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Research article

Ruminal pH in cattle (*Bos primigenius* f. *taurus*) and moose (*Alces alces*) under different feeding conditions: a pilot investigation

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Abstract
Feeding recommendations for captive wild ruminants strictly restrict the use of high-starch/low fibre concentrates and fruits and vegetables, because of their potential to induce acidotic conditions in the forestomach. Nevertheless, such items are still used, and actual measurements documenting the consequences are rare. We used a captive moose (*Alces alces*) and two domestic cows (*Bos primigenius* f. *taurus*), equipped with intraruminal pH sensors, to monitor the short-term effects of five diets (a 'natural diet' of browse for moose and grass hay for the cows; a grass diet; an alfalfa hay diet; and a diet where concentrates, apples and carrots were offered, along with an ad libitum roughage source, at two increasing levels – ration 1 and 2, respectively). Lowest mean pH and highest pH variability were measured on ration 2. The provision of concentrates/produce in two meals per day (0800 and 1600) resulted in distinct pH differences between day and night periods. Differences in the amount of roughages accepted (for example, the moose refused the freshly cut grass, and the cows had low intakes on the alfalfa hay offered) could explain differences in the level and course of pH observed between diets. No particular species differences were noted that did not relate to roughage acceptance. These results underline that using roughages, and restricting/avoiding the use of concentrates and produce, will result in more stable forestomach conditions that are possibly favourable for ruminant health.

Introduction

In 1970, Dirksen summarised the pathogenesis of acute rumen acidosis in cattle and mentioned the syndrome of 'chronic compensated acidosis' as a milder and prolonged form. This subacute rumen acidosis (SARA) is characterised by a transient drop of rumen pH to values around 5.5 (Nordlund and Garrett 1994; Garrett et al. 1999) and is caused by lack of structural fibre and/or an excessive intake of concentrates such as rapidly fermentable carbohydrates (reviewed e.g. in Kleen and Cannizzo, 2012). Several health consequences, including reduced feed intake, rumenitis, liver abscesses, abomasal displacement, laminitis, diarrhoea and reduced fertility, and altered milk composition, are discussed and labelled responsible for great financial losses in the cattle industry (reviewed in e.g. Krause and Oetzel 2006; Plaizier et al. 2008; Enemark 2009; Kleen and Cannizzo 2012).

However, SARA has not only been described in domestic ruminants but has also occurred in captive wild ruminant species. Elze et al. (1978) describe rumen acidosis as the most common cause of alimentary digestive disorders in several captive specimens of muskoxen (*Ovibos moschatus*), Père David's deer (*Elaphurus davidianus*) and giraffe (*Giraffa camelopardalis*), and Marholdt (1991) reported acidosis-induced lesions of the rumen mucosa in a large set of wild ruminants kept in zoos. The suggestion that SARA might contribute to compromised hoof health observed in zoo ruminants (Clauss and Kiefer 2003) was corroborated by Zenker et al. (2009), who observed a connection between offering higher proportions of concentrated feed, a lower ruminal pH, and impaired claw health when comparing groups of Himalayan tahr (*Hemitragus jemlahicus*) and blackbuck antelope (*Antilope cervicapra*). Acidotic conditions in the rumen compromise the mucosal integrity, so that bacterial toxins can pass into the bloodstream and cause damage at predilection sites such as the hoof capillaries. Similar reports on high amounts of easily fermentable carbohydrates as a risk factor for hoof problems exist in captive giraffe (Hummel et al. 2006b) and moose (*Alces alces*) (Clauss et al. 2009). Recently, Schilcher et al. (2013) reported histological evidence for rumen acidosis in four captive wild ruminant species fed diets high in easily fermentable carbohydrates. Other studies mention rumen acidosis in farmed elk (*Cervus elaphus*) (Woodbury et al. 2005) or free-ranging ruminants such as white-tailed deer (*Odocoileus virginianus*) (Wobeser and Runge 1975; Woolf and

Kradel 1977), roe deer (*Capreolus capreolus*) (Sugár 1983) and moose (Butler et al. 2008), often associated with supplemental feeding.

As in cattle, the consumption of highly fermentable feedstuffs is suspected to be the primary cause for rumen acidosis in captive wild ruminant species, although studies providing actual evidence are scarce. Nevertheless, feeding recommendations suggest trying to avoid acidosis induced by concentrates in supplementary feeding of free-ranging wild ruminants (Woolf and Kradel 1977; Rehbinder and Ciszuk 1985), and in feeding captive specimens (Clauss and Dierenfeld 2008). Ruminant species adapted to a natural diet of browse appear to be particularly susceptible to rumen digestive problems, acidosis and reduced longevity (Marholdt 1991; Hofmann and Nygren 1992; Clauss et al. 2003; Clauss and Dierenfeld 2008; Müller et al. 2011). The reasons for this may lie in these animals' general reluctance to ingest grass hay or other roughages readily available in zoos, with a consequent disproportionately high concentrate intake. Therefore, it has become current practice to produce pelleted feeds high in fibre particular targeted at these species (Clauss and Dierenfeld 2008; McCusker et al. 2011). Whether other differences in the digestive physiology of wild ruminants (Clauss et al. 2008; Codron and Clauss 2010) result in a difference in acidosis susceptibility remains to be investigated.

