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ORIGINAL PAPER

Forestomach pH in hunted roe deer (*Capreolus capreolus*) in relation to forestomach region, time of measurement and supplemental feeding and comparison among wild ruminant species

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Abstract There is a debate whether supplemental feeding of deer bears the risk of inducing health problems, in particular acidosis. Here, the pH values of forestomach contents of free-ranging roe deer (*Capreolus capreolus*) shot in areas with and without supplemental winter feeding were compared. pH was similar in the dorsal and ventral rumen, but lower at these sites than in the *Atrium ruminis*, where it was again lower than in the reticulum; this pattern corresponds to expectations based on differences in the presence of saliva at the different sites of the forestomach. pH was lower with increasing time that elapsed between death of the animal and measuring pH in unsupplemented animals and was lower in unsupplemented animals in May/June than later in the year. Animals with supplemental winter feeding had significantly lower rumen pH (5.5) than animals without food supplementation (5.7). These data suggest that supplemental feeding of roe deer has the potential to lower forestomach pH. Although pH values measured in supplemented animals in this study would be considered indicative of rumen acidosis in domestic cattle, they are within the range previously measured in various free-ranging Odocoilid species, including roe deer; were of a similar magnitude as the May/June values of unsupplemented roe deer in this study; and must be considered with respect to potentially rapid declines in pH between death of the animal and pH measurement. Given methodological problems, analyses of literature data from free-ranging wild ruminants provide little evidence for a systematic variation of rumen pH with feeding type and body mass, but lead to the hypothesis that some New World cervids, including the roe deer, might either naturally have lower pH values than other ruminants or rumen contents whose pH drops rapidly after death.

Keywords Rumen · Reticulum · Acidosis · Feeding · Wildlife · Cervid

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Introduction

Microbial digestion in the forestomach of ruminants leads to the production of pH-active substrates, in particular short-chain fatty acids (SCFA) such as acetate, propionate and butyrate; these substrates serve as the main energy sources for the animal (Stevens and Hume 1998). Because the viability of the microbial population depends, among other factors, on a constant and rather neutral pH, regulating mechanisms such as secretion of buffering saliva during ingestive mastication and rumination, and constant absorption of SCFA by the rumen mucosa, are important for the stability of the rumen ecosystem (Van Soest 1994). In veterinary medicine, the pH of the rumen contents is an important measure of ruminant health, with low values (acidosis) usually indicating the excessive consumption of highly digestible feeds (Smith 1996; Dirksen et al. 2006; Radostits et al. 2007). Acidosis can be acute, resulting mostly in death, or chronic (subacute ruminal acidosis), resulting in damage to the rumen mucosa (parakeratosis), generalised or localised (hepatic) inflammation and abscesses, laminitis (inflammation of the digital laminae of the hoof, leading to lameness), food intake depression and low milk fat (Kleen and Cannizzo 2012). For domestic ruminants, pH values of 5.5 to 7.2 in cattle (Smith 1996; Dirksen et al. 2006; Radostits et al. 2007) and 6.2 to 7.0 in sheep (Behrens et al. 2001) are considered normal, whereas specific references for goats are lacking. In cattle, values below 5.5 are considered indicative for subacute acidosis, and values below 5.0 are typical for acute cases (Dirksen et al. 2006; Radostits et al. 2007; Kleen and Cannizzo 2012), whereas in small ruminants, values below 6.0 are mostly considered as acidotic (Bostedt and Dedié 1996; Behrens et al. 2001).

Because of differences in the nutrient composition of the natural diet, as well as differences in the throughput of saliva through the rumen, a systematic variation of rumen pH with body mass and with feeding type could be expected under natural conditions. Larger animals usually consume food of lower nutritive quality (Owen-Smith 1988; Codron et al. 2007), which should translate into a lower SCFA production and higher pH readings. Although a decrease in the concentration of SCFA in the rumen (Clemens and Maloiy 1983) and a decrease of SCFA production rates with increasing body mass (Hoppe 1977; Gordon and Illius 1994) were demonstrated in free-ranging wild ruminants, no corresponding relationship between rumen pH and body mass has been found so far (Giesecke and Van Gylswyk 1975; Maloiy et al. 1982).

Hoppe (1984) suggested that increased absorption of the fermentation products in the smaller species guaranteed the stability of the rumen pH. Similarly, the concept that browsing or 'concentrate selector' species (Hofmann 1989) should have higher proportions of SCFA in their rumen contents because of a putatively higher nutritive value of their natural diet was apparently corroborated (Clemens and Maloiy 1983; note that the result could not be repeated by Clauss et al. 2008 using the same dataset). A lower rumen pH in browsing species could also be explained by the hypothesis that several browser species have a 'moose-type' forestomach physiology that is characterised by a low fluid and hence saliva throughput (Clauss et al. 2010b, 2011a), which might therefore be less well buffered than the forestomach of 'cattle-type' ruminants that is characterised by a high fluid and hence saliva throughput. However, comparative pH measurements failed to document a difference between various African browsers and grazers (Giesecke and Van Gylswyk 1975; Maloiy et al. 1982; cf. Table 1). In contrast, Jones et al. (2001) found that the rumen fluid of some African browsers (giraffe *Giraffa camelopardalis*, greater kudu *Tragelaphus strepsiceros*, eland *Taurotragus oryx*, grey duiker *Sylvicapra grimmia*), for which they did not give species-specific data, was about 5.78, whereas that of grazers (wildebeest *Connochaetes taurinus*, sheep) was about 6.68.

Studies on individual species yielded similar values—for example, white-tailed deer (*Odocoileus virginianus*) reportedly had rumen pH values of 5.7–5.8 (Short et al. 1969a; Woolf and Kradel 1977), mule deer (*Odocoileus hemionus*) values of 5.5 (Short et al. 1966) and bushbuck (*Tragelaphus scriptus*) values of 5.6 (Odendaal 1976). Short et al. (1969b) demonstrated that free-ranging white-tailed deer feeding on browse had a higher rumen pH (but still low when compared to domestic cattle) of 5.9, whereas animals feeding on acorns had low values of 5.5. Published measurements in roe deer are also mostly lower than values considered typical in small domestic ruminants. Whereas in feeding experiments with captive roe deer, rumen pH values of 5.9–6.4 were measured, depending on the level of concentrate supplement (Enzinger and Hartfiel 1998), values measured in free-ranging roe deer varied from 5.5 in spring to 6.3 in winter (Djordjević et al. 2006; Popović et al. 2009). In six free-ranging roe deer considered healthy in southern Germany, rumen pH was 5.55±0.18 (range 5.27–5.77) directly after death, whereas a 1-year-old male found in a comatose state, a pH of 4.7 was measured and considered indicative of acute rumen acidosis (Immekus, personal observation). These reports suggest that differences between individual ruminant species may occur and that 'normal' reference ranges should not be transferred automatically between species.

