

The effect of feeding live yeast cultures on ruminal pH and redox potential in dry cows as continuously measured by a new wireless device

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ABSTRACT: An experiment was carried out on four dry Holstein cows fitted with rumen cannulas that were divided into two groups. The crossover design experiment was divided into 4 periods of 3 weeks. Each period consisted of a 17-day preliminary period followed by a 4-day experimental period. Cows were fed twice daily the total mixed ration based on maize silage and concentrate. Control cows (Control) received the basal diets while experimental animals (Yeast) received the basal diet supplemented with 3.0 g of live yeast (BIOSAF Sc 47, Lesaffre, France) at each feeding. During each experimental period ruminal pH and redox potential (Eh) were monitored continuously using a developed wireless probe. Further, in each experimental period five samples of ruminal fluid were taken at 6:30, 8:30, 10:30, 13:30 and 16:30 h to determine the content of volatile fatty acids, lactic acids and ammonia. On the last day of each period, blood samples were taken for determination of blood parameters and acid-base balance. Average daily dry matter intake throughout the experiment was 8.2 kg/day and was not affected by the treatment. The average ruminal pH in Control was 6.16 that was significantly lower than in Yeast, being 6.26 ($P < 0.001$). The diurnal pattern of ruminal pH showed a similar trend in both groups. Mean Eh in Control (–210 mV) differed significantly from Yeast (–223 mV, $P < 0.001$). The mean value of rH (Clark's Exponent) calculated for Control (5.33) was higher than that calculated for Yeast (5.09, $P < 0.001$). Total VFA concentrations were on average 40.8mM in Control and 57.2mM in Yeast ($P > 0.05$). Lactate and ammonia concentrations at individual sampling times and overall mean did not differ significantly between treatments ($P > 0.05$). Blood pH and CO₂ were not affected by the treatment.

Keywords: rumen; physicochemical parameters; Clark's Exponent; wireless measurement

Over the last two decades, the use of live yeast in ruminant diets has been reported as beneficial due to the increased number of total ruminal bacteria and cellulolytic bacteria (Newbold et al., 1995), increased proportion of propionate (Mutsaers et al., 1992; Newbold et al., 1995) and decreased concentration of lactate (Newbold et al., 1990) resulting in the stabilization of ruminal pH (e.g. Bach et al., 2007; Marden et al., 2008; Thrane et al., 2009).

Although the mode of live yeast action on the rumen microbial ecosystem has not been completely clarified yet, Jouany (2001) suggested that live yeast cells, as aerobes, use oxygen present in traces in ingested feed particles for sugar and other nutrient metabolism. The removal of oxygen improves anaerobiosis resulting in better conditions for the growth and multiplication of anaerobic bacteria (Jouany, 2006; Chaucheyras-Durand et al., 2008).

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Further, end-products of nutrient metabolism of yeast cells can be used by other bacteria associated with yeasts in the trophic chain that is physically organized in micro-consortium structures that prevent the dilution of these metabolites in the ruminal fluid (Jouany, 2006). However, the beneficial effects of yeast products depend on lactation stage (Wohlt et al., 1998), diet characteristics (Piva et al., 1993) and environmental conditions (Schingoethe et al., 2004; Chaucheyras-Durand et al., 2008).

Measurement of the ruminal fluid redox potential (Eh) represents a better insight into fermentation processes in the rumen providing a different view on the mechanisms involved in the stabilization of ruminal pH. Under normal conditions, the ruminal fluid Eh is markedly negative, reflecting the anaerobiosis and strong reducing power of the rumen milieu (e. g. Mathieu et al., 1996; Chaucheyras-Durand and Fonty, 2002; Marden et al., 2005, 2008). Due to the entry of oxygen during feed and water intake and rumination the Eh slowly increases with subsequent decline that could be explained by the rapid uptake of O₂ by microorganisms to maintain anaerobic conditions of the rumen (Broberg, 1957; Barry et al., 1977). Although reported numerical values of Eh differ in dependence on sampling and measuring techniques (discussed in details in Marden et al., 2005), the live yeast has been reported as a balancer of the ruminal fluid redox potential (Marden et al., 2008). Mathieu et al. (1996) and Marden et al. (2008) confirmed *in vivo* that the addition of live yeasts significantly lowered the redox potential in the rumen by up to –20 mV or –34 mV, respectively.

