Institut für Tierernährung
der Vetsuisse-Fakultät Universität Zürich

Direktorin: Prof. Dr. med. vet. Annette Liesegang

Vitamin D status in growing dairy goats and sheep: Influence of UVB radiation on bone metabolism and calcium homeostasis

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Malin Veronique Nemeth

Tierärztin
von Winterthur, ZH

genehmigt im Antrag von

Prof. Dr. med. vet. Annette Liesegang, Referentin

2017

Institut für Tierernährung
der Vetsuisse-Fakultät Universität Zürich

Direktorin: Prof. Dr. med. vet. Annette Liesegang

Vitamin D status in growing dairy goats and sheep: Influence of UVB radiation on bone metabolism and calcium homeostasis

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Malin Veronique Nemeth

Tierärztin
von Winterthur, ZH

genehmigt im Antrag von

Prof. Dr. med. vet. Annette Liesegang, Referentin

2017

Inhaltsverzeichnis

Summary

The aim of this study was to investigate how UVB irradiation in combination with reduced nutritional vitamin D supply affects the vitD status and Ca metabolism of growing goats and sheep. Sixteen lambs and fourteen goat kids aged 14 weeks were housed in an UVB free environment and fed with hay and vitD free concentrate for 13 weeks. One group of each species was daily exposed to UVB lamps, the other served as controls. Serum, urine and faeces samples were taken in monthly intervals. Serum was analyzed for vitD metabolites, bone markers, GH, IGF- I, Ca and P. Apparent digestibility and urinary excretion of Ca and P were determined. Bone mineral density of the metatarsus was analyzed before starting and at the end of the trial. In the 13th week, all animals were slaughtered and samples of skin, rumen, duodenum, kidney and bone were collected for further analyses (sterols of vitD in the skin, Ca flux rates in rumen and duodenum, VDR expression in duodenum and VDR, Calb-D28k, 1α-OHase and 24-OHase in kidney). UVB exposure lead to lower 7-DHC content in skin and higher serum 25-OH-D. However the dropped vitD status in the control groups did not affect the Ca homeostasis and no signs of vitD deficiency were detected. Differences between the two species were seen in bone metabolism and sterol contents in the skin. The results indicated that growing lambs and goat kids are able to fully compensate a vitD reduced diet by cutaneous vitD synthesis when exposed to UVB irradiation.

Keywords: Bone metabolism, calcium, skin, vitamin D.

Zusammenfassung

In dieser Studie wurde untersucht wie UVB Strahlung in Kombination mit reduziertem VitD in der Nahrung den VitD Status und Ca-Haushalt von wachsenden Ziegen und Schafen beeinflusst. Sechszehn Schaf- und vierzehn Ziegenlämmern wurden in einem UVB freiem Stall gehalten und mit Heu und Kraftfutter (ohne VitD) gefüttert. Die Hälfte der Tiere wurde täglich mit UVB Lampen bestrahlt, während die anderen Tiere als Kontrolle dienten. Blut, Urin und Kotproben wurden in monatlichen Abständen entnommen. Im Serum wurde der Gehalt von VitD-Metaboliten, Knochenmarker, GH, IGF-I, Ca und P bestimmt. Die Verdaulichkeit und die renale Ausscheidung von Ca und P wurden bestimmt. Die Knochendichte (Metatarsus) wurde am Anfang und nach Versuchsende gemessen. In der dreizehnten Woche wurden die Tiere geschlachtet und Proben von Haut, Pansen, Duodenum, Niere und Knochen wurden entnommen und weitere untersucht (Haut VitD-Sterole, Pansen und Duodenum Ca-Fluxe, VDR expression im Duodenum, VDR, Calb-D28k, 1α-OHase und 24-OHase in der Niere). Die Bestrahlung führte zu niedrigeren Haut 7-DHC Werte und zu höheren Serum 25-OH-D Werte. Die Kontrolltiere zeigten trotz geringerem vitD Status keine Mangelanzeichen und der Ca-Haushalt wurde nicht beeinflusst. Im Knochenmetabolismus und den Haut VitD-Sterol Werte wurden Speziesunterschiede gesehen. Die Resultate zeigten dass Schaf- und Ziegenlämmer das fehlende VitD in der Nahrung über die Eigenproduktion in der Haut mittels UVB-Strahlung kompensieren können.

Schlüsselwörter: Calcium, Haut, Knochenmetabolismus, Vitamin D.

Manuskript

Diese Arbeit wurde im Journal of Dairy Science zur Publikation akzeptiert: J Dariy Sci 2017.
DOI: 10.3168/jds.2017-13061

Interpretive Summary

Are growing goats and sheep able to cover their vitamin D demand by cutaneous synthesis alone?

By Nemeth et al.

In dairy research it is important to elucidate species differences in vitamin D metabolism. In this study the effect of UVB irradiation on the metabolism of sheep and goats depleted of dietary vitamin D was investigated with the goal to optimize the health in the future and increase the available vitamin D in products for human nutrition. It was demonstrated that as cows, sheep and goats are capable of covering their vitamin D demand by endogenous production within their skin.

Running head: ULTRAVIOLET B EFFECT ON VITAMIN D METABOLISM

Vitamin D status in growing dairy goats and sheep: Influence of UVB radiation on bone metabolism and calcium homeostasis

M. V. Nemeth*†, M. R. Wilkens‡ and A. Liesegang*†§[1]

*Institute of Animal Nutrition, Vetsuisse-Faculty, University of Zurich, Switzerland
† Centre for Clinical Studies, Vetsuisse-Faculty, University of Zurich, Switzerland
‡Department of Physiology, University of Veterinary Medicine, Foundation Hannover, Germany
§ Center for Applied Biotechnology and Molecular Medicine, University of Zurich, Zurich, Switzerland

[1] Corresponding author: Prof. Dr. med. vet. Annette Liesegang, Institute of Animal Nutrition, Vetsuisse-Faculty, University of Zurich, Winterthurerstrasse 270, CH-8057 Zurich, Phone: +4144 635 88 23, Fax: +4144 635 89 39, Mail: aliese@nutrivet.uzh.ch

ABSTRACT

The aim of this study was to investigate how controlled UVB irradiation in combination with reduced nutritional vitamin D (vitD) supply affects the vitD status and Ca metabolism of growing goats and sheep. The hypothesis was that like dairy cows, they are able to compensate the missing nutritional supply of vitD through endogenous production in the skin, with the consequence of a high vitD status and a balanced Ca homeostasis. Sixteen lambs and fourteen goat kids aged three and a half month were housed in an UVB free environment and fed with hay and vitD free concentrate over a period of 13 weeks. One group of each species was daily exposed to UVB lamps during individual feeding, the other groups served as controls. Serum, urine and faeces samples were taken at the start and in a monthly interval. Serum was analyzed for vitD metabolites, bone markers, growth hormone, insulin-like growth factor I, Ca and P. Apparent digestibility and urinary excretion of Ca and P were determined. The left metatarsus was analyzed by peripheral quantitative computer tomography for bone mineral density before starting and at the end of the trial. In the 13th week, all animals were slaughtered and samples of skin, rumen, duodenum, kidney and bone (metatarsus) were collected. Content of sterols of vitD synthesis in the skin, Ca flux rates in rumen and duodenum, expression of vitD receptor in duodenum and kidney, renal and intestinal gene expression of Ca transport proteins and renal enzymes related to vitD metabolism were determined. UVB exposure led to lower 7-dehydrocholesterol content in skin and a better vitD status (higher serum 25-hydroxyvitamin D), but no signs of vitD deficiency were seen in the control groups and no effect of irradiation was detected in the analyzed parameters of Ca homeostasis. Differences between the two species were detected: lambs had a higher increase of bone mineral density, lower values of bone markers, growth hormone and insulin-like growth factor I in serum and higher tachysterol and lower lumisterol content in skin compared to goat kids. The results indicated that growing lambs and goat kids are able to compensate a vitD reduced diet by cutaneous vitD synthesis when exposed to UVB irradiation and therefore to keep a high vitD status. In contrast, when a reduced vitD diet is combined with a missing UVB exposure, the vitD status drops, but the experimental time was probably too short to induce a vitD deficiency or an effect on Ca homeostasis.

Key Words

Bone metabolism, calcium, skin, vitamin D.

INTRODUCTION

Endogenous synthesis of vitamin D (**vitD**) in the skin or nutritional supply is needed to meet vitD requirements of humans and animals. In human skin, when ultraviolet radiation (**UVB**, wavelengths 290-315 nm) is absorbed, 7-dehydrocholesterol (**7DHC**) is photoisomerized to pre-vitamin D_3 (**pre-D3**). This unstable product can be thermally isomerized to vitamin D_3 (**vitD3,** cholecalciferol) or reversibly photoisomerized to the inactive storage forms lumisterol and tachysterol (MacLaughlin et al., 1982; Webb and Holick, 1988; Holick, 2004). Afterwards vitD3 is transferred in to the blood circulation and transported by vitD binding protein to the liver where, together with the vitamin D_2 (**vitD2,** ergocalciferol) coming from nutrition**,** the enzyme vitamin-D-25-hydroxylase (**25-OHase**) transforms them into 25-hydroxyvitamin D (**25vitD**) (Haddad et al., 1993; Holick, 2008; Jones, 2013). 25vitD can be stored in tissues as well as transported to the kidney where it is converted to the active product 1,25-dihydroxyvitamin D (**1,25vitD**, calcitriol) by the enzyme 25-hydroxyvitamin-D-1α-hydroxylase (**1α-OHase**; (Horst and Reinhardt, 1983; Holick, 2008; Dittmer and Thompson, 2011). The amount of 1,25vitD is controlled by its strictly regulated production by 1α-OHase and by the degradation through the enzyme 24-hydroxylase (**24-OHase**; (Horst and Reinhardt, 1983; Holick, 2008). High Ca and P serum levels decrease serum 1,25vitD, while parathyroid hormone (**PTH**), which is secreted when Ca serum level is low, as well as growth hormone (**GH**) and insulin-like growth factor I (**IGF-I**) increase 1,25vitD in serum. (Horst and Reinhardt, 1983; Nesbitt and Drezner, 1993; Tryfonidou et al., 2003; Dittmer and Thompson, 2011).

In ruminants, with sufficient dietary Ca supply, vitD stimulates bone formation and mineralization, whereas if Ca serum level drops, vitD enhances mobilization from the bones and intestinal Ca absorption (Liesegang and Risteli, 2005; Schroeder and Breves, 2006; Dittmer and Thompson, 2011).

It could be demonstrated that humans as well as a number of different species (including cows) are able to produce vitD in their skin (Holick et al., 1979; Hymøller and Jensen, 2010), in contrast to other species like cats and dogs who depend entirely on dietary supply (How et al., 1994; Morris, 1999). With the experiment of Kovács et al. (2015) it could be demonstrated that goats and sheep are able to produce vitD within their skin, but its importance compared to nutritional supply is still unclear and available data are conflicting (Hidiroglou and Karpinski, 1989; Kohler et al., 2013).

