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An epidemiological study of calcium metabolism in non-paretic postparturient Holstein cows

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Abstract

Data from 1021 lactations of non-paretic Holstein cows followed in 14 Québec dairy herds were used to describe calcium 'metabolism' after calving in healthy cows. Serum total calcium, phosphorus, magnesium, potassium, albumin, and glucose were measured on the first and seventh days post-calving. The distributions were described and compared between the first and seventh day postpartum. The relationships between serum calcium on the one hand and the other serum metabolites and the cow's age on the other hand were assessed using a general linear model. Serum calcium and phosphorus values were lower on the first day postpartum than a week later (2.03±0.26 vs 2.26±0.18 mmol/l, 1.78±0.48 vs 1.93±0.39 mmol/l, respectively), whereas it was the opposite for glucose, magnesium, and potassium (3.98±0.95 vs 3.12±0.60 mmol/l, 1.01±0.35 vs 0.95±0.13 mmol/l, 4.84±0.40 vs 4.69±0.38 mmol/l, respectively). Albumin values were similar (25.7±3.3 vs 25.2±3.4 g/l). On the first day postpartum, serum calcium was associated in a curvilinear fashion with age, phosphorus and albumin. It also was associated, but to a lesser extent, with glucose and magnesium, whereas it was not associated with potassium. On the seventh day postpartum, calcium was associated with age, phosphorus and with an increased importance of albumin. The results are discussed with regard to postpartum hypocalcemia, the interpretation of serum metabolite values after calving, and the use of the physiological stress at calving. We concluded that (1) postpartum hypocalcemia was an event to be expected, especially for the older cow, (2) a multivariable approach should be used to interpret biochemical profiles after calving, and (3) such profiles could be used to better assess the cow's health. © 1998 Elsevier Science B.V.

Keywords: Cattle-metabolism; Lactation; Calcium metabolism; Biochemical profile; Multivariable association

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1. Introduction

Parturition is a normal event occurring during a cow's life, but it is nonetheless a stressful one. A cow has to perform two important tasks around calving: parturition and lactogenesis, which need her energy and calcium metabolisms to be greatly accelerated (Kronfeld, 1971). Lactogenesis involves an important demand on the calcium metabolism. To date, many clinical studies on calcium metabolism at parturition were done on parturient paretic cows (cows with 'clinical milk fever'). This provided for a point of view where diseased animals were investigated in order to find the relationship of parturient paresis to calcium metabolism. Also, experimental physiological studies were conducted on calcium metabolism using non-parturient, non-lactating cows. But, few studies and few animals were used to investigate serum calcium and the health status of cows which did not show clinical signs of parturient paresis (Risco et al., 1994). Thus, only a simple view of calcium metabolism in cows at parturition is available.

When considering calcium metabolism at calving, the most important change is the onset of the flow of calcium to the mammary gland associated with a greater mobilisation of calcium from the bone and an acceleration of intestinal absorption (Kronfeld, 1971). The calcium level in the cow's serum is associated with age (Hansard et al., 1954; Montgomery, 1985) and with changes in serum levels of phosphorus (Kronfeld, 1971) and albumin (Carlstrom, 1970). Although the individual relationships of calcium to age, phosphorus and albumin have been studied (usually only on a small number of animals), no study has reported their joint relationship.

More recently, ionised calcium has been associated with a messenger role at the cellular level (Rasmussen, 1986; Walsh, 1991). Ionised calcium is also related to muscular contraction, neurosecretion, hepatocyte glycogenolysis function and insulin modulation (Rasmussen, 1986; Walsh, 1991). This additional cellular role of calcium must now also be taken into account whenever calcium metabolism is discussed.

Diagnosis is based on the comparison of a questionable or new case to a reference or normal group of animals and to a diseased group. In order to improve on the value of the comparison, it is important to have an excellent description of the reference population. For postpartum serum calcium evaluation, many variables have been associated with the calcium metabolism. It is then important to describe the reference population with respect to those variables. In a following step, it could be used to provide optimal cutoff values for the medical decision making which is diagnosis.

We investigated the metabolism of 1021 Holstein cows at calving and early postpartum with the objective of better describing the normal or reference population. In the present paper, we study the calcium metabolism and describe the serum level of total calcium in healthy (i.e. non-paretic) postpartum Holstein cows and its joint relationship with age and other serum metabolites. The consequences of the findings when interpreting biochemical profiles from cows around calving are discussed.

