Evaluation of a device for continuous measurement of rumen pH and temperature considering localization of measurement and dietary concentrate proportion

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Abstract:

Continuous rumen pH and temperature measurement may be useful tools for diverse purposes including the detection of subacute ruminal acidosis. The objectives of the present study were to evaluate a device for continuous monitoring of rumen pH and temperature of cattle focussing on rumen pH determination and to test the effects of measurement localization as well as dietary concentrate proportion on rumen pH and temperature. Four rumen-fistulated cows were fed on two diets containing 0 and 40 % concentrate. Measurement was executed for two days per cow and diet. One probe was inserted each in the dorsal and ventral rumen sac to measure pH and temperature. Manual temperature determination and pH measurement were performed postprandial in direct proximity to the probes at preset short term intervals. PH sensors were tested for drift. The pH sensor drift was inconsistent with a considerable individual variation. A moderate correlation between manual and continuous measurement of pH ($r = 0.59$, $p < 0.001$) and temperature ($r = 0.46$, $p < 0.001$) was calculated. A Bland-Altman comparison of both methods indicated moderate agreement. A bias effect of probe pH determination with a pH overestimation in the range of low rumen pH below 6.0 and an underestimation of higher rumen pH was observed. Rumen pH was not affected by the localization of measurement but by diet and time after feeding. Significant effects of localization, diet and time and an interaction of localization and diet on rumen temperature were found. In conclusion, the evaluated technique was promising. Indications of inaccuracy of probe pH measurement suggested the need of further improvement.

Keywords: *continuous rumen pH measurement, rumen temperature, concentrate proportion*

Zusammenfassung

Evaluierung eines Gerätes zur kontinuierlichen Messung von Pansen-pH und -temperatur unter Berücksichtigung von Messort und Kraftfutteranteil der Ration

Die Messung von Pansen-pH und -temperatur kann zur Detektion einer subakuten Pansenazidose beitragen. Ziel dieser Arbeit war die Evaluierung eines Gerätes zur kontinuierlichen Bestimmung beider Parameter bei variierendem Messort und Kraftfutteranteilen von 0 und 40 % in der Ration. Vier am Pansen fistulierte Kühe wurden mit jeweils einem Gerät im dorsalen und ventralen Pansen ausgerüstet. Zusätzlich erfolgten zu definierten Zeitpunkten manuelle Messungen in unmittelbarer Nähe der Geräte. Die pH-Drift der Sensoren war bei den einzelnen Geräten uneinheitlich mit einer hohen Variation. Eine moderate Korrelation lag zwischen manueller und kontinuierlicher pH- ($r = 0{,}59$) und Temperaturmessung ($r = 0{,}46$) vor. Ein Bland-Altman Vergleich deutete auf eine mäßige Übereinstimmung der pH-Messungen hin. Es wurden Hinweise auf Ungenauigkeiten der kontinuierlichen pH-Bestimmung mit einer Überschätzung niedriger Werte unter 6,0 und einer Unterschätzung hoher Werte über 6,5 beobachtet. Der Pansen-pH wurde durch die Ration und die Zeit nach der Fütterung nicht aber durch den Messort beeinflusst. Signifikante Effekte des Messortes, der Ration und der Zeit nach der Fütterung auf die Pansentemperatur und eine Interaktion des Messortes und der Ration wurden ermittelt. Die evaluierte Messtechnik hat ein hohes Potential, Indikationen von Messungenauigkeiten weisen jedoch auf die Notwendigkeit einer Verbesserung der pH-Bestimmung hin.

Keywords: *kontinuierliche Pansen-pH-Messung, Pansentemperatur, Kraftfutteranteil*

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1 Introduction

Subacute ruminal acidosis (SARA) is a metabolic disorder affecting rumen fermentation and functionality, animal health and productivity of dairy cows with a considerable prevalence in European herds (Kleen et al., 2009; Morgante et al., 2007). It may be induced by the consumption of diets containing high amounts of easily fermentable carbohydrates, particularly in combination with a rumen environment insuffi-ciently adapted to such diets as frequently occurring in post-partum periods (Kleen et al., 2003). Feeding high amounts of grain results in increased production of short chain fatty acids (Bauman et al., 1971) and decreased rumen pH, whereas especially in early lactation the rumen mucosal papillae are short with a small surface for the absorption of short chain fatty acids.

Various thresholds of rumen pH have been discussed to indicate the onset of SARA. Garrett at al. (1999) suggested a critical value of 5.5 and discussed the determination of pH in rumen fluid from group subsamples as a potential tool to detect SARA in dairy cow herds. In a recent study, the onset of SARA was supposed to be characterized by a rumen pH below 5.6 for at least three hours per day (Gozho et al., 2005). The validity of results obtained by pH measurement in rumen fluid gained either by means of rumenocentesis, via oro-ruminal probes or through a rumen cannula as frequently executed in research is discussed to be limited. Restricted times of sampling, sample and animal number as well as sampling sites in the rumen or saliva contamination may affect the significance of obtained results. Furthermore the mentioned methods can hardly be applied by farmers. Enemark (2008) discussed the continuous monitoring of rumen pH instead of spot sampling as a promising measure to contribute to SARA diagnosis. One opportunity to realize a continuous pH measurement may be the use of indwelling rumen probes, which would allow the animal to move freely and undisturbed and would offer the benefit of sampling rumen pH at programmed intervals and thus give the chance to closely follow the course of rumen pH as influenced by different feeding regimes (Enemark, 2008). After the beginning of continuous rumen pH determination by means of intraruminal devices (Dado and Allen, 1993) various probes were evaluated for the application both in small ruminants (Penner et al., 2009) and cattle (Enemark et al., 2003; Penner et al., 2006; Phillips et al., 2010). Effects of the sites of sampling on pH of withdrawn rumen fluid were reported, mean pH values at the cranial-dorsal rumen were slightly but not significantly lower than those at the cranial-ventral rumen (Duffield, 2004; Li et al., 2009). Dietary concentrate propor-tion is well known to affect rumen pH (AlZahal et al., 2009; Mishra et al., 1970), though small alterations of the forage: concentrate ratio in dairy cow diets do not necessarily influence mean rumen pH (Maekawa et al., 2002). Information about interactions of the localization of pH measurement in the rumen and the amount of concentrate fed is rare but may be valuable for the interpretation of pH data determined by indwelling rumen probes.

