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Effect of anionic salts in concentrate mixture and magnesium intake on some blood and urine minerals and acid-base balance of dry pregnant cows on grass silage based feeding

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Twenty Friesian cows were randomly assigned to one of four prepartum diets in a 2 x 2 factorially designed experiment to determine the effect of anionic salts contained in a concentrate mixture and magnesium (Mg) intake on some blood and urine minerals in cows fed a grass silage based diet. Four diets provided either 16 g or 33 g total dietary Mg/day, and had either a low or high cation-anion difference. Dietary cation-anion balance (DCAB) of the diets, calculated as milliequivalents [(Na^+ + K^+) – (Cl^- + S^{2-})], was +31 mEq/kg dry matter (DM) in the low DCAB group and +340 mEq/kg DM in the high DCAB group. DCAB was formulated using NH_4Cl, $(NH_4)_2SO_4$ and $MgCl_2$ as anionic salts. Cows received grass silage (5.2 kg DM), hay (1.0 kg DM) and concentrate mixture (1.5 kg DM) until calving. Blood and urine samples were collected 4, 3, 2 and 1 week before the expected calving date, at calving, the day after calving and 1 week following calving. Cows fed the low DCAB diet had a lower urinary pH ($P<0.05$) and excreted more Ca in the urine ($P<0.05$) throughout the study. During the experimental period, Mg intake did not affect any parameters measured in plasma or urine. It was concluded that there was no benefit of additional Mg over Finnish recommendations (17 g Mg/d) when using MgO as a source of Mg for silage based diets. In addition, reducing DCAB within positive a range may not be sufficient, since urinary pH was relatively high and no changes in blood Ca^{2+} were observed.

Keywords: calcium, cation-anion balance, cows, magnesium, minerals, metabolism

Introduction

Previous studies have shown that factors other than dietary calcium can also influence the occurrence of periparturient hypocalcaemia (Braak van de et al. 1986, Hollis et al. 1981). Some studies have reported a connection between a high incidence of milk fever and insufficient supply of magnesium (Mg) during the dry period (Bar-

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ber et al. 1983, Braak, van de et al. 1987a, Samson et al. 1983). According to Braak van de et al. (1987b), low dietary magnesium (16.6 g Mg/d) resulted in lower bone calcium resorption rates and a smaller exchangeable calcium pool compared with high dietary magnesium (71.0 g/d) in cows at parturition. Additionally, Conteras et al. (1982) found that magnesium-deficient cows (10.6 g Mg/d) had smaller pools of exchangeable calcium which limited their ability to maintain blood calcium levels.

According to Finnish feeding standards for dairy cows (Tuori et al. 1995), the recommendation for dietary Mg intake is 17 g Mg/d during the dry period. In typical feeding of dry cows in Finland this means about 0.2% Mg of diet dry matter. However, Dutch experiments (Breukink 1993) have indicated recommended levels as high as 0.4% of diet dry matter.

Although many trials evaluating the effect of anionic salts in preventing parturient paresis have shown encouraging results (Block 1984, Goff et al. 1991, Oetzel et al. 1988), very little is known about the effects of dietary magnesium on calcium metabolism of dry cows when given as anionic salts in concentrate mixtures for grass silage based diets. The main objective of the present experiment was to study this and to evaluate the effect of a moderately high dietary cation-anion balance (DCAB) on acid base balance. Furthermore, measurements of blood and urine mineral concentrations allowed the reliability of Finnish dietary Mg allowances to be assessed.

Material and methods

Experimental design and treatments

Twelve Friesian cows (age 67±10 months) and eight heifers (age 26±1 months) were selected from the University of Helsinki research farm. The cows weighed 647±51 kg at the beginning of the trial and were randomly assigned to one of four dietary treatment groups with 3 cows and 2 heifers per diet. Animals received grass silage (5.2 kg dry matter (DM)/d), hay (1.0 kg DM/d) and an experimental concentrate mixture (1.5 kg DM/d). The feeding period started 4 weeks before the expected calving date and ended at parturition. Immediately after parturition, cows entered the normal nutrition and management program applied at the University of Helsinki research farm.