2. Materials and methods

A list of all herds from the target population was constituted; they were herds which participated in a milk production recording program (Programme d'Amélioration des Troupeaux Laitiers du Québec, PATLQ) and were situated within a 30 km radius from Saint-Hyacinthe, Québec, Canada. A random cluster sample of those herds was produced and 20 farms were contacted of which 15 agreed to participate. Those who refused did so because of the degree of involvement required by the research and were otherwise comparable in size and milk production. After 6 months, one herd was lost because it was sold. Therefore, all cows from 14 herds were followed from October 1987 to January 1990 and provided data from 1145 lactations and 893 cows. The unit of concern was 'lactation' and it was defined as the period extending from one calving to the next, or to death, culling, or the end of the project (whichever came first). Blood samples were taken from every available cow within the 24 h postpartum and on the seventh day after calving. Health and reproductive events were noted by the farmers and collected by a technician on farm visits (at least once a week) during which blood samples were taken. The herd nutritional program was submitted for evaluation by a nutritionist to insure that the animals were on an adequately balanced diet.

From the 1145 lactations, 10.8% were rejected because the cows were paretic or received calcium because they looked dull. Thus, data from 1021 lactations without any suspicion of milk fever were analyzed. The rejection criterion was likely to be severe since the milk fever prevalence in the region was only 6% according to previously-obtained data (unpublished).

Blood samples were drawn from the tail. During summer, blood samples were transported in an ice box. All samples were centrifugated immediately after clotting had occurred. Blood chemistry was done on a BM/HITACHI 705 system with the ortho-creosol-phtalein-complex method for calcium, the ammonium molybdate method for inorganic phosphorus, the biuret method for total protein, and 4-aminophenazone phenol method for glucose. Albumin was obtained as a percentage of total protein by electrophoresis on agarose gel (Corning) and were reported in g/l by multiplying the percentage to the total protein value measured. Potassium was measured using a selective electrode potentiometer Beckman E4A, and magnesium was measured by atomic absorption on a Perkin–Elmer 1100-b.

Descriptive statistics and histograms were obtained for all variables. Paired *t*-test was used to assess the difference between the first and the seventh day postpartum. In order to compare our results to those in the literature, Pearson's correlations were calculated between calcium and albumin for days one and seven postpartum. Scatter diagrams of calcium with age, phosphorus and albumin were produced and visual assessment indicated a curvilinear relationship of calcium to age and phosphorus. Ordinary least-square multiple regression was done to relate calcium to phosphorus, albumin, age, glucose, potassium and magnesium for the first- and seventh-day post-calving serum samples separately. The nominal variable herd was included in all models as a fixed effect to account for the clustering of the sample. The same models were also estimated using 'herd' as a random effect but produced similar results and are not reported. A second-order polynomial model with interaction was used as a starting model to account for the observed curvilinear nature of some of the relationships. A final model was obtained by using a backward hierarchical algorithm with a *p*-value of 0.05 as a removal threshold. Residual analysis was done to insure that the fit was adequate and assumptions were met

(Daniel and Woods, 1980). Calculations were done using the SAS GLM procedure (SAS Institute, version 6.12).

3. Results

Cows that were younger than 4 years of age at calving represented 49% of the animals included in this study; the others were distributed in decreasing frequency with increasing age. The frequency distribution of serum calcium at calving was asymmetrical (Fig. 1). Values were more dispersed and showed a tendency to be lower than at one week postpartum. The median for serum calcium values at calving was 2.03±0.26 (sd) mmol/l, while it was 2.26±0.18 mmol/l one week later. This was in agreement with the drop in serum calcium that was expected to occur in the first day postpartum with the onset of milk production (Ramberg et al., 1984). Inorganic phosphorus levels in the serum showed a mean value of 1.78±0.48 mmol/l at calving and a value of 1.93±0.39 mmol/l seven