Nevertheless, and also in spite of current feeding recommendations (Oftedal et al. 1996; Lintzenich and Ward 1997; Hummel and Clauss 2006), pelleted feeds high in starch, and fruits and vegetables high in sugar, are still widespread in captive wild ruminant husbandry (Clauss et al. 2002; Flores-Miyamoto et al. 2005; Hummel et al. 2006b; Wright et al. 2011; Schilcher et al. 2013; Taylor et al. 2013). A common husbandry procedure is to provide such items as a morning and an afternoon feed in the daily routine – a practice that should, theoretically, lead to two separate peaks of fermentation, and hence acid production and low pH, in the ruminants (Hummel et al. 2006a). We applied intra-ruminal pH meters in a captive moose and two cattle to record the course of pH measurements across the day on different diets, and to corroborate the suspicion that the moose – a browser in natural conditions – might be more susceptible to acidotic conditions in the rumen than cattle.

Methods

Animals

Two lactating, non-pregnant, multiparous Holstein Friesian cows (Cow 1: 720 kg, 4.6 years; Cow 2: 770 kg, 5.9 years) without clinical aberrations from normal eating and ruminating behaviour were kept in a tie stall at the Vetsuisse Faculty, University of Zürich, Switzerland, and milked only once a day in order to keep daily production below 10 litres, to allow a less biased comparison with the non-lactating moose. The bull moose (200 kg, 2 years) at the Wildnispark Zürich, Switzerland, was kept individually in an outdoor pen with access to a stable. The study was conducted under Animal Care and Use Committee License Number 84/2010.

Feeding regime

Before the insertion of the rumen bolus, the animals were kept for 14 days on a constant diet. This was grass hay only for the cows, and fresh browse (consisting mainly of *Salix* spp.) for the moose. After the insertion of the rumen bolus, the animals were first tested on the respective adaptation diets ('natural diet'); subsequently, they received a typical 'zoo ration' ('ration 1') consisting of a roughage source ad libitum (grass hay for the cows, alfalfa chaff for the moose), combined with a limited amount of pelleted feeds, apples and carrots (Table 1). In a third step ('ration 2'), the amounts of concentrates, apples and carrots were doubled. Afterwards, both cattle and moose received a 'fresh' source of grass (ensiled for cattle, freshly cut for moose; 'grass diet'), followed by a final period of alfalfa hay only ('alfalfa diet').

Table 1. The composition of the five different diets offered to the two cows (*Bos primigenius* f. *taurus*) and the moose (*Alces alces*).

Diet name	Species/ individual	Composition		Consumption[1]	Days offered
Natural diet	Moose	Browse		not measured	7
	Cow 1	Grass hay		17	
	Cow 2			20	
Ration 1	Moose	Alfalfa chaff	Concentrate, apples and carrots[2]	7	4
	Cow 1	Grass hay		9	
	Cow 2			8	
Ration 2	Moose	According to Ration 1[3]		6	4
	Cow 1			6	
	Cow 2			5	
Grass diet	Moose	Freshly cut grass		not measured[4]	4
	Cow 1	Grass silage		22	
	Cow 2			32	
Alfalfa diet	Moose	Alfalfa chaff		7	4
	Cow 1	Alfalfa hay		7	
	Cow 2			7	

[1]Amount of roughage eaten per animal and day in kg as fed.
[2]Concentrate 38 g/kg metabolic body mass, apples and carrots each 94g/kg metabolic body mass (which corresponded to the normal allowance fed to the moose).
Composition and content of the concentrates: *UFA 743 Wildfutter 13% RP, composition* = 5.5% crude ash, 13% crude protein, 2.5% crude fat, 10.5% crude fibre, 33.4% NDF, 16.1% ADF, 5.1% ADL; contents = pomace, barley, oat, soybean meal, minerals, wheat bran, grain milling residues, beet molasses, corn gluten meal, wheat starch, refining fatty acids, wheat, herbs. *ISO-HORSE COMPLETE 8MM: composition* = 9% crude ash, 10% crude protein, 3% crude fat, 15% crude fibre, 43.5% NDF, 20.1% ADF, 3.7% ADL; contents = wheat bran, cereal straw, oat bran, oat, barley, alfalfa meal, dried beet pulp, beet molasses, TradiLin 135 (linseed, wheat barn, middlings extruded), sunflower expeller cake, calcium carbonate, sodium chloride, whey feed flour, monocalcium phosphate, plant oil, magnesium oxide.
[3]Twice the dosages of concentrate, apples and carrots compared to ration 1.
[4]The visual impression was that the moose did not consume any of the grass offered.

