The effect of individuality of animal on diurnal pattern of pH and redox potential in the rumen of dry cows

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ABSTRACT: The aim of this study was to continuously monitor ruminal pH and redox potential of individual dry cows using a newly developed wireless device. Three dry Holstein cows fitted with rumen cannulas were used for the individual measurement of ruminal pH and redox potential (Eh) using a newly developed wireless device. The experiment was carried out in the period of 14 days consisting of a 10-day preliminary period followed by a 4-day measurement period. Cows were fed twice daily the diet based on maize silage, lucerne hay and concentrate. During the measurement period ruminal pH and redox potential were monitored continuously using a developed wireless probe. Average daily feed intake throughout the experiment was 8.2 kg/day. The mean ruminal pH was almost identical in Cows 21 and 25, being 6.79 and 6.75, respectively, and was lower than in Cow 26 (6.86; $P < 0.05$). The mean Eh of the ruminal fluid was –274 mV in Cow 21 and 26 and –270 mV in Cow 25, while the results did not differ significantly ($P > 0.05$). The diurnal pattern of ruminal pH and Eh showed a similar trend in all animals. Mean values of rH (Clark's exponent) calculated for Cows 21 and 25 being 4.43 and 4.48, respectively, were lower than the value calculated for Cow 26 (4.59; $P < 0.05$). This method may be useful for investigating factors affecting the dynamics of ruminal fermentation and may also help in the identification of variables associated with various metabolic disorders.

Keywords: redox potential; pH; rumen; measurement *in vivo*; wireless device

The rumen with its physicochemical conditions, i.e. temperature 39–40°C, mean pH close to neutrality, anaerobiosis characterised by very low redox potential (Eh) values (Hobson, 1997) represents an excellent environment for the development and maintenance of a dense and diverse microbial community. Measurements of pH and Eh in rumen content can contribute to the understanding of the microbiological activity and dynamics of fermentation (Broberg, 1957a). The redox potential is an important parameter describing the mode of action of factors that show the ability to influence the reducing power of the rumen as mentioned in the study of Marden et al. (2008). The authors evaluated the capacity of sodium bicarbonate and live yeast in optimizing ruminal pH with simultaneously decreasing the redox potential in dairy cows. From this aspect the Eh values appear to be important parameters complementing pH measurements. For correct measurement, it is important to observe strictly anaerobic conditions to avoid a considerable error resulting from changes in equilibrium between the ruminal liquid phase and the gas mixture in the rumen headspace (Marden et al., 2005).

There are several techniques of pH and Eh measurements reported in the literature, such as *ex vivo* measurement of ruminal fluid samples collected by an oral probe or by ruminal puncture or collected by a suction-strainer device from ruminally cannulated animals (Duffield et al., 2004). However, during these procedures the ruminal fluid is in contact

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with air that can modify the original ruminal physicochemical characteristics (Marden et al., 2005). In other studies (e.g. Müller and Kirchner, 1969; Barry et al., 1977) measurements were realised directly in the rumen through a well-closed rumen fistula. These techniques require the connection of electrodes to a data-logger located outside the rumen that can be a source of frequent defects. Recently, Marden et al. (2005) tested a new sampling and measuring device that enables to determine ruminal pH and Eh in the absence of air contamination. Their method with a complex of pipes, pumps and measuring devices requires continuous supervision.

The aim of this study was to continuously monitor ruminal pH and redox potential of individual dry cows using a newly developed wireless device that can solve all above-mentioned problems.

MATERIAL AND METHODS

Animals and feeding

Three dry Holstein cows fitted with rumen cannulas were used for the measurement of ruminal pH and Eh using a newly developed wireless device. The measurement was carried out in the period of 14 days consisting of a 10-day preliminary period followed by a 4-day experimental period during which the continuous 24-h measurement was realised. During the whole measurement cows were fed the basal diet based on maize silage, lucerne hay and concentrate (Table 1). The diet was fed as a total mixed ration (TMR) and was divided into two equal portions administered at 6:30 and 16:30 h.