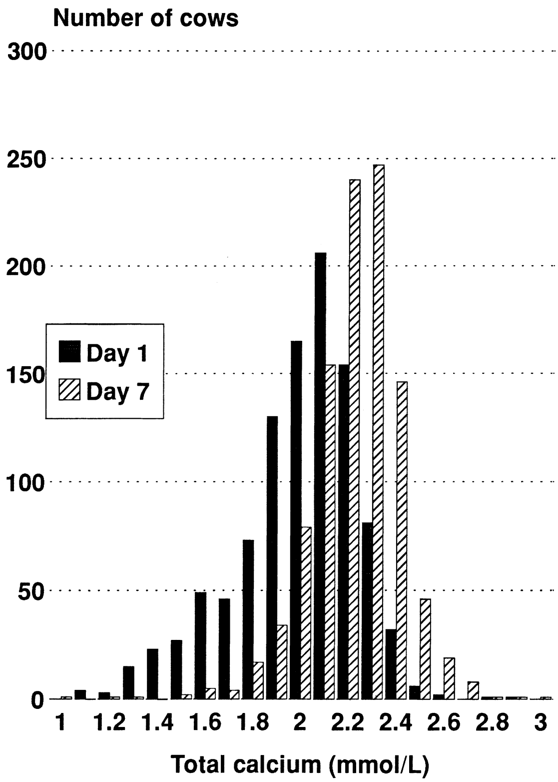

Fig. 1. Frequency distribution of serum total calcium in 1021 non-paretic Holstein cows on the first and on the seventh day postpartum (Québec, 1990).

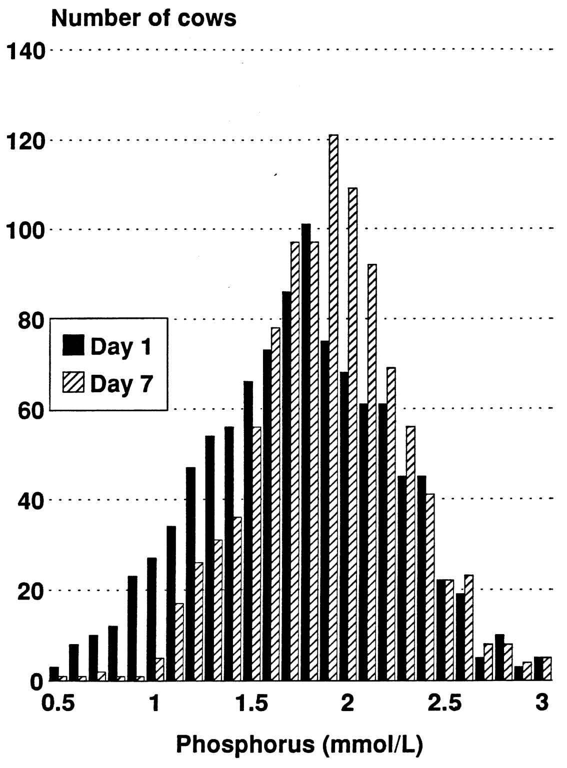

Fig. 2. Frequency distribution of serum inorganic phosphorus in 1021 non-paretic Holstein cows on the first and seventh day postpartum (Québec, 1990).

days later. This parameter showed a tendency to be slightly asymmetrical towards lower values (Fig. 2). The values for albumin indicated that there were little changes in mean albumin values between calving and one week later. The mean values were 25.7±3.3 g/l at calving and 25.2±3.4 g/l one week later with a similar dispersion. Our data showed that mean values at calving compared to one week later were significantly ($p<0.0001$) higher for glucose (mean ± sd: 3.98±0.95 mmol/l and 3.12±0.60 mmol/l, respectively), magnesium (1.01±0.35 mmol/l and 0.95±0.13 mmol/l, respectively), and potassium (4.84±0.40 mmol/l and 4.69±0.38 mmol/l, respectively). Serum calcium and albumin were correlated on the seventh day postpartum (Pearson's $r=0.31$; $p<0.001$) but not at calving (Pearson's $r=-0.05$; $p=0.131$).

At calving, serum total calcium was significantly associated with age, phosphorus, and albumin (Table 1). Inorganic phosphorus was associated with serum calcium at calving in a curvilinear fashion; also, this relationship was age-dependent (Fig. 3). The regression coefficients for age showed that serum total calcium values decreased with age until age was 12 years. It included a simple positive linear relationship of calcium to glucose and potassium, while magnesium was not retained as an explanatory variable. The final model

Table 1
Regression of serum total calcium on age, serum inorganic phosphorus, albumin, glucose, and potassium in Holstein cows on the first day postpartum controlling for herd (n=1018) (Québec, 1990)

Parameters	b	Se(b)	p-value
Herd	–	–	0.0001
Age (yr)	−0.164	0.020	0.0001
Age * age	0.006	0.001	0.0001
Phosphorus (mmol/l)	0.594	0.094	0.0001
Pho. * pho.	−0.140	0.021	0.0001
Age * pho.	0.027	0.007	0.0001
Albumin (g/l)	0.006	0.002	0.0012
Glucose (mmol/l)	0.032	0.007	0.0001
Potassium (mmol/l)	0.052	0.015	0.0007
Intercept	1.232	0.149	0.0001
Model	r^2=0.495	F=46.66	p-value=0.0001

