

# VARIATIONS IN SERUM CALCIUM, INORGANIC PHOSPHORUS AND MAGNESIUM LEVELS DUE TO STAGE OF LACTATION, SEASON, AND AGE IN AYRSHIRE COWS INJECTED WITH VITAMIN D<sub>3</sub> PRIOR TO CALVING

VAPPU KOSSILA, PAAVO NIEMELÄ and ERKKI KOSKENKORVA

*Department of Animal Husbandry, University of Helsinki*

Received October 22, 1969

Lactational disturbances (parturient paresis, grass tetany, acetonemia) can be reduced to a minimum by proper nutrition and care of dairy cows. For instance parturient paresis, which is characterized by low serum calcium and phosphorus levels and often also by somewhat elevated magnesium levels, occurs mainly only in mature, aged cows, which are inefficient in calcium absorption from their feed, in mobilizing their bone stores, and in retaining their endogenous calcium balance (HANSARD et al. 1954). Heavily lactating cows usually maintain a negative calcium and magnesium balance (Lomba et al. 1968, PAQUAY et al. 1968). The phosphorus balance, in turn, tends to be positive if the cows are fed according to their requirements except when they lose weight, which is usually the case during heavy lactation (ref. STOTT 1968).

Parturient paresis is not solely caused by drainage of calcium into the milk as was once believed, since serum calcium appears to be depressed (NIEDERMEIER et al. 1949) and paresis may occur (ref. STOTT 1968, p. 157) even in mastectomized cows at calving. Even though udder inflation alleviates the symptoms of paresis (GREIG 1930) (decreases loss of calcium, phosphorus and proteins from blood to milk), colostrum excretion of calcium in paretic cows is not higher than in normal cows (HIBBS 1948, NURMIO 1968). Grain supplement (KENDALL et al. 1966), a high phosphorus-low calcium diet fed from 4 to 6 weeks prior to parturition (BODA & COLE 1954), and feeding (HIBBS & POUNDEN 1955, HIBBS & CONRAD 1960) or injections (PALOHEIMO 1968, PAYNE 1968) of vitamin D shortly before calving have proved beneficial in preventing milk fever. A negative calcium balance was noted before calving in cows developing milk fever at parturition (WARD et al. 1952). However, a high calcium diet does not prevent this disturbance (BODA & COLE 1954),

and in fact such a diet may provoke paresis in healthy cows (ALBRIGHT & BLOSSER 1957, ENDER et al. 1962).

STOTT (1968) believes that the amount of calcium and phosphorus mobilizable from the bones at calving is an essential factor, a high calcium-low phosphorus diet leading to a state of »bone starvation».

A decrease in the appetite (MOODIE & ROBERTSON 1961) as well as in the efficiency of the intestinal absorption of calcium (NURMIO 1968, p. 90) at calving may also play a part in the development of paresis.

Endocrine factors (thyroid, parathyroid, pituitary, adrenal cortical and estrogenic hormones) are apparently also involved in the development of lactational disturbances the incidence of which may be increased by excess or deficiency in the secretion rate of one or several hormones.

The parathyroid hormone, which is important in the regulation of the plasma calcium level, was at one time believed to be of importance in the development of milk fever. However, this hormone was found to be of dubious value in preventing and curing milk fever (LITTLE & MATTIC 1933). JÖNSSON (1960) failed to find any histological indication of parathyroid insufficiency in paretic cows. Parathyroidectomized cows failed to develop milk fever at parturition (STOTT & SMITH 1957). Estrogens (STOTT 1968) and thyrocalcitonin (TCT) (BARLET 1967, YOUNG & CAPEN 1967) may be involved, both have properties that are contrary to the effects of parathormone. Estrogen titers are very high at the time of parturition but rapidly decrease after the event (TURNER 1966). TCT injected at a rate of 100  $\mu\text{g}/\text{min}$  produced signs of milk fever in cows (CARE 1968). Decrease in the number of secretory granules of the C-cells and in the TCT content of the thyroid observed in paretic cows suggest that there might be an acute discharge of TCT at calving (ANAST 1968).

Due to effective physiological regulation systems in the body, the serum calcium level is not readily influenced by the intake of calcium from the diet. The inorganic phosphorus level in serum, in turn, is more readily affected by the amount of phosphorus in the diet and a shortage of this element eventually leads to a depression of the serum level. The serum magnesium level depends also on the continuous intake of this element from the diet, since the amount of mobilizable magnesium that can be stored in the cow's body, is very small. Acute hypomagnesaemia is frequently seen in cows put on grass heavily fertilized with ammonium salts, especially if in combination with sulphur or potash, and sudden changes in environmental climate and feeding (ref. ROOK & STORRY 1962), as well as insufficient energy (BREIREM & HVIDTEN 1966) and sodium (BUTLER 1963) intakes, appear to increase susceptibility to this disorder.

The purpose of this study was to find out, by means of investigating the serum calcium, inorganic phosphorus and magnesium levels, whether these levels: a) fluctuate during the pre- and postpartum periods in well-fed, high-producing Ayrshire cows injected with vitamin D prior to calving, b) are affected by the season of the year and by the age of the cow, and c) are indicative of possible shortages in the ratios of the cows in the herd under observation.

#### *Material*

During the indoor feeding as well as the grazing periods in 1963, altogether 207 blood samples were obtained from 41 either dry and pregnant or lactating, nonpregnant Ayrshire

cows of the dairy herd of the Viik Experimental Farm (Tables 1, 2 & 4). All cows had been injected with vitamin D prior to parturition. The entire herd of about 65 cows

Table 1. Serum calcium, inorganic phosphorus and magnesium levels in Ayrshire cows before and after calving (1963).