Table 1. Composition of the diet on dry matter basis

Component	
Maize silage (g/kg)	477
Lucerne hay (g/kg)	416
Concentrate (g/kg)	107
Composition of supplemental mixture[1]	
Wheat (g/kg)	250
Barley (g/kg)	200
Soy extract meal (g/kg)	125
Sunflower extract meal (g/kg)	125
Maize (g/kg)	100
Sunflower expellers (g/kg)	50
Malt sprouts (g/kg)	50
Calcium salt of fatty acids (g/kg)	30
Linseed (g/kg)	20
Dicalcium phosphate (g/kg)	20
$CaCO_3$ (g/kg)	20
NaCl (g/kg)	5
$MgPO_4$ (g/kg)	5
Dry matter (g/kg)	430
Organic matter (g/kg)	929
Crude protein (g/kg)	151
NDF[2] (g/kg)	388
PDIN[3] (g/kg)	96.4
PDIE[3] (g/kg)	85.6
NEL[4] (MJ/kg)	5.87

[1]supplemental mixture was completed: 12 000 IU/kg vitamin A, 2 000 IU/kg vitamin D_3, 50 mg/kg vitamin E and 27 mg/kg $CuSO_4 \times 5H_2O$
[2]neutral detergent fibre
[3]digestible protein in the intestine when rumen fermentable N supply or energy supply are limiting, respectively
[4]net energy of lactation

Measurements

During each experimental period ruminal pH and Eh were monitored using a developed wireless probe that allowed continuous measurements of Eh inside the rumen under anaerobic conditions (Figure 1). The probe was made on the basis of patent applications No. PV 2009–220 (Křížová et al., 2009) and PV 2009–224 (Richter et al., 2009) and utility model No. UV 19727 (Křížová et al., 2009) and UV 19728 (Richter et al., 2009). The developed device consists of a measuring probe that is anchored to the cannula lid via an antenna cable. The probe is composed of a hermetically sealed cylindrical stainless steel enclosure with front and end plate cover. In the end cover there is a cable grommet for the passage of the antenna cable that is taken out of the rumen through the cannula lid and terminated with a transmitter. In the front cover there are cable grommets for the passage of a combined glass electrode with a reference gel electrode and redox potential platinum electrode

Figure 1. Probe for continuous measurements of pH and Eh inside the rumen of cattle

(Elektrochemické detektory, Ltd. Turnov, Czech Republic). The front cover is protected with a shield with perforations large enough to allow the ruminal fluid to percolate freely and which prevented the electrode from contacting the ruminal epithelium. A stabilizing lead is added to the shield to keep the probe positioned in the ventral sac resulting in a total probe weight of 1.7 kg. Inside the probe there are two electronic modules, batteries and a bag with silica gel. The data measured inside the rumen are wirelessly transmitted from the probe antenna to a receiver that is via interface and USB port connected to a computer. Ruminal pH and Eh were measured every 20 s, and averages over 1-min intervals. The probe was inserted into the ventral sac of the rumen of each cow through the cannula on day 10 of the preliminary period. The probe positioning was checked twice daily after feeding. Ruminal pH and Eh were measured continuously for 4 days, starting on day 10 (24:00 h) to day 14 (24:00 h). After the measurement probes were removed. Before inserting the probes into the rumen and after their removal, probes were checked for accuracy with Zobell's redox potential standard and pH 4.0 and 7.0 standards.

Calculations

Because the reference electrode in the actual measurements was not a hydrogen electrode, all records of the potential difference were corrected using the formula:

$$Eh = E0 + C$$

where:
E0 = potential of the platinum electrode
C = potential of the reference electrode relative to the standard hydrogen electrode (i.e. +199 mV at 39°C; Nordstrom, 1977)

Clark's exponent (rH), which gives a true index of the reducing power in the rumen, was calculated by means of Nernst's equation (Marounek et al., 1987) according to the following formula:

$$rH = Eh\ (mV)/30 + 2\ pH$$

where:
Eh = potential difference (mV) between a platinum electrode and a standard hydrogen electrode
pH = pH value in the rumen

Average daily pH and Eh values and the respective Max and Min values were compared using the following model:

$$Y_{ij} = \mu + C_i + D_j + \varepsilon_{ij}$$

where:
μ = general mean
C_i = effect of cow (i = 3)
D_j = effect of day (l = 4)
ε_{ij} = residual error

RESULTS

Average daily dry matter intake was 8.2 kg and the diet provided 125% of the NEL requirement. No changes in the position of the probe inside the rumen were noted during the measurement. No differences were found during the calibration of probes before and after measurement.