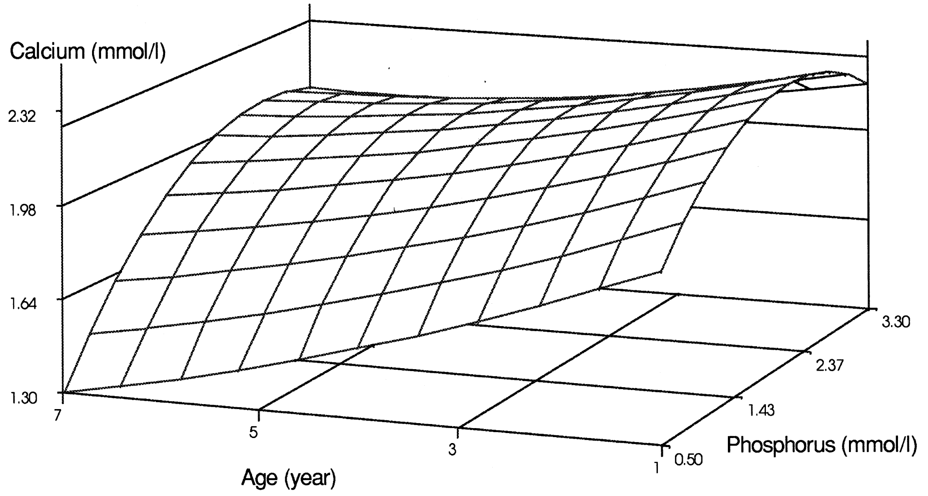

Fig. 3. Graphical representation of the relationship between age, serum total calcium, and inorganic phosphorus in non-paretic Holstein cows on the first day postpartum. (CALCIUM=1.236 −0.164*AGE +0.006*AGE^2 +0.594*PHOSPHORUS − 0.14*PHOSPHORUS^2 +0.027*AGE*PHOSPHORUS +0.007*25.7+0.038*3.98+ 0.052*4.84).

obtained for the seventh day postpartum was very different from that at calving with regard to the regression coefficients of the main variables and to the occurrence of the different second-order and interaction terms (Table 2). However, serum total calcium values were still a decreasing function of age. The r^2 of the seventh-day model was less than about half that of the model at calving.

Table 2
Regression of serum total calcium on age, serum inorganic phosphorus, albumin, glucose, and potassium in non paretic Holstein cows on the seventh day postpartum controlling for herd (n=1003) (Québec, 1990)

Parameters	b	Se(b)	p-value
Herd	–	–	0.0001
Age (yr)	−0.012	0.003	0.0001
Phosphorus (mmol/l)	0.909	0.116	0.0001
Pho. * pho.	−0.121	0.022	0.0001
Albumin (g/l)	0.083	0.014	0.0001
Alb. * alb.	−0.001	0.0002	0.0006
Alb. * pho.	−0.013	0.003	0.0001
Glucose (mmol/l)	0.047	0.009	0.0001
Potassium (mmol/l)	0.468	0.197	0.0176
Pot. * pot.	−0.041	0.020	0.0431
Intercept	−1.332	0.516	0.0099
Model	r^2=0.314	F=20.43	p-value=0.0001

4. Discussion

Data came from all the dairy cows from 14 herds; this number of herds was used to insure a satisfactory number of animals and a diversity of nutritional programs and field conditions. Thus, the results should be representative of normal Holstein cows in a usual Québec situation.

4.1. Serum calcium at calving

The distribution of serum calcium at calving appeared to be different from that observed one week later or in non-parturient cows (Horst, 1986) because of lower central tendency and higher variation. Indeed, the distribution of serum calcium at calving showed an important group of the cows in this study (approximately 25%) that was below the 1.87 mmol/l threshold suggested by Carlstrom (1970) and Oetzel (1988) for the diagnosis of milk fever (Fig. 1). Based on calcium levels, these cows would have been diagnosed as hypocalcemic and it would be suggestive of milk fever, although they might not show clear clinical signs. Since the present database considered only animals free of clinical diagnosis of milk fever, this large group of Holstein cows can represent a disease-free group. Since specificity is the probability of being test-positive given that the animal is disease-free, then, in the present situation, one minus the probability of being hypocalcemic is an estimate of the specificity of calcemia on the first day postpartum as a test for milk fever (75%). This indicated that a medical inference of milk fever based strictly on hypocalcemia would not be very specific. The regression models for serum calcium values at calving gave some insight on the presence of hypocalcemic animals among our clinically normal cows (reference population), and, more generally, on calcium metabolism at calving and soon afterwards.

