

Influence of Anionic Salts on Bone Metabolism in Periparturient Dairy Goats and Sheep

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ABSTRACT

The purpose of the present study was to investigate the influence of diets supplemented with anionic salts on bone metabolism of dairy goats and sheep. Twelve Saanen goats and 12 Ostfrisean milk sheep (fourth lactation) were divided into 2 groups each [sheep control (SC), goat control (GC); sheep anionic salts (SA), goat anionic salts (GA)]. Each group was fed a different diet in the last 10 d of gestation. Groups SC and GC received a normal diet according to the requirements of goats and sheep in this stage of gestation. Groups SA and GA received supplemental anionic salts. The dietary cation-anion difference (DCAD) was +524 (SC) and +515 (GC) vs. –163 (SA) and –164 (GA) mEq/kg of dry matter. Blood and urine samples were collected daily until parturition. Serum Ca, P, Mg, serum crosslaps (CTX), osteocalcin, 1,25-dihydroxy-vitamin D (VITD), urinary pH, and urinary Ca concentrations were analyzed. Bone mineral density and bone mineral content were measured with peripheral quantitative computer tomography. The bone resorption marker CTX showed significant differences between the animals supplemented with anionic salts and the control animals in goats, but not in sheep. The goats receiving anionic salts had greater CTX concentrations throughout the administration of the salts. In sheep, a difference was only observed on the day of parturition. Similar observations were made in VITD concentrations, although a significant difference between the goat groups was only observed 3 d prepartum. The bone formation marker osteocalcin was lower prepartum in the animals supplemented with anionic salts. The urinary pH was lower in the SA and GA animals, whereas urinary Ca concentrations were greater. Bone mineral content and bone mineral density decreased in all groups around parturition. In conclusion, this experiment showed that the addition of anionic salts in goats led to greater bone resorption rates while on this feeding regimen. It can

be concluded that the anionic salts induced a mild metabolic acidosis with all its effects on calcium metabolism. These effects were not evident in milk sheep.

Key words: anionic salt, dairy goat, milk sheep, bone metabolism

INTRODUCTION

At the initiation of lactation, Ca homeostatic mechanisms have to react to a tremendous increase in demand for Ca. Mobilization of Ca from bone and increased absorption from the gastrointestinal tract are required to reestablish homeostasis. Milk fever or periparturient paresis in cows is a disorder of Ca metabolism, in which Ca homeostatic mechanisms fail to maintain normal plasma Ca concentrations at the beginning of lactation (Goff and Horst, 1997). The physiological control of calcium metabolism and skeletal remodeling is normally under regulation of systemic hormones, especially calcitonin, parathyroid hormone (PTH), and 1,25 dihydroxyvitamin D (VITD; Russell, 2001). Calcitonin released by the thyroid in response to hypercalcemia reduces Ca resorption from bone and increases Ca excretion. In the study of Capen and Young (1967), histological evidence indicated that calcitonin secretion was increased at parturition in cows with milk fever. Interestingly, the measurement of calcitonin around parturition in cows with or without milk fever did not demonstrate a role of calcitonin in the development of hypocalcemia at calving (Goff, 2000). Different studies (Gardner and Park, 1972; Block, 1984; Abu Damir et al., 1994) have suggested that the response of kidney and bone to PTH is impaired in cows showing periparturient hypocalcemia. This responsiveness of the target organs can be modified by prepartum diets. It was shown that dietary anions play an important role in the prevention of milk fever by mobilizing Ca from bone and by increasing Ca absorption in the GI tract (Horst et al., 1997; Goff and Horst, 2003).

Monitoring bone metabolism is possible with different proteins or enzymes released during bone formation or resorption. A marker of bone formation is osteocalcin (OC), whereas a marker of bone resorption is an epitope of the carboxyterminal telopeptide of type I collagen

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Table 1. Daily allowance (kg of original substance) and nutrient intake (daily intake in g or MJ) of the experimental diets

Ingredient	Until parturition				From parturition	
	SA	SC	GA	GC	Sheep	Goats
Daily allowance						
Hay ¹ (kg/d)	1.4	1.4	1.4	1.4	2.0	2.0
Concentrate ² (kg/d)	0.5	0.5	0.4	0.4	0.5	0.55
Anionic salt (g/d) (ammonium chloride)	37.0	—	36.5	—	—	—
Nutrient intake						
DM	1,672	1,672	1,584	1,584	2,200	1,760
NE _L , ³ MJ	11	11	10	10	14	14
Absorbable protein (AP)	164	164	156	156	216	221
Calcium	12.8	12.8	12.0	12.0	16.5	17.0
Phosphorus	6.6	6.6	5.6	5.6	7.7	7.9
Magnesium	3.9	3.9	3.5	3.5	4.7	4.8
Sodium	1.7	1.7	1.4	1.4	1.9	1.9
Potassium	28.3	28.3	27.6	27.6	39.0	39.3
Sulfur	2.2	2.2	2.0	2.0	2.7	2.82
Chloride	29.2	4.8	28.7	4.6	6.4	6.4
DCAD (mEq/kg of DM)	-163	+524	-164	+515	+728	+733

¹Hay (barn-dried, balanced in grasses and leguminoses): 6.1 MJ of NE_L/kg of DM, 99 g of AP/kg of DM, 7 g of Ca/kg of DM, 3.1 g of P/kg of DM.

²Concentrate: 7 MJ of NE_L/kg of DM (main source: barley), 96.5 g of AP/kg of DM, 9.6 g of Ca/kg of DM, 5.1 g of P/kg of DM (UFA 765, UFA AG, Sursee, Switzerland).

³Calculated according to RAP (1999).

(measured as crosslaps). Osteocalcin is a small protein that is synthesized by mature osteoblasts (Russell, 2001). The protein is characterized by the presence of 3 γ -carboxyglutamic acids, which are responsible for the binding of the protein to hydroxyapatite and maintaining its secondary structure. Osteocalcin has been used in humans and several animal species as a promising bone formation marker (Liesegang et al., 1998, 2000; Russell, 2001). Type I collagen represents more than 90% of the organic matrix of bone. One degradation product of mature collagen type I is an epitope of the carboxyterminal telopeptide of type I collagen (CTX). This marker has been used in several studies in animal species and has been evaluated for goats and sheep (Liesegang and Risteli, 2005). Dairy goats seem to react similarly to cows according to bone markers around parturition, whereas milk sheep do not show the same extent of bone turnover as dairy goats and dairy cows (Liesegang et al., 2000; Liesegang and Risteli, 2005). From these studies, it can be concluded that dairy goats may be an appropriate animal to model calcium metabolism in dairy cows.

The purpose of the present study was to investigate the influence of diets supplemented with anionic salts on bone metabolism of dairy goats and sheep. These results may provide further evidence as to whether dairy goats and dairy cows react similarly with respect to Ca metabolism.