Experimental diets were arranged 2 x 2 factorially as follows: Diet 1, high DCAB, normal Mg (0.21%, 16 g/d); Diet 2, high DCAB, high Mg (0.44%, 33 g/d); Diet 3, low DCAB, normal Mg (0.21%, 16 g/d); and Diet 4, low DCAB, high Mg (0.44%, 33 g/d). Cows were divided into two blocks according to age. Within each block, cows were randomly assigned to one of four treatments in groups of four animals according to expected calving date. The low DCAB diet contained added chlorine (Cl) and sulphur (S), supplied primarily by adding chlorides of ammonium and magnesium and ammonium sulphate. A mixture of different salts was used to avoid potential toxicity of using only one acidifying salt. Anionic salts were included in the concentrate mixture which was pelletted. The composition of the experimental diets and the concentrate mixtures are shown in Table 1. Using the formula $[(Na^+ + K^+) - (Cl^- + S^{2-})]$ mEq/kg DM the high DCAB diet contained +340 mEq/kg DM, and the low DCAB diet contained +31 mEq/kg DM. Ammonium sulphate was included to avoid an excessive Cl content. Tucker et al. (1991) have also demonstrated that the effect of S on the systemic acid-base status in lactating cows is similar to the effect of Cl. The high Mg level was achieved by adding 35 g/d magnesium oxide (MgO) to the ration. Dietary energy content expressed as feed units (1 FU=1 kg barley with 11.7 MJ metabolizable energy according to MAFF 1975) was formulated to meet a moderate feed intake (i.e. 1.2 times maintenance) as recommended by van de Braak et al. (1986). Chemical analysis of the experimental diets is shown in Table 2.

Cows were housed, fed, weighed and body condition scored as previously described by Tau-

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Table 1. Ingredient composition of the concentrate mixture and experimental diets[1].

Concentrate mixture Ingredient, %	High DCAB[2)]		Low DCAB	
Molasses	18.16		16.31	
Wheat	29.51		26.50	
Barley	29.51		26.50	
Wheat bran	6.81		6.12	
Beet pulp	6.81		6.12	
Pelleted hay meal	3.41		3.06	
NH_4Cl	1.14		6.63	
$MgCl_2$	1.14		1.22	
$(NH_4)_2SO_4$	–		4.18	
NaH_2PO_4	3.52		3.36	
Diet Ingredient, %	**Normal Mg**	**High Mg**	**Normal Mg**	**High Mg**
Grass silage	66.58	66.32	66.84	66.58
Hay	13.21	13.13	13.11	13.03
Concentrate mixture	19.82	19.70	19.66	19.54
$CaCO_3$	0.39	0.39	0.39	0.39
MgO	–	0.46	–	0.46

[1)] Dry matter basis.
[2)] Dietary cation-anion balance.

Table 2. Dry matter intake, energy content, chemical composition[1)] and dietary cation-anion differences of experimental diets.

	High DCAB[2)]		Low DCAB	
	Normal Mg	High Mg	Normal Mg	High Mg
DMI[3)], kg/d	7.57	7.61	7.63	7.67
ME[4)], MJ/kg DM	10.54	10.49	10.51	10.33
Crude protein, %	12.02	11.97	14.27	14.21
Crude fiber, %	26.08	25.86	25.86	25.75
ADF[5)], %	28.71	26.22	26.17	26.06
NDF[6)], %	48.69	48.28	48.13	47.92
Ca,%	0.69	0.71	0.66	0.69
P, %	0.44	0.44	0.40	0.40
Mg, %	0.21	0.43	0.21	0.43
K, %	2.55	2.54	2.50	2.49
Na, %	0.29	0.29	0.24	0.24
Cl, %	1.13	1.13	1.76	1.75
S, %	0.19	0.19	0.35	0.35
DCAB[7)], mEq/kg DM	+341	+339	+31	+31

[1)] Expressed on a dry matter basis.
[2)] Dietary cation-anion balance.
[3)] Dry matter intake.
[4)] Metabolizable energy calculated according to MAFF (1975).
[5)] Acid detergent fibre.
[6)] Neutral detergent fibre.
[7)] Dietary cation-anion balance calculated as milliequivalents $(Na^+ + K^+) - (Cl^- + S^{2-})$ per kg dry matter.

riainen et al. (1998). Samples of grass silage, hay and concentrate were taken and handled according to Tauriainen et al. (1998).