Serum mineral	Days pre partum				Days post partum				75 < (29)
	38-31 (7)	30-16 (11)	15-0 (17)	0-15 (34)	16-30 (34)	31-45 (31)	46-60 (22)	61-75 (22)	
Ca mg %	9.85 ± 0.19	9.21 ± 0.25	9.67 ± 0.22	9.33 ± 0.12	9.48 ± 0.10	9.63 ± 0.12	9.70 ± 0.15	10.00 ± 0.15	10.09 ± 0.10
P »	4.44 ± 0.31	5.20 ± 0.43	6.02 ± 0.42	6.15 ± 0.23	5.54 ± 0.26	5.31 ± 0.24	5.48 ± 0.22	5.16 ± 0.24	5.34 ± 0.23
Mg »	1.60 ± 0.14	1.92 ± 0.17	1.82 ± 0.09	1.67 ± 0.07	2.01 ± 0.08	1.69 ± 0.07	1.72 ± 0.09	1.64 ± 0.08	1.72 ± 0.07

Number of cases in brackets.

All cows received vitamin D injections as explained in text.

Table 4. Effects of season and age on serum calcium, inorganic phosphorus, magnesium, blood hemoglobin and hematocrit values in Ayrshire cows (1963).

Month	Group I (younger cows)						Group II (older cows)					
	N	C	Ca mg %	P mg %	Mg mg %	Hb Hc	N	C	Ca mg %	P mg %	Mg mg %	Hb Hc
April	16	9	9.54 ± 0.19	5.07 ± 0.24	1.71 ± 0.07	9.81 31.94	22	14	9.33 ± 0.16	5.04 ± 0.37	1.77 ± 0.09	9.80 32.47
May	17	9	9.68 ± 0.13	5.66 ± 0.10	1.45 ± 0.09	9.83 33.25	25	16	9.35 ± 0.09	5.47 ± 0.22	1.45 ± 0.06	10.12 32.90
July	21	17	9.92 ± 0.12	5.95 ± 0.23	1.89 ± 0.16	10.10 34.12	23	17	10.03 ± 0.09	4.69 ± 0.25	1.66 ± 0.09	10.32 34.04
August	25	13	10.13 ± 0.09	5.58 ± 0.21	1.93 ± 0.07	10.36 33.86	16	12	10.22 ± 0.14	4.66 ± 0.16	1.76 ± 0.08	10.16 33.53

N = number of samples, C = number of cows, Hb = hemoglobin g/100 ml, Hc = hematocrit value.

Table 2. Serum calcium, inorganic phosphorus and magnesium levels in three cows with lactational disturbances (1963).

Cow	mg %					
AINI		(- 1)		(+ 19)	(+ 33)	(+ 40) (+ 62)
	Ca	7.82		8.22	8.74	9.62 9.34
	P	6.21		9.95	7.35	6.40 6.03
	Mg	2.53		3.72	2.55	1.95 2.07
PONSI				(+ 22)	(+ 36)	
	Ca			4.81	9.14	
	P			10.53	8.92	
	Mg			3.53	1.70	
ETTI		(- 7)	(+ 6)	(+ 7)	(+ 14)	
	Ca	9.02	8.62	8.94	10.02	
	P	6.40	7.56	6.30	5.80	
	Mg	1.14	0.12	0.49	0.61	

Numbers in brackets indicate days before (-) or after (+) parturition.

Table 3. Changes in serum mineral composition during late dry and early lactation periods in control and vitamin D injected cows (1969).

Group	Days <i>pre partum</i>		Days <i>post partum</i>			
	< 6	6-0	0-5	6-10	10-15	16 <
<b>Vitamin D</b>	(13)	(4)	(7)	(4)	(4)	(8)
Ca mg %	9.48 ± 0.12	9.69 ± 0.75	9.50 ± 1.28	10.33 ± 0.11	10.70 ± 0.01	10.72 ± 0.12
P »	5.28 ± 0.37	5.35 ± 0.65	4.25 ± 1.03	5.62 ± 0.55	5.94 ± 0.24	5.26 ± 1.11
Mg »	1.20 ± 0.11	1.18 ± 0.17	1.17 ± 0.02	1.15 ± 0.02	1.16 ± 0.02	1.12 ± 0.02
<b>Control</b>	(7)	(2)	(6)	(6)	(5)	(6)
Ca mg %	9.68 ± 0.65	10.28 ± 0.91	9.75 ± 2.03	9.37 ± 2.31	9.60 ± 0.68	9.32 ± 1.05
P »	5.26 ± 0.87	5.65 ± 1.70	3.88 ± 0.74	4.32 ± 0.70	4.52 ± 0.76	4.33 ± 0.55
Mg »	1.33 ± 0.02	1.16 ± 0.00	1.24 ± 0.01	1.08 ± 0.01	1.04 ± 0.01	1.12 ± 0.01

Numbers in brackets indicate the No. of samples analysed.

produced an average of 5095 kg milk and 232 kg butterfat during the control year 1962-63, and 5416 and 249 kg respectively during 1963-64.

During April-May in 1969, 72 additional blood samples were taken from 12 cows shortly before and after parturition (Table 3). 6 of the cows were treated with vitamin D and 6 were left as controls.

The feeding and management of the herd has been recently described in detail by KOSSILA (1967). Parturient paresis has been prevented successfully by means of injecting vitamin D<sub>3</sub> intramuscularly twice into the cows, 5 million I.U. each dose, the first dose administered approximately 7 days before and the second dose on the calculated date of delivery (PALOHEIMO 1968).