MATERIALS AND METHODS

Animals

Twelve Saanen goats and 12 Ostfrisean milk sheep were used in this study. The goats and sheep were all in their fourth lactation and weighed 91 ± 2 kg and 102 ± 4 kg, respectively. Animals were housed in individual pens bedded with sawdust and animals were randomly divided into 2 groups with 6 animals each (SC, GC; SA, GA), where S and G indicate sheep and goats, respectively, C represents control, and A indicates treatment with anionic salts. The animals of the different groups (SC, GC and SA, GA) were fed different diets during the adaptation period and the last 10 d before parturition. The animals were gradually adapted to the diets for 14 d. In addition, the animals of the treatment group (GA, SA) received gradually increasing amounts (increase of about 2.5 g/d) of anionic salts with the concentrate until 10 d prepartum. From 10 d prepartum until parturition, the entire dose of salts was added to the concentrate and fed individually to the animals of the treatment groups. The mixed hay (balanced in grasses and legumes) was offered individually in a hay rack and the concentrate was offered subsequently in a bowl by hand to each animal. The animals of the control groups SC and GC received a normal diet without any supplementation according to the requirements and weight of goats and sheep in this stage of gestation (Table 1), whereas the animals of the groups SA and GA

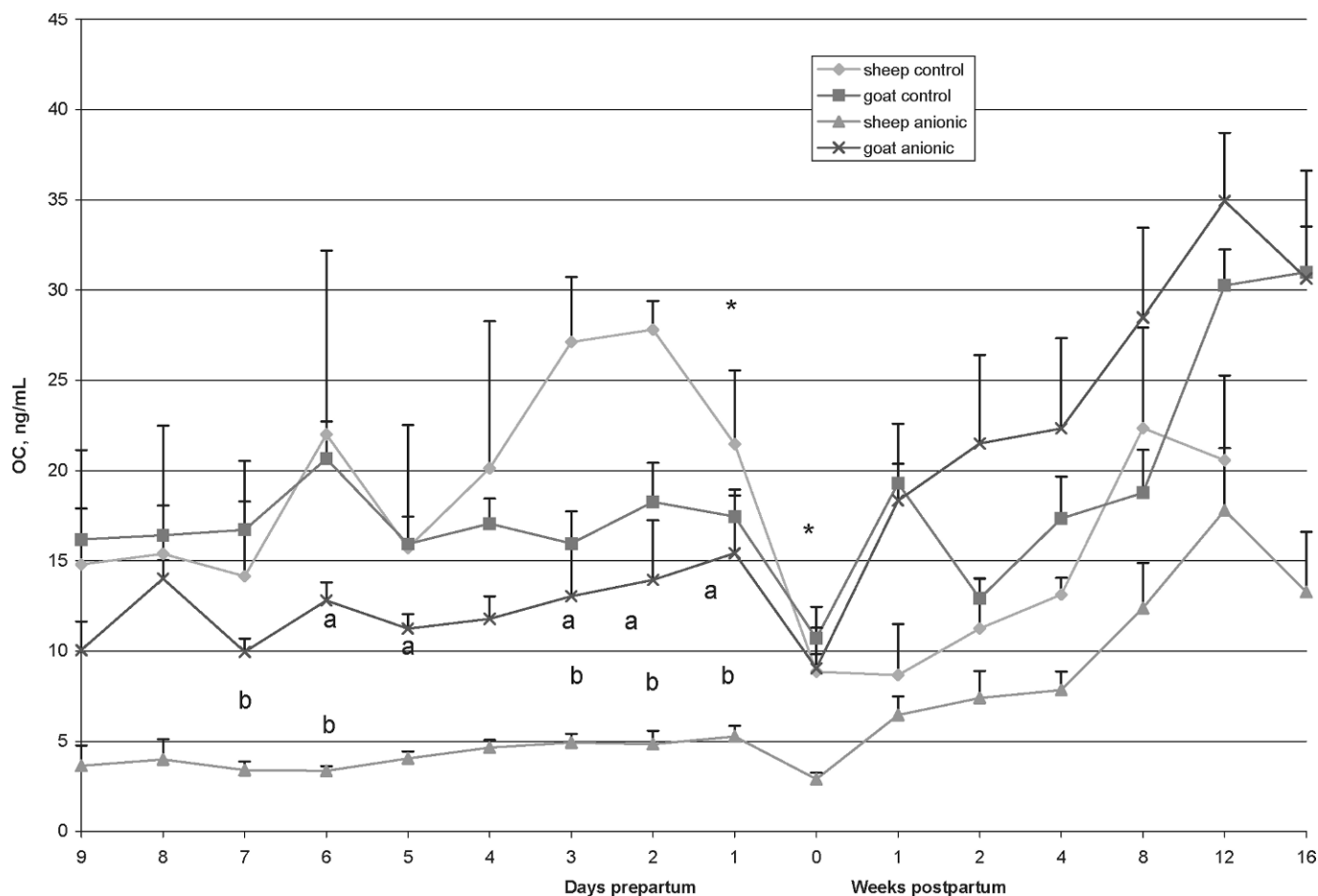


Figure 1. Serum concentrations of osteocalcin (OC, mean \pm SE) for 10 d prepartum and 16 wk postpartum. *Indicates a significant time effect from one asterisk to the next; a and b indicate significant treatment effects in goats and sheep, respectively.

received, in addition to the individually offered normal common diets, the anionic salt (36.5 g of ammonium chloride per goat; 37 g of ammonium chloride per sheep). The anionic salt was administered to each animal of the SA and GA group orally and individually for 10 d (until parturition with a difference of ± 2 d) with the concentrate after an adaptation period of 2 wk. The sheep and goat groups were very similar according to age and weight (Table 1). The DCAD was calculated using the formula $\text{DCAD (mEq/kg of DM)} = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$. All animals gave birth to 2 kids or lambs. After parturition, all groups received the same ration with higher calcium mineral supplement to meet the requirements associated with lactation (Table 1). All animals had free access to water and no refusals were left during the trial. The diet was offered twice daily in equal meals.

Collection of Blood and Urine Samples

Blood and urine samples were collected daily beginning 9 d before parturition, on the day of parturition,

and then once per week at 1, 2, 4, 8, 12, and 16 wk after parturition. Blood samples were collected from the jugular vein into tubes without additives. Blood was centrifuged ($1,500 \times g$, 10 min) within 30 min of collection. Spontaneous urine samples were collected manually into plastic tubes without additives in the morning on the same days. Urinary pH measurements were made immediately after collection. The pH was determined using a pH meter (Metrohm 632, Herisau, Switzerland). The volume of urine was not recorded. Serum and urine were stored at -20°C until the analyses were performed.