Ration 1 was chosen as a typical diet usually used for moose in zoos or parks. It consisted of 2 kg concentrated feed, 5 kg chaffed apples and 5 kg chaffed carrots per day, of which one half was offered around 0800 and the other half at 1600. Additionally, alfalfa chaff was offered ad libitum. The concentrated feed contained a mixture of a quarter each of corn pellets and malt dry marc pellets, and two commercial products (UFA 743 Wildfutter 13% RP, UFA AG, Switzerland and Iso-Horse Complete 8mm, Provimi Kliba SA, Switzerland; composition is given in Table 1). For the cows, the dosage of the same concentrate feeds, apples and carrots was adjusted on the basis of metabolic body mass ($BM^{0.75}$), resulting in about 5.5 kg concentrate, 13.5 kg chaffed apples and 13.5 kg chaffed carrots per day, with grass hay ad libitum. Ration 2 consisted of the same components as ration 1, but with twice the

amount of concentrates, apples and carrots. Water was offered ad libitum during the whole trial, and all food offered ad libitum was weighed to record the amount consumed (Table 1). Whereas the cows consumed noticeably less food during the alfalfa diet, the moose seemed to eat hardly any fresh grass during the grass diet period, as the material in the racks appeared untouched the next day. Unfortunately, no objective measurements on food consumption are available for the natural and the grass diet in the moose. During ration 1 and 2, the moose consumed more or less the same amount of roughage as the cows, despite its distinctly smaller body mass. Due to a misunderstanding, no samples of the diet items fed were conserved for nutrient analysis. The lack of nutrient composition of the actual diet items used in this study, in particular the roughages, represents a major constraint for interpreting the results.

Technical measurement devices

Rumen boli (Kahne Bolus, Kahne Ltd, New Zealand) were used to record pH data from the animals' rumens. The boli were calibrated as recommended in the user manual prior to insertion and then administered orally. For this procedure, the moose was anesthetised by blowpipe with 1 ml of a mixture of etorphine and acepromazine (Immobilon) and 1.8 ml xylazine (2%), antagonised with 2 ml diprenorphine and 2 ml atipamezol, whereas the cows were restrained manually. Every ten seconds, data were captured and transmitted instantaneously to a portable computer by means of a yagi antenna (Kahne Receiver KR2002). Afterwards, data were exported from the software (Kahne System V5.2.4) as excel files. The percentage of missing datapoints (i.e. interruption of the 10 sec-transmission potentially due to transmission failure) averaged 28 ± 13% of measurements for the trial periods (cf. 'missing' datapoints in Fig. 1). The cows were slaughtered after the trial, allowing confirmation of the normal function of the rumen boli until the end of data capture.

Data analysis

Because the sample in this dataset is large (more than 180,000 observations across only three test subjects), standard errors around the means of treatment groups were very small. Statistical tests on these data would thus result in an increased likelihood of detecting significant differences of little relevance. To avoid this, we summarised the data by calculating the mean pH measured over each hour of the day, for each animal on each diet treatment. This procedure resulted in a dataset of 360 observations, which was then subject to statistical analysis. We used a three-way ANOVA, including all 2nd-degree interaction terms, with the time slot and diet treatment (ration) as fixed effects and animal (moose, cow 1 or cow 2) as a random effect, to evaluate effects on pH. Two models were used: in the first, time slot was a two-level effect (day or night, defined as the period from 0800:00 to 2159:59, and between 2200 and 0759:59, respectively). In the second version, the day was sub-divided into five time slots, representing two feeding periods (0800:00-1059:59 and 1600:00-1959:59), two post-feeding periods (1100:00-1559:59 and 2000:00-2359:59), and night time (0000:00-07:59:59). Significance levels were set at α=0.05. Where necessary, multiple comparisons were made using the Bonferroni *post hoc* test. The ANOVAs were carried out using the General Linear Models module of STATISTICA v8.0 (Statsoft Inc, 2007). Variances in rumen pH within and between diets for the subject animals were compared using an *F*-test, where

$$F = \frac{s.d.^2_{\text{group with higher s.d.(i)}}}{s.d.^2_{\text{group with lower s.d. (j)}}}$$

distributed with *i*-1, *j*-1 degrees of freedom.

To further characterise pH patterns of the different diets, a command line script (cross platform Perl script) was developed to count the number of readings below certain pH thresholds, and the number of consecutive readings for certain time windows (5 and 10 min, respectively) that pH measures were below certain thresholds. Additionally, we calculated the difference in pH for the day/night comparison and the difference between the maximum and minimum pH value allocated to one of the five time slots into which the day was divided. These measures were not subjected to statistical analysis.

Results

Visual inspection of the data indicated that pH levels were notably higher on grass for the moose, and on alfalfa for the two cows, compared with the other diet treatments (Fig. 1) – corresponding to the low food intakes the animals showed on these respective diets. Whereas differences across the day appeared less pronounced for the roughage-only diets (natural diet, grass diet, alfalfa diet), they appeared to be distinct for ration 1 and 2, with a clear drop in pH after the onset of feeding in the morning and a second drop after the afternoon feeding (Fig. 1). In the cows, feeding bouts, as indicated by drops in pH, appeared more frequent and shorter on the grass silage as compared to grass hay (Fig. 1).

Plotting the data for each hour of the day, for each animal and on each ration, pH levels were again clearly higher on grass for the moose and on alfalfa for the two cows, compared with the other diet treatments (Fig. 2). There were few or no consistent differences between the other diets. Visually it also appeared that night time hours (particularly between 0000 and 0800, and for the ration 1 and 2) were associated with generally higher pH levels compared with daytime hours (Fig. 2). These trends become clearer when the day is subdivided into means for day and night hours (Fig. 3).