To prevent hypomagnesaemic tetany, the cows have received magnesium containing mineral salt mixture during the last two to three weeks of the stall-feeding period and during the entire grazing period. The summer in 1963, during which part of the data was obtained for this study (see Table 4), was unusually dry and from the beginning of July the cows received hay twice daily in addition to the pasture grass. Thus the differences

in feeding conditions between the stall and the grazing periods were somewhat smaller than usually.

### Methods

Jugular blood was drawn into the heparinized test tubes. The hematocrit (Hc) values were estimated by the micromethod using an International capillary centrifuge (14000 RPM, 5 ½ minutes). The absorbance of hemoglobin (Hb) in the whole blood was read directly at 540 m $\mu$  by a Beckman B spectrophotometer after first treating the blood sample with dilute ammonia solution.

From the blood samples collected in 1963, the serum calcium was estimated by titrating the diluted serum sample (1 ml serum, 3 ml distilled water) with 0.01-N EDTA solution using murexide as an indicator. The total amount of calcium plus magnesium was estimated from the serum sample mixed with ammonia-NH<sub>4</sub>Cl-buffer solution (pH 11.1—11.6) by titration with EDTA solution using eriochrome black T as an indicator. The magnesium content was obtained from the difference (Mg + Ca) — Ca = Mg.

From the blood samples obtained in 1969 the serum calcium and magnesium values were estimated with an atomic absorption spectrophotometer and several calcium analyses were carried out simultaneously using the EDTA method, which tended to give somewhat lower values compared to the atomic absorption method.

The serum inorganic phosphorus was estimated according to TAUSKY & SHORR (1953), this method involving precipitation of plasma proteins with trichloroacetic acid, addition of ferrous sulphate — ammonium molybdate reagent to the supernatant liquid, and measuring the intensity of the blue colour with a Beckman B spectrophotometer at 840 m $\mu$ .

All analyses were carried out in duplicates. Statistical calculations were made according to CROXTON & COWDEN (1955).

### Results

**Effect of stage of lactation.** The data obtained from 41 cows and consisting of 207 blood samples was divided into nine parts according to the stage of lactation as shown in Table 1. The mean calcium, inorganic phosphorus and magnesium levels in the serum of the cows during the nine stages of lactation are given in the same Table.

The serum calcium level was lowest during the days 30—16 *pre partum* (9.21 mg %) rising to 9.67 mg % on days 15—0 *pre p.* During the days 0—15 *post p.*, the calcium level was quite low again (9.33 mg %), however, from this stage the calcium level increased consistently with advancing lactation (the peak yield is usually reached between 35—45 days *post p.* in the herd) the increase from days 0—15 to days 61—75 being highly significant ( $P < 0.001^{***}$ ).

The inorganic phosphorus level, was relatively low during the earliest phase of the dry period (4.44 mg %), whereas relatively high values were noted during the days 15—0 *pre p.* (6.02 mg %) and 0—15 *post p.* (6.15 mg %); thereafter a fairly significant ( $P < 0.02^*$ ) decrease to 5.31 mg % by the days 31—45 *post p.* (peak lactation) was noted. From this stage on, inconsistent non-significant variation occurred in the phosphorus level with advancing lactation.

The serum magnesium level was relatively low during the first phase of the dry

period (1.60 mg %) the level increasing, however, before parturition. Even though the serum magnesium level as a whole tended to be lower in lactating than in dry cows, the highest mean value (2.01 mg %) was noted during the days 16—30 *post p.*, this value being significantly ( $P < 0.002^{**}$ ) higher than those obtained during other phases after calving (Table 1).

None of the 41 cows exhibited signs of parturient paresis at calving even though two cows had a calcium level as low as 7.80 mg % (see cow AINI in Table 2). Definite signs of some lactational disturbance were noted in three cows during the indoor feeding period, i.e. in cow ETTI 5 days *post p.* and in the cows AINI and PONSI 18—20 days *post p.* The serum mineral values obtained for these cows are given in Table 2 and the case histories below:

AINI (4th calving). — Day 1 *pre p.*: Ca level quite low. Days 17—19 *post p.*: Rothera test, urine + + +, milk +, serum Ca slightly below normal, inorg. P and Mg elevated. Day 25: received Borocalcium Myrilas 200 ml, Dextrasol 500 ml and Ketocystin 750 ml intrav. Day 27: Dexta-Korti 10 ml intram. Day 40: cow appeared healthy, the milk yield had increased markedly, the serum mineral levels had returned to normal (Table 2).

PONSI (5th calving) — Day 20 *post p.*: Rothera test, urine + + +, milk +. Day 22: serum Ca critically low, inorg. P and Mg elevated. Day 26: Borocalcium Myrilas 200 ml subcutaneously into neck. Day 28: Borocalcium Myrilas 150 ml + Dextrasol 500 ml + Ketocystin 750 ml intrav. Day 30: Dexta-Korti 10 ml intram. Day 32: Dextrasol 500 ml intrav. Day 35: Borocalcium Myrilas PMD 400 ml + Dextrasol 500 ml intrav. Day 36: serum Ca and Mg quite normal, inorg. P elevated. Within three days the appetite grew worse. Day 39: the cow was too weak to get up — she was slaughtered.