Analysis of Serum Samples

Serum Ca, P, CTX, OC, and VITD were analyzed. Minerals were determined by colorimetry with an autoanalyzer (Cobas Mira Roche autoanalyzer, Hoffmann-La Roche Ltd., Basel, Switzerland), using commercial kits as described previously (Liesegang et al., 2003). Analyses were based on the following methods:

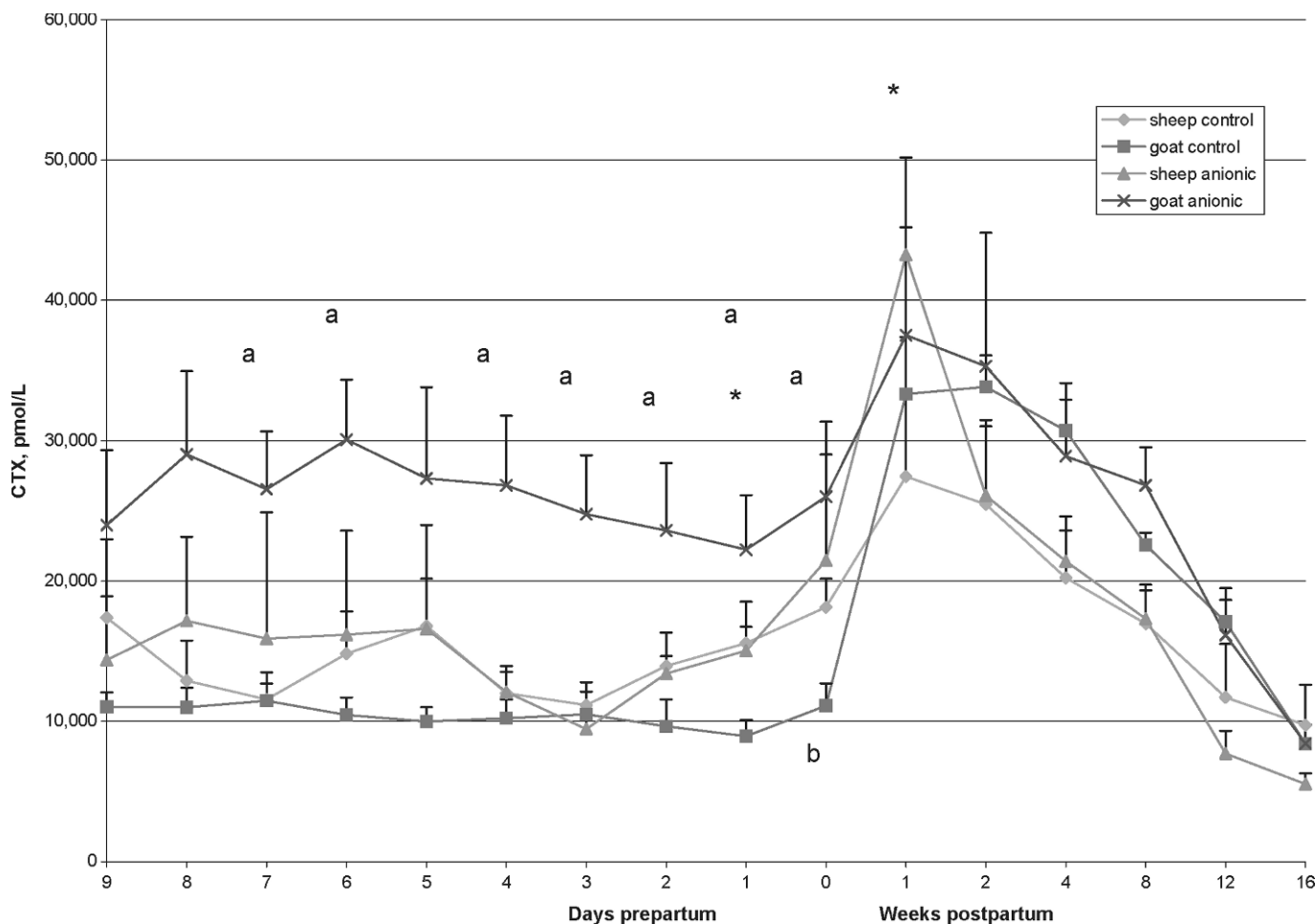


Figure 2. Serum concentrations of the epitope of carboxyterminal telopeptide of type-I collagen (CTX, mean \pm SE) for 10 d prepartum and 16 wk postpartum. *Indicates a significant time effect from one asterisk to the next; a and b indicate significant treatment effects in goats and sheep, respectively.

methylthymol blue for Ca, phosphomolybdate without precipitation of proteins for P (Liesegang et al., 1998). Serum concentrations of OC were measured using a commercially available RIA (Nichols Diagnostics, San Juan Capistrano, CA). Serum CTX concentrations were measured as described previously (Liesegang and Risteli, 2005). Serum concentrations of VITD were measured with a commercially available RIA using a sheep anti-1,25-dihydroxyvitamin D antibody (Nichols Diagnostics).

Bone Mineral Density and Content

After measuring the length of the metatarsus of the living animals, total bone mineral density (BMD) and content (BMC) were measured in the middle of the left metatarsus with peripheral quantitative computer tomography (Stratec XCT 2000 bone scanner, Stratec Medizinaltechnik GmbH, Pforzheim, Germany). The

measurements were taken in the middle of the diaphyses. Cortical BMC and BMD (cortical mode 2; threshold for cortical bone >640 mg/cm³) were calculated by automated computation.

Analysis of Urine Samples

Calcium in urine was determined by colorimetry with the same autoanalyzer used for quantification of Ca in serum, using a commercial kit as mentioned above. Analyses were based on the methylthymol blue method. Creatinine in urine was measured using an autoanalyzer (Cobas Mira Roche autoanalyzer, Hoffmann-La Roche Ltd.) and a commercial kit based on the Jaffé method (DiaSys, Diagnostic Systems GmbH, Holzheim, Germany; Liesegang et al., 1998). Because urine was not collected for a 24-h period, the Ca concentrations in urine were corrected for creatinine with the formula