There is a continuous effort to optimize the dairy production and the composition of animal sources for human nutrition, for example the increase of vitD content in the milk (Kohler et al., 2013; Jakobsen et al., 2015; Weiss et al., 2015). The actual need of the commonly used highly vitD supplemented diets in ruminants nutrition need to be questioned. Indeed if it can be proved that they are capable of covering their vitD demand by endogenous production through UVB exposure, even in the situation of challenged Ca homeostasis because of the growing process, the livestock husbandry can be optimized.

The aim of our study was to investigate, based on the results of Kovács et al. (2015), how controlled UVB irradiation in combination with reduced nutritional vitD supply affects the vitD status and Ca regulation (including intestinal Ca absorption, renal excretion and bone metabolism) of healthy growing goats and sheep of dairy breeds with special attention on species-specific differences. Our hypothesis was that they are able to fully compensate the missing nutritional vitD supply through endogenous production in the skin while exposed to UV light. This will lead to a vitD status comparable to a status when vitD is supplemented, and consequently to a well balanced Ca homeostasis and a healthy growing process.

MATERIALS AND METHODS

Sixteen East Frisian milk sheep lambs (eight females and eight males) and fourteen Saanen Dairy goat kids (all males) were included in this study. At the beginning (week 0) the mean age was three and a half month and the mean body weight was 20.8 ± 0.7 kg. All lambs were shorn before starting the experiment.

Over the trial time of 13 weeks, the animals were housed in groups, in pens, on wood shavings, in a UVB free environment. At the end, in the thirteenth week, all animals were slaughtered.

They were fed individually twice a day with hay of the second cut (at the beginning for growing, stade 3) and an especially for these trial designed concentrate without vitD (Food 2921 Without Vitamin D Addition, KLIBA NAFAG, Kaiseraugst, Switzerland, Table 1). They had access to NaCl licking bowl (UFA Salzleckstein, Sodium Chloride (38.5 % Sodium) and Iodine (100 mg/kg), UFA AG, Herzogenbuchsee, Switzerland) and water ad libitum. Weighing was performed every week and the feed ration was individually adapted. The ration started at daily 700 g hay and 250 g concentrate for lambs with 20 kg body weight and 600 g hay and 200 g concentrate for goat kids with 20 kg body weight and increased continuously till daily 900 g hay and 450 g concentrate for lambs and 750 g hay and 375 g

concentrate for goat kids respectively when reaching 35 kg body weight. To evaluate the intake of each animal, refusals were weighed 60 min after feeding. All nutrient requirements were covered except for vitD (Agroscope, 2013).

The lambs and goat kids were randomly assigned to two groups for each species, after balancing for gender and weight. Half of the animals, **LUV** (UVB Exposed Lambs) and **GUV** (UVB Exposed Goat Kids), were daily exposed to UVB irradiation of UV lamps (OSRAM Ultra Vitalux, 300 Watt, OSRAM GmbH, München, Germany) for 30 min during feeding. Irradiation was monitored each morning on the animals back level with a UVB-Radiometer (Solarmeter 6.2, Solartech, Glenside, Pennsylvania, USA; response range of 280–320 nm) to ensure a minimum irradiation of 20 $\mu W/cm^2$. With this dosage the 30 min of lamps irradiation correspond to 55 min of sun exposure in summer near Zürich (calculated with the mean daily UVB dose measurements of Kohler et al. (2013)). The rest of the animals served as controls, and were assigned to the groups **LC** (Lambs Control) and **GC** (Goat Kids Control).

All the procedures on this experiment were approved by the respective Swiss authority for animal welfare (approval number 162/2012, Kantonales Veterinäramt Zürich, Switzerland) in accordance with the animal welfare law of Switzerland.

Table 1. Food composition

	Hay	Concentrate
Dry matter (%)	93.6	91.9
Crude Fiber (% DM)	21.8	6.46
Crude Protein (% DM)	13.5	12.1
Crude Fat (% DM)	2.09	3.73
ADF (% DM)	28.4	10.3
ADL (% DM)	3.74	1.99
NDF (% DM)	50.2	30.2
Crude Ash (% DM)	8.22	6.72
HCL insoluble Ash (% DM)	1.43	0.58
Ca (g/kg OM)	8.93	12.3
P (g/kg OM)	2.66	3.9
Mg (g/kg OM)	2.73	3.26
Vitamin A (IU/kg)[1]	-	24000
Vitamin D (IU/kg)[1]	-	0

[1] Amount of additives according to manufacturer's declaration (KLIBA NAFAG, Kaiseraugst, Switzerland).

Sample collection

Samples of urine, blood and faeces were collected before the start of the experiment (week 0), and afterwards in weeks 3, 7 and 11. Spontaneous morning urine was collected on the sampling day before feeding and immediately cooled at 5 °C. Before the samples were stored at -20 °C, pH was measured (827 pH Lab, Metrohm AG, Herisau, Switzerland). Venal blood was taken from the jugular vein (Greiner Bio-One VACUETTE Z Serum Clot Activator, 5 ml, St. Gallen, Switzerland) two and six hours after morning feeding or feeding with irradiation and was centrifuged (3000 *g*, 15 min) within 30 min after sampling. Serum was then stored at -20°C and -80°C. Feces samples were collected directly from the anus, pooled over one week per animal and also stored at -20°C.

Finally after slaughtering different samples were taken. Within 10 min, samples of the rumen (ventral ruminal sac) and duodenum (directly after pancreatic duct entrance) were taken to perform modified Ussing chamber technique as described by Sidler-Lauff et al. (2010). The net Ca ion fluxes (**Jnet**) were calculated from the mean detected unidirectional fluxes (**Jms** fluxes from mucosal to serosal side of the epithelium, **Jsm** fluxes from serosal to mucosal) with the formula Jnet = Jms - Jsm (nmol/h/cm^2). Additionally, mucosa samples from duodenum as well as kidney (cross section) samples were collected as described in Kovács et al. (2015) and stored at -80 °C.

Skin samples of five different localizations (forehead, neck, antebrachium, back and kneefold), shaved in advance, were taken within 5 min after slaughtering, frozen in liquid nitrogen and stored at -80 °C.

The left metatarsal bones were cleaned form adherent tissues and stored at -20 °C.

Additionally, random food samples of hay and concentrate were taken during the experiment, pooled over time and analyzed.

Serum and urine sample analyses

Serum levels of following parameters were analyzed in samples taken two hours after morning feeding or feeding with irradiation using commercial kits as described by Kovács et al. (2015): 25vitD (25-hydroxyvitamin D RIA, Immunodiagnostics Systems GmbH, Frankfurt am Main, Germany; intra- and interassay CVs 5.3% and 8.1%, sensitivity < 3 nmol/l; cross reactivity with 25-hydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3 100%, with 25-hydroxyvitamin D_2 75%, with vitD3 < 0.01% and with vitD2 < 0.3 %), 1,25vitD (1,25-

Dihydroxy VitD RIA, Immunodiagnostics Systems GmbH, Frankfurt am Main, Germany; intra- and interassay CVs 9.1% and 9.6%, sensitivity < 8 pmol/l; cross reactivity with 1,25-dihydroxyvitamin D_3 100%, with 1,25-dihydroxyvitamin D_2 97%, with 24,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3 < 0.01%), GH (Sheep somatotropin (GH1) ELISA Kit and goat growth hormone (GH) ELISA Kit, LuBioScience GmbH, Lucerne, Switzerland; intra- and interassay for both CVs 15% and 15%, sensitivity 0.675 ng/ml for sheep and 3.120 ng/ml for goats), IGF-I (IGF-I ELISA of human insulin-like growth factor-I – IGFBP-blocked, Mediagnost/TECOmedical AG, Sissach, Switzerland; intra- and interassay CVs 6.8% and 6.7%, sensitivity 0.09 ng/ml), serum crosslaps (**SCL,** Serum CrossLaps ELISA, Immunodiagnostic Systems GmbH, Frankfurt am Main, Germany; intra- and interassay CVs 1.7% and 2.5%, sensitivity 0.020 ng/ml) and osteocalcin (**OC**, MikroVue^{TM} Osteocalcin EIA Kit; Quidel Corporation, San Diego, CA, USA; intra- and interas- say CVs 4.8% and 4.8%, sensitivity 0.45 ng/ml).

In serum and urine samples, Ca and P levels were determined by colorimetry using an autoanalyzer (Cobas Mira Roche-autoanalyzer, F. Hoffman-La Roche Ltd., Basel, Switzerland) and commercial testkits (Ca, testkit DIA00460, Cresolphthalein complexone; P, testkit DIA00620, ammonium molybdate; Diatools AG, Villmergen, Switzerland, intra- and interassay CVs 0.62% and 1.66% for Ca, and 0.86% and 1.07% for P, sensitivity 0.050 mmol/l for Ca and 0.065 mmol/l for P). In serum samples, the mean values of the two measurements two and six hours after morning feeding or feeding with irradiation were calculated. Urine creatinine (**Crea**) was also analyzed with the same method using another testkit (Crea Jaffe, DIA00540; Diatools AG, Villmergen, Switzerland, intra- and interassay CVs 0.83 % and 0.85 %, sensitivity 18 µmol/l.), and the results of Ca and P were related to these values.

Quantitative RT-PCR

In samples of the kidney, mRNA expression of vitD receptor (**VDR**), calbindin D_{28k} (**Calb-D28k**), 1α-OHase (CYP27B1) and 24-OHase (CYP24A1) was analyzed. The RNA extraction was performed following the TRI Reagent protocol (TRI Reagent Solution, Ambion, Rotkreuz, Switzerland) and RNA concentration were measured by spectrophotometry using NanoDrop 2000 (Thermo Scientific – Thermo Fisher Scientific, Waltham, Massachusetts, USA). The RNA samples were than reverse transcribed in cDNA using a commercial kit

(QuantiTect Reverse Transcription Kit, Qiagen, Hilden, Germany), according to manufacturer's instructions using 1 µg RNA in a reaction volume of 20µl.

For quantification of RNA expression of VDR, Calb-D28k, CYP27B1 and the housekeeping genes β-actin (for VDR) and glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**, for Calb-D28k, CYP27B1) we used specific primers and probes (Table 2). A reaction mixture (20µl) containig TaqMan^{TM} Universal PCR Master Mix (Applied Biosystems, Darmstadt, Germany), specific primers (1µM), specific probe (0.75µM) and reverse transcribed RNA (50 ng) was prepared. After the PCR products have been amplified (95°C, 2 s, 45 cycles of 95°C, 3 s and 60°C, 30 s) they were analyzed on a 7500 Fast Real Time PCR System (ABI 7500Fast Sequence Detection System, Life Technologies, Waltham, USA). CT-values were determined with a threshold of 0.1.

Expression of CYP24A1 was determined using SYBR Green PCR assays (with specific primer, Table 2) using a real-time PCR cycler (CFX96TM; Bio-Rad, Munich, Germany) as described by Herm et al. (2015). Absolute copy numbers were determined using calibration curves generated with cloned PCR fragment standards as described by (Wilkens et al., 2009).

