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LONG TERM STUDIES ON BONE MINERAL CHANGES DURING DIFFERENT LACTATIONS IN SWEDISH DAIRY CATTLE

By

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HOLMBERG, T., B. BERGLUND, G. RAL and B. ÅHMAN: *Long term studies on bone mineral changes during different lactations in Swedish dairy cattle.* Acta vet. scand. 1985, *26*, 49—60. — Changes in the bone mineral content (BMC) during the first 4 lactations were continuously studied between July 1977 and February 1982 in a total number of 103 dairy cows of different breeds and rearing intensities. The BMC was measured by dichromatic photon absorptiometry in 2 coccygeal vertebrae. Although great individual variations were found, the changes in BMC during the lactation followed a typical pattern with high values during the dry period and low values after calving and during mid-lactation. The influence of body weight on the BMC value is discussed. The changes in BMC were more accentuated during the first lactation, as compared to the subsequent lactations. This could indicate a metabolically more active skeleton in younger cows. In this study no difference in BMC pattern was observed between cows affected or not affected by parturient paresis.

bone mineral; dairy cows; milk production; parturient paresis.

In dairy cattle the demands of milk production for calcium is met by an increased gastro-intestinal absorption but also by an increased release of calcium from bone (e.g. *Ramberg et al.* 1970, *Black et al.* 1973). The bone mineral content (BMC) of the skeleton during lactation is of clinical interest since a high milk production in dairy cows often seems to be associated with symptoms of calcium deficiency, i.e. hypocalcemia during early lactation which could be a result of an insufficient release of calcium from the bone.

An accurate method for determination of BMC by dichromatic photon absorptiometry (*Judy* 1971, *Dissing* 1974, 1975) in cattle was presented by *Zetterholm & Dalén* (1978). This method was used to measure variations in BMC during a single lactation period in dairy cows of the Swedish Red and White breed (SRB) (*Zetterholm* 1978 a, b, c). A study of the variations in BMC during several lactations was however not performed. The aim of the present investigation, therefore, was to make such a long term study on a large number of Swedish dairy cows of different breeds and rearing intensities.

MATERIALS AND METHODS

Animals and management

The cows studied were of the following dairy breeds: Swedish Red and White (SRB), Swedish Friesian (SLB), Swedish Jersey (SJB) and reciprocal F_1-crosses between SRB and SLB (SRB × SLB). A total of 103 cows were included in this study. The distribution in breed groups and lactations is showed in Table 1. The study was performed between July 1977 and February 1982 and is part of an extensive study of longevity traits, the outlines of which were published by *Berglund et al.* (1980). The calves were recruited before 2 months of age from the university experimental herd or from milk recording herds utilizing AI. At 14—17 weeks of age the breed groups were equally divided between 2 levels of feeding, normal (N) and high (H) energy intake, with the same protein allowance per energy unit. The average daily gain from 60 days of age to first calving was, in the heavier breeds (SRB, SLB and crosses SRB × SLB), 500—550 g for the N group and 610—680 g for the H group. First calving occurred at about 28 months of age for the N group and at about 25 months

Table 1. Number of animals in each breed group during lactations 1—4.

	Lactation number			
Breed	1	2	3	4
SRB	36	32	17	8
SRB × SLB	16	20	17	8
SLB	16	14	10	2
SJB	14	16	10	6
	82	82	54	24

for the H group. The average daily gain for SJB during the same period was about 420 g for the N group and about 490 for the H group. First calving in this breed occurred at about 26 and 23 months of age, respectively.

After calving, animals were fed according to Swedish standards (*Eriksson et al.* 1976). Roughage (equal amounts of dry matter from hay and grass silage) were given in a ration of 1.5—1.7 kg dry matter per 100 kg live weight. The concentrate mixture contained 2 % dicalcium phosphate. Three weeks before calving the concentrate ration was increased and immediately after calving all cows received concentrate (5.6 kg) for a production of 11 kg fat-corrected milk. During the first weeks of lactation concentrate feeding was increased by 0.4 kg per day, and thereafter given according to milk production.

