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Original article

Rumination time as an indicator of stress in the first thirty days after calving

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Abstract

The objectives of this study were to examine the option of being able to use rumination time (RT) as a form of stress indicator in the first thirty days after calving, and to determine the relationship between rumination time, blood cortisol levels, and lactate concentration levels in dairy cows during the first thirty days after calving.

Ninety cows which produced milk (DIM) within 1-30 days were selected and categorised into the following groups: the first group (1) fell within 1-7 days after parturition (dpp) (n=30); the second group (2) fell within 8-14dpp (n=30); and the third group (3) fell within 15-30dpp (n=30) after calving. The cows were milked using Lely Astronaut® A3 milking robots with free traffic. The blood samples were tested using the fluorescence enzyme immunoassay method for cortisol analysis. Lactate concentrations were tested with a Lactate Pro2®.

The RT increased during all of the exploratory periods (with readings between 1.12-4.90%). A decrease was also observed in the lactate levels (by 1.10 times) and cortisol levels (by 1.98 times, $p < 0.05$) of cows which fell within the 8-14dpp group, when compared to an average of 1-7dpp in the previous study period (15-30dpp). However, lactate concentrations increased (by 1.84 times, $p < 0.05$) as well as cortisol levels (by 2.09 times, $p < 0.01$) when compared with a figure between 8-14 dpp on the average. The results obtained indicate that, RT increased during all exploratory periods, while a decrease by 1.10 times and 1.98 times was observed in lactate levels and cortisol levels, respectively. During the entire period of the study RT was positively correlated with the lactate concentration levels, and negatively correlated with cortisol levels. Within a period of 1-14 days, a negative correlation was determined with lactate levels along with a 15-30dpp-positive correlation coefficient. In conclusion, RT can be used as a kind of stress indicator for cows in the first thirty days after calving; however, further research is required to ascertain this conclusion.

Key words: rumination time, cortisol, lactate, cow

Introduction

An increase in rumination time (RT) in dairy cattle has been associated with increased saliva production and improved rumen health. Most estimates of rumination are based on direct visual observations which provide useful information regarding the cows' health. The eating and rumination behaviour of sick cattle are of primary importance from a clinician's standpoint, and these activities are routinely monitored in sick cows during and after treatment (Braun et al. 2013). Observing the eating and rumination behaviour of individual animals is difficult in large herds, especially if specific information regarding the duration of these behaviours, the number of eructated cuds per unit time, or the number of chewing cycles per cud is being sought (Braun et al. 2013). Recently, an electronic system that allows the automated monitoring of rumination in cattle was developed (Schirmann et al. 2009). Monitoring RT around calving and, in particular, during the first week of lactation, has been proposed as being an effective means of identifying those cows that are at a greater risk of developing disease in early lactation (Isehunwa et al. 2017). According to Schirmann et al. (2009), further research is required to gain a better understanding of the changes in and relationship between rumination time (RT) and disease after calving.

Paudyal (2016) reported that the early detection of clinical and subclinical disease through rumination monitoring would allow producers to more rapidly begin remedial therapies that could reduce the costs that are associated with later treatments and more severe production losses. Furthermore, the time required for the normalisation of eating and rumination behaviour in a sick animal has a prognostic value. This may be taken as a parameter of the effectiveness of the applied treatment (Braun et al. 2013). According to Reiter et al. (2018), the agreement between the Smart bow system and video analyses was excellent from a practical and clinical point of view. The detected differences were negligible. However, researchers suggested the need to conduct further research to test the system under various field conditions and to evaluate the benefits of incorporating rumination data into herd management decisions. Rumination times in the electronic system highly correlates with those that were taken from direct observation, indicating that the electronic system was an accurate tool for monitoring this behaviour in dairy cows (Schirmann et al. 2009). The automatic measurement of RT is useful for predicting calving time, and also in quickly obtaining information on the health status of the animals within a period which is as critical as the transition phase (Soriani et al. 2012). Schirmann et al. (2012) found that rumination time can be used

to estimate individual cow variations in feeding behaviour and intake, but daily summaries of rumination behaviour are a poor indicator of days in milk (DIM) production. The rumination times which were obtained from the electronic system correlated highly with those that were taken from direct observations, indicating that the electronic system was an accurate tool for monitoring this behaviour in dairy cows (Soriani et al. 2012).