Efficiency of the different PCR assays tested in advance for dilutions of intestinal or renal cDNA and cloned standard ranged from 90 to 120%. Specificity of the amplicons was verified using NCBI Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Parallel PCR assays for each gene were performed with cDNA samples, plasmid standards and a no-template control containing water. Each series of experiments was carried out twice.

Table 2. Primers and probes used for TaqMan^{TM} assays (VDR (vitamin D receptor), Calb-D28k (calbindin D_{28k}), CYP27B1 (1α-hydroxylase), β-actin and GAPDH (glyceraldehyde 3-phosphate dehydrogenase)), synthetized by Mycrosynth AG (Balgach, Switzerland) and for SYBR Green PCR assays (CYP24A1, 24-hydroxylase, source: Herm et al. (2015)) synthetized by TIB MOLBIOL (Berlin, Germany).

Gene	Sense and antisense primers (5′→ 3′)	Probes
VDR	cagatcgtgctgctgaagtc	FAM cgacatgtcctggacctgtg BHQ-1
	acctggaacttgatgagggg	
Calb-D28k	cagaatcccacctgcaatca	FAM ctggcttcatttcgacgctga BHQ-1
	tcaagttctgaagctcctttc	
CYP27B1	cactggtcactctgtgtcact	FAM acttcaagggaccctgccca BHQ-1
	cgcttgccaaagccaaagg	
β-actin	actgggacgacatggagaag	FAM caccttctacaacgagctgc BHQ-1
	gtcatcttctcacggttggc	
GAPDH	gtcaccagggctgcttttaa	FAM aagttccacggcacagtcaa BHQ-1
	atctcgctcctggaagatgg	
CYP24A1	gaggcctcaagaaacagcac	
	ctgaccctctgccagtcttc	

Western blot analysis

In samples of the duodenum VDR expression on protein level as well as GAPDH as housekeeping protein was analyzed by western blot analysis as described by Kovács et al. (2015). The used antibodies for VDR were an antibody against VDR (Abcam AB54387; Sigma- Aldrich ChemieGmbH, Buchs, Switzerland) and a HRP-conjugated polyclonal anti-rat IgG (A9037; Sigma-Aldrich Chemie GmbH) and those for GAPDH were monoclonal anti-GAPDH (G8795; Sigma-Aldrich Chemie GmbH) and polyclonal anti-mouse IgG-specific peroxidase antibody (A2304; Sigma-Aldrich Chemie GmbH). Signal visualization was performed using enhanced chemiluminescence (Pierce; Thermo Scientific, Bonn, Germany) and a ChemiDoc system (Bio-Rad, München, Germany). For normalization, the ratio of protein expression of VDR to the GAPDH was calculated.

Skin sample analysis

Skin samples were analyzed by HPLC at 254 nm after being prepared as described by Morris (1999) with little modifications (Kovács et al., 2015). Using a Nucleosil silica column (EC250/4.6 Nucleosil 100-5C18; Macherey-Nagel, Düren, Germany) 7DHC, pre-D3, lumisterol, tachysterol and vitD3 were determined.

pQCT

Peripheral quantitative computed tomography (XCT 960 A Knochen Scanner; Stratec Medizinaltechnik, Pforzheim, Germany) of the left metatarsus was performed before starting the experiment (week 0) and after slaughtering (week 13). The length of the metatarsus was measured, and total, cortical and trabecular bone mineral density (**BMD**) as well as cortical bone thickness (**CBT**) were determined (cortical mode 2, threshold for cortical bone > 640 mg/cm^3) distal in the metaphysis (10 % of the metatarsus lengths) and in the middle of the diaphysis (50 % of the metatarsus lengths) as described by Liesegang and Risteli (2005).

Feed and feces analysis

The DM content of feed and feces (%) was determined by drying of 3-5 g feed samples and 3-5 g pooled feces samples at 103 °C up to weight constancy. Afterwards, samples were ashed at 550 °C in a muffle furnace, hydrolyzed in hydrochloric acid 0.8 % and analyzed for content of Ca and P by colorimetry using the same testkits and autoanalyzer as for urine and serum

samples. The amount of crude ash, HCl insoluble ash, crude fat, crude protein and crude fiber in feed was determined by proximate analysis. In feed and feces ADL and ADF, as well as NDF in feed, were analysed as described by Van Soest et al. (1991).

Apparent digestibility (**AD**) of Ca and P were calculated by indicator method using ADL as indicator and the formula (Stangl, 2014):

$$\text{AD}\ (\%) = 100 - \frac{\%\ \text{indicator in feed}}{\%\ \text{indicator in faeces}}\ x\ \frac{\%\ \text{mineral in faeces}}{\%\ \text{mineral in feed}}\ x\ 100$$

Statistical analysis

The experimental design was a 2-factorial design with the factors irradiation (UV or C) and species (L or G) that built the four groups LUV, LC, GUV and GC.

The statistical analyses were performed with SYSTAT (Version 13 for Windows, Systat Software Inc., San Jose, California, USA). To test the differences between the time-dependent patterns (for blood and urine samples, apparent digestibility, bone densities and body weights) and between the localization -dependent patterns (skin samples) in the groups, a multivariate analysis of variance for repeated measurements (MANOVA) was performed, after testing the data for normal distribution by Shapiro-Wilk test. If the data were not normally distributed, results were verified by non-parametric tests: Kruskal-Wallis was used to investigate the difference between the groups, and a Wilcoxon signed-rank test for paired samples to compare the time dependent effect within each group. To exclude a possible influence of the gender on the irradiation treatment within the lambs, a 2way ANOVA (gender, irradiation and gender x irradiation) was performed for all analysed parameters. No interaction between irradiation and gender was found in any of the investigated parameters.

Additionally pearson's correlations coefficient between serum 25vitD levels and sterol contents in the skin was calculated and significance was evaluated by t-test (zero correlation).

All data are reported as means ± standard error. Differences were considered significant at $p < 0.05$ for all test.

RESULTS

Body Weight

The body weight increased gradually indicating normal growth, in lambs from 21.2 ± 0.58 kg in week 0, to 34.2 ± 0.85 kg in week 13 and in goat kids from 20.2 ± 1.25 kg in week 0, to 29.7 ± 1.53 kg in week 13. The mean weekly increase was higher ($p < 0.001$) in lambs (0.996 ± 0.032 kg/week) compared to goat kids (0.748 ± 0.036 kg/week), but there was no influence of the irradiation on the mean weekly increase and we observed no significant difference in the body weights between the groups at any time point.

Skin

Concentration of 7DHC in skin was influenced by irradiation (Table 3). In lambs 7DHC values were significantly lower in LUV than in LC in skin samples from the back ($p = 0.013$) and the neck ($p = 0.027$), whereas in goat kids it was lower in GUV than in GC in samples from the back ($p = 0.012$), the neck ($p = 0.003$) and the antebrachium ($p = 0.001$). In both species the lowest concentrations overall were discovered in samples of the back, followed by the neck and the highest were in the skin of the forehead ($p < 0.001$). The 7DHC concentration of the back and the neck correlated negatively with the 25vitD serum concentration in week 11 ($r = - 0.547$; $p = 0.012$ and $r = - 0.461$; $p = 0.004$ respectively, Figure 1). No species difference could be detected.

The concentration of pre-D3 in the skin was influenced neither by irradiation, species nor localization.

VitD3 could not always be detected in each skin sample of the different locations in lambs as well as in samples of forehead of goat kids, whereas in samples of all other localizations of the goat kids vitD3 was detectable. Again no influence of irradiation, species or location was found.

We observed a species difference in concentrations of tachysterol and lumisterol in the skin. Lumisterol was generally lower in lambs than in goats kids (significant only in samples of the back and the kneefold, with $p = 0.002$ and $p = 0.010$ respectively), and again was not detected in all lamb samples. On the other hand tachysterol was generally lower in goats kids than in lambs (significant in samples of the head, the antebrachium and the kneefold with $p = 0.004$, $p = 0.004$ and $p < 0.001$ respectively), and was not always detected in goat kids samples. Neither for tachysterol nor for lumisterol an irradiation effect was detected.

Table 3. Effect of irradiation on mean (± SE) content of sterols in skin of lambs and goat kids after slaughter (µg/g skin).

	Group[1]				
	LUV	LC	GUV	GC	p-Value group[2]
7-Dehydrocholesterol					
Forehead	64.9 ± 5.46	60.2 ± 1.75	47.9 ± 7.74	47.5 ± 6.31	n.s.[3]
Neck	13 ± 1.66[a]	22.8 ± 3.4[b]	18.1 ± 2.66[a]	30.0 ± 1.82[b]	0.001
Antebrachium	31.5 ± 4.33	37.3 ± 4.03	20.5 ± 2.3[a]	34.0 ± 1.88[b]	0.013
Back	9.64 ± 1.05[a]	17.1 ± 2.32[b]	10.7 ± 1.19[a]	18.6 ± 2.25[b]	0.004
Kneefold	28.5 ± 2.91	30.7 ± 3.78	20.3 ± 2.21	31.9 ± 3.34	n.s.
Pre-vitamin D_3					
Forehead	2.71 ± 1.28	2.83 ± 1.3	0.8 ± 0.05	0.86 ± 0.1	n.s.
Neck	1.17 ± 0.21	1.37 ± 0.73	1.03 ± 0.18	0.9 ± 0.13	n.s.
Antebrachium	1.46 ± 0.34	1.61 ± 0.62	0.96 ± 0.17	1.01 ± 0.18	n.s.
Back	1.16 ± 0.24	1.47 ± 0.56	2.11 ± 0.56	2.53 ± 0.62	n.s.
Kneefold	2.59 ± 0.54	2.39 ± 0.82	1.47 ± 0.21	1.45 ± 0.35	n.s.
Vitamin D_3					
Forehead	1.11 ± 0.86	1.67 ± 0.86	0.13 ± 0.06	0.45 ± 0.31	n.s.
Neck	0.17 ± 0.04	0.15 ± 0.05	0.31 ± 0.09	0.32 ± 0.11	n.s.
Antebrachium	0.41 ± 0.25	0.68 ± 0.26	0.37 ± 0.14	0.16 ± 0.05	n.s.
Back	0.18 ± 0.08	0.29 ± 0.06	0.7 ± 0.27	0.93 ± 0.39	n.s.
Kneefold	0.25 ± 0.04	0.31 ± 0.11	0.5 ± 0.13	0.24 ± 0.05	n.s.
Lumisterol					
Forehead	1.45 ± 0.81	0.53 ± 0.08	1.32 ± 0.23	1.35 ± 0.21	n.s.
Neck	0.52 ± 0.05	0.9 ± 0.27	1.14 ± 0.21	1.03 ± 0.18	n.s.
Antebrachium	0.98 ± 0.16	0.55 ± 0.1	1.39 ± 0.3	1.3 ± 0.24	n.s.
Back	0.46 ± 0.06[m]	0.5 ± 0.14[m]	3.35 ± 1.21[n]	2.73 ± 0.52[n]	0.021
Kneefold	0.56 ± 0.16[m]	1.01 ± 0.36	2.43 ± 0.67[n]	1.54 ± 0.2	0.028
Tachysterol					
Forehead	0.41 ± 0.07	0.34 ± 0.07[m]	0.2 ± 0.06	0.12 ± 0.03[n]	0.031
Neck	0.23 ± 0.06	0.24 ± 0.09	0.1 ± 0.03	0.09 ± 0.02	n.s.
Antebrachium	0.33 ± 0.06[m]	0.31 ± 0.09	0.13 ± 0.02[n]	0.09 ± 0.01	0.045
Back	0.3 ± 0.07	0.23 ± 0.09	0.17 ± 0.06	0.3 ± 0.12	n.s.
Kneefold	0.37 ± 0.06[m]	0.24 ± 0.06[m]	0.12 ± 0.03[n]	0.08 ± 0.01[n]	0.001

[1] LUV: UVB exposed lambs; LC: lambs control; GUV: UVB exposed goat kids; GC: goat kids control.