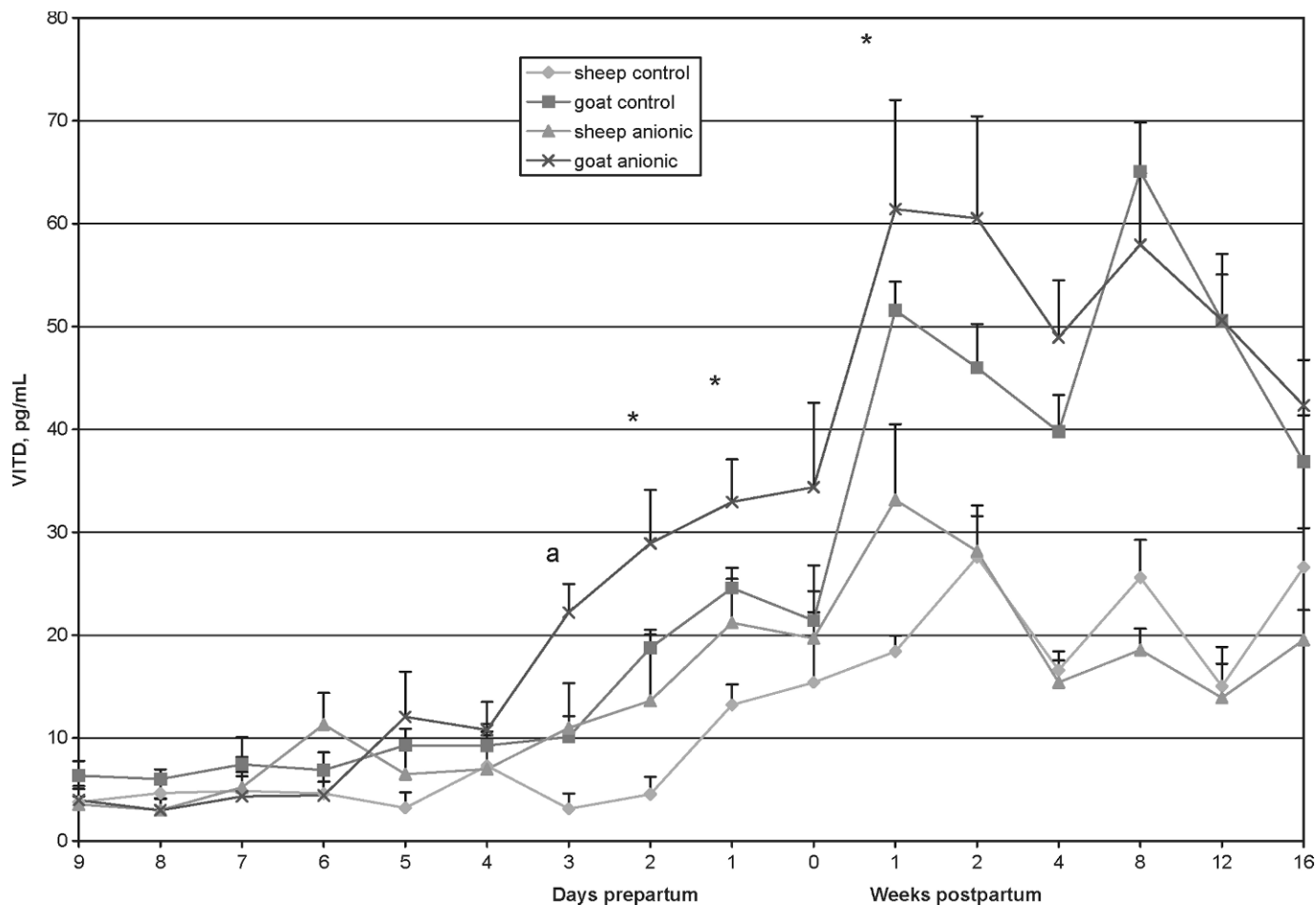


Figure 3. Serum 1,25-dihydroxyvitamin D (VITD) concentrations for 10 d prepartum and 16 wk postpartum. *Indicates a significant time effect from one asterisk to the next; a and b indicate significant treatment effects in goats and sheep, respectively. ^{a,b}Indicate significant differences ($P \leq 0.05$) between treatments (treatment effect at different time points within a species).

Ca (mmol/L)/creatinine in mmol/L = Ca corrected (mmol/mmol of creatinine) (Liesegang et al., 2007).

Statistical Analysis

A multivariate ANOVA for repeated measures (MANOVA, test within subjects) and a trend analysis (TREND, linear, quadratic, and cubic) were performed by the use of statistical software (Systat version 8.0, SPSS Inc., Chicago, IL). The factor GROUP (species and treatment: groups SC, SA, GC, GA) was included in the model. The test within subjects indicates whether significant changes occurred during the whole period for all animals. The factor GROUP tests whether the changes are different between species or treatment within the species. The goat and sheep were analyzed within one species (SC vs. SA; GC vs. GA) and additionally compared with the other species (SC vs. GC and GA; SA vs. GC and GA). The trend analysis is a breakdown of

the test within subject and gives an indication about the form of the changes during time. To avoid false conclusions because of a violation of the assumption of compound symmetry, a Huynh-Feldt correction was performed. The difference between groups at chosen times was tested with a Mann-Whitney U test for independent samples. The differences were considered statistically significant if the P -value was < 0.05 . If several tests were performed for one parameter, a Bonferroni adjustment of the significance level (P divided by the number of tests) was performed. All data were presented graphically as means (± 1 SE).

RESULTS

The time-dependent pattern of all the variables in the 4 groups are presented in Figures 1 through 7.

Average milk yield of all the animals during lactation ($n = 12$) and Ca content in milk are listed in Table 2.

