Article

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Simple Summary: In 2006 to 2014, 10 cases of urolithiasis in yaks, with calcium carbonate uroliths, were confirmed in Switzerland. From 6 problem and 4 control farms a total of 99 animals were examined. In addition, roughage, soil and water samples were analyzed. The farms are distributed within different regions in Switzerland. The study revealed different Ca : P ratio from 1.56 to 7.74 : 1 in the forages and mild *hypercalcemia* in the animals of problem farms. In the univariate analysis, problem versus control farms, about 20 other significant factors emerged. The multivariate analysis has shown that altitude, CP (crude protein), P, Mg, NDF (neutral detergent fibre) and ADF (acid resistant detergent fibre) in the diet are important factors. Based on the analysis of the pedigree there was no evidence for an obvious genetic background of *urolithiasis*. The question of whether *urolithiasis* in yaks in alpine Switzerland, poses a husbandry risk, can be answered affirmatively. The calcium-rich forages play a crucial role in this multifactorial process.

Abstract: Background: In 2006 to 2014, 10 cases of urolithiasis in yaks, with calcium carbonate uroliths, were confirmed in Switzerland and at the same time a 6-fold calcium overhang in roughage in an affected farm was shown. Methods: Blood samples from 99 animals were examined from 10 farms (n=6 problem farms; n=4 control farms). In addition, roughage, soil and water samples were analyzed. The farms are distributed within different regions in Switzerland. Results: The study revealed different Ca : P ratio from 1.56 to 7.74 : 1 in the forages and mild hypercalcemia in the animals of problem farms. In the univariate analysis, problem versus control farms, about 20 other significant factors emerged. The multivariate analysis has shown that altitude, CP (crude protein), P, Mg, NDF (neutral detergent fibre) and ADF (acid resistant detergent fibre) in the diet are important factors. Based on the analysis of the pedigree there was no evidence for an obvious genetic background of urolithiasis. Limitations: Limitation is the small number of Yaks in Switzerland. Conclusion: The question of whether *urolithiasis* in yaks in alpine Switzerland, poses a husbandry risk, can be answered affirmatively. The calcium-rich forages play a crucial role in this multifactorial process.

Keywords: yak; hypercalcemia; urolithiasis

Introduction

Yak farming is becoming increasingly important in Switzerland. Yaks can replace cows in the higher regions. The yaks can graze on both the abandoned cattle and sheep pastures. In addition, the wolf (*Canis lupus*) normally does not harm the yaks. Since up to 1 km^2 of pastureland is now available per yak, depending on the alp, they are often overfed. In the Valais, the traditional Eringer cows have become heavier to perform better in traditional cow fights and grazing is therefore becoming a challenge for the pastures. Cows of other breeds are grazed up to 2000 meters above sea level (m a.s.l.). Up to 2400 m a.s.l., In the past, Eringer cows were grazed even up to the highest vegetation

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level at 3000 m a.s.l., but today it is no longer possible due to higher damage to the pastures because of the weight. Consequently, pastures are abandoned between 2400 and 3000 m a.s.l. and represent an increased avalanche danger for the villages and traffic in Valais in winter. Thus, yak farming represents ecologically sensible animal husbandry, but overfeeding in summertime might be a problem reed.

The yak (*Bos grunniens*) is an Asian wild and domestic cattle, classified as ruminants (*Ruminantia*) and horned bearers (*Bovidae*), in the subfamily *Bovinae*, the genus (*Bos*).

Very cold winters and cool summers (hardly above 10 to 15°C daily maximum temperature) determine the vegetation in Tibet, the origin of the yak.

In 2021, there were 75 yak keepers with around 1000 animals in Switzerland [1].

The yaks are mainly kept for breeding, meat production, trekking and as hobby animals for extensive landscape maintenance.

The formation of kidney stones is a multifactorial process. Current theories assume a nidus-associated stone formation of calcium-based *urolithiasis*, whereby an unfavorable ratio of promoters to inhibitors of lithogenesis also plays an important role beside of the pH. In addition, there are also indications of a genetic component and gender [2–4]. Single genes were identified, which increase the risk for stone formation in dogs [5] and cows [6].

