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Effects of a reduced nitrogen diet on calcitriol levels and calcium metabolism in growing goats☆

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ABSTRACT

For monogastric animals, changes in dietary protein content modulate calcium (Ca) metabolism by changing parathyroid hormone and calcitriol concentrations. However, the effects of dietary nitrogen (N) restriction on Ca metabolism are not known in ruminants. Since ruminants express endogenous recycling mechanisms very efficiently to save N, it is known that these recycling mechanisms protect ruminants against N depletion in times of dietary N restriction. Therefore, consequences on Ca metabolism induced by reduction of dietary N supply as observed in monogastric animals should not occur in ruminants. Due to this specific metabolic feature, a reduction of dietary N intake can be used to diminish environmental N pollution. The aim of the present study was to determine the consequences of a reduced N intake on Ca homeostasis and respective regulatory hormone concentrations in ruminants. Growing goats fed with a reduced N diet showed a decrease in ionised calcium (Ca^{2+}) and total Ca concentrations while bone resorption marker carboxyterminal cross-linked telopeptide of type I collagen increased in plasma. Unexpectedly, despite hypocalcemia, concentrations of calcitriol were decreased in the animals of the N reduction group whereas calcidiol levels were not affected. From this data, it can be concluded that the Ca metabolism of growing goats can be modulated by changes of dietary N content like in monogastric animals.

1. Introduction

In monogastric animals and humans, changes in dietary protein supply provided evidence that protein metabolism is closely related to calcium (Ca) homeostasis. In monogastric animals and humans nourished with a low protein diet, a decline in plasma calcitriol (1,25-dihydroxyvitamin D_3) concentrations was observed, which caused a reduction of intestinal Ca absorption [1,2]. Besides an intestinal reduction of Ca absorption, an abrupt rise in serum parathyroid hormone concentration was observed in humans, too [2,3] which might have severe consequences for Ca homeostasis and health, especially bone and kidney functions. Therefore, an interaction between protein and Ca metabolism was discussed in monogastric animals and humans [4].

In ruminant feeding, controlled reduction of dietary intake would be desirable to reduce nitrogen (N) emission into the environment because of contribution of manure to high eutrophication of aquatic environments. Dietary total N intake is the major factor causing N excretion with the manure in cows [5]. Ruminants are unique in their ability to save electrolytes and N by endogenous recycling mechanisms especially during times of protein and electrolyte scarcity [6–8]. The consequences of a reduction of dietary N intake can be prevented, thereby maintaining N homeostasis but reducing N excretion.

Therefore, it was hypothesised that the potential interaction between low protein dietary intervention and Ca homeostasis as observed in monogastric animals is unlikely in ruminants. The objective of this study was to examine potential effects of dietary N reduction on Ca metabolism and respective hormone concentrations like calcidiol (25-hydroxyvitamin D_3) and calcitriol in growing goats.

2. Materials and methods

2.1. Animals and diets

All animal handling and experimental conditions were approved and its conduct was supervised by the Animal Welfare Commissioner of the University of Hannover according to the German Animal Welfare Law.

A total number of 13 male White Saanen goats, about 2 months of age and weighing approximately 16.3 ± 2.3 kg live weight, were subdivided into two different feeding regimes with adequate ($n = 7$ animals) or reduced N supply ($n = 6$ animals). Each feeding group

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Table 1
Components and composition of the pelleted concentrate diets[a].

	Control ($g\,kg^{-1}$)	Reduced nitrogen ($g\,kg^{-1}$)
Dry matter	966	961
Crude ash	56	86
Crude protein ($N \times 6.25$)	193	67
Crude fibre	71	64
Crude fat	22	18
Urea	31	nd
Calcium	6.9	4.4
Phosphorus	2.7	2.0
Vitamin D_3	nd	nd
DCAD (mEQ/kg DM)	210	200
Diets		
Beet pulp	425	455
Tapioca	407	452
Soybean meal	108	33
Soybean oil	10	10
Mineral–vitamin mix[b]	10	10
$MgHPO_4 \times 3\,H_2O$	4	6
Urea	30	–
Sipernat 22S	6	34

nd: not detected.
[a] Composition expressed as fed.
[b] Per kg mineral–vitamin mix: 180 g Ca, 60 g P, 100 g Na, 30 g Mg, 500,000 IU vitamin A, 80,000 IU vitamin D_3, 300 mg vitamin E, 4200 mg Zn, 900 mg Mn, 16 mg Co, 20 mg I and 44 mg Se.