Sample collection

Blood samples were collected from the jugular vein of each cow before afternoon feeding, at 4, 3, 2 and 1 week prepartum, on the day of calving and at 1 d and 7 d postpartum. Samples were placed on crushed ice immediately after sampling. One sample was taken into a heparinized vacuum tube for measurements of acid-base status. After immediate analysis of blood gases and haemoglobin of whole blood, the remaining sample was centrifuged twice (3000 g for 5 min) and the plasma was stored frozen for determination of Na, K, Cl, Ca and Mg. Another heparinized sample was taken into a vacuum tube for the determination of blood Ca ion concentration within 24 hours of collection. The body temperature of experimental cows was measured at each sampling.

Urine samples were taken at 4, 3, 2, and 1 weeks prepartum, on the day of calving and at 1 d and 7 d postpartum. Urine samples were obtained by vulval stimulation and frozen for subsequent measurement of pH, creatinine, hydroxy (OH) proline. Five ml of urine were pipetted into one tube containing 0.5 ml of 12 N HCl and frozen for later determination of Ca, Mg, K and Na.

Laboratory analysis

The Cl content of the grass silage was determined according to standard procedures (AOAC 1984) while methods used for all other chemical analysis have been previously reported (Tauriainen et al. 1998).

Statistical analysis

The data were analysed in two parts: 1. prepartum; from 4 weeks to 1 week before the expected calving date, 2. peripartum; from 1 week before expected calving to 1 week after calving. Plasma and urinary data were analysed by a repeated measures analysis of variance within the SAS (1985) general linear model procedure for a complete block design including the effects of age, dietary Mg level, DCAB and their interactions in the model. Since there was no interaction between treatments and age, this term was excluded from the model. Because preliminary analysis of raw data indicated heterogenous of variation for Mg in plasma, OH-proline, Ca/creatinine, Ca FE%, K FE% and Mg FE%, these variables were logarithmically transformed to achieve a more homogeneous variance. A one-way analysis of variance of the four treatment groups was carried out for data collected at 4 weeks before the expected calving date to assess initial differences between experimental groups. Due to significant ($P<0.05$) differences between urinary K and OH-proline/creatinine at the start of the trial, pre-treatment values were used as covariates.

Results

Cows were fed a fixed ration throughout the experiment. The palatability of the low DCAB concentrate mixture was good. Only grass silage was refused (0.10 kg DM/d) by all cows. Body condition of all cows at parturition was satisfactory (3.1), indicating that the low feeding level (1.19 x maintenance) during the dry period had no visible adverse effects.

There were no interactions between treatments for any blood or urine parameters measured. The concentration of Ca ions was not affected by DCAB or parity, but tended to be lower in cows fed diets high in Mg prepartum (Table 3). None of the cows showed clinical signs of milk fever around parturition. A subclinical hypocalcaemia (Ca^{2+} < 1.00 mmol/l, Radostits et al.1994) at calving occurred in three cows fed Diets 2, 3 and 4, respectively. Plasma Mg con-

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Table 3. Effect of dietary cation-anion balance (DCAB) and Mg intake on mean concentrations of blood Ca ions and plasma Mg.

	Factor	Time from parturition							Significance[2)]	
		–4 wk	–3 wk	–2 wk	–1 wk	0	+1 d	+1 wk	Prepartum	Peripartum[3)]
Calcium^{2+} mmol/l	High DCAB	1.27	1.22	1.22	1.22	1.12	1.14	1.20		
	Low DCAB	1.26	1.22	1.24	1.23	1.16	1.10	1.23		
	sem[1)]	0.011	0.013	0.019	0.011	0.044	0.029	0.043		
	Normal Mg	1.28	1.23	1.25	1.24	1.15	1.13	1.22		
	High Mg	1.25	1.22	1.21	1.21	1.13	1.10	1.21		
	sem	0.011	0.013	0.019	0.011	0.042	0.029	0.043		
									ns	ns
Magnesium mmol/l	High DCAB	0.97	0.97	0.98	0.96	1.00	1.05	0.98		
	Low DCAB	1.01	1.01	1.00	0.98	0.99	1.07	0.93		
	sem	0.027	0.031	0.021	0.027	0.033	0.045	0.034		
	Normal Mg	0.97	0.97	0.96	0.98	0.99	1.02	0.97		
	High Mg	1.01	1.00	1.02	0.97	1.00	1.10	0.94		
	sem	0.027	0.031	0.021	0.027	0.033	0.045	0.034		
									ns	ns