Hereditary Calcium Oxalate Urolithiasis, Type 1 (CaOx1) and Type 2 (CaOx2), are two different autosomal recessive genetic disorders that greatly increase the risk for formation of CaOx stones in the urinary tract.

Urinary drainage disorders are much more common in male domestic ruminants. This fact can be explained by the special anatomy of the *urethra* in male ruminants. Predisposed anatomic locations for urinary obstructions in the male are the *sciatic arcus* and the *flexura sigmoidea*.

In this study, the question of whether *urolithiasis* in yaks in the alpine regions of Switzerland poses a husbandry risk was investigated. The hypothesis was that elevated calcium levels in roughage led to *hypercalcemia* and thus to the formation of calcium carbonate stones.

Materials and Methods

Ten farms were visited between the beginning of December 2016 and mid-April 2017 and roughage, water, soil, urine, faecal and blood samples were examined. Since there are only about 70 - 80 farms with totally about 1000 animals in Switzerland, it was not possible to make a randomized selection of farms. Initially, 4 problem farms, i.e., farms in which *urolithiasis* in the last 11 years had been detected and 6 farms without *urolithiasis* were included in the study. During the study, two farms switched from control farms to problem farms (6 problem farms and 4 control farms) due to the extended anamnesis.

Since the problem cases all occurred during the winter feeding, the sampling was also placed in this period. Winter feeding is defined in this study as not going to pasture and eat roughage compared to eating variable amounts and quality of grass on the pasture. Additionally, drinking water is more available in summer than in winter in the study region of Switzerland due to freezing water. If minerals are given in the right amount could not be evaluated sufficiently. In some farms, winter feeding starts at the beginning of October and lasts until the beginning of June (33 weeks). Except for one farm, which has imported part of its basic feed component from Germany, all of them feed their own or regional roughage. The selected farms are located at an altitude of between 488 and 1777 m a.s.l..

The aim was to sample 10 animals per farm, ideally male and female of all ages.

At the time of the farm visit, there were 361 animals on the 10 farms, with 150 animals in the largest and 9 in the smallest. Blood samples were collected and examined from 94 animals. Very uncooperative animals were excluded from the investigations for safety reasons for humans and animals. The youngest animal was 14 months old at the time of the blood sampling, the oldest 188 months. The median age was 45 months.

Urine could only be collected from 6 animals by means of spontaneous urine. Catheterization for urine collection and an ultrasound examination of the kidneys could not have been established due to excessive use of force in relation to the increase in knowledge, in accordance with Swiss animal welfare law.

Vacutainer® sterile (Becton-Dicinson AG, Allschwil, Switzerland; 10 ml; 367896) with potassium oxalate (368921) and sodium fluoride (2 ml; 367764) and with potassium EDTA (5/10 ml; 366643) were used for blood sampling.

The urine analysis was performed on a Cobas Mira S® (Roche, Basel) in the laboratory of the Department of Farm Animals, Department of Outpatient and Population Medicine, Vetsuisse Faculty of the University of Zurich. They included the following parameters:

Na (sodium): Flame photometer IL 243®. Urine previously diluted 1:2 with 1N HCL

K (potassium): Flame photometer IL 243®. Urine previously diluted 1:2 with 1N HCL

Mg (magnesium): photometric determination. Cobas Mira S®. Roche Diagnostics GmbH, Mannheim, Germany.

Ca, P, protein, pH and sediment in the urine were not determined in this analysis.

Most of the blood samples for collecting serum or plasma were centrifuged and pipetted on site within 6 hours after sampling and then sent by express mail with a cold bag to the laboratory of the Department of Farm Animals, Vetsuisse Faculty of the University of Zurich.