of animals were housed separately. The day's supply was given in three equal portions. Feed refusals were recorded three times daily and used to estimate mean daily intake level "per animal" of nutrients and macro minerals over the entire experimental period. Water was available at all times. The reduction of N supply was maintained for at least 6–8 weeks. The feed content of dry matter, crude ash, crude fibre, crude fat and crude proteins ($N \times 6.25$) were determined by the standard procedure according to the methods of the Association of German Agricultural Investigation and Research Center (VDLUFA) [9]. The components and composition of the diets are presented in Table 1. Both diets were isoenergetic, containing approximately 12.1 ± 0.7 kJ metabolisable energy (ME)/kg diet. The goats were weighed once a week about 3 h after the morning feed. Blood samples were taken 24 h before slaughter by venipuncture from the vena jugularis with lithium heparinate covered syringes. Blood plasma was separated by centrifugation at $2000 \times g$ at room temperature for 15 min and stored at $-20\,°C$. Urine samples were obtained at time of slaughter by aspiration from the bladder.

2.2. Biochemical determinations

Plasma urea concentrations were determined using a commercial kit (R-Biopharm, Darmstadt, Germany) to define N status of the animals. Ionised calcium (Ca^{2+}) was measured immediately after taking in whole blood samples with an ion sensitive electrode (Chiron Diagnostics GmbH, Wiesentheid, Germany). Concentrations of inorganic phosphate (P_i) and total Ca were measured colorimetrically in plasma and urine by standard spectrometric techniques [10,11]. The amount of calcidiol was determined by a competitive enzyme immunoassay (Immundiagnostik AG, Bensheim, Germany). Calcitriol concentrations were measured by a commercial radioreceptor assay (Immundiagnostik AG, Bensheim, Germany). The calcitriol assay systems had already been used in other studies to detect caprine hormone levels [12,13]. The concentration of plasma carboxyterminal cross-linked telopeptide of type I collagen (CTX) was determined using the commercial kit Serum CrossLaps® ELISA (Immundiagnostic Systems, Frankfurt, Germany). The cross-reactivity for goats had been tested by the company [14]. The inter- and intra-assay CVs of the kit were <10% and <6%. Plasma and urine creatinine was analysed by an enzymatic method, the creatinine PAP method [15].

2.3. Statistical analysis

Data are provided as means ± SD; *n* represents the number of animals. The data were tested for significance using the unpaired Student's *t*-test and $P < 0.05$ was considered statistically significant. To test for linear relationship between two variables, a simple correlation analysis with Pearson's correlation coefficient was calculated. All statistical analyses were performed by using GraphPad Prism version 4.0 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

3. Results

3.1. Daily weight gain, body weight, daily feed intake, intake of N and Ca

The animals were clinically healthy throughout the study. Daily weight gains (control group $100 \pm 39\,g\,d^{-1}$ versus reduced N diet $59 \pm 32\,g\,d^{-1}$) and average body weight of the goats (control group 21.8 ± 1.7 kg versus reduced N diet 19.8 ± 3.1 kg) were not affected significantly. Mean daily feed, N and Ca intake was estimated from group mean values. The daily feed intake of the control goats on a "per animal basis" was significantly higher than in the goats of N reduced diet ($598 \pm 43\,g\,d^{-1}$ versus $431 \pm 70\,g\,d^{-1***}$). Consequently, the control group had a significantly higher intake of N (18.7 ± 1.4 g N versus 5.5 ± 0.8 g N^{***}) and Ca (4.5 ± 0.3 g Ca versus 2.3 ± 0.3 g Ca^{***}) per day.