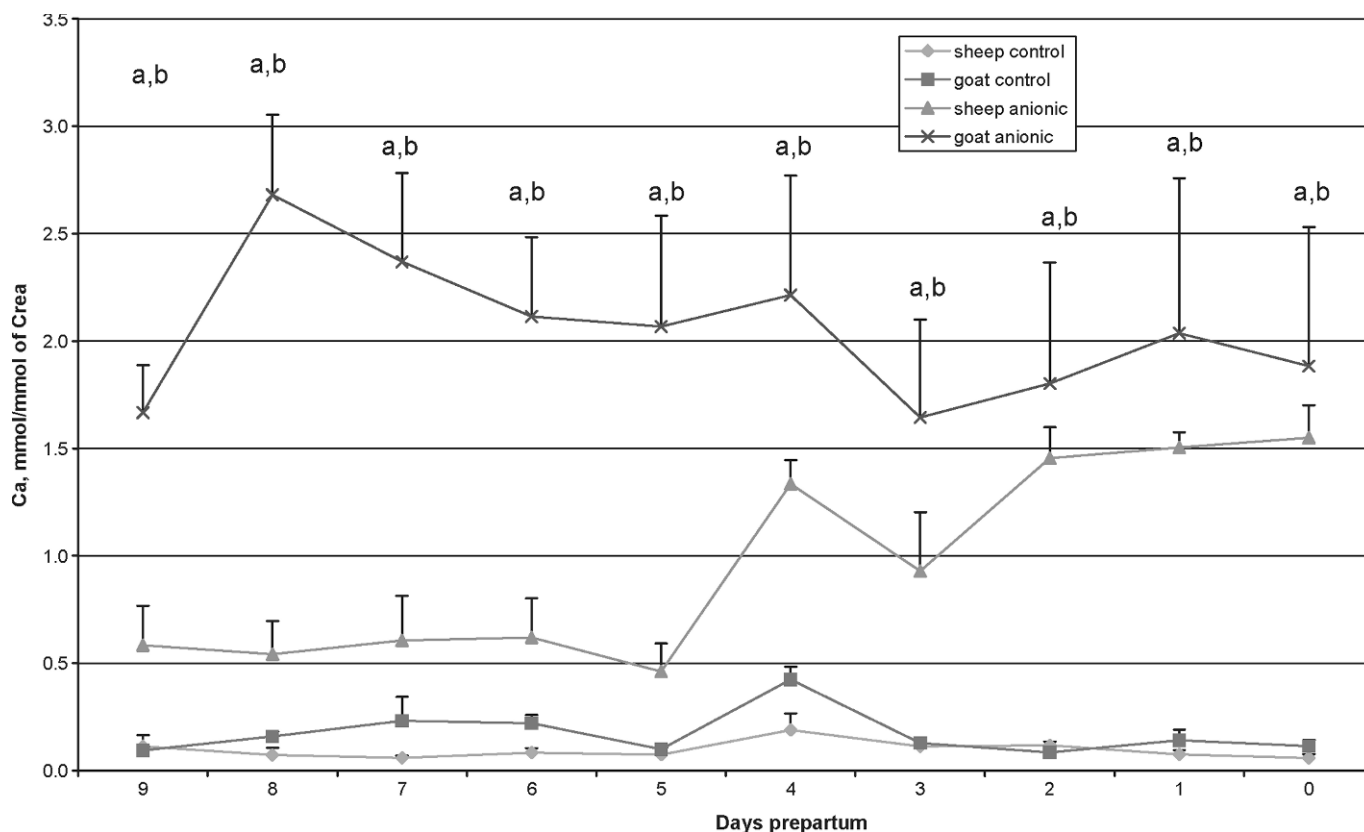


Figure 4. Urinary Ca concentrations (mmol/mmol of creatinine (Crea); mean \pm SE) for 10 d prepartum. Letters a and b indicate significant treatment effects in goats and sheep, respectively.

The milk of both species had the greatest Ca content per day at parturition. At 2 mo of lactation, sheep and goats had the greatest milk yield.

The mean Ca concentrations (Table 3) decreased significantly in both goat groups ($P < 0.0001$) from the beginning of the experiment (GA: 2.41 ± 0.06 mmol/L; GC: 2.35 ± 0.07 mmol/L) to parturition in GA (2.08 ± 0.05 mmol/L), and in GC (2.11 ± 0.07 mmol/L). No significant treatment effect was evident. After the nadir, the Ca concentrations increased significantly ($P \leq 0.05$) and reached levels as at the beginning in wk 1 postpartum (GA: 2.46 ± 0.05 mmol/L; GC: 2.38 ± 0.05 mmol/L; Table 3; reference range for goat: 2.2 to 2.8 mmol/L). The sheep treatment groups revealed no fluctuations in total Ca concentrations (reference range for sheep: 2.1 to 2.7 mmol/L; Kraft and Dürr, 1999). The mean P concentrations always stayed within the normal reference ranges of 1.4 to 2.3 mmol/L and 1.3 to 1.9 mmol/L in goats and sheep, respectively (Kraft and Dürr, 1999).

Bone Formation Marker

A significant group effect (SA vs. SC, GA vs. GC) was observed within sheep and goats at time points 6, 5, 3,

2, 1 d prepartum and 7, 6, 5, 4 d prepartum, respectively (Figure 1). The animals of the groups with anionic salts (SA, GA) had lower concentrations compared with the control groups of the same species (SC, GC). Mean OC concentrations significantly decreased ($P = 0.01$) from d 1 prepartum until parturition (Figure 1) in groups GC ($P = 0.005$), GA ($P = 0.002$), and SC ($P = 0.043$). Concentrations OC also decreased in the SA group, but not significantly ($P = 0.1$). The concentrations of OC increased in all groups from parturition until the end of the experiment at 16 wk postpartum.

Bone Resorption Marker

Concentrations of the bone resorption marker CTX increased significantly ($P = 0.001$) after parturition in all groups (Figure 2). After the peak, the CTX concentrations decreased continuously until wk 16 postpartum. Mean CTX concentrations showed significant differences between the goats supplemented with anionic salts and the control animals from d 7 prepartum to parturition. Treatment did not alter CTX in sheep. The goats receiving anionic salts had greater concentrations throughout the administration of anionic salts. In