The serum or plasma tests were carried out on a Cobas Mira S® (Roche, Basel) in the aforementioned laboratory and included the following parameters:

FFA (free fatty acids): enzymatic colour test, Wako 994 - 75409 D

BHB (beta-hydroxybutyric acid): enzymatic determination. Sigma Diagnostics, Procedure No. 310-UV

Ca (calcium): methylthymol blue reaction. Cobas Mira S®. Roche Diagnostics GmbH, Mannheim, Germany

P (Phosphor): Phosphomolybdat, UV-Test. Cobas Mira S®. Roche Diagnostics GmbH, Mannheim, Deutschland

Mg (magnesium): photometric determination using Mg-Kit Bio Mérieux SA, Lyon

GLDH (Glutamate Dehydrogenase): Optimized Standard Method of the German Society for Clinical Chemistry with Oxalglutarate +NADH+NH4

GGT (gamma-glutamyl-transferase): kinetic color test DGKC with L-gamma-glutamyl-3-carboxy-4-nitoanilide + glycilglycerin.

The following investigations were carried out by SYNLAB. Vet. GmbH, Augsburg, Germany:

Hb (hemoglobin): Photometric measurement of the reaction product cyanmethemoglobin, using ADVIA from Siemens

GSH-Px (glutathione peroxidase): photometric measurement, AU680 from Beckman Coulter, with the Randox reagent kit.

The following investigations were carried out at the laboratory of the Institute of Animal Nutrition and Dietetics, Vetsuisse Faculty University of Zurich (www.tierer.uzh.ch). The laboratory-kits were evaluated for bovine.

OC (osteocalcin): competitive enzyme immunoassay, using MicroVue Osteocalcin EIA Kit, Quidel Corporation, Athens, USA

PTH (Parathormon): competitive enzyme immunoassay, using MicroVue Human PTH Kit, Quidel Corperation, Athens, USA

25-hydroxy-Vitamin-D: radioimmunassay, using 25-Hydroxy-Vitamin-D RIA, Immunodiagnostic System Farm, Newcastle, GB.

During the farm visits faecal samples were obtained rectally. The farmers had been instructed not to deworm their animals before the study. The periods since the last anthelmintic treatments comprised at least 6 months. The faecal material was stored at 4°C and examined within one week after sampling with exception of the Baermann technique[7], which was done at the same or the following day. The samples were additionally analysed with the combined sedimentation/flotation technique, the McMaster method (sensitivity 50 eggs per gram [epg]) and the Ziehl-Neelsen staining [7,8] Faecal cultures for differentiation of strongyle larvae were done with faeces of maximal 6 animals each (MAFF, 1986) [7].

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Genomic DNA of all yaks was isolated using the Qiagen DNeasy Blood & Tissue Kit (Qiagen 69504, Germany). Pedigree-Viewer software was used to prepare a pedigree of yak family [9].

On average, two to three roughage or hay samples per farm were collected during the farm visits. They were analyzed at the laboratory of the Institute of Animal Nutrition and Dietetics, Vetsuisse Faculty University of Zurich (http://www.nutrivet.uzh.ch) sensory, microscopic and botanical investigations were performed. Furthermore, a proximate analysis and a van Soest fibre analysis was carried out.

The minerals and trace elements were analyzed at UFAG Laboratorien AG, Sursee, Switzerland. Detailed description of the methods on www.ufag-laboratorien.ch (17.9.23).

In order to compare the farms with each other, the mean values of the individual farms were used.

The soil samples were taken by the yak farmers themselves at several locations on the roughage pastures. The soil analyses were carried out by the Laboratory for Soil and Environmental Analysis, Thun. Detailed description of the methods can be found on www.lbu.ch (17.9.23).

The following parameters were analyzed in the soil, according to the method described by lbu, (Labor für Bodenanalyse und Umweltanalytik, Thun, Switzerland) www.lbu.ch (17.9.23):

Humus, clay, silt (Touch test)

pH: pH (1:2.5 H_2O)

Available (water-soluble) nutrients: nitrate, phosphorus, potassium, calcium, magnesium

Reserve nutriments: phosphorus, potassium, calcium, magnesium

The water samples were taken by the yak farmers themselves, while the water from the drinking facilities was microbiologically and physico-chemically analyzed by the laboratory of the Food Safety and Veterinary Office of the Canton of Fribourg, www.fr.ch/lsvw.