The individual mean and average Min and Max values of ruminal pH, Eh and rH are given in Table 2. The mean ruminal pH was almost identical in Cows 21 and 25, being 6.79 and 6.75, respectively, and was lower than the value determined in

Table 2. Effect of cow on pH, redox potential (Eh) and rH values of ruminal fluid (n = 4 days)

Item	Cow 21	Cow 25	Cow 26	SEM	P
pH					
Mean	6.79[a]	6.75[a]	6.86[b]	0.026	0.049
Max[1]	7.05[a]	7.04[a]	7.21[b]	0.020	0.002
Min[1]	6.54[a]	6.32[b]	6.56[a]	0.046	0.020
Eh (mV)					
Mean	–274	–270	–274	1.18	0.103
Max[1]	–256[a]	–227[b]	–244[c]	1.08	< 0.001
Min[1]	–287	–287	–291	1.20	0.118
rH					
Mean	4.43[a]	4.48[ab]	4.59[b]	0.029	0.023
Max[1]	4.81[a]	5.60[b]	5.35[ab]	0.130	0.013
Min[1]	4.06[ab]	3.75[a]	4.24[b]	0.082	0.015

[1]Min and Max values were calculated as a mean from the daily Min and Max values

Cow 26 (6.86; $P < 0.05$). Similarly, mean Max values in Cows 21 and 25, which were almost the same, were significantly lower than in Cow 26 ($P < 0.05$). The lowest Min pH value of 6.32 was found in Cow 25, which was significantly different from the other two cows ($P < 0.05$). The diurnal pattern of ruminal pH as presented in Figure 2 showed a similar trend in all cows, with the rapid drop in pH value within 1 h after feeding.

The mean Eh of the ruminal fluid was –274 mV in Cow 21 and 26 and –270 mV in Cow 25 ($P > 0.05$). Similarly, the mean Min Eh values did not differ significantly ($P > 0.05$) among cows. The Max Eh values were significantly different ($P < 0.05$) with