Recently, Kaur et al. (2010) tested commercially available devices for rumen pH, pressure and temperature measurement with the ability of telemetric data transfer (KB 1101 bolus, Kahne Limited, New Zealand). They observed a weak relationship between bolus and manual pH measurement in withdrawn rumen fluid and a steadily increasing pH sensor drift. Bolus pH determination was performed via ISFET (ion-selective field-effect transistor) sensors, which may show long-term drift and low performance in comparison to glass electrodes (Oelssner et al., 2005). Meanwhile, a renewed successor of these probes is available using a glass membrane sensor for pH measurement. Though an improved accuracy and agreement with manual rumen pH determination may be expected, reliable information about the actual performance of that transformed probe is required. Furthermore, rumen temperature measurement may aid in the detection of SARA since a close inverse correlation with rumen pH was reported (AlZahal et al., 2008). Forestomach temperature was found to be strongly correlated with rectal temperature (Bewley et al., 2008; Burns et al., 2002) and may also be a useful diagnostic parameter for the detection of estrus, heat stress or infectious diseases in dairy cows (Fordham et al., 1988; Kadzere et al., 2002; Martello et al., 2010). The aim of the present study was to evaluate new commercially available devices for pH, temperature and pressure measurement in the rumen of cattle primarily focussing on the examination of pH measurement and to test potential effects of the localization of measurement in the rumen as well as dietary concentrate proportion on rumen pH and temperature.

2 Materials and Methods

2.1 Animals and feeding

The present study was performed at the Experimental Station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health in Braunschweig, Germany, in compliance with the European Union Guidelines concerning the protection of experimental animals. Four non-lactating German Holstein cows with a mean initial bodyweight of 614 ± 76 kg equipped with large rubber cannulas in the dorsal rumen sac were used in the experiment, which was divided into two successive periods. Due to illness one animal had to be replaced by a lactating cow before adaptation feeding of diet 2 was started. The animals were kept in a tethered barn with individual troughs and free access to water. Two diets were fed successively after three weeks of adaptation each. Feeding was performed *ad libitum* twice daily at 05:15 and 15:15 h. Daily individual dry matter intake was recorded to calculate organic matter intake. Diet 1 was composed of 60 % maize silage and 40 % grass silage on a dry matter (DM) basis. Diet 2 was designed as a Total Mixed Ration (TMR) containing 36 % maize silage, 24 % grass silage and 40 % concentrate. Concentrate was composed of 50.0 % wheat grain, 26.8 % soybean meal, 20.8 % corn grain and 2.4 % mineral and vitamin premix. Feed samples were collected daily to produce an aggregate sample for analysis of the chemical composition. Diet crude nutrient contents were analyzed according to the suggestions of the Verband Deutscher Landwirtschaftlicher Unter-

suchungs- und Forschungsanstalten (Naumann and Bassler, 1993). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to Goering and Van Soest (1970) and expressed without residual ash. The chemical composition of the diets is presented in Table 1.

Table 1
Chemical composition (g/ kg DM) and energy content (MJ/ kg DM) of experimental diets

Parameter	Diet 1	Diet 2
Organic matter	948	948
Crude protein	97	144
Ether extract	35	33
Crude fibre	227	149
ADF	245	164
NDF	447	333
ME[1]	10.4	11.6
NE_L[1]	6.2	7.1

[1]Tabular values were used to estimate silage and concentrate digestibility (DLG, 1997). Metabolizable Energy (ME) and Net Energy Lactation (NEL) were calculated as following (GfE, 2001): ME [MJ/kg] = 0.0312* DEE [g/kg] + 0.0136* DCF [g/kg] + 0.0147* (DOM - DEE – DCF) [g/kg] + 0.00234* CP [g/kg], where DEE = digestible ether extract; DCF = digestible crude fibre; DOM = digestible organic matter.