Rumen acidosis has not only been described in captive wild ruminant species, where browsers appear to be particularly susceptible to the problem (Marholdt and Hofmann 1991; Hofmann and Nygren 1992; Clauss et al. 2003; Woodbury et al. 2005), but also sporadically in free-ranging animals such as moose (*Alces alces*) (Butler et al. 2008), white-tailed deer (Wobeser and Runge 1975) and roe

Table 1 Literature data on pH on rumen contents of free-ranging ruminant species

Species		BM (kg)[a]	%grass[a]	pH	Source[b]
Impala	*Aepyceros melampus*	48	60	6.41	a, b, g
Hartebeest	*Alcelaphus buselaphus*	136	96.7	6.40	c
Sringbok	*Antidorcas marsupialis*	39	30	6.50	a
Bison	*Bison bison*	647	84	6.51	h
Spanish ibex	*Capra pyrenaica*	60	60	6.34	j
Roe deer	*Capreolus capreolus*	21	9	5.89	k, l, m
Red deer	*Cervus elaphus*	154	47	6.35	n, o
Sika deer	*Cervus nippon*	50	50	6.44	p, q, r, s
Sambar deer	*Cervus unicolor*	212	45	6.40	t
Wildebeest	*Connochaetes taurinus*	148	90	6.63	a, c, e
Fallow deer	*Dama dama*	52	46	6.10	k
Topi	*Damaliscus lunatus*	126	99.3	6.10	c
Thomson's gazelle	*Gazella thomsoni*	21	75	6.04	b
Giraffe	*Giraffa camelopardalis*	904	0.2	6.50	f
Waterbuck	*Kobus ellypsiprymnus*	160	80	6.50	f
Gerenuk	*Litocranius walleri*	43	0	6.30	f
Dikdik	*Madoqua kirki*	5.2	17	6.40	d
Grant's gazelle	*Nanger granti*	50	50	6.01	b
Suni	*Nesotragus moschatus*	6.2	0	6.35	d
Mule deer	*Odocoileus hemionus*	59	9	5.50	u
White-tailed deer	*Odocoileus virginianus*	56	11	5.80	v
Oryx	*Oryx beisa*	145	83	6.70	f
Gemsbok	*Oryx gazella*	182	82	6.40	a
Reindeer	*Rangifer tarandus*	120	36	6.20	n
Chamois	*Rupicapra rupicapra*	32	74	6.47	o
African buffalo	*Syncerus caffer*	493	90	6.53	a, f, w
Eland	*Taurotragus oryx*	366	50	6.40	f
Bushbuck	*Tragelaphus scriptus*	43	10	5.56	x
Kudu	*Tragelaphus strepsiceros*	194	5	6.40	a

[a]Data on mean body mass and %grass from Clauss et al. (2009c, 2010a, 2011b)

[b]Sources for pH: a (Giesecke and Van Gylswyk 1975), b (Hoppe et al. 1977a), c (Hoppe et al. 1977b), d (Hoppe et al. 1983), e (Kreulen and Hoppe 1979), f (Maloiy et al. 1982), g (Booyse and Dehority 2011), h (Towne et al. 1989), j (de la Fuente et al. 2009), k (Hennig et al. 1988), l (Djordjević et al. 2006), m (Popović et al. 2009), n (Hobson et al. 1975/76), o (Drescher-Kaden 1981), p (Li and Qin 1992), q (Tung et al. 1996), r (Ichimura et al. 2004), s (Ito et al. 1993), t (Tung et al. 1995), u (Short et al. 1966), v (Short et al. 1969a), w (Van Hoven 1980), x (Odendaal 1976)

deer (*Capreolus capreolus*) (Sugár 1983), in association with overfeeding on grains. Such grains may be available from cultured crops, or from supplementary feeding programs for other species such as wild fowl or wild boar, or from supplementary feeds intentionally fed to the ruminant species in question. It is generally recommended that triggering acidosis by supplementary feeding regimes should be avoided (Woolf and Kradel 1977; Rehbinder and Ciszuk 1985), but studies on the effect of supplementary feeding on rumen pH and rumen health are rare.

Interpreting measurements of rumen pH from shot, free-ranging animals can be complicated by at least two different factors. On the one hand, fermentation processes in the rumen are not terminated after death, but absorption of the fermentation products is. Therefore, rumen pH may well decrease over time, and pH values may reflect the time that elapsed between death and measuring pH. Such observations, albeit without accompanying data, were for example stated by Gasaway and Coady (1974). Because pH measurements from the literature are commonly given without an indication of the time elapsed between death and actual measurement, the comparability of data from different studies, or even from data within the same study, may be compromised.

Additionally, the pH of the ruminant forestomach will vary according to the region in the forestomach that is sampled. Forestomach contents of ruminants are subject to varying degrees of stratification, which is more pronounced in 'cattle-type' ruminants and less pronounced to absent in 'moose-type' ruminants (Clauss et al. 2010b). Regardless of this physiological distinction, the contents of the reticulum—which is in communication with the rumen—are invariably more moist than those of the dorsal rumen (Clauss et al. 2009a, b), indicating a higher proportion of salivary fluid at this site. Because saliva acts as a buffer, one would expect a higher pH in reticular contents than in those from the rumen, as the results in red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) from Prins and Geelen (1971) suggest. Because

fluid from the reticulum spills back into the *Atrium ruminis* during reticular contractions, it appears logical to expect a pH at this site that is intermediate between the reticulum and the dorsal rumen. In cattle, where the stratification of rumen contents is very pronounced (Hummel et al. 2009), differences in the pH of rumen contents have been reported depending on the site of sampling (Duffield et al. 2004; Tafaj et al. 2004), and also higher pH values in more cranial rumen regions that are closer to the oesophagus and hence to the inflow of buffering saliva (Li et al. 2009). Thus, measuring pH without reference to a certain location within the forestomach may also compromise the comparability of results between and within studies.