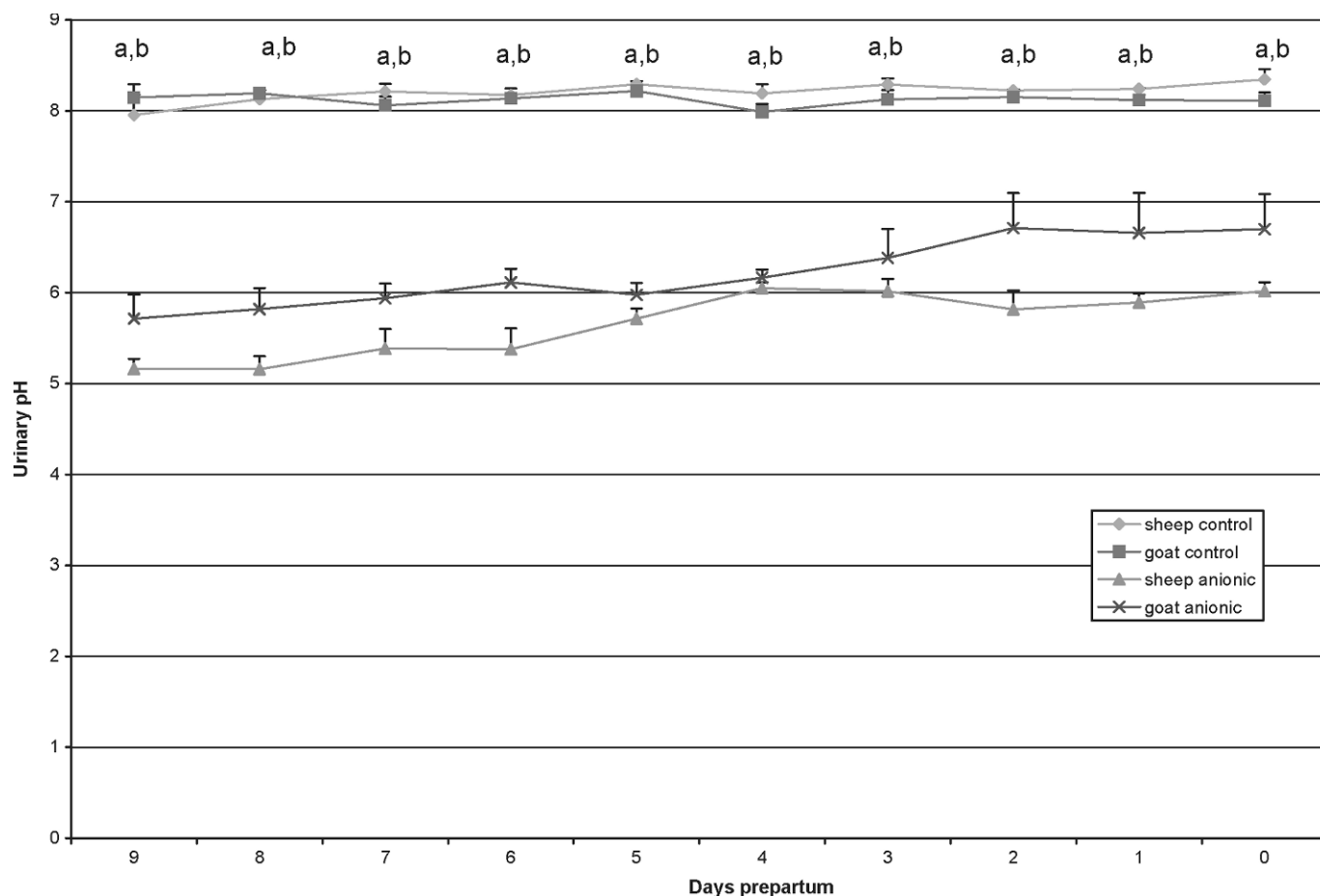


Figure 5. Urine pH values (mean \pm SE) for 10 d prepartum. Letters a and b indicate significant treatment effects in goats and sheep, respectively.

sheep, a difference was only observed on the day of parturition.

Vitamin D, Ca in Urine, and Urine pH

The VITD concentrations in all 4 groups increased during the experiment. In group SC, VITD increased from 2 d prepartum until 2 wk postpartum (from 4.35 ± 1.7 to 27.6 ± 3.8 pg/mL). In groups SA, GA, and GC, VITD concentrations increased from the day of parturition until wk 1 postpartum. In goats the VITD levels remained elevated throughout the experiment, whereas in sheep the VITD concentrations showed fluctuations. A significant group effect was only observed between goats on d 3 prepartum (Figure 3).

Calcium concentrations in urine were increased in groups SA and GA. In GA, the concentrations were increased from the first sampling until the day of parturition. In SA the concentrations were increased after 4 d. In the 2 control groups, the concentrations were not increased throughout the sampling period (Figure 4).

Urinary pH in groups SA and GA were significantly lower compared with SC and GC throughout the sampling of urine (Figure 5).

BMC and BMD

Total BMC (Figure 6) and BMD (Figure 7) decreased from the day before parturition until 1 wk postpartum in both species. The mineralized bone area declined, whereas the corresponding marrow area was elevated. Afterwards, BMC increased until 1 mo postpartum in goats and 2 wk postpartum in sheep. The BMD of sheep and goats returned to gestation levels during lactation by 2 wk postpartum. The sheep had significantly greater BMD throughout the experiment. Total BMC was significantly greater in sheep in wk 1 postpartum. No significant treatment effect due to anionic salts within the species was observed.

DISCUSSION

The goal of the present study was to investigate the influence of feeding an anionic salt during late gestation

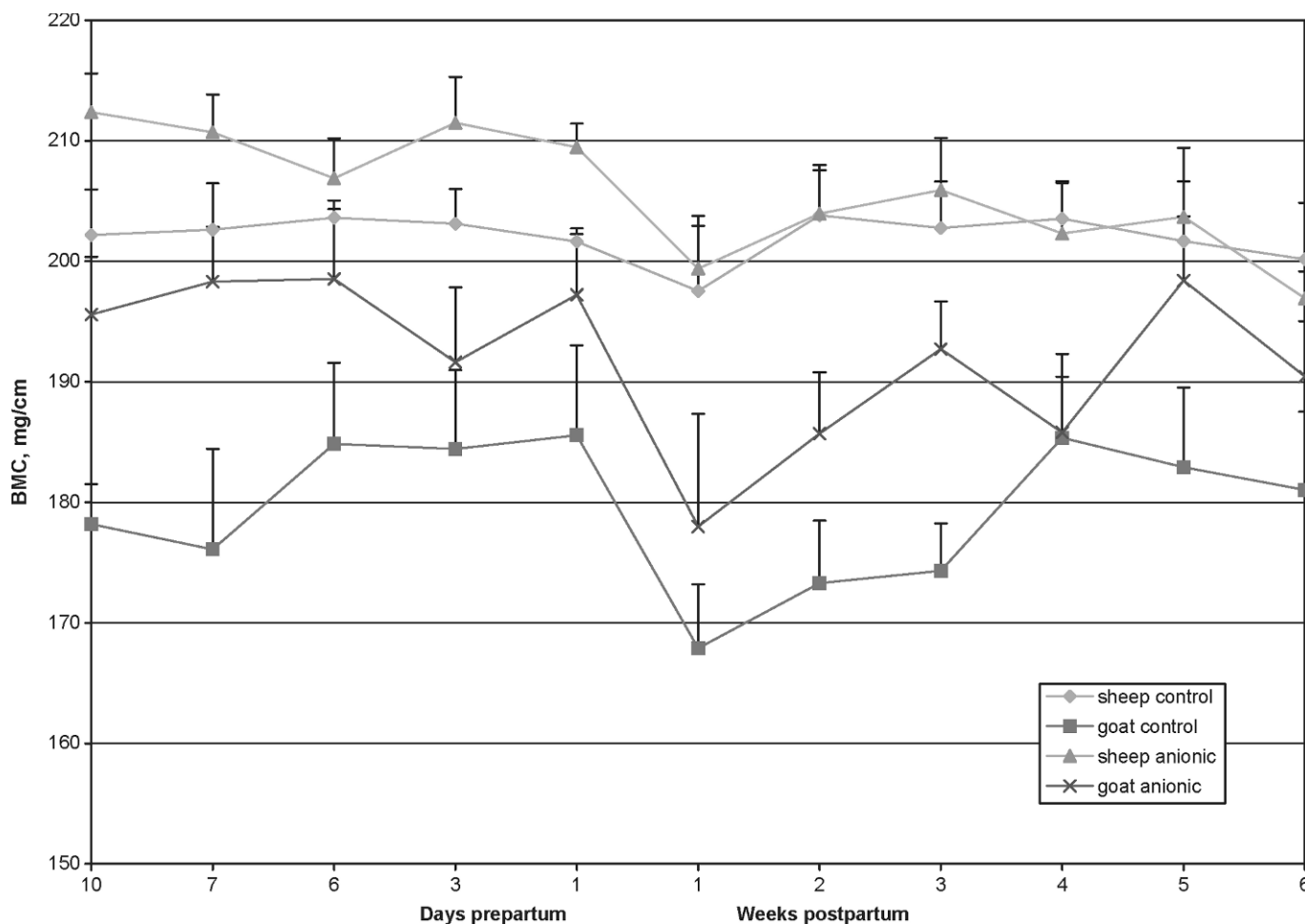


Figure 6. Bone mineral content (BMC; mean \pm SE) for 10 d prepartum and 6 wk postpartum ($n = 6$ for each group).

