	1.33^{b}	.04
Phosphorus, %	0.78^{a}	0.77^{ab}	0.74^{b}	0.77^{ab}	.01
Calcium:Phosphorus	2.02	1.71	1.73	1.73	
Magnesium, %	0.29	0.26	0.27	0.29	.04
Sodium, %	0.36^{a}	0.15^{b}	0.15^{b}	0.15^{b}	.00
Potassium, %	1.55	1.60	1.60	1.60	.07
Chloride, %	0.24^{ab}	0.26^{a}	0.21^{b}	0.23^{b}	.01
Sulfur, %	0.15^{a}	0.45^{b}	0.49^{c}	0.56^{d}	.00
Iron, %[2]	0.025	0.025	0.028	0.031	

[1] Values expressed as LSMeans ± standard error of LSMeans
[2] Calculated from tabular data (NRC, 1978)
^{a,b,c,d} Values within rows with different superscripts differ ($P<0.05$)

sodium (Na) was higher ($P<0.05$) in the control diet than in diets 1,2 and 3. The content of chloride (Cl) was higher in diet 1 than in diets 2 and 3 but similar to the control diet. The concentration of sulfur (S) was the highest ($P<0.05$) in diet 3 and the lowest ($P<0.05$) in the control diet. Content of S in diet 1 was lower ($P<0.05$) than diet 2, with diet 2 having a lower ($P<0.05$) content of S than diet 3.

3. Daily mineral intake and absorption

Daily intake of minerals (g/day) varied according to treatments (Table 8). Daily intake of Ca was lower ($P<0.05$) for cows fed diet 2 than for cows fed control and diet 3, with no difference ($P>0.05$) between control, diet 1 and diet 3. Daily intake of phosphorus was lower ($P<0.05$) for cows fed diet 2 than for cows fed diet 3, with no difference ($P>0.05$) between control, diet 1 and diet 3. Ratio between daily intake of Ca and P was the highest in the control group (2.01:1) and the lowest in treatment groups 2 and 3 (1.72:1, 1.73:1, respectively). Daily intake of Mg was lower ($P<0.05$) for cows fed diet 2 than for cows fed diets 1 and 3, with no difference ($P>0.05$) between control, diet 1 and diet 3. The daily intake of anion-cation balance in milliequivalents (meq), was more positive ($P<0.05$) for cows fed control diet than for cows fed diets 1, 2 and 3, with the least positive ($P<0.05$) for cows fed diet 3. No differences ($P>0.05$) were

TABLE 8. Daily intake of minerals for cows fed diets with different anion-cation balances from day-45 prepartum to parturition

	Daily intake of minerals (g)[1]				
Mineral	Control	Diet 1	Diet 2	Diet 3	S.E.
Calcium	188.03[a]	165.22[ab]	148.99[b]	172.69[a]	6.53
Phosphorus	93.55[ab]	92.10[ab]	86.53[a]	99.83[b]	0.35
Ratio Ca:P	2.01	1.79	1.72	1.73	
Magnesium	33.97[ab]	36.42[a]	30.58[b]	37.11[a]	1.91
Sodium	42.74[a]	18.00[bc]	17.25[b]	20.05[c]	1.05
Potassium	186.16[a]	182.59[a]	184.36[a]	208.60[b]	7.33
Chloride	28.30[a]	29.95[a]	24.78[b]	29.75[a]	0.99
Sulfur	18.41[a]	54.23[b]	57.74[b]	72.69[c]	1.95
Anion-cation[2] (meq)	4674[a]	1226[b]	1165[b]	835[c]	122

[1] Values expressed as LSMeans ± standard error of LSMeans
[2] Calculated as daily intake of milliequivalents [(Na+K)-(Cl+S)]
[a,b,c] Values within rows with different superscripts differ ($P<0.05$)

obtained between diets 1 and 2. However, when individual minerals comprising the anion-cation balance are examined (Na,K, Cl and S), the daily intake of each mineral varied. Daily intake of Na was higher ($P<0.05$) for cows fed control diet than for cows fed diets 1, 2 and 3, with cows fed diet 2 having a lower ($P<0.05$) intake than cows fed diet 3. Daily intake of K was higher ($P<0.05$) for cows fed diet 3 than for cows fed control, diet 1 and 2. Daily intake of Cl was lower ($P<0.05$) for cows fed diet 2 than for cows fed control and diets 1 and 3. Daily intake of S was lower ($P<0.05$) for cows fed control diet than for cows fed diets 1, 2 and 3 with cows fed diet 3 having the highest ($P<0.05$) intake. However, due to the difference between treatments in the percentage of apparent absorption of minerals (see Digestibility Study section), the daily quantity of apparently absorbed minerals differed from the daily intake of minerals (Table 9). The daily quantity of absorbed Ca was higher ($P<0.05$) for cows fed control diet than for cows fed diets 1, 2 and 3. There were no differences ($P>0.05$) between diets in the daily quantity of absorbed P. The ratio of the daily quantity of absorbed Ca and P was greatest ($P<0.05$) for cows fed control diet and lowest for cows fed diets 2 and 3. Even if the trend was similar as for the ratio of daily intake of Ca and P, absolute values of Ca and P were lower for the ratio of the daily quantity of absorbed Ca and P, especially for diets 2 and 3. Daily quantity of absorbed Mg was higher ($P<0.05$) for

TABLE 9. Daily quantity of apparent absorbed minerals in cows fed diets with different anion-cation balances from day-45 prepartum until parturition

	Daily quantity of absorbed minerals (g)[1]				
Mineral	Control	Diet 1	Diet 2	Diet 3	S.E.
Calcium	105.02[a]	79.65[b]	71.04[b]	73.83[b]	4.22
Phosphorus	55.55	55.33	49.83	52.87	2.50
Ratio Ca:P	1.89	1.44	1.43	1.40	
Magnesium	22.91[a]	19.07[ab]	15.50[b]	19.00[b]	1.45
Sodium	32.41[a]	12.44[bc]	10.72[b]	14.00[c]	0.97
Potassium	168.88[a]	165.09[a]	163.85[a]	186.80[b]	6.78
Chloride	20.33[a]	21.52[a]	16.07[b]	20.09[a]	0.84

[1] Values expressed as LSMeans ± standard error of LSMeans

[a,b,c] Values within rows with different superscripts differ ($P<0.05$)

cows fed control diet than for cows fed diets 2 and 3. Daily quantity of absorbed Na was higher ($P<0.05$) for cows fed control diet than for cows fed diets 1, 2 and 3, with cows fed diet 2 having a lower ($P<0.05$) quantity of absorbed Na than cows fed diet 3. Daily quantity of absorbed K was higher ($P<0.05$) for cows fed diet 3 than for cows fed control and diets 1 and 2. Daily quantity of absorbed Cl was lower ($P<0.05$) for cows fed diet 2 than for cows fed control and diets 1 and 3.

4. Incidence of milk fever

Observations were made on 20 parturitions of 20 animals. According to the recognition of two or more symptoms, milk fever was diagnosed in 4 cases out of 5 in the control group, and in 3 cases out of 5 in treatment groups 1, 2 and 3. Thus, a 25% reduction in the incidence of milk fever in cows fed diets 1, 2 and 3 was observed, compared to cows fed the control diet. Injections of a calcium solution were necessary more than once for 50% of the milk fever cases in the control group and for 67% of the milk fever cases in treatment groups 1, 2 and 3. Symptoms of milk fever recognized were similar for both groups.

5. Plasma macrominerals

5.1. Plasma calcium

The average value of concentration of plasma Ca (Table 18) in cows fed control diet and diet 3 was lower ($P<0.05$) than in cows fed diets 1 and 2. The average of the initial concentration of plasma Ca for all groups was 8.56 mg/dl. Concentration of plasma Ca decreased from day-45 to day-35 prepartum by 8.8% in cows fed control diet and diets 1 and 2, and by 4.4% in cows fed diet 3 (Figure 1). A comparaison of the lowest concentration of plasma Ca of each group (Table 10) showed that cows fed control diet and diet 3 had a lower ($P<0.05$) value than cows fed diets 1 and 2. However, the lowest concentration of the plasma Ca did not occur at the same time postpartum (Figure 2); in fact, cows fed the control diet had their lowest value at 12 hours postpartum, cows fed diets 2 and 3 at 24 hours postpartum and cows fed diet 1 at 60 hours postpartum. The concentration of plasma Ca at parturition expressed as a percentage of the values obtained at day-45 prepartum, were approximately twice as high in cows fed diets 1, 2 and 3 than in cows fed the control diet (Table 11). However, when the lowest values obtained for concentration of plasma Ca were expressed as a percentage of the values obtained at the day-45 prepartum, a

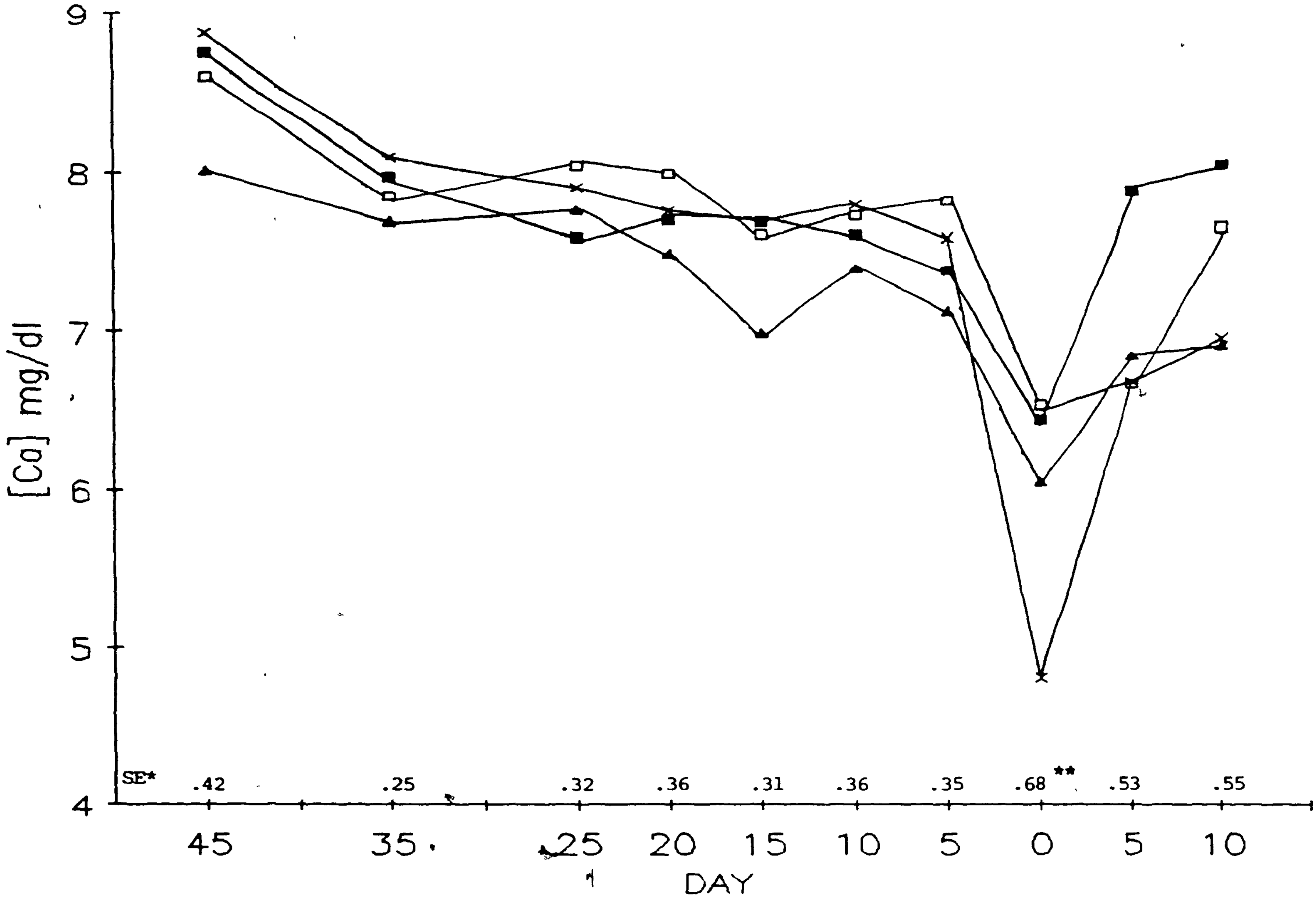
TABLE 18. Average concentration of plasma minerals from day-45 pre- to day-10 post-partum in cows fed diets with different anion-cation balances.

Plasma minerals[1]	Treatment				
	Control	Diet 1	Diet 2	Diet 3	S.E.
Calcium, mg/dl	6.62[a]	7.21[b]	7.29[b]	6.79[a]	.09
Phosphorus, mg/dl	8.27[a]	6.86[bc]	6.58[b]	7.36[c]	.16
Magnesium, mg/dl	1.95[a]	2.02[a]	2.18[b]	2.26[c]	.03
Sodium, meq/l	126.98[a]	117.89[b]	119.84[b]	123.53[a]	1.38
Potassium, meq/l	4.40[a]	4.02[b]	4.72[c]	4.68[c]	.07
Chloride, meq/l	86.38[a]	94.71[b]	92.68[b]	88.64[a]	.80
Sulfate, mmol/l	1.66[a]	1.81[b]	1.88[b]	1.88[b]	.04

[1] Values expressed as LSMeans ± standard error of LSMeans
[a,b,c] Values within rows with different superscripts differ ($P<0.05$)

Figure 1. Concentration of plasma calcium from day-45 prepartum to day-10 postpartum in cows offered the control diet (X), diet 1 (□) diet 2 (■) and diet 3 (▲).

* Standard error
** Values differ significantly ($P<0.05$)



9
8
7
6
5
4
[Ca] mg/dl
SE*
.42
.25
.32
.36
.31
.36
.35
.68 **
.53
.55
45
35
25
20
15
10
5
0
5
10
DAY

TABLE 10. Lowest concentration of plasma calcium (Ca) that occurred during the period of the trial (day-45 prepartum to day-10 postpartum in cows fed diets with different anion-cation balance[1]

Treatment	Lowest concentration of plasma Ca (mg/dl)	
	Mean	S.E.
Control	4.00[a]	.88
Diet 1	5.43[b]	.90
Diet 2	5.55[b]	.88
Diet 3	4.46[a]	.64

[1] Values expressed as LSMeans ± standard error of LSMeans
[a,b] Values within column with different superscripts differ ($P<0.05$)

Figure 2. Concentration of plasma calcium from 48 hours prepartum to 60 hours postpartum in cows offered the control diet (X), diet 1 (□ diet 2 (■) and diet 3 (▲).
* Standard error
** Values differ significantly (P<0.05)

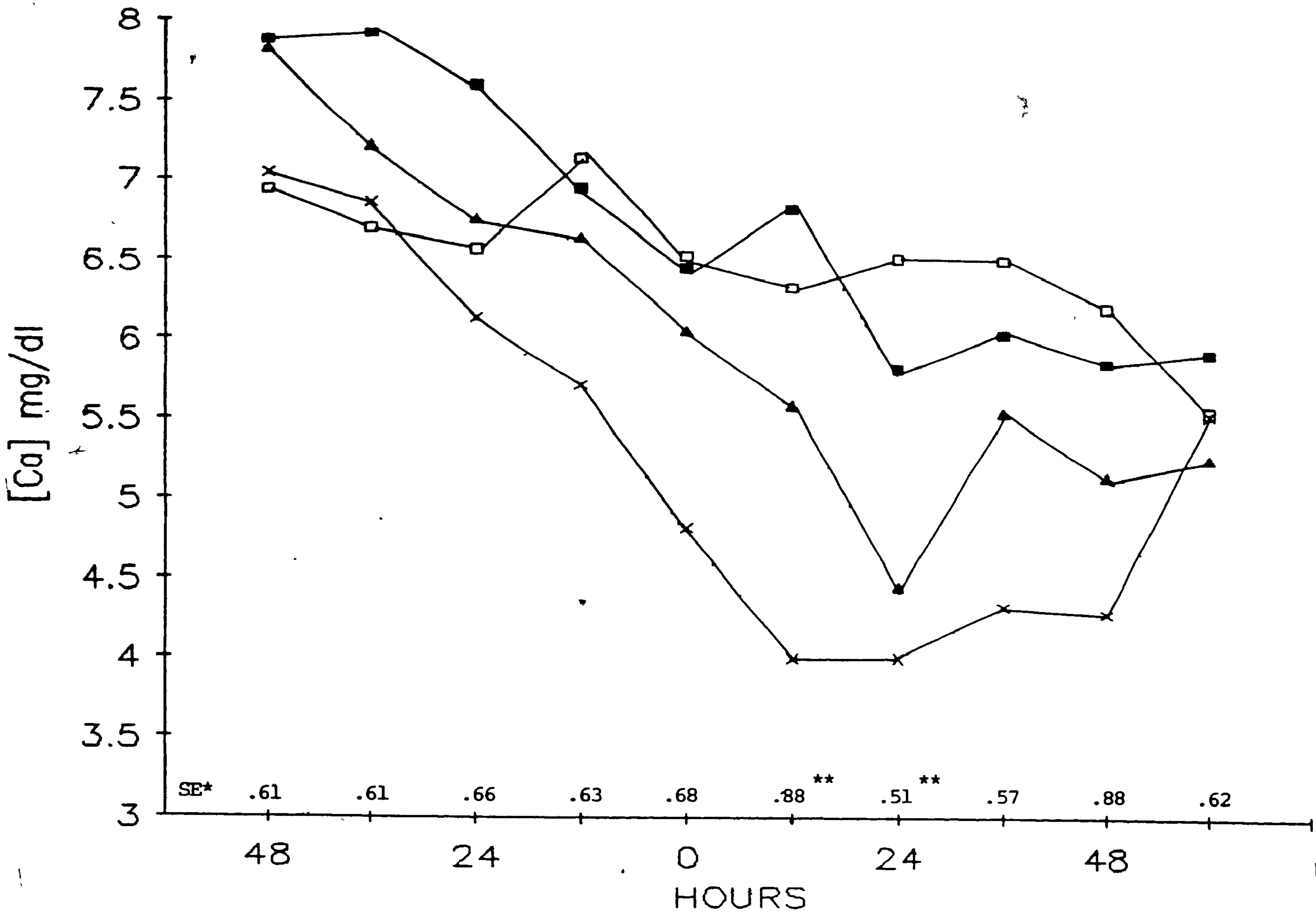

[Ca] mg/dl
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
SE* .61 .61 .66 .63 .68 .88 ** .51 ** .57 .88 .62
48
24
0
24
48
HOURS