In this study, we aimed at measuring the pH of rumen contents of roe deer in relation to the site of sampling in the rumen, in relation to the time elapsed between death and measurement, and in relation to the presence or absence of supplementary feeding. We hypothesised that pH would be higher in the reticulum as compared to the dorsal and ventral rumen, that pH would decrease with increasing time interval between death and pH measurement, and that pH would be lower in animals receiving supplemental feeds.

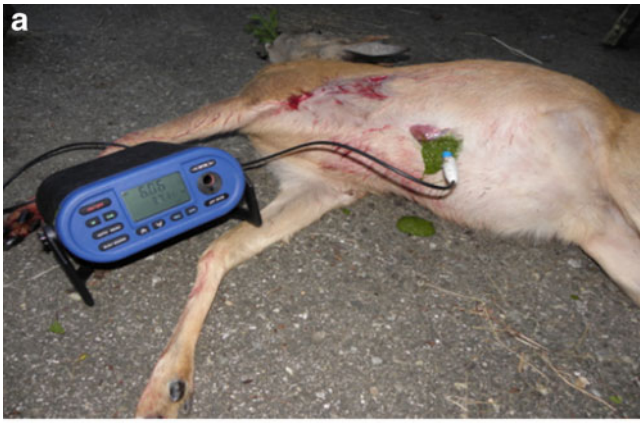
Material and methods

Pilot observations

In a pilot investigation, a female and a male roe deer were shot in a hunting area in southern Germany; the animals were left intact apart from for a small opening of the left body wall to insert the probe of a pH-meter (pH 1970i, WTW, Weilheim, Germany) into the rumen (Fig. 1a). pH readings were taken at successive time intervals, indicating a decrease in pH with increasing time after death (Fig. 1b). Based on these results, it was decided to always record the time that elapsed between death and pH measurement in this study.

Roe deer study

Measurements were taken on 101 male (mean ± SD body mass 23.3±4.1 kg) and 112 female (20.2±3.7 kg) roe deer hunted from hides or by stalking in seven different hunting districts (covering a total of 4,881 hectares including 2,712 hectares of forest) in northern Lower Austria between 2010 and 2011. The average hunting yield in these areas is 5.7 roe deer per 100 hectares per year. Animals were shot during May–October when no feeding supplements were provided, and during November when such feeds were provided in some, but not all districts. Supplementary feed consisted of a mixture (in percent as fed) of ensiled apple pomace (20.7 %), sugar beets (20.7 %), oats (20.3 %), sesame expeller (10.0 %), triticale (8.3 %), peas (8.3 %), maize (8.3 %) and rye (3.4 %). Approximately 120 tons of this material was distributed, from the end of September to the end of March, at 105 feeding stations.



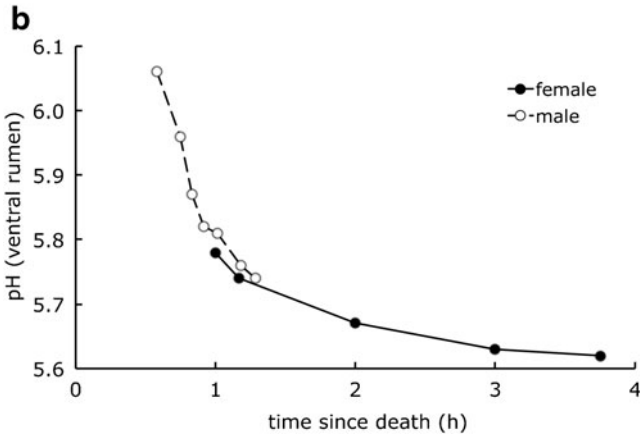


Fig. 1 Pilot observation investigating the effect of time since death on pH measurements. **a** Carcass left intact with pH probe inserted for repeated measurements; **b** results from two roe deer measured in this way

The time between the death of the animal and the measurement of pH was recorded to the closest minute. Shot animals were weighed and dissected, and the pH and temperature of forestomach contents were measured using a 206-pH1 set (Testo, Lenzkirch, Germany). Measurements were taken from the dorsal rumen, the ventral rumen, the *Atrium ruminis*, and the reticulum (Fig. 2). Environmental temperature, as a putative mediator of rumen temperature, was not recorded in this study.

Statistical analyses

Our first objective was to determine how pH and temperature differs across forestomach compartments. We compared pH and temperature in the ventral rumen, dorsal rumen, *Atrium ruminis* and reticulum with repeated measure ANOVAs, and Tukey's post hoc tests for multiple comparisons. Our second,

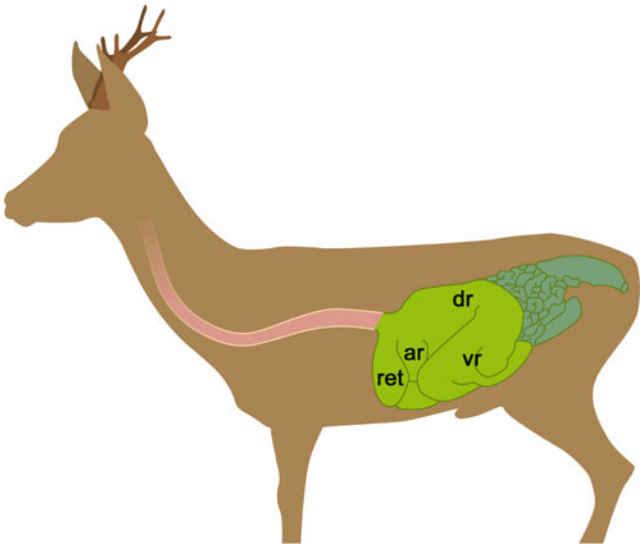


Fig. 2 Schematic representation of sampling sites in the roe deer forestomach—*dr* dorsal rumen, *vr* ventral rumen, *ar Atrium ruminis*, *ret* reticulum. Modified from Hofmann (1985) by Jeanne Peter

and main, objective was to determine whether supplemental feeding had an effect on forestomach pH, accounting for contributions of other physiological and ecological factors to total variation in pH. Factors we investigated were season (month of sampling), the origin of the animals sampled (hunting area), sex and body mass (kilogram), which could also serve as a proxy to differentiate between juvenile and adult animals. We included these effects, as well as supplemental feeding or not, and postmortem effects (time since death, or temperature changes in the forestomach) as additive effects in a general linear model. Using stepwise addition and backwards removal of various parameters, we compared all possible combinations to determine the model(s) that are best-supported by our data. Model selection is based on Akaike's Information Criterion (AIC), and we consider only those models with a ΔAIC ($AIC_{candidate\ model}$−minimum AIC)≤2 as being well-supported by our data (Burnham and Anderson 2001, 2002).