The daily milk yield was recorded once a week. The total milk production in the first lactation was, for the heavier breeds (SRB, SRB × SLB and SLB), about 4,300 kg milk with 4.2 % fat. In the third and fourth lactation it had increased to about 5,500 kg milk with 4.1 % fat. The corresponding values for SJB were about 2,900 kg milk with 6.4 % fat in the first lactation and 3,500 kg milk with 6.4 % fat in the third and fourth lactation.

The cows were kept tethered indoors at the university research station in Uppsala. No grazing was thus allowed, but the animals were exercised outdoors in grass-free paddocks twice a week during the period of April-October. All disturbances affecting the cows were recorded in the university herd register, from where information about the cows affected by parturient paresis was obtained.

Bone mineral determination

The bone mineral content was determined in 2 coccygeal vertebrae (V and VI or VI and VII), by dichromatic photon absorptiometry, using the technique described by *Zetterholm & Dalén* (1978). The equipment consisted of an isotope holder with 2 gamma radiation sources (^{125}I and ^{241}Am) with energies of 27 and 60 KeV, respectively. Absorption was measured by a scintillator photomultiplic detector. The isotope holder and the detector were attached to a U-shaped mount to ensure parallel movements during the scanning procedures. The instrument (Bone Scanner 7102, AB Atomenergi, Studsvik, Nyköping, Sweden) was used for scanning the whole vertebrae by 12—15 parallel scans. By this scanning procedure the mineral content of each vertebrae was calculated and expressed as g/vertebrae. The mineral content in the 2 vertebrae were then added to form the BMC-value which was used in the statistical analysis.

Before each measurement the instrument was equilibrated in air and water and checked against a standard, i.e. an aluminium alloy with absorption properties similar to that of bone. After epidural anesthesia the cow's tail was fixed in a special holder and the bone scanner was raised to a suitable height behind the animal (Fig. 1). The location of the 2 measured coccygeal vertebrae was marked by tattooing the skin over the most cranial of the 3 intervertebrae discs.

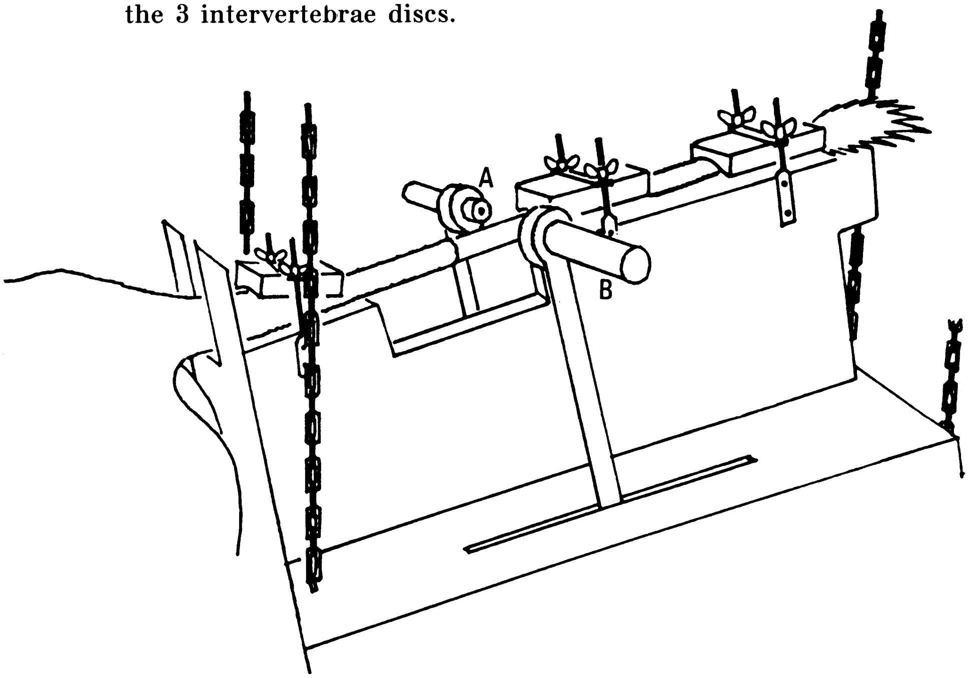

Figure 1. The tail holder. A = gamma-ray source, B = scintillator detector (*Zetterholm & Dalén* 1978).