TABLE 11. Concentration of plasma calcium at parturition expressed as a percentage of the values obtained at day-45 prepartum (% of day-45) in cows fed diets with different anion-cation balances

Treatment	% of day-45 prepartum[1]	
	Mean	S.E.
Control	52.45[a]	8.74
Diet 1	75.19[b]	6.29
Diet 2	73.98[b]	7.37
Diet 3	75.11[b]	6.29

[1] Values expressed LSMeans ± standard error of LSMeans

[a,b] Values within column with different superscripts differ ($P<0.05$)

larger decrease was observed for cows fed control and diet 3 than for cows fed diets 1 and 2 (Table 12). Concentration of plasma Ca during the periparturient period (48 hours pre- to 60 hours post-partum) are shown in Table 13. Concentration of plasma Ca in cows fed diets 1 and 2 at 12 hours and 36 hours postpartum was higher ($P<0.05$) than in cows fed the control diet. Concentration of plasma Ca was not different ($P>0.05$) between cows fed diets 1, 2 and 3, except at 24 hours postpartum where values were higher ($p<0.05$) for cows fed diet 1 than for cows fed diet 3. Concentration of plasma Ca was not different ($P>0.05$) between groups on day-10 postpartum. Concentration of plasma Ca was negatively correlated with the level of dietary anion-cation balance (meq/kg ration DM) (Table 14). The correlation obtained for the entire trial was weaker ($r=-0.17416$, $P=0.0001$) than the correlation obtained for the periparturient period of day-2 pre- to day-1 post-partum ($r=-0.37467$, $P=0.0001$) with the highest correlation being on the day of parturition ($r=-0.58980$, $P=0.0127$) (Table 15). Correlations observed between concentration of plasma Ca and the daily quantity of absorbed minerals are shown in Tables 16 and 17. Negative correlations were obtained between concentration of plasma Ca and daily quantity of absorbed Ca, P, Mg, Na and Cl for the first and second week prepartum. However, no correlation was observed for the third week prepartum, and for the fourth

TABLE 12. Lowest concentration of plasma calcium expressed as a percentage of the values obtained at day-45 prepartum (% of day-45) in cows fed diets with different anion-cation balances[1]

Treatment	% of day-45 prepartum	
	Mean	S.E.
Control	46.81	8.31
Diet 1	66.13	11.84
Diet 2	64.44	8.49
Diet 3	55.73	7.24

[1] Values expressed as LSMeans ± standard error of LSMeans

TABLE 13. Concentration of plasma calcium during the periparturient period (48 hours pre- to 60 hours post-partum) in cows fed diets with different anion-cation balances from day-45 prepartum until parturition

Treatment	Plasma calcium (mg/dl)[1]									
	Hours prepartum				Parturition	Hours postpartum				
	48	36	24	12	0	12	24	36	48	60
Control	7.04	6.86	6.13	5.70	4.82	4.00[a]	4.00[a]	4.33[a]	4.29	5.53
S.E.	.61	.61	.66	.55	.68	.57	.88	.51	.57	.88
Diet 1	6.94	6.69	6.57	7.13	6.53	6.32[b]	6.51[b]	6.50[b]	6.19	5.55
S.E.	.44	.45	.57	.55	.59	.57	.64	.51	.68	.88
Diet 2	7.88	7.92	7.60	6.95	6.45	6.81[b]	5.81[ab]	6.03[b]	5.85	5.92
S.E.	.44	.45	.57	.63	.59	.57	.64	.44	.49	.63
Diet 3	7.32	7.21	6.75	6.64	6.05	5.58[ab]	4.46[a]	5.54[ab]	5.14	5.26
S.E.	.44	.45	.57	.55	.59	.49	.64	.44	.49	.63

[1] Values expressed as LSMeans ± standard error of LSMeans
[a,b] Values within columns with different superscripts differ ($P<0.05$)

TABLE 14. Correlations between the level of dietary anion-cation balance (meq/kg ration DM) and the concentration of plasma calcium, phosphorus, magnesium, sodium, potassium, chloride and sulfate for the trial (day-45 pre- to day-10 post-partum).

Mineral	Coefficients of correlation	Probability (P>F)
Calcium	-0.17416	0.0001
Phosphorus	+0.16556	0.0003
Magnesium	-0.22771	0.0001
Sodium	+0.03836	0.3763
Potassium	+0.00102	0.9813
Chloride	-0.20629	0.0001
Sulfate	-0.17517	0.0001

TABLE 15. Correlations between the concentration of plasma calcium and the level of dietary anion-cation balance (meq/kg ration DM) during the periparturient period (48 hours pre- to 36 hours postpartum)

Hours	Coefficients of correlation	Probability (P>F)
48 prepartum	-0.47134	0.0483
36 prepartum	-0.37877	0.1211
24 prepartum	-0.33415	0.1620
12 prepartum	-0.46315	0.0458
0 parturition	-0.54812	0.0151
12 postpartum	-0.58980	0.0127
24 postpartum	-0.28732	0.2476
36 postpartum	-0.45493	0.0578

TABLE 16. Correlations between the concentration of plasma calcium and the daily quantity of apparent absorbed calcium, phosphorus, magnesium, sodium, potassium and chloride for the last three weeks prepartum.

Absorbed minerals (g/day)	Coefficients of correlation		
	Weeks prepartum		
	1	2	3
Calcium	-0.18300[a]	-0.22019[a]	-0.09643
Phosphorus	-0.13551[b]	-0.16384[b]	-0.06674
Magnesium	-0.21095[a]	-0.14693	-0.08662
Sodium	-0.13894[b]	-0.24530[a]	0.07840
Potassium	-0.11324	-0.14637	-0.09519
Chloride	-0.20015[a]	-0.17336[b]	-0.01017

a P<0.05
b P<0.1

TABLE 17. Correlations between the concentration of plasma calcium and the daily quantity of apparently absorbed calcium, phosphorus, magnesium, sodium, potassium and chloride for the fourth, sixth and seventh weeks prepartum.

Absorbed minerals (g/day)	Coefficients of correlation		
	Weeks prepartum		
	4	6	7
Calcium	-0.09176	0.17332	0.22642
Phosphorus	-0.08281	-0.06370	-0.17011
Magnesium	0.48470[a]	0.20573	0.48187[b]
Sodium	-0.01899	0.13891	0.57704[a]
Potassium	-0.12576	0.16511	-0.28214
Chloride	0.09087	0.04674	0.03029

a $P<0.05$
b $P<0.1$

week prepartum, concentration of plasma Ca was positively correlated with the quantity of Mg absorbed daily. Concentration of plasma Ca was also positively correlated with the quantities of Mg and Na absorbed daily for the seventh week prepartum. No correlation was observed between concentration of plasma Ca and the quantity of K absorbed daily. Paretic cows tended to show a higher concentration of plasma Ca than non-paretic cows during the prepartum period (day-45 to day-5 prepartum) being significant (P<0.05) at days 12 and 5 prepartum (Figure 3). However, during the periparturient period, concentration of plasma Ca of paretic cows tended to be lower than non-paretic cows (Figure 4). In fact, concentration of plasma Ca in paretic cows decreased from 48 hours pre- to 24 hours post-partum by 35% while in non-paretic cows concentration of plasma Ca decreased by 26%. Even though the concentration of plasma Ca was not different (P>0.05) between paretic and non-paretic cows at the day of parturition, it was lower (P<0.05) at days 3 and 5 postpartum in paretic cows compared to non-paretic cows.

5.2. Plasma phosphorus

The average concentration of plasma phosphorus (Table 18) in cows fed the control diet was higher (P<0.05) than in cows fed diets 1, 2 and 3, with cows fed diet 2 having a lower (P<0.05) concentration of plasma P than cows fed diet 3.

Figure 3. Concentration of plasma calcium from day-45 prepartum to day-10 postpartum in paretic cows (□) and in non-paretic cows (■).

* Standard error
** Values differ significantly (P<0.05)

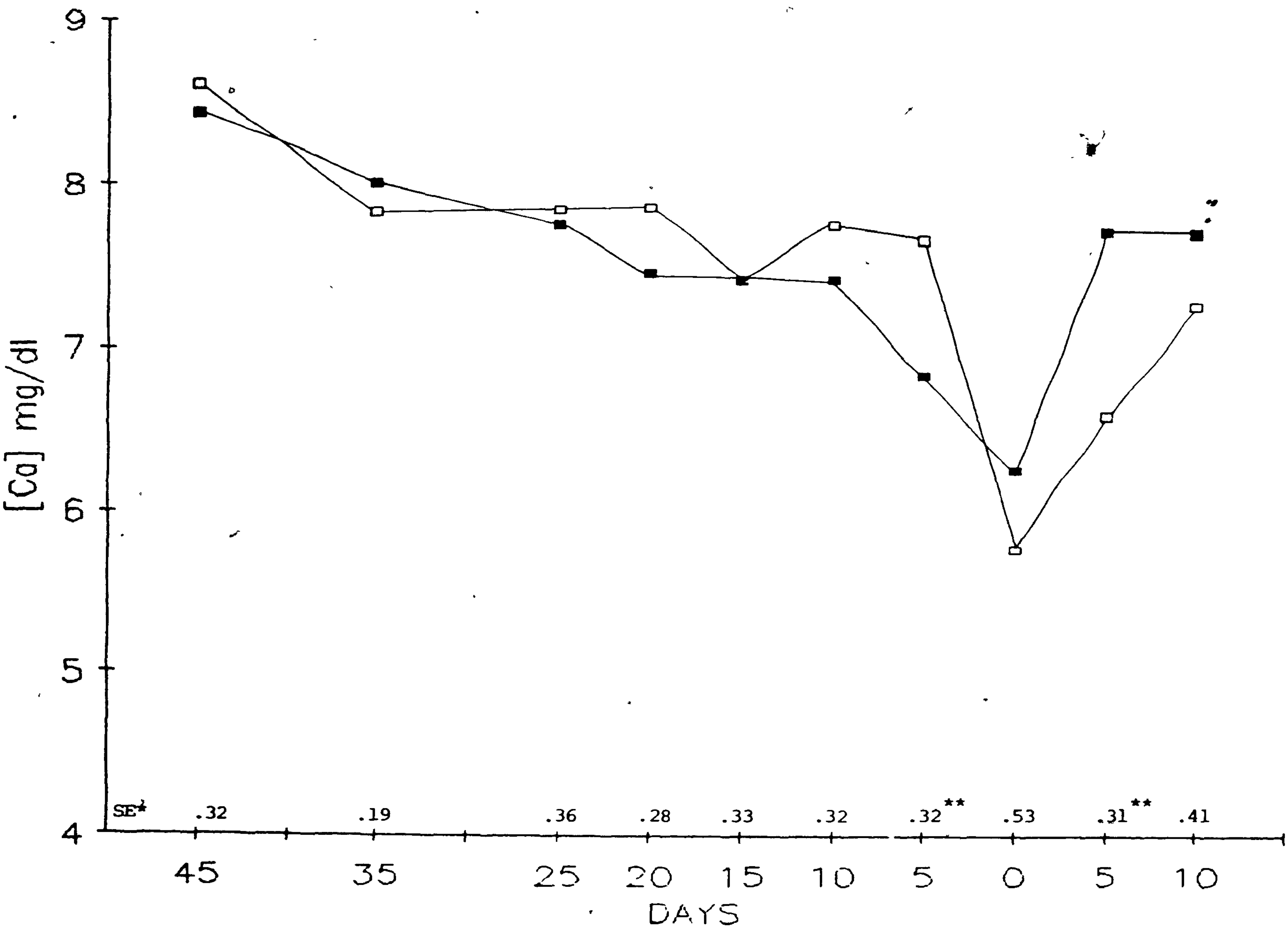
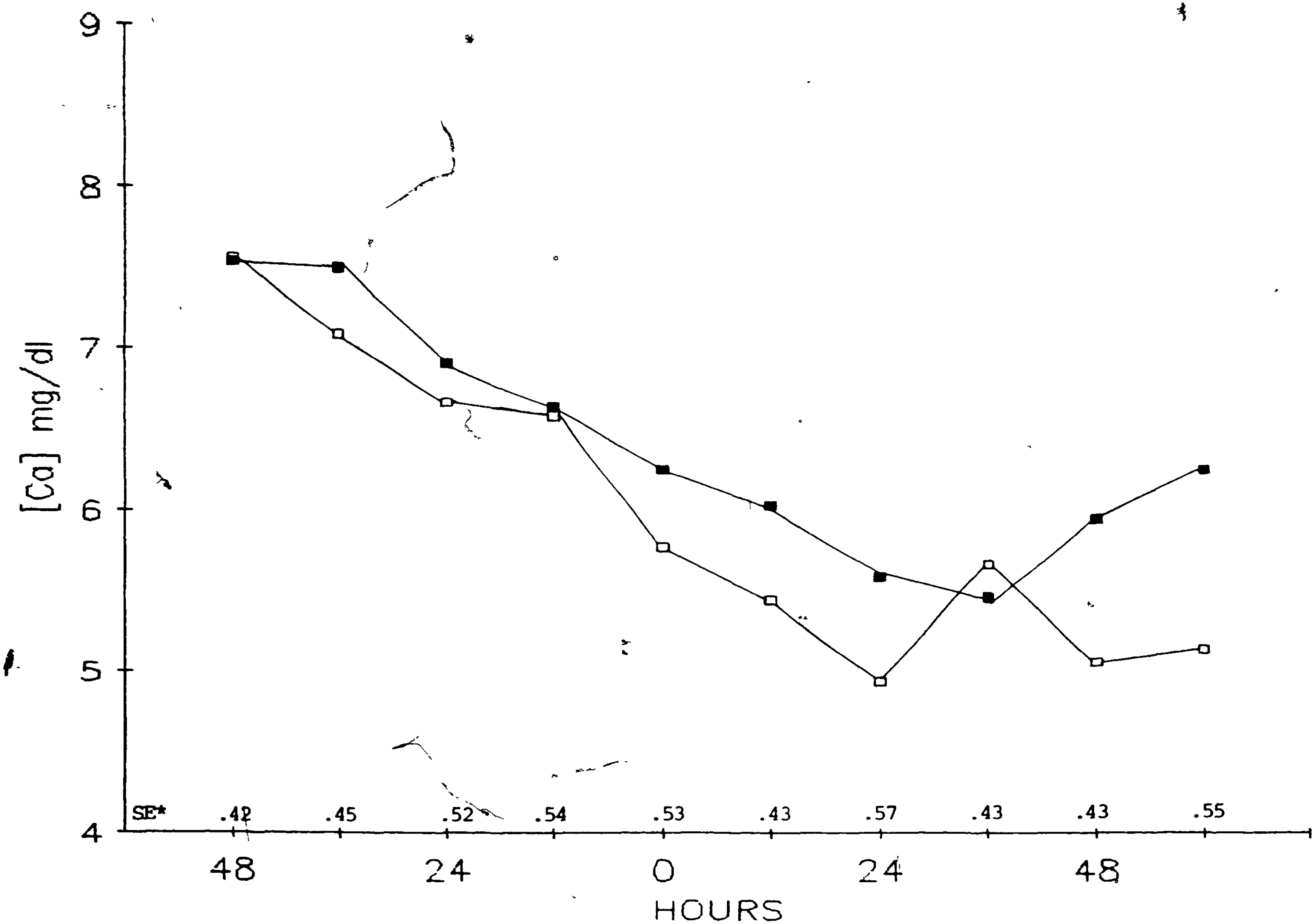

[Ca] mg/dl
9
8
7
6
5
4
SE*
.32
.19
.36
.28
.33
.32
.32**
.53
.31**
.41
45
35
25
20
15
10
5
0
5
10
DAYS

Figure 4. Concentration of plasma calcium from 48 hours prepartum to 60 hours postpartum in paretic cows (□) and in non-paretic cows (■

* Standard error



9
8
7
6
5
4
[Ca] mg/dl
SE*
.42
.45
.52
.54
.53
.43
.57
.43
.43
.55
48
24
0
24
48
HOURS

During the prepartum period (Figure 5), on days 25, 5 and 3 prepartum, concentration of plasma P in cows fed the control diet was higher (P<0.05) than in cows fed diets 1 and 2. At 48 hours and 24 hours prepartum, cows fed control diet had an higher (P<0.05) concentration of plasma P than cows fed diet 1. However, there were no differences between groups on the day of parturition (Figure 6). At day-10 postpartum, concentration of plasma P in cows fed diet 3 was lower (P<0.05) than in cows fed diets 1 and 2. The positive correlation obtained for the entire trial between the concentration of plasma P and the level of dietary anion-cation balance (meq/kg ration DM) was weaker (r= 0.16556, P=0.0003) than the correlation obtained on day-2 postpartum (r= 0.56284, P=0.0232) with no correlation observed at parturition. Correlations between concentration of plasma P and the quantities of Ca, P and Mg absorbed daily are shown in tables 19 and 20. The quantity of P absorbed daily was negatively correlated to the concentration of plasma P on the second, third and seventh week prepartum with no correlation observed for the first, fourth and sixth week prepartum. Concentration of plasma P was only negatively correlated to the quantity of Ca absorbed daily on the second and third week prepartum. No correlation was observed between concentration of plasma P and the quantity of Mg absorbed daily.