Three-way ANOVAs confirmed that differences in pH across diet treatments were different between subject animals (animal–ration interaction $F_{8,338}$=65.480, P<0.0001 for the first model, and $F_{8,317}$=67.623, P<0.0001 for the second). Thus, while pH levels were not significantly different between animals (P=0.661 and 0.643 in the two models), or between diets (P=0.062 in both models), subjects clearly responded differently to the different diets. On all but the alfalfa diet, the moose typically had higher rumen pH levels than the two cows (Table 2; Bonferonni *post hoc* P<0.001 compared with cow 2 on the natural diet; P<0.0001 compared with both cows on ration 1, and on grass; and P<0.0001 compared with cow 2 on ration 2). The pattern was reversed for the alfalfa diet, on which the two cows had higher pH levels than the moose (P<0.0001). Only on the natural diet did rumen pH for the two cows differ, with cow 1 being significantly higher than cow 2 (P=0.039). Variability in rumen pH also differed across diets, depending on the test subject (Table 2). In the moose, variance was lowest on grass compared with all other diets ($F_{23,23}$= 2.281 to 3.486; P=0.002 to 0.027). In the cows, variance was lowest on the alfalfa diet ($F_{23,23}$=2.391 to 38.351; P<0.0001 to 0.021). Furthermore, in cow 1, ration 1 and 2 were associated with significantly higher variance than were the grass and natural diets (P<0.0001 and 0.004), and a similar although not consistently significant trend was observed for cow 2 (P<0.0001 to 0.064).

Different associations between diet and rumen pH were also recorded between subjects. In the moose, the highest pH levels were associated with the grass diet, whereas the highest pH levels in both cows were linked to the alfalfa diet (P<0.05). In addition, variance in rumen pH was lower for moose than for cows on the grass diet ($F_{23,23}$=2.996 and 2.660, P=0.006 and 0.011, respectively), but higher for moose than cows on the alfalfa diet ($F_{23,23}$=6.389 and 2.905, P<0.0001 and =0.007, respectively; Table 2). The lowest

pH levels in all animals were associated with the natural diet and ration 2 ($P<0.05$). Ration 2 was associated with the highest proportion of low pH measurements, and also with the highest number of 5- and 10-minute time periods during which the pH was consistently low in all three animals – followed by ration 1 and the natural diet (Table 2). On these three diets, variance in pH was less in the moose than in the two cows ($P<0.05$ in all cases, except that variances for moose and cow 1 on the natural diet were similar, $P=0.221$). In no cases did the two cows differ in terms of variance in rumen pH ($P=0.090$ to 0.486), except that on alfalfa cow 2 was slightly more variable (s.d.=0.08 and 0.06, respectively; $F_{23,23}=2.199$, $P=0.032$).