1) sem = standard error of means
2) P > 0.05 = ns
3) These peripartum means were based on nine rather than ten observations and the sem given should be multiplied by 1.052 when making comparisons with other values.

centration did not differ among treatments, but heifers had a lower plasma Mg concentration than cows. Concentrations of total Ca, Cl, Na or K in plasma were unaffected by treatments during the trial and varied within reference range (Radostitis et al. 1994). Blood pH, HCO_3^- and base excess were not influenced by DCAB or parity throughout the experiment (P>0.10). Thus, all cows were acid-base balanced (Radostitis et al. 1994).

Urinary calcium excretion was higher (P<0.05, Table 4) and urinary pH much lower (prepartum P<0.05; peripartum P<0.01) in cows fed the low DCAB diet than for cows fed the high DCAB diet. Heifers excreted more calcium in the urine peripartum in comparison with cows (P<0.05). Treatments did not influence urinary excretion of Mg, K, Na, or urine FE% of Mg, K, and Na. Heifers had a lower K FE% peripartum than cows (P<0.05). The urine FE% of Mg tended to be higher among heifers than cows (P>0.05) during the trial, but heifers excreted more OH-proline in the urine prepartum than cows (P<0.01).

Discussion

In the present study the low DCAB diet was more positive (+31 mEq/kg DM) than expected, due ta a higher content of K in silage and hay fed during the trial than indicated by preliminary analysis. Thus, the low DCAB diet was ineffective in causing metabolic acidosis and increasing the ability of cows to maintain blood Ca^{2+} concentration at parturition, as found in previous studies with lower DCAB (Phillippo et al. 1994, Schonewille et al. 1994, Tauriainen et al. 1998).

In the current study, increasing Mg intake above recommended levels (Tuori et al. 1995) did not affect plasma Mg concentration. How-

Table 4. Effect of dietary cation-anion balance (DCAB) and Mg intake on mean urinary pH and Mg and Ca excretion.

		Time from parturition							Significance[2]	
	Factor	–4 wk	–3 wk	–2 wk	–1 wk	0	+1 d	+1 wk	Prepartum	Peripartum[3]
Urinary pH	High DCAB	8.47	8.45	8.40	8.39	8.46	8.54	8.49		
	Low DCAB	8.50	7.88	8.21	7.47	7.39	8.30	8.06		
	sem[1]	0.032	0.191	0.151	0.250	0.200	0.124	0.167		
	Normal Mg	8.48	8.11	8.37	7.96	7.88	8.43	8.28		
	High Mg	8.50	8.22	8.25	7.90	7.98	8.41	8.26		
	sem	0.032	0.191	0.151	0.250	0.200	0.124	0.167		
									DCAB[4] *	DCAB **
Mg/creat.[5]	High DCAB	0.69	1.08	0.99	0.85	0.58	0.65	1.13		
	Low DCAB	0.75	0.87	0.84	0.87	0.75	0.72	0.85		
	sem	0.137	0.124	0.112	0.082	0.089	0.102	0.241		
	Normal Mg	0.83	0.89	0.89	0.85	0.64	0.59	0.80		
	High Mg	0.62	1.07	0.94	0.87	0.69	0.78	1.18		
	sem	0.137	0.124	0.112	0.082	0.089	0.102	0.241		
									ns	ns
Mg FE%[6]	High DCAB	9.59	15.13	14.55	13.42	9.10	8.98	14.86		
	Low DCAB	9.52	11.72	11.63	12.95	10.65	9.08	10.61		
	sem	1.401	1.623	1.373	1.062	1.309	1.351	3.415		
	Normal Mg	10.78	12.18	12.86	12.59	9.45	7.95	9.69		
	High Mg	8.33	14.67	13.31	13.77	10.29	10.12	15.78		
	sem	1.401	1.623	1.373	1.062	1.309	1.351	3.415		
									ns	ns
Ca/creat.	High DCAB	0.30	0.33	0.21	0.14	0.07	0.05	0.40		
	Low DCAB	0.27	0.75	0.91	1.31	0.31	0.11	0.14		
	sem	0.087	0.107	0.108	0.394	0.082	0.032	0.083		
	Normal Mg	0.27	0.54	0.64	1.07	0.21	0.04	0.24		
	High Mg	0.30	0.54	0.48	0.38	0.17	0.12	0.30		
	sem	0.087	0.107	0.108	0.394	0.082	0.032	0.083		
									DCAB *	DCAB *
Ca FE%	High DCAB	1.48	1.73	1.21	1.08	0.46	0.32	1.90		
	Low DCAB	1.38	4.12	4.86	4.53	1.79	0.66	0.66		
	sem	0.382	0.544	0.525	0.696	0.464	0.198	0.342		
	Normal Mg	1.34	2.73	3.45	3.08	1.26	0.25	1.14		
	High Mg	1.51	3.12	2.62	2.53	0.99	0.74	1.42		
	sem	0.382	0.544	0.525	0.696	0.464	0.198	0.342		
									DCAB **	