All data were collected using an Excel® spreadsheet and transferred in STATA (StataCorp., 2017; Stata Statistical Software®: Release 15.1; College Station, TX, USA: StataCorp LP). All data were checked for normal distribution using the Shapiro-Wilk test. Non-normally distributed data has been transformed where possible. Normally distributed data were given as mean ± standard deviation, and non-normally distributed data as median, minimum, and maximum.

The significant and tendentious differences in the continuous data were tested using analysis of variance (ANOVA), linear regression and one-way ANOVA with Bonferroni post-hoc testing. In the univariate models, the unpaired two-sided t-test or the paired two-sided t-test was performed for continuous data. For categorical data, the chi-square test was applied at $n \geq 5$ / cell and the Fisher's exact test at $n < 5$ / cell. In addition, "General Linear Model" (GLM) was applied. The Akaike Information Criterion (AIC) was used to optimize the models. The inclusion criterion for the step back procedure was a p-value of < 0.2. A p-value of ≤ 0.05 was considered for the final model (Altmann et al., 1994) [10]. Basically, a p-value of ≤ 0.05 was considered significant and a p-value of $0.05 < p < 0.2$ was considered a tendency.

Results

All 7 stones that could be examined were calcium carbonate (n = 5) or calcite stones (n = 2).

The problem farms are located exclusively in mountain zones III (farms on high altitude and other factors) and IV (farms on very high altitude and other factors), the control farms in all cadastral zones. The location of farms is shown in Table 1 and Figure 1. There is a significant correlation between m a.s.l. in problem and control farms.

Table 1. Cadastral zone distribution and sampled yaks.

Zone	Total Farms	Control Farms	Yaks	Problem Farms	Yaks
Valley	1	1	10	0	0
Mountain I	1	1	9	0	0
Mountain I – II	1	1	10	0	0
Mountain II – IV	1	1	10	0	0

Mountain III – IV	1	0	0	1	5
Mountain IV	5	0	0	5	50
Total	10	4	39	6	55

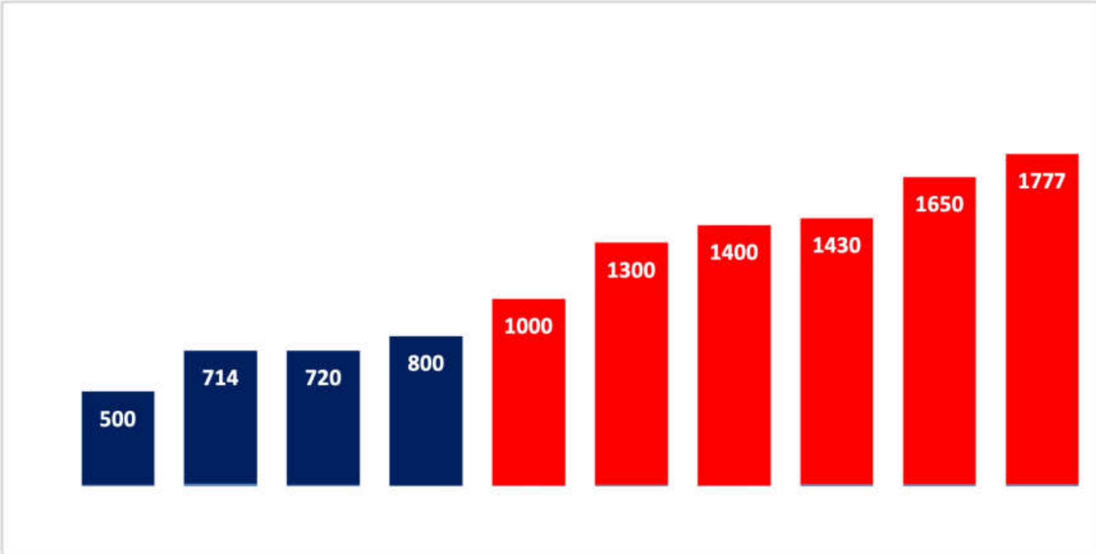

Figure 1. Altitude of farms (m a.s.l.); blue = control farms, red = problem farms.

The duration of the urolithiasis problem at the beginning of study varies between one and eleven years. In the case of two farms, problems only arose after the sample had been collected.