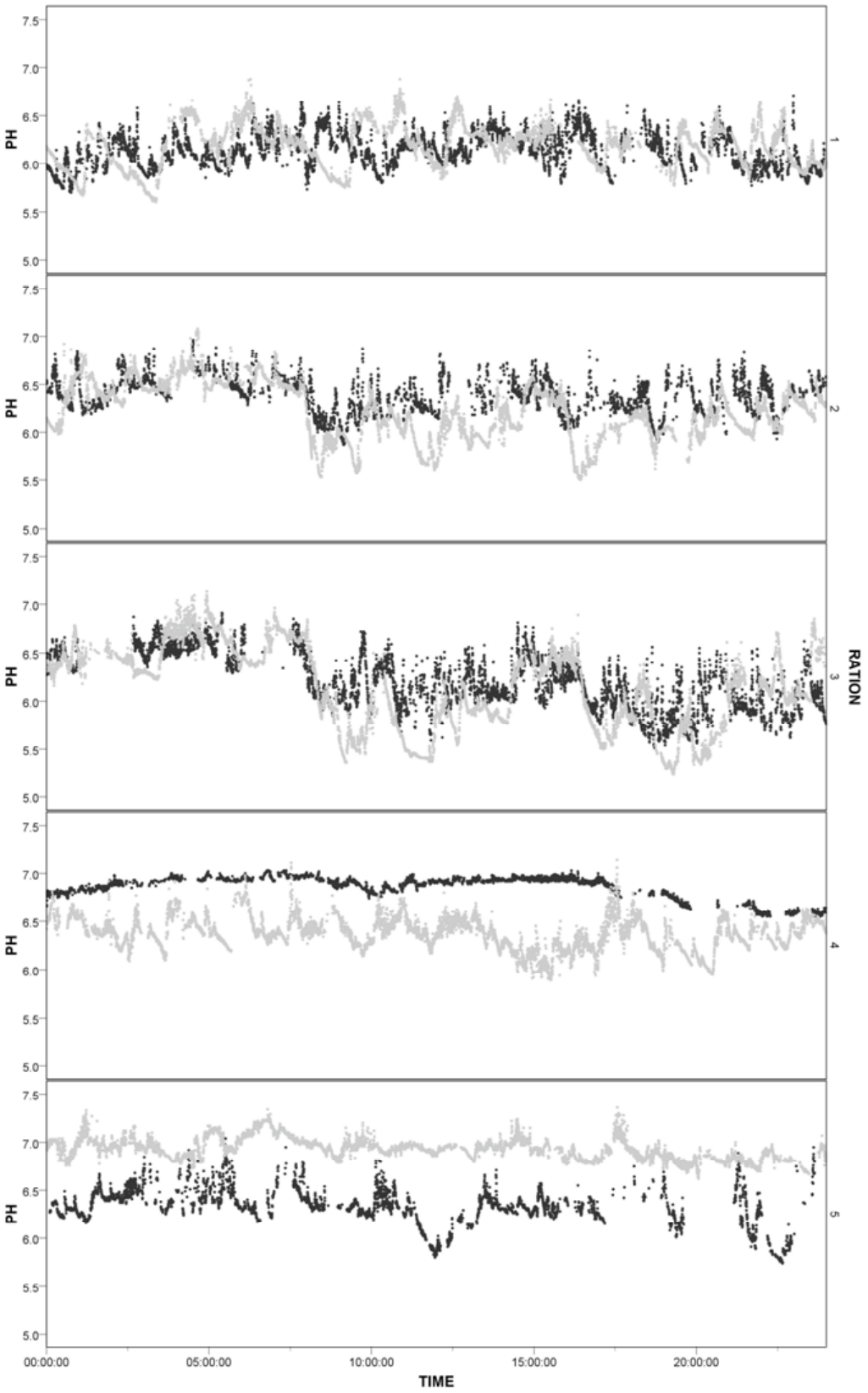

Figure 1. Examples of the daily course of rumen pH in the moose (black dots) and one of the cows (grey dots) during one day each when fed 1 'natural diet' (browse for moose, grass hay for cows), 2 'ration 1', 3 'ration 2', 4 'grass diet' and 5 'alfalfa diet'.

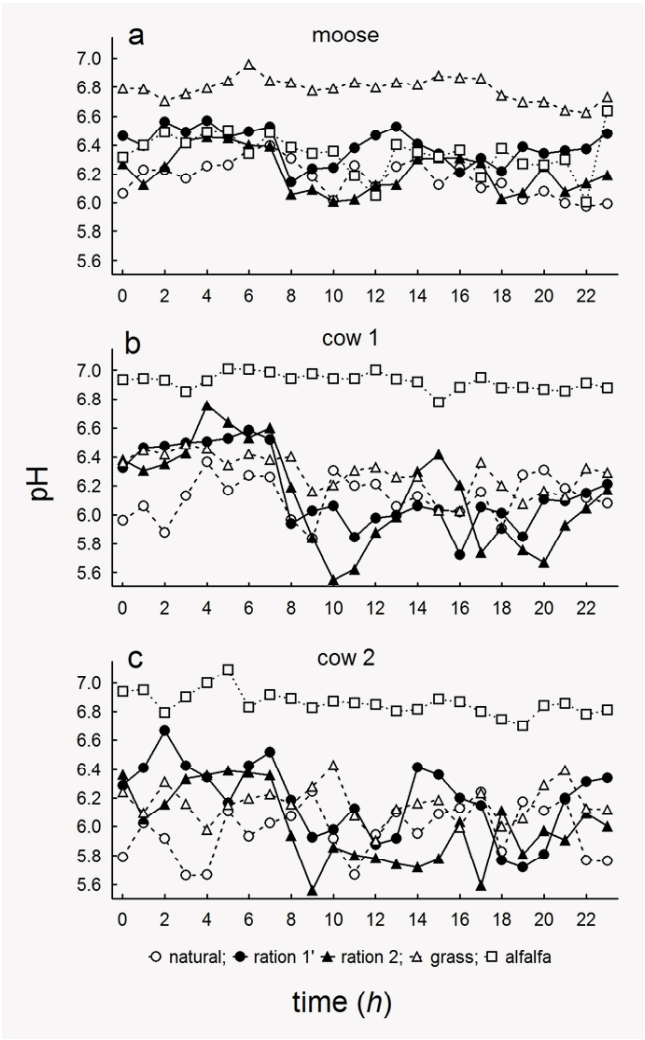

Figure 2. Changes in pH of test subjects on five diet treatments throughout the day. Symbols represent means for each hour of the day.

