

## Identification of behavioural patterns associated with acidosis in dairy cows

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Acidosis is a major health and welfare issue, especially in high-producing dairy cows. During acidosis, rumination is affected, as early as a few days before diagnosis. For this reason, behavioural patterns can be considered as promising indicators for the early detection of acidosis. However, the power of behaviour analysis to predict acidosis can go much further, and behavioural patterns of healthy dairy cows, weeks or months before the onset of acidosis, could be used as a risk factor for the disease. The aim of this study was to determine whether cows that will subsequently develop acidosis, show early distinctive behavioural patterns associated with the disease compared to healthy cows. Daily hours spent standing, lying down, walking, ruminating and eating were recorded from calving to 15d prior acidosis using accelerometer collars on two commercial farms in Spain and two others in Italy. The acidosis group (n=10) included cows that suffered an episode of acidosis during lactation. Acidosis was diagnosed by veterinary records in both countries. In Spanish farms, acidosis cases were confirmed by rumen pH measured by boluses (smaXtec Animal Care GmbH, Graz, Austria; a cow was confirmed as suffering from acidosis when exhibiting a rumen pH below 5.6 for at least 50 min/day). A control group of healthy cows (n=10) balanced for parity and lactation stage was constituted afterwards. The daily milk production before the diagnosis of acidosis was similar in both groups. The group effect (acidosis vs. healthy) on the intercept and the slope of the regression curve of each individual cow for all behaviours was analyzed using ANOVA. The group significantly affected the intercept of the regression curve for the ruminating, lying down and eating duration showing that cows in the acidosis group spent more time lying (10.55 vs. 10.22 h/d) and eating (3.55 vs 3.41 h/d) 85 days before the onset of acidosis ( $P < 0.001$ ), compared to control cows. In addition, the rumination duration of acidosis cows was higher (7.73 vs. 6.86 h/d) 115 d before the onset of acidosis, but lower (7.33 vs 7.81 h/d) 35 d before the onset of acidosis, compared to the control cows. The trend in rumination duration over time prior to acidosis was also different as shown by the lower slope coefficient for acidosis cows ( $P < 0.001$ ). This study suggests that cows that will subsequently suffer from acidosis might expressed distinct behavioural patterns (e.g. rumination) already months prior to the onset of acidosis. Further research with a larger sample size is needed to confirm whether differences in behavioural patterns can be considered as promoters of acidosis. This study was conducted within the ClearFarm project which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 862919.

### Abstract

*Keywords: Dairy cows, acidosis, behavioural activities, time-budget.*

## Introduction

Acidosis is a metabolic disease caused by an accumulation of organic acids (mainly lactic acids and volatile fatty acids) which is not counterbalanced by a sufficient rumen buffering inducing a low ruminal pH for several hours per day (Plaizier *et al.*, 2009). Acidosis is a major health and welfare issue in dairy cows since it has many negative impacts. Indeed, it affects feed intake, milk production, rumen digestion and microflora, and can lead to rumen mucosal damages, diarrhoea, lameness, and even death in the most acute cases.

Some management practices are considered as risk factors for acidosis. The ruminal pH depression observed in case of acidosis is favoured in dairy cows fed with concentrate-rich and fibre deficient diets (Plaizier *et al.*, 2009) or by fewer concentrate distribution per day (Yun and Han, 1989). This feeding practice of offering a concentrate-rich diet is commonly used to meet the high requirements of early-lactating cows, which means that this period is a risk period for acidosis (Penner *et al.* 2007).

However, even when dairy cows are fed and managed similarly, individuals can exhibit different degree of the disease because of individual factors. The individual variations for acidosis susceptibility are not totally elucidated, but behavioural factors could likely, at least in part, explain this individual variability. For instance, it has been suggested that the risk of acidosis increase in dairy cows that typically sort their feed in favour of short particles and against long fibre particles, or who exhibit a high eating rate since it results in of lower feed ensalivation (Beauchemin and Penner, 2009).

For this reason, behavioural patterns can be considered as promising indicators for the early detection of acidosis (Gomez *et al.*, 2022). However, the power of behaviour analysis to predict acidosis can go much further, and behavioural patterns of healthy dairy cows, weeks or months before the onset of acidosis, could be considered as a risk factor.