Because of imbalances in our sample, we excluded all interaction terms from the above models. In particular, both sexes were not sampled in all months, and only females received the supplement, and then only in November and December. Thus, we repeated the analyses including only females (omitting sex as a factor), and then again including only females sampled during November and December (omitting also month as a factor) to check if significance of parameters and/or goodness-of-fit differed from the results based on the whole dataset. To test whether supplemental feeds influenced the effects of individual parameters, we plotted data visually (for month and sex, using 95 % confidence intervals, to guide interpretation of statistically different subgroups), or two-way ANOVAs (for hunting area) and separate-slopes ANCOVAs (for body mass and time since death/temperature) to test for interactions between each parameter and diet supplementation. The latter models were also rerun including only females, and again including only females sampled during November and December. All analyses were carried out in STATISTICA v8.0 (Statsoft_Inc 2007). Statistical significance was evaluated based on F ratios, with critical α (two-tailed) set at 0.05.

Comparative literature study

Data on the pH of rumen contents of wild ruminant species were collated from the literature (Table 1). Means of all data given were recorded. Data on the percentage of grass in the species' natural diets and on their body mass were also taken from the literature. Using simple correlation analysis and a general linear model (GLM), we evaluated whether body mass or the proportion of grass (%grass) in the natural diet systematically influenced rumen pH.

Results

Roe deer study

The total pH range in roe deer forestomach compartments was broad, varying from strongly acidic at 4.64, to almost neutral at 6.77, with a mean of 5.69 (SD=0.33). This total range did not seem to differ across compartments (ventral rumen 4.64 to 6.59; dorsal rumen 4.83 to 6.77; *Atrium ruminis* 4.96 to 6.66; reticulum 5.05 to 6.70). However, apart from a few extreme values, variance around the means was relatively narrow (SD<0.35 in all compartments), such that 95 % confidence intervals were within the range of only 5.57 to 5.83 in each case (Table 2). Nevertheless, there were significant variations in pH both within and between the four compartments.

Repeated measures ANOVA revealed significant differences between stomach compartments ($F_{3,\ 636}$=118.027; p< 0.0001). The lowest mean pH values were recorded in the ventral and dorsal rumen (5.62 and 5.61, respectively; Tukey's post hoc p=0.971), significantly lower than that of the *Atrium ruminis* (5.73; p<0.0001) which, in turn, was lower than the mean of the reticulum (5.79; p< 0.0001). As expected, forestomach pH declined post mortem, evinced by the negative relationships between pH and across-individual variation in the time between death and sampling (Fig. 3a; note this effect is not significant for pH of the reticulum, p=0.158). Postmortem effects did not, however, influence the pattern of lowest pH in the ventral and dorsal rumen, followed by higher values in the *Atrium ruminis*, and highest values in the reticulum (95 %

Table 2 Comparison of pH and temperature (°C) between forestomach regions of roe deer

Forestomach region	Number	Mean	SD	−95 % CI	+95 % CI
pH[a]					
Ventral rumen (a)	213	5.62	0.3173	5.5768	5.6625
Dorsal rumen (a)	213	5.61	0.3188	5.5717	5.6578
Atrium ruminis (b)	213	5.73	0.3284	5.6881	5.7768
Reticulum (c)	213	5.79	0.3319	5.7405	5.8302
Temperature[b]					
Ventral rumen (a)	211	31.9	5.0058	31.1989	32.5576
Dorsal rumen (a)	213	32.4	4.6861	31.7469	33.0127
Atrium ruminis (a)	212	32.0	4.4865	31.4028	32.6176
Reticulum (b)	213	29.4	5.1608	28.6626	30.0567

Homogeneous groups are marked with the same letters enclosed in parenthesis

[a] Repeated measures ANOVA $F_{3,\ 636}=118.027$; $p<0.0001$

[b] Repeated measures ANOVA $F_{3,\ 627}=84.009$; $p<0.0001$

confidence intervals for all slopes shown in Fig. 3a were overlapping).

Postmortem changes in temperature appeared more pronounced than were changes in pH (slopes ranged between −2.739 and −3.263, compared with a range of −0.032 to −0.055 for pH; Fig. 3b). Differences in temperature between forestomach components were significant (RM ANOVA $F_{3,\ 627}=84.009$; $p<0.0001$). However, the patterns did not consistently match those recorded for pH, because while mean temperature of the dorsal and ventral rumen were similar (31.9 and 32.4 °C, respectively; Tukey's post hoc $p=0.086$), the *Atrium ruminis* did not differ from either of these regions (32.0 °C; $p=0.924$ and 0.308, respectively; Table 1). Mean temperature of the reticulum (29.4 °C) was significantly lower than in all parts of the rumen ($p<0.0001$).

Animals that received supplemental feeds had lower mean forestomach pH (average for all compartments combined=5.50±0.34 SD) compared with animals that had no supplements (5.73±0.29 SD). This effect was significant even after accounting for the effects of season, area of origin, sex, body mass and postmortem changes in the analyses ($p<0.001$ to 0.028; Table 3). The only other factor that influenced pH was time of year, with pH of animals sampled during May and June generally lower than animals sampled during other months ($p<0.05$; Fig. 4a). When only females were considered in the analysis, the consistently significant effect of feeding supplementation persisted, and only temperature had any additional influence, and then only in the reticulum (Table 3).

