



Short communication

# Daily rumen pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation

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## Abstract

The aim of this study was to continuously monitor rumen pH of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. Three multiparous lactating rumen-cannulated cows receiving the same basal ration once daily were supplemented with 5 g/d (equivalent to  $10^{10}$  CFU/d) of *Saccharomyces cerevisiae* strain CNCM I-1077 (Levucell SC2, Lallemand, Toulouse, France) alternately for periods of 2 weeks following a cross-over design. The three cows were maintained in loose-house conditions and milked with a robotic milking system. Cows consumed a fraction of their ration in the feed bunk as a mixed ration and about 3 kg/d of concentrate during milking at the robotic milking unit. During the last 8 d of each period, rumen pH was monitored every 15 min. However, the rumen was accessed only once every 3 days. These pH measurements were recorded with a pH meter capable of storing pH values automatically that was placed inside a custom-made polyvinyl cylinder with about 300 g of lead to ensure that the device remained in the ventral part of the rumen throughout all readings. Individual feed intake and feeding patterns were also recorded. The data were analyzed using a mixed-effects model with repeated measures. Average rumen pH was greater ( $P<0.01$ ) when yeast was supplemented than when no yeast was provided. Ruminal pH decreased ( $P<0.001$ ) as time since last basal ration bout increased, and this decrease was more ( $P<0.05$ ) pronounced when no

*Abbreviations:* YS, yeast supplementation; NY, no yeast supplementation

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yeast was supplemented. Yeast-supplemented cows had a greater ( $P < 0.05$ ) meal frequency than the unsupplemented cows. The results indicate that live yeasts have a beneficial effect on ruminal pH of cows kept in loose-house conditions. Furthermore, yeast effects on rumen pH may be evident starting 1 week after supplementation.

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## 1. Introduction

Several studies have shown that live yeast and yeast culture supplementation may increase feed intake and milk production of dairy cows (Robinson and Garrett, 1999; Dann et al., 2000; Nocek et al., 2003). However, all research relating to yeast supplementation on rumen fermentation has been conducted in vitro (Nisbet and Martin, 1991; Newbold et al., 1995; Lila et al., 2004) or with cows maintained in tie-stalls (Yoon and Stern, 1996; Kung et al., 1997; Robinson and Garrett, 1999; Dann et al., 2000; Nocek et al., 2003). However, most commercial dairy enterprises keep their cows in loose-house conditions. Because eating pattern may be different in loose-house conditions compared with cows in tie-stalls due, for example, to social dominance (Livshin et al., 1995), ruminal response to yeast supplementation may also be different. Therefore, the aim of this study was to evaluate the changes on rumen pH of lactating dairy cattle maintained in loose-house conditions as affected by feeding pattern and the supplementation of live yeast.

## 2. Materials and methods

### 2.1. Animals and treatments

Three multiparous lactating rumen-cannulated cows (body weight =  $732 \pm 28$  kg; days in milk =  $335 \pm 42$  d) maintained in loose-house conditions were supplemented (YS) or not (NY) with live yeast for two periods of 2 weeks each, following a cross-over design. In period 1, two cows were YS, and one was NY, and in period 2, two cows were NY and one was YS. The yeast supplement consisted on top-dressing the basal ration (Table 1) with a single dose of 5 g/d (equivalent to  $10^{10}$  CFU/d) of *Saccharomyces cerevisiae* strain CNCM I-1077 (Levucell SC2, Lallemand, Toulouse, France). The three cows were in a group of 50 cows in total, with access to 28 feeding spaces. All animals were handled under the Animal Care Committee of IRTA (Bellaterra, Spain) supervision. Cows were milked with a robotic milking system (VMS, DeLaval, Tumba, Sweden). In addition to the basal ration, during milking, cows received 1.5 kg of concentrate (Table 1). If cows were milked more than twice daily, they would not receive concentrate beyond the second milking (maximum amount of concentrate was 3 kg/d). During the last 8 d of each period, rumen pH was monitored every 15 min, using a pH meter (X-Mate Pro MX 300, Mettler-Toledo, Barcelona, Spain) capable of recording and storing pH values automatically. The pH meter was placed inside a custom-made polyvinyl chloride cylinder (170 mm long and 75 mm of diameter) with a

Table 1  
Ingredient and nutrient composition of the basal ration and the concentrate offered during milking