The aim of this study was to continuously monitor the ruminal redox potential and pH of dry cows as affected by live yeast supplementation using a newly developed wireless device.

MATERIAL AND METHODS

Animals and treatments

Four dry Holstein cows (705 kg of live weight) fitted with rumen cannulas were used in an experiment. The experiment was carried out in a cross-over design where 2 cows were control (Control, without yeast supplement) and the other 2 cows received a control diet supplemented with the recommended dose of yeast (Yeast). In each subsequent period the experimental treatment was changed in

Table 1. Composition of the diet on DM basis

Item	
Maize silage (g/kg)	628
Concentrate ¹ (g/kg)	372
Nutrient analysis	
Dry matter (g/kg)	502
Organic matter (g/kg)	942
Crude protein (g/kg)	139
Starch (g/kg)	290
NDF ² (g/kg)	325
ADF ³ (g/kg)	156
PDIN ⁴ (g/kg)	89.3
PDIE ⁴ (g/kg)	91.3
NEL ⁵ (MJ/kg)	7.13

¹concentrate contains (in g/kg): maize grain 492; wheat feeding flour 323; soybean meal 168; sodium chloride (NaCl) 4.2; dicalcium phosphate (DCP) 7.0; limestone (CaCO₃) 5.0; microelements and vitamin mixture 0.8

²neutral detergent fibre

³acid detergent fibre

⁴digestible protein in the intestine when rumen fermentable N supply or energy supply are limiting, respectively

⁵net energy of lactation

such a way to have 2 replications of the treatment in each group. The experiment was divided into 4 periods of 3 weeks (it is in total 84 days). Each period consisted of 17 days of a preliminary period followed by a 4-day experimental period.

During the experiment the cows were fed a basal diet based on maize silage and concentrate (Table 1). The diet was fed as a total mixed ration and was divided into two equal portions given at 6:30 and 16:30 h. Control cows (Control) received basal diets while experimental animals (Yeast) received the basal diet supplemented with 3.0 g of yeast (BIOSAF Sc 47, Lesaffre, France) at each feeding. Feed intake and respective refusals were monitored daily.

Experimental procedure

During each experimental period ruminal pH and redox potential were monitored using a developed wireless probe described in details in a previous

study (Richter et al., 2010) that allowed continuous measurements of pH and Eh inside the rumen under anaerobic conditions. Ruminal pH and redox potential were measured every 20 s, and averaged over 1-min intervals. The probe was inserted into the ventral sac of the rumen of each cow through the cannula on day 12 of the preliminary period. Ruminal pH and redox potential were measured continuously for 4 days, starting on day 17 (24:00 h) to day 21 (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.

Sampling and analyses

Feeds and refusals

In each experimental period representative samples of feeds and respective refusals were taken for the determination of chemical composition. Dry matter (DM) was determined by drying at 55°C for 24 h, followed by milling through a 1-mm screen and drying for another 4 h at 103°C. Contents of crude protein (CP), crude fibre (CF), ash and fat were estimated according to AOAC (1984). Neutral detergent fibre (NDF, with α -amylase) was estimated according to Van Soest et al. (1991), ash-free acid detergent fibre (ADF) was estimated according to Goering and Van Soest (1970).

Ruminal fluid

During the experimental period (4 days) five samples of ruminal fluid were collected at 6:30, 8:30, 10:30, 13:30 and 16:30 h. Immediately after sampling, pH was measured using an accurate pH-meter. Samples for the determination of NH_3 and lactic acid (preserved with 2 ml of 50% H_2SO_4) and volatile fatty acids (VFA) were kept frozen at -20°C until subsequent analyses. Prior to analyses, samples within the cow and period from the same time interval were thawed, mixed thoroughly and an aliquot was taken to obtain a representative sample for each time interval per cow and period. Ruminal fluid was centrifuged at 3 000 g for 20 min and the supernatant was used for analyses. VFA were determined using gas chromatography on a CHROM-5 gas chromatograph (Laboratorní přístroje Praha, CR) fitted

with glass column, packed with 80/120 Carbowax B-DA/4% CARBOWAX 20 M. Trimethylacetic acid was used as an internal standard, nitrogen was the carrier gas. Results were evaluated by CSW 1.5 programme method with internal standard ISTD-2. Lactic acid was determined using an optimised colorimetric method (Madrid et al., 1999) and ammonia content was determined by the Conway microdiffusion method (Conway, 1962).