2.2 Rumen probes

The experiment was performed using two rumen probes (KB 3/04 bolus, Kahne Limited, New Zealand). The boluses were a successor of the technique described by Kaur et al. (2010) and were constructed to measure rumen pH, temperature and pressure in an adjustable frequency of 10 to 59 seconds or 1 to 255 minutes. The probes were constructed as a copolymer barrel of 145 mm length and 27 mm diameter with wings of altogether 185 mm attached to the tapered top. For pH determination a glass membrane pH sensor was incorporated in the bottom of the boluses. Pre- and post-use storage of the pH sensor was performed as recommended in a 3 molar potassium chloride solution. Kahne Data Processing System V 5.1 software was used for the calibration of the boluses and to download and export data for evaluation. The pH sensors were calibrated according to Kaur et al. (2010) in a water bath of 40 ± 0.1 °C in standard buffer solutions with a pH of 7.0 (first) and 4.0 (second), respectively (ZMK-Analytik-GmbH, Bitterfeld-Wolfen, Germany). A Kahne KR 2001 transceiver was connected to a computer by USB cable to transfer the calibration and setting instructions. Both temperature and pressure sensor were integrated in the probe enclosure. No manufacturer information about construction and functionality of the temperature sensor were available. Measured data were stored in an integrated memory card for later download with a maximum storage capacity of 11.955 data points per bolus, according to manufacturer. Data could be transferred to a computer in real time simultaneously. Logged bolus data transmission was initialized on demand using a handheld trigger device (Kahne Wand KW1, frequency 134.2 KHz). Data were received by a KR 2002 receiver (frequency 433.9 MHz) including antenna with a range of up to 30 meters, according to manufacturer.

2.3 Sampling

Bolus and manual measurement was performed simultaneously for two consecutive days per cow and diet. Manual rumen pH and temperature as well as rectal temperature were determined 15, 45, 75, 135, 195, 255, 315, 375, 435 and 495 minutes after morning feeding. Two boluses were set to measure every 5 minutes. The probes were fixed to a coated cord tied to the inner cannula and weighed down by a galvanised iron weight of approximately 500 g in such a way to place one bolus in the dorsal and ventral rumen sac, respectively. The dorsal bolus was immersed approximately 10 cm in the rumen content at pre feeding level in the morning. Bolus data were downloaded using the trigger device. To minimize the impact of short term variation bolus rumen pH and bolus rumen temperature were calculated by taking the arithmetic mean of all recorded values within ± 15 minutes from preset manual measurement times. To investigate pH sensor drift boluses were removed at the end of both eight days periods followed by pH measurement in unused standard buffer solutions at 40 °C as utilized for calibration. This procedure was followed by bolus recalibration. Temperature sensors were not subjected to drift examination as their drift was reported to be negligible in a former study conducted by Kaur et al. (2010). Pressure data were not included in the evaluation due to expected data falsification by the rumen cannula and the necessity of opening it during the experiment.

Manual temperature measurement was performed via digital thermometer (Digitemp Servoprax E315, Servopax GmbH, Wesel, Germany) rectally and in the rumen in direct proximity of the two boluses. For manual pH measurement rumen content was withdrawn from the localizations of manual temperature determination. Obtained samples were squeezed immediately trough a close meshed nylon net followed by pH measurement in the gained fluid using a pH meter (WTW pH 530 BCB, LAT Labor- und Analysenbedarf, Garbsen, Germany).

2.4 Statistical analyses

Statistical analyses were performed utilizing the software package SAS version 9.1 (SAS, 2004). Pearson correlation coefficients between rumen pH, rumen temperature and rectal temperature data were calculated using the procedure "CORR". Linear regression analysis was executed by means of the "REG" procedure to compare bolus and manual pH measurement. The method described by Bland and Altman (1986) was used to assess the agreement between both techniques, differences between bolus and manual pH were plotted against the arithmetic mean for the pairs at each measurement point. The bias as the mean difference (d) including 95 % confidence interval (CI) and the standard deviation of the differences (s)

were calculated. The upper and lower limits of agreement were defined as d ± 1.96 s and used to summarize the level of agreement between both methods.

The procedure "MIXED" was applied to analyze rumen pH and temperature data. Diet, localization in the rumen, method and time were considered as fixed effects. Interactions between theses variables were investigated. Rumen temperature and rumen pH were included as covariates assessing rumen pH and temperature, respectively. The "random" statement was utilized for the individual cow effect. The restricted maximum likelihood method (REML) was used to evaluate variances. Degrees of freedom were calculated by the Kenward-Roger method. To investigate differences between least square means, the "PDIFF" option was used applying a Tukey-Kramer test for post-hoc analysis. Values used to quantify the effects of the mentioned variables were presented as LS means. Differences were considered to be significant at p <0.05.

Table 2
Correlation coefficients of rumen pH and rumen temperature among methods and localizations of measurement

				Temperature			
				Bolus		Manual	
				Ventral	Dorsal	Ventral	Dorsal
pH	Bolus	Ventral	r	0.07	-0.35	-0.01	-0.26
			p	0.385	<0.001	0.936	0.001
		Dorsal	r	0.06	-0.20	0.01	-0.16
			p	0.463	0.012	0.895	0.040
	Manual	Ventral	r	0.05	-0.43	-0.09	-0.37
			p	0.553	<0.001	0.272	<0.001
		Dorsal	r	0.01	-0.30	0.11	-0.36
			p	0.935	<0.001	0.165	<0.001

3 Results

3.1. General results

The mean daily organic matter intake per cow was 9.6 kg for diet 1 and 17.3 kg for diet 2. One bolus had to be replaced after feeding diet 1 due to technical disturbances which occurred after sampling. The operation of trigger device, receiver and software was easy to handle and appropriate for the download of data under the given experimental conditions, though the trigger had to be used in direct proximity of the animals. The receiver was able to receive data continuously and directly after the record of each data point from a distance of approximately 5 meters, whereat the manufacturer's data of a range of up to 30 meters was not verified. A total of 315 paired samples were available each for the comparison of pH and temperature data obtained by bolus and manual measurement, respectively.