on Ca metabolism in dairy goats and milk sheep. Calcium concentrations in animals under normal conditions are maintained at normocalcemic levels by calcitonin, VITD, and PTH (Goff and Horst, 1997). Bone resorption and intestinal absorption of Ca are regulated by the latter 2 hormones. When serum Ca is decreased, PTH and VITD production are increased. The consequences are greater bone resorption rates, decreased excretion of Ca via the kidney, and increased absorption of Ca in the intestines (Goff and Horst, 1997). In the present study, a significant decrease of serum Ca concentrations was observed at parturition, but only in goats. This may be due to the fact that the goats lose about 7.5 g of Ca/d, and sheep about 5.5 g of Ca/d at parturition via the milk. The Ca loss from the serum pool must be replaced by increasing intestinal Ca absorption or increasing bone Ca resorption, or both. Because CTX and VITD concentrations were increased and OC concentrations, BMD, and BMC were de-

creased, it can be concluded that both processes were activated and functioned to increase serum Ca levels in all groups independent of the DCAD of the diet. The mechanisms of anionic salts are not totally understood (Horst et al., 1994, 2005; Goff et al., 2004). The DCAD concept is based on the theory of strong ion difference, as described by Stewart (1983). In this theory, the difference in the number of cation and anion equivalents in a diet available for absorption determines the acid-base status of the animal. The animals become acidotic if the absorbable anions predominate, as done by the addition of an anionic salt in the present trial. This results in a reduction in pH in blood and urine. Urinary pH between 5.5 and 6.2 is associated with effective administration of anions (Oetzel et al., 1991, 1988) in cows. In the current study, urine pH was decreased in both species with anionic salts. The exact mechanism of how dietary anions work is still unresolved. It is suggested that the responsiveness of the target tissues,

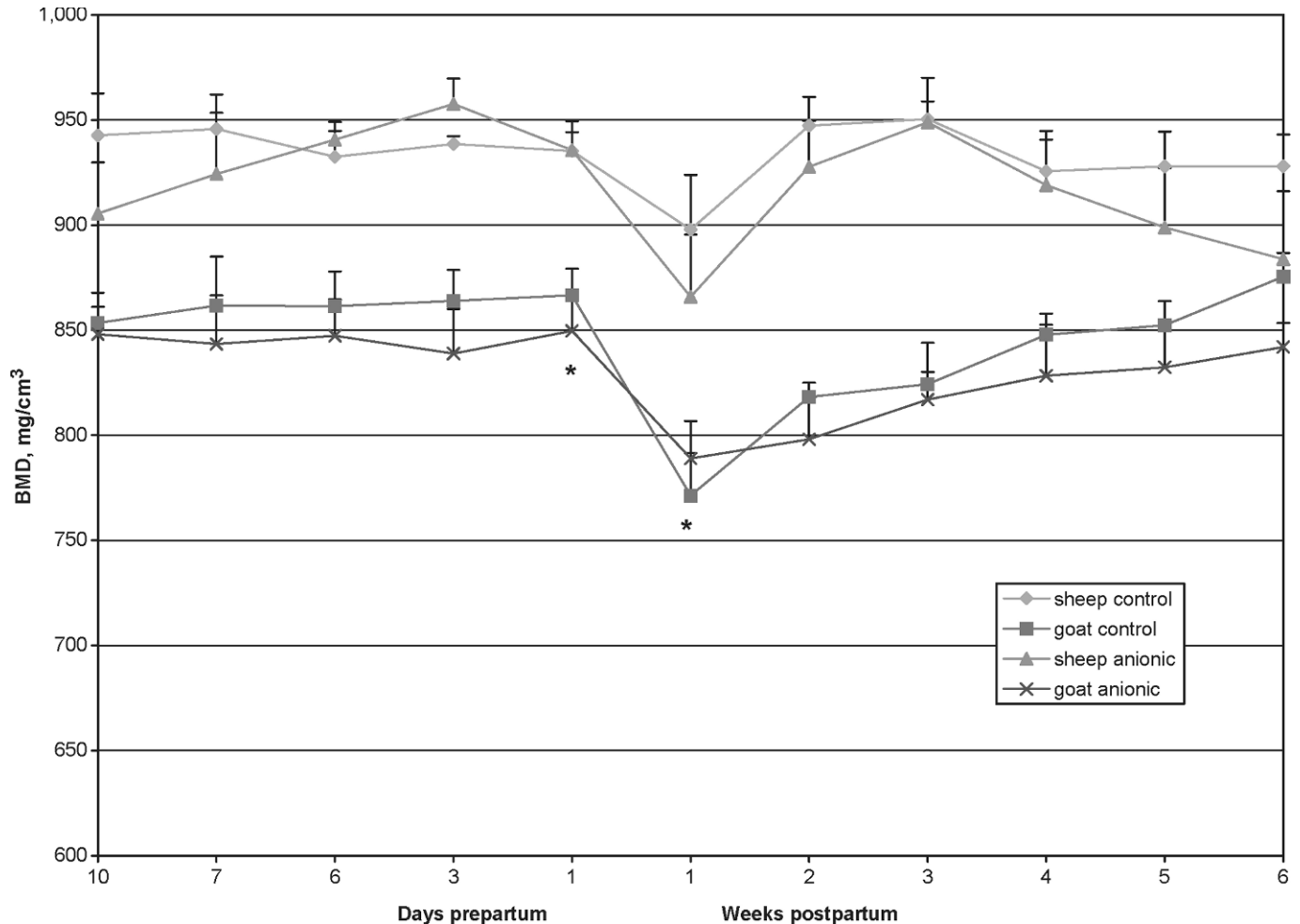


Figure 7. Bone mineral density (BMD; mean \pm SE) for 10 d prepartum and 6 wk postpartum ($n = 6$ for each group). *Indicates a significant time effect from one asterisk to the next.

such as kidney or bone tissue, is increased by inducing a mild acidosis (Goff, 1999). Concentrations of VITD were increased in all groups and bone resorption was increased in all groups around parturition. But in the goat group with anionic salts, VITD and CTX concentrations were significantly greater compared with those in the control goats before parturition. Interestingly, BMD

and BMC were not significantly lower. This may be because trabecular bone was resorbed to a greater extent in bones other than the metatarsus midshaft. Greater Ca urinary excretion and lower pH in the groups supplemented with anionic salts demonstrated that there is a significant effect of these salts on the kidney. Block (1984) hypothesized that at least part of