Figure 5. Concentration of plasma phosphorus from day-45 prepartum tc
to day-10 postpartum in cows offered the control diet (X), diet 1 (□
diet 2 (■) and diet 3 (▲).

* Standard error
** Values differ significantly (P<0.05)

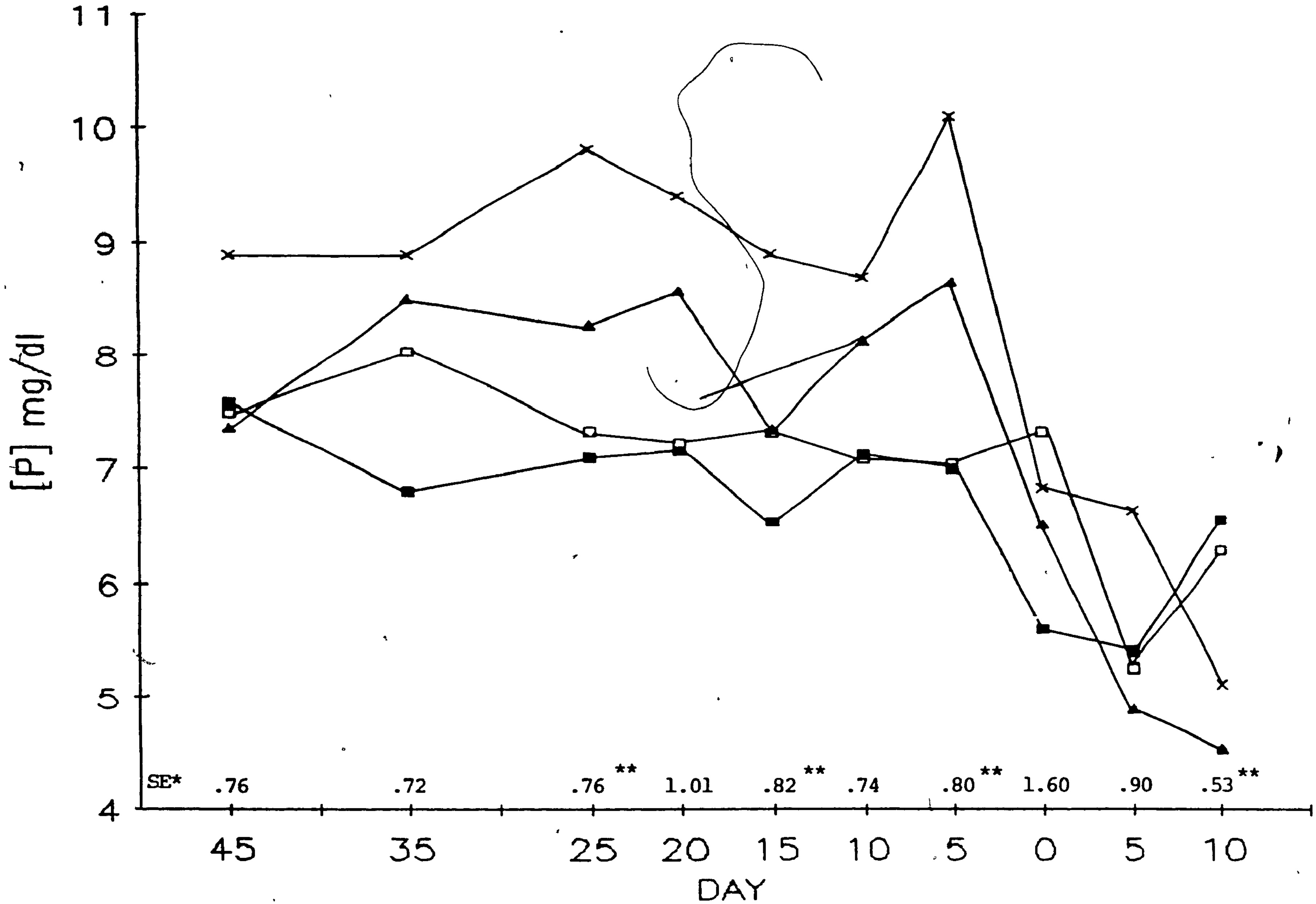
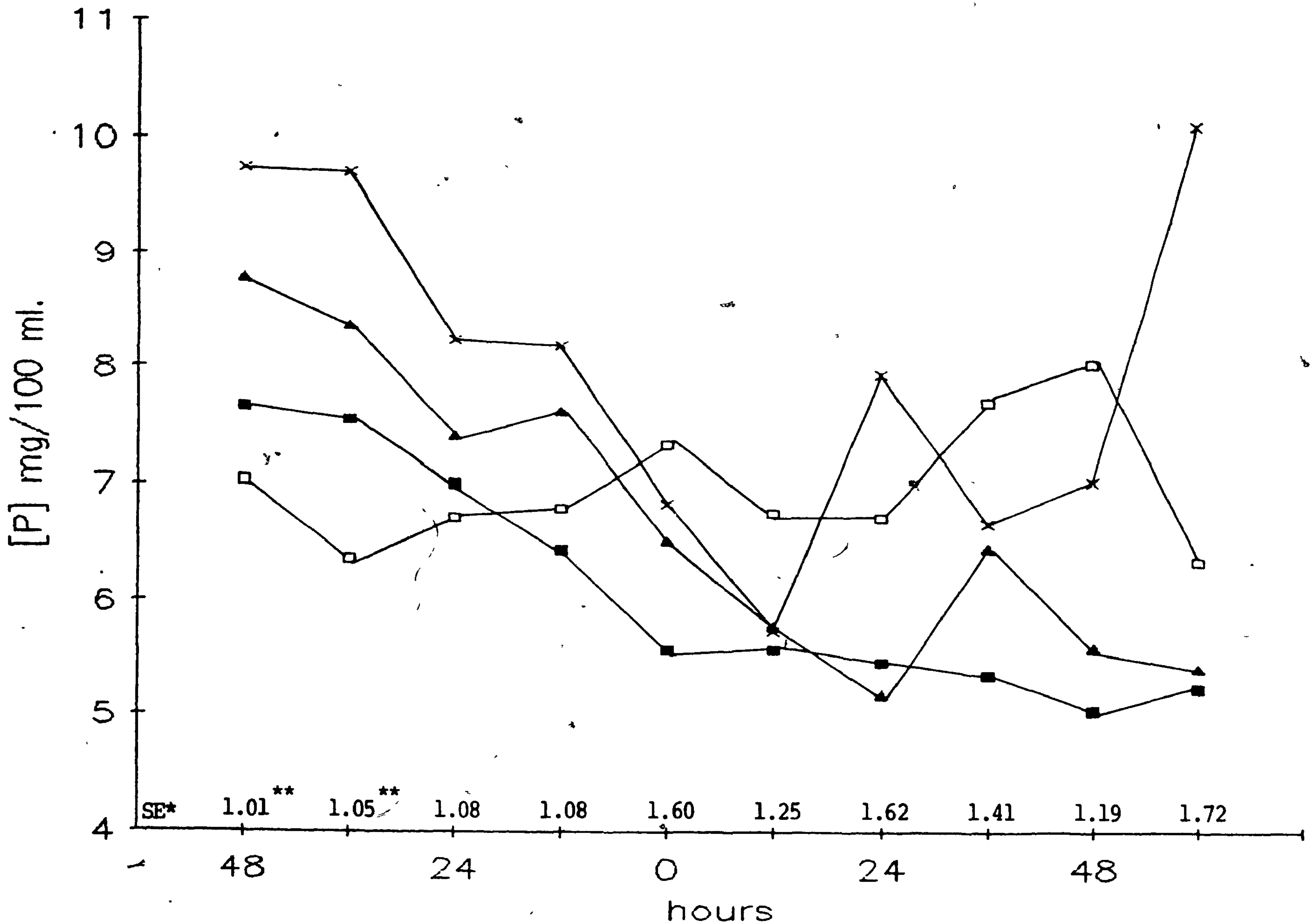

11
10
9
8
7
6
5
4
[P] mg/dl
SE* .76 .72 .76** 1.01 .82** .74 .80** 1.60 .90 .53**
45 35 25 20 15 10 5 0 5 10
DAY

Figure 6. Concentration of plasma phosphorus from 48 hours prepartum to 60 hours postpartum in cows fed the control diet (X), diet 1 (□ diet 2 (■) and diet 3 (▲).

* Standard error

** Values differ significantly ($P<0.05$)



[P] mg/100 ml.
11
10
9
8
7
6
5
4
SE*
1.01**
1.05**
1.08
1.08
1.60
1.25
1.62
1.41
1.19
1.72
48
24
0
24
48
hours

TABLE 19. Correlations between concentration of plasma phosphorus and the quantity of calcium, phosphorus, magnesium, sodium, potassium and chloride apparently absorbed daily for the last three weeks prepartum.

Absorbed minerals (g/day)	Coefficients of correlation — Weeks prepartum		
	1	2	3
Calcium	-0.0[illegible]738	-0.21114[a]	-0.27558[b]
Phosphorus	-0.04091	-0.17227[b]	-0.26492[b]
Magnesium	-0.03940	-0.01419	-0.11364
Sodium	-0.05870	-0.18195[b]	-0.13593
Potassium	-0.03205	-0.13375	-0.31680[a]
Chloride	-0.06391	-0.17534[b]	-0.33080[a]

a P<0.05
b P<0.1

TABLE 20. Correlations between the concentration of plasma phosphorus and the quantity of calcium, phosphorus, magnesium, sodium, potassium and chloride apparently absorbed daily for the fourth, sixth and seventh week prepartum.

Absorbed minerals (g/day)	Coefficients of correlation weeks prepartum		
	4	6	7
Calcium	-0.13563	0.00864	-0.19879
Phosphorus	-0.31439	0.20308	-0.43010[b]
Magnesium	0.01115	-0.25938	0.08129
Sodium	0.15176	0.21569	-0.07066
Potassium	-0.31649	-0.04841	-0.43270[b]
Chloride	-0.22861	-0.11636	0.03543

a $P<0.05$
b $P<0.1$

5.3. Plasma magnesium

Average concentration of plasma Mg was lower ($P<0.05$) in cows fed control and diet 1 than in cows fed diets 2 and 3, with the highest ($P<0.05$) value in cows fed diet 3 (Table 18). All four groups had their lowest concentration of plasma Mg at day-5 postpartum (Figure 7), with no difference ($P>0.05$) between groups. During the periparturient period (Figure 8), concentration of plasma Mg in cows fed diets 2 and 3 tended to be higher ($P<0.1$) than in cows fed control and diet 1, with significance ($P<0.05$) being obtained only for the day of parturition. All groups, except treatment group 3, showed a stable concentration of plasma Mg through the periparturient period, however, cows fed diet 3 increased their concentration of plasma Mg with the highest value at 24 hours postpartum. At day- 10 postpartum, concentration of plasma Mg was not different ($P>0.05$) between groups. Concentration of plasma Mg was negatively correlated ($r=-0.22771$, $P=0.0001$) to the level of dietary anion-cation balance (meq/kg ration DM). Correlations were stronger from day-2 prepartum to day-10 postpartum (Table 21) with the highest correlation observed on the day of parturition ($r=-0.56333$, $P=0.0120$). Correlations between concentration of plasma Mg and the daily quantity of absorbed minerals are

Figure 7. Concentration of plasma magnesium from day-45 prepartum to day-10 postpartum in cows offered the control diet (×), diet 1 (□), diet 2 (■) and diet 3 (▲).

* Standard error
** Values differ significantly ($P<0.05$)

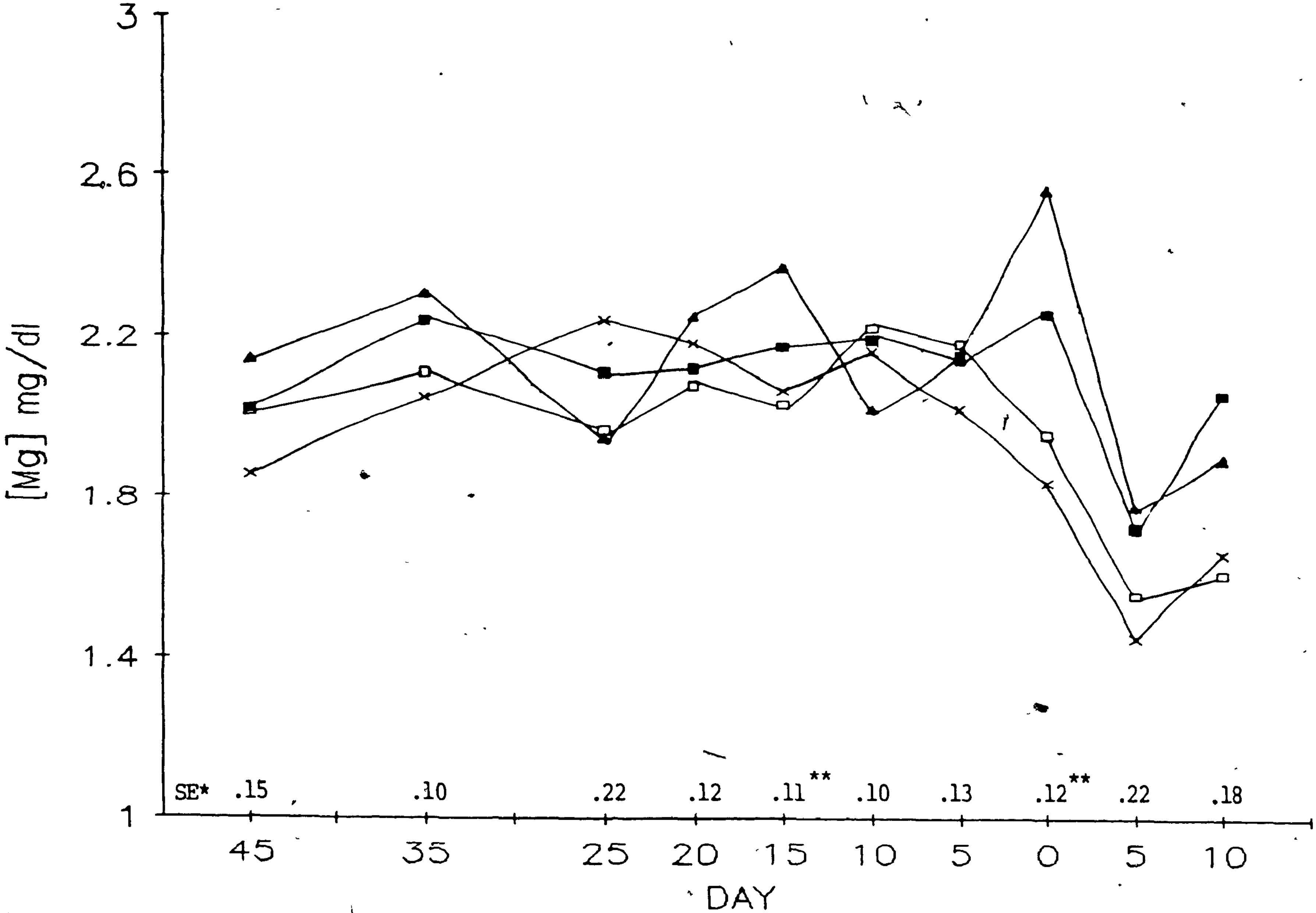
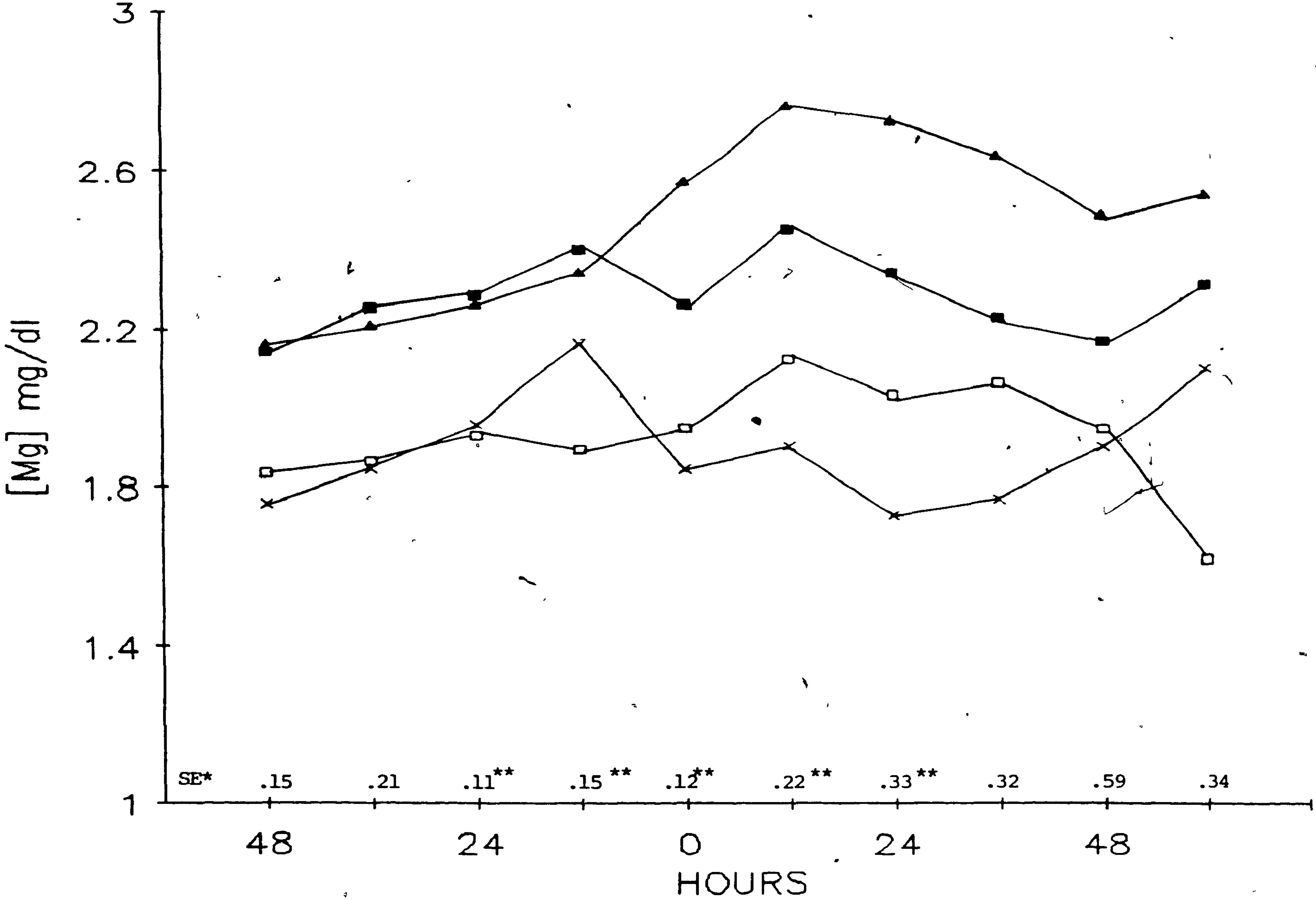

[Mg] mg/dl
3
2.6
2.2
1.8
1.4
1
SE* .15 .10 .22 .12 .11** .10 .13 .12** .22 .18
45 35 25 20 15 10 5 0 5 10
DAY

Figure 8. Concentration of plasma magnesium from 48 hours prepartum to 60 hours postpartum in cows offered the control diet (×), diet 1 (□), diet 2 (■) and diet 3 (▲).