Given these results, it is not surprising that the effect of feed supplementation appeared in virtually all well-supported models (ΔAIC≤2.0) describing sources of variation in forestomach pH, whereas other environmental and biological effects did not contribute to the best models consistently (Table 4). In addition to this, forestomach pH was not differently affected by supplementation across animals from different hunting areas, of different body masses, rumen temperature or times since death (Fig. 4b–e; interaction effects $p=0.058$ to 0.612). Only when just females were included in the analysis was the effect of postmortem temperature changes on pH of the ventral rumen different for animals that received the supplement (more positive; $p=0.013$ and $p=0.017$ when only animals sampled during November and December were considered).

Similarly, interaction effects between diet supplementation and other parameters were seldom significant in other forestomach compartments. Results for the dorsal rumen mirrored those of the ventral rumen and the reticulum, whereas there appeared to be a positive

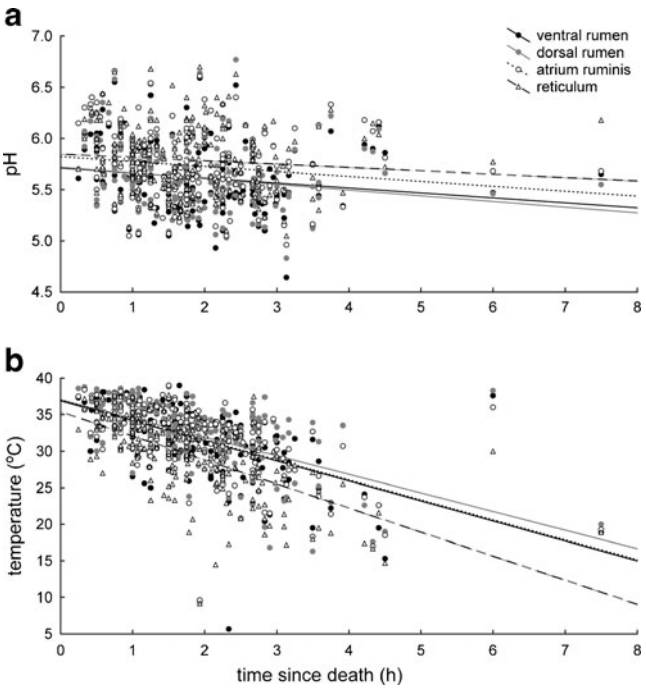


Fig. 3 Changes in forestomach pH (**a**) and temperature (**b**) of roe deer in relation to the time lapsed between death and sampling. Four forestomach regions are compared (ventral rumen, dorsal rumen, *Atrium ruminis* and reticulum). Fit lines are simple linear regressions: (a) ventral rumen $y=5.706–0.048x$, $r^2=0.023$, $p=0.027$; dorsal rumen $y=5.713–0.055x$, $r^2=0.030$, $p=0.021$; *Atrium ruminis* $y=5.818–0.048x$, $r^2=0.021$, $p=0.034$; reticulum $y=5.843–0.032x$, $r^2=0.010$, $p=0.158$; (b) ventral rumen $y=36.896–2.739x$, $r^2=0.297$, $p<0.0001$; dorsal rumen $y=37.030–2.537x$, $r^2=0.293$, $p<0.0001$; *Atrium ruminis* $y=37.012–2.730x$, $r^2=0.368$, $p<0.0001$; reticulum $y=35.293–3.263x$, $r^2=0.402$, $p<0.0001$

Table 3 General linear models testing additive effects of feed supplementation, season (month), sex, locality, body mass and postmortem changes (time since death or temperature changes) on forestomach pH

Effect	*df*	pH ventral		pH dorsal		pH *Atrium*		pH reticulum	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*P*
Whole dataset									
Postmortem effect is time									
Suppl	1	7.950	0.0054	5.975	0.0155	11.251	0.0010	4.886	0.0284
Month	7	2.095	0.0463	2.093	0.0466	2.524	0.0169	2.112	0.0446
Sex	1	0.886	0.3477	0.956	0.3296	3.143	0.0780	2.830	0.0943
Area	6	1.246	0.2848	1.757	0.1106	1.474	0.1896	1.577	0.1564
M	1	0.059	0.8077	0.015	0.9031	0.031	0.8603	0.169	0.6815
t	1	1.186	0.2777	2.088	0.1503	0.782	0.3778	0.001	0.9706
Postmortem effect is temperature									
Suppl	1	7.087	0.0085	6.117	0.0143	11.570	0.0008	5.111	0.0250
Month	7	2.226	0.0342	2.122	0.0435	2.579	0.0148	2.197	0.0365
Sex	1	0.860	0.3551	0.946	0.3320	3.135	0.0783	2.902	0.0902
Area	6	1.113	0.3566	1.719	0.1188	1.498	0.1812	1.616	0.1450
M	1	0.026	0.8718	0.020	0.8868	0.031	0.8597	0.168	0.6820
T°C	1	0.064	0.8001	0.000	0.9840	0.189	0.6641	2.563	0.1111
Females only									
Postmortem effect is time									
Suppl	1	7.675	0.0068	5.676	0.0194	12.191	0.0008	4.708	0.0328
Month	6	0.524	0.7886	0.648	0.6914	0.654	0.6870	0.749	0.6119
Area	6	1.909	0.0883	1.750	0.1190	1.537	0.1756	1.374	0.2342
M	1	0.000	0.9943	0.023	0.8797	0.002	0.9615	0.000	0.9936
t	1	0.512	0.4760	0.162	0.6888	1.429	0.2352	0.530	0.4685
Postmortem effect is temperature									
Suppl	1	6.960	0.0099	6.005	0.0162	12.498	0.0007	5.067	0.0269
Month	6	0.570	0.7528	0.676	0.6693	0.594	0.7343	0.797	0.5747
Area	6	1.811	0.1063	1.806	0.1070	1.518	0.1818	1.444	0.2070
M	1	0.002	0.9682	0.024	0.8769	0.004	0.9488	0.000	0.9843
T°C	1	0.986	0.3236	1.861	0.1760	1.392	0.2413	4.413	0.0385
Females from November/December only									
Postmortem effect is time									
Suppl	1	7.798	0.0069	5.648	0.0205	12.773	0.0007	5.326	0.0243
Area	6	2.174	0.0569	1.714	0.1320	1.714	0.1320	1.784	0.1165
M	1	0.132	0.7180	0.226	0.6360	0.102	0.7502	0.041	0.8394
t	1	2.074	0.1547	1.469	0.2300	3.557	0.0638	0.358	0.5520
Postmortem effect is temperature									
Suppl	1	6.830	0.0112	5.861	0.0183	12.771	0.0007	5.897	0.0179
Area	6	1.965	0.0838	1.737	0.1264	1.667	0.1435	1.935	0.0883
M	1	0.109	0.7419	0.223	0.6382	0.090	0.7656	0.039	0.8435
T°C	1	0.280	0.5987	1.324	0.2540	0.986	0.3244	5.124	0.0269

suppl supplemental feed provided or not, *area* locality, *M* body mass (kilogram), *t* time since death, *T°C* temperature

relationship between body mass and pH of the *Atrium ruminis* only in animals that received the diet supplement (see Supplementary material).