3.2. Biochemical parameters of blood plasma and urine

The plasma concentrations of urea decreased significantly by reduced N-feeding regimes ($5.05 \pm 1.58\,mmol\,L^{-1}$ versus $0.78 \pm 0.29\,mmol\,L^{-1}$) whereas concentrations of plasma creatinine increased (Table 2). The decline in plasma urea concentration was positively related with ionised Ca^{2+} levels (Fig. 1). Total Ca and ionised Ca^{2+} levels were significantly reduced (Table 2), while plasma P_i concentrations were not affected ($1.71 \pm 0.60\,mmol\,L^{-1}$ versus $1.89 \pm 0.43\,mmol\,L^{-1}$). Total and ionised Ca levels were positively correlated ($r = 0.8335$, $P = 0.0004$) while ionised Ca^{2+} and P_i were negatively related ($r = -0.6629$, $P = 0.0135$). The amount of plasma CTX, as a bone resorption marker, was significantly elevated in the goats of the N reduced diet (Table 2). Urinary Ca concentration was significantly diminished in goats fed a reduced N diet (Table 2).

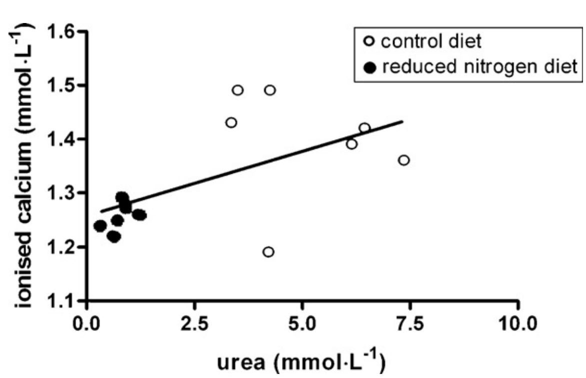

Fig. 1. Relationship between plasma urea and ionised calcium in goats fed a reduced nitrogen diet ($r = 0.5738$, $P = 0.0403$). The level of significance according to Pearson's correlation of coefficient was set to $\alpha = 0.05$.

Table 2
Effects of a reduced nitrogen diet in growing goats on calcitriol, carboxyterminal cross-linked telopeptide of type I collagen (CTX), creatinine, total and ionised Ca concentrations in plasma and total Ca concentrations in urine.

	Control diet	Reduced nitrogen diet	*P*-value
Plasma			
Calcitriol (pg mL^{-1}) (n = 6/group)	46.80 ± 11.73	31.63 ± 8.88*	0.0301
CTX (ng mL^{-1}) (n = 6/group)	0.5014 ± 0.233	0.9320 ± 0.386*	0.0414
Creatinine (μmol L^{-1}) (n = 6 to 7/group)	74.49 ± 6.551	88.22 ± 7.618**	0.005
Total calcium (mmol L^{-1}) (n = 6 to 7/group)	2.657 ± 0.244	2.320 ± 0.148*	0.0133
Ionised calcium (mmol L^{-1}) (n = 6 to 7/group)	1.396 ± 0.103	1.257 ± 0.026**	0.0082
Urine			
Total calcium (mmol L^{-1}) (n = 6/group)	1.074 ± 0.624	0.3375 ± 0.236*	0.0222

Results are given as means ± SD (n = number of animals).
* Significance levels for the effect of a low nitrogen level were $P < 0.05$.
** Significance levels for the effect of a low nitrogen level were $P < 0.01$.

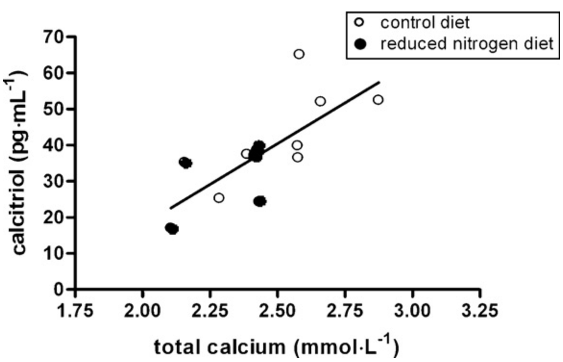

Fig. 2. Relationship between plasma total calcium and calcitriol in goats fed a reduced nitrogen diet (r = 0.7327, P = 0.0067). The level of significance according to Pearson's correlation of coefficient was set to α = 0.05.