* Standard error
** Values differ significantly (P<0.05)



[Mg] mg/dl
3
2.6
2.2
1.8
1.4
1
SE*
.15
.21
.11**
.15 **
.12**
.22 **
.33**
.32
.59
.34
48
24
0
24
48
HOURS

TABLE 21. Correlations between the level of dietary anion-cation balance (meq/kg ration DM) and the concentration of plasma magnesium during the periparturient period (48 hours pre- to 36 hours post-partum).

Hours	Coefficients of correlation	Probability (P>F)
48 prepartum	-0.46169	0.0538
36 prepartum	-0.16542	0.5118
24 prepartum	-0.41292	0.0789
12 prepartum	-0.15699	0.5210
0 parturition	-0.56333	0.0120
12 postpartum	0.05608	0.8251
24 postpartum	-0.41816	0.0842
36 postpartum	-0.37136	0.5844

shown in Tables 22 and 23. Correlations between concentration of plasma Mg and the quantities of Ca and P absorbed daily were observed for the second and third weeks prepartum. Correlations were observed with the quantities of K and Cl absorbed daily for the third week prepartum with no correlation obtained between concentration of plasma Mg and the quantity of Mg absorbed daily. A weak correlation was also observed between concentration of plasma Mg and the quantity of Na absorbed daily for the third week prepartum.

5.4. Plasma sodium

Average value of concentration of plasma Na (Table 18) was higher (P<0.05) in cows fed the control diet and diet 3 than in cows fed diets 1 and 2, which was more evident during the prepartum period (day-45 to day-5 prepartum) (Figure 9). During the periparturient period (day-2 pre- to day-2 post-partum) (Figure 10), concentration of plasma Na was not different (P>0.05) between groups, except for the day of parturition where cows fed the control diet had a higher (P<0.05) concentration of plasma Na than cows fed diet 3. No correlation was obtained between concentration of plasma Na and the level of dietary anion-cation balance (meq/kg ration DM) (Table 14).

TABLE 22. Correlations between the concentration of plasma magnesium and the quantity of calcium, phosphorus, magnesium, sodium, potassium and chloride apparently absorbed daily for the last three weeks prepartum.

Absorbed minerals (g/day)	Coefficients of correlation		
	Weeks prepartum		
	1	2	3
Calcium	-0.11093	-0.18608[b]	-0.40563[a]
Phosphorus	-0.09913	-0.16835[b]	-0.34933[a]
Magnesium	-0.04204	-0.09881	-0.01399
Sodium	0.03089	-0.11963	-0.25873
Potassium	-0.10192	-0.15394	-0.37524
Chloride	-0.05104	-0.13401	-0.37304[a]

a $P<0.05$
b $P<0.1$

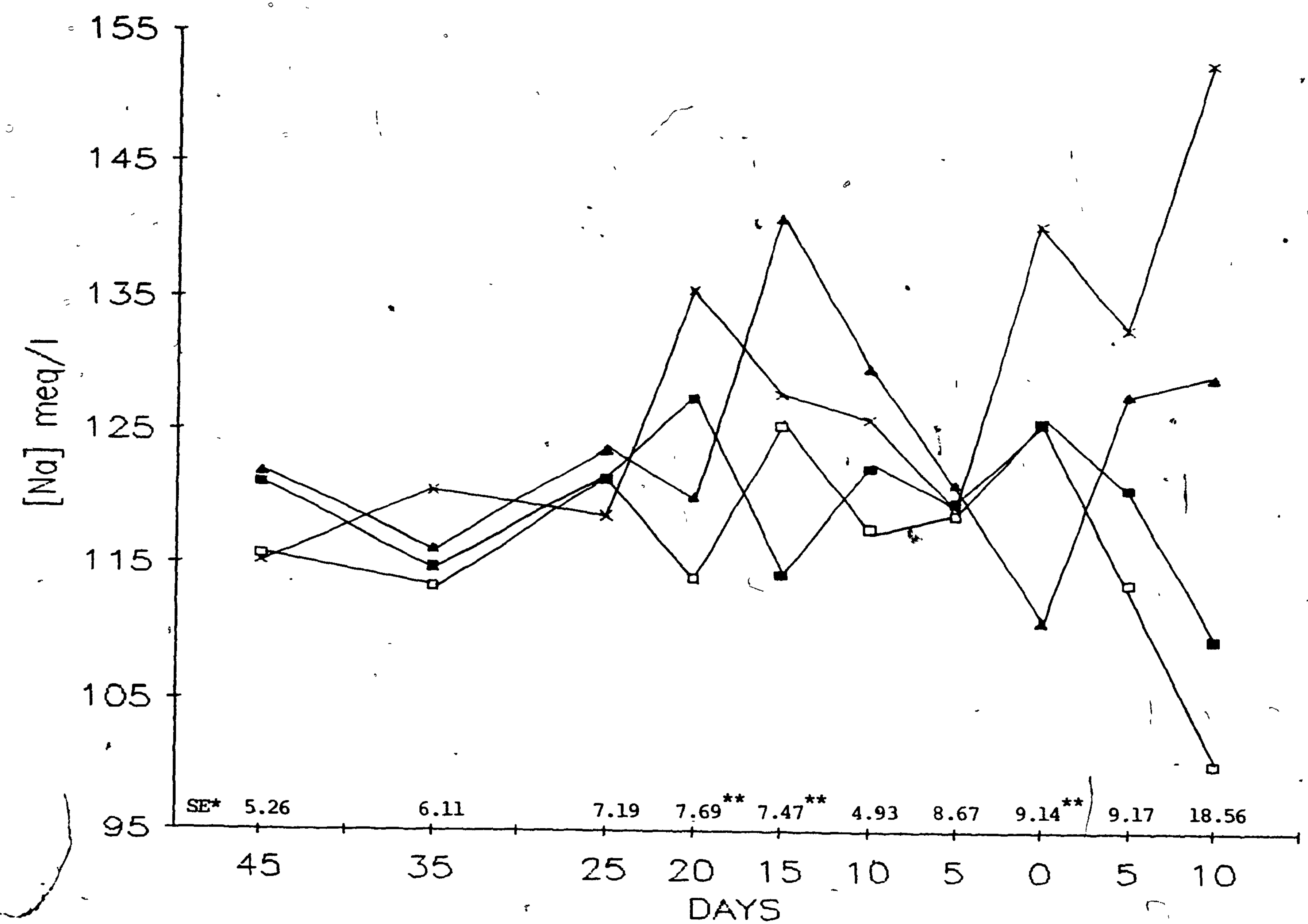
TABLE 23. Correlations between the concentration of plasma magnesium and the quantity of calcium, phosphorus, magnesium, sodium, potassium and chloride apparently absorbeb daily for the fourth, sixth and seventh weeks prepartum.

Absorbed minerals (g/day)	Coefficients of correlation Weeks prepartum 4	6	7
Calcium	0.21506	-0.12648	-0.23950
Phosphorus	-0.04372	-0.09473	0.12776
Magnesium	0.17165	-0.05015	-0.31464
Sodium	0.13144	0.01753	-0.26907
Potassium	-0.29311	-0.35358	-0.16779
Chloride	0.02739	0.05075	-0.17340

a $P<0.05$
b $P<0.1$

Figure 9. Concentration of plasma sodium from day-45 prepartum to day-10 postpartum in cows offered the control diet (X), diet 1 (□) diet 2 (■) and diet 3 (▲).

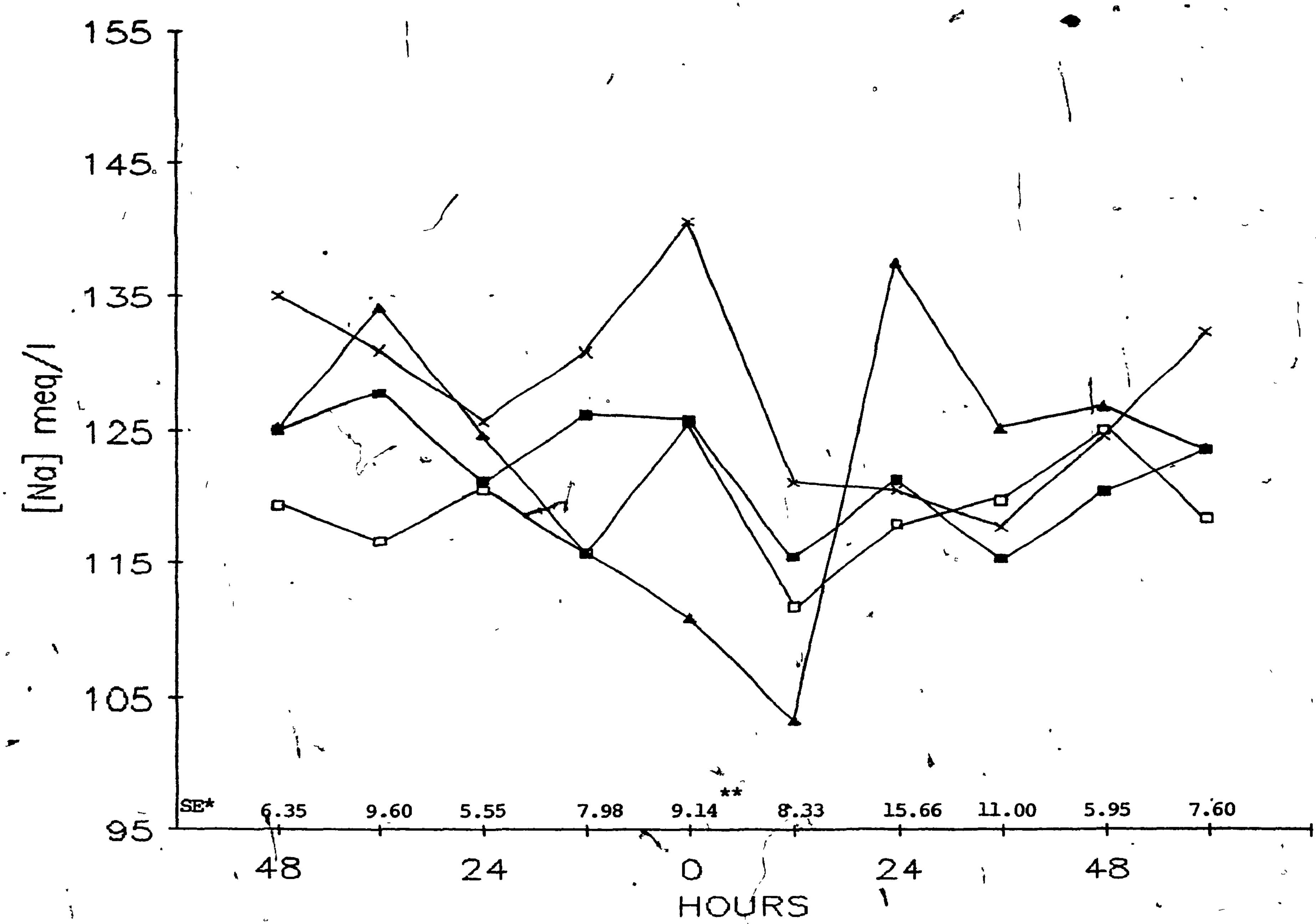
* Standard error
** Values differ significantly (P<0.05)



155
145
135
125
115
105
95
[Na] meq/l
SE* 5.26 6.11 7.19 7.69** 7.47** 4.93 8.67 9.14** 9.17 18.56
45 35 25 20 15 10 5 0 5 10
DAYS

Figure 10. Concentration of plasma sodium from 48 hours prepartum to 60 hours postpartum in cows offered the control diet (X), diet 1 (□), diet 2 (■) and diet 3 (▲).

*** Standard error**
**** Values differ significantly (P<0.05)**



155
145
135
125
115
105
95
[Na] meq/l
SE*
6.35
9.60
5.55
7.98
9.14
**
8.33
15.66
11.00
5.95
7.60
48
24
0
24
48
HOURS

5.5. Plasma potassium

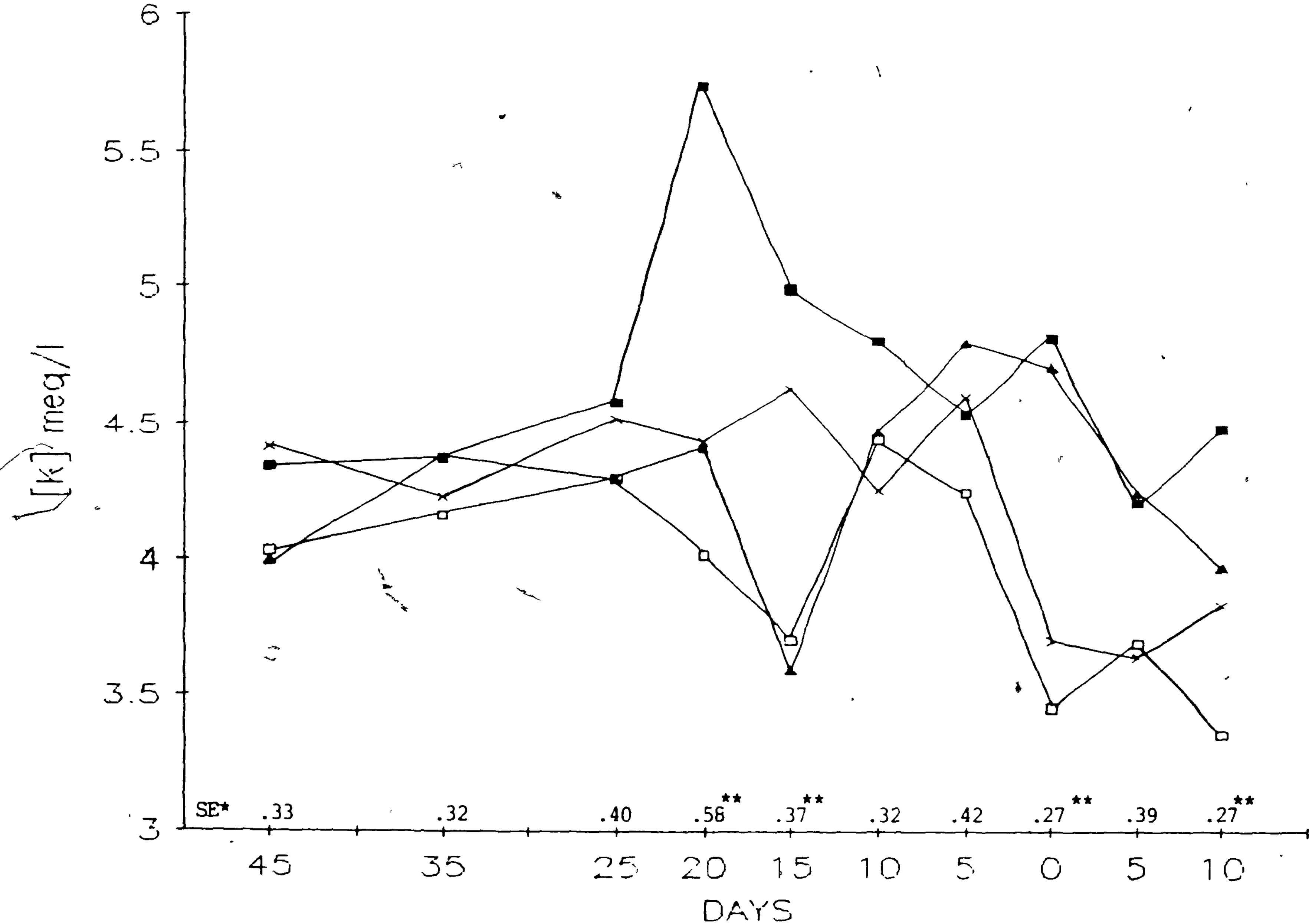
Average concentration of plasma K was lower (P<0.05) in cows fed diet 1 than in cows fed the control diet and diets 2 and 3 with cows fed diets 2 and 3 having a higher (P<0.05) value than cows fed the control diet (Table 18). Cows fed diet 2 had a higher (P<0.05) concentration of plasma K than cows fed diet 1 at days 15 and 20 prepartum (Figure 11). During the periparturient period (Figure 12), concentration of plasma K was higher (P<0.05) in cows fed diet 3 than in cows fed the control diet on days 2 and 1 prepartum and on the day of parturition. No correlation was obtained between concentration of plasma K and the level of dietary anion-cation balance (meq/kg ration D.M.) (Table 14).