The analytical error of the method was calculated using the difference between duplicate determinations of the coccygeal vertebrae, and expressed as a coefficient of variation (c.v.):

$$\text{c.v.}\ (\%) = \frac{\sqrt{\frac{\Sigma d^2}{2n}} \cdot 100}{\bar{x}}$$

where
d = difference between duplicate measurements
n = number of duplicate determinations
$\bar{x}$ = overall mean of the measurements.

The recordings were performed approximately 3 weeks prior to expected day of calving and thereafter 3, 6 and 9 months after calving. This schedule was repeated during all lactations.

Statistical analysis

To evaluate the effect of animal, breed and registration number on BMC-value, the method of least-squares analysis was used, as applied in Harvey's LSML76 program (*Harvey* 1977).

The following full model was assumed to describe the data:

$$y_{ijklmn} = \mu + c_i + a_{ij} + d_k + s_l + r_m + b_i(x_{ijklmn} - \bar{x}) + e_{ijklmn} \quad \text{model (1)}$$

where

y_{ijklmn} = the ijklmnth observation
μ = least-squares mean
c_i = effect of the ith breed ($i = 1,2,3,4$)
a_{ij} = effect of the jth animal within the ith breed
d_k = effect of the kth recording year ($k = 1,2,\ldots,6$)
s_l = effect of the lth season ($l = 1,2$)
r_m = effect of the mth registration ($m = 1,2,\ldots,17$)
b_i = partial regression coefficient on weight within breed
x_{ijklmn} = weight of the ijklmnth animal
$\bar{x}$ = mean weight
e_{ijklmn} = residual effect with mean = 0 and variance = σ_e^2.

To solve the normal equations the restrictions $\sum_i c_i = \sum_k d_k = \sum_l s_l = \sum_m r_m = 0$ were imposed on the model.

All effects, except the animal effect and the residual effect were considered as fixed. The year was divided into 2 seasons, October-March and April-September. The effect of rearing intensity did not significantly ($P > 0.05$) influence the BMC-value and was therefore ignored.

Model 1 was also used to calculate the relationship between different milk traits and BMC. The following milk traits were used: Milk yield in kg/day and corresponding fat percentages recorded the same week the BMC was measured, total milk yield in kg during the whole lactation and corresponding mean fat percentage, maximum daily milk yield in kg, milk yield in kg during the first 200 days and finally persistency in milk yield using the definition by *Johansson & Hansson* (1940), i.e. the ratio between production in the 101—200th day and production during 1—100th day.

To study the difference between cows affected or not affected by parturient paresis model 1 was also used.

To be able to study the correlation between weight and BMC-value the effect of weight was excluded in model 1. This model is referred to in the text as model 2.

$$y_{ijklmn} = \mu + c_i + a_{ij} + d_k + s_l + r_m + e_{ijklmn} \qquad \text{model (2)}$$

where the effects were the same as those already mentioned.

The coefficient of determination, R^2, was adjusted for degrees of freedom according to the formula:

$$R_a^2 = 1 - \frac{N\text{-}1}{N\text{-}p} \cdot \frac{\text{SS Residual}}{\text{SS Corrected total}}$$

where

N = number of observations

p = degrees of freedom for the model

SS Residual = sums of squares for residual

SS Corrected total = total sums of squares corrected for the mean.

RESULTS

The analytical error (c.v.) for the measurements in this study was 1.9 %. The results of the analysis of variance are given in

Table 2. Least-squares analysis of BMC-value (model 1).

Source of variation	df	Mean squares
Breed	3	1925749n.s.
Animal within breed	99	1632032***
Recording year	5	200454***
Season	1	79674*
Registration	16	339058***
Linear regression on weight	1	32951***
Linear regression on weight within breed	3	157035***
Residual	754	17519
Coefficient of determination, R_a^2, %		94.9

n.s. = (not significant) $P > 0.05$; * = $P \leq 0.05$; *** = $P \leq 0.001$.

Table 2 (model 1). All sources of variation considered in the model, except breed, significantly influenced BMC. The most important factor was the individual animal, which accounted for more than 50 % of the total variation in the model.

The BMC is highly influenced by the live weight of the animal at the time of recording. Regression coefficients of BMC-values on live weight were positive for all breeds (Table 3). Using model 2 the correlation between BMC and weight of the animal was calculated as 0.18 ($P \leq 0.001$).