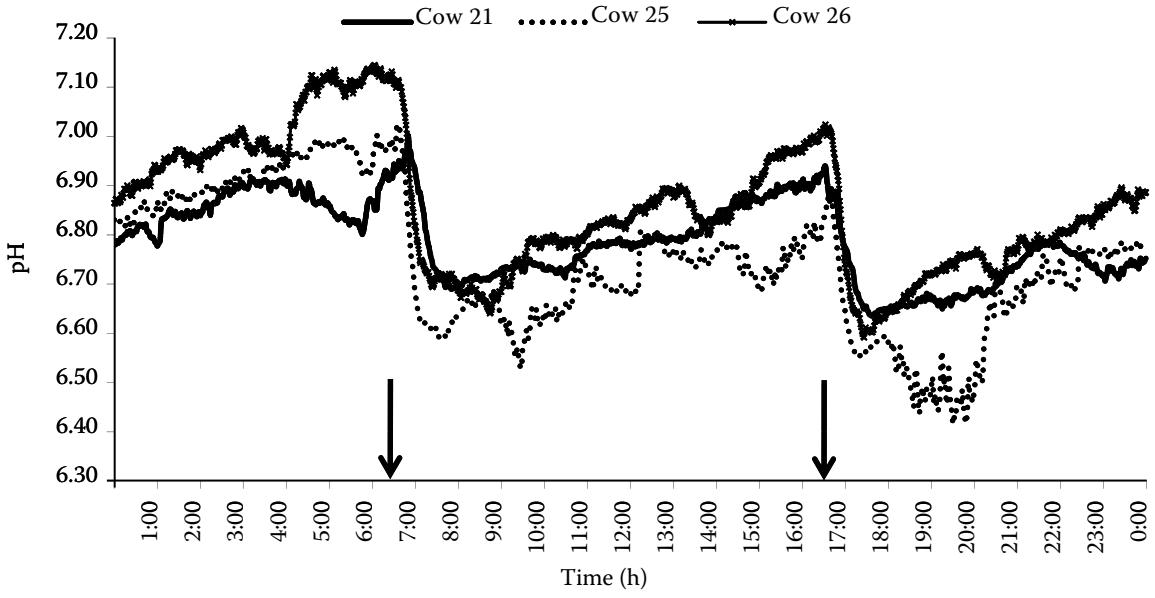

Figure 2. Effect of cow on the mean diurnal pattern of ruminal pH

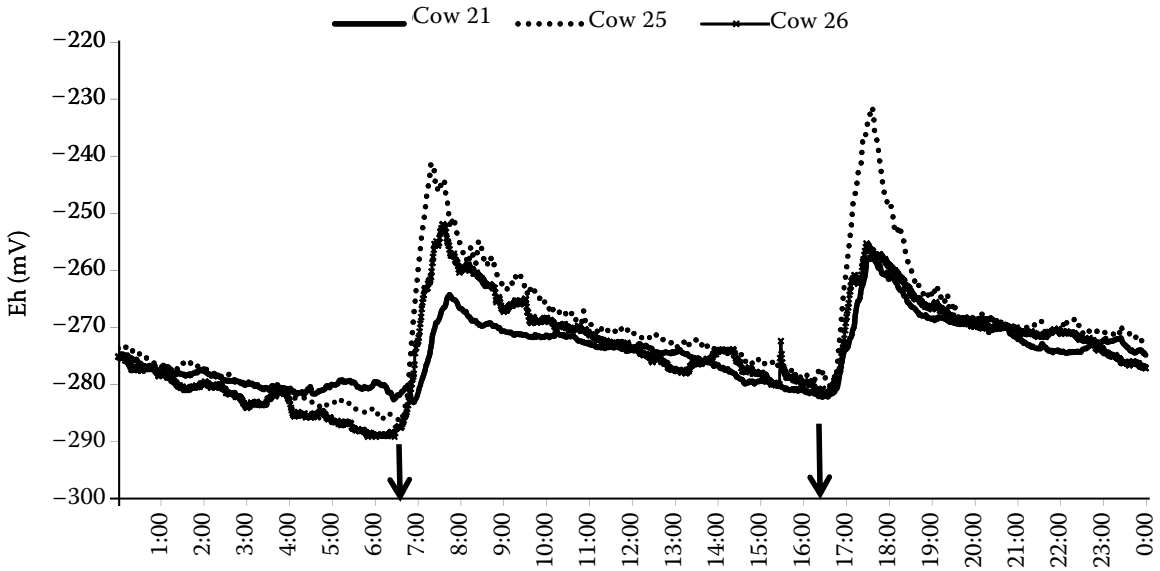

Figure 3. Effect of cow on the diurnal pattern of ruminal oxidative redox potential (Eh)

the highest Max value (–227 mV) determined in Cow 25 and the lowest one in Cow 21 (–256 mV). The diurnal pattern of ruminal Eh is presented in Figure 3. The Eh values of the rumen fluid showed a similar trend in all cows and were low before feeding and then increased, reaching a maximum 1 h after feeding, after which they decreased until the subsequent meal.

Mean rH calculated for Cows 21 and 25 being 4.43 and 4.48, respectively, were lower than the value calculated for Cow 26 (4.59; $P < 0.05$). The highest Max value was found in Cow 25 and was significantly higher than in Cow 21 ($P < 0.05$). Min Eh in Cow 25 was lower than in Cow 26 ($P < 0.05$) and did not differ from Cow 21.

DISCUSSION

During the measurement, no changes in the probe location in the rumen were noted suggesting that the weight of the probe was sufficient enough to ensure a stable and permanent measuring position in the ventral sac of the rumen during the measurement.

The mean ruminal pH measured in our study ranged from 6.75 (Cow 25) to 6.86 (Cow 26) and is in agreement with the data obtained e. g. by Marden et al. (2005). Similarly, the diurnal pattern of ruminal pH agrees with other studies (e.g. Duffield et al., 2004 or Marden et al., 2005).