[2] Differences in groups reflect both differences between species as well as irradiation treatment.

[3] Not significant ($p > 0.05$): n.s.

[ab] Different superscript within a row and the same species indicate a significant influence by irradiation treatment ($p < 0.05$).

[mn] Different superscript within a row indicate a significant influence by species ($p < 0.05$)

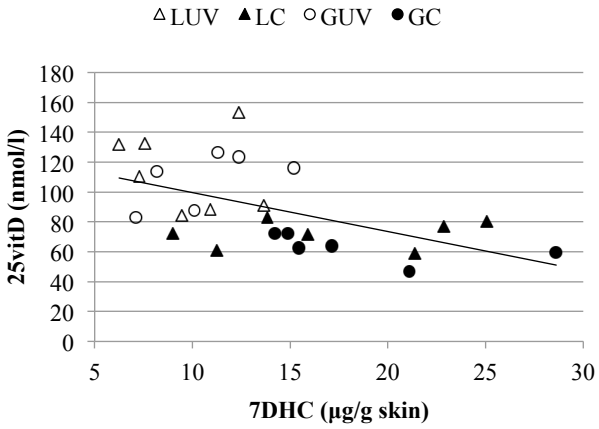

Figure 1: Correlation between 25-hydroxyvitamin D (25vitD) in serum (week 11) and 7-Dehydrocholesterol (7DHC) in the Back skin of lambs and goat kids. LUV: UVB exposed lambs; LC: lambs control; GUV: UVB exposed goat kids; GC: goat kids control. Pearson's correlation coefficient r = -0.547, p = 0.012.

Serum

Serum 25vitD levels were significantly higher in LUV than LC over the whole experiment (Table 4). In goat kids the difference of GUV having higher levels than GC was significant from week 3 till the end of the experiment. Only in week 0 there was a species-specific difference, with goat kids having higher 25vitD levels compared to lambs. Over time, serum 25vitD levels increased significantly in the first 3 weeks in lambs ($p = 0.012$ for LUV and $p = 0.017$ for LC), and decreased significantly in both control groups from week 3 to 11 (LC $p = 0.012$, and GC $p = 0.018$), whereas in irradiated groups they remained higher with fluctuations.

Serum 1,25vitD levels decreased significantly over time only in the control groups (Table 4), in LC from week 0 to 7 ($p = 0.036$), and in GC from week 0 to 11 ($p = 0.018$). Anyway this decrease was not enough to create a significant difference between the groups at any time point.

As well no irradiation dependent effect was detected in serum for the analyzed minerals (Ca, P), the bone parameters (OC, SCL) and IGF-I. Serum Ca levels were higher ($p < 0.001$) in lambs (range 2.47–3.46 mmol/l) than in goat kids (range 2.28– 2.82 mmol/l) during the entire experiment. No time dependent effect was detected. Serum P level of goat kids increased over time with a significant increase from week 7 to 11 ($p = 0.003$), whereas in lambs it did not vary significantly. At the last sampling in week 11 goat kids had significant higher levels of serum P levels than lambs ($p < 0.001$). The range during the whole experiment was 1.47–2.49 mmol/l for lambs and 1.49–2.65 mmol/l for goat kids. Serum OC levels (Table 4) in lambs

decreased significantly over time ($p = 0.001$) and were lower than in goat kids over the whole experiment ($p < 0.001$). Also SCL levels (Table 4) were lower in lambs than in goat kids through the second half of the experiment (weeks 7 and 11, $p < 0.001$) because of a time dependent increase from week 0 to 11 in goat kids ($p = 0.001$). Serum IGF-I levels increased significantly from week 0 to 11 in all groups (lambs $p = 0.012$, LC $p = 0.012$, GUV = 0.018 and GC $p = 0.018$, Table 4). Over the whole time goat kids had higher serum levels than lambs, but it reached statistical significance only in weeks 0, 7 and 11 ($p < 0.001$, $p = 0.025$ and $p = 0.005$).

Also values of serum GH were always significantly higher in goat kids than in lambs ($p < 0.001$, Table 4), but showed different time courses. While in lambs and GC the significant decrease until week 7 ($p < 0.001$ and $p = 0.028$ respectively) was followed by an increase until week 11 (significant only in lambs, $p = 0.002$), in GUV there was a peak in week 3 ($p = 0.028$) followed by a decrease until week 11 ($p = 0.028$). Consequently, we saw an irradiation effect in week 3 and 7 ($p = 0.021$ and $p = 0.003$), where GC had significantly lower GH levels in serum than GUV.

Table 4. Effect of irradiation on mean (± SE) serum levels of 25-hydroxyvitamin D (25vitD), 1,25-dihydroxyvitamin D (1,25vitD), Serum Crosslaps (SCL), Osteocalcin (OC), Growth Hormone (GH) and insulin-like growth factor I (IGF-I) in lambs and goat kids.

	Group[1]				
	LUV	LC	GUV	GC	p-value group[2]
25vitD (nmol/l)					
week 0	92.9 ± 2.37^{amx}	81.5 ± 4.21^{bmx}	127 ± 7.77^{nx}	109 ± 8.16^{nx}	< 0.001
week 3	130 ± 3.76^{ay}	103 ± 5.5^{by}	126 ± 8.4^{axz}	100 ± 5.96^{bx}	0.002
week 7	100 ± 4.27^{ax}	61.9 ± 2.47^{bz}	87.8 ± 8.13^{ay}	54.5 ± 3.29^{by}	< 0.001
week 11	110 ± 9.16^{a}	69.4 ± 3.9^{bz}	107 ± 6.52^{az}	60.9 ± 3.85^{by}	< 0.001
p-value time[3]	0.006	< 0.001	< 0.001	< 0.001	
1,25vitD (pmol/l)					
week 0	143 ± 15.2	142 ± 13.9^{x}	176 ± 29.8	157 ± 17.3^{x}	n.s.[4]
week 3	109 ± 13.4	115 ± 9.48^{y}	115 ± 11.4	121 ± 14.3^{y}	n.s.
week 7	114 ± 21	108 ± 13.1^{y}	140 ± 4.11	139 ± 21.8	n.s.
week 11	117 ± 25.5	119 ± 10.9	137 ± 13.2	102 ± 12.9^{y}	n.s.
p-value time	n.s.	0.035	n.s.	0.008	
SCL (ng/ml)					
week 0	1.11 ± 0.21	1.35 ± 0.31	0.92 ± 0.09^{xy}	0.87 ± 0.06^{x}	n.s.
week 3	0.62 ± 0.06	0.89 ± 0.11	0.88 ± 0.1^{x}	0.82 ± 0.06^{x}	n.s.
week 7	0.68 ± 0.05^{m}	0.7 ± 0.06^{m}	1.18 ± 0.1^{ny}	1.15 ± 0.08^{ny}	< 0.001
week 11	0.8 ± 0.06^{m}	0.91 ± 0.07^{m}	1.52 ± 0.08^{nz}	1.61 ± 0.08^{nz}	< 0.001
p-value time	n.s.	n.s.	< 0.001	< 0.001	

OC (ng/ml)					
week 0	197 ± 8.86^{mw}	169 ± 12.5^{mx}	290 ± 25.3^{n}	271 ± 21.1^{n}	< 0.001
week 3	157 ± 6.71^{myx}	160 ± 6.28^{mx}	222 ± 18.3^{n}	228 ± 22.8^{nx}	0.001
week 7	129 ± 4.41^{my}	124 ± 8.09^{my}	232 ± 27.7^{n}	231 ± 13.1^{nx}	< 0.001
week 11	147 ± 8.97^{mz}	128 ± 4.39^{my}	273 ± 16.7^{n}	307 ± 20.1^{ny}	< 0.001
p-value time	< 0.001	< 0.001	n.s.	0.002	
GH (ng/ml)					
week 0	2.97 ± 0.13^{mx}	2.91 ± 0.03^{mx}	6.64 ± 0.17^{nx}	6.63 ± 0.18^{nx}	< 0.001
week 3	2.83 ± 0.07^{mx}	2.77 ± 0.07^{mxz}	7.08 ± 0.2^{any}	6.26 ± 0.23^{bnx}	< 0.001
week 7	2.5 ± 0.05^{my}	2.52 ± 0.05^{my}	6.41 ± 0.13^{anx}	5.84 ± 0.09^{bny}	< 0.001
week 11	2.73 ± 0.1^{mx}	2.64 ± 0.06^{mz}	6.06 ± 0.16^{nx}	5.89 ± 0.14^{ny}	< 0.001
p-value time	0.002	< 0.001	0.001	0.001	
IGF-I (ng/ml)					
week 0	145 ± 11.6^{mx}	135 ± 18.3^{mx}	280 ± 50.9^{nx}	263 ± 27.6^{nx}	0.002
week 3	190 ± 7.81^{y}	186 ± 26.9^{y}	240 ± 37.5^{x}	240 ± 37.5^{x}	n.s.
week 7	218 ± 13.1^{y}	207 ± 41.2^{my}	240 ± 37.5^{x}	346 ± 36.9^{nx}	0.032
week 11	335 ± 20.7^{mz}	285 ± 50^{mz}	443 ± 45.8^{ny}	476 ± 69.6^{ny}	0.031
p-value time	< 0.001	0.005	< 0.001	0.015	

[1] LUV: UVB exposed lambs; LC: lambs control; GUV: UVB exposed goat kids; GC: goat kids control.

[2] Differences in groups reflect both differences between species as well as irradiation treatment.

[3] Differences in time points reflect the differences between measurements in week 0, 3, 7 and 11.

[4] Not significant ($p > 0.05$): n.s.

[ab] Different superscript within a row and the same species indicate a significant influence by irradiation treatment ($p < 0.05$).

[mn] Different superscript within a row indicate a significant influence by species ($p < 0.05$).

[wxyz] Different superscript within a column (group) indicate a significant influence by time ($p < 0.05$).