Although sex was included as a factor in some of our analyses, comparisons are difficult because there are only a few months in which multiple specimens of both sexes were sampled. Nevertheless, the finding of a lack of differences between sexes in GLMs, and the fact that this effect was not consistently prominent in the best-supported combinations of parameters, persisted even within months: for May and June, 95 % confidence limits for forestomach pH in both sexes overlapped strongly (females 5.42 to 5.66, females 5.55 to 5.78; Fig. 4a). In a similarly restricted comparison, females

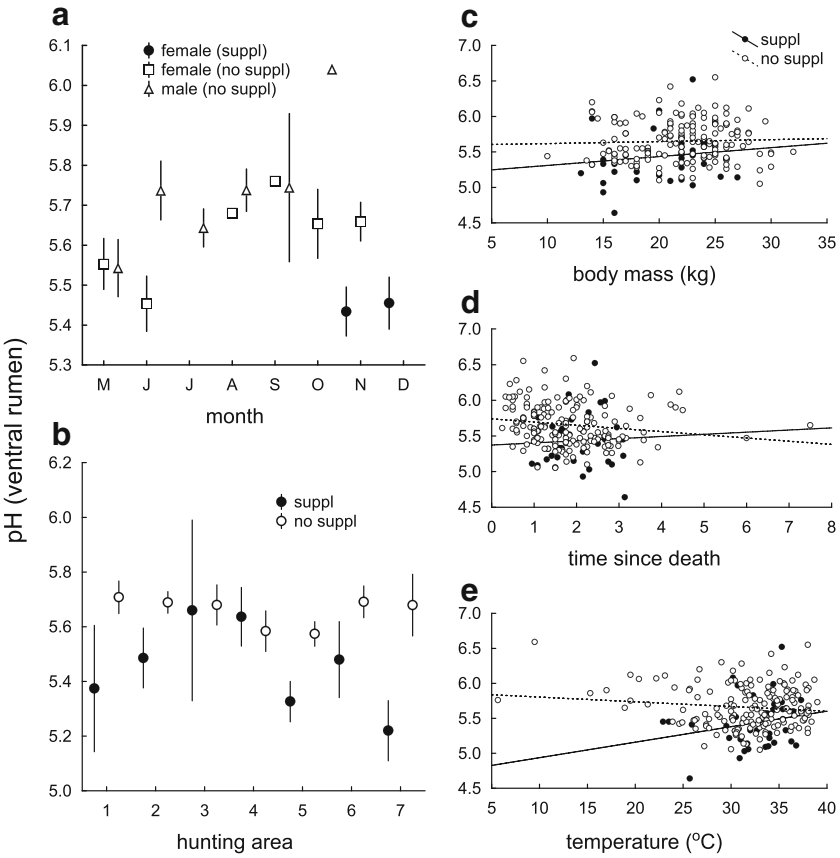


Fig. 4 Influence of supplemental feeding on environmental and biological effects on forestomach pH of roe deer (only data from the ventral rumen are shown; see Supplementary Figs. S1, S2 and S3). Symbols in **a** and **b** are means, vertical bars are ±1 SEM. In months with sufficient sample, 95 % confidence intervals of females and males overlapped (**a**; note that the sample did not include any males that received the supplemental feeding). Interaction effects were not significant in all cases (**b**–**e**; p= 0.058 to 0.612); however, the temperature effect (**e**) had a significantly more positive slope among animals that had access to supplemental feeds when only females were included in the analysis (p= 0.013), and when only females sampled during November and December were included (p= 0.017)

sampled during November and December that received diet supplements had consistently lower mean forestomach pH (5.44) than females from the same time period but which did not receive supplements (5.66). The 95 % confidence intervals of these two groups of females never overlapped (e.g. for the ventral rumen 5.32 to 5.55 and 5.56 to 5.76, respectively), further validating the strong influence of diet supplementation on these data.

Comparative literature study

In the complete species dataset (n=29), the relationship between body mass (BM) and rumen pH tended towards significance (r=0.346, p=0.066; Fig. 5a), and there was a significant correlation between %grass in the diet and rumen pH (r=0.437, p=0.018, Fig. 5b). The GLM with %grass and BM as covariables indicated a significant influence of the first on rumen pH and again a tendency for the second measure (%grass: $F_{1,\ 26}$=5.943, p=0.022; BM: $F_{1,\ 26}$= 3.865, p=0.060; r^2=0.30). If the four species that visually appear as outliers (three New World cervids and the bushbuck *Tragelaphus scriptus*) were excluded from the dataset (n=25), these relationships disappeared. Neither %grass (r= 0.117, p=0.579) nor BM (r=0.339, p=0.097) was significantly correlated to pH, and the GLM also indicated no significant influence of these covariables (%grass: $F_{1,\ 22}$= 0.351, p=0.560; BM: $F_{1,\ 22}$=3.262, p=0.085; r^2=0.14).