As can be seen in Table 2, the average duration of winter feeding in the problem farms (28.0 ± 5.0 weeks) is on average seven weeks longer than in the control farms (20.5 ± 1.7 weeks), with three of the problem farms having relatively short winter-feeding periods (not significant).

Urine samples from six yaks were examined. Of these, five were taken in problem farms and one in a control farm. All values are within the reference range for cattle (Table 2).

Table 2. Summary of urine analysis.

Parameter	mean	sd	min	p50	max	N	Benchmarks
Na, mmol/L	15.0	2.0	13.0	14.5	18.0	6	>10
K, mmol/l	292.0	72.0	176.0	299.0	384.0	6	<400
Mg, mmol/l	37.1	9.2	25.2	39.3	49.1	6	>5 - 10

N: samples; SD: standard deviation; Calcium could not be analyzed.

Three problem farms have significantly higher blood levels for Ca on average. In the case of P, a problem farm has a significantly higher mean value than the reference value for cattle. Two control farms and one problem farm are at the upper limit of the reference value (Table 3). Except for one control farm, all farms have significantly higher values for Mg than the reference values for cattle.

Table 3. Blood values.

Parameter	Case			Control			difference	reference
	Mean	SD	N	Mean	SD	N		
Ca, mmol/l	2.5	2.3	56	2.2	0.5	39	***	A: 2.3 – 2.6 B: 1.48 - 3.10
P, mmol/l	2.3	2.3	56	2.3	0.6	39	n.s.	A: 1.3 - 2.4 B: 0.81 – 4.43

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Mg, mmol/l	**1.6**	**1.4**	**56**	**1.3**	0.3	**39**	***	A: 0.80 - 1.0 B: 0.37 – 1.00
OC, g/ml	124.2	113.6	56	100.2	110.6	39	n.s.	
PTH, pg/ml	190.7	208.2	47	222.7	381.8	34	n.s.	
25-OH-Vit-D, nmol/l	134.8	127.1	56	114.5	29.0	39	n.s.	
BHB, µmol/l	358.6	332.5	56	284.7	133.9	39	**	A: < 900.00
FFA, mEa/l	**0.25**	0.30	**56**	**0.38**	0.20	**39**	***	A: < 0.100
GGT, U/l	11.4	16.9	45	23.2	22.9	38	n.s.	A: < 25
GLDH, U/l	**38.9**	54.1	**39**	**76.2**	62.9	**55**	***	A: < 10

N: samples; SD: standard deviation; n.s. = P>0.05; * = P < 0.05, ** = P < 0.01, *** = P < 0.001; A: reference of the section for herd health, University of Zurich; B: International Species Information System[14]; bold: above reference, italic: below reference.

At BHB, all concentration at farm level are within the reference range for cattle. However, the values are significantly higher for the problem farms than for the control farms. On the other hand, all values for FFA are above the reference value for cattle. Overall, the values of the control farms are significantly higher.

The values for GGT were increased for two control farms and the values for GLDH are increased for all farms. The activity of GLDH was significantly higher in the control farms than in the problem farms. It is worth mentioning that one of the control farms had a known problem with *Fasciola hepatica*. As expected, the values of this farm were well above the normal values.

For the feed samples, two to three samples were analyzed per farm. At the control farms, only grass-rich samples were found. For dry matter (DM; p<0.01) and for CF (crude fibre), NDF (neutral detergent fibre) and ADF (acid resistant detergent fibre; p<0.001) had significantly higher mean values than the problem farms. The problem farms, on the other hand, show significantly (p<0.001) higher mean values for CP (crude protein), SF (Soxhlet fat); and ADL (acid detergent lignin; Table 4). If the Ca values of roughage are compared at farm level, only one control farm that obtains its roughage from abroad is in the reference range. Two control farms had values that are too low and all problem farms are higher than the reference range. The Ca value in the feed was clearly dependent on the altitude m a.s.l. of the farm. In the case of the P-values, both the mean value of the problem farms and that of the control farms are below the reference value (Table 4). In the study, the control farms show a slightly significantly higher value than the problem farms. On our farms, there was no correlation between the P-value in the feed and the altitude. Figure 2 shows the high Ca – P ratios of the problem farms as a result of the high Ca and low P values. All values for K in roughage are within the reference range which is higher in Switzerland than in other countries. For Na, on the other hand, the value is well below the reference range for all farms which is a frequent finding in Switzerland. In the case of the S, only one problem operation is in the reference range, the others are all too low. For Mn, Cu, Zn, Se, Co, J, Fe and Mo there are large differences between the farms, but no difference between the case and control farms.