Urine

Urinary Ca and P content were not influenced by irradiation. Over time values of Ca/Crea content in lambs increased from 0.051 ± 0.007 mmol/mmol Crea in week 0 to 0.153 ± 0.020 mmol/mmol Crea in week 11 ($p < 0.001$) whereas in goat kids it increased from 0.178 ± 0.028 mmol/mmol Crea in week 0 till 0.389 ± 0.076 mmol/mmol Crea in week 3 ($p = 0.013$) and decreased again till 0.117 ± 0.017 mmol/mmol Crea in week 11 ($p = 0.005$). Consequently goat kids Ca/Crea content in urine was higher in week 0 and 3 and lower in week 7 compared to lambs ($p < 0.001$, $p = 0.001$ and $p = 0.001$ respectively). The content of P/Crea in urine did not vary significantly over time in any of the groups (from 0.097 ± 0.004 mmol/mmol Crea in week 0 to 0.111 ± 0.015 mmol/mmol Crea in week 11), but in week 11 we found lower levels in goat kids than in lambs ($p = 0.011$, 0.071 ± 0.008 and 0.144 ± 0.024 mmol/mmol Crea respectively).

Ussing Chamber

We observed a net active Ca absorption in rumen and a net active Ca secretion in duodenum (Figure 2). In goat kids a significant irradiation dependent difference was found: the absorption in rumen as well as the secretion in duodenum ($p = 0.009$ and $p = 0.001$, respectively) was lower in GUV than in GC. A species-specific effect was detected in both localisations, in rumen with GUV lower than LUV ($p = 0.004$) and in duodenum with GC higher ($p = 0.004$) than LC.

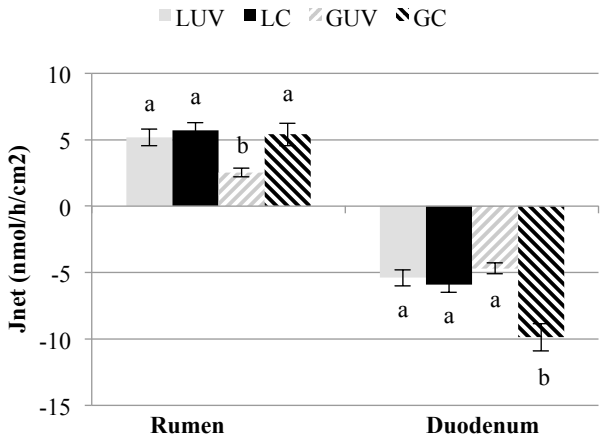

Figure 2: Results of Ussing Chamber Technique: net Ca ion fluxes (Jnet) trough rumenal and duodenal mucosa of goat kids and lambs. Calculated from the mean detected unidirectional fluxes (Jms fluxes from mucosal to serosal side of the epithelium, and Jsm fluxes from serosal to mucosal) with the formula: Jnet = Jms - Jsm (nmol/h/cm^2). LUV: UVB exposed lambs; LC: lambs control; GUV: UVB exposed goat kids; GC: goat kids control. Groups within one location with different letters differ significantly ($p < 0.050$). Mean ± SE.

Feces

In feces samples we observed neither an irradiation dependent effect nor a time effect for AD of Ca and P (Table 5). The species difference showed higher AD of Ca in goat kids than in lambs in week 3 ($p = 0.006$) and 7 ($p < 0.001$), and a higher AD of P in lambs than in goat kids at those time points ($p < 0.001$ and $p = 0.001$ respectively).

Table 5. Effect of irradiation on mean (± SE) apparent digestibility of Ca and P calculated with acid detergent lignin (ADL) used as indicator, in lambs and goat kids.

	Group[1]				
	LUV	LC	GUV	GC	p-Value group[2]
Calcium %					
week 3	18.4 ± 2.53[m]	25.7 ± 3.21	29.9 ± 2.39[n]	35.1 ± 2.98	0.002
week 7	20.6 ± 3.5[m]	23.3 ± 4.34[m]	38 ± 2.62[n]	37.1 ± 3.41[n]	0.002
week 11	24.4 ± 2.7	26.1 ± 6.78	32.6 ± 3.46	37.6 ± 3.2	n.s.[3]
p-value time[4]	n.s.	n.s.	n.s.	n.s.	
Phosophor %					
week 3	9.76 ± 3.73 [m]	10.67 ± 3.47 [m]	12.76 ± 6.46 [n]	-4.58 ± 4.68 [n]	0.003
week 7	9.61 ± 3.02 [m]	14.69 ± 5.04 [m]	-4.9 ± 5.27 [n]	-3.83 ± 4.44 [n]	0.009
week 11	13.94 ± 3.19	11.15 ± 5.97	5.44 ± 7.17	-0.04 ± 6.17	n.s.
p-value time	n.s.	n.s.	n.s.	n.s.	

[1] LUV: UVB exposed lambs; LC: lambs control; GUV: UVB exposed goat kids; GC: goat kids control.

[2] Differences in groups reflect both differences between species as well as irradiation treatment.

[3] Not significant ($p > 0.05$): n.s.

[4] Differences in time points reflect the differences between measurements in week 3, 7 and 11.

[mn] Different superscript within a row indicate a significant influence by species ($p < 0.05$).

Table 6. Effect of irradiation on mean (± SE) RNA expression of VDR (vitamin D receptor), Calb-D28k (calbindin D_{28k}), CYP27B1 (1α-hydroxylase), CYP24A1 (24-hydroxylase) normalized to either β-actin (VDR) or GAPDH (glyceraldehyde 3-phosphate dehydrogenase, Calb-D28k, CYP27B1 and CYP24A1) in kidneys of goat kids and lambs. Effect of irradiation on mean (± SE) protein expression of VDR (vitamin D receptor) normalized to GAPDH (glyceraldehyde 3-phosphate dehydrogenase) in duodenum of lambs and goat kids determined with western blot.

	Group[1]		
Lambs	LUV	LC	p-value group[2]
Kidney			
VDR	0.64 ± 0.17	1 ± 0.18	n.s.[3]
Calb-D28k	1.45 ± 0.77	1 ± 0.25	n.s.
CYP27B1	0.87 ± 0.42	1 ± 0.43	n.s.
CYP24A1	1.85 ± 0.42	1 ± 0.16	n.s.
Duodenum			
VDR	1.27 ± 0.13	1 ± 0.2	n.s.
Goat kids	GUV	GC	
Kidney			
VDR	1.37 ± 0.23	1 ± 0.25	n.s.
Calb-D28k	1.64 ± 0.45	1 ± 0.21	n.s.
CYP27B1	0.76 ± 0.34	1 ± 0.34	n.s.
CYP24A1	0.43 ± 0.15	1 ± 0.38	n.s.
Duodenum			
VDR	1.22 ± 0.14	1 ± 0.1	n.s.

[1] LUV: UVB exposed lambs; LC: lambs control; GUV: UVB exposed goat kids; GC: goat kids control.

[2] Reflects differences between irradiation treatment within one species.

[3] Not significant ($p > 0.05$): n.s.

PCR/WB

In the kidneys, expression of VDR, Calb-D28k, 1α-OHase and 24-OHase on RNA level was compared within one species in relation to the respective control group. No irradiation dependent effect was found (Table 6).

Expression on protein level of VDR in duodenum within one species was not influenced by irradiation (Table 6).

pQCT

At that the end of the experiment we found no irradiation dependent effect on any of parameters mesured by pQCT (Table 7). Total BMD (diaphysis and metaphysis) as well as cortical BMD and CBT increased significantly over time in all groups ($p < 0.014$) except in GC where the increase of total BMD and CBT in diaphysis did not reach significance. In the trabecular BMD the time dependent effect not significant.

In the diaphysis we found significant higher values of cortical and total BMD in lambs compared to goad kids at the end of the experiment ($p < 0.001$). A species-specific difference in the metaphysis was found in week 0, where the total and trabecular BMD was significantly lower in lambs compared to goat kids ($p < 0.022$). Also LUV had lower values of total and trabecular BMD in week 0 compared to LC. These differences in the metaphysis disappeared until the end of the trial.

CBT was significantly higher in week 0 in goat kids compared to lambs ($p = 0.001$) whereas at the end of the experiment, in week 13, the situation was inversed ($p < 0.001$).

Table 7. Effect of irradiation on mean (± SE) metatarsal bone mineral density (BMD) and cortical bone thickness (CBT) in lambs and goat kids.

	group[1]				
	LUV	LC	GUV	GC	p-value group[2]
Total BMD diaphysis[3] (mg/cm^3)					
week 0	593 ± 21.8	609 ± 18.7	572 ± 29.4	605 ± 26.9	n.s.[4]
week 13	801 ± 11.3^{m}	791 ± 20^{m}	659 ± 15^{n}	639 ± 18.2^{n}	< 0.001
p-value time[5]	< 0.001	< 0.001	0.014	n.s.	
Total BMD metaphysis[6] (mg/cm^3)					
week 0	321 ± 8.03^{am}	361 ± 7.06^{bm}	440 ± 23.6^{n}	419 ± 15.6^{n}	< 0.001
week 13	465 ± 14.8	451 ± 9.03	479 ± 16.7	475 ± 12.9	n.s.
p-value time	< 0.001	< 0.001	0.014	0.002	
Trabecular BMD metaphysis (mg/cm^3)					
week 0	313 ± 12.1^{am}	369 ± 8.86^{b}	402 ± 37.7^{n}	387 ± 18.9	0.032
week 13	354 ± 18.6	327 ± 15.9	379 ± 27.7	372 ± 18.9	n.s.
p-value time	n.s.	0.044	n.s.	n.s.	
Cortical BMD diaphysis (mg/cm^3)					
week 0	913 ± 15.2	928 ± 8.97	868 ± 24	904 ± 15.3	n.s.
week 13	1078 ± 6.35^{m}	1070 ± 6.95^{m}	996 ± 5.48^{n}	979 ± 13.2^{n}	< 0.001
p-Value time	< 0.001	< 0.001	0.001	< 0.001	
CBT diaphysis (mm)					
week 0	2.44 ± 0.07^{m}	2.46 ± 0.04^{m}	2.76 ± 0.08^{n}	2.84 ± 0.18^{n}	0.018
week 13	3.34 ± 0.07^{m}	3.27 ± 0.08^{m}	2.94 ± 0.07^{n}	2.99 ± 0.1^{n}	0.004
p-value time	< 0.001	< 0.001	0.026	n.s.	

[1] LUV: UVB exposed lambs; LC: lambs control; GUV: UVB exposed goat kids; GC: goat kids control.

[2] Differences in groups reflect both differences between species as well as irradiation treatment.

[3] Measuring point at 50% of metatarsus length.

[4] Not significant ($p > 0.05$): n.s.

[5] Differences in time points reflect the differences between measurements in week 0 and 13.

[6] Measuring point at 10% of metatarsus length.

[ab] Different superscript within a row and the same species indicate a significant influence by irradiation treatment ($p < 0.05$).