[1] sem = standard error of mean.
[2] ns P>0.05, P < 0.05 *, P < 0.01 **, P < 0.001 ***
[3] These peripartum means were based on nine rather than ten observations and the sem given should be multiplied by 1.052 when making comparisons with other values.
[4] DCAB = high DCAB vs. low DCAB.
[5] Mineral/creatinine, mmol/mmol
[6] Fractional excretion

ever, three cows had a plasma Mg concentration under 0.85 mmol/l at calving which is considered to be too low (Samson et al. 1983). Two of these cows belonged to the high Mg group. Thus, it seems that the availability of Mg provided as MgO was unsatisfactory. According to Braak van de et al. (1986) 0.22% Mg of the DM was inadequate to meet Mg requirements during the dry

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period. They concluded that even in the absence of subclinical hypomagnesaemia, a low Mg intake (17 g Mg/d) can decrease the ability of a cow to mobilise calcium at parturition. On the basis of these facts we suggest that it may be beneficial to increase Finnish Mg recommendations.

Different diets did not influence the plasma concentrations of Na, K or Cl which is consistent with a previous study (Tauriainen et al. 1998). Plasma Na concentration varies within relatively narrow range. Even a mild acidosis with associated changes in plasma bicarbonates can exist without significant changes in Na content. Cl concentration tends to vary inversely with bicarbonate concentration, since bicarbonates can be exchanged for Cl (Carlson 1989). However, DCAB in the present study was too high and therefore did not alter the acid-base balance of cows.

Cows fed the high Mg diet tended to increase Mg excretion with no change in urinary excretion of Ca, although urinary excretion of Ca and Mg have been shown to be interrelated (Halse 1984). In addition, cows fed anionic salts tended to decrease Mg excretion in the urine in the current experiment. Metabolic acidosis usually increases urinary excretion of Mg (Fredeen et al. 1988, Gaynor et al.1989, Horst & Jorgensen 1973). In the present study cows in the low DCAB group were all acid-base balanced. Therefore, possibly due to inefficiency of the positive DCAB, there was no clear interrelation between urinary excretion of Ca and Mg. These findings may also be due to age, since heifers excreted more Ca in the urine peripartum than cows ($P<0.05$). This observation was also noticed by Tucker et al. (1992). During the week after calving urinary excretion of Mg tended to be lower in cows fed a normal Mg diet than that of cows fed a high Mg diet. However, in the present trial the difference was not as clear as in the study of Braak van de et al. (1986). This cannot be explained by the source or intake of Mg because these were comparable between studies.

In the current experiment urinary calcium excretion was increased ($P<0.05$) in the low DCAB group although DCAB was positive. This is consistent with findings of Delaquis & Block (1995) who demonstrated that differences in DCAB even within a positive range affected acid-base status and water metabolism of dry cows. However, at the same time urine pH was over 7.4 throughout the current study. According to Jardon (1995), the optimum level of urine pH should be 6–7 for prevention of milk fever. Consequently it appears that the low DCAB diet fed to cows in the present study failed to greatly reduce cation-anion balance.