ETTI (3rd calving) — Day 7 *pre p.*: serum mineral levels were normal. Day 5 *post p.*: signs of grass tetany, Rothera tests negative, cow was treated with Borocalcium Myrilas 120 ml intram., Borocalcium Myrilas PMD 800 ml + Borocalcium Myrilas 200 ml intrav. Day 6: Ca and inorg. P quite normal, Mg greatly depressed, blood Hb (15.15) and Hc (48.5) somewhat elevated, received Borocalcium Myrilas PMP 400 ml intrav. + Digitalis 6 ml intram. Day 7: cow's condition had improved, serum Mg had risen slightly. Day 10: appetite had decreased. Day 15: further normalization in the blood mineral levels. Day 16: body temp. 41° C, received penicillin, further decrease in appetite. Day 20: cow in poor condition, Rothera test, urine + +, Day 21: cow was slaughtered, pathological changes were noted in heart, kidneys, liver, and intestines.

Serum mineral changes in control and vitamin D treated cows. During stall feeding period in April-May 1969, a further study on serum calcium, inorg. phosphorus, and magnesium was carried out, in which six cows received two vitamin D injections both in conjunction with 1.5 million units of vitamin A and 250 mg vitamin E and six cows were left as controls. Both groups consisted of cows similar in respect of age, feeding, and level of milk yield. The results of this study have been summarized in Table 3.

As Table 3 indicates, the control group has a somewhat higher mean serum calcium level during the dry period and during the period right after calving, but a lower level during the periods beginning from the 6th day *post p.* than the vitamin D group, respectively. In the latter, the serum calcium level increased consistently after calving whereas in control cows it remained more or less constant.

In both groups the serum inorg. phosphorus level was markedly lower right after calving than during the late dry period. However, after calving the prepartum phosphorus level was rapidly regained in the vitamin D group whereas in the control group the level remained rather low (Table 3).

The serum magnesium level was similar in both groups and was slightly higher during the dry period than after calving. As a whole, the serum magnesium level was lower in this study (Table 3) compared to the results obtained earlier from the same herd (Table 1).

The daily milk yield was somewhat higher (by 1.5—2.0 kg) in the vitamin D group than in the control group.

Several cows in both groups had various disorders after calving. The type of disorder and number of cows suffering from it have been listed below:

Disorder	Control	Vitamin D
Retained placenta	4	2
Parturient paresis	1	1*
Mastitis	3	2
Ketosis	1***	4**
Lymphadenosis	1	—

\* vitamin D injections were given too early, the second one 2 weeks before calving

\*\* two cows had previous histories of ketosis

\*\*\* apparently secondary type of ketosis

In addition to the above specifications, two cows in the control group and one in the vitamin D group gave birth to twins. An interesting case history of one cow in the control group is given below:

JUOMA (6th calving April 4, 1969) calved three weeks too early, twins dead at birth (20 ♂ + 20 ♀ kg), and had retained placenta. Day 7 *post p.*: signs of paresis, received medication, had also mastitis. Day 10: no appetite, milk yield decreased from about 20 kg to 5 kg/day, cow was treated for acetoneemia and seemed to recover. Day 17: milk yield had increased to 25 kg/day. Day 80: milk yield rapidly decreased to 1 kg/day after which the cow died. Post mortem examination revealed signs of malignant lymphoma (internal hemorrhages, greatly enlarged lymphnodes and spleen, numerous pea-sized tumours in the uterus, pathological changes in the heart). The mineral values obtained for this cow after the 6th calving are given below (compare Table 2):

	(+ 2)	(+ 7)	(+ 17)	(+ 31)	(+ 36)
JUOMA Ca mg %	7.84	6.35	7.92	10.51	10.15
P »	4.45	3.53	5.20	2.90	3.88
Mg »	1.17	1.10	1.15	0.98	1.20
Hb g/100 ml	12.40	10.00	9.40	8.90	7.80

As the above values indicate, the serum Ca level first remained low after calving but returned to normal by the 31st day *post p.* The serum inorg. P level was mostly below the average of the control group (compare Table 4), whereas the serum Mg although low, was close to the group average. The blood Hb value decreased consistently from 12.40 to 7.80 during the study period, while the mean Hb value of the control group remained between 10.20 and 11.09 g/100 ml, respectively. During the period June 1966—June 1969, the total of leucocytes and the percentage of lymphocytes in the blood of this cow were estimated in the State Veterinary Institute twice yearly the number of lymphocytes varying from 5800 to 9100 per ml. Thus, according to Bendixen's classification (ref. SCHALM 1965, p. 488), this cow has been either suspect or positive in respect of lymphosarcoma. Two weeks before the death of the cow, the total leucocyte count of her blood was only 6100; the percentage of lymphocytes was not estimated at this time. Previously, two of JUOMA's sisters from the dam's side were lost from the same herd because of the same disease, while her dam, although over 10 years old at slaughter, appeared normal at post mortem examination. JUOMA was one of the best milk producers in the herd.

**E f f e c t s o f s e a s o n a n d a g e.** The data obtained in 1963 was first divided into two groups according to the age of the cows, group I included animals with one or two calvings and group II older ones. Both groups were further divided according to the month during which the blood samples had been taken. The results thus obtained have been summarized in Table 4.

The serum calcium level rose from May to August fairly significantly in group I ( $P < 0.01^{**}$ ) and very significantly in group II ( $P < 0.001^{***}$ ). Group I had a higher calcium level in April-May but a lower one in July-August than group II, but the difference between the groups was significant ( $P < 0.05^{*}$ ) only in May.

The inorg. phosphorus level rose in both groups from April to May, the rise being significant ( $P < 0.05^{*}$ ) in group I. In group I the inorg. phosphorus level remained rather high during the grazing period (July-August in Table 4), whereas in group II a significant ( $P < 0.01^{**}$ ) drop was noted from May to August. In July-August, group I had a significantly ( $P < 0.002^{**}$ ) higher serum inorg. phosphorus level than group II. As a whole, older cows had significantly ( $P < 0.001^{***}$ ) lower mean inorg. phosphorus levels than younger cows.