Blood

On the last day of each experimental period, blood samples were taken into heparinised tubes from the jugular vein for determination of blood parameters and acid-base balance. pH and CO_2 were analyzed within 2 h on a Rapidlab 855 analyser (BAYER Corp., NY). Immediately after obtaining blood, the samples for determination of blood metabolites were centrifuged at 1 500 g for 15 min and analysed using standard enzymatic method kits (Randox Laboratories, Břeclav, Czech Republic for BHB and NEFA and Roche, s.r.o., Praha, Czech Republic for the remaining metabolites) adapted to the HITACHI 902 analyser (Roche Diagnostics GmbH, Mannheim, Germany).

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

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

where:

Eh = potential difference (mV) between the platinum electrode and standard hydrogen electrode

pH = pH value in the rumen

Table 2. Effect of live yeast on ruminal pH, redox potential (Eh) and rH values ($n = 6$)

Item	Control	Yeast	SEM	<i>P</i>
pH	6.16	6.26	0.003	< 0.001
Eh (mV)	−210	−223	0.243	< 0.001
rH	5.33	5.09	0.008	< 0.001

Control = control diet; Yeast = Control + 6 g/day of live yeast (BIOSAF Sc 47)

Eh = redox potential

rH = Clark's Exponent

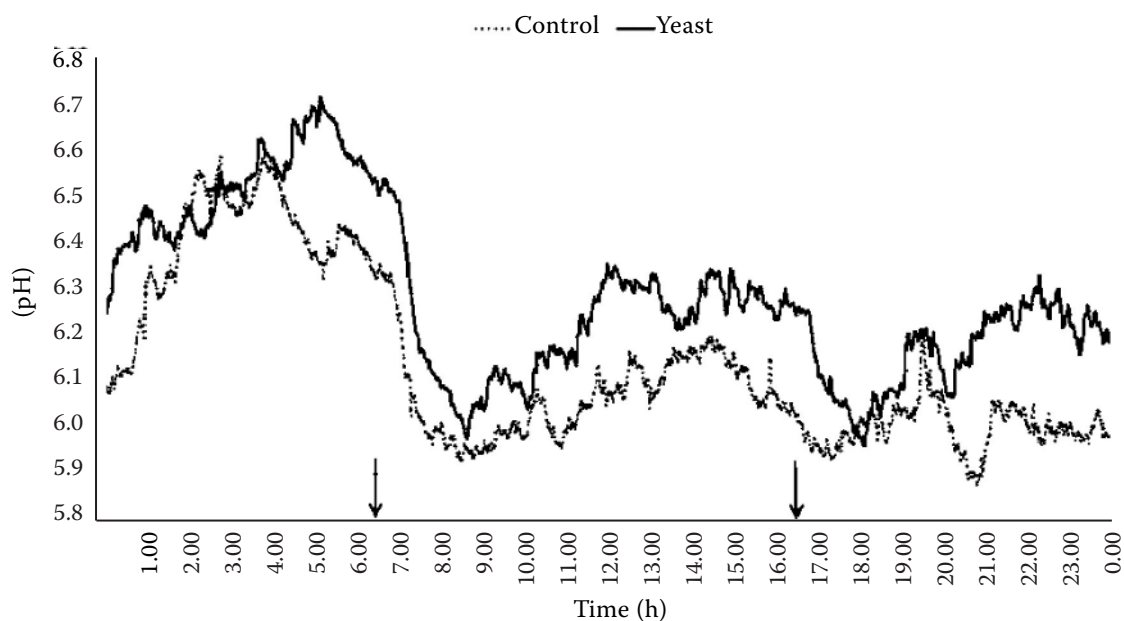


Figure 1. Effect of live yeast on the diurnal pattern of ruminal pH (feeding time is marked with arrows)

RESULTS

One animal had to be removed from the evaluation due to health problems.

Average daily DM intake throughout the experiment was 8.2 kg/day and was not affected by the treatment. Ruminal pH was measured continuously for 4 days of each period starting on day

17 (24:00 h) to 21 (24:00 h), using the invented probe inserted into the rumen of each cow through the ruminal cannula. No differences were found during the calibration of probes before and after measurement.