5.6. Plasma chloride

Average concentration of plasma Cl was higher (P<0.05) in cows fed diets 1 and 2 than in cows fed the control diet and diet 3 (Table 18). Cows fed diet 1 had a higher (P<0.05) concentration of plasma Cl than cows fed diets 2 and 3 at day-6 prepartum and than cows fed control diet at day-8 prepartum (Figure 13). The average initial concentration of plasma Cl (day-45 prepartum) was higher (P<0.05) in cows fed

Figure 11. Concentration of plasma potassium from day-45 prepartum to day-10 postpartum in cows offered the control diet (X), diet 1 (□) diet 2 (■) and diet 3 (▲).

* Standard error
** Values differ significantly (P<0.05)



[K] meq/l
6
5.5
5
4.5
4
3.5
3
SE* .33 .32 .40 .56** .37** .32 .42 .27** .39 .27**
45 35 25 20 15 10 5 0 5 10
DAYS

Figure 12. Concentration of plasma potassium from 48 hours prepartum to 60 hours postpartum in cows offered the control diet (X), diet 1 (□), diet 2 (■) and diet 3 (▲).

* Standard error
** Values differ significantly (P<0.05)

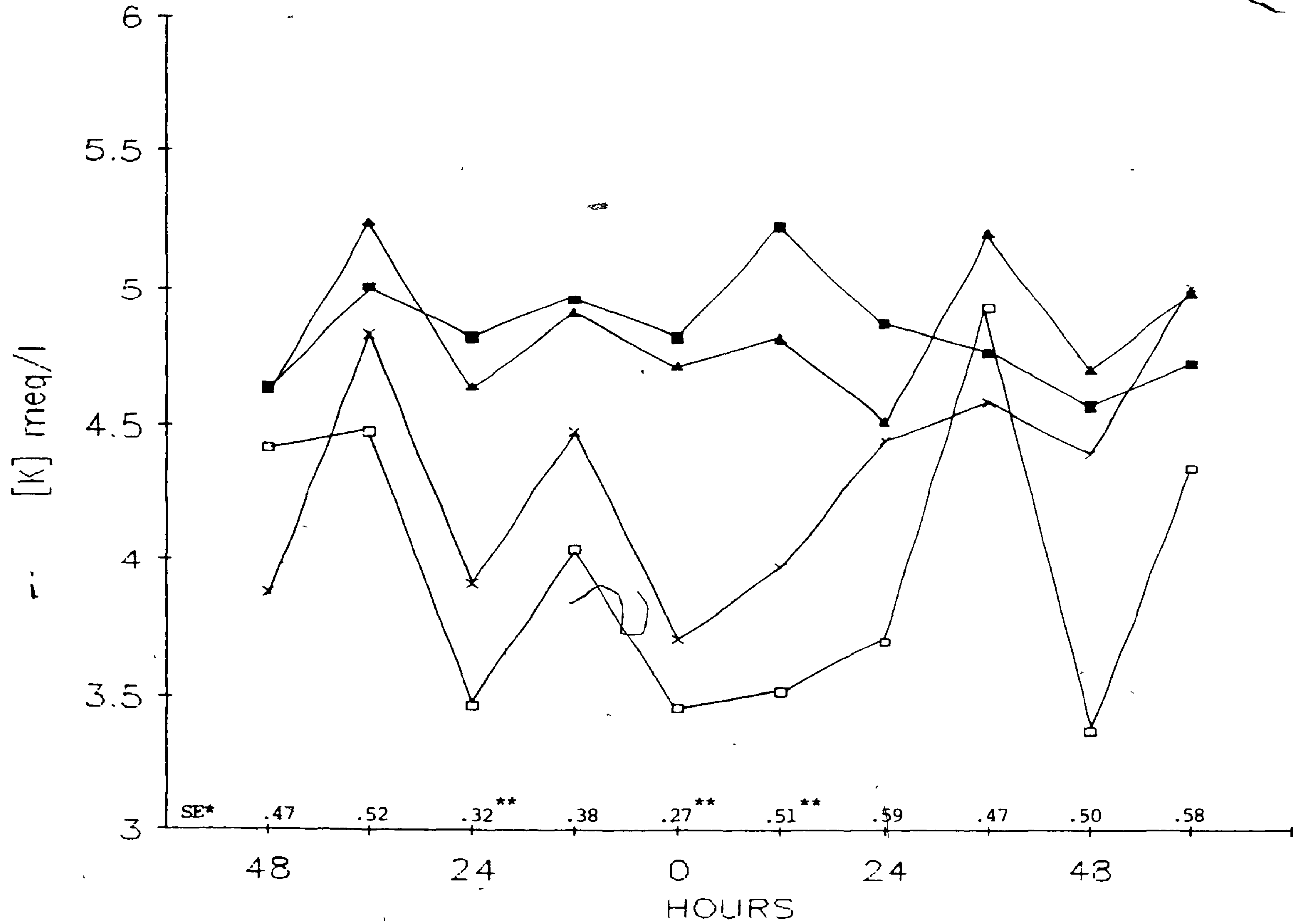
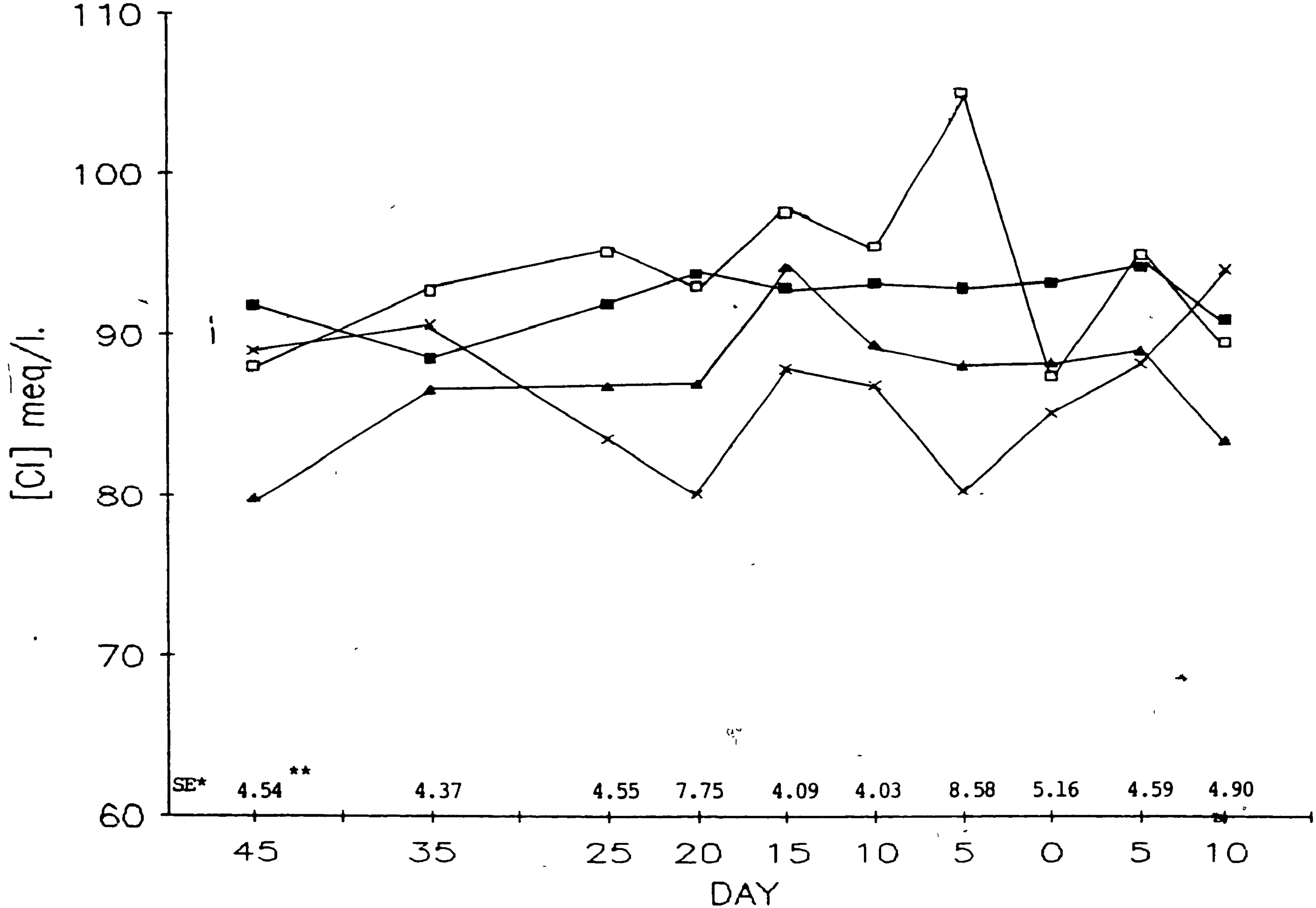

[K] meq/l
6
5.5
5
4.5
4
3.5
3
SE* .47 .52 .32** .38 .27** .51** .59 .47 .50 .58
48
24
0
24
48
HOURS

Figure 13. Concentration of plasma chloride from day-45 prepartum to day-10 pospartum in cows offered the control diet (X), diet 1 (□), diet 2 (■) and diet 3 (▲).

* Standard error
** Values differ significantly (P<0.05)



[Cl] meq/l.
110
100
90
80
70
60
SE*
**
4.54
4.37
4.55
7.75
4.09
4.03
8.58
5.16
4.59
4.90
45
35
25
20
15
10
5
0
5
10
DAY

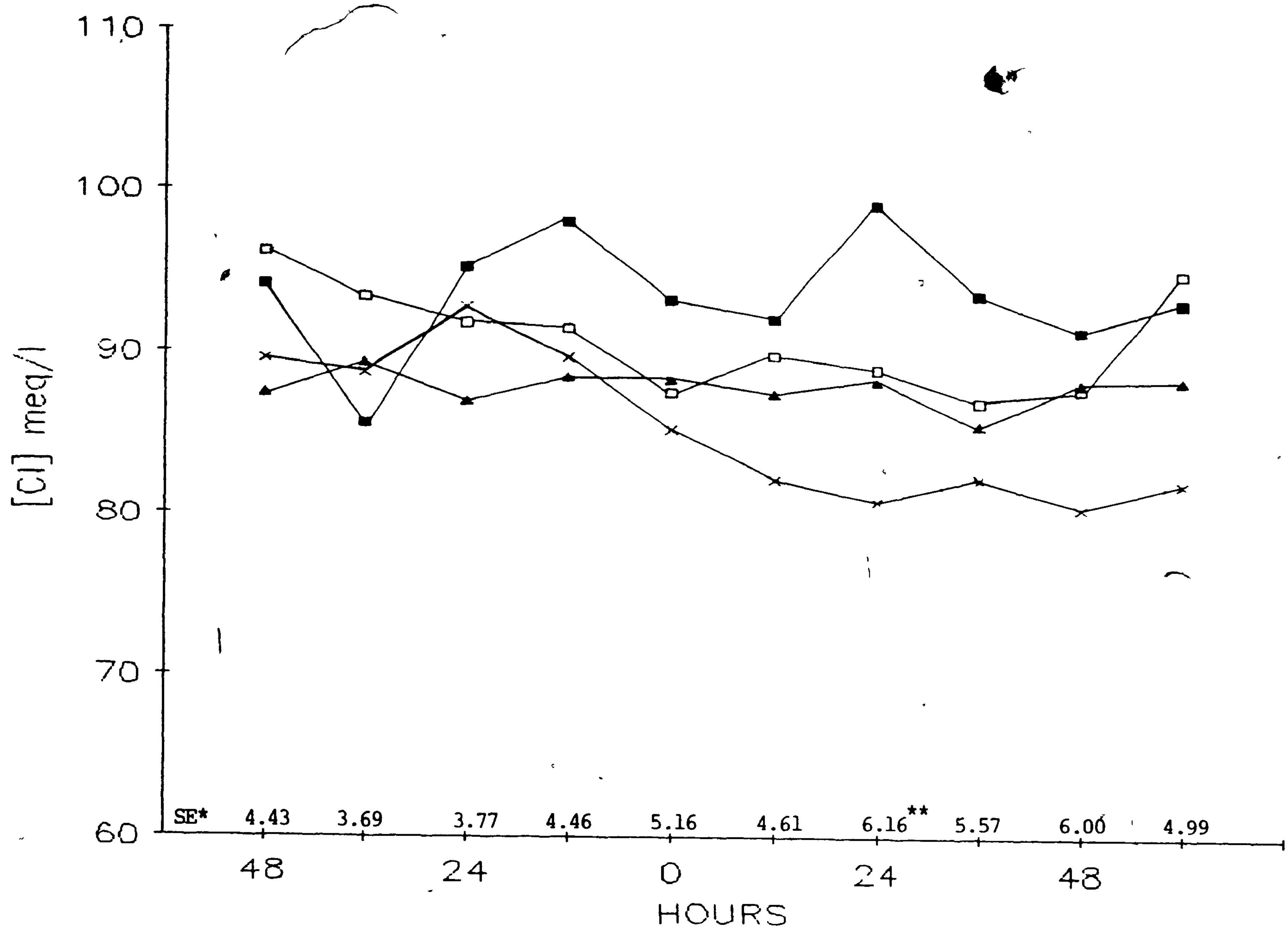
diet 2 than in cows fed diet 3. Concentration of plasma Cl was not different ($P>0.05$) between groups during the periparturient period except on day-1 postpartum where concentration of plasma Cl in cows fed diet 2 was higher ($P<0.05$) than in cows fed the control diet (Figure 14). Negative correlation ($r=-0.20629$, $P=0.0001$) was obtained between concentration of plasma Cl and the level of dietary anion-cation balance (meq/kg ration D.M.) (Table 14) with the highest correlation obtained at day-9 prepartum ($r=-0.53501$, $P=0.0221$).

5.7. Plasma sulfate

Average concentration of plasma sulfate (SO_4) was lower ($P<0.05$) in cows fed the control diet than in cows fed diets 1, 2 and 3 (Table 18). Cows fed diets 2 and 3 had a higher ($P<0.05$) concentration of plasma SO_4 than cows fed the control diet at days-25 and 20 prepartum (Figure 15). Concentration of plasma SO_4 in cows fed diets 2 and 3 was higher ($P<0.05$) during the periparturient period than in cows fed the control diet for day-1 prepartum and for day-2 postpartum with cows fed diet 3 having a higher ($P<0.05$) value than cows fed the control diet for the day of parturition (Figure 16). At day-10 postpartum, concentration of plasma SO_4 was not different ($P>0.05$) between groups.

Figure 14. Concentration of plasma chloride from 48 hours prepartum to 60 hours postpartum in cows offered the control diet (X), diet 1 (□), diet 2 (■) and diet 3 (▲).

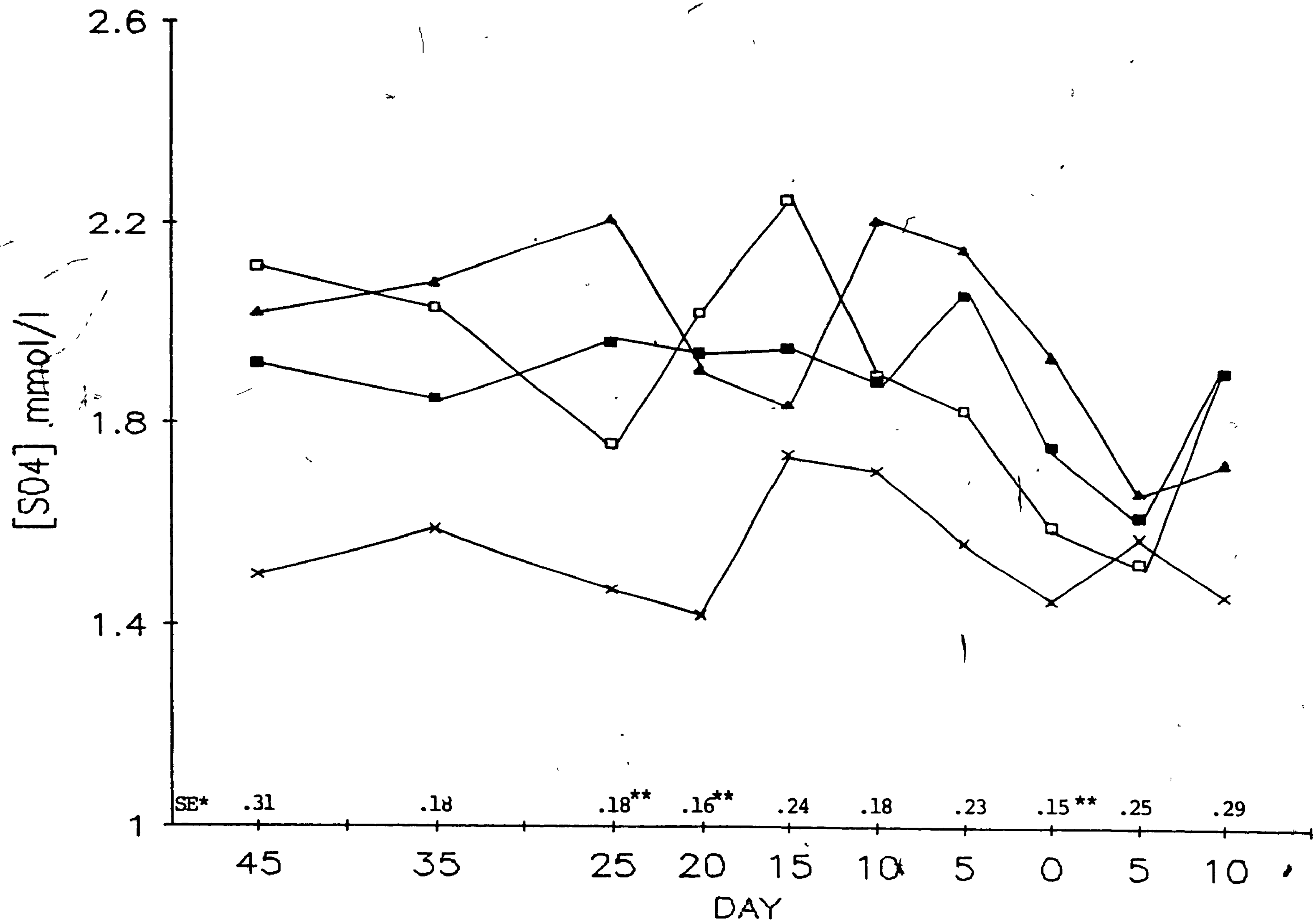
* Standard error
** Values differ significantly ($P<0.05$)



110
100
90
80
70
60
[Cl] meq/l
SE* 4.43 3.69 3.77 4.46 5.16 4.61 6.16** 5.57 6.00 4.99
48
24
0
24
48
HOURS

Figure 15. Concentration of plasma sulfate from day-45 prepartum to day-10 postpartum in cows offered the control diet (X), diet 1 (□), diet 2 (■) and diet 3 (▲).