Cows in group I had higher mean serum calcium and inorg. phosphorus levels during the dry period as well as during the first 10 weeks of lactation compared to the cows in group II. This is demonstrated in the following compilation.

	Dry period			Early lactation period		
	N	Ca mg %	P mg %	N	Ca mg %	P mg %
Group I	16	9.61	6.33	67	9.69	5.65
Group II	19	9.52	4.70	65	9.48	5.31

As the above values indicate, the effect of age was most pronounced in the case of the inorg. phosphorus level during the dry period.

The magnesium level decreased from April to May, this decrease occurring already before the beginning of the grazing period (Table 4). The magnesium values increased significantly in group I ( $P < 0.001^{***}$ ) and group II ( $P < 0.01^{**}$ ) from May to August. Group I had a somewhat higher magnesium level during the grazing period than group II.

The hemoglobin and hematocrit values were higher in both groups during the grazing than during the indoor feeding periods. This seasonal trend was not significant, however, neither was the difference between group I and II (Table 4).

The mean daily milk yield of the cows in group II was 24.74 kg, and that of the cows in group I 17.51 kg, the difference being highly significant ( $P < 0.001^{***}$ ).

#### Discussion

As a whole, the serum calcium and inorganic phosphorus levels in the cows of the present study were similar to those reported earlier in the literature, whereas the magnesium levels were lower (HIBBS & POUNDEN 1955, CARLSTRÖM 1961a, CARLSTRÖM 1961b, MOODIE & ROBERTSON 1961 p. 220).

The amount of vitamin D<sub>3</sub> used in this study effectively prevented milk fever, and the serum calcium level decreased only slightly after calving in vitamin D treated cows (Tables 1 & 3). HIBBS & POUNDEN (1955) noted a decrease in the serum calcium level at



12 and 24 hours after calving even in cows fed with 30 million units of vitamin D during 3—7 days prior to calving. In their experiment the vitamin D treated cows had considerably higher calcium and phosphorus levels shortly after parturition as compared to the control cows.

In this study the serum calcium as well as the inorg. phosphorus levels of the vitamin D treated cows tended to increase during the early lactation period, whereas both the calcium and phosphorus levels of the control cows tended to remain lower, respectively (Table 3). According to MUIR et al. (1968), vitamin D fed cows recovered faster from hypocalcemia induced by EDTA infusion than normal cows or cows with history of milk fever.

It seems that for Finnish Ayrshire cows, in which the paresis incidence is about 9.98 % (ref. NURMIO 1968 p. 83), the amount of vitamin D<sub>3</sub> used in this study (2 injections 5 mill. I.U. each) is sufficient in reducing significantly this lactational disturbance. Larger vitamin D amounts may be harmful or even fatal and cannot be recommended. Furthermore, the estimation of the best time for the administration of massive vitamin D doses (by injection or feeding) is often difficult due to the fact that the calving date cannot be predicted exactly. According to PAYNE (1968), the toxic side effect can be prevented with an addition of vitamin A or thyroxine, but the mixtures are not as effective as vitamin D<sub>3</sub> alone. PAYNE & MANSTON (1967) noted also that if the diet included adequate phosphorus and magnesium, the vitamin D<sub>3</sub> was less likely to induce metastatic calcification. Vitamin D<sub>3</sub> has been used in the dairy herd of the Viik Experimental Farm since 1956, first alone, and during the last three years in conjunction with vitamins A and E. Over 200 cows have been discarded from this herd since 1956 and none of them had signs of pathological calcification at post mortem examination. However, in order to avoid toxic side effects of massive doses of vitamin D, milk fever could possibly be prevented in practical farming conditions merely by means of adjusting the Ca/P of the dry period ration to an optimum level. Incidence of paresis has been noted to be lower in cows receiving a diet with Ca/P of 2.3:1 compared to cows receiving a diet with Ca/P 0.95:1 (KENDALL et al. 1969a) or 4.3:1 (KENDALL et al. 1968) during the last three weeks of gestation. Furthermore, also acid dietary additives (NH<sub>4</sub>Cl) appear to help in maintaining the serum calcium level high at calving (KENDALL et al. 1969b).

In the data presented in Table 1, the cows had the highest inorg. phosphorus level during the two closest 15-day periods immediately before and after calving, whereas in the later data (Table 3) the phosphorus level was depressed after calving (particularly in cows not receiving vitamin D). This difference in the results of the two studies has probably been due to the fact that in the former 33 % of the cows were first calvers, whereas in the latter all cows had calved at least twice.

As a whole, the serum magnesium levels were relatively low (Table 1) especially in the data obtained in 1969 (Table 3). The blood samples in the latter were taken in April-May. As Table 4 indicates, the serum magnesium level has been low in May also in the data obtained in 1963. In addition to the effect of the season, the low magnesium values in the latter data may have been partly due to the different method used in the estimation of the serum magnesium content. None of the cows exhibited signs of magnesium deficiency during the study period in 1969. According to ROOK & STORRY (1962 p. 1064), the magnesium level may decrease to 0.5 mg % without clinical signs. Furthermore, high producers have usually lower magnesium levels than low producers. Age had only a slight effect on

the magnesium level in the said herd. In 1963 the mean magnesium level of 104 young cows (group I) was  $1.79 \pm 0.03$  mg %, while that of 103 old cows (group II) was  $1.73 \pm 0.05$  mg %.