Table 4. Feed analyses of hay [15,18,19].

Parameter	Case			Control			difference	reference
	Mean	SD	N	Mean	SD	N		
DM %	94.2	94.8	18	95.7	2.2	10	**	-
CP g/100g DM	*10.3*	*10.2*	18	*8.4*	*2.4*	10	***	A: 10.4 –14.9
CF g/100g DM	27.3	27.9	18	**29.5**	2.6	10	***	A: 23.9 – 27.3
SF g/100g DM	*1.9*	1.6	18	0.5	*1.2*	10	***	LO: 2.3 – 3.0
NDF g/ 100g DM	**57.5**	58.6	18	**61.4**	3.6	10	***	A: 49.8 – 55.0
ADF g/ 100g DM	**35.1**	35.3	18	**36.6**	2.7	10	***	A: 28.2 – 31.6
ADL g/ 100g DM	6.2	5.9	18	5.5	0.5	10	***	N: 3.8 – 17.1
Ca g/kg DM	**9.1**	**7.9**	18	5.1	**2.4**	10	***	TD: 4.5– 6.5
P g/kg DM	*1.5*	1.6	18	*1.7*	0.4	10	n.s.	TD: 3.0– 4.0

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Mg g/kg DM	2.9	2.5	18	*1.7*	0.8	10	***	TD: 2.0– 3.0
K g/kg DM	18.5	20.3	18	22.1	4.5	10	n.s.	U: 15.0 –30.0
Na g/kg DM	*0.12*	*0.09*	18	*0.05*	*0.11*	10	n.s.	U: 2.0 – 3.0
S g/kg DM	*1.7*	*1.7*	18	*1.5*	0.3	10	n.s.	U: 2.0 – 4.0

N: samples; SD: standard deviation; n.s. = P>0.05; * = P < 0.05, ** = P < 0.01, *** = P < 0.001; A = average value Agridea 201619; LO=Upper Austrian Chamber of Agriculture31; N= Nater S. et al. 200632; U=Target area UFAG33; bold: above reference, italic: below reference; TD: Laboratory of the Institute of Animal Nutrition and Dietetics, Vetsuisse Faculty, University of Zurich (www.tierer.uzh.ch); DM: dry matter; CP: crude protein; CF: crude fibre; SF: Soxhlet fat; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin.