Although pH plays an important role in regulating the microbial ecosystem in the rumen (Russell and Wilson, 1996), it is not the sole parameter affecting the bacterial activity (Offer, 1990). The metabolic activity of ruminal anaerobic bacteria depends also upon Eh, the parameter describing the reducing characteristics of the rumen milieu. Published results concerning the measurement of ruminal Eh are scarce and inconsistent. The discrepancy between published data depends largely on the technique of measurement (Marden et al., 2005). Further, there is no comparable study with the continuous 24-h measurement on dry cows, but several studies obtained their data from the continuous measurement made from 1 h before feeding to 8 or 7 h after feeding (e.g. Barry et al., 1977; Mathieu et al., 1996; Marden et al., 2005, 2008).

Individual mean Eh values in the present experiment were –274, –270 and –274 mV in Cows 21, 25 and 26, respectively. These values were lower than those obtained by Marden et al. (2005) in dry cows ranging from –173.5 to –216.8 mV when testing a new device for the continuous measurement of ruminal parameters in the absence of oxygen. Barry et al. (1977) reported a range of Eh in sheep from –150 to –260 mV, while Mathieu et al. (1996) or Sar et al. (2005) presented lower values ranging from –319 to –290 mV. However, data presented in the latter studies are not fully comparable with results of Marden et al. (2005) and ours because they ex-

pressed Eh as a potential difference (E) between the platinum electrode and reference electrode, i.e. calomel or silver:silver chloride. Due to the fact that the Eh is a potential difference between the platinum electrode and standard hydrogen electrode (The International Hydrogen Zero) their data should be corrected using the potential of the reference electrode used relative to the standard hydrogen electrode (Sauer and Teather, 1987) as mentioned in the Material and Methods chapter.

The diurnal pattern of Eh observed in our experiment is in accordance with previously published studies, e.g. Marden et al. (2005, 2008) in cows or Barry et al. (1977) or Mathieu et al. (1996) in sheep. In contrast to the above-mentioned studies, where the ruminal Eh increased within 3 or 5 hours after feeding, respectively, a rapid increase in Eh was noted in the present experiment during the first hour after feeding. This increase is related to the entry of oxygen while the probe position was checked through the cannula rather than to the oxygen supply directed towards the rumen during feed and water intake and mastication. The subsequent decline in Eh is associated with the rapid uptake of oxygen by microorganisms to maintain anaerobic conditions of the rumen (Broberg, 1957b)

In the present experiment, mean rH calculated for individual animals ranged from 4.43 (Cows 21) to 4.59 (Cow 26). There are no comparable data concerning rH in dry cows. However, our data are lower than data that can be calculated from the mean pH and range of Eh in Marden et al. (2005) ranging somewhere between 5.3 and 7.3. In their latter study, Marden et al. (2008) calculated mean rH of 8.05 in control lactating dairy cows fed a high-concentrate diet. Higher rH values were also reported by Barry et al. (1977) in sheep (8.0 and 5.0) or by Marounek et al. (1982) in goats (from 6.3 to 8.6).

With respect to the used measuring technique (without air contamination) and animal category (dry cows) the best comparable results are those published by Marden et al. (2005). An advantage of the device described in this study is that it enables continuous measurement over 24-h periods in comparison with 10-h periods used in the above-mentioned work or in recent study of Marden et al. (2008). Lower mean values of pH (6.52 vs 6.80) and higher values of Eh (–195 vs –273 mV) published by Marden et al. (2005) were probably induced using a higher content of concentrate in TMR (213 vs 107 g/kg DM) in comparison with our experiment. The correct comparison of mentioned methods requires to conduct an experiment using the same animals and diet.

CONCLUSION

A technique for the measurement of ruminal pH and redox potential using a newly developed device with wireless data transmission presented in this study enables long-term continuous 24-h measurement directly inside the rumen under strictly anaerobic conditions. To our knowledge, up to date there have not been data in the literature describing the variability in the mentioned parameters in individual cows. This method may be useful for investigating factors affecting the dynamics of ruminal fermentation and may also help in the identification of variables associated with various metabolic disorders.