Urine pH decreased prepartum when anionic salts were fed ($P<0.05$), but the rise noticed after two weeks of feeding was unexpected. This variation can be only partly explained by the source of Mg. In the study of Erdman et al. (1982) MgO increased rumen and faecal pH. MgO is a buffer commonly fed in rations for lactating cow. It would therefore have been better to use an alternative source of Mg when conducting studies using anionic salts with dry cows, althoughits current use was based on practical and economical reasons.

Heifers excreted more OH-proline in the urine prepartum than cows. This in in agreement with the findings of Mosel et al. (1994) which, in addition, confirmed these results with bone histomorphometric measurements. It could be speculated that the effects due to age are is probably due to a higher bone turnover rate in younger animals. Calcium intake and DCAB could have been too high to stimulate OH-proline excretion in the current study, since there was no difference between dietary treatments in OH-proline excretion. In experiments where OH-proline excretion has been reported (Gaynor et al. 1989, Goff et al. 1991), sample collection has been carried out more often and closer to the parturition than in the current study and may account for discrepancies between these observations.

This trial failed to show any advantage in raising Mg supply when given as MgO as there was no interaction between treatments for any of the blood parameters. There may be no need to raise Mg supply when using anionic salts for silage based diets. The current results agree with those of Wang & Beede (1992) who conducted a trial with a very low DCAB diet (-296 mEq/kg DM), and found no advantage with regard to Ca metabolism when increasing Mg intake by raising Mg concentration from 0.20 to 0.37% DM. However, it is concluded that it might be useful to increase Finnish Mg recommendations because a few subclinical hypomagnesemia cases were noticed in the present study. Probably these findings could have been even more clear if cows had been older. It is doubful if DCAB +31 mEq/kg DM elicited sufficient improvement on calcium metabolism in cows fed a grass silage based diet, since urine pH was relatively high and there was no noticable change in blood Ca^{2+}.

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SELOSTUS

Kationi-anionitasapaino ja magnesiumin saanti ummessaolevien lypsylehmien säilörehuruokinnassa

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Tutkimuksessa selvitettiin ummessaolevien lehmien kationi-anionitasapainon alentamisen vaikutuksia, kun magnesiumin saanti oli joko suomalaisten kivennäisnormien (16 g Mg/pv, 0.2% kuiva-aineesta) tai hollantilaisten kivennäisnormien mukaista (33 g/Mg, 0.4% kuiva-aineesta). Kationi-anionitasapaino laskettiin $[(Na^+ + K^+) - (Cl^- + S^{2-})]$ mEq/kg kuiva-ainetta (ka). Se oli joko +340 tai +31 mEq/kg ka. Magnesiumlisä annettiin magnesiumoksidina. Suoloina käytettiin magnesiumkloridia, ammoniumkloridia ja -sulfaattia. Lehmät saivat säilörehua (5.2 kg ka), heinää (1.0 kg ka) ja täysrehua (1.5 kg ka) neljä viikkoa ennen odotettua poikimista poikimispäivään saakka. Veri- ja virtsanäytteitä otettiin 4, 3, 2 ja 1 viikkoa ennen odotettua poikimista, poikimispäivänä sekä 1 vrk ja 1 viikko poikimisen jälkeen. Tulosten mukaan magnesiumoksidista ei ollut hyötyä ummessolevien lehmien magnesiumlisänä. Nykyistä suomalaista magnesiumsuositusta tulisi silti tarkistaa, koska kokeessa ilmeni muutamia hypomagnesiatapauksia poikimisvuorokautena. +31 mEq/kg ka ei aiheuttanut muutoksia verestä ja virtsasta mitattuihin parametreihin. Se ainoastaan lisäsi kalsiumin eritystä virtsaan ja alensi virtsan pH-arvoa verrattuna positiivisempaan kationi-anionitasapainoon (+340 mEq/kg/ka). Näin ollen kationi-anionitasapaino tulisi säätää pienemmäksi, jotta siitä olisi hyötyä poikimisen aikaiseen kalsiumaineenvaihduntaan.