The aim of the present study was to determine whether cows that developed acidosis, show early distinctive behavioural patterns associated with the disease compared to healthy cows, that it could be considered as a risk factor for acidosis..

## Material and methods

### Data collection

This study was carried out based on data acquired from January 2021 to October 2022 in two commercial farms in Spain and two others in Italy. All cows were Holstein Friesian dairy cows. Their diet consisted of a total mixed ration or a partial mixed ration and concentrates. Dairy cows ( $n = 422$ ) were equipped with accelerometer collars dedicated to monitor their activity along the day (Ida collars, Connecterra, Amsterdam, The Netherlands). In the two Spanish farms, some cows ( $n = 17$  in the first one and  $n = 12$  in the second one) were equipped with rumen boluses (smaXtec Animal Care GmbH, Graz, Austria) which continuously measured rumen pH each 10 min (only for a duration of approximately 6 months after the placement of the bolus).

The data obtained from the dairy farms was from individual animals related to production (lactation rank, calving date, days in milk and daily milk production), dairy cow activities recorded by the accelerometer collars (daily hours spent standing, lying down, walking, ruminating and eating), and rumen pH for the cows equipped with rumen boluses. Acidosis cases were identified by veterinary records in the four farms. For the cows equipped with rumen boluses, acidosis were confirmed by rumen pH, when rumen pH was below 5.6 for at least 50 min/day

An acidosis group was constituted by including all cows that suffered from only one episode of acidosis during lactation. This group contained 10 dairy cows. A control group of healthy cows (n=10) balanced for parity and lactation stage was constituted afterwards by creating matching pairs of one acidosis and one control dairy cows. The two groups was composed of 5 primiparous and 5 multiparous each. The daily milk production before the diagnosis of acidosis was similar in both groups.

### Group constitution

For cows from the acidosis group, only data corresponding to the period from calving to 15d prior acidosis were kept for the statistical analyses in order to remove the sickness behaviour observed before the diagnosis of acidosis and analyse only the behaviour of dairy cows when they are not experiencing acidosis. In cows from the control group only data from calving to the day in milk corresponding to 15 days prior acidosis of its matching acidosis cow were kept for the statistical analyses. Before analyses, the day before acidosis and the behavioural data were mean-centered and reduced.

### Statistical analysis

All statistical analyses were performed using R software version 4.1.1. (R Development Core Team, Vienna, Austria). The evolution of the time per day spent doing each behaviour (i.e. standing, lying down, walking, ruminating and eating) relative to the day before acidosis was modelled for each cow using generalized linear mixed models thanks to the lmer function from the lme4 package. This permitted to estimate the intercept and the slope of the regression curve for the time spent doing each behaviour relative to the day before acidosis for each individual cow. Then, the group effect (acidosis vs. control) on the intercept and the slope of the regression curve of each individual cow for all behaviours was analyzed an ANOVA using the lm function from the stats package.

The statistical significance threshold was set at  $P \leq 0.05$ , and the trend-level significance was defined as  $0.05 < P \leq 0.10$ .

The intercept of the regression curves of each behaviour relative to the day before acidosis corresponded to 85 days before acidosis. The group significantly affected the intercept of the regression curve for the standing, lying, walking, ruminating and eating duration (Table 1). This showed that cows in the acidosis group spent more time lying (10.55 vs. 10.22 h/d), walking (3.50 vs. 2.83 h/d), ruminating (7.57 vs. 7.25 h/d) and eating (3.55 vs 3.41 h/d), and less time standing (9.97 vs. 10.94 h/d) 85 days before the onset of acidosis ( $P < 0.001$ ), compared to control cows.

## Results

The trend in the time spent lying and eating over time prior to acidosis was also different as shown by the lower slope coefficients for acidosis cows ( $P < 0.001$ ).