The drift of the pH sensors after both eight days periods resulted in a mean bias of 0.04 ± 0.12 (Mean ± s.d.) in pH 4 buffer solution and -0.02 ± 0.15 in pH 7 buffer solution. One sensor showed a minimal positive drift in pH 4 buffer solution (Diet 1: 0.02, diet 2: 0.01) but slightly negative drift in pH 7 buffer solution (Diet 1: -0.03, diet 2: -0.08). However the other two sensors drift was either positive (Diet 1: pH 4: 0.19, pH 7: 0.20) or negative (Diet 2: pH 4: -0.04, pH 7: -0.16). Due to the partially undirected pH drift bolus pH data were not subjected to drift correction.

Slightly negative overall Pearson correlation coefficients between rumen pH and rumen temperature were calculated for both bolus (r = -0.11, p = 0.052) and manual (r = -0.25, p <0.001) measurement. A closer inverse relationship was found between rumen pH at both localizations of measurement and dorsal rumen temperature compared with ventral rumen temperature (Table 2). The correlations between pH values and rectal temperature (Bolus: r = -0.06, p = 0.323. Manual: r = -0.14, p = 0.002, respectively) and between rumen and rectal temperature (Bolus: r = 0.15, p = 0.09. Manual: r = 0.11, p = 0.053, respectively) were not existent or minimal.

3.2 Comparison of methods

The mean (± s.d.) total rumen pH was 6.39 ± 0.38 for bolus and 6.31 ± 0.57 for manual measurement. The number of recorded data points with a pH below 6.0 were in total n = 50 for bolus and n = 144 for manual reading. The correlation coefficients between the evaluated methods were r = 0.59 (p <0.001) for rumen pH and r = 0.46 (p <0.001) for rumen temperature. A closer correlation was found between ventral bolus pH and both ventral and dorsal manual pH (r = 0.80, p <0.001 and r = 0.74, p <0.001, respectively) than between dorsal bolus pH and ventral and dorsal manual pH (r = 0.51, p <0.001 and r = 0.45, p <0.001, respectively). The intra method correlation between pH determination at the ventral and dorsal rumen sac, respectively, was r = 0.59 (p <0.001) for bolus and r = 0.85 (p <0.001) for manual sampling.

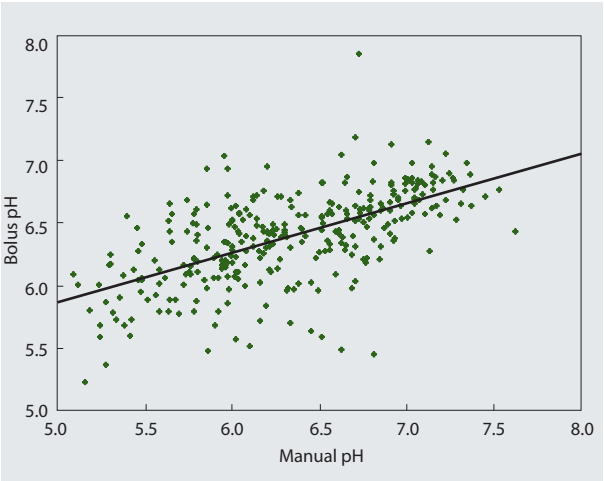

Figure 1
Relationship between manual and bolus pH measurement. y = 0.3938x + 3.9011, r^2 = 0.3436, N = 315.

Performing linear regression analysis the relationship between bolus and manual pH measurement was characterized by a considerable variation around the regression line with a relatively low coefficient of determination of $r^2 = 0.3436$ (Figure 1). The Bland-Altman comparison indicated a bias effect of bolus pH measurement with the tendency of pH overestimation in the range of low rumen pH below 6.0 and a pH underestimation at rumen pH above 6.5 (Figure 2). The estimated limits of agreement between bolus and manual pH measurement were -0.83 to 0.97.

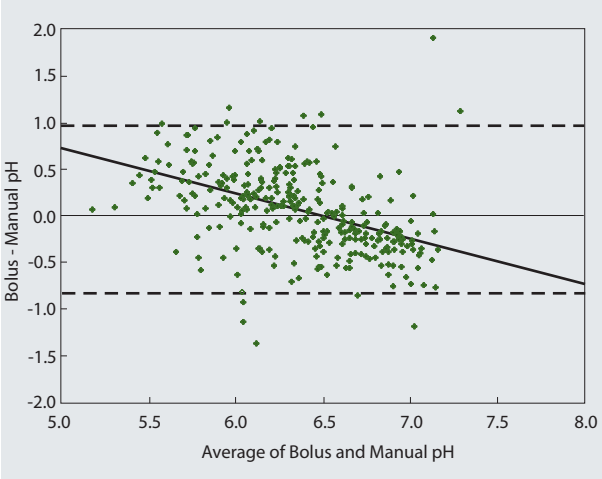

Figure 2
Differences of bolus and manual rumen pH measurement versus their mean. N = 315. Bias: 0.07, 95 % confidence interval: 0.03 to 0.11. Dotted lines show limits of agreement. A best fit line has been added to show change in bias with pH.