Serum calcium values were associated with age with a curvilinear relationship which was negative for the observed range of age in our cows. According to our model, a

2-year-old cow would have approximately 0.3 mmol/l less of blood calcium in five years time compared to its present value. Various authors have also found an inverse relationship of serum calcium to age, some in association with decreased bone resorption efficiency (Van de Braak and Van't Kloester, 1987; Anderson, 1991), and others in association with dry matter intake (Marquardt et al., 1977) or intestinal absorption efficiency (Hansard et al., 1954; Anderson, 1991). The number of osteoblasts and osteoclasts diminish with age in humans (Anderson, 1991). In a review article, Reinhardt et al. (1988) reported that bone calcium content diminished with age and that peripartum hypocalcemia should be considered normal for older cows. In agreement with these authors, it could be speculated that peripartum hypocalcemia was, at least partially, a consequence of the aging process. This suggests that the interpretation of serum total calcium value of cows should use threshold values corrected for age for parturient Holstein cows and, probably, including those suspected of milk fever.

Other factors can also explain periparturient hypocalcemia. A 'lag time' in the calcium homeostatic mechanisms was reported to indicate that cows cannot react fast enough to meet the increased demand for calcium, resulting in hypocalcemia in 2–3 days (Littledike, 1976; Goff et al., 1986a). Oestrogens are higher during the last five days prepartum (Goff et al., 1986a) and decrease feed intake (Muir et al., 1972). This would tend to force the animal on the first day postpartum to go from passive to active intestinal absorption of calcium and phosphorus. Oestrogens also inhibit bone resorption (Riggs et al., 1976), diminish bone responsiveness to the parathyroid hormone and decrease the absorbtion of calcium from the intestine (Riggs et al., 1976; Sechen et al., 1988). These effects coupled with the increased demand of the mammary glands on calcium could explain why some of our animals were hypocalcemic. These effects also supported the observation that hypocalcemia was a postpartum event to be expected.

Serum calcium exists in three states: protein bound, complexed and ionised (Kaneko, 1989). Bound calcium is usually tied to serum protein (mainly albumin) (Carlstrom, 1970). Actually, Kvart and Larsson (1978) reported a correlation coefficient of 0.33 between serum total calcium and albumin in normal cows outside the peripartum period. Our results showed a similar correlation between serum calcium and albumin one week postpartum but not on the first day postpartum. Kaneko (1989) suggested that total calcium should be adjusted for serum albumin. In contrast, our results also showed that the association between serum total calcium and albumin was weak at calving, thus no correction should then be made. Carlstrom (1970) reported (using an in vitro evaluation) that the calcium-binding capacity of a protein fraction containing mainly albumin diminished at calving. This may explain the discrepancy between the day 1 and day 7. A mechanism related to blood acid–base balance could probably explain the different behaviour of the albumin fraction of total serum protein at that period (Brenner and Rector, 1991). This implies that total calcium in serum samples taken during early postpartum should not be adjusted for albumin and that serum components are different in the day following calving, especially with respect to calcium.

At calving, the onset of lactation drives calcium metabolism to change and increase in order to provide the mammary glands with the needed calcium (Kronfeld, 1971; Horst, 1986). This output of calcium from the blood is balanced mainly by a greater demand on the bone tissue and an increased intestinal absorption. Osteolysis occurs at the cellular level, sending to the blood not only calcium, but also phosphorus. Therefore, serum calcium and phosphorus values are expected to be associated. Our model for serum calcium at calving confirmed this by showing a curvilinear relationship between serum total calcium and phosphorus. Hypocalcemia could be associated with either high phosphorus values far above their physiological range or, more probably, with hypophosphatemia as was reported by Kronfeld (1971). The interaction term between age and phosphorus in our model of serum calcium at calving also showed that the curvilinear relationship between calcium and phosphorus is age-dependent. In particular, this means that old cows would have especially low calcium values compared to young cows, when both cows have low levels of phosphorus in the serum. For example, the expected calcium-to-phosphorus ratio for a 9-year-old cow with 25 g/l of albumin varied from 0.6 to 2.5 when serum phosphorus decreased from its higher to its lower observed values. This interaction between age and phosphorus also implied that the hypothesized effects of the aging process were different on calcium and phosphorus metabolisms. We suggest that a multivariable approach should be used when interpreting biochemical profiles, especially when setting the threshold values for different animals.