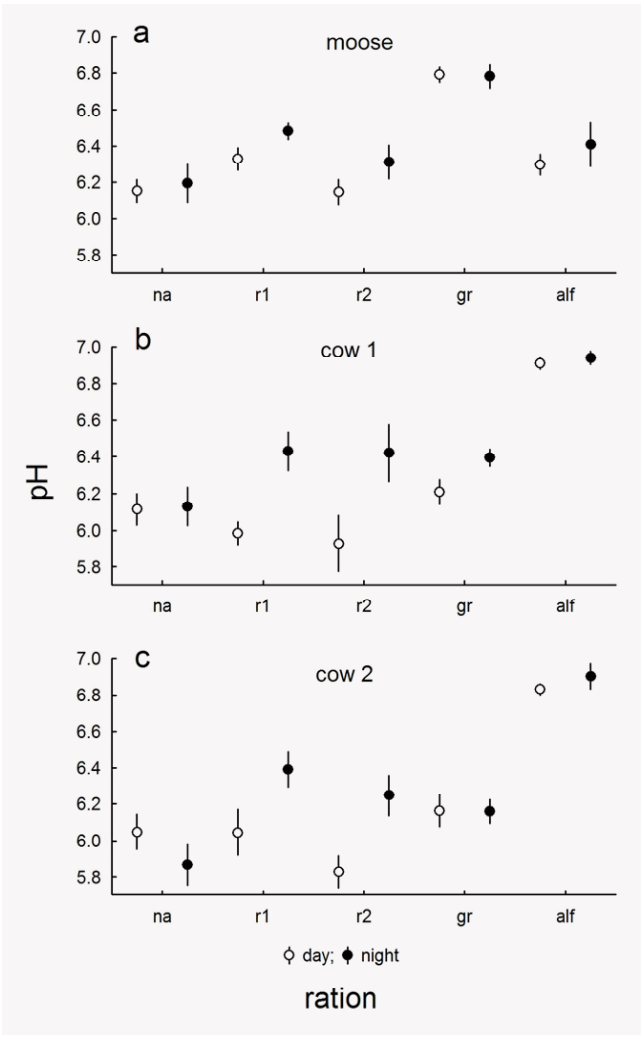

Figure 3. Comparison of mean pH levels of each animal, across the five diet treatments, between day (0800 to 2200) and night (2200 to 0800). Error bars are 1 standard error around the means. Diet treatments: na – natural; r1 – ration1; r2 – ration2; gr – grass; alf – alfalfa.

The ANOVA models also concurred with the interpretation that pH levels were generally higher at night than during the day ($F_{1,338}$=13.056, P=0.062 with only two time slots considered, but $F_{4,317}$=12.731, P=0.002 when five time slots were considered). However, the interaction between time of day and animal was also significant in both models ($F_{2,338}$=7.655, P<0.001, and $F_{8,317}$=2.584, P=0.01, respectively). This occurred because, whereas the moose had a higher pH during the day than the two cows (6.34±0.26 compared with 6.23±0.39 and 6.18±0.38 in the cows; P<0.05; note that daytime means for the two cows were similar, P=0.907), pH levels of all animals increased during night hours, so that the moose and at least one cow had similar means at night (6.43±0.23 and 6.46±0.30, P=0.999). Even with the day subdivided into five time slots, there remained few or no consistent significant differences across time slots, except between the night (mean for 0000 to 0800 slot = 6.43±0.30) and day (means for all other time slots ranged between 6.24 and 6.25; P<0.05). It is worth noting, however, that whereas night time pH levels of both cows were always higher than during the day, night values for the moose only differed from the time slot between 1900 and 0000 (P<0.001; P=0.691 to 0.999 for all other comparisons), indicating that temporal shifts in the moose were smaller than in the cows.

We also found significant interaction effects of time slot and diet treatment in both models ($F_{4,338}$=26.268, P<0.0001, and $F_{16,317}$=6.711, P<0.0001, respectively). Multiple comparisons tests revealed this occurred mainly because pH levels were highest on alfalfa, and less so grass, during the day (P<0.05), but in the night time slot the mean pH for alfalfa was not different from ration 1 and 2 (P=0.181 to 0.999). This pattern reflects the high pH levels of moose and cows on grass and alfalfa, respectively, and the smaller increase in pH from day to night in the moose compared with that observed in the two cows. In all three animals, the difference in pH between day and night were highest on ration 2, followed by ration 1 and the natural diet (Table 2).

When comparing the pH on ration 1 and 2 between the three animals in relation to the ratio of concentrates (including apples and carrots, on an estimated dry matter content of 15%) to roughages, there does not appear to be a systematic difference between moose and cows (Fig. 4). Differences in mean pH, on rations 1 and 2, appear to correspond to differences in the actually ingested proportion of concentrates and roughages.

Discussion

This study confirms that the diet fed can significantly influence not only the average pH in the forestomach of ruminants, but also daily pH fluctuations. Recording the variation of pH over time showed

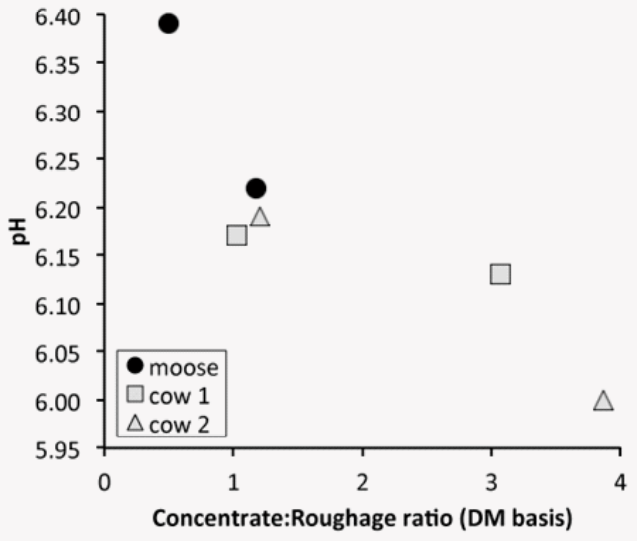

Figure 4. Relationship between the ratio of concentrates (including apples and carrots) to roughage intake in the three animals, with the mean rumen pH for the two rations representative for zoo diets. Note that higher pH in the moose is associated with a lower concentrate:roughage ratio, due to a higher roughage intake in the moose compared to the cows.