[mn] Different superscript within a row indicate a significant influence by species ($p < 0.05$).

DISCUSSION

Vitamin D status and synthesis in the skin

In this study, we investigated the hypothesis that growing lambs and goat kids of dairy breeds are able to fully compensate a reduced vitD content in food by producing vitD within their skin. Level of 25vitD in serum is recognized to be the most reliable indicator for vitD status (Adams et al., 1982). In the testkit use to determine 25vitD level in serum, there is no differentiation between 25-dihydroxyvitamin D_2 (coming from the vitD2 acquired through nutrition), 25-dihydroxyvitamin D_3 (coming from endogenous production) and 24,25-dihydroxyvitamin D, and therefore all this storage forms are included independently of their origin (Horst and Reinhardt, 1983). Both irradiated groups (LUV and GUV) had a better vitD status with higher 25vitD serum levels, compared to the control groups (LC and GC). In the experiment of Kovács et al. (2015), lambs and goat kids were irradiated in the same way as in the present study, but had a high VitD supply in the diet. The values of 25vitD at the end of that trial were in a similar range as the values of our irradiated groups (LUV and GUV), while the levels of 25vitD of our control groups (LC and GC) without any vitD source were clearly lower. This shows that the irradiation was sufficient to compensate the lack of vitD and maintain the serum 25vitD levels. Additionally the drop of the initially high serum 25vitD levels in our control groups confirms that the natural sources of vitD in the feedstuffs in this study, where not sufficient to cover the vitD requirements of the animals.

The natural content of vitD in hay depends on composition, season and climatic conditions of the region as well as drying method. Therefore there is a high variation in the vitD contents described in the literature. Jäpelt et al. (2011), for example, found levels between 2.8 and 255.6 IU of vitD2/Kg organic matter (analyzed by liquid chromatography tandem mass spectrometry) in different ryegrass over the seasons in Denmark, while Kohler et al. (2013) found a higher level of 2508 IU of vitD2/Kg dry matter in lowland hay in Switzerland (analyzed by HPLC). Considering that the recommended vitD supply, to reach a normal vitD status, is between 120 and 500 IU/day for sheep, and between 250 and 1500 IU/day for goats respectively (Agroscope, 2013), the mean content of vitD in the hay used must have been under 153 IU vitD/kg dry matter. Therefore the absorbed vitD naturally present in the hay did not cover the vitD requirement of the animals (which explains the dropped vitD levels in the control groups), but still provided a significant vitD amount, which prevented the drop of the vitD levels in a vitD deficient range.

In most species (including humans) the normal range of 25vitD in serum is considered to bee between 75 and 150 nmol/l, while levels between 25 and 75 nmol/l are defined as vitD insufficiency (meaning that endocrine function such as immune regulation and cell differentiation are compromised), and levels < 25 nmol/l are defined as actual vitD deficiency with negative effects on the classical vitD function (such as bone metabolism and calcium homeostasis investigated in this study) (Norman, 2011; Weber et al., 2014; Nelson et al., 2016). Different authors also described lower ranges of 25vitD in lambs and calves (compared to adult animals) with good health and no signs of classical vitD deficiency (Horst and Littledike, 1982; Handel et al., 2016; Nelson et al., 2016).

The animals in this study had a normal initial vitD status thanks to the high vitD intake due to the vitD content in the normal concentrate fed before starting the experiment and the vitD intake via milk before weaning. Indeed for new born ruminants, in contrast to other species, milk can be a adequate source of vitD under the condition of a high vitD status in combination with a moderate milk yield of the dam (Kohler et al., 2013; Weiss et al., 2015; Hymøller et al., 2017). The 25vitD can than be stored in the body tissue (primary fat, muscle and liver) to help preventing a fast drop of vitD status (Hidiroglou, 1987; Hidiroglou and Karpinski, 1989). Therefore, also the short duration of the experiment and the healthy initial vitD status helped preventing a real vitD deficiency in our control groups.

The levels of the active metabolite 1,25vitD in serum on the other hand, where neither influenced by irradiation nor by species difference. After three months of observation, only a decrease of 1,25 levels in control groups could be observed. Therefore the inexplicable higher serum 1,25vitD levels in irradiated goats kids found by Kovács et al. (2015) could not be confirmed. The production of 1,25vitD from 25vitD by the enzyme 1α-OHase is inhibited by the final product 1,25vitD as well as by Ca and P, and stimulated by calcitonin, IGF-I and PTH (Nesbitt and Drezner, 1993; Holick, 2008; Dittmer and Thompson, 2011; Jones, 2013). The levels of Ca and P in serum remained stable in a normal range (Pugh, 2002) during the whole experiment and IGF-I increased in a similar way in all the groups due to the physiological growing process (Žofková, 2003). Consequently, neither the amount of the enzyme 1α-OHase nor 24-OHase in the kidney was altered. In future studies it would be interesting to analyze the reaction in an additional situation of reduced Ca supply.

Considering therefore that the difference in 25vitD status between the groups was not caused by a different degradation and as the husbandry conditions and the feeding where identical for all groups, the difference must have been caused by the production with irradiation of the

skin. These findings are in line with previous studies that showed a better vitD status in sheep exposed to UVB irradiation (Quarterman, 1964; Smith, 1980; Hidiroglou and Karpinski, 1989; Kovács et al., 2015) and evidence the parallelism to the endogenous vitD production in dairy cows (Hymøller et al., 2009; Hymøller and Jensen, 2010; Hymøller and Jensen, 2012; Hymøller et al., 2017). Also the lower content of 7DHC in the skin of the irradiated animals compared to the control group supports our hypothesis. This goes along with the findings described by Kovács et al. (2015) and is caused by the transformation of 7DHC into pre-D_3, as it has been proven in vitro for human skin (Holick et al., 1981). On the other hand we couldn't find an irradiation dependent increase of the concentration of the photoproducts (preD3, lumisterol, tachysterol, vitD3) in skin samples. Kühn et al. (2015) investigated the effect of UVB irradiation in chicks skin and found no significant effect on 7DHC concentrations (but a slight irradiation dependent decrease), and an irradiation dependent increase on vitD 3 concentrations. An explanation for the lack of accumulation of the produced vitD3 in the analyzed skin samples is that it has been released to circulation (Holick et al., 1980). In fact the amount of 7DHC in the skin parts most exposed to UVB irradiation (back and neck) was negatively correlated to the 25vitD serum level. The lack of an increase of lumisterol and tachysterol remains unclear but it could be hypothesized that the amount of UVB reaching the skin layers, which can be influenced by factors like skin pigmentation or thickness as well as hair coat density, was not high enough for their production or that other storage forms such as suprasterol I, suprasterol II ore 5,6-transvitamin D3 are involved (Forrest and Fleet, 1985; Webb and Holick, 1988; Handel et al., 2016). To clarify interspecies differences in those storage forms further studies are needed. So far we could show that lambs have higher concentration of tachysterol and goat kids higher concentration of lumisterol within their skin. Also the influence of the amount of 7DHC in the skin, as part of the cholesterolgenic pathway needs to be further investigated in both species. In fact we could not confirm that sheep have 10 times lower concentration of 7DHC in their skin compared to goats as showed by Kohler et al. (2013) in adult animals, and for unclear reasons our 7DHC levels were higher compared to those measured in the study of Kovács et al. (2015).

Effect of vitamin D status on bone metabolism, Ca absorption and Ca homeostasis

A sufficient vitD status combined with a normal Ca availability, leads to the embedment of the minerals in the bone and these, to an increase of BMD. In all groups BMD increased over the trial time which is part of a normal physiological ageing process (Liesegang et al., 2013),

although the increase was higher in lambs than in goat kids. This agrees with the results of Kovács et al. (2015) and supports the suggestion of Liesegang and Risteli (2005) that growing sheep react faster in embedding minerals in the bone compared to growing goats. No difference between irradiated and control groups was seen in the BMD at the end of the experiment and the animals of the control groups did not show any signs of rickets or poor bone mineralization. This means that all the animals continued their normal bone growth and that the reduced vitD status in the control groups was not yet to low to influence bone formation (Van Saun, 2004; Dittmer et al., 2011; Dittmer and Thompson, 2011).

The concentrations of bone formation marker (OC) as well as bone resorption marker (SCL) in serum were higher in goat kids than in lambs, which leads to a higher bone turnover and is conform with reduced mineralization of the bones (Lips and van Schoor, 2011). Also IGF-I and GH influence bone remodeling during the growth process. The pituitary gland produces and releases GH that stimulates the production of IGF-I in the liver and other tissues, which then increases osteoclastic and osteoblastic activity. It has been demonstrated in young men that IGF-I correlates well with bone turnover (Fatayerji and Eastell, 1999), therefore it was not surprising that, like in Kovács et al. (2015), goat kids had also higher GH and IGF-I levels compared to lambs. However this needs to be carefully interpreted, because two different biases could be responsible for that significant interspecies difference: the pulsatile excretion of the two hormones during the day (which can also be affected by interspecies differences), and the high difference in sensitivity of the methods used for the different species.

In the gastrointestinal tract we investigated the active Ca absorption with the background of a sufficient Ca supply. Therefore the paracellular absorption was probably sufficient to cover the Ca demand. Accordingly, even if goat kids had higher AD of Ca during the whole experiment, they did not have higher active Ca net fluxes compared to lambs. We detected an active Ca net absorption in rumen, and an active Ca net secretion in duodenum, which goes along with previous studies (Schröder et al., 1999; Sidler-Lauff et al., 2010; Kovács et al., 2015). Ruminal Ca absorption plays an important role in Ca homeostasis in ruminants, but the underling mechanisms are still not fully understood. It was previously demonstrated that, as in our study, the active trans-epithelial Ca absorption is not influenced by 1,25vitD (Schröder et al., 1999; Sidler-Lauff et al., 2010; Wilkens et al., 2011; Wilkens et al., 2012), but can be increased for example by alimentary administration of anionic salts, short-chain fatty acids or Cl (Leonhard-Marek et al., 2007; Wilkens et al., 2016). As all the groups received the same diet, the reason why the control group (GC) had an unexpected higher active ruminal Ca

absorption compared to GUV cannot be explained. Whereas the higher active net Ca secretion in duodenum in GC goes conform with the results of Kovács et al. (2015), and could be a mechanism to compensate the excess of Ca absorbed in the rumen to keep the Ca blood level constant. This supports the theory of Schroeder and Breves (2006) who showed that the net absorption in the forestomachs and abomasum is negatively correlated to the intestinal net Ca absorption in cows, indicating a kind of compensatory mechanism that still needs to be investigated. The differences between the groups that we found in goat kids but not in sheep, also indicate that probably goats adapt faster through the intestine in a situation of challenged Ca homeostasis and profit more from intestinal Ca absorption compared to sheep (Wilkens et al., 2012; Kovács et al., 2015).