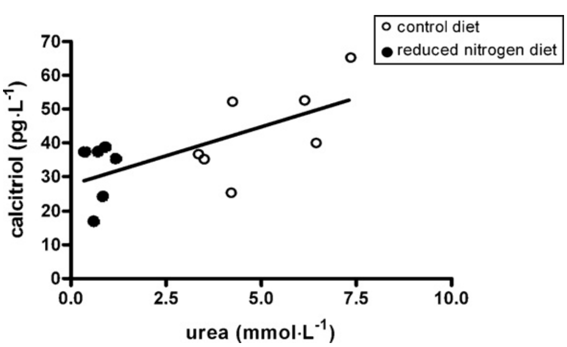

Fig. 3. Relationship between plasma urea as an indirect indicator of dietary nitrogen supply and calcitriol in goats fed a reduced nitrogen diet (r = 0.6712, P = 0.012). The level of significance according to Pearson's correlation of coefficient was set to α = 0.05.

3.3. Vitamin D_3 metabolites in blood plasma

Calcidiol levels in blood plasma were not changed in both feeding groups (14.66 ± 5.33 nmol L^{-1} versus 18.22 ± 5.57 nmol L^{-1}) while the concentrations of calcitriol were significantly reduced in the goats fed a reduced N diet (Table 2). Calcidiol levels were inversely related to ionised Ca^{2+} levels (r = −0.5969, P = 0.0313) while calcitriol was positively correlated with ionised Ca^{2+} (data not shown), total Ca plasma levels (Fig. 2) and with plasma urea (Fig. 3).

4. Discussion

Despite effective N recycling pathways, dietary N restriction affects Ca homeostasis in goats. A significant decrease in plasma total Ca and ionised Ca^{2+} concentrations could be observed. This interaction of N and Ca metabolism was also indicated by a linear relationship between plasma urea levels, which are used as an indirect parameter for N intake, and plasma ionised Ca^{2+} concentrations (Fig. 1). However, an adaptive increase in calcitriol concentrations – as it could be expected due to hypocalcemia – could not be observed. In contrast, calcitriol concentrations were even decreased in response to a reduced N diet like in monogastric animals. As a consequence of reduced plasma calcitriol concentrations, the intestinal absorption of Ca, which is assumed to be calcitriol-dependent like in monogastric animals [16], could be diminished. That could be a reason for the significant decrease in plasma total and ionised Ca^{2+} levels in the goats. Additionally, in the reduced N group a further aspect has to be taken into account. The dietary Ca intake was decreased due to the diminished food intake, which could be a consequence of decreased microbial metabolism caused by low N levels in rumen [17]. Reduced food intake and lower levels of Ca in the ration could also promote the development of hypocalcemia. However, dietary Ca restriction only caused an increase in calcitriol levels in goats [18]. This adaptive response was not observed in this study, indicating that the mechanisms induced by N restriction have a stronger influence on Ca metabolism than pathways stimulated by dietary Ca restriction only. This hypothesis is confirmed by findings in rats fed a low protein diet without changes in dietary Ca intake. In these rats, a decline in gastrointestinal Ca absorption was observed, concomitantly to the reduction of plasma calcitriol concentrations [1]. Such a decrease in intestinal Ca absorption was also reported for humans consuming a low protein diet [2]. The mechanism that underlies the decrease in intestinal Ca absorption in response to reduced dietary N is not completely understood.