3.3 Effects of diet, localization and time

A significant diet effect on rumen pH was observed (Diet 1: 6.61. Diet 2: 6.16. p <0.001, Figure 3). The localization of measurement in the rumen did not influence pH values (Dorsal: 6.39. Ventral: 6.37. p Figure 2: Differences of bolus and manual rumen pH measurement versus their mean. N = 315. Bias: 0.07, 95 % confidence interval: 0.03 to 0.11. Dotted lines show limits of agreement. A best fit line has been added to show change in bias with pH >0.05), whereas the method of pH determination had a significant effect (p = 0.004). Rumen pH was affected by time after feeding (p <0.001, Figure 3). It decreased postprandial and recovered approximately to the initial value within the sampling period of 495 minutes. Though the interaction of diet and localization was significant (p = 0.014), the nominal effects were marginal. Dorsal and ventral rumen pH were nearly equal feeding diet 1 (Dorsal: 6.58, ventral: 6.63) and differed only slightly feeding diet 2 (Dorsal: 6.20, ventral: 6.12). No interactions were found between diet and time (p >0.05) and localization and time (p >0.05), respectively.

Rumen temperature was influenced by diet and increased due to feeding low fibre but high energy (Diet 1: 38.9 °C, Diet 2: 39.3 °C. p <0.001). The localization of measurement had a significant effect on rumen temperature (p <0.001). Ventral and dorsal temperature were almost equal feeding diet 1 (Ventral: 39.0 °C, dorsal: 38.9 °C), however a temperature gradient was observed for diet 2 (Ventral 39.0 °C, dorsal: 39.7 °C. Localization x diet: p <0.001). The applied methods did not affect temperature values (p = 0.115). A time effect on rumen temperature (p <0.001) was investigated. The significant interaction between diet and time was characterized by a time dependent decline of rumen temperature feeding diet 1 with the nadir after 75 minutes and a full recovery within the sampling period (p <0.001, Figure 4). Localization and time did not interact significantly (p >0.05).

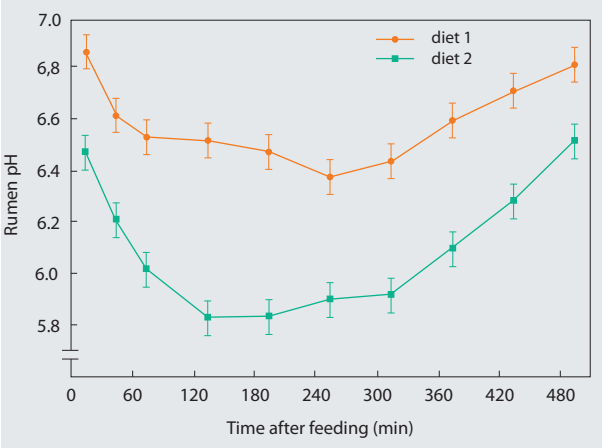

Figure 3
Rumen pH depending on time after feeding (min) and diet. Ventral and dorsal pH values were pooled and presented as means ± standard error.

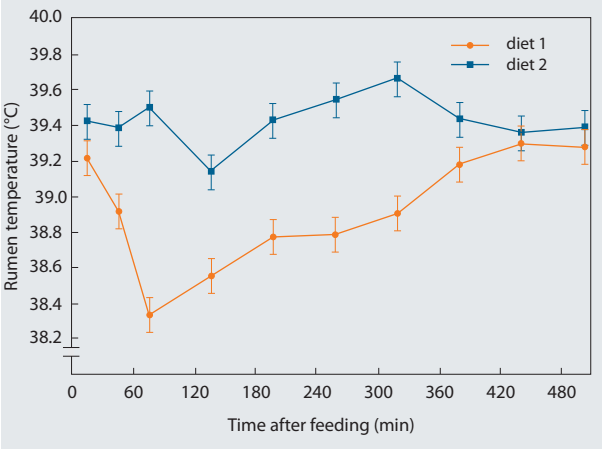

Figure 4
Rumen temperature depending on time after feeding (min) and diet. Ventral and dorsal pH values were pooled and presented as means ± standard error.

4 Discussion

A close relationship between rumen pH and temperature as indicated by AlZahal et al. (2009) would be expected to be inverse and to arise especially during intensive postprandial

fermentation reflecting both decreasing rumen pH but increasing rumen temperature. In the present study, the correlations between rumen pH and temperature were considerably lower than those reported in earlier experiments. An inverse relationship of r = -0.39 between rumen pH obtained by manual determination in fluid withdrawn from the ventral rumen sac of sheep and bolus temperature measured in vivo was found by Kaur et al. (2010). AlZahal et al. (2008) observed a correlation of r = -0.46 between ventral rumen pH and temperature utilizing an indwelling electrode measuring both parameters simultaneously in lactating dairy cows, whereas the direct proximity of both sensors may have contributed to the close relationship. The authors developed an equation for the prediction of rumen pH from rumen temperature and thus discussed the potential of rumen temperature for the detection of SARA in cattle. In the present experiment, rumen pH measured via both methods was distinctly closer correlated to dorsal than to ventral temperature. Thus, under the present conditions, the potential contribution of rumen temperature measurement to the prediction of rumen pH and the use as an indication of SARA may depend on the site of intraruminal temperature determination.

A close correlation (r = 0.65) between reticulum temperature measured by an indwelling probe and rectal temperature of intact dairy cows was observed by Bewley et al. (2008) and based on a large amount of paired samples which were taken during several seasons. Burns et al. (2002) used the same technique in cows near the occurrence of oestrus and reported a relationship of r = 0.50 between reticular and rectal temperature. The restricted postprandial sampling times may have contributed to the low relationship of rumen and rectal temperature in the present trial. Though after feeding a time effect was observed for rumen temperature, rectal temperature may have been less affected causing a low correlation. Uncertainty exists whether an outflow of heat through the perforating rumen cannula has contributed to the low relationship of the two parameters. However a close correlation between rumen and rectal temperature (r = 0.92) was reported for rumen-fistulated lactating cows in a former study using a prototype rumen bolus (Sievers et al., 2004).