Figure 1 shows the evolution of the time spent ruminating over time before acidosis. The trends of the curves differed between groups ( $P < 0.001$ ). The regression curves of rumination duration before acidosis of both groups intersect 57 days before acidosis diagnosis. This showed that cows from acidosis group spent more time ruminating from the beginning of the lactation to 57 days before acidosis compared to cows from the control group. However, from 57 to 15 days before acidosis, they spent less time ruminating compared to control cows. We thus have re-ran the analysis of the effect on the group on the intercept for the two different periods: before 57 d prior to acidosis and from 57 d to 15 d prior to acidosis. The group significantly affected the intercept of the regression curve showing that the rumination duration of acidosis cows was

Table 1. Effect of the group (acidosis vs. control) on the intercept of the regression curve of time spent by each cow doing each behaviour. The intercept corresponds to 85 days before acidosis.

Behaviour	Group		P-value
	Acidosis	Control	
Standing	9.97	10.94	< 0.001
Lying	10.55	10.22	< 0.001
Walking	3.50	2.83	< 0.001
Ruminating	7.57	7.25	< 0.001
Eating	3.55	3.41	< 0.001

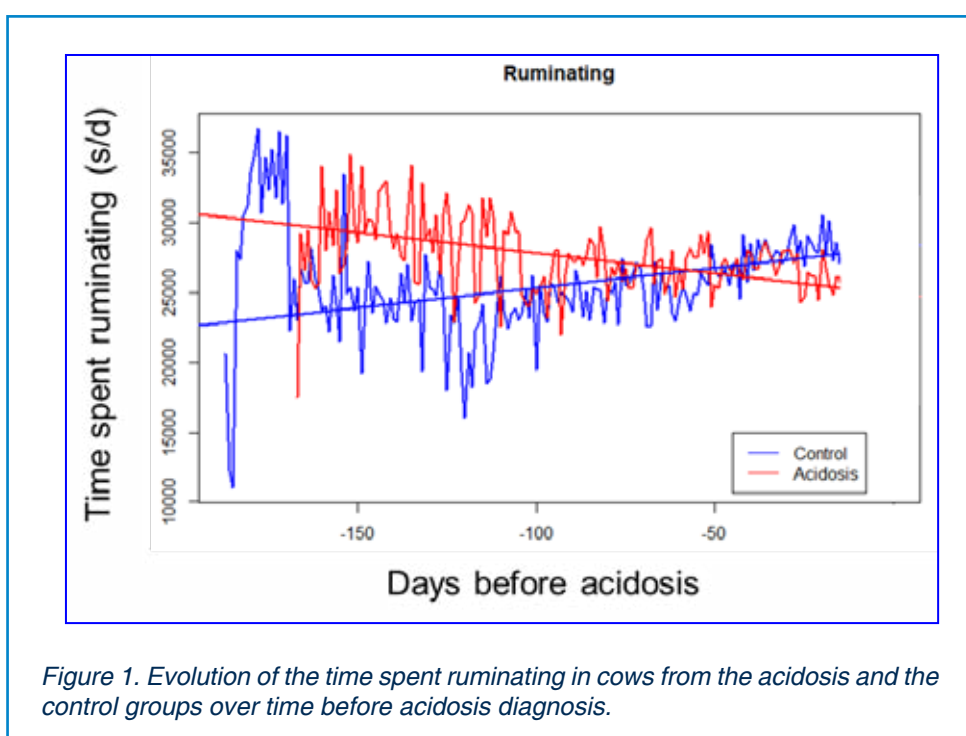


Figure 1. Evolution of the time spent ruminating in cows from the acidosis and the control groups over time before acidosis diagnosis.

higher (7.73 vs. 6.86 h/d) 115 d before the onset of acidosis, but lower (7.33 vs 7.81 h/d) 35 d before the onset of acidosis, compared to the control cows.

## Discussion and conclusions

The purpose of the present study was to analyze the differences in time-budget (time spent standing, lying down, walking, ruminating and eating per day) between dairy cows that will subsequently suffer from acidosis and dairy cows that stay healthy during the entire lactation. The present study showed that cows suffering from acidosis might expressed different time-budget already few months prior to the onset of acidosis. Therefore, the time-budget of dairy cows could be used as a risk factor of acidosis. This was already suggested by previous studies showing that cows that high acidosis risk did, on average, spend less time ruminating than the cows with a low acidosis risk (DeVries *et al.*, 2009; Coon *et al.*, 2019).

Further research with a larger sample size is needed to confirm distinct behavioural patterns associated with the subsequent development of acidosis.

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