Likewise, the decrease in plasma calcitriol concentrations cannot be explained yet. However, there are some potential mechanisms indicated by studies from the literature. A fall of glomerular filtration rate (GFR) could be induced by a reduction of dietary N as it has been observed in goats [19], sheep [20,21], and in cattle [22,23]. Reduction of GFR could be one of the reasons for the significant decrease in calcitriol levels in the goats of the N reduced group of this study. A reduction in GFR was indicated by an increase of plasma creatinine levels under reduced N feeding. Lower GFR diminishes the activity of renal 1-alpha-hydroxylase. This enzyme converts calcidiol to calcitriol [24]. In humans, a negative correlation between GFR and plasma calcitriol concentrations was shown [25]. The GFR might be influenced by IGF-1 [26] which is reduced under low dietary protein supply in humans, thereby causing a decrease in GFR [27]. Confirmingly, a reduction of calcitriol concentrations was also observed in rats fed a low protein diet [1]. In humans, a dietary protein based downregulation of calcitriol synthesis was proposed. Therefore, calcitriol synthesis should be diminished by the decreased renal function and mass following low dietary protein intake [28]. This positive relation between calcitriol concentrations and plasma urea levels, as an indirect parameter of dietary N intake, was also observed in goats (Fig. 3). The complex homeostatic system that controls extracellular Ca involves primary kidney and bone. Bone resorption activity increased in goats with a reduced N diet while the renal excretion of Ca decreased. This hypocalciuria could be a compensatory effect induced by the hypocalcemia.

In conclusion, an interaction of N and Ca metabolism occurred in growing goats like in monogastric animals.

Conflict of interest

The authors have declared no conflict of interest.