Observations concerning sensor drift of indwelling devices for rumen pH measurement were diverse. Penner et al. (2006) reported partially undirected but not significant pH drift after 72 hours using an encapsulated electrode for pH measurement in the ventral rumen sac of dairy cows. In a former study, Enemark et al. (2003) utilized devices which were initially developed for marine animals and observed a slightly positive electrode drift after ten days of continuous application in the reticulum of cows. In a study of Kaur et al. (2010) a predecessor generation of probes for intraruminal pH, temperature and pressure measurement produced by the same manufacturer was evaluated utilizing fistulated sheep in ten day periods. Bolus pH sensor drift was visually apparent after 48 h and increased steadily from that time. The technical comparability of both probe series may be limited due to constructional differences, as the former boluses were equipped with ISFET sensors for pH measurement, which may exhibit long-term drift and low performance in comparison to glass electrodes (Oelssner et al., 2005). The inconsistent and partially undirected pH sensor drift in the present study and the wide range reported for sensors in earlier experiments suggested the need to assess occurrence and direction of pH sensor drift individually and depending on application time. Therefore further investigations of direction, amount and time-dependence of pH sensor drift seemed to be recommended prior to longer-term application of the evaluated boluses in intact animals.

Differing correlations between pH measurement via intraruminal probes and manual determination were reported in earlier studies. Duffield et al. (2004) observed varying but mainly weak relationships between pH measurement via a device placed in the ventral sac of the rumen of cows and manual pH determination in the second 200 ml of rumen fluid gained by a tube-like probe through a rumen cannula from different intraruminal sites (Cranial-ventral: r = 0.25, Caudal-ventral: r = 0.24, Central: r = 0.58, Cranial-dorsal: r = 0.53). A rather low correlation (r = 0.46) between pH readings gained by the former probe generation produced by the same manufacturer as the boluses used in the present study and manual measurement was investigated by Kaur et al. (2010). The closer correlation between bolus and manual pH data in the present study may be due to the better performance of the used glass membrane pH sensors and the potentially wider distance of probe and manual sampling in the former experiment. Other workers have proved closer correlations of r = 0.85 (Dado and Allen, 1993), r = 0.88 (AlZahal et al., 2007), r = 0.88 as well as r = 0.98 (Penner et al., 2006) and r = 0.98 (Phillips et al., 2010) between various indwelling probes and in vitro pH measurement in withdrawn rumen fluid samples.

The minor correlations between dorsal bolus pH and manual pH readings and the low intra method relationship between bolus pH measurement at the dorsal and ventral rumen sac, respectively, may be due to methodological reasons. The probe used to measure dorsal rumen pH was fixed to a cord to be immersed in the rumen content for approximately 10 cm at pre morning feeding level and may have protruded into the rumen gas phase for several short times during the sampling periods and thus may have produced a distortion of dorsal bolus pH data.

Though in the present trial the different standard deviations of pH values determined by both methods of rumen pH measurement indicated an unequal variation, the mean bolus pH was only slightly higher than the mean manual pH. That is consistent with results of Kaur et al. (2010), who found the probe pH to be 0.05 to 0.21 pH units higher than the manual pH measured by a pH meter, depending on diet. Contradictory findings were observed in earlier studies, were manual pH values were reported to be higher in rumen fluid removed through a cannula than the pH measured in vivo in the rumen (Dado and Allen, 1993) or the reticulum (Enemark et al., 2003) of dairy cows. The distance between the probe location and the site of rumen fluid sampling was discussed as a possible explanation for the differences in pH (Enemark et al., 2003).

The significance of the Pearson correlation coefficient as a tool of method comparison is limited as it is a measure of the linearity between two variables, not of the agreement between them. No indication of how the plotted data deviate from the line y = x can be derived (Lin, 1992) and data which seem to be in poor agreement can produce high correlations (Bland and Altman, 1986). Furthermore it may be difficult to assess differences between methods by a simple plot of the results of one method against those of the other. The method described by Bland and Altman involved plotting the difference (y-axis) of the paired measurements against their arithmetic mean (x-axis). The true value is unknown and the mean is the best available estimate (Bland and Altman, 1986). In the present study a deficient accuracy of manual pH determination cannot be excluded and may be caused by inherent bias like temperature effects (Meinrath and Spitzer, 2000). Such potentially restricted accuracy of manual pH measurement may have been responsible for the limited agreement between both methods. The positive bias of 0.07 in the Bland-Altman analysis would suggest the probes typically gave slightly higher results than the standard manual method. However the trend line indicated a lack of bias consistency with a pH overestimation in the range of rather low rumen pH below 6.0 and a pH underestimation at higher rumen pH above 6.5. This is congruent with the lower number of total data points of rumen pH below 6.0 detected by bolus measurement (n = 50) in comparison with the manual technique (n = 144) and may be misleading especially in interpreting rumen pH values to obtain indications of SARA. The calculated limits of agreement were – 0.83 to 0.97 indicating rather low agreement within the given experimental design. This range is expected to include 95 % of the values and its magnitude can be utilized to assess the utility of an alternative method (MacFarlane et al., 2010). Bland and Altman (1986) suggested that the acceptable range of the limits of agreement should be based on the clinical impact of the results within this range. Though it would be difficult to define suitable limits of agreement for rumen pH measurement, the determination of rumen pH requires a high accuracy to ensure reliable results for both research and diagnostic purposes.