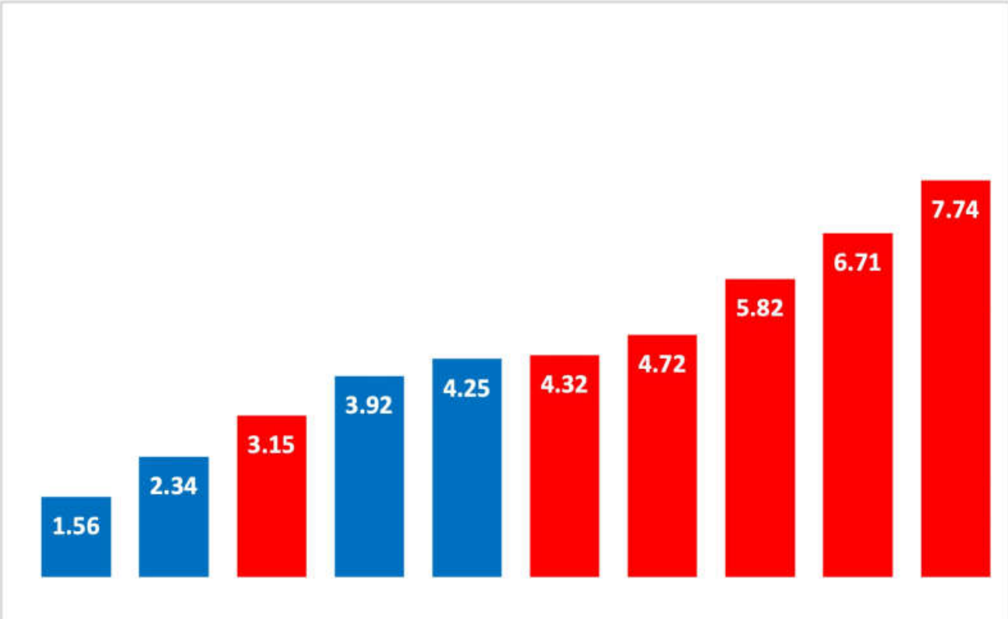

Figure 2. Ca: P ratio in roughage; red = problem farms, blue = control farms.

In the soil samples, humus, clay, silt, soil pH, available N, available P, available K, available Ca, available Mg, reserve P, reserve K, reserve Ca**, r**eserve Mg, soil Bo, soil Mn, soil Cu and soil Fe differences between farms were evaluated, but no difference is found between the case and control farms.

For the minerals of the water and the degree of hardness, the control farms show significantly higher values than the control farms (Table 5; p < 0.05). The water is softer in the control farms than in the problem farms.

Table 5. Tap Water analyses.

Parameter	Case			Control			difference
	Mean	SD	N	Mean	SD	N	
Ca mg/l	44	50.77	6	60	25.11	4	**
Mg mg/l	7	10.32	6	15	9.62	4	***
fH° water hardness	14.62	17.02	6	20.83	9.95	4	***

N: samples; SD: standard deviation; ** = P < 0.01, *** = P < 0.001.

By means of a stepback procedure taking into account the AIC (Akaike information criterion), the multivariate model (GLM, general linear model) with variance function Gaussian, shows that the altitude, crude protein, NDF, ADF, ADL, Ca in the feed, Mg in the feed, and P in the feed differ significantly in the control and problem farms. As a result of the loss of degrees of freedom, subgroups such as water-, soil-, blood- and roughage-parameters had to be set up first with altitude

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as a fixed term. From these variables, an overall model for GLM was created. All interactions had to be eliminated due to colinearity.

Genomic DNA could be isolated for all yaks and may be analyzed later if candidate genes are recognized, or DNA regions of interest will be identified by genome-wide association mapping. A pedigree of the yaks was prepared, starting from the animals affected by *urolithiasis*. The analysis of the pedigree did not reveal evidence for a simple mendelian inheritance of the trait.

Discussion

We could demonstrate, that *urolithiasis* is a problem for yak farming in Switzerland and that there is a connection between the increased cases of *urolithiasis* in the alpine regions and the high calcium and magnesium levels in roughage.

Yak farming in Switzerland is certainly a niche sector in agriculture. However, the animals thrive quite well here despite the environmental factors that vary from their area of origin. Originally from a semi-arid continental area and significantly higher above sea level than in the Swiss Alps and a nomadic life that involves a lot of exercise, they have adapted to our temperate climate, lower altitude, and reduced movement to a certain extent. The lush vegetation for yaks, even on the very highest pastures in Switzerland, leads to more frequent adipose yaks compared to domestic cattle. As a result of high protein content in the forage at higher altitudes, the freely available mineral content changes. Proteins as ampholytes can bind minerals differently. This alters the bioavailability of the minerals and can affect the risk of *urolithiasis*. In humans, it is generally assumed that there is a genetic predisposition to *urolithiasis*.

The problem farms differ significantly in their altitude from the control farms. This has an influence on the feed composition.

Since almost all cases of *urolithiasis* occurred during winter feeding, it was reasonable to assume that the water uptake, even in the mild winter climate of Swiss Alps was low. Additionally, the main feeding in winter is hay and in summer gras. Hay has a dry matter content of around 90%, whereas gras has a contend of 20%. Water-availability can be restricted I wintertime due to freezing conditions. This can lead to poorer renal clearance for calcium. It turns out that the duration of the winter-feeding period does not depend solely on the altitude. On the one hand, some farms leave their animals longer on the farm pastures around the winter quarters in autumn, on the other hand, depending on the sun exposure, the pastures can also be used earlier in spring.