The high magnesium level noted during the 2nd and 3rd weeks after parturition, i.e. during the period when acetonemia has most frequently occurred (see Tables 1 & 2), are so far unexplained.

Simultaneously, elevated magnesium and inorg. phosphorus levels were noted in two cows (AINI, Mg 3.72, P 9.95 mg %, PONSI Mg 3.53 and P 10.53 mg %) suffering from acetonemia during the 3rd week of lactation (Table 2). Cow Aini who recovered had a nearly normal serum calcium level, whereas the cow Ponsi, who did not recover had a markedly reduced serum calcium level, which however rose to normal after medication. Cow Etti, who apparently suffered from a magnesium deficiency, had greatly depressed magnesium, normal phosphorus and slightly below normal calcium values. Had she been treated with magnesium she probably would have recovered. However, she was treated with Ca-solutions which apparently were of little value in this case.

In the study carried out in 1969, cow Juoma suffering from malignant lymphoma, appeared to have difficulties in maintaining normal calcium and inorg. phosphorus levels in serum after calving, whereas the magnesium level was similar to other cows in the same group. The hemoglobin content of her blood decreased markedly after calving apparently partly as a result of internal hemorrhages. However, it was noted earlier in the same herd that even in apparently healthy cows the Hb value, which was relatively high at calving, tended to decrease during the ascending phase of lactation and that in older cows there was often a greater decrease than in the younger ones.

The serum mineral concentrations reflect to a certain degree the mineral balance in the cow's body. The serum inorg. phosphorus and magnesium levels are influenced more readily by the amount of these elements in the diet than the serum calcium level. Nevertheless, in this study the serum calcium level increased in young as well as old cows during the grazing period (Table 4), whereas the inorg. phosphorus level decreased in older cows (group II) but was not much affected in younger cows (group I). The seasonal trends noted in the serum calcium and inorg. phosphorus levels suggest that the Ca/P of the ration of the cows has been higher during the grazing than during the indoor feeding period. Since there is not enough information on the calcium, phosphorus and magnesium contents of hay, silage, and pasture grass fed to the herd in question, it has not been possible to obtain reliable information on the amounts of these minerals ingested by cows at various stages of lactation and during different seasons. It seems likely, however, that the Ca/P of the ration of dry pregnant cows (fed mainly with hay + silage during indoor feeding period and with pasture grass ad lib. during summer), has been somewhat higher than the Ca/P of the ration of lactating cows (fed with concentrates in addition to roughages or pasture grass) in spite of the fact that mineral salt mixtures have been used in the concentrate mixtures. According to SAARINEN (1950), the blood calcium level of lactating cows was significantly influenced by the Ca/P of the diet while the effect of the calcium intake was nonsignificant. Furthermore, the blood inorganic phosphorus level was significantly affected by the phosphorus intake from the diet while the effect of Ca/P on the diet remained nonsignificant.

The serum magnesium level began to decrease already in May, i.e. even before the

cows were put on pasture (Table 4). No reliable explanation can be offered at the moment for this phenomenon. Younger cows had somewhat higher mean serum magnesium levels than older cows during summer. In both age groups the magnesium levels tended to increase towards the end of the grazing period probably as a result of the changes in the mineral composition of the herbage.

If the serum mineral values are taken to indicate the mineral balance in the cow's body, the results of this paper will lead to a few suggestions which might prove helpful in increasing the resistance of cows to lactational stresses in the said herd. An increase in the amount of dietary phosphorus during the dry period and grazing periods, especially in case of older cows, is recommended, also an increase in the amount of dietary magnesium during the spring months (April-May) in the ration of all cows. It seems, on the other hand, that the calcium intake of the cows has been well above the recommended requirements (*Nutrient Requirements* 1965, p. 29—30).

### Summary

Blood mineral studies were carried out in the dairy herd of the Viik Experimental Farm, in which parturient paresis has been successfully prevented by intramuscular injections of vitamin D<sub>3</sub> (2 × 5 million I.U.). The material consisted of 41 cows from which 207 blood samples had been taken in 1963 and 12 cows (6 controls, 6 vitamin D injected cows) from which 72 blood samples had been taken in 1969. All cows were either dry and pregnant or lactating and nonpregnant.

Compared to values obtained during the dry period, the serum calcium level was only slightly lower shortly after calving. The calcium level increased in the vitamin D treated cows during the ascending phase of lactation, whereas both the calcium and inorganic phosphorus levels remained relatively low in the control cows, respectively. The highest mean magnesium levels occurred during the 2nd and 3rd weeks of lactation.

The serum calcium level was higher during the grazing period than during the indoor feeding period. Inorganic phosphorus remained at a relatively high level in younger cows during the grazing period, whereas a significant decrease was noted in the phosphorus level of older cows, respectively. The lowest magnesium values were noted in May, i.e. already before the beginning of the pasture season, the concentration increasing towards the end of the grazing period especially in younger cows.

As a whole, younger cows had slightly higher mean serum calcium and magnesium levels and significantly higher inorganic phosphorus levels compared to older cows. The effect of age on the inorg. phosphorus level appeared to be more marked in dry cows and during the grazing period than in lactating cows and during the winter feeding period.

Special attention has been paid to the blood mineral values of four individual cows suffering from various illnesses during the study period.