	Basal ration	Concentrate
Ingredient composition (g/kg as fed)		
Ryegrass silage	600	–
Distillers dried grains	–	99
Alfalfa hay	91	–
Barley grain	–	33
Cottonseed whole	52	–
Citrus pulp	26	–
Corn grain	131	296
Soybean meal	37	296
Corn gluten feed	57	165
Soybean hulls	–	66
Molasses, sugar cane	–	20
Sodium bicarbonate	–	16
Calcium carbonate	2.3	–
Dicalcium phosphate	0.5	–
Magnesium oxide	–	3.3
Salt	2.1	6.6
Micromineral-Vitamin Premix	0.5	–
Nutrient composition (DM basis)		
Energy <sup>a</sup> (MJ/kg NE <sub>L</sub> )	6.53	7.91
Crude protein (g/kg)	156	258
Ether extract (g/kg)	49	29
Neutral detergent fibre <sup>b</sup> (g/kg)	354	217
Absorbable calcium <sup>c</sup> (g/kg)	4	2
Absorbable phosphorous <sup>b</sup> (g/kg)	3	4

<sup>a</sup> Estimated at a level of intake equivalent to 23.5 kg of DM/d following the NRC (2001) model.

<sup>b</sup> Crude protein was determined using the AOAC (988.05 1990), ether extract was determined using the AOAC (920.39 1990), and neutral detergent fibre was determined with sodium sulphite and heat stable alpha-amylase (Van Soest et al., 1991).

<sup>c</sup> Estimated following the NRC (2001) model.

12-mm diameter opening to allow the pH probe to have direct contact with rumen fluid. The cylinder contained the pH meter and about 300 g of lead to ensure that the device sunk and remained in the caudo-ventral sac of the rumen throughout all readings. The device was removed from the rumen every 3 d (except for the last sampling) to retrieve the pH measurements stored in the memory of the pH meter and returned to the rumen within 30 min. Also, when the device was removed from the rumen, the pH meter was checked for accuracy and re-calibrated, if needed, with pH 4.0 and 7.0 standards. Therefore, the rumen cannulae were only open at the beginning of the sampling period, and 3, 6, and 8 d later (Fig. 1).

The basal ration and the concentrate offered during the milking were analyzed for dry matter (24 h at 103 °C), crude protein (AOAC, 1990), neutral detergent fibre (Van Soest et al., 1991), and ether extract (AOAC, 1990). Total DMI and milk production were recorded daily. Also, attendance to the feed bunk was continuously monitored throughout the study using a computerized system described elsewhere (Bach et al., 2004).