The effect of live yeast supplement to basal diet on pH, Eh and rH of ruminal fluid is given in Table 2. The ruminal pH in Control varied from

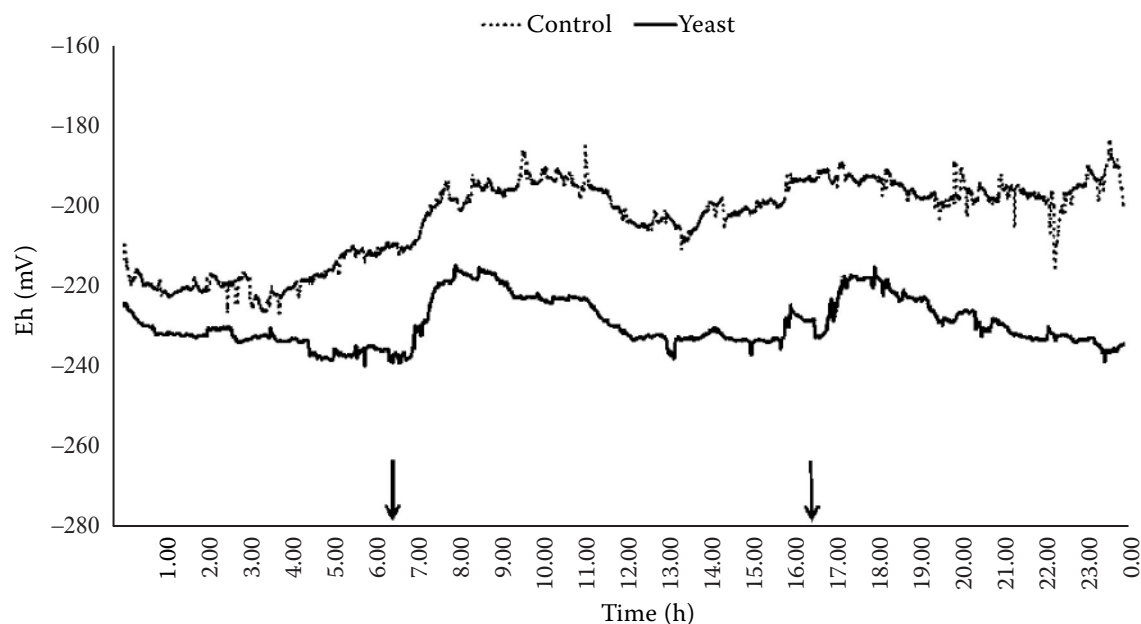


Figure 2. Effect of live yeast on the diurnal pattern of ruminal redox potential Eh (feeding time is marked with arrows)

Table 3. Effect of live yeast on ruminal volatile fatty acids (VFA) and lactate concentrations ($n = 6$)

Item	Control	Yeast	SEM	<i>P</i>
Total VFA (mM)	40.8	57.2	4.89	0.760
Acetate (mM)				
0 h	10.7	14.8		0.346
2 h	22.4 ^a	42.1 ^b		0.013
4 h	18.7 ^a	28.8 ^b		0.028
7 h	14.2 ^a	30.3 ^b		0.032
10 h	11.5	21.0		0.079
Mean	15.5 ^a	27.4 ^b	2.13	0.037
Propionate (mM)				
0 h	5.2	6.0		0.511
2 h	20.5 ^a	30.5 ^b		0.037
4 h	15.3	16.4		0.623
7 h	9.6	13.4		0.101
10 h	7.1	9.1		0.204
Mean	11.6	15.1	1.90	0.532
Butyrate (mM)				
0 h	8.5	7.7		0.599
2 h	16.3 ^a	19.9 ^b		0.015
4 h	17.5	15.8		0.199
7 h	14.0	17.3		0.147
10 h	12.1	12.8		0.702
Mean	13.7	14.7	1.00	0.798
Lactate (mM)				
0 h	4.69	4.04		0.712
2 h	4.72	5.27		0.747
4 h	3.99	3.86		0.952
7 h	4.13	4.03		0.950
10 h	4.80	3.77		0.657
Mean	4.47	4.19	0.48	0.736
Ammonia (mg/l)				
0 h	48.88	42.51		0.501
2 h	89.52	79.63		0.199
4 h	56.74	53.89		0.714
7 h	24.67	18.78		0.089
10 h	33.31	27.34		0.495
Mean	50.60	44.43	2.30	0.125