that, whereas the average pH did not differ significantly between a 'natural' diet (of browse for moose, and of grass hay for cattle) and a typical 'zoo' diet (ration 1), there were clear differences in the variability of pH measurements over time. The 'zoo' diets (ration 1 and 2) resulted in a distinct difference between day and night time pH levels (Fig. 3), and in greater difference between minimum and maximum pH in the cattle (Tab. 2). Thus, the results of this study confirm the often-voiced warning that traditional 'zoo' diets based on concentrates and particularly fruits/non-leafy vegetables can lead to comparatively acidic states in the rumen (Oftedal et al. 1996; Hummel et al. 2006a; Clauss and Dierenfeld 2008).

Apart from the evident limitation of the low number of individuals investigated in this study, and the lack of information on the nutritional composition of the diet items used, additional constraints relate to the short time periods available for measurement of the different diets and the habituation of the animals to the diets used. Therefore, the recorded pH values represent data from ruminant animals and microbial populations not very well adapted to the diets used (except for the first diet). The short time periods for the various diets were determined by the method used, as manufacturer information suggested that intraruminal pH loggers would safely yield reliable measurements for approximately 2–3 weeks, after which accuracy could no longer be guaranteed. Because the loggers used in the cattle, which could be retrieved after this time period, showed no deviation in pH readings compared to the beginning, we consider this information to be correct. Potentially, longer use might have been possible. Due to the stratification of rumen contents, in particular in 'cattle-type' ruminants (Clauss et al. 2010), the position of the bolus in the reticulorumen can influence the measurement (reviewed in Ritz et al. 2013); the position of the bolus could not be modified and may in particular have varied between the dorsal and the ventral rumen contents (at slaughter, the bolus was in the fibre mat in the cows).

One evident difference between the moose and the cows was that the moose had been exposed, for long periods of his life, to a diet that resembled the 'zoo' diet (ration 1), whereas the

Table 2. pH measurements in two cows (*Bos primigenius* f. *taurus*) and one moose (*Alces alces*) on different diets (see Table 1), including the proportion of time recorded below three pH thesholds, the number of uninterrupted time intervals below two pH thresholds, and the difference in pH measurements between day and night averages and between the highest and lowest mean of one of the five time periods of the day (see Methods).

		pH				pH<6.0		pH<5.8				pH<5.5				pH difference	
Diet	Animal	mean	SD	min	max	%	min/d	%	min/d	>5 min	>10 min	%	min/d	>5 min	>10 min	day/night	min/max
Natural diet	Moose	6.17^{a}	0.21^{a}	5.67	6.75	22.3	321.3	2.2	32.1	6	2	0.0	0.0	0	0	0.04	0.23
	Cow 1	6.12^{a}	0.14^{ab}	5.46	7.51	30.6	440.4	9.3	134.5	9	8	0.3	3.9	1	0	0.02	0.17
	Cow 2	5.97^{b}	0.18^{b}	5.26	6.94	49.7	716.0	30.5	439.3	20	14	6.1	87.6	3	2	0.18	0.19
Ration 1	Moose	6.39^{a}	0.12^{a}	5.87	7.11	1.5	21.8	0.0	0.0	0	0	0.0	0.0	0	0	0.15	0.29
	Cow 1	6.17^{b}	0.26^{b}	5.5	7.08	31.5	454.2	13.6	195.7	18	12	0.0	0.0	0	0	0.45	0.56
	Cow 2	6.19^{b}	0.25^{b}	5.22	7.55	26.4	380.3	14.9	214.8	8	8	0.1	83.5	3	3	0.34	0.38
Ration 2	Moose	6.22^{a}	0.15^{a}	5.49	7.56	22.6	324.8	7.0	100.9	14	2	0.0	0.1	0	0	0.16	0.30
	Cow 1	6.13^{b}	0.35^{b}	5.16	7.14	40.0	575.4	22.9	330.2	22	13	7.8	112.5	8	5	0.49	0.64
	Cow 2	6.00^{b}	0.26^{b}	4.97	7.2	45.2	650.5	29.9	430.5	22	16	8.3	119.6	11	4	0.42	0.53
Grass diet	Moose	6.79^{a}	0.08^{a}	6.51	7.04	0.0	0.0	0.0	0.0	0	0	0.0	0.0	0	0	0.01	0.15
	Cow 1	6.29^{b}	0.14^{b}	5.6	7.14	12.3	176.5	3.1	45.0	4	3	0.0	0.0	0	0	0.19	0.22
	Cow 2	6.16^{b}	0.13^{b}	5.46	7.56	22.4	321.8	7.0	100.2	5	3	0.8	11.5	2	1	0.00	0.21
Alfalfa diet	Moose	6.34^{a}	0.14^{a}	5.74	7.04	4.2	59.8	0.3	4.8	1	0	0.0	0.0	0	0	0.11	0.17
	Cow 1	6.92^{b}	0.06^{b}	6.56	7.37	0.0	0.0	0.0	0.0	0	0	0.0	0.0	0	0	0.03	0.07
	Cow 2	6.86^{b}	0.08^{c}	6.44	7.34	0.0	0.0	0.0	0.0	0	0	0.0	0.0	0	0	0.07	0.13