Table 3. Linear regression coefficients and standard errors (SE) of BMC-values on live weight for SRB, SLB, SRB×SLB and SJB (model 1).

Breed	No. of measurements	Linear regression coefficients	SE
Total	883	1.24587	0.28738
SRB	359	0.89956	0.32254
SLB	212	0.48489	0.33634
SRB×SLB	152	2.28635	0.31873
SJB	160	1.31267	0.55380

Least-squares means and standard errors of BMC-values for the 4 different breeds are shown in Table 4 (model 1). SRB× SLB had the highest BMC followed by SLB. SJB and SRB had very similar mean values.

Table 4. Least-squares means and standard errors (SE) for BMC-values for SRB, SLB, SRB×SLB and SJB (model 1).

Breed	No. of measurements	LS-means	SE
SRB	359	2940[a]	175
SLB	212	3076[b]	195
SRB×SLB	152	3211[c]	215
SJB	160	2982[a]	444

a, b, c, Figures with the same superscript are not significantly ($P > 0.05$) different from each other.

Least-squares means for the different registrations are presented in Fig. 2 (model 1). Within all lactations the BMC was highest prior to calving. The values declined 3—6 months after calving and thereafter increased again. The changes in BMC followed the same pattern in all lactations but were more accentuated, especially the decrease in BMC after parturition, in the first lactation. The BMC rose slowly with increasing lactation

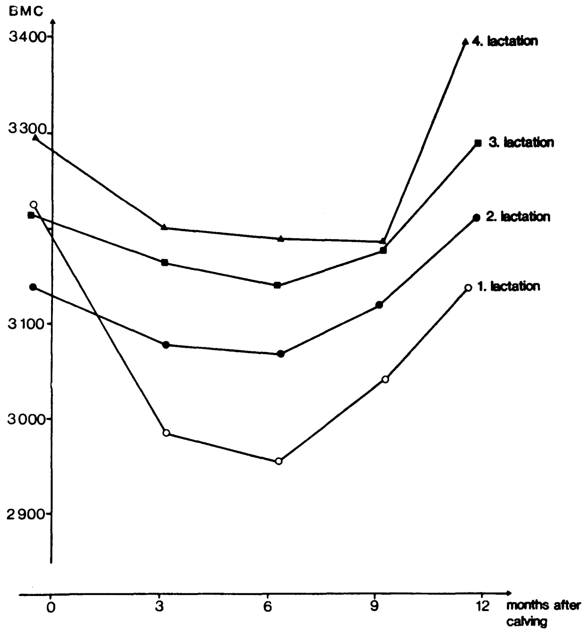

Figure 2. BMC-values corrected for effects in model 1 for the 4 different lactations.

number, but not until 3 weeks prior to the fourth lactation the BMC exceeded the value it had before the first calving.

There was a significant negative correlation (model 1) between BMC and the amount of milk at the week of recording (Table 5). There were no significant correlations between BMC-values and the total amount of milk during the lactation. Persistency in milk yield during the lactation was negatively correlated with the BMC-values.

Table 5. Correlations between BMC-value and different milk traits (model 1; 877 observations).

Milk trait	Coefficient of correlation
Milk, kg (week of recording)	—0.10**
Fat, % (week of recording)	—0.03n.s.
Milk/lactation, kg	—0.06n.s.
Fat/lactation, %	—0.08*
Max. milk yield, kg	0.01n.s.
Milk 200 days, kg	—0.03n.s.
Persistency	—0.07*

n.s. = (not significant) $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$.

The bone mineral changes during lactations were also studied in all cows affected by parturient paresis. No clear difference between cows affected (n = 26) or not affected, at any occasion during the study by parturient paresis, could be seen. There was a tendency, however, for cows affected by parturient paresis to have lower BMC-values in lactations 1 and 2. The number of recordings made in lactations 3 and 4 were too small for this group to give reliable estimates.

DISCUSSION

A long term study over several lactations on bone mineral changes in dairy cows, as presented in this paper, was never carried out before. The method, dichromatic photon absorptiometry, used for measurements of BMC in coccygeal vertebrae as introduced by *Zetterholm & Dalén* (1978), has proved to give a good picture of the changes in BMC in dairy cows during lactations. The analytical error in this study, as in the study presented by *Zetterholm & Dalén* (1978), was quite low (1.9 %).