The small number of urine samples does not allow a statistically stable statement. Five of the six samples came from problem farms. Protein in the urine can have various causes and, as mucoprotein, promote the formation of urinary stones. These parameters would certainly be interesting to investigate and compare in a future study.

Although the total of the data per parameter was subjected to a normality test and showed a satisfiable normal distribution, the division into cases and controls showed skewed distributions on several occasions.

Hypercalcemia and hypermagnesemia were detected. In our study, the elevated serum concentrations show a correlation with the elevated Ca and Mg levels in roughage. There is also a correlation with the altitude of the farm [11,12].

The soil conditions have an influence on the P-value in the feed. Fine-grained, clayey raw soils have the highest P values in the feed, podsols the lowest [22]. The roughage from higher altitudes (> 1000 m a.s.l.) has a high proportion of herbs, which contain significantly more P than grasses, but at the same time massively more Ca. The concentration in the feed correlates with the values in the blood. The values for P were below the reference values. The reason for this could be the less intensive farming, especially of mountain organic farms. The less intensive fertilization and use, as well as the later stage of development of the plants during the later prescribed harvest, are decisive here. Although the control farms are also organic farms, they are located in lower regions with a different climate and botanical composition. Only in two of the control farms the Ca : P ratio is close to the target range of 2 : 1 for ruminant feed. The largest difference in the Ca : P ratio was 8 : 1 in problem farms [13].

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The influence of 25-Hydroxy-Vitamin-D is unclear. The 25-Hydroxy-Vitamin-D values in the problem farms are on average higher than in the control farms, but there is no significant difference.

The quality of the roughage was good. The samples from the control farms contained only grass-rich roughage. No poisonous plants that may play a role in calcium metabolism were found in the feed samples.

The values for the minerals Ca and Mg as well as the degree of hardness in the water samples are higher in the control farms. Since the yaks absorb rather small amounts of water compared to cattle, the amount of minerals in the water probably has a small influence. The amount of water absorbed has not been determined.

It has been shown that at both locations, in Switzerland and in China, Ca : P ratios between 5 : 1 – 11 : 1 occur at times and the magnesium values in China are also 3 times higher in winter than in summer, while the value is similar to our samples [13]. Another major difference is to be found mainly in the protein content, and to a lesser extent in the crude fibre content of the feed. On average, the roughage in the problem farms examined was higher in protein and lower in crude fibre than that of the control farms and in some cases also than the samples examined from the area of origin, in the winter period.

In this study, the influence of parasites on the general health of yaks in Switzerland was also investigated to rule out parasites as a possible confounder of urolithiasis [16,17,20,21] The presence of parasite load did not affect the incidence of urolithiasis. Results will be published elsewhere.

We can conclude from this study that the broad Ca : P ratio and the protein-rich and low-crude fibre feed rations are decisive for the formation of urinary stones for the animals in Switzerland if one compares the problem farms with the control farms. This was also shown by the multivariate analysis, in which the factors altitude, CP, NDF, ADF, ADL, as well as Ca, Mg and P values in roughage significantly influence the presence of *urolithiasis* in yaks in Switzerland. The altitude directly or indirectly influences most of the values in the feed. Since it is probably a multifactorial event, other factors that are not related to the feed are involved in the formation of urinary stones.

To avoid further cases in the future, yak owners should increasingly have their roughage analyzed in order to compensate for one-sided surpluses or deficiencies in minerals and be aware of water supplementation. The anion gap has to be controlled for to stabilize pH.

Dominant animals may hinder other animals from the mineral sources in yaks has generally been understudied [14]. Efforts should be made to encourage the animals to exercise more during the winter period. Many yaks are obese as a result of the good feed quality and the large pastures in summer, up to one km^2 per yak, although the additional influence of castration is still unclear.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Ethics Statement: For the collection of the urine and blood samples, an animal experiment permit was issued by the Cantonal Veterinary Office of Valais, Switzerland, which was also valid for all other cantons in Switzerland (VS 04/16). All procedures were fulfilling European Animal Welfare legislation.

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