### REFERENCES

- ALBRIGHT, J. L. & BLOSSER, T. H. 1957. Blood levels and urinary excretion of calcium in normal cows and cows with parturient paresis after intravenous injections with calcium borogluconate. *J. Dairy Sci.* 40: 477—486.
- ANAST, C. S. 1968. (Personal communication) Univ. Missouri, Columbia, Mo. U.S.A.
- BARLET, J.-P. 1967. Effects of thyrocalcitonin on blood Ca and P in the dairy cow. *C.r. hebd. Séanc. Acad. Sci., Ser. D, Paris* 265: 1075—1082.

- BODA, I. M. & COLE, H. H. 1954. The influence of dietary calcium and phosphorus on the incidence of milk fever, *J. Dairy Sci.* 39: 360—372.
- BREIREM, K. & HVIDSTEN, H. 1966. Hypomagnesaemia bei Wiederkäuern. *Kraftfutter* 49: 59.
- BUTLER, E. J. 1963. The mineral element content of spring pasture in relation to the occurrence of grass tetany and hypomagnesaemia in dairy cows. *J. Agric. Sci.* 60: 329.
- CARE, J. 1968. (Personal communication) Univ. Missouri Medical Center, Columbia, Mo. U.S.A.
- CARLSTRÖM, G. 1961a. Studies on parturient paresis in dairy cows. *Acta Vet. Scand.* 2: 330—349.
- »— 1961b. Phosphorus, magnesium and pH in bovine blood serum normally, after parturition and in parturient paresis. *Ibid.* 2: 350—356.
- CROXTON, F. E. & COWDEN, D. J. 1955. *Applied general statistics*. 2nd ed. XVI + 843 pp. London.
- ENDER, F., DISHINGTON, I. W. & HELGEBOSTAD, A. 1962. Parturient paresis and related forms of hypocalcemic disorders induced experimentally in dairy cows. II. Studies on the etiological importance of feeding prepartal diets high in calcium and low or normal in phosphorus in relation to development of milk fever — effect of high and low alkalinity of diets. *Acta Vet. Scand. Suppl.* 1, vol. 3.
- GREIG, J. R. 1930. Studies in the prevention of milk fever. *Vet. Rec.* 10: 301.
- 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.
- HIBBS, J. W. 1948. *Abstr. Doctoral Dissertations*, Columbus, Ohio State Univ. Press. 54: 163.
- »— & CONRAD, H. R. 1960. Studies on milk fever in cows. VI. Effect of three prepartal dosage levels of vitamin D on milk fever incidence. *J. Dairy Sci.* 43: 1124—9.
- »— & POUNDEN, W. D. 1955. Studies on milk fever in dairy cows. IV. Prevention by short-time, prepartum feeding of massive doses of vitamin D. *Ibid.* 38: 65—72.
- JÖNSSON, G. 1960. On the etiology and pathogenesis of parturient paresis in dairy cows. *Diss. Acta Agric. Scand. Suppl.* 8.
- KENDALL, K. A., HARSHBARGER, K. E., HAYS, R. L. & ORMISTON, E. E. 1966. Preventing parturient paresis in the parturient suspect through grain feeding. *J. Dairy Sci.* 49: 720.
- »— 1969a. Blood serum composition associated with two levels of monosodium phosphate feeding. *Ibid.* 52: 931.
- »— 1969b. Some responses of dairy cows to acid or alkaline dietary additives. *Ibid.* 52: 931.
- »— HAYS, R. L. & ORMISTON, E. E. 1968. Postpartum serum calcium and phosphorus levels associated with calcium carbonate and monosodium phosphate feeding. 63rd Ann. Meeting. American Dairy Sci. Ass. Columbus, Ohio, June 16—19, Program p. 74.
- KOSSILA, V. 1967. On the weight and basic structural components of the thyroid in dairy cattle. *Acta Agral. Fenn.* 109. 2. 115 pp., p. 32—36.
- LITTLE, W. L. & MATTIC, E. C. W. 1933. *Vet. Rec.* 13: 238.
- LOMBA, F., PAQUAY, R., BIENFET, V. & LOUSSE, A. 1968. Statistical research on the fate of dietary mineral elements in dry and lactating cows. II. Magnesium. *J. Agric. Sci., Camb.* 71: 181—188.
- MOODIE, E. W. & ROBERTSON, A. 1961. Dietary intake of the parturient cow. *Res. Vet. Sci.* 2: 217—226.
- MUIR, L. A., HIBBS, J. W. & CONRAD, H. R. 1968. Effect of vitamin D on the ability of cows to mobilize blood calcium. *J. Dairy Sci.* 51: 1046—1050.
- NIEDERMEIER, R. P., SMITH, V. R. & WHITEHAIR, C. K. 1949. Parturient paresis. III. A study of various blood constituents at parturition in mastectomized cows. *Ibid.* 32: 927—934.
- NURMIO, P. 1968. On plasma calcium regulation in paresis puerperalis hypocalcemia in cattle. *Acta Vet. Scand. suppl.* 26.
- The Nutrient Requirements of Farm Livestock. 1965. No. 2. Ruminants. Technical Reviews and Summaries. Agr. Res. Council, London pp. XI + 264.
- PALOHEIMO, L. 1968. D-vitamiini ja poikimahalvauksen torjunta. (Vitamin D and prevention of milk fever). *Karjalalous* 44: 136.
- PAQUAY, R., LOMBA, F., LOUSSE, A. & BIENFET, V. 1968. Statistical research on the fate of dietary mineral elements in dry and lactating cows. I. Calcium. *J. Agric. Sci., Camb.* 71: 173—180.
- PAYNE, J. M. 1968. Milk fever. *Outl. Agric.* 5: 266—72.
- »— & MANSTON, R. 1967. The safety of massive doses of vitamin D<sub>3</sub> in the prevention of milk fever. *Vet. Rec.* 81: 214.