Due to its association to calcium, serum phosphorus could be used to investigate calcium metabolism at calving, especially with regard to hypocalcemia. The importance of phosphorus as an indicator of health status at calving has already been suggested. Goff et al. (1989) and Reinhardt et al. (1988) suggested that in downer cows, the kidney was not sensitive to the parathyroid hormone. Tanaka and DeLuca (1973), on the other hand, suggested that intracellular phosphorus levels controlled the activation of vitamin D by the kidney. Welsh et al. (1991) in an article on signal transduction pathways indicated the complexity of the effects of the parathyroid hormone. Also, Goff et al. (1986a) reported that when treating animals with vitamin D, the urinary excretion of phosphorus was increased. More important still, they found a large variation between the response of individual animals. Our results supported these hypotheses and suggested that the serum calcium-to-phosphorus ratio was an indicator of renal function efficiency. This was in agreement with Anderson (1991), who recommended further studies to better understand the influence of phosphorus and proteins on calcium metabolism. This would be in agreement with the cation–anion balance of the diet as a potential cause of milk fever (Beede et al., 1992). An unbalanced input of sodium and potassium from the diet would have an impact on the renal function and be associated with metabolic alkalosis (Goff and Horst, 1996). We suggest that the calcium–phosphorus ratio corrected for age should be investigated as an indicator of renal function.

Our model showed a positive linear relationship of serum total calcium to glucose and potassium, but no association with magnesium (Table 1). Although significant, the associations with glucose or potassium were weak and not useful to explain the variation of serum total calcium. In contrast, Kronfeld (1971) reported different values of serum glucose, potassium or magnesium for cases of parturient paresis and an association between hyperglycemia and hypermagnesemia. Goff and Horst (1996) indicated that dietary calcium (as opposed to potassium) had little impact on the risk of clinical milk fever. Since the objective of this paper was the description of clinically normal cows, our results support a role of potassium and renal function but cannot confirm the effects in parturient cows.

4.2. Serum calcium at one week postpartum

The situation appeared very different one week postpartum. Much less variation in serum calcium was observed between cows. This was an expected finding since at that time calcium homeostasis is much more stable (Kronfeld, 1971; Littledike, 1976; Goff et al., 1986b). Calcium was still related to phosphorus in a curvilinear fashion, but the relationship was more important and more linear than at calving (Tables 1 and 2) as can be seen from the coefficients of the respective models. Age showed a 7 times smaller simple linear relationship and albumin's importance was increased and its relationship was curvilinear. The expected level of protein-bound calcium was similar to levels reported by Carlstrom (1970) and the association was of the order of magnitude (r=0.33) reported by Kvart and Larsson (1978). But there was an antagonistic interaction between albumin and phosphorus. This was understandable since the calcium homeostatic mechanisms (especially the renal ones) keep serum total values inside their physiological range (Ramberg et al., 1984). The model (Table 2) had a lower r^2 than that for the first day postpartum, but there was also less between-animals variation to explain (Fig. 1).

4.3. Relationship to milk fever

Our results showed that serum total calcium values lower than the threshold for defining milk fever could be observed in non-paretic dairy cows at calving. The probability of this event even increased with the age of the animal. They also showed that hypocalcemia and hypophosphatemia were associated at calving as they were in other studies (Littledike, 1976; Ramberg et al., 1984; Goff et al., 1986b; Reinhardt et al., 1988). Since parturient paresis usually happened during the first day postpartum, and since hypocalcemia was found to be a common occurrence especially for older cows soon after calving, the nature of the relationship between these events might not be causal as usually agreed upon. It might in fact be coincidental. The aging process which potentially causes a decreased serum calcium at the lactogenesis onset could also play a role in the pathogenesis of milk fever.

Our model for non-paretic cows showed a small association of serum calcium with serum glucose, potassium, and no association with magnesium. In contrast, serum glucose, potassium or magnesium values were reported to be different in occurrences of parturient paresis and hypocalcemia was then associated with hyperglycemia and hypermagnesemia in paretic parturient cows (Kronfeld, 1971). This difference in metabolism of calcium and other serum metabolites might provide a better basis for medical inference of parturient paresis. Goff and Horst (1996) suggested something similar especially regarding potassium. These results also provided a basis for questioning the tissue-level calcium homeostasis hypothesis usually accepted for parturient paresis. Al-Eknah and Noakes (1989) have provided evidence that induced signs of hypocalcemia can be produced either by blocking calcium homeostasis using EDTA or by blocking cellular calcium mechanisms using nefidipine indicating that another biological pathway exists to induce at least the principal clinical signs of parturient paresis.