Discussion

Methodological aspects of pH measurements in wild ruminants

This study confirms the predictions from the 'Introduction' that in measuring pH, both the exact locality of measurement in the forestomach, and the time between death and measurement (albeit not statistically significant for roe deer provided supplemental feed), are relevant details that need to be recorded for truly comparable results. Even within the small forestomach of roe deer (with a capacity of approximately 1.5–2 kg), small differences in the location of the measurement, as between the ventral rumen and the *Atrium ruminis*, led to differences of a decimal (5.6 vs. 5.7) in pH. The location of measurement may have an even larger effect on the data in other species. In roe deer, the contents of the rumen itself show a low degree of stratification (Clauss et al. 2009a). This is reflected in the fact that pH did not differ between dorsal and ventral contents in this study, suggesting that measurements from different roe deer studies are

Table 4 Best-supported models (i.e. models with ΔAIC≤2.0) of effects of feed supplementation, locality, season (month), sex, body mass and postmortem changes (time since death or temperature changes) on forestomach pH

pH ventral		pH dorsal		pH *Atrium*		pH reticulum	
Model	ΔAIC	Model	ΔAIC	Model	ΔAIC	Model	ΔAIC
Whole dataset							
Postmortem effect is time							
Suppl+*t*	0.00	Suppl+mo+*t*	0.00	Suppl+sex	0.00	Suppl	0.00
Suppl	0.92	Suppl+sex+mo+*t*	0.26	Suppl+*t*	0.27	Suppl+sex	0.38
Suppl+sex	1.51	Suppl+*t*	1.49	Suppl	0.69	Suppl+*M*	1.16
Suppl+sex+*t*	1.60	Suppl+mo+*M*+*t*	1.99	Suppl+sex+*t*	0.89	Suppl+*t*	1.18
Suppl+*M*+*t*	1.66			Suppl+*M*+*t*	2.00		
Postmortem effect is temperature							
Suppl	0.00	Suppl	0.00	Suppl+sex	0.00	Suppl+T°C	0.00
Suppl+sex	0.44	Suppl+sex	0.68	Suppl	0.86	Suppl	1.12
Suppl+*M*	1.37	Suppl+*M*	1.75	Suppl+T°C	2.00	Suppl+sex+T°C	1.42
Suppl+T°C	1.97	Suppl+T°C	1.91	Suppl+*M*	2.00	Suppl+sex	1.52
Females only							
Postmortem effect is time							
Suppl+area	0.00	Suppl	0.00	Suppl	0.00	Suppl	0.00
Suppl	0.27	Suppl+area	0.66	Suppl+*t*	1.30	Suppl+*M*	1.85
Suppl+area+*t*	1.33	Suppl+*M*	1.82	Suppl+area	1.95	Suppl+*t*	1.92
Suppl+area+*M*	1.66	Suppl+*t*	2.00	Suppl+*M*	1.98		
Postmortem effect is temperature							
Suppl	0.00	Suppl+T°C	0.00	Suppl	0.00	Suppl+T°C	0.00
Suppl+area	0.29	Suppl	1.43	Suppl+T°C	0.05	Suppl	0.86
Suppl+T°C	0.68	Suppl+area+T°C	1.71			Suppl+*M*+T°C	1.98
Suppl+area+T°C	1.14	Suppl+*M*+T°C	1.74				
Suppl+*M*	1.99	Suppl+area	1.98				
Females from November/December only							
Postmortem effect is time							
Suppl+area+*t*	0.00	Suppl	0.00	Suppl+*t*	0.00	Suppl	0.00
Suppl+area	0.36	Suppl+area	1.09	Suppl+area+*t*	0.13	Suppl+area	0.59
Suppl+*t*	0.61	Suppl+*t*	1.19	Suppl	0.56	Suppl+*t*	1.82
Suppl+area+*M*+*t*	1.82	Area+*t*	1.25	Suppl+area+*M*+*t*	1.98	Suppl+*M*	1.98
Suppl	1.88	Suppl+area+*t*	1.48	Suppl+*M*+*t*	2.00		
		Suppl+*M*	2.00				
Postmortem effect is temperature							
Suppl+area	0.00	Suppl+T°C	0.00	Suppl+T°C	0.00	Suppl+area+T°C	0.00
Suppl	0.62	Suppl	1.05	Suppl	0.18	Suppl+T°C	1.53
Suppl+T°C	1.33	T°C	1.40	Suppl+area	1.57	Suppl+area+*M*+T°C	1.56
Suppl+area+T°C	1.72	Suppl+*M*+T°C	1.87	Suppl+*M*+T°C	1.88		
Suppl+area+*M*	1.91	Suppl+area	1.99				

suppl supplemental feed provided or not, *area* locality, *mo* month, *M* body mass (kilogramme), *t* time since death, *T°C* temperature

probably comparable, if one assumes that sampling took place at either the dorsal or the ventral rumen. Such an assumption is reasonable as these sites are usually accessed first when opening the forestomach. In species with more stratified rumen contents such as red deer or bison (*Bison bison*) (Clauss et al. 2009b), defined measurement locations will be even more necessary than in roe deer.

That continuing fermentation processes after the cessation of the absorption of the end products will influence pH measurements was confirmed in both the pilot observation

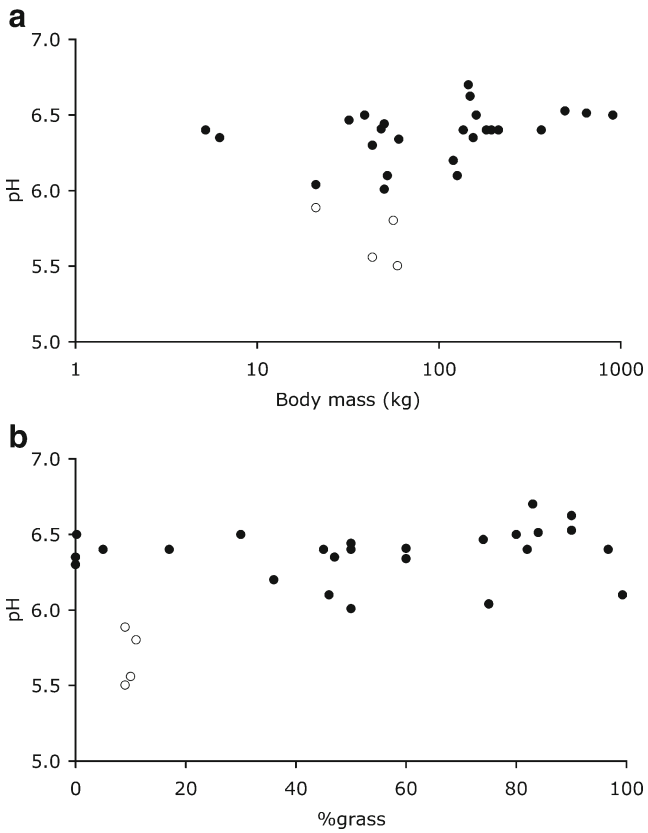


Fig. 5 Relationship of the pH of rumen contents to **a** body mass and **b** the percentage of grass in the natural diet in various wild ruminant species (data from Table 1). The *open symbols* denote the roe deer (*Capreolus capreolus*), white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*) and the bushbuck (*Tragelaphus scriptus*)