- ROOK, J. A. F. & STORRY, J. E. 1962. Magnesium in the nutrition of dairy cows. *Nutr. Abstr. & Revs.* 32: 1055—1077.
- SAARINEN, P. 1950. A statistical study of the effect of excessive feeding of calcium and phosphorus supplements on the blood calcium and phosphorus in dairy cows. *J. Sci. Agr. Soc. Finl.* 22: 122—131.
- SCHALM, D. W. 1965. *Veterinary hematology* p. 488. 2nd. ed. Philadelphia.
- STOTT, G. H. 1968. Dietary influence on the incidence of parturient paresis. *Fed. Proc.* 27: 156—161.
- & SMITH, V. R. 1957. Parturient paresis. VIII. Results of parathyroidectomy of cows. *J. Dairy Sci.* 40: 897—904.
- TAUSSKY, H. H. & SHORR, E. 1953. A microcolorimetric method for the determination of inorganic phosphorus. *J. Biol. Chem.* 202: 675—685.
- TURNER, C. W. 1966. What causes high production? *Mo. Agr. Exp. Sta. Bull.* 836.
- WARD, G. M. & BLOSSER, T. H. & ADAMS, M. F. 1952. The relation of prepartal and postpartal mineral balances to the occurrence of parturient paresis in dairy cows. *J. Dairy Sci.* 35: 587.
- YOUNG, D. M. & CAPEN, C. C. 1968. Thyrocalcitonin biologic assay and ultrastructure of thyroid C-cells of cows with parturient paresis and hypocalcemia. *Proc. Symp. on Thyrocalcitonin and C-cells.* London.

## SELOSTUS

LAKTAATIOKAUDEN VAIHEEN, VUODENAJAN JA IÄN VAIKUTUS VERISEERUMIN  
KALSIUMIN, EPÄORGAANISEN FOSFORIN JA MAGNESIUMIN MÄÄRIIN  
AYRSHIRE-KARJASSA, JOSSA POIKIMAHALVAUSTA ON TORJUTTU  
D-VITAMIINIRUISKEELLA

VAPPU KOSSILA, PAAVO NIEMELÄ ja ERKKI KOSKENKORVA

*Helsingin yliopiston kotieläintieteen laitos*

Veren kivennäiskoostumuksen vaihteluita tutkittiin Viikin opetus- ja koetilan karjassa, jossa poikimahalvausta on torjuttu vitamiini D<sub>3</sub> ruiskeilla. Tutkimusaineisto oli kerätty vuonna 1963 (41 lehmää, 207 verinäytettä) ja vuonna 1969 (12 lehmää, 72 verinäytettä). Lehmät olivat joko tiineitä, ummessaolevia tai ei tiineitä maitoa tuottavia.

Tutkimuksessa todettiin mm. että laktaatiokauden alkuvaiheessa veriseerumin kalsiumtaso ei merkittävästi poikennut ummessaolokauden arvoista. Kalsiumin tason todettiin kohoavan laktaatiokauden alkupuolella D-vitamiinia saaneilla lehmillä. Edellä mainittuihin verrattuna kontrollilehmillä (eivät saaneet D-vitamiinia) sekä kalsium- että epäorgaaninen fosforitaso oli laktaatiokauden alussa selvästi alhaisempi. Seerumin magnesiumtaso oli maidontuotantokautena yleensä jonkin verran alhaisempi kuin ummessaolokautena lukuunottamatta laktaation 2. ja 3. viikkoa, jolloinka magnesiumtaso oli keskim. korkein ja jolloinka lehmillä esiintyy herkimmin asetonitautia.

Seerumin kalsiumtaso oli laidunkautena korkeampi kuin sisäruokintakautena. Nuoremmilla lehmillä (1—2 kertaa poikineet) seerumin epäorg. fosforin määrä pysytteli suhteellisen korkeana laidunkautenakin kun taas vanhemmilla lehmillä (yli 2 kertaa poikineet) epäorg. fosforin määrä oli merkittävästi alhaisempi verrattuna sisäruokintakauden viimeisen kuukauden fosforitasoon. Veriseerumin magnesiumtaso oli suhteellisen alhainen jo toukokuussa (jo ennen laidunkauden alkua). Magnesiumtaso kohosi laidunkauden loppua kohden ja kohoaminen oli nuoremmilla lehmillä selvempi kuin vanhemmilla.

Nuoremmilla lehmillä veriseerumin kalsium- ja magnesiumtaso oli keskim. jonkin verran korkeampia epäorg. fosforin määrä merkittävästi korkeampi kuin vanhemmilla lehmillä. Iän vaikutus epäorgaanisen fosforin määrään seerumissa oli ummessaolevilla lehmillä ja laidunkauden aikana selvempi kuin maidontuotantokautena ja sisäruokinnan aikana. Koska tiedot ko. karjan ruokinnassa käytettyjen rehujen (erityisesti heinän, säilörehun ja laidunruohon) kivennäispitoisuuksista ovat puutteelliset, ei eri ruokintaluokkiin kuuluvien lehmien dieetin kivennäismääriä ole voitu saada selville riittävän luotettavasti. Näyttää kuitenkin siltä, että esim. ravinnon kalsium-fosfori suhde on ollut korkeampi sekä ummessaoloaikana että laidunkautena kuin maidontuotannon aikana ja sisäruokinta kautena.

Tutkimuksessa on kiinnitetty erityistä huomiota myös neljän tutkimusaikana sairaaksi todetun lehmän veriseerumin kivennäisarvoihin.