The evaluation of the accuracy of continuous measurement via indwelling rumen probes using aggregated bolus data may be adequate for a comparison with spot sampling techniques same as the described manual method. Actually such method comparison did not consider the option of continuous measurement being the primarily advantage of the investigated boluses. According to Gozho et al. (2005), SARA in cattle should be defined as a depression of rumen pH below 5.6 for three or more hours per day. In intact animals, such intervals are hardly detectable with spot sampling techniques.

Rumen pH decreased significantly due to feeding concentrate as previously reported for steers (Jaakkola and Huhtanen, 1993; Owens et al., 2008) and cows fed high amounts of grain (Mishra et al., 1970). Besides the effects of dietary concentrate, the feeding of diet 2 was characterized by a higher OM intake level which may have contributed to the calculated diet effect on rumen pH. The localization of measurement in the rumen did not influence pH readings significantly ($p > 0.05$). Though a considerable pH gradient would be expected to emerge especially between reticulum and rumen, earlier experiments partially proved effects of the site of measurement in the rumen on mean pH values (Duffield et al., 2004; Li et al., 2009). Despite the absence of such a pH gradient between dorsal and ventral rumen sac in the present experiment, its possible emergence should be taken into consideration for potential on-farm and research application of the evaluated boluses or similar unfixed devices for intraruminal usage, as the probes may move through the reticulorumen if used in intact animals without certainty of the exact site of measurement.

In the current study rumen temperature was affected by diet, however a rise in mean rumen temperature associated with the feeding of concentrate was primarily observed at the dorsal rumen. Similar to the evaluation of diet effects on rumen pH, the higher intake level of diet 2 may have increased the described effect on rumen temperature. AlZahal et al. (2009) reported a significant increase of rumen temperature due to feeding high amounts of grain in comparison to a mixed hay diet. Such effects may depend on the amount of concentrate fed as in a study of Gasteiner et al. (2009) differences in rumen temperature of steers were not significant between a 100 % hay and a 50 % concentrate diet. The postprandial development of a temperature gradient in the rumen of dairy cows with an increased dorsal temperature due to feeding high-concentrate diets was coherent as, first, concentrate would be expected to be subjected to a faster ruminal degradation than forage and, secondly, the materials in the top stratum of the rumen digesta are recently ingested and are subjected to a higher fermentative activity than those contained in the middle and bottom strata of the rumen (Martin et al., 1999; Tafaj et al., 2004).

5 Conclusion

The evaluated devices showed a moderate linear relationship and agreement of pH measurement with the applied manual method, a fact that may as well be due to a deficient accuracy of manual pH determination. Varying and partially undirected but low pH sensor drift was observed. Indications for an inconsistent bias of bolus pH determination were found. Effects of the site of measurement in the rumen were not observed for pH but for temperature, may interact with the diet fed and should be taken into consideration for a potential use of indwelling devices. Though the described indications of inaccuracy of bolus pH measurement suggested the need of further improvement, the technique was promising especially due to the option of continuous intraruminal measurement.

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References

AlZahal O, Rustomo B, Odongo NE, Duffield TF, McBride BW (2007) Technical note : a system for continuous recording of ruminal pH in cattle. J Anim Sci 85:213-217

AlZahal O, Kebreab E, France J, Froetschel M, McBride BW (2008) Ruminal temperature may aid in the detection of subacute ruminal acidosis. J Dairy Sci 91:202-207

AlZahal O, Steele MA, Valdes EV, McBride BW (2009) The use of a telemetric system to continuously monitor ruminal temperature and to predict ruminal pH in cattle. J Dairy Sci 92:5697-5701

Bauman DE, Davis CL, Buchholtz HF (1971) Propionate production in rumen of cows fed either a control or high-grain, low-fibre diet. J Dairy Sci 54:1282-1287

Bewley JM, Einstein ME, Grott MW, Schutz MM (2008) Comparison of reticular and rectal core body temperatures in lactating dairy cows. J Dairy Sci 91:4661-4672

Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 8476:307-310

Burns P, Wailes W, Baker P (2002) Changes in reticular und rectal temperature during the periestrous period in cows. J Anim Sci 80(Suppl 2):128-129

Dado RG, Allen MS (1993) Continuous computer acquisition of feed and water intakes, chewing, reticular motility and ruminal pH of cattle. J Dairy Sci 76:1589-1600

DLG (1997) DLG-Futterwerttabellen Wiederkäuer. Frankfurt a M : DLG-Verl, 212 p

Duffield T, Plaizier JC, Fairfield A, Bagg R, Vessie G, Dick P, Wilson J, Aramini J, McBride B (2004) Comparison of techniques for measurement of rumen pH in lactating dairy cows. J Dairy Sci 87:59-66

Enemark JMD (2008) The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA) : a review. J Vet Med A 176:32-43

Enemark JMD, Peters G, Jorgensen RJ (2003) Continuous monitoring of rumen pH : a case study with cattle. J Vet Med A 50:62-66

Fordham DP, Rowlinson P, McCarthy TT (1988) Estrus detection in dairy cows by milk temperature-measurement. Res Vet Sci 44:366-374