In the duodenum of monogastric animals the effect of 1,25vitD on the active Ca absorption is mediated by a VDR dependent genomic action (Bronner, 2003), and in vitD deficient rats a reduction of VDR in duodenum was found (Zineb et al., 1998). In contrast, even if VDR was detected in the duodenum of ruminants (Boos et al., 2007; Riner et al., 2008), the transcellular Ca transport that is influenced by 1,25vitD is less important (Schröder et al., 1997; Sidler-Lauff et al., 2010; Wilkens et al., 2011). Indeed we found no difference between the groups in VDR expression on protein level, probably also because the vitD status of the control group was not as low as in a situation of vitD deficiency.

Considering the AD of P, we only found lower AD in goat kids compared to lambs. This agrees with previous studies that showed that in goats fecal P losses might well be so high that apparent digestibility results to be negative, but this does not reflect a negative true P digestibility. The amount of AD of P is linearly correlated to the dietary P intake, and the fecal P losses are also influenced by the dietary P intake, the salivary excretion, as well as the dry matter intake and crude fiber content in feed (Bravo et al., 2003; Tayo et al., 2009).

The excretion of Ca and P through the kidneys was also not influenced by irradiation or vitD status. As already showed in earlier studies, in ruminants in contrast to monogastric animals, the kidneys are not a main site for the regulation of Ca homeostasis (Hoenderop et al., 2005; Herm et al., 2015).

In this study healthy animals with a good initial vitD status were used to demonstrate that the irradiation can prevent the drop of the vitD status even if the nutritional vitD supply is missing and therefore keep the healthy growing going, instead of curing a initial vitD deficiency which is rare considering the currently used highly vitD supplemented diets. In summary, during these experiment the animals were in a situation of challenged Ca

homeostasis because of the growing process, but the AD of Ca and P as well as the Ca and P content in serum remained constant in both species over the whole time, and therefore the 25vit D levels and the absorbed Ca were sufficient to permit an embedment in the bones and a normal growth with increasing of BMD. Even if the vitD status in the control groups was declining, the period of the experiment was not sufficient to really achieve a vitD deficiency and therefore no negative effects on Ca homeostasis were detected. However an effect of the declined vitD status on other physiological processes, as for example on the immune system or on cell differentiation (Holick, 2004; Dittmer and Thompson, 2011), that were not analyzed in these study cannot be excluded. In ruminants it was demonstrated that vitD status interferes with reproductive performance in an observational study done in a wild Soay sheep population (Handel et al., 2016), and that vitD improves the immune function in cows (Nelson et al., 2010; Vieira-Neto et al., 2017). Therefore future studies should include effects on fertility and the immune system.

CONCLUSIONS

In conclusion we could demonstrate that, like cows, growing dairy goats and sheep are able to compensate a vitD reduced diet by producing vitD within their skin while exposed to UVB irradiation an therefore to keep a high vitD status and a balanced Ca homeostasis. In contrast, the vitD status of the control groups with no UVB exposure dropped, but not enough to induce an actual vitD deficiency and therefore no effects on Ca homeostasis were yet detected. In future studies, with a prolonged observation time, it should be clarified which is actually the ideal vitD status in young sheep and goats to ensure a healthy growing process and a balanced Ca homeostasis and how much UVB irradiation is needed to achieve that goal with the practical consequences in livestock farming.

ACKNOWLEDGEMENTS

The authors thank Ines Mittner, Susanne von Waldow and Reto Mühlemann for their technical assistance. The laboratory work was partly performed using the logistics of the Centre for Clinical Studies at the Vetsuisse Faculty of the University of Zurich.

REFERENCES

Adams , J. S., T. L. Clemens , J. A. Parrish , and M. F. Holick 1982. Vitamin-D Synthesis and Metabolism after Ultraviolet Irradiation of Normal and Vitamin-D-Deficient Subjects. New Engl. J. Med. 306(12):722-725.

Agroscope. 2013. Fütterungsempfehlungen für Wiederkäuer (Grünes Buch). Accessed 5 Aug. 2014: http://www.agroscope.admin.ch/futtermitteldatenbank/04834/index.html?lang=de. Agroscope, Posieux, Schweiz.

Boos, A., K. Riner, M. Hässig, and A. Liesegang. 2007. Immunohistochemical Demonstration of Vitamin D Receptor Distribution in Goat Intestines. Cells Tissues Organs. 186(2):121-128.

Bravo, D., D. Sauvant, C. Bogaert, and F. Meschy. 2003. III. Quantitative aspects of phosphorus excretionin ruminants. Reprod. Nutr. Dev. 43(3):285-300.

Bronner, F. 2003. Mechanisms and functional aspects of intestinal calcium absorption. J Exp Zool A Comp Exp Biol. 300A(1):47-52.

Dittmer, K. E., E. C. Firth, K. G. Thompson, J. C. Marshall, and H. T. Blair. 2011. Changes in bone structure of Corriedale sheep with inherited rickets: A peripheral quantitative computed tomography assessment. Vet J. 187(3):369-373.

Dittmer, K. E. and K. G. Thompson. 2011. Vitamin D Metabolism and Rickets in Domestic Animals: A Review. Vet. Pathol. 48(2):389-407.

Fatayerji, D. and R. Eastell. 1999. Age-Related Changes in Bone Turnover in Men. J Bone Miner Res. 14(7):1203-1210.

Forrest, J. W. and M. R. Fleet. 1985. Lack of tanning in white Merino sheep following exposure to ultraviolet light. Aust Vet J. 62(7):244-246.

Haddad, J. G., L. Y. Matsuoka, B. W. Hollis, Y. Z. Hu, and J. Wortsman. 1993. Human plasma transport of vitamin D after its endogenous synthesis. J. Clin. Invest. 91(6):2552-2555.

Handel, I., K. A. Watt, J. G. Pilkington, J. M. Pemberton, A. Macrae, P. Scott, T. N. McNeilly, J. L. Berry, D. N. Clements, D. H. Nussey, and R. J. Mellanby. 2016. Vitamin D status predicts reproductive fitness in a wild sheep population. Sci Rep. 6:18986.

Herm, G., A. S. Muscher-Banse, G. Breves, B. Schröder, and M. R. Wilkens. 2015. Renal mechanisms of calcium homeostasis in sheep and goats. J. Anim. Sci. 93(4): 1608–1621.

Hidiroglou, M. 1987. Kinetics of Intravenously Administered 25-Hydroxyvitamin D3 in Sheep and the Effect of Exposure to Ultraviolet Radiation. J Anim Sci. 65(3):808-814.

Hidiroglou, M. and K. Karpinski. 1989. Providing Vitamin D to Confined Sheep by Oral Supplementation vs Ultraviolet Irradiation. J. Anim. Sci. 67(3):794-802.

Hoenderop, J. G. J., B. Nilius, and R. J. M. Bindels. 2005. Calcium Absorption Across Epithelia. Physiol. Rev. 85(1):373-422.

Holick, M., J. MacLaughlin, and S. Doppelt. 1981. Regulation of cutaneous previtamin D3 photosynthesis in man: skin pigment is not an essential regulator. Science 211(4482):590-593.

Holick, M. F. 2004. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am. J. Clin. Nutr. 80(6 Suppl):1678S-1688S.

Holick, M. F. 2008. Sunlight, vitamin D and health: A D-lightful story. Solar Radiation and Human Health. The Norwegian Academy of Science and Letters.147-166.

Holick, M. F., J. A. MacLaughlin, M. B. Clark, S. A. Holick, J. T. Potts, Jr., R. R. Anderson, I. H. Blank, J. A. Parrish, and P. Elias. 1980. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science. 210(4466):203-205.

Holick, M. F., N. M. Richtand, S. C. McNeill, S. A. Holick, J. E. Frommer, J. W. Henley, and J. T. Potts, Jr. 1979. Isolation and identification of previtamin D3 from the skin of rats exposed to ultraviolet irradiation. Biochemistry. 18(6):1003-1008.

Horst, R. L. and E. T. Littledike. 1982. Comparison of plasma concentrations of vitamin D and its metabolites in young and aged domestic animals. Comp. Biochem. Physiol. B 73(3):485-489.

Horst, R. L. and T. A. Reinhardt. 1983. Vitamin D Metabolism in Ruminants and Its Relevance to the Periparturient Cow. J. Dairy Sci. 66(4):661-678.

How, K. L., H. A. W. Hazewinkel, and J. A. Mol. 1994. Dietary Vitamin D Dependence of Cat and Dog Due to Inadequate Cutaneous Synthesis of Vitamin D. Gen. Comp. Endocrinol. 96(1):12-18.

Hymøller, L. and S. K. Jensen. 2010. Vitamin D3 synthesis in the entire skin surface of dairy cows despite hair coverage. J. Dairy Sci. 93(5):2025-2029.

Hymøller, L. and S. K. Jensen. 2012. 25-Hydroxycholecalciferol status in plasma is linearly correlated to daily summer pasture time in cattle at 56°N. Br J Nutr. 108(04):666-671.

Hymøller, L., S. K. Jensen, P. Kaas, and J. Jakobsen. 2017. Physiological limit of the daily endogenous cholecalciferol synthesis from UV light in cattle. J. Anim. Physiol. Anim. Nutr. 101(2):215-221.

Hymøller, L., S. K. Jensen, H. Lindqvist, B. Johansson, M. O. Nielsen, and E. Nadeau. 2009. Supplementing dairy steers and organically managed dairy cows with synthetic vitamin D3 is unnecessary at pasture during exposure to summer sunlight. J Dairy Res. 76(03):372-378.

Jakobsen, J., S. K. Jensen, L. Hymøller, E. W. Andersen, P. Kaas, A. Burild, and R. B. Jäpelt. 2015. Short communication: Artificial ultraviolet B light exposure increases vitamin D levels in cow plasma and milk. J. Dairy Sci. 98(9):6492-6498.

Jäpelt, R. B., T. Didion, J. Smedsgaard, and J. Jakobsen. 2011. Seasonal Variation of Provitamin D2 and Vitamin D2 in Perennial Ryegrass (Lolium perenne L.). J Agric Food Chem. 59(20):10907-10912.

Jones, G. 2013. Extrarenal Vitamin D Activation and Interactions Between Vitamin D2, Vitamin D3, and Vitamin D Analogs. Annu. Rev. Nutr. 33:23-44.

Kohler, M., F. Leiber, H. Willems, L. Merbold, and A. Liesegang. 2013. Influence of altitude on vitamin D and bone metabolism of lactating sheep and goats. J. Anim. Sci. 91(11):5259-5268.

Kovács, S., M. R. Wilkens, and A. Liesegang. 2015. Influence of UVB exposure on the vitamin D status and calcium homoeostasis of growing sheep and goats. J. Anim. Physiol. Anim. Nutr. 99:1-12.

Kühn, J., A. Schutkowski, F. Hirche, A. C. Baur, N. Mielenz, and G. I. Stangl. 2015. Non-linear increase of vitamin D content in eggs from chicks treated with increasing exposure times of ultraviolet light. J. Steroid Biochem. Mol. Biol. 148:7-13.