Other biological pathways include ionised calcium which has a messenger role at the cellular level related to muscular contraction, neurosecretion, hepatocyte glycogenolysis function and insulin modulation (Rasmussen, 1986; Walsh, 1991). Our results lead us to speculate that the cellular messenger role played by calcium could in fact provide an alternative causal pathway that connected calcium to parturient paresis. If the messenger role of calcium was impaired, it would result in symptoms associated at the cellular level with a deficient energy metabolism as well as muscle weakness (Rasmussen, 1986; Walsh, 1991). Our earlier speculation about a role for the renal function is also in agreement with a cellular calcium messenger problem. The messenger role would also help in understanding the genetic component that was associated with parturient paresis (Grohn et al., 1986; Erb and Grohn, 1988). Moreover, Aiumlamai et al. (1992) suggested a possible role of endotoxins in spontaneous paresis in cows, but they do not suggest any pathway for the action of the endotoxins. It is also likely that, in some cases, ration deficiencies with respect to calcium and phosphorus could be responsible for accidental parturient paresis (Jorgensen, 1974; Shappell et al., 1987; Barnouin and Chassagne, 1991: Oetzel, 1991), or more likely with dietary cation–anion imbalance (Beede et al., 1992; Goff and Horst, 1996).

5. Conclusion

This epidemiologic study of calcium metabolism in non-paretic parturient Holstein cows suggests that a multivariable approach is useful when setting threshold values and interpreting biochemical profiles especially from serum taken in early postpartum. Clinical epidemiology should be developed in accordance with these findings and then, maybe, clinical biochemistry could be useful other than for diagnostic confirmation. The findings suggested that profiles containing measures of calcium, phosphorus, albumin and age could be developed to assess the health status of the cow, especially the renal integrity. Our observations on normal metabolic behaviour under field conditions lead us to state that hypocalcemia, as a tissue-level homeostasis problem, is a situation to be expected at calving, its severity increasing with age.

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References

Aiumlamai, S., Fredriksson, G., Kindhal, H., Edqvist, L.E., 1992. A possible role of endotoxins in spontaneous paretic cows around parturition. J. Vet. Med. A39, 57–68.

Al-Eknah, M.M., Noakes, D.E., 1989. A preliminary study on the effect of induced hypocalcemia and nefidipine on uterine activity in the parturient cow. J. Vet. Pharmacol. Therap. 12, 237–239.

Anderson, J.J.B., 1991. Nutritional biochemistry of calcium and phosphorus. J. Nutr. Biochem. 2, 300–307.

Barnouin, J., Chassagne, M., 1991. An aetiological hypothesis for the nutrition induced association between retained placenta and milk fever. Ann. Rech. Vet. 22, 331–343.

Beede, D.K., Risco, C.A., Donovan, G.A., Wang, C., Archbalb, L.F., Sanchez, W.K., 1992. Nutritional management of the late pregnant dry cow with particular reference to dietary cation–anion difference and calcium supplementation. Proc. Am. Ass. Bovine Practioners Convention 24, 51–55.

Brenner, B.M., Rector, F.C., 1991. The Kidney, Vol.2., 4th edn., W.B. Saunders, pp. 1551–1591.

Carlstrom, G., 1970. Studies on parturient paresis in dairy cows: V. On the composition and calcium binding capacity of two bovine serum protein fractions with special regard to parturient paresis. Acta Vet. Scand. 11, 89–102.

Daniel, C., Woods, F.C., 1980. Fitting Equations to Data. Computer Analysis of Multifactor Data. 2nd edn., John Wiley, New York, 458pp.

Erb, H.N., Grohn, Y.T., 1988. Epidemiology of metabolic disorders in the periparturient dairy cow. J. Dairy Sci. 71, 2557–2571.

Goff, J.P., Horst, R.L., 1996. Potassium, not calcium induces milk fever: Addition of potassium or sodium, but not calcium to prepartum rations induces milk fever in dairy cows. Proc. Am. Ass. Bovine Practioners Convention 29, 170.

Goff, J.P., Horst, R.L., Littledike, E.T., Boris, A., Uskokovic, M.R., 1986a. Bone resorption, renal function and mineral status in cows treated with 1,25 dihydroxycholecalciferol and its 24 fluoro analogues, J. Nutr. 116, pp. 1500–1510.

Goff, J.P., Littledike, E.T., Horst, R.L., 1986b. Effect of synthetic bovine parathyroid hormone in dairy cows: Prevention of hypocalcemic parturient paresis, J. Dairy Sci. 69, pp. 2278–2289.

Goff, J.P., Reinhardt, T.A., Horst, R.L., 1989. Recurring hypocalcemia of bovine parturient paresis is associated with failure to produce 1,25 dihydroxyvitamin D. Endocrinology 125, 49–53.