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References

[1] E. Orwoll, M. Ware, L. Stribrska, D. Bikle, T. Sanchez, M. Andon, H. Li, Effects of dietary protein deficiency on mineral metabolism and bone mineral density, Am. J. Clin. Nutr. 56 (2) (1992) 314–319.
[2] J.E. Kerstetter, K.O. O'Brien, K.L. Insogna, Dietary protein affects intestinal calcium absorption, Am. J. Clin. Nutr. 68 (4) (1998) 859–865.
[3] J.E. Kerstetter, K.O. O'Brien, K.L. Insogna, Low protein intake: the impact on calcium and bone homeostasis in humans, J. Nutr. 133 (3) (2003) 855S–861S.
[4] A.D. Conigrave, H.C. Mun, L. Delbridge, S.J. Quinn, M. Wilkinson, E.M. Brown, L-amino acids regulate parathyroid hormone secretion, J. Biol. Chem. 279 (37) (2004) 38151–38159.
[5] A.R. Castillo, E. Kebreab, D.E. Beever, J.H. Barbi, J.D. Sutton, H.C. Kirby, J. France, The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets, J. Anim. Sci. 79 (1) (2001) 247–253.
[6] J. Harmeyer, H. Martens, Aspects of urea metabolism in ruminants with reference to the goat, J. Dairy Sci. 63 (10) (1980) 1707–1728.
[7] K. Huber, C. Walter, B. Schroder, G. Breves, Phosphate transport in the duodenum and jejunum of goats and its adaptation by dietary phosphate and calcium, Am. J. Physiol. Regul. Integr. Comp. Physiol. 283 (2) (2002) R296–302.
[8] K. Huber, U. Roesler, A. Holthausen, E. Pfeffer, G. Breves, Influence of dietary calcium and phosphorus supply on epithelial phosphate transport in preruminant goats, J. Comp. Physiol. B 177 (2) (2007) 193–203.
[9] C. Naumann, R. Bassler, Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. Methodenbuch Band III. Die chemische Untersuchung von Futtermitteln. Mit Ergänzungslieferungen 1983, 1988, 1993, 1997, VDLUFA-Verlag, Darmstadt, 1976.
[10] B.C. Sarkar, U.P. Chauhan, A new method for determining micro quantities of calcium in biological materials, Anal. Biochem. 20 (1) (1967) 155–166.
[11] J.D. Kruse-Jarres, Klinische Chemie, Vol. II. Spezielle klinisch-chemische Analytik, Fischer, Stuttgart, 1979.
[12] I. Widiyono, K. Huber, K. Failing, G. Breves, Renal phosphate excretion in goats, Zentralbl. Veterinarmed. A 45 (3) (1998) 145–153.
[13] A. Muscher, J. Hattendorf, E. Pfeffer, G. Breves, K. Huber, Hormonal regulation of phosphate homeostasis in goats during transition to rumination, J. Comp. Physiol. B 178 (5) (2008) 585–596.
[14] P. Chavassieux, P. Garnero, F. Duboeuf, P. Vergnaud, F. Brunner-Ferber, P.D. Delmas, P.J. Meunier, Effects of a new selective estrogen receptor modulator (MDL 103,323) on cancellous and cortical bone in ovariectomized ewes: A biochemical, histomorphometric, and densitometric study, J. Bone Miner. Res. 16 (1) (2001) 89–96.
[15] K. Jung, C. Wesslau, F. Priem, G. Schreiber, A. Zubek, Specific creatinine determination in laboratory animals using the new enzymatic test kit "Creatinine-pap", J. Clin. Chem. Clin. Biochem. 25 (6) (1987) 357–361.
[16] O. Hattenhauer, M. Traebert, H. Murer, J. Biber, Regulation of small intestinal Na-P(i) type IIb cotransporter by dietary phosphate intake, Am. J. Physiol. 277 (4 Pt 1) (1999) G756–G762.
[17] J.L. Firkins, Z. Yu, M. Morrison, Ruminal nitrogen metabolism: perspectives for integration of microbiology and nutrition for dairy, J. Dairy Sci. 90 (2007) E1–E16.
[18] B. Schröder, H. Käppner, K. Failing, E. Pfeffer, G. Breves, Mechanisms of intestinal phosphate transport in small ruminants, Br. J. Nutr. 74 (5) (1995) 635–648.
[19] M.H. Valtonen, A. Uusi-Rauva, L. Eriksson, The effect of protein deprivation on the validity of creatinine and urea in evaluation of renal function. An experimental study in the goat, Scand. J. Clin. Lab. Invest. 42 (6) (1982) 507–512.
[20] J.H. Gans, P.F. Mercer, Effect of osmotic diuretics and vasopressin on renal function in the sheep, Am. J. Vet. Res. 23 (1962) 230–235.
[21] L. Rabinowitz, R.A. Gunther, E.S. Shoji, R.A. Freedland, E.H. Avery, Effects of high and low protein diets on sheep renal function and metabolism, Kidney Int. 4 (3) (1973) 188–207.
[22] C.B. Bailey, Function in cows with particular reference to the clearance of silicic acid, Res. Vet. Sci. 11 (6) (1970) 533–539.
[23] G.D. Phillips, E.C. Pickering, K. Wilkinson, Renal responses of the cow to alteration of the dietary intake of nitrogen and sodium chloride, J. Physiol. 245 (2) (1975) 95P–96P.
[24] M.R. Hughes, D.J. Baylink, P.G. Jones, M.R. Haussler, Radioligand receptor assay for 25-hydroxyvitamin D_2/D_3 and 1α, 25-dihydroxyvitamin D_2/D_3, J. Clin. Invest. 58 (1) (1976) 61–70.
[25] R.L. Prince, B.G. Hutchison, J.C. Kent, G.N. Kent, R.W. Retallack, Calcitriol deficiency with retained synthetic reserve in chronic renal failure, Kidney Int. 33 (3) (1988) 722–728.
[26] R. Hirschberg, J.D. Kopple, Effects of growth hormone and IGF-I on renal function, Kidney Int. Suppl. 27 (1989) S20–S26.
[27] L. Fontana, E.P. Weiss, D.T. Villareal, S. Klein, J.O. Holloszy, Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans, Aging Cell 7 (5) (2008) 681–687.
[28] B. Hess, D. Ackermann, M. Essig, R. Takkinen, P. Jaeger, Renal mass and serum calcitriol in male idiopathic calcium renal stone formers: role of protein intake, J. Clin. Endocrinol. Metab. 80 (6) (1995) 1916–1921.