^{a,b}means in the same row followed by different superscripts differ ($P < 0.05$)

Control = control diet; Yeast = Control + 6 g/day of live yeast (BIOSAF Sc 47)

6.60 and 5.90 (Figure 1) with an average pH of 6.16, which was significantly lower than that recorded in Yeast, being on average 6.26 ($P < 0.001$) and ranging from 5.9 to 6.7. The diurnal pattern of ruminal pH showed a similar trend in both Control and Yeast with the drop in pH value during 1 h postfeeding that reached minimum at 2 h postfeeding.

The diurnal pattern of ruminal Eh is presented in Figure 2. The Rh of the ruminal fluid varied between –185 and –228 mV in Control and between –215 and –241 mV in Yeast. Mean Eh in Control (–210 mV) differed significantly from Yeast (–223 mV, $P < 0.001$, Table 2). The mean value of rH calculated for Control (5.33) was higher than that calculated for Yeast (5.09, $P < 0.001$).

Total VFA concentrations were on average 40.8 mM in Control and 57.2 mM in Yeast ($P > 0.05$, Table 3). Average acetate concentration determined in Yeast group 2, 4 and 7 h after feeding was significantly higher than in Control ($P < 0.05$). Overall mean acetate concentration in Yeast was higher (27.4 mM) than in Control (15.5 mM, $P < 0.05$). Mean propionate concentration in Yeast was significantly higher compared to Control only in samples taken 2 h after feeding (30.5 vs. 20.5 mM, respectively, $P < 0.05$). Overall mean concentration of propionate was not affected by the treatment ($P > 0.05$). Butyrate, lactate and ammonia concentrations at individual sampling times and overall mean did not differ significantly between treatments ($P > 0.05$).

The blood parameters describing acid-base balance, i.e. blood pH and CO_2 , were not affected by

the treatment (Table 4). Plasma metabolites did not differ significantly between treatments, except BHB, which was higher in Yeast in comparison to Control ($P < 0.05$).

DISCUSSION

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

In the present experiment, no differences in average daily DM intake were observed. Similar responses to yeast supplementation in early and late lactation dairy cows were reported in recent studies of Schingoethe et al. (2004), Bach et al. (2007) or Thrune et al. (2009). On the other hand, Erasmus et al. (1992) or Dann et al. (2000) observed enhanced DM intake after live yeast supplementation.

The dry cows in our experiment were fed a diet containing 37.2% of concentrate on DM basis resulting in 148% of the NEL requirement (calculated according to Sommer, 1994).

The mean ruminal pH in Control was 6.16, so the animals showed signs of subclinical ruminal acidosis (mean rumen pH < 6.25 ; Sauvant et al., 1999). However, a drop in the pH value below 6 lasted only for approximately 1.5 h after each feeding, starting 1.5 h after morning meal and within 0.5 h after evening meal. The dietary application of live yeast resulted in significant stabilization of pH that increased to the mean value of 6.26 ($P < 0.001$). Our results are in agreement with other studies focused on the effect of live yeast on ruminal pH (e.g. Erdman, 1988; Fiems et al., 1993; Bach et al., 2007; Marden et al., 2008; Thrune et al., 2009). Although not significant, a higher content of total VFA concentrations in Yeast in comparison with Control was observed in our study. Similar findings were reported by Marden et al. (2008) in lactating dairy cows. The average acetate concentration determined in Yeast group 2, 4 and 7 h after feeding and overall mean were significantly higher than in Control ($P < 0.05$). This is in accordance with Marden et al. (2008). The propionate concentration did not differ between Control and Yeast except 2 h postfeeding when a higher value of propionate was found in Yeast ($P < 0.05$).