and the dataset on non-supplemented animals in this study. Particularly low pH results from the literature, such as in mule deer or bushbuck (Table 1), had been suspected by their respective authors to be a result of such a delay between the time of death and the pH reading. The regression equations for Fig. 4d suggest that in this study, a pH drop of about 0.05 should be expected for each hour that elapses between the time of death and pH reading; however, the data from the pilot observation in Fig. 1b indicate that the most dramatic effect will occur directly after death, where pH may drop in a non-linear fashion by several decimals within less than half an hour. As the shortest time difference between death and measurements was 15 min (Fig. 3)—a time span in which the pH might drop from 6.06 to 5.87, as indicated by the difference in pH between the reading 30 and 45 min after death in the pilot observation (Fig. 1b), we must consider the possibility that the data gained from measurements in shot roe deer (in this and other studies) are not representative of live animals. For the purpose of a comparison between different localities in the forestomach, or between different treatment groups (as in this study), this need not be considered detrimental when accounting for time since death. However, linking the results to threshold values, as available for domestic ruminants, is currently not feasible. Even worse, without detailed studies on the influence of even short delays between death and measurement in many species, comparative analyses of rumen pH between ruminant species, and from various literature sources, are highly problematic.

Finally, seasonal aspects on rumen pH need to be considered. Similar to previous studies in roe deer (Djordjević et al. 2006; Popović et al. 2009), there was a difference in pH between seasons, with lower values, indicating higher quality diets that have higher fermentation rates, during the vegetative period (May/June in this study).

Comparative aspects of wild ruminant forestomach pH

Given these methodological constraints, interpreting patterns in data collated from the literature cannot be more than explorative and hypothesis-generating. When data for all species were used, effects of body size (as a proxy for selectivity and hence diet quality) and the percentage of grass in the natural diet (as a proxy for the proportion of more slowly fermenting material) were as predicted. This effect occurred, however, only because of the inclusion of four species—Odocoiline cervids, and the bushbuck. Note, for example, that the rumen pH reported for very small, non-grazing ruminants, the dikdik (*Madoqua kirki*) and the suni (*Neotragus moschatus*), were of the same magnitude as many larger, more grazing species (Table 1, Fig. 5). The existing data raise the somewhat intriguing question why the pH is low in the four mentioned outlier species. If one assumes similar methodological constraints for all ruminant species, i.e. assuming that conditions with respect to time of death and time of measurement were comparable—in most cases, the measurements were taken in animals shot in the wild—then these animals may indeed be peculiar. Alternatively, one could hypothesise that differences in the degree by which rumen pH drops between death and measurements between different species might cause these outliers to be different. In general, in spite of the finding of Jones et al. (2001) that browsers had lower rumen pH (in the range of our roe deer) as opposed to grazers (see 'Introduction'), existing data so far do not allow a clear distinction between feeding types, or the demonstration of a systematic effect of body mass. Such effects would have to be investigated in a study that applies a consistent sampling regime and covers a large range of species.

Supplemental feeding of free-ranging wild ruminants

While it is generally accepted that supplementary feeding of free-ranging wildlife should not jeopardise animal health (Woolf and Kradel 1977; Rehbinder and Ciszuk 1985),

studies that focus on this aspect are rare—in contrast to studies on the effect of supplementary feeding on forest damage or population development (Putman and Staines 2004). To our knowledge, no scientific review of the supplementary feeding practices in roe deer exists; however, a review on such practices for red deer recorded a large variety of regimes, ranging from hay-only supplements to mixtures of various energy-dense feeds such as those used in this study (Putman and Staines 2004). Supplementary feeding serves to maintain body mass and, via a reduction of winter mortality and increased fecundity, high population densities, to increase trophy sizes, and to prevent forestry or agriculture damage (Bartoskewitz et al. 2003; Putman and Staines 2004; Peterson and Messmer 2007); therefore, natural selection is reduced in such populations (Schmidt and Hoi 2002).

The selection of supplement diets ranges between two extremes: Low-quality roughage diets are maybe used to avoid concentrate-associated acidosis (Rehbinder and Ciszuk 1985), with the potential problem that such diets are either not accepted by the animals (Ouellet et al. 2001; Clauss et al. 2003) or even lead to digestive impactions (Schoonveld et al. 1974). Diets higher in energy are more readily accepted, achieve most management goals to a higher degree, but incur the danger of ruminal acidosis (Wobeser and Runge 1975; Woolfe 1977). The results of this study clearly demonstrate that supplemental feeding led to a lower rumen pH in the investigated roe deer populations. The magnitude of the effect was comparatively small (from 5.7 to 5.5), but the mean pH reached in the supplemented group is at the threshold for the diagnosis of subacute, chronic acidosis in domestic cattle (see 'Introduction'). There are, however, several indications that this need not directly be associated with a potential danger in roe deer. The low pH value in the supplemented animals is within the range reported for non-supplemented individuals of other studies (Djordjević et al. 2006; Popović et al. 2009) and also within the range of non-supplemented roe deer of this study from May/June (Fig. 4a and Supplements). In domestic ruminants, there is limited evidence that the goat has a higher resistance to rumen acidosis than cattle (Mgasa and Mbassa 1988), with rumen pH as low as 5.2 not associated with clinical signs or rumen pathology (Mgasa and Arnbjerg 1993). Again, this emphasises the concept that pH measurements alone are not sufficient to determine whether a health problem occurs, unless extremely low values indicative of acute acidosis (below 5.0) are measured shortly after death. The suitability of the supplementation regime used in this study, therefore, would have to be evaluated for example by histological examinations of the rumen mucosa. As a precautionary measure, the inclusion of a more fibrous component in the overall diet mixture, such as chopped lucerne hay or sunflower seed hulls (Baker and Hobbs 1985), would probably reduce the effect of the diet on rumen pH.

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