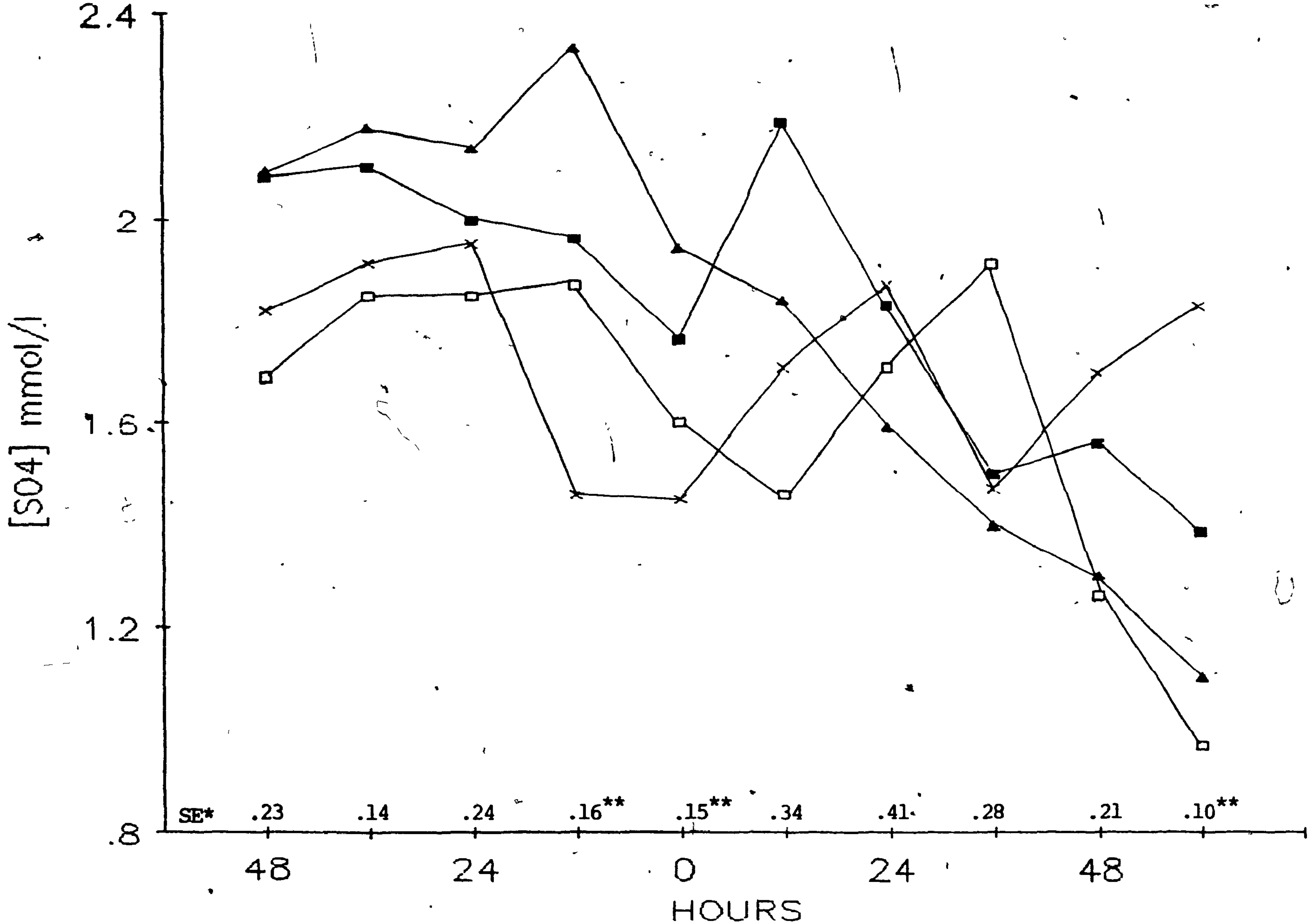
* Standard error
** Values differ significantly (P<0.05)



2.6
2.2
1.8
1.4
1
[SO4] mmol/l
SE* .31 .18 .18** .16** .24 .18 .23 .15** .25 .29
45 35 25 20 15 10 5 0 5 10
DAY

Figure 16. Concentration of plasma sulfate from 48 hours prepartum to 60 hours postpartum in cows offered the control diet (X), diet 1 (□), diet 2 (■) and diet 3 (▲).

* Standard error
** Values differ significantly (P<0.05)


2.4
2
1.6
1.2
.8
[SO4] mmol/l
SE*
.23
.14
.24
.16**
.15**
.34
.41
.28
.21
.10**
48
24
0
24
48
HOURS

Negative correlations were obtained between concentration of plasma SO_4 and the level of dietary anion-cation balance (meq/kg ration D.M.) (Table 14). Correlations were negative during the prepartum period but positive during the postpartum period (Table 24). The strongest correlation observed was for day-1 prepartum (r=-0.71301, P=0.0009) with correlations becoming weaker at the day of parturition. On day-2 postpartum, a positive correlation was obtained between concentration of plasma SO_4 and the level of dietary anion-cation balance (r= 0.58604, P=0.0276).

6. Interaction between plasma macrominerals and plasma calcium

Positive correlations were obtained for the entire trial between concentration of plasma Ca and concentration of plasma P, K, Cl and SO_4 with the strongest correlation being with concentration of plasma SO_4 (r=0.34298, P=0.0001). A weak correlation was observed between concentration of plasma Ca and concentration of plasma Mg (Table 25). However, during the periparturient period (Table 26), the correlation between concentration of plasma Ca and concentration of plasma P, K and SO_4 were stronger than for the entire trial. The only significant (P<0.05) correlation obtained during the periparturient period was between the concentration of plasma Ca and the concentration of plasma Mg was on day-2 prepartum

TABLE 24. Correlations between the concentration of plasma sulfate and the level of dietary anion-cation balance (meq/kg ration DM) during the periparturient period (48 hours pre- to 60 hours postpartum).

Hours	Coefficients of correlation	Probability (P>F)
48 prepartum	-0.40482	0.1070
36 prepartum	-0.43431	0.0815
24 prepartum	-0.29047	0.2581
12 prepartum	-0.71301	0.0009
0 parturition	-0.51019	0.0305
12 postpartum	-0.40077	0.1109
24 postpartum	-0.21516	0.4236
36 postpartum	-0.09129	0.7367
48 postpartum	0.29990	0.2422
60 postpartum	0.58604	0.0276

TABLE 25. Correlations between the concentration of plasma calcium and the concentration of plasma phosphorus, magnesium, potassium, chloride and sulfate during the trial (day-45 pre- to day-10 post-partum).

Mineral	Coefficient of correlation	Probability (P>F)
Phosphorus	0.28459	0.0001
Magnesium	-0.04456	0.3004
Potassium	0.12048	0.0053
Chloride	0.15362	0.0004
Sulfate	0.34298	0.0001

TABLE 26. Correlations between the concentration of plasma calcium and the concentration of plasma phosphorus, magnesium, potassium, and sulfate during the periparturient period (48 hours pre- to 60 hours post-partum).

Minerals	Coefficients of correlation									
	prepartum					postpartum				
	48	36	24	12	0	12	24	36	48	60
Phosphorus	.25	.52[a]	.43[b]	.36	.64[a]	.29	.24	.06	.07	-.18
Magnesium	.68[a]	.36	.20	-.11	.24	.19	-.31	.01	-.10	-.03
Potassium	.20	.42[b]	.48[a]	.26	.42[b]	.49[a]	.33	-.06	-.29	.25
Sulfate	.71[a]	.55[a]	.57[a]	.51[a]	.17	-.42[b]	.45[b]	.52[a]	.04	.17

[a] $P<0.05$
[b] $P<0.1$

(r= 0.68108, P=0.0019). No correlation was observed between the concentration of plasma Ca and the concentration of plasma Cl during the periparturient period. At parturition, correlations were only observed between the concentration of plasma Ca and the concentration of plasma K and P. Concentration of plasma P was not correlated to the concentration of plasma Ca on days 1 and 2 postpartum. A negative correlation was obtained for day-3 postpartum (Table 27) between the concentration of plasma Ca and the concentration of plasma P.

7. Digestibility study

7.1. Period 1 (day-24 to day-21 prepartum)

Values for digestibility of DM (%) and apparent absorption of minerals (%) are shown in Table 28. There were no difference (P>0.05) between groups for digestibility of DM (%) and for apparent absorption of Ca, P, Mg K, Na and Cl (%). Positive correlations (Table 29) were observed between the level of dietary anion-cation balance (meq/kg ration DM) and the percent apparent absorption of Ca, P, Mg, K, Na and Cl with the strongest being with percent apparent absorption of Mg (r=0.40419, P=0.0001). Correlations between percent absorption of minerals and the daily intake of minerals

TABLE 27. Correlations between the concentration of plasma calcium and the concentration of plasma phosphorus, potassium and sulfate during the postpartum period (day-3 to day-10 postpartum).

Minerals	Coefficients of correlation — Days postpartum 3	5	10
Phosphorus	-0.62347[a]	-0.14639	0.61726[a]
Potassium	0.31626	0.45052	0.02575
Sulfate	0.36774	0.21153	0.32788

[a] $P<0.05$
[b] $P<0.1$

TABLE 28. Digestibility of dry matter (DM) and apparent absorption of calcium, phosphorus, magnesium, sodium, potassium and chloride for period 1 (day-24 pre- to day-21 pre-partum) in cows fed diets with different anion-cation balance.[1]

Nutrient digestibility (%)	Treatment				
	Control	Diet 1	Diet 2	Diet 3	S.E.
Dry matter	74.12	70.65	69.26	68.37	2.50
Calcium	52.48	46.47	45.10	42.72	4.46
Phosphorus	57.32	55.40	57.73	57.04	3.36
Magnesium	58.14	51.77	49.02	47.94	5.85
Sodium	73.05	54.77	59.58	55.83	7.40
Potassium	93.49	92.88	91.66	90.51	1.52
Chloride	67.84	64.06	60.28	58.20	4.03

[1] Values expressed as LSMeans ± standard error of LSMeans

TABLE 29. Correlations between the apparent absorption of calcium, phosphorus, magnesium, sodium, potassium and chloride and the level of dietary anion-cation balance (meq/kg ration DM) for period 1 (day-24 to day-21 prepartum).

Mineral absorption (%)	Coefficients of correlation	Probability (P>F)
Calcium	0.26141	0.0001
Phosphorus	0.15978	0.0002
Magnesium	0.40419	0.0001
Sodium	0.30067	0.0001
Potassium	0.37605	0.0001
Chloride	0.24384	0.0001

(g/day) are shown in Table 30. The percent apparent absorption of Ca was not correlated with daily intake of any mineral (g/day). The percent apparent absorption of Mg was only correlated with the daily intake of Mg. The percent apparent absorption of P, K and Cl were not correlated with daily intake of any mineral. The percent apparent absorption of Na was only correlated with the daily intake of Mg. Correlations between percent apparent absorption of Ca and percent apparent absorption of each minerals are shown in Table 31. Correlation was only observed between percent apparent absorption of Ca and P.

7.2. Period 2 (day-7 prepartum to day-1 postpartum)

Values for digestibility of DM (%) and apparent absorption of minerals (%) are shown in Table 32. There was no difference ($P<0.05$) between groups for the digestibility of DM (%) and for the percent apparent absorption of P, Na, K and Cl. The percent apparent absorption of Ca was lower ($P<0.05$) in cows fed diets 2 and 3 than in cows fed the control diet. The percent apparent absorption of Mg was higher ($P<0.05$) in cows fed the control diet than in cows fed diets 1, 2 and 3. Similar to period 1, positive correlations were observed between the level of dietary anion-cation balance (meq/kg ration D.M.) and percent apparent absorption

TABLE 30. Correlations between the apparent absorption of calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K) and chloride (Cl) and the daily intake of these minerals for period 1 (day-24 to day-21 prepartum).

Minerals absorption (%)	Coefficients of correlation minerals intake (g/day)						
	Ca	P	Mg	Na	K	Cl	S
Calcium	.04	.07	-.37	.29	.01	-.08	-.21
Phosphorus	-.14	-.11	-.14	-.00	.03	-.10	-.03
Magnesium	.41[b]	.14	.61[a]	.33	.02	.29	-.32
Sodium	.41[b]	.14	.47[a]	.43[b]	-.22	.17	-.34
Potassium	.08	-.14	.28	.18	-.11	.07	-.33
Chloride	.12	-.01	.08	.33	-.09	.12	-.31

[a] $P<0.05$
[b] $P<0.1$

TABLE 31. Correlations between the apparent absorption of calcium and the apparent absorption of phosphorus, magnesium, sodium, potassium and chloride for period 1 (day-24 to day-21 prepartum) and for period 2 (day-7 prepartum to day-1 postpartum).

Minerals absorption (%)	Coefficients of correlation	
	Period 1	Period 2
Phosphorus	0.65734[a]	0.54000[a]
Magnesium	-0.08869	0.42973[b]
Sodium	0.20012	-0.15314
Potassium	0.22043	0.02590
Chloride	0.36200	0.35298

[a] $P<0.05$
[b] $P<0.1$

TABLE 32. Digestibility of dry matter (DM) and apparent absorption of calcium, phosphorus, magnesium, sodium, potassium and chloride for period 2 (day-7 prepartum to day-1 postpartum) in cows fed diets with different anion-cation balances.

Nutrient digestibility (%)[1]	Treatment				
	Control	Diet 1	Diet 2	Diet 3	S.E.
Dry matter	74.95	70.19	69.35	71.07	2.24
Calcium	59.42[a]	48.18[ab]	46.65[b]	42.21[b]	4.30
Phosphorus	64.75	58.93	57.58	52.02	4.75
Magnesium	70.07[a]	43.18[b]	46.99[b]	48.88[b]	6.43
Sodium	74.20	66.63	63.22	68.57	7.81
Potassium	88.95	89.46	88.29	89.47	3.45
Chloride	71.56	71.63	66.18	67.34	2.53

[1] Values expressed as LSMeans ± standard error of LSMeans

[a,b] Values within rows with different superscripts differ ($P<0.05$)

of Ca, P, Mg, Na, K and Cl (Table 33). Compared to period 1, correlations obtained during period 2 were stronger for percent apparent absorption of Ca, Mg, P and Na. Correlations between percent apparent absorption of every macrominerals and the daily intake of every mineral are shown in Table 34. The only correlation observed was between the percent apparent absorption of Mg and the daily intake of S ($r=-0.48331$, $P=0.0494$). Correlations between percent apparent absorption of Ca and percent apparent absorption of others minerals are shown in Table 31. As for period 1, the only correlation observed was between percent apparent absorption of Ca and P. However, there was a weak correlation ($P<0.1$) between the percent apparent absorption of Ca and Mg for period 2. No differences ($P>0.05$) were found between periods for the digestibility of DM (%) and for the percent apparent absorption of Ca, P, Mg, Na, K and Cl.

TABLE 33. Correlations between the apparent absorption of calcium, phosphorus, magnesium, sodium, potassium and chloride and the level of dietary anion-cation balance (meq/kg ration DM) for period 2 (day-7 prepartum to day-1 postpartum).

Minerals absorption (%)	Coefficients of correlation	Probability (P>F)
Calcium	0.61036	0.0001
Phosphorus	0.45062	0.0001
Magnesium	0.56707	0.0001
Sodium	0.48138	0.0001
Potassium	0.14773	0.0008
Chloride	0.27569	0.0001

TABLE 34. Correlations between the apparent absorption of calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K) and chloride (Cl) and the daily intake of minerals for period 2 (day-7 prepartum to day-1 postpartum).