Table 2. Average milk yield, Ca concentration, and Ca yield of sheep and goats ($n = 12$) during lactation

Variable	Parturition	2 mo	3 mo	4 mo	5 mo	6 mo
Sheep						
Milk yield (kg/d)	2.1 \pm 0.5	2.9 \pm 0.6	1.8 \pm 0.7	1.6 \pm 0.9	1.5 \pm 0.4	1.1 \pm 0.4
Ca content (g/L)	2.5 \pm 0.4	1.7 \pm 0.2	1.8 \pm 0.1	1.9 \pm 0.1	1.8 \pm 0.2	1.8 \pm 0.2
Total Ca yield (g/d)	5.4 \pm 0.2	4.6 \pm 0.1	3.2 \pm 0.1	3.0 \pm 0.1	2.7 \pm 0.1	2.0 \pm 0.1
Goat						
Milk yield (kg/d)	2.9 \pm 0.4	3.5 \pm 0.6	2.9 \pm 0.4	2.7 \pm 0.5	2.2 \pm 0.6	1.9 \pm 0.7
Ca content (g/L)	2.6 \pm 0.3	1.3 \pm 0.1	1.2 \pm 0.1	1.2 \pm 0.4	1.3 \pm 0.1	1.1 \pm 0.2
Total Ca in milk (g/d)	7.5 \pm 0.1	4.6 \pm 0.1	3.5 \pm 0.04	3.2 \pm 0.2	2.9 \pm 0.1	2.1 \pm 0.1

Table 3. Mean total Ca concentrations (\pm SE) in serum (mmol/L)

Group ¹	Day prepartum				Week postpartum					
	9	5	1	Parturition	1	2	4	8	12	16
SC	2.49 ± 0.09	2.48 ± 0.07	2.51 ± 0.08 ^A	2.54 ± 0.04 ^A	2.46 ± 0.12	2.66 ± 0.08	2.65 ± 0.09	2.55 ± 0.10	2.53 ± 0.07	2.66 ± 0.05
	2.41 ± 0.04	2.45 ± 0.07	2.41 ± 0.11 ^A	2.47 ± 0.13 ^A	2.46 ± 0.08	2.47 ± 0.07	2.54 ± 0.06	2.57 ± 0.05	2.46 ± 0.11	2.63 ± 0.07
	2.35 ± 0.07*	2.35 ± 0.09	2.20 ± 0.06 ^B	2.11 ± 0.07* ^{AB}	2.60 ± 0.08*	2.73 ± 0.10	2.64 ± 0.09	2.59 ± 0.12	2.56 ± 0.06	2.38 ± 0.05
	2.41 ± 0.06*	2.29 ± 0.08	2.16 ± 0.04 ^B	2.08 ± 0.05* ^{AB}	2.48 ± 0.11*	2.57 ± 0.08	2.66 ± 0.06	2.63 ± 0.07	2.58 ± 0.10	2.46 ± 0.05

^{A,B}Indicates significant difference between species ($P > 0.05$).¹SC = sheep control; GC = goat control; SA = sheep with anionic salts; GA = goat with anionic salts.*Indicates significant time effect ($P > 0.05$) within species, no significant effect of treatment.

the greater urinary Ca loss in cows fed with anionic salts may be attributed to increased bone mobilization of Ca. However, in the present study with goats and sheep, no greater bone mobilization rate was evident when measuring metatarsus BMD and BMC. Increased intestinal absorption of Ca could contribute to urinary Ca (Verdalis and Evans, 1976; Horst, 1986; Fredeen et al., 1988; Horst et al., 1994). The animals of SA and GA in this study did not have significantly greater VITD concentrations, despite the level observed for group GA on d 3. However, a tendency to greater concentrations compared with the other treatment group within the species was evident throughout the administration of anionic salts. In a previous report, Schonewille et al. (1994b) noted that a decrease of urinary pH from 8.68 to 7.97 increased urinary Ca excretion, but also intestinal Ca absorption. These authors hypothesized that with diets rich in anions, unaltered bone resorption with decreased bone accretion could explain increased urinary excretion (van Mosel et al., 1993; Schonewille et al., 1994b), because the increased Ca absorption did not fully account for the increase in urinary Ca loss. This hypothesis cannot be adopted for the present study, because there was a difference between the groups according to bone resorption in goats and bone formation in both species. Beck and Webster (1976) reported that in rats induced metabolic acidosis increased the renal excretion of calcium, despite there being no change or a decrease in the filtered load. This suggests that urinary Ca excretion was augmented by a depression of the renal tubular resorption of Ca induced by acidosis (van Mosel et al., 1993). The different diets induced only significant differences between the different diet groups for urinary Ca content and urinary pH. Because it is assumed that Ca in cows fed an anionic diet is bound to proteins to a lesser degree, paracellular transport is enhanced (Moore, 1970).

There are, in fact, many theories on how DCAD really functions, and contradictory results are found in the literature. Calcium intake may play an important role in the reduction of milk fever incidence when anionic diets are used. It is very difficult to compare all the results found in the literature, because various DCAD and Ca contents were used in the experiments. In the literature, not many studies have been performed measuring specific bone parameters. Abu Damir et al. (1994) examined bone histology and found increased woven bone structures after administering anionic salts. This may lead to the assumption that more bone is resorbed when anionic diets are fed. Other studies (Block, 1984; Goff et al., 1991) measured hydroxyproline in urine, which is not specific for bone resorption, and found increased concentrations of this parameter. The anionic salt diets did induce greater Ca excretion

via urine and lower pH in urine. This may lead to the conclusion that anionic salts could play an important role in the prevention of milk fever, as stated by Schoenewille et al. (1994a).

In other studies (Liesegang et al., 2006a,b) it was shown that dairy goats reacted more intensely compared with milk sheep. From these results, it may be concluded that dairy goats may be a better model for the dairy cow, because these 2 species seem to react more strongly to changes in Ca demand compared with milk sheep. In the present study, the bone resorption marker CTX showed significant differences between the goats supplemented with anionic salts and the control animals, but not in sheep. Similar observations were made for VITD concentrations, although a significant difference between the goat groups was only observed 3 d prepartum. In conclusion, these data showed that the addition of anionic salts in goats led to greater bone resorption rates while on this feeding regimen in contrast to sheep. In addition, greater bone resorption rates were observed in goats compared with sheep. It can be concluded that the anionic salts induced a mild metabolic acidosis with all its effects on calcium metabolism. In contrast, in milk sheep these effects were not evident.

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