Leonhard-Marek, S., G. Becker, G. Breves, and B. Schröder. 2007. Chloride, Gluconate, Sulfate, and Short-Chain Fatty Acids Affect Calcium Flux Rates Across the Sheep Forestomach Epithelium. J. Dairy Sci. 90(3):1516-1526.

Liesegang, A., D. Hüttenmoser, J. Risteli, F. Leiber, M. Kreuzer, and M. Wanner. 2013. Influence of high-altitude grazing on bone metabolism of growing sheep. J. Anim. Physiol. Anim. Nutr. 97(1):58-66.

Liesegang, A. and J. Risteli. 2005. Influence of different calcium concentrations in the diet on bone metabolism in growing dairy goats and sheep. J. Anim. Physiol. Anim. Nutr. 89(3-6):113-119.

Lips, P. and N. M. van Schoor. 2011. The effect of vitamin D on bone and osteoporosis. Best Pract. Res. Clin. Endocrinol. Metab. 25(4):585-591.

MacLaughlin, J. A., R. R. Anderson, and M. F. Holick. 1982. Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. Science 216(4549):1001-1003.

Morris, J. G. 1999. Ineffective Vitamin D Synthesis in Cats Is Reversed by an Inhibitor of 7-Dehydrocholestrol-Δ7-Reductase. J. Nutr. 129(4):903-908.

Nelson, C. D., J. D. Lippolis, T. A. Reinhardt, R. E. Sacco, J. L. Powell, M. E. Drewnoski, M. O'Neil, D. C. Beitz, and W. P. Weiss. 2016. Vitamin D status of dairy cattle: Outcomes of current practices in the dairy industry. J. Dairy Sci. 99(12):10150-10160.

Nelson, C. D., T. A. Reinhardt, T. C. Thacker, D. C. Beitz, and J. D. Lippolis. 2010. Modulation of the bovine innate immune response by production of 1α,25-dihydroxyvitamin D3 in bovine monocytes. J Dairy Sci. 93(3):1041-1049.

Nesbitt, T. and M. K. Drezner. 1993. Insulin-like growth factor-I regulation of renal 25-hydroxyvitamin D-1-hydroxylase activity. Endocrinology. 132(1):133-138.

Norman, A. W. 2011. Vitamin D nutrition is at a crossroads. Public Health Nutr. 14(4):744-745.

Pugh, D. G. 2002. Sheep and Goat Medicine. 1st edn. W. B. Saunders Company, Philadelphia, USA.

Quarterman, J. D. A. C., Adam A. 1964. Some factors affecting the level of vitamin D in the blood of sheep. Brit. J. Nutr. 18:79-89.

Riner, K., A. Boos, M. Hässig, and A. Liesegang. 2008. Vitamin D receptor distribution in intestines of domesticated sheep Ovis ammon f. aries. J Morphol. 269(2):144-152.

Schröder, B., I. Rittmann, E. Pfeffer, and G. Breves. 1997. In vitro studies on calcium absorption from the gastrointestinal tract in small ruminants. J Comp Physiol B. 167(1):43-51.

Schröder, B., S. Vössig, and G. Breves. 1999. In vitro studies on active calcium absorption from ovine rumen. J Comp Physiol B 169:487-494.

Schröder, B. and G. Breves. 2006. Mechanisms and regulation of calcium absorption from the gastrointestinal tract in pigs and ruminants: comparative aspects with special emphasis on hypocalcemia in dairy cows. Anim. Health Res. Rev. 7(1-2):31-41.

Sidler-Lauff, K., A. Boos, M. Kraenzlin, and A. Liesegang. 2010. Influence of different calcium supplies and a single vitamin D injection on vitamin D receptor and calbindin D9k immunoreactivities in the gastrointestinal tract of goat kids. J. Anim. Sci. 88(11):3598-3610.

Smith, B. S. W., Wright H. 1980. The response of serum 25-hydroxyvitamin D concentrations to vitamin D intake and insolation in sheep. Br. J. Nutr (43):533.

Stangl, G.I. 2014. Die Verdauung. Pages 27-46 in Tiernährung. 14th edn. Kirchgessner, M.; Stangl, G.I..; Schwarz, F.J.; Roth, F.X.; Südekum, K.H.; Eder, K. (eds.). DLG-Verlag GmbH, Frankfurt am Main, Germany.

Tayo, G. O., S. X. Tang, Z. L. Tan, Z. H. Sun, M. Wang, C. S. Zhou, and X. F. Han. 2009. Determination of endogenous faecal phosphorus loss in goats. Arch Anim Nutr. 63(2):104-111.

Tryfonidou, M. A., M. S. Holl, M. Vastenburg, M. A. Oosterlaken-Dijksterhuis, D. H. Birkenhäger-Frenkel, W. E. van den Brom, and H. A. W. Hazewinkel. 2003. Hormonal regulation of calcium homeostasis in two breeds of dogs during growth at different rates. J Anim Sci. 81(6):1568-1580.

Van Saun, R. J. 2004. Vitamin D-responsive rickets in neonatal lambs. Can Vet J. 45(10):841-844.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. 74(10):3583-3597.

Vieira-Neto, A., I. R. P. Lima, F. Lopes Jr, C. Lopera, R. Zimpel, L. D. P. Sinedino, K. C. Jeong, K. Galvão, W. W. Thatcher, C. D. Nelson, and J. E. P. Santos. 2017. Use of calcitriol to maintain postpartum blood calcium and improve immune function in dairy cows. J Dairy Sci. 100:1-19.

Webb, A. R. and M. F. Holick. 1988. The role of sunlight in the cutaneous production of vitamin D3. Annu. Rev. Nutr. 8:375-399.

Weber, G. M., A.-K. M. Witschi, C. Wenk, and H. Martens. 2014. TRIENNIAL GROWTH SYMPOSIUM— Effects of dietary 25-hydroxycholecalciferol and cholecalciferol on blood vitamin D and mineral status, bone turnover, milk composition, and reproductive performance of sows. J Anim Sci. 92(3):899-909.

Weiss, W. P., E. Azem, W. Steinberg, and T. A. Reinhardt. 2015. Effect of feeding 25-hydroxyvitamin D3 with a negative cation-anion difference diet on calcium and vitamin D status of periparturient cows and their calves. J. Dairy Sci. 98(8):5588-5600.

Wilkens, M. R., C. Kunert-Keil, H. Brinkmeier, and B. Schröder. 2009. Expression of calcium channel TRPV6 in ovine epithelial tissue. Vet J. 182(2):294-300.

Wilkens, M. R., N. Mrochen, G. Breves, and B. Schröder. 2011. Gastrointestinal calcium absorption in sheep is mostly insensitive to an alimentary induced challenge of calcium homeostasis. Comp Biochem Physiol B Biochem Mol Biol. 158(3):199-207.

Wilkens, M. R., C. Praechter, G. Breves, and B. Schröder. 2016. Stimulating effects of a diet negative in dietary cation–anion difference on calcium absorption from the rumen in sheep. J Anim Physiol Anim Nutr. 100(1):156-166.

Wilkens, M. R., J. Richter, D. R. Fraser, A. Liesegang, G. Breves, and B. Schröder. 2012. In contrast to sheep, goats adapt to dietary calcium restriction by increasing intestinal absorption of calcium. Comp Biochem Physiol B Biochem Mol Biol. 163(3–4):396-406.

Zineb, R., B. Zhor, W. Odile, and R.-R. Marthe. 1998. Distinct, Tissue-Specific Regulation of Vitamin D Receptor in the Intestine, Kidney, and Skin by Dietary Calcium and Vitamin D. Endocrinology. 139(4):1844-1852.

Žofková, I. 2003. Pathophysiological and Clinical Importance of Insulin-Like Growth Factor-I with Respect to Bone Metabolism. Physiol. Res. 52:657-679.

Danksagung

"Alle Hindernisse und Schwierigkeiten sind Stufen, auf denen wir in die Höhe steigen."

- Friedrich Nietzsche-

Durch die Stufen dieser Arbeit in die Höhe zu steigen, war nur möglich weil ich die Unterstützung von vielen wunderbaren Menschen hatte, dehnen ich an dieser Stelle von herzen danken möchte:

Prof. Dr. med. vet. A. Liesegang für die Möglichkeit diese Arbeit durchzuführen und die Bereitstellung des Themas. Danke für die Betreuung, die reichen Anregungen, sowie für die wissenschaftliche Durchsicht der Arbeit.

PD Dr. med. vet. habil. M. Wilkens sowie ihren Mitarbeitern für die Analyse der Proben, für die konstruktive Anregungen und fachliche Unterstützung.

Ines Mittner, Susanne von Waldow und Reto Mühlemann für die Durchführung der Laborarbeiten, für die Geduld, die Unterstützung und die guten Zeiten im Labor.

Den Metzgern Paul Müller und Peter Bänziger für die Hilfe bei den Schlachtungen.

Den Tierpflegern Holger Born, Joanna Schmidt, Silvana Ressegatti und Hanspeter Müller für die gute Gesellschaft im Stall.

Besonders danken möchte ich meinen Mitdoktoranden Elisabeth Kolp, Vivien Rampling und Monika Bieri für grosse Unterstützung bei der Versuchsdurchführung, für die gute Stimmung und Hilfsbereitschaft im Team und für die Freundschaft.

Zum Schluss möchte ich noch dehnen danken die mir diese Arbeit ermöglicht haben indem sie immer an mich geglaubt haben und auf deren liebevolle Unterstützung ich immer zählen konnte: meinen Eltern Gianna Würgler und Tibor Nemeth († 12.04.2006), meinen Schwestern Annik, Svenja und Kerstin Nemeth und meinem Lebenspartner Christophe Benz.

Lebenslauf

Vorname Name	Malin Veronique Nemeth
Geburtsdatum	08.09.1989
Geburtsort	Grabs
Nationalität	CH
Heimatort bei Schweizern	Winterthur

09/1995 – 06/2000	Scuola elementare, Claro, Schweiz
09/2000 – 06/2004	Scuola media, Castione, Schweiz
09/2004 – 06/2008	Liceo Cantonale Bellinzona, Bellinzona, Schweiz
30.06.2018	Attestato di Maturià**,** Liceo Cantonale di Bellinzona, Bellinzona, Schweiz
09/2008 – 09/2013	Studium Veterinärmedizin, Vetsuisse-Fakultät der Universität Zürich, Zürich, Schweiz
30.12.2013	Abschlussprüfung vet. med., Vetsuisse-Fakultät der Universität Zürich, Zürich, Schweiz

02/2014 – 07/2017	Anfertigung der Dissertation unter Leitung von Prof. Dr. med. vet. Annette Liesegang am Institut für Tierernährung der Vetsuisse-Fakultät Universität Zürich Direktorin Prof. Dr. med. vet. Annette Liesegang
02/2014 – 08/2015	Doktorandin am Institut für Tierernährung, Vetsuisse-Fakultät der Universität Zürich, Zürich
10/2016 – Dato	Tierärztin bei Tierarztpraxis Bachtelwald AG, Wald ZH, Schweiz