REFERENCES

Barry T.N., Thompson A., Armstrong D.G. (1977): Rumen fermentation studies on two contrasting diets. 1. Some characteristics of the *in vivo* fermentation, with special reference to the composition of the gas phase, oxidation/reduction state and volatile fatty acid proportions. Journal of Agricultural Science, Cambridge, 89, 183–195.

Broberg G. (1957a): Oxygen's significance for the ruminal flora as illustrated by measuring the redox potential in rumen contents. Nordisk Veterinaer Medicin, 9, 57–60.

Broberg G. (1957b): Measurements of the redox potential in rumen contents. I. *In vitro* measurements on healthy animals. Nordisk Veterinaer Medicin, 9, 918–931.

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

Hobson P.N. (1997): Introduction. In: Hobson P.N., Stewart C.S. (eds.): The Rumen Microbial Ecosystem. Chapman and Hall, London, UK, 1–9.

Křížová L., Třináctý J., Richter M. (2009): Device for measurement of ruminal fluid pH. Utility model, No. UV 19727.

Křížová L., Třináctý J., Richter M. (2009): Device for measurement of ruminal fluid pH. Patent Application, No. PV 2009–220.

Marden J.P., Bayourthe C., Enjalbert F., Moncoulon R. (2005): A new device for measuring kinetics of ruminal

pH and redox potential in dairy cow. Journal of Dairy Science, 88, 277–281.

Marden J.P., Julien C., Monteils V., Auclair E., Moncoulon R., Bayourthe C. (2008): How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? Journal of Dairy Science, 91, 3528–3535.

Marounek M., Bartoš S., Kalachnyuk G.I. (1982): Dynamics of the redox potential and rH of the rumen fluid of goats. Physiologia Bohemoslovaca, 31, 369–374.

Marounek M., Roubal P., Bartoš S. (1987): The redox potential, rH and pH values in the gastrointestinal tract of small ruminants. Physiologia Bohemoslovaca, 36, 71–74.

Mathieu F., Jouany J.P., Senaud J., Bohatier J., Bertin G., Mercier M. (1996): The effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on fermentations in the rumen of faunated and defaunated sheep: Protozoal and probiotics interactions. Reproduction Nutrition Development, 36, 271–287.

Müller R., Kirchner I. (1969): Determination of hydrogenion concentration and redox potential in the rumen content of the cattle *in vitro* and *in vivo*. 3. Continuous measurements *in vivo*. Zeitschrift für Thierphysiologie Tierernahrung und Futtermittelkunde, 25, 242–247.

Nordstrom D.K. (1977): Thermochemical redox equilibria of ZoBell's solution. Geochimica et Cosmochimica Acta, 41, 1835–1841.

Offer N.W. (1990): Maximizing fiber digestion in the rumen: The role of yeast culture. In: Lyons T.P. (ed.): Biotechnology in the Feed Industry. Alltech Technical Publications. Nicholasville, USA, 79–86.

Richter M., Kadlec R., Třináctý J., Svobodová J. (2009): Device for measurement of ruminal fluid redox-potential. Patent Application, No. PV 2009-224.

Richter M., Kadlec R., Třináctý J., Svobodová J. (2009): Device for measurement of ruminal fluid redox-potential. Utility model, No. UV 19728.

Russell J.B., Wilson D.B. (1996): Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? Journal of Dairy Science, 79, 1503–1509.

Sar C., Mwenya B., Pen B., Takaura K., Morikawa R., Tsujimoto A., Kuwaki K., Isogai N., Shinzato I., Asakura Y., Toride Y., Takahashi J. (2005): Effect of ruminal administration of *Escherichia coli* wild type or a genetically modified strain with enhanced high nitrite reductase activity on methane emission and nitrate toxicity in nitrate-infused sheep. British Journal of Nutrition, 94, 691–697.

Sauer F.D., Teather R.M. (1987): Changes in oxidation-reduction potentials and volatile fatty acid production by rumen bacteria when methane synthesis is inhibited. Journal of Dairy Science, 70, 1835–1840.

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