Grohn, Y., Saloniemi, H., Syvajarvi, J., 1986. An epidemiological and genetic study on registered diseases in Finnish Ayrshire cattle. III Metabolic diseases. Acta Vet. Scand. 27, 209–222.

Hansard, S.L., Comar, C.L., Plumlee, M.P., 1954. The effects of age upon calcium utilization and maintenance requirements in the bovine. J. Anim. Sci. 13, 25–32.

Horst, R.L., 1986. Regulation of calcium and phosphorus homeostasis in the dairy cow. J. Dairy Sci. 69, 604–616.

Jorgensen, N.A., 1974. Combating milk fever. J. Dairy Sci. 57, 933–944.

Kaneko, J.J., 1989. Clinical Biochemistry of Domestic Animals. 4th edn., Academic Press, New York, 932pp.

Kronfeld, D.S., 1971. Parturient hypocalcemia in dairy cows. Adv. Vet. Sci. Comp. Med. 15, 133–157.

Kvart, C., Larsson, L., 1978. Studies on ionized calcium in serum and plasma from normal cows. Acta Vet. Scand. 19, 487–496.

Littledike, E.T., 1976. Relationship of milk secretion to hypocalcemia in the dairy cow. J. Dairy Sci. 59, 1947–1953.

Marquardt, J.P., Horst, R.L., Jorgensen, N.A., 1977. Effect of parity on dry matter intake at parturition in dairy cattle. J. Dairy Sci. 60, 929–934.

Montgomery, M.E., 1985. Sources of variation in the blood chemistry of Holstein–Friesian dairy cows. MSc Thesis, University of Guelph, Guelph, ON, 154pp..

Muir, L.A., Hibbs, J.W., Conrad, H.R., Smith, K.L., 1972. Effect of estrogen and progesterone on feed intake and hydroxyproline excretion following induced hypocalcemia in dairy cows. J. Dairy Sci. 55, 1613–1620.

Oetzel, G.R., 1988. Parturient paresis and hypocalcemia in ruminant livestock. Vet. Clin. North America: Food Anim. Practice 4, 351–364.

Oetzel, G.R., 1991. Analysis of nutritional risk factors of milk fever in dairy cattle. J. Dairy Sci. 74, 3900–3912.

Ramberg Jr, C.F., Johnson, E.K., Fargo, R.D., Kronfeld, D.S., 1984. Calcium homeostasis in cows with special reference to parturient hypocalcemia. Am. J. Physiol. 15, R698–R704.

Rasmussen, H., 1986. The calcium messenger system. New England J. Med. 314, 1094–1101.

Reinhardt, T.A., Horst, R.L., Goff, J.P., 1988. Calcium, phosphorus and magnesium homeostasis in ruminants. Vet. Clin. North America: Food Anim. Practice 4, 331–349.

Riggs, B.L., Jowsey, J., Kelly, P.J., Arnaud, C.D., 1976. Role of hormonal factors in the pathogenesis of postmenopausal osteoporosis. Israel J. Med. Sci. 12, 615–619.

Risco, C.A., Drost, M., Ttcher, W.W., Savio, J., Tatcher, M.J., 1994. Effects of claving-related disorders on prostaglandin, calcium, ovarian activity and uterine involution in postpartum dairy cows. Theriogenology 42, 183–203.

Sechen, S.J., Brenel, R.D., Jorgensen, N.A., 1988. Prolactin, estradiol, and progesterone changes in paretic and non-paretic cows during the peripartum period. Am. J. Vet. Res. 49, 411–416.

Shappell, N.W., Herlein, J.H., Deftos, L.J., Aiello, R.J., 1987. Effects of dietary calcium and age on parathyroid hormone, calcitonin and serum and milk minerals in the periparturient cow. J. Nutr. 117, 201–207.

Tanaka, Y., DeLuca, H.F., 1973. The control of 25 hydroxyvitamin D metabolism by inorganic phosphorus. Arch. Biochem. Biophys. 154, 566–569.

Van de Braak, A.E., Van't Kloester, A.T.H., 1987. Effects of calcium and magnesium intakes and feeding level during the dry period on bone resorption in dairy cows at parturition. Res. Vet. Sci. 43, 7–12.

Walsh, M.P., 1991. Calcium-dependent mechanisms of regulation of smooth muscle contraction. Biochem. Cell Biol. 69, 771–800.

Welsh, J.E., Weaver, V., Simboli-Campbell, M., 1991. Regulation of renal 25(OH)D_3 1 –hydroxylase: Signal transduction pathways. Biochem. Cell Biol. 69, 768–770.