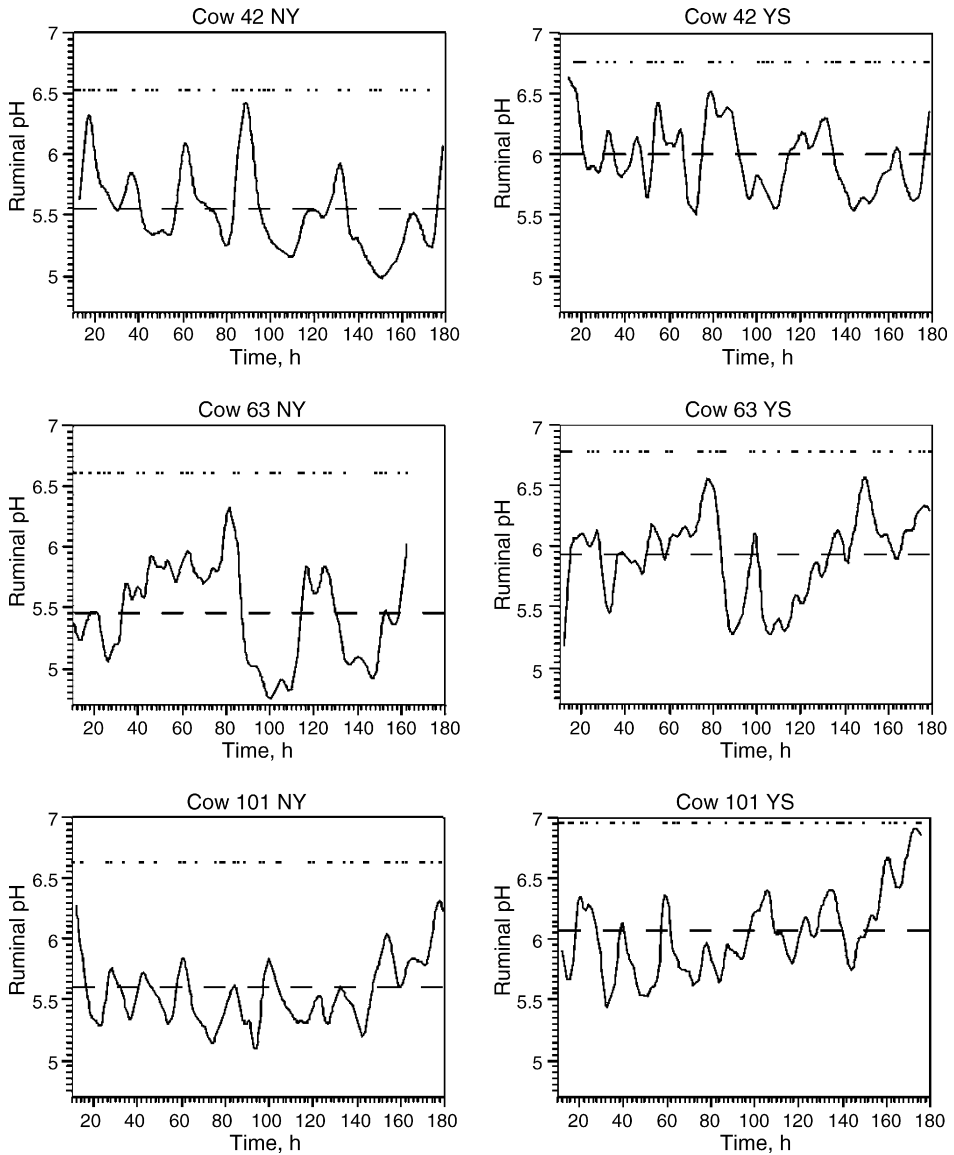


Fig. 1. Individual ruminal pH fluctuation pattern (solid line) during the 8 days of sampling as affected by supplementation (YS) or not (NY) of yeast. The dashed line depicts average ruminal pH. The dots indicate the beginning of a meal.

## 2.2. Calculations and statistical analyses

Rumen pH data were analyzed using a mixed-effects model with repeated measures with a compound symmetry variance–covariance structure:

$$\begin{aligned}
\text{Rumen pH} = & \mu + \text{Cow}_i + \text{Seq}_j + \text{Period}_k + \text{Treatment}_l + \text{Day}_{m(l)} \\
& + \text{TimeSinceBR}_n + \text{TimeSinceConcentrate}_o + \text{CumBR}_p \\
& + \text{Treatment}_l \times \text{Day}_{m(l)} + \text{Treatment}_l \times \text{TimeSinceBR}_n \\
& + \text{Treatment}_l \times \text{TimeSinceConcentrate}_o + \text{Treatment}_l \\
& \times \text{CumBR}_p + \varepsilon_{ijklmnop}
\end{aligned}$$

where  $\mu$  represented the overall mean, Cow accounted for the random effect of each individual animal, Period represented the period (1 or 2), Seq accounted for the effect of sequence (Cow(Seq) was used as random effect), Treatment accounted for the fixed effect of yeast supplementation, Day (repeated measure) represented the fixed effect of day of sampling within treatment, TimeSinceBR represented the time (min) since last basal ration eating bout, TimeSinceConcentrate depicted the time (min) since previous grain consumption during milking, CumBR represented the cumulative amount of basal ration consumed every day, and  $\varepsilon$  accounted for the unexplained random error. Both, TimeSinceBR and TimeSinceConcentrate were transformed to a logarithmic scale prior statistical analysis to obtain normal distributions.

To assess the severity of subclinical acidosis, the area under the pH curve was calculated by adding the absolute values of negative deviations in pH from pH 5.6 or 6.0 for each 15-min interval. The area under the pH curve was analyzed using a mixed-effects model with repeated measures (with the independent variables and random factors as defined above):

$$\begin{aligned}
\text{Area under the pH curve} = & \mu + \text{Cow}_i + \text{Seq}_j + \text{Period}_k + \text{Treatment}_l \\
& + \text{Day}_{m(l)} + \text{Treatment}_l \times \text{Day}_{m(l)} + \varepsilon_{ijk}
\end{aligned}$$

Also, to group consecutive visits to the feed bunk into a single meal, meal criterion (maximum amount of time between visits to the feedbunk to consider a visit as a part of the same meal) was calculated as described by Bach et al. (2006). The same model used for the area under the pH curve was also used to determine the effects of YS on feeding behaviour.