Minerals absorption (%)	Coefficients of correlation intake of minerals (g/day)						
	Ca	P	Mg	Na	K	Cl	S
Calcium	.01	-.09	-.11	.29	-.15	-.08	-.44[b]
Phosphorus	-.26	-.30	-.28	-.01	-.23	-.19	-.41
Magnesium	.04	-.14	.39	.32	-.25	-.09	-.48[a]
Sodium	-.06	-.18	.32	.06	-.09	-.06	.03
Potassium	-.29	-.33	.04	-.29	-.32	-.27	-.22
Chloride	-.07	-.11	.20	-.09	-.10	.02	-.12

[a] $P<0.05$
[b] $P<0.1$

V. DISCUSSION

1. Experimental diets

All diets contained high levels of Ca and P in order to obtain rations that will induce milk fever. In fact, prepartal diets of less than 100 g of Ca are recommended to minimize the incidence of milk fever (Jorgensen,1974) and prepartum diets low in P (10 g daily), in the presence of prepartum diets high in Ca, will help to prevent milk fever (Kichura et al,1982). On the other hand, a high level of intake P is known to suppress kidney formation of 1,25$(OH)_2D_3$ (Reinhardt and Conrad,1980). Our control diet had a higher Ca content than diets 1,2 and 3 due to the difference between calculated and analyzed values. The use of some commercial mineral salts, such as limestone and gypsum, prevented us from meeting our expected values for Ca. This uncontrolled mistake emphasizes the need for using pure minerals when experiments are conducted, even with large animals. However, the difference in the Ca content between the control diet and diets 1, 2 and 3 should not have influenced the results since Ca content in the latter diets ($\geq$ 150 g daily) was already high enough to induce milk fever. In fact, a prepartal diet delivering 150 g of Ca per day has been shown to reduce the resorptive ability of bone during parturition, which is an important factor in the development of severe hypocalcemia (Black et al,1973). Magnesium content of the diets was not different between groups and was at a higher level than NRC (1978) requirements at more than 0.25% of the ration DM with the reasonning that high levels of dietary Ca may cause a reduced availability of dietary Mg (Sansom et al,1983). Additionnally, diets high in sulfate may reduce concentration of plasma Mg if the Mg content of the diet is less than 0.25% of the ration DM (Kappel et al,1983). Dietary levels of Na, K, Cl and S have been calculated to meet the four different anion-cation balances of +400, +200, +100 and +50 meq/kg ration DM, however, as previously mentioned, dietary anion-cation balances differed somewhat from calculated values because of the differences between calculated and analyzed values. This difference did not prevent us from observing the effects of treatment on the various parameters.

2. Dry matter intake

A reduced dry matter intake expressed as a percentage (%) of body weight (BW) observed in cows fed diet 2 compared to cows fed the control diet and diets 1 and 3 is not supported by Block (1984). This result cannot be explained by the quality of the forages since forages fed to cows were similar in all diets. Mineral content of diet 2 was similar to diet 3, except for the sulfur content. In fact, sulfur content of diet 2 was lower than diet 3 but higher than the level of 0.35% DM, which depressed DM intake when given to lactating cows (Bouchard and Conrad,1974). However, cows fed diet 3 did not show a reduced DM intake even with a higher sulfur content in the diet than diet 2. This is supported by other researchers who did not find any harmful effects when feeding relatively high (up to 1.72 % of ration DM) sulfur levels to ruminants (Chalupa et al,1971; Gawthorne and Nader,1976). Feed intake at the day of parturition was not influenced by treatments but was greatly reduced for cows fed all diets. This was reported as a normal event at parturition and related to a rise of plasma estrogen concentration (Braithwaite,1976).

3. Incidence of milk fever

Reducing the dietary anion-cation balance from +394.31 (control diet) to +120.65 meq/kg ration DM (diet 1) had reduced the incidence of milk fever by 25%. However, as we reduced the anion-cation balance from +120.65 (diet 1) to +62.20 meq/kg ration DM (diet 3), no further reduction in the incidence of milk fever was observed. It seemed that either a difference of more than 60 meq/kg ration DM is needed between treatments to observe an effect on the incidence of milk fever or, that dietary anion-cation balance should be less than +62.20 meq/kg ration DM to have a further reduction in the incidence of milk fever. Even though we observed a reduction in the incidence of milk fever between cows fed the control diet and cows fed diets 1,2 and 3, the number of cows was too small to be conclusive. A prepartum dietary anion-cation balance intake of approximately +4486 meq/day (+330 meq/kg ration DM) induced milk fever in 47% of dairy cows (Block,1984) while in a study by Dishington (1975), 86% of the cows consuming a prepartum dietary anion-cation balance of +3875 meq daily contracted milk fever. In our trial, a prepartum dietary anion-cation balance intake (+4674 meq/day) similar to literature previously cited, induced milk fever in 80% of the cows, which supported results obtained by

Dishington (1975). A number of factors could have contributed to the lower incidence of milk fever observed in the trial by Block (1984). Even though our Ca:P ratio was similar to the one in Block's (1984) study, our values of Ca and P intake during the prepartum period were much greater. Our dietary prepartum anion-cation balance was also slightly higher which could explain a slight increase of the incidence of milk fever, but not the large difference observed between both studies. The 60% incidence of milk fever encountered in cows fed diets 1, 2 and 3 could also be explained through the elevated intake of Ca and P during the prepartum period and the positive dietary anion-cation balance. Block (1984) prevented milk fever in 100% of the cows when cows received a negative dietary prepartum anion-cation balance of -128.5 meq/kg ration DM, and Dishington (1975) observed an incidence of milk fever in 8% of the cows when cows received a negative prepartum anion-cation balance of -255 to -385 meq daily.

Factors influencing the incidence of milk fever (age, breed and milk production) did not represent the difference observed between treatments, since cows were assigned within blocks according to their age and milk production, and breed was not different between treatments. Susceptibility of cows to milk fever may be increased if cows are hypomagnesemic (Davies et al,1978), however, in our study, plasma Mg concentration of all groups was maintained near normal, therefore, none of the cows were considered hypomagnesemic.

4. Alterations of plasma calcium, inorganic phosphorus and magnesium

4.1. Plasma calcium

At the beginning of the trial, concentration of plasma Ca averaged 8.56 mg/dl in all cows. This value is similar to values reported by others workers for dry cows (Block,1984; Verdaris and Evans,1975). The decrease in concentration of plasma Ca observed from day-45 to day-35 prepartum in cows fed all different diets could not be due to the high Ca and high P content of the diets since workers (Black et al,1973; Kichura et al,1982) observed either an increase or no change in serum Ca concentration when they started to feed a prepartum diet high in Ca and P to dairy cows. However, the decrease was less important in cows fed diet 3 since these cows did not have to decrease their initial plasma Ca concentration as much as cows fed other diets to reach the similar, and quite stable, plasma Ca concentration for the remainder of the prepartum period (day-35 to day-5 prepartum). Prepartum concentration of plasma Ca was maintained lower than in a trial by Kichura _et al_. (1982) even with an intake of Ca twice as high and approximately the same intake of P. The quantity of absorbed Ca (g/day) during the prepartum period (day-45 to day-5 prepartum) was not correlated to plasma Ca concentration. This was unexpected, since workers found a response of plasma Ca concentration to dietary intake of Ca within 3 days (Green et al,1981). However, the range of the quantity of absorbed Ca was not very large, and could have explained the lack of relation with plasma Ca concentration.

If we refer to the weak correlation obtained for the entire trial between the level of dietary anion-cation balance and plasma Ca concentration, it appears that treatment did not influenced plasma Ca concentration. However, during the periparturient period (day-2 pre- to day-2 post-partum), which is the critical period because this is the period of the highest incidence of milk fever, treatment was effective. In fact, we observed a strong negative correlation between the level of dietary anion-cation balance and plasma Ca level during the periparturient period, especially at parturition. Cows fed rations with a less positive anion-cation balance (diets 1,2 and 3) had a hypocalcemia that was less severe than cows fed the control diet. Therefore reducing the dietary anion-cation balance reduced the incidence of the disease because milk fever is related to a severe hypocalcemic status. This was supported by an experiment by Block (1984) who observed a severe parturient hypocalcemia in cows fed a positive anion-cation balance similar to our control diet but a normocalcemic plasma level with no cases of milk fever in cows fed the negative anion-cation balance. It also appeared that treatment influenced the time that cows exhibited their lowest plasma Ca concentration, since cows fed the less positive anion-cation balances (diets 1,2 and 3) exhibited their lowest plasma Ca concentration at a later time postpartum than cows fed the control diet. This response was also observed by Takagi and Block (1986, a), using sheep fed reduced anion-cation balanced diets and induced experimentally to have hypocalcemia by infusing a solution of Ethylene diamine tetra-acetate (EDTA) intravenously. Plasma Ca concentration of animals fed the low anion-cation balanced diet and infused with EDTA showed a resistance to the reduction of plasma Ca caused by EDTA compared to control sheep, which was explained by a higher Ca mobilization capacity by sheep fed the low anion-cation balanced diet. This less rapid decline of plasma Ca concentration could be a determinant in the incidence of milk fever since we observed, as did Van Soest and Blosser (1954), that paretic cows showed a more rapid decline in their plasma Ca concentration than non-paretic cows. Absolute plasma Ca concentration could also be an important factor; the lowest plasma Ca level was below 5 mg/dl in paretic cows and above 5 mg/dl in non-paretic cows, as it was reported by Mayer et al (1966). We did not measure plasma ionic Ca concentration, however, we can speculate that it constantly followed along with the change of total plasma Ca concentration. In fact, strong positive correlations were obtained between both values in normocalcemic, hypocalcemic and paretic cows (Littledike et al,1969; Schroeder,1973; Dauth et al,1984).

There are three controlling mechanisms for counterbalancing disturbances in Ca stress and maintaining plasma Ca homeostasis: intestinal Ca absorption; bone Ca removal; and renal Ca reabsorption (Ramberg et al,1984). In our study, apparent Ca absorption, which did not account for the endogenous loss of fecal Ca, may not represent the possible source of Ca for maintaining plasma Ca homeostasis in cows fed the lower anion-cation balanced diets. In fact, cows fed the less positive anion-cation balances (diets 1,2 and 3) had a smaller quantity of Ca apparently absorbed than cows fed the control diet. A reduced apparent absorption percent of Ca could have involved a higher endogenous fecal Ca loss with an increase or no change in the true Ca absorption percent. However, research conducted with lambs receiving NH_4Cl (Braithwaite,1972) showed no effect of the experimental diet on endogenous fecal Ca excretion, but did show a higher percent true absorption of Ca indicating a higher percent apparent absorption of Ca. Increased percent apparent absorption of Ca was also observed when non-lactating goats received a ration containing 1.2% HCl

(Freeden et al,1984), or when dairy cows were fed ration containing 115 or 230 ml of concentrated HCl (Hart et al,1931). However, when lambs were fed diets with a reduced anion-cation balance, percent apparent absorption of Ca was not affected by treatment (Takagi and Block, 1986, b). From these studies, we can speculate that addition of acid to diets may act differently on intestinal absorption of Ca than reducing the dietary anion-cation balance. The effect of HCl and NH_4Cl may be through the liberation of hydrogen and/or chloride ion in the gastrointestinal tract that can alter the absorption of Ca by modifying the pH of the gastrointestinal tract. In fact, the upper small intestine of ruminants, which is the main site of absorption of Ca, has a limited capacity to neutralize acidic digesta from the abomasum (Wheeler,1980). Thus, addition of acid to the diet may decrease pH of the small intestine thereby increasing the solubility of Ca. The experiment of Verdaris and Evans (1975) may support this hypothesis since they observed a stimulating effect of decreasing pH of the diet on absorption of Ca. It is unlikely that our diets affected the environmental pH of the gastrointestinal tract because no increase of absorption of Ca was observed when animals received a reduced dietary anion-cation balance.

We did not measure urinary Ca excretion, however, according to literature the loss of Ca via this route may be higher in cows fed less positive anion-cation balances (diets

1,2 and 3) than in cows fed the control diet. In fact, higher excretion of urinary Ca was observed by Takagi and Block (1986, b) when lambs received a less positive dietary anion-cation balance compared to a control diet. Increased urinary excretion of Ca was also reported in non-lactating goats (Freeden et al,1984), in lactating dairy cows (Hart et al,1931) and in sheep (Braithwaite,1972) when acid was added to the diet as well as when inorganic sulfate was included to diets of adult rats (Whiting and Cole,1986). Furthermore, the addition of acid to the diet (Braithwaite,1972) and feeding a decreased anion-cation balance (Takagi and Block, 1986,b) resulted in a decreased urinary pH. Net acid excretion has been mentioned as being responsible for the increased urinary excretion of Ca (Whiting and Cole,1986) encountered when adult rats received a diet high in inorganic sulfate or chloride. It is then logical to propose that in our study, cows fed the less positive anion-cation balance (with the higher Cl and SO_4 content) had a decreased urinary pH. Also, because it was demonstrated in poultry (Mongin,1980) that a reduced anion-cation balance (excluding the sulfur content) could created a metabolic acidotic animal, it is quite possible that cows on our trial fed a reduced anion-cation balance had developed a mild metabolic acidosis. Therefore, plasma Ca homeostasis of cows fed the less positive anion-cation balances appeared to be maintained at a higher level via bone resorption since percent apparent absorption of Ca was decreased and urinary excretion of Ca may have been increased. Bone resorption could be stimulated during a chronic metabolic acidosis to supply buffering materials. In fact, Barzel and Jowsey (1969) had argued that metabolic acidosis might stimulates the 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. However, Braithwaite (1972) using lambs, and Newell and Beauchene (1975) using rats, found that metabolic acidosis did not alter the resorption rate of bone. On the other hand, diets containing an acid load of inorganic sulfate (1.42%) fed to adult rats produced an enhanced bone resorption for a period of 2 months (Whiting and Draper,1981); in an experiment with prepartum dairy cows (Block, 1984) plasma hydroxyproline levels showed an earlier enhancement of bone resorption in cows fed a reduced anion-cation balance compared to cows fed increased anion-cation balance during the periparturient period.

4.2. Plasma inorganic phosphorus

As reported by Schroeder (1973), plasma inorganic phosphorus (Pi) fluctuated more than plasma Ca concentration, probably because of the tighter regulation of plasma Ca through its homeostatic mechanism (Barton et al., 1981). Plasma Pi concentration is affected by patterns of feed consumption as it has been shown to increase markedly for 2 hours post-feeding, then declines (Forar et al., 1982). Our plasma Pi values were slightly higher than the range of normal values seen in the literature (Verdaris and Evans, 1975; Barton et al.,1981). However, these high values could be partly explained by the time of blood sampling (2 hours after the morning feeding), and partly by the elevated intake of P by cows fed all diets. In fact, Kichura et al. (1982) and Forar et al. (1982) reported that as intake of P increased, plasma Pi concentration will also increase. However, the influence of dietary intake of P on plasma Pi concentration observed by Kichura et al.(1982) was effective during the prepartum period, but not during the periparturient period, as in our experiment. This was evidenced by finding no correlation between the quantity of apparently absorbed P and plasma Pi concentration during the last week preceding parturition. The lower prepartum plasma Pi concentration observed in cows fed reduced anion-cation balance compared to cows fed the control diet could be related to a reduced demand for P due to a lower Ca retention in cows fed diets 1,2 and 3. In fact, Takagi and Block (1986, b) had observed a lower Ca retention, as a proportion of Ca intake, and a net loss of P (g/7 days) in sheep fed reduced anion-cation balance compared to sheep fed control diet with an anion-cation balance of +314 meq/kg ration DM. Thus, it is probable that cows fed diets 1,2 and 3 had a lower percent of skeletal Ca retention than cows fed control diet. Because of the close association of Ca and P in bone the skeletal Ca retention is related to the skeletal P retention (Braithwaite, 1984). Thus, a reduced skeletal P retention will reduce the demand for P and may explain the net loss of P demonstrated by Takagi and Block (1986, b), as well as the reduced concentration of plasma Pi prepartum in cows fed reduced anion-cation balance observed in our study.

During the periparturient period, plasma Pi concentration paralleled plasma Ca concentration, as it did in a trial by Schroeder (1973). Several authors found positive correlations between plasma Pi and plasma ionized Ca and also with total plasma Ca (Blum et al., 1972; Daniel and Moodie, 1979). A decreased plasma ionized Ca will stimulate parathyroid hormone secretion in cows (Fisher et al., 1973) and may explain the reduction of plasma Pi concentration since parathyroid hormone lowers plasma Pi either by increasing renal phosphate excretion (Mayer et al., 1966, b) and/or by increasing the salivary secretion of phosphate (Clarke et al., 1975). The positive correlation observed in our study between plasma Pi and plasma Ca concentration from day-2 prepartum to parturition supported results obtained by Daniel and Moodie (1979). We did not find any correlation for day-1 and day-2 postpartum probably due to the high variation between hypocalcemic cows preceding paresis, as shown by Daniel and Moodie (1979). The negative correlation obtained at day-3 postpartum agrees with the finding of Byrne et al. (1971) that plasma Pi concentration continued to decrease for one week postcalving while plasma Ca concentration was increasing.

4.3. Plasma magnesium

Average plasma Mg values determined in this study were slightly lower than normal values reported in the literature (Byrne et al., 1971; Littledike et al., 1970). As increased chloride and sodium intake have been shown to increase urinary Mg excretion, and urinary Mg loss was not influenced by the amount of digestible Mg (Lomba et al., 1968), it was possible that high chloride intake in our four different diets had affected the plasma Mg concentration, particularly in the cows fed the control diet since Na intake was also elevated in this group. In fact, negative correlations were observed during the third week preceding parturition between plasma Mg concentration and quantity of Na and Cl absorbed.