During acute acidosis, the concentration of lactate in rumen can exceed 50 mM (Dunlop, 1972;

Table 4. Effect of live yeast on acid-base balance and blood parameters of dry cows

Item	Control	Yeast	SEM	<i>P</i>
pH	7.42	7.42	0.006	0.565
CO_2	6.40	6.30	0.225	0.760
Total protein	76.04	74.86	0.69	0.290
BHB	0.34 ^a	0.40 ^b	0.012	0.018
NEFA	0.31	0.34	0.026	0.512

Control = control diet; Yeast = Control + 6 g/day of live yeast (BIOSAF Sc 47)

BHB = β -hydroxybutyrate

NEFA = nonesterified fatty acids

^{a,b}means in the same row followed by different superscripts differ ($P < 0.05$)

Nagaraja et al., 1985) while during subacute acidosis the concentrations of lactate are very low, rarely exceeding 10mM (e.g. Hristov et al., 2001; Ghorbani et al., 2002; Beauchemin et al., 2003; Bevans et al., 2005). Similarly, ruminal lactate concentrations determined in our study were low, being 4.47mM in Control and 4.19mM in Yeast ($P > 0.05$).

Although not significant, lower ammonia concentrations were determined in Yeast at all sampling times in comparison with Control ($P > 0.05$). Similar findings were also reported in other studies, e.g. by Erasmus et al. (1992), Chaucheyras-Durand and Fonty (2001) or Moallem et al. (2009). Based on their *in vitro* study, Chaucheyras-Durand et al. (2005) supposed that yeast supplementation decreased the activity of ruminal proteolytic bacteria resulting in lower ammonia production. On the other hand, Erasmus et al. (1992) suggested that a lower ammonia concentration found in yeast-fed cows was associated with the increased incorporation of ammonia into microbial protein.

Published results concerning the measurement of ruminal Eh are scarce because such a measurement requires strictly anaerobic conditions (Marden et al., 2005). Eh values in the present experiment were –210 mV in Control and –223 mV in Yeast. The Control values were higher 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. In their subsequent experiment on early lactating dairy cows, Marden et al. (2008) found Eh of –115 mV in the control and of –149 mV in yeast diet ($P < 0.001$). Similarly to Marden et al. (2008), the Eh values determined in Yeast in our study were lower than those in Control ($P < 0.001$). Barry et al. (1977) reported that Eh values in sheep fed control or concentrate diets ranged between –150 and –260 mV with the evident diurnal cycle that was the most oxidizing just prior to feeding and the most reducing just after feeding. A similar diurnal pattern in Eh was observed in our study. Marden et al. (2005, 2008) in cows or Matieu et al. (1996) in sheep also obtained similar curves during their measurement from 1 h prior to feeding to 8 or 7 h postfeeding, respectively.

Calculated rH in our experiment was 5.33 in Control and decreased to 5.09 in Yeast ($P < 0.001$). This is in accordance with findings of Marden et al. (2008), who observed a significant increase in rH values after the application of live yeast to a high concentrate diet of lactating dairy cows.

Based on the data of Marden et al. (2005), the rH value in dry cows can range somewhere between 5.3 and 7.3 (calculated from the mean pH and range of Eh in the latter study). According to Barry et al. (1977), rH in sheep varied from 8.0 to 5.0 units. On the other hand, Marounek et al. (1982) reported higher values from 6.3 to 8.6 in goats. rH calculated in our experiment was slightly lower than the above-mentioned levels. The differences in Eh and rH values between Control and Yeast groups in our study show an evidence of the ability of live yeast to strengthen the reducing power of the rumen via improvement in the growth and activity of lactate-consuming (Rossi et al., 1995) and cellulolytic bacterial populations (Chaucheyras et al., 1997).

As expected, under described conditions no differences in blood pH and CO₂ were found in our experiment. This is in agreement with other studies (e.g. Ghorbani et al., 2002, Bevans et al., 2005) confirming the fact that blood pH is very resistant to fluctuations because the acid-base balance is highly regulated and blood is saturated with bicarbonate. However, during metabolic acidosis, the concentration of blood CO₂ increases due to the reduction of the blood bicarbonate ion (Owens et al., 1998). Thus, a lower concentration of CO₂ can be taken as an indicator of reduced risk of metabolic acidosis (Brown et al., 2000).

CONCLUSION

The newly developed wireless device used in the present experiment enables to carry out long-term continuous measurements of ruminal pH and redox potential under strict anaerobic conditions. The supplementation of live yeast to the diet with a high proportion of concentrate for dry cows resulted in a significant increase in ruminal pH and decrease in the ruminal redox potential.

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