^{a,b}Different superscripts within a diet indicate significant differences between individuals in the mean pH or the variability of the pH value during this feeding regime.

specific concentrates, and particularly the apples and carrots, were novel diet items for the cows. This did not affect the intake of these items, but might have had an influence on the mode of intake, the corresponding large drop in pH, and reduction of concomitant grass hay intake. In cattle, Bevans et al. (2005) showed that a rapid adaptation to a high-concentrate diet led to more variation in ruminal pH than a gradual adaptation. The remarkable drop in grass hay intake in the cows when introduced to the concentrates/apples/carrots may represent a consequence of the rapid introduction to these items, with the low pH values potentially suppressing hay intake. Potentially, a longer adaptation period would have led to an increase in hay intake in these animals as well.

The level and course of forestomach pH in domestic ruminants is influenced by various factors. These include the absolute amount ingested of a diet – higher intakes are usually associated with lower pH measurements (Robinson et al. 1986), a fact which might have contributed to the difference in pH between the two cows on the natural diet; the proportion of easily fermentable, 'concentrate' feed (Beckman and Weiss 2005); and the number of feeding bouts /feeding frequency (Robles et al. 2007). On the lowest food intake, the moose (on the grass diet) and the cows (on the alfalfa diet) had very high, and very stable, pH measurements; changing the animals to more readily accepted roughages (alfalfa for the moose, grass silage for cattle) led to a lower average pH, and a higher pH variability (Table 2). The roughage with a probably even higher acceptance in moose, fresh browse, caused an even lower average pH with more variability in this animal. For cattle, the major difference between the grass silage and hay diets was the intake level (which might be related to the difference in moisture content; Jackson and Forbes 1970), with higher dry matter intakes on the grass hay (due to the moisture content in silage), which was also associated with a lower average pH but no evident difference in variability. The addition of concentrates/apples/carrots evidently increased pH variability due to the two distinct feeding events (as compared to the constant ad libitum access to the roughages), and led to a lower average pH at the higher feeding level (Table 2). These changes occurred more or less in parallel in the moose and the cows, with differences adequately explained by differences in roughage acceptance. Finally, there may be intra-individual variation, such as the lower pH measured in cow 2 as compared to cow 1 in terms of the time the measure was below certain thresholds (Table 2).

In theory, a difference in the susceptibility to low pH between cattle and moose could have been expected based on a fundamental physiological difference (Clauss et al. 2010): whereas the moose has a typical 'moose-type' forestomach physiology characterised by an absence of stratification of rumen contents and a low fluid throughput through the rumen, cattle have a typical 'cattle-type' forestomach physiology, characterised by clearly stratified rumen contents and a high fluid throughput through the rumen. Because fluid throughput is mainly a function of saliva production, and saliva acts as a buffer for the rumen contents (Van Soest 1994), one could have expected cattle to have a generally more stable rumen pH. This could also explain the observation that roe deer (*Capreolus capreolus*), another ruminant with a 'moose-type' forestomach physiology, and some other browsing ruminants, have low rumen pH levels in the wild when compared to other free-ranging ruminants (Ritz et al. 2013). Some evidence supporting this concept was provided by Estell and Galyean (1985) who found, in an evaluation of data from several feeding trials with cattle, a (weak) positive relationship between the fluid dilution rate in the rumen and rumen pH, indicating that a higher fluid throughput would favour higher pH levels. However, the limited results of this study suggest that when comparing moose and cattle, differences in the diets actually ingested are far more likely to explain differences in rumen pH than putative effects of their different physiology. The challenge in feeding 'moose-type' ruminants thus is probably not their digestive physiology per se, but the unpredictability of what roughages - other than browse - they will accept sufficiently well.

Moose are often particularly difficult in this respect (reviewed in Clauss et al. 2013). The good acceptance of alfalfa hay by the moose in this study corresponds to a similar observation in four other moose from the same facility (Clauss et al. 2013). In contrast, whereas another moose in this facility had readily ingested freshly cut grass from a mixed meadow (Clauss et al. 2013) and successful experimental feeding of moose with grass silage has been reported (Lechner et al. 2010), our individual refused the freshly cut grass more or less completely. This observation emphasises the effect of individual preferences on the feeding regimes possible in captivity, perhaps also influenced by past husbandry practices and diets used. Providing herbivores with adequate roughage sources that they accept readily remains one of the major challenges of zoo animal husbandry (Clauss and Dierenfeld 2008).

To conclude, this study confirms suspicions that diets high in concentrates and produce will induce low and less stable pH levels in ruminants, in particular in conjunction with distinct feeding times. The husbandry of ruminants should aim at providing adequate feeds with constant provision to maintain stable rumen pH levels. Potential consequences of chronic low rumen pH could not be evaluated in this study, but can be derived by transferring literature results on domestic animals to captive wild ruminants (e.g. Kleen and Cannizzo 2012).

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