### 3. Results and discussion

#### 3.1. Performance and intake

There were no differences in milk yield ( $18.8 \pm 2.31$  kg/d) nor dry matter intake ( $18.4 \pm 4.24$  kg/d) between treatments. Also, concentrate consumption ( $2.78 \pm 0.72$  kg/d) during milking was not different between treatments. Meal criteria were not affected ( $P=0.37$ ) by yeast supplementation, although they were numerically shorter with YS than with NY (11.47 min *versus* 14.47 min, respectively). Meal length (Table 2) was not affected ( $P=0.82$ ) by yeast supplementation, and it was within the range described by some authors (Tolkamp et al., 2000; DeVries et al., 2003), but smaller than values reported by others (Melin et al., 2005). Meal size (amount of basal ration consumed in every meal without considering concentrate consumption at the robotic milking system) was not affected ( $P=0.87$ ) by yeast

Table 2  
Effects of yeast supplementation on dry matter intake and feeding pattern of dairy cows<sup>a</sup>

	NY	YS	S.E.	P-value <sup>b</sup>
Meal criterion <sup>c</sup> (min)	14.47	11.47	1.862	0.37
Meal length <sup>c</sup> (min)	28.43	31.33	8.424	0.82
Interval between meals <sup>c</sup> (h)	4.03	3.32	0.107	0.02
Meal size <sup>c</sup> (kg/meal)	2.94	2.68	1.086	0.87
Total DMI (kg/d)	18.34	18.48	4.239	0.59

<sup>a</sup> NY: no yeast supplementation; YS: yeast supplementation.

<sup>b</sup> Effect of yeast supplementation.

<sup>c</sup> These parameters refer only to basal ration consumption (DM basis). The consumption of concentrate in the robotic milking unit was ignored for these calculations.

supplementation. Cows receiving NY ate more ( $P<0.05$ ) sparsely (on average every 4.03 h) than YS cows (on average every 3.32 h), or in other words, YS consumed the basal ration more frequently than NY cows.

### 3.2. Rumen pH

Average rumen pH was lower ( $P<0.01$ ) with NY than with YS (Table 3). This response was consistent in all three cows. The improvement in rumen pH with YS is consistent with observations reported elsewhere (Michalet-Doreau and Morand, 1996; Nocek et al., 2002) in dairy cows, although results are inconsistent with other observations reported *in vivo* (Doreau and Jouany, 1998; Robinson and Garrett, 1999; Ghorbani et al., 2002) and *in vitro* (Sullivan and Martin, 1999) with either live yeast or yeast cultures. Average maximum and minimum rumen pH for YS cows was greater ( $P<0.001$ ) than for NY cows (Table 3). The areas under the curve of pH 5.6 and 6.0 were greater ( $P<0.05$ ) with NY than with YS cows, suggesting a greater severity of subclinical acidosis in NY than in YS cows.

As time since cows last consumed the basal ration from the feedbunk increased, rumen pH decreased ( $P<0.001$ ). The decrease in rumen pH associated with basal ration consumption was more ( $P<0.05$ ) pronounced in NY than in YS cows ( $-0.083$  pH units/log min *versus*  $-0.019$  pH units/log min since last basal ration consumption, respectively). The cumulative amount of basal ration consumed throughout the day affected ( $P<0.05$ ) negatively rumen pH in both NY and YS cows, indicating that the greater the amount fermentable mass that reaches the rumen the lower the ruminal pH, independently of yeast supplementation.

Table 3  
Effects of live yeast supplementation on rumen pH of loose-housed dairy cows<sup>a</sup>

	NY	YS	S.E.	P-value <sup>b</sup>
Average pH	5.49	6.05	0.051	0.01
Average maximum pH	6.22	6.77	0.063	<0.001
Average minimum pH	5.11	5.44	0.08	0.04
Area, pH < 5.6 × h/d	4.0	1.3	0.78	0.021
Area, pH < 6.0 × h/d	9.5	4.1	1.25	0.006

<sup>a</sup> NY: no yeast supplementation; YS: yeast supplementation.

<sup>b</sup> Effect of yeast supplementation.

#### 4. Conclusion

Live yeast supplementation of lactating cows in loose-house conditions improved average rumen pH within the following week after the beginning of supplementation. These positive effects were not due to changes in meal size or meal length. However, yeast-supplemented cows showed a more frequent consumption of basal ration compared with unsupplemented cows. Thus, the mechanisms involved in the reduction of subclinical acidosis associated with live yeast supplementation need to be elucidated, but meal frequency may be an important factor to consider.

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