Higher plasma Mg concentration observed during the periparturient period in cows fed diets 2 and 3 compared to cows fed the control diet and diet 1, could be explained through an increased bone resorption which was predicted previously in the plasma Ca section. This difference in plasma Mg concentration between groups could not have been related to higher absorption of Mg since Lomba et al. (1968) demonstrated that only the Mg and Ca intake will have an enhanced effect on percent apparent absorption of Mg. These mineral intakes were not higher in cows fed diets 2 and 3 than in cows fed control and diet 1. Furthermore, percent apparent absorption of Mg was lower in the former groups than in the control group. Urinary Mg excretion was not measured in our experiment, however, according to the results of Takagi and Block (1986, b), reduced dietary anion-cation balance did not alter urinary Mg excretion in lambs. Higher plasma Mg concentrations were observed when cows were fed a prepartum diet low in Ca compared to a prepartum diet high in Ca (Verdaris and Evans, 1975). It is now evident that prepartum diets low in Ca enhances bone resorption during the periparturient period to maintain plasma Ca concentration (Green et al., 1981; Kichura et al., 1982). Plasma Mg concentration could also be increased through an enhanced bone resorption, since bone contains 60% of the total body Mg with a labile portion (Aikawa, 1981) that can be mobilized during Mg deficiency (McAleese et al., 1960). Furthermore, a close relationship exists between Mg and Ca homeostasis because both can be released from bone through a stimulation by parathyroid hormone (Koo, 1984). Therefore, we suspect that increased bone resorption during the periparturient period was the cause of the elevated plasma Mg concentration observed in cows fed reduced anion-cation balances. The positive correlations expected between plasma Ca and plasma Mg concentration were found only on day-2 prepartum, probably because of the high variation in plasma Mg between cows in response to hypocalcemia as stated by Daniel (1980). As observed by others (Allcroft and Godden, 1934; Schroeder, 1973), a decrease in plasma Mg concentration was found up to day-5 postpartum in cows fed all diets. This can be related to a general negative Mg balance occurring in the early part of the postpartum period in lactating cows because higher dietary energy content, higher dry matter intake, nitrogen and fat have been shown to reduce Mg balance (Lomba et al., 1968).

5. Alterations of other plasma macrominerals

5.1. Plasma sodium

Our values for plasma Na were lower than normal values in dairy cows as reported by Fettman et al. (1984) and Escobosa et al. (1984). This was probably due to the elevated urinary Na excretion related to the expected high urinary Ca excretion; there is an interdependence in the renal handling of Ca and Na (Sutton and Dirks, 1978). Higher average plasma Na concentration in cows fed control diet confirmed the finding of Cohen and Hurwitz (1974) as well as Block (1984) that, an increase in dietary Na will increase the plasma level of Na. On the other hand, the elevated plasma Na concentration in cows fed diet 3 cannot be explained through a higher intake of Na compared to cows fed diet 1 and 2, thus, the response of this group remains unclear.

5.2. Plasma potassium

As observed by Block (1984), average plasma K concentration in cows fed elevated anion-cation balance (control and diet 1) was lower than in cows fed reduced anion-cation balance (diet 2 and 3). However, the amount of K apparently absorbed was higher in cows fed diet 3. This is unlikely to affect the plasma K concentration since the animal will efficiently control body K content by excretion in the urine of the excess absorbed K (Field, 1964). Block (1984) explained the lower plasma K concentration in cows fed the cationic diet (+330 meq/kg ration DM) by a possible interaction betweem K and Na, especially when the diet is low in Cl. This interaction was observed by Cohen and Hurwitz (1974) in an experiment with poultry. Although Na intake was higher in cows fed the control diet, this reason does not explain the response of cows fed diet 1, since their Na intake was not higher than cows fed diets 2 and 3. Furthermore, Cl intake was similar in all groups. Therefore, it is difficult to related our results to the interaction between Na and K.

Interesting positive correlations were obtained between plasma K and plasma Ca concentration from day-2 prepartum to day-5 postpartum. This supported the finding that plasma K concentration may fall during natural cases of milk fever as reported by Ward (1956) and Littledike et al. (1969). Daniel (1980) also observed a drop in plasma K concentration accompanying the decrease in plasma Ca concentration induced experimentally in cows. He stipulated that stress, associated with the onset of hypocalcemia and recumbency in the cows, may result in the release of ACTH (Daniel, 1977), which may be partially responsible for the depressions in plasma K concentration. This statement is supported by Waage (1984) who had reported a higher plasma concentration of cortisol in cows that contracted milk fever than in healthy cows.

5.3. Plasma chloride

Our values for plasma Cl are slightly lower than normal values in ruminant (Burkhalter et al., 1980; Fettman et al.,

1984) probably because the intake of Ca, P and Mg were similarly higher than requirements (NRC, 1978) in all groups. The intake of these minerals has been reported to increase urinary excretion of Cl in cows (Paquay et al., 1969), which may affect the plasma level of Cl since urinary excretion is the main homeostatic mechanism of Cl in ruminants (Coppock et al., 1979). Moreover, we observed a lower average plasma Cl concentration in cows fed the control diet and diet 3 compared to cows fed diets 1 and 2. We propose that the higher amount of Ca and Na absorbed in cows fed the control diet contributed to reducing the plasma Cl concentration since the intake of these minerals has been reported to increase urinary excretion of Cl (Paquay et al., 1969). On the other hand, the higher amount of K absorbed in cows fed diet 3 compared to cows fed other diets could explain the lower plasma Cl concentration observed in the former group since intake of K was positively correlated to urinary excretion of Cl (Paquay et al., 1969).

5.4. Plasma sulfate

The higher plasma sulfate (SO_4) concentration observed in cows fed reduced anion-cation balance (diet 1,2 and 3) could be related to the higher dietary intake of sulfur (S) compared to the control group. In fact, concommittant increases in serum SO_4 and increases in urinary SO_4 excretion have been reported in a cysteine-supplemented group of infants (Cole and Zlotkin, 1983). Furthermore, urinary SO_4 excretion was increased when dietary inorganic SO_4 was increased in rats (Whiting and Draper, 1981). These results indicate that an increase of plasma SO_4 occurs when dietary SO_4 is increased. This is supported by Kennedy et al. (1974) who observed this expected response when cattle were fed different amounts of S. The strong positive correlation observed during the periparturient period between plasma Ca and SO_4 concentration may be related to the effect of SO_4 on Ca metabolism. In fact, the anion SO_4 has been demonstrated to decrease the reabsorption of Ca from the kidney tubules in rats possibly by forming an insoluble complex (Bushinsky et al., 1982), and to increase bone resorption (Whiting and Draper, 1981); both effects should increase plasma Ca concentration.

6. Absorption of calcium, phosphorus and magnesium

Our values of percent apparent absorption of minerals were higher than normal values encountered in the literature (Mayer et al., 1967; Bushman et al., 1968; Verdaris and Evans, 1975). This overestimation was probably related to the use of chromic sesquioxide (Cr_2O_3) as a marker for the indicator method to estimate digestibility. Usually, digestibility studies conducted with Cr_2O_3 have been reported to underestimate the percent apparent absorption of minerals, mainly because of the incomplete recovery of Cr_2O_3 in the feces (Stevenson, 1962; Field, 1964). However, the duration of our preliminary and collection periods (Stevenson, 1962) with the mixing of Cr_2O_3 in the feed (Crampton and Lloyd, 1951) should have improved the recovery of Cr_2O_3 in the feces and, therefore, attenuated the usual underestimation of the apparent absorption of minerals. We estimate that the loss of some unknown quantity of Cr_2O_3 given in the feed during mixing and feeding, was more important than the incomplete recovery of Cr_2O_3 in the feces.This could explain our elevated values of percent apparent absorption of minerals.

The reduced percent apparent absorption of Ca observed in cows fed the lower anion-cation balances (diets 2 and 3) is suspected to involve a higher endogenous fecal excretion of Ca with no change or a reduced percent true absorption of Ca. Because urinary excretion of Ca was increased and Ca retention was decreased when lambs were fed diets with reduced anion-cation balances (Takagi and Block, 1986, b) we can expect the same response in cows fed diets with reduced anion-cation balances. Negative correlations obtained between urinary Ca excretion and percent apparent absorption of Ca in dry cows (Paquay et al.,1968) further supports this hypothesis. Endogenous excretion of fecal Ca may be considerable and variable (Mayer et al.,1967) but it is not affected by intake of Ca (Braithwaite, 1982) and it increased when excretion of urinary Ca and bone resorption increased following the administration of parathyroid extract to parathyroidectomized cows (Mayer et al., 1967). In this latter case, the percent true absorption of Ca was increased. Although, our study did not involve the use of parathyroid extract to increase plasma Ca concentration, the lower apparent absorption of Ca observed, as well as the expected enhanced urinary Ca excretion and bone resorption could have led to an increased endogenous excretion of fecal Ca. An increase in the size of the exchangeable pool of Ca as suggested when sheep were fed diets with reduced anion-cation balance (not including S in the calculation) (Freeden et al., 1984) may also support our idea concerning the endogenous excretion of fecal Ca.

The percent apparent absorption of P, per se, was not altered by treatment; however, because we expected a lower Ca retention in cows fed diets with reduced anion-cation balances (see plasma P section) compared to control cows, we propose that this latter group had a lower endogenous fecal excretion of P and a higher percent true absorption of P than the former group, as reported by Braithwaite (1984) where sheep exhibited a higher Ca retention. Furthermore, the elevated endogenous fecal excretion of P suspected in cows fed diets with reduced anion-cation balances may involve a very high portion of the P coming from the saliva, since the salivary glands have been proposed as the major route of endogenous P excretion in ruminants (Horst, 1986).

The lower percent apparent absorption of Mg observed during the period including parturition (day-7 prepartum until day-1 postpartum) in cows fed the diets with reduced anion-cation balances (diets 1, 2 and 3) compared to cows fed the control diet, and the lack of change in the apparent absorption of Mg between groups during the prepartum period could be explained by an increased endogenous fecal excretion of Mg resulting from the suspected enhancement of bone resorption during the periparturient period. The net amount of absorbed dietary Mg is assumed to be directly related to the intake of Mg (Aikawa, 1981). However, the intake of Mg was not higher in cows fed the diets with reduced anion-cation balances than in cows fed the control diet, whereas the apparent absorbed amount of Mg was lower in cows fed diets with reduced anion-cation balances. Therefore, it is proposed that the decreased percent apparent absorption of Mg observed in cows fed diets with reduced anion-cation balances during the period including parturition was due to a higher endogenous fecal Mg excretion. The strong positive correlation obtained between apparent absorption of Ca and apparent absorption of P in our study, as well as in the work of Takagi and Block (1986, b) could be explained through the strong relation existing between Ca retention and P retention in sheep (Braithwaite, 1975). It may also suggest that reducing the dietary anion-cation balance had an indirect effect on percent apparent absorption of P by reducing the Ca retention by the animal.

There appears to be an interrelationship between the absorption of Mg and Ca in the rat (Alcock and MacIntyre, 1962) and in the cow (Lomba et al., 1968; Paquay et al. 1968), with a common mechanism for transporting Ca and Mg across the intestinal wall (Hendrix et al., 1963). The tendancy ($P<0.1$) observed in our study, of having a positive correlation between the percent apparent absorption of these two minerals further substantiates this claim.

VI. SUMMARY

Reducing the dietary anion-cation balance from +394.31 to +120.65 meq/kg ration DM decreased the incidence of milk fever by 25% but no further reduction was observed when dietary anion-cation balance was decreased to +62.20 meq/kg ration DM. However, number of cows was too small to be conclusive.

A strong negative correlation was observed during the periparturient period, especially at parturition, between the level of dietary anion-cation balance and the concentration of plasma calcium.

Reducing the level of dietary anion-cation balance delayed the time of cows showing their lowest concentration of plasma calcium.

Reducing the level of dietary anion-cation balance decreased the severity of the decline of plasma calcium during the periparturient period.

Concentration of plasma phosphorus was weakly correlated to the level of anion-cation balance during the periparturient period but strongly correlated to the concentration of plasma calcium.

Reducing the level of dietary anion-cation balance increased the concentration of plasma magnesium during the periparturient period.

A strong negative correlation was observed during the periparturient period, especially at parturition, between the level of dietary anion-cation balance and the concentration of plasma magnesium.

Positive correlations were obtained between the concentration of plasma calcium and the concentration of plasma potassium during the periparturient period.

Reducing the level of dietary anion-cation balance increased the concentration of plasma sulfate.

Strong positive correlations were obtained during the periparturient period between the concentration of plasma calcium and the concentration of plasma sulfate.

Reducing the dietary anion-cation balance did not influence the dry matter digestibility either during period 1 (day-24 to day-21 prepartum) or during period 2 (day-7 prepartum to day-1 postpartum).

Apparent absorption of calcium, phosphorus, magnesium, sodium, potassium and chloride was not influenced by the level of dietary anion-cation balance during period 1.

Apparent absorption of phosphorus, sodium, potassium and chloride was not influenced by the level of dietary anion-cation balance during period 2.

Reducing the dietary anion-cation balance decreased the apparent absorption of calcium during period 2.

Reducing the level of dietary anion-cation balance decreased the apparent absorption of magnesium during period 2.

A strong positive correlation was observed between the apparent absorption of calcium and the apparent absorption of phosphorus.

VII. CONCLUSION

Reducing the level of dietary anion-cation balance decreased the severity of the decline of plasma Ca during the periparturient period, thus, tended to diminish the incidence of milk fever. However, a dietary anion-cation balance of +62.20 meq/kg ration DM (diet 3) is too high to be an efficient tool in the prevention of milk fever. It is suspected that a negative anion-cation balance will be more appropriate. Because of the strong correlation existing between the concentration of plasma Ca and plasma P as well as between the apparent absorption of both minerals, phosphorus metabolism is more related to the calcium metabolism than to the level of dietary anion-cation balance.

Because of the importance of the dietary balance between the anions and the cations in acid-base balance of the animal, it is suspected that the mode of action of the reduced dietary anion-cation balance is through an impairment of the acid-base status of the animal toward a metabolic acidosis. This status may have affected the kidney as well as the resorptive ability of the bone by lowering the reabsorption of urinary calcium and by increasing the bone resorptive process. The suspected increased fecal excretion of endogenous calcium would be an indirect response caused by the increased exchangeable pool of calcium. This mechanism would be further accentuated during the periparturient period probably because of the higher calcium stress on the animal. An increased bone resorption would explain the increased concentration of plasma magnesium as well as the less severe hypocalcemia observed during the periparturient period in cows fed reduced anion-cation balance.

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APPENDIX

Table I. Composition of Cal-Dextrose used to treat cows diagnosed with milk fever.

Ingredients	Composition (%)
Calcium gluconate	1.70
Phosphorus	0.96
Magnesium	0.40
Dextrose	16.50
Boric acid	< 0.004
Bromine	< 0.0014
Sodium	< 0.0004

Table II. Composition of Cal-Mag-K used to treat cows diagnosed with milk fever.

Ingredients	Composition (%)
Calcium borogluconate	23
Dextrose monohydrate	25
Magnesium chloride hexahydrate	4.50
Sodium hypophosphite	3.43
Potassium chloride	0.20
Formaldehyde(preservative)	0.20

TABLE III. Correlations between the concentration of plasma sodium and the quantity of calcium, phosphorus, magnesium, sodium, potassium and chloride apparently absorbed daily for the first, second, third, fourth, sixth and seventh weeks prepartum.

Absorbed minerals (g/day)	Coefficient of correlation — Weeks prepartum					
	1	2	3	4	6	7
Calcium	-.16[a]	.10	-.06	-.15	-.27	-.24
Phosphorus	-.15[a]	.09	-.16	.15	-.31	-.30
Magnesium	-.14[b]	-.02	-.08	-.24	-.42[b]	-.05
Sodium	-.03	.00	.21	.03	-.07	.04
Potassium	-.14[b]	.04	-.27[b]	-.03	-.07	-.50[a]
Chloride	-.15[a]	.06	-.20	-.27	.01	-.17

[a] $P<0.05$
[b] $P<0.1$

TABLE IV. Correlations between the concentration of plasma potassium and the quantity of calcium, phosphorus, magnesium, sodium, potassium and chloride apparently absorbed daily for the first, second, third, fourth, sixth and seventh weeks prepartum.

Absorbed minerals (g/day)	Coefficient of correlation					
	Weeks prepartum					
	1	2	3	4	6	7
Calcium	.18[a]	.24[a]	.09	.11	.07	-.30
Phosphorus	.20[a]	.29[a]	.14	.37	-.01	-.48[b]
Magnesium	.18[a]	.09	.15	.21	.26	-.40
Sodium	-.08	.00	.01	-.10	.00	-.41
Potassium	.21[a]	.30[a]	.19	.26	.15	-.27
Chloride	.21[a]	.30[a]	.15	.19	.12	-.32

[a] $P<0.05$
[b] $P<0.1$

TABLE V. Correlations between the concentration of plasma chloride and the quantity of calcium, phosphorus, magnesium, sodium, potassium and chloride apparently absorbed daily for the first, second, third fourth, sixth and seventh weeks prepartum.

Absorbed minerals (g/day)	Coefficient of correlation					
	Weeks prepartum					
	1	2	3	4	6	7
Calcium	-.11	-.18[a]	-.03	.01	.31	.40
Phosphorus	-.03	-.11	-.07	.31	.28	.30
Magnesium	-.07	-.05	.10	.02	.06	.38
Sodium	-.02	-.30[a]	-.03	-.01	.38	.33
Potassium	-.01	-.05	-.22	-.19	.43[b]	.51[a]
Chloride	-.03	-.08	-.09	.00	.13	.28

[a] $P<0.05$
[b] $P<0.1$

TABLE VI. Correlations between the concentration of plasma sulfate and the quantity of calcium, phosphorus, magnesium, sodium, potassium and chloride apparently absorbed daily for the first, second, third, fourth, sixth and seventh weeks prepartum.

Absorbed minerals (g/day)	Coefficient of correlation					
	Weeks prepartum					
	1	2	3	4	6	7
Calcium	-.08	-.07	-.05	.11	.16	.64[a]
Phosphorus	-.09	-.02	-.12	.43[b]	.00	.62[a]
Magnesium	-.08	.00	-.07	.08	.02	.57[a]
Sodium	.03	-.04	.08	.22	.22	.47
Potassium	-.08	-.01	-.25	.29	-.19	.51[b]
Chloride	-.09	-.02	-.12	.05	-.11	.69[a]

[a] $P<0.05$
[b] $P<0.1$