Although there are obviously great individual variations among the cows in this study, the changes in BMC during the lactation periods show a typical pattern (Fig. 2). This influence of milk production on the mineral content in the dairy cow's skeleton has been only partially described before (*Sansom* 1969, *Rowland et al.* 1972, *Zetterholm* 1978 a, b, c). In the present study the decrease in BMC seems to be a quite slow process with a calculated minimum value somewhat between 3—6 months after parturition. The daily milk yield had already reached it's maximum before 3 months. The fact that the calcium removal from bone is a slow process, however, is in accordance with other observations (e.g. *Ramberg et al.* 1970).

It has been known for a long time that the mobilizable fraction of Ca in bones diminishes as cows grow older. In young cattle 60 % of the bone Ca is reported to be available, but in old cows only 5 % (*Hansard et al.* 1954). The more dramatic change in BMC during the first lactation seen in the present study therefore, is probably due to a more active skeleton with a larger amount of mobilizable bone Ca.

In a radiographic study (*Holmberg et al.* 1984) of cows from the same experimental herd a higher daily growth rate coincided with an earlier start of the closure of the growth plates. In animals on the high level of feeding thicker bone cortices, i.e. high cortical index, were found during the rearing period. In the present study the differencies in feeding and daily gain between heifers of the 2 rearing intensities seemed not to affect the skeletons capacity to mobilize Ca during the first or the following lactations. After first calving the feeding regimes from the rearing period were changed and all cows were fed according to milk production.

In man an interrelation between body weight and BMC in e.g. vertebrae was demonstrated by *Dalén & Jacobsson* (1973). In growing animals *Zetterholm* (1978 a) found a positive interrelation between weight and BMC-value. This is in agreement with the results found in the present study subjecting all lactations to the analysis of variance. The BMC rose slowly with increasing age during the 4 lactation periods (24—72 months of age) coinciding with an increase in the cow's body weight. Within lactations no such interrelation between live weight and BMC could be found. When studying variations of the BMC-value within lactations it is found to be more influenced by milk yield than by live weight. The weight of the cow during a lactation period is also highly affected by stage of pregnancy.

Through the years a common opinion about the etiology of parturient paresis has been that cows suffering from this disease at the onset of lactation have some kind of metabolic disorder e.g. parathyroid/thyroid gland insufficiency in mobilizing Ca from the bone to compensate for the loss of Ca to milk production (e.g. *Hibbs* 1950, *Payne* 1964, *Ender et al.* 1971). Although no definite conclusions on this subject can be drawn from the present study, the results obtained do not support this theory. No difference in the pattern of changes in BMC during lactations was observed in this study when comparing cows affected and not affected by paresis. Although cows of the Jersey breed are more prone to paresis (*Hibbs* 1950, *SHS* 1983), they did not show any different BMC pattern as compared to other breeds. It should however be pointed out that this study did not include more than 4 measurements of BMC during the whole lactation period, and therefore the intensive calcium kinetics just around calving escaped evaluation.

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SAMMANFATTNING

Långtidsstudier av benmineralförändringar under olika laktationer hos svensk mjölkboskap.

Förändringarna i benmineralmängden (BMC) studerades kontinuerligt under de första 4 laktationerna mellan juli 1977 och februari 1982 på totalt 103 svenska mjölkkor av olika ras och uppfödningsintensitet. Benmineralmängden mättes i två svanskotor med hjälp av dikromatisk foton absorptiometri. Trots stora individuella variationer följde förändringarna i benmineralmängd ett typiskt mönster under laktationerna med höga värden under sintiden och låga värden efter kalvningen och i mitten av laktationen. Sambandet mellan kroppsvikt och uppmätt benmineralmängd diskuteras. Förändringarna i benmineralmängd var mer uttalade under första laktationen jämfört med de efterföljande laktationerna, vilket kan tyda på ett mer metaboliskt aktivt skelett hos yngre kor. Ingen skillnad beträffande benmineralförändringar sågs i denna studie mellan friska kor och kor som drabbades av puerperal pares.

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