Garrett EF, Pereira MN, Nordlund KV, Armentano LE, Goodger WJ, Oetzel GR (1999) Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. J Dairy Sci 82:1170-1178

Gasteiner J, Fallast M, Rosenkranz S, Hausler J, Schneider K, Guggenberger T (2009) Measuring rumen pH and temperature by an indwelling and wireless data transmitting unit and application under different feeding conditions. Wien Tieraerztl Monatsschr 96:188-194

GfE (2001) Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder 2001. Frankfurt a M : DLG-Verl, 136 p, Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere 8

Goering HK, Van Soest PJ (1970) Forage fibre analyses : (apparatus, reagents, procedures and some applications). Washington DC : Agric Res Service, USDA, 20 p, Agric Handbook 379

Gozho GN, Plaizier JC, Krause DO, Kennedy AD, Wittenberg KM (2005) Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J Dairy Sci 88:1399-1403

Jaakola S, Huhtanen P (1993) The effects of forage preservation and proportion of concentrate on nitrogen digestion and rumen fermentation in cattle. Grass Forage Sci 48:146-154

Kadzere CT, Murphy MR, Silanikove N, Maltz E (2002) Heat stress in lactating dairy cows : a review. Livestock Prod Sci 77:59-91

Kaur R, Garcia SC, Horadagoda A, Fulkerson WJ (2010) Evaluation of rumen probe for continuous monitoring of rumen pH, temperature and pressure. Anim Prod Sci 50:98-104

Kleen JL, Hooijer GA, Rehage J, Noordhuizen JPTM (2003) Subacute ruminal acidosis (SARA) : a review. J Vet Med A 50:406-414

Kleen JL, Hooijer GA, Rehage J, Noordhuizen JPTM (2009) Subacute ruminal acidosis in dutch dairy herds. Vet Rec 164:681-684

Li M, Penner GB, Hernandez-Sanabria E, Oba M, Guan LL (2009) Effects of sampling location and time, and host animal on assessment of bacterial diversity and fermentation parameters in the bovine rumen. J Appl Microbiol 107:1924-1934

Lin LIK (1992) Assay validation using the concordance correlation coefficient. Biometrics 48:599-604

MacFarlane PD, Grint N, Dugdale A (2010) Comparison of invasive and noninvasive blood pressure monitoring during clinical anaesthesia in dogs. Vet Res Commun 34:217-227

Maekawa M, Beauchemin KA, Christensen DA (2002) Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. J Dairy Sci 85:1165-1175

Martello LS, Savastano H, Silva SL, Balieiro JCC(2010) Alternative body sites for heat stress measurement in milking cows under tropical conditions and their relationship to the thermal discomfort of the animals. **Int J Biometereol** 54:647-652

Martin C, Devillard E, Michalet-Doreau B (1999) Influence of sampling site on concentrations and carbohydrate-degrading enzyme activities of protozoa and bacteria in the rumen. J Anim Sci 77:979-987

Meinrath G, Spitzer P (2000) Uncertainties in determination of pH. **Mikrochimica Acta** 135:155-168

Mishra M, Martz FA, Stanley RW, Johnson HD, Campbell JR, Hilderbr E (1970) Effect of diet and ambient temperature-humidity on ruminal pH, oxidation reduction potential, ammonia and lactic acid in lactating cows. J Anim Sci 30:1023-1028

Morgante M, Stelletta C, Berzaghi P, Gianesella M, Andrighetto I (2007) Subacute rumen acidosis in lactating cows : an investigation in intensive Italian dairy herds. J Anim Physiol Anim Nutr 91:226-234

Naumann C, Bassler R (1993) Die chemische Untersuchung von Futtermitteln : Methodenbuch ; Bd. 3 mit Ergänzungslieferungen 1983, 1988, 1993, 1997, 2004 und 2006. Darmstadt : VDLUFA

Oelssner W, Zosel J, Guth U, Pechstein T, Babel W, Connery JG, Demuth C, Ganseve MG, Verburg JB (2005) Encapsulation of ISFET sensor chips. Sensors actuators B 105:104-117

Owens D, McGee M, Boland T, O'Kiely P (2008) Intake, rumen fermentation and nutrient flow to the omasum in beef cattle fed grass silage fortified with sucrose and/or supplemented with concentrate. Anim Feed Sci Technol 144:23-43

Penner GB, Beauchemin KA, Mutsvangwa T (2006) An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J Dairy Sci 89:2132-2140

Penner GB, Aschenbach JR, Gabel G, Oba M (2009) Technical note : evaluation of a continuous ruminal pH measurement system for use in noncannulated small ruminants. J Anim Sci 87:2363-2366

Phillips N, Mottram T, Poppi D, Mayer D, McGowan MR (2010) Continuous monitoring of ruminal pH using wireless telemetry. Anim Prod Sci 50:72-77

SAS (2004) SAS/STAT User's Guide : version 9.1. Cary, NC, USA

Sievers AK, Kristensen NB, Laue HJ, Wolffram S (2004) Development of an intraruminal device for data sampling and transmission. J Anim Feed Sci 13(Suppl 1):207-210

Tafaj M, Junck B, Maulbetsch A, Steingass H, Piepho HP, Drochner W (2004) Digesta characteristics of dorsal, middle and ventral rumen of cows fed with different hay qualities and concentrate levels. Arch Anim Nutr 58:325-342