Stressful situations which cows may experience have been observed as one of the factors responsible for the reduction in rumination time. Farm animals are faced with many stressors around the feeding environment such as temperature, the feed itself, and the number of animals per unit area. Cortisol is well known as a stress marker of the endocrine system (Takeshi Ito et al. 2017). The hypothalamic-pituitary-adrenocortical axis releases glucocorticoids as part of the endocrine mechanism for self-protection of the body against any stressor. The quantification of cortisol or its metabolites is a physiological indicator for assessing stress (Giancarlo Bozzo et al. 2018). Cortisol also plays a role in cases of acute or chronic stress, whereby it mobilises energy reserves through the conversion of glycogen into energy. Stress increases cortisol concentrations in the blood and triggers a depletion of glycogen reserves in muscles. This can lead to a decrease in lactic acid production and a resultant high pH value. During short-term stress, glucocorticoids improve fitness by means of energy mobilisation, and may influence behaviour such as rumination. However, severe chronic stress (prolonged periods of high cortisol concentrations) may decrease the fitness level of each animal by means of immunosuppression and atrophy in the tissues. In addition, the reproductive success of the animal is decreased. There are also indications that stereotypy might be related to stress (Möstl et al. 2002). A fluctuating lactate level would have been rapidly elevated by physical effort, including the behavioural reaction to stress (Choe et al. 2014). Lactate is not only an anaerobic metabolic end product, it also signals the unfavourable situation of the animal in question. In healthy individuals, there is a continuous cycle of milk production and metabolism, which ensures that blood lactate concentrations are kept low (Isehunwa et al. 2017). Cortisol may cause a rise in glucose levels through the use of gluconeogenic substrates such as lactates, amino acids, and fatty acids (Isehunwa et al. 2017). At the same time, Cortisol-induced hyperglycaemia caused an increase in blood lactate levels when compared with cortisol levels, which confirm that the beta adrenergic receptors are involved in and play a dominant role in cortisol-induced hyperglycaemia (Isehunwa et al. 2017). Milk cortisol could be used as an indicator

of painful symptoms, such as lameness. Higher values in milk cortisol levels during the first week of lactation should be taken into consideration for the purpose of interpretation (Gerlich et al. 2015).

A plasma L-lactate concentration may be a useful predictor of productive outcomes in cows with right-sided abomasal disorders (Figueiredo et al. 2006). Despite the low pH levels of rumen and the high blood D-lactate levels (with a peak of 4.8mM, at 26h), the animals experienced only a mild acid-base disturbance which was accompanied by a six-fold increase in the rate of D-lactate absorption, whereas, L-lactate absorption increased by only 70% despite higher rumen levels (Harmon et al. 1985). Rumination time in cattle decreases during rumen acidosis. DeVries et al. (2009) observed that high-risk cows spent less time ruminating than the low-risk cows.

The objectives were to determine the possibility of using rumination time as a form of stress indicator in the first thirty days after calving, and to determine the relationship between rumination time, blood cortisol levels, and lactate concentrations in dairy cows during the first thirty days after calving.

Materials and Methods

Location, animals, and experimental design

The experiment was carried out on a dairy farm in the eastern region of Europe at 56 00 N, 24 00 E. Lithuanian Black-and-White fresh dairy cows were selected, and only those which fitted the profile of having had two or more lactations as well as being clinically healthy (with an average rectal temperature of 38.8°C, a rumen motility of 5-6 times in every three minutes, and having no signs of mastitis, lameness, or metritis) were chosen. The study was carried out on ninety dairy cows out of a herd of 1,300 cows. The cows were kept in a loose housing system, and were given a feed ration throughout the year at the same time, being balanced according to their physiological needs. Cow feeds were given daily at 06:00 and 18:00.

Ninety cows were selected between 1-30 days of milk (DIM) production and were categorised into the following groups: the first group (1) covered 1-7dpp (n=30) after calving; the second group (2) covered 8-14dpp (n=30); and the third group (3) covered 15-30dpp (n=30).

The cows were milked with Lely Astronaut® A3 milking robots with free traffic. To motivate the cows to visit the robot, a total of 2kg/d of concentrates were fed to them by the milking robot. The blood samples were tested for cortisol using the fluorescence enzyme immunoassay method by Tosh Corporation AIA-360 (USA).

Lactate concentration levels were tested with a Lactate Pro2® using the enzyme electrode method. Compatible reagents were Lactate Pro 2 Test Strips (sensors with an electrode for measuring lactates in the blood) by Arkray, Japan. Postpartum health checks were carried out daily according to general clinical investigation guidelines. Diseases which occurred after calving (including metritis, LDA, ketosis, and milk fever) were diagnosed by a general clinical examination and when searching for specific clinical signs. All the cows were ascertained to be clinically healthy during the study period.

The results obtained were analysed using the SPSS application (Statistical Package for the Social Sciences, 20.0). The results were expressed as the mean (M) ± standard error of the mean (SEM), with a 95% confidence interval (CI). The Pearson correlation (r) was calculated to determine the statistical relationship between those traits that were being investigated. The average reliability of the data was tested using the one-sample Kolmogorov-Smirnov test. The differences in the mean values of normally distributed variables were analysed by means of the Student's T-Test. The results were considered to be statistically significant when $p < 0.05$.

Results

The average rumination time for the animals being investigated was 435.46 ± 7.277 minutes, with a mean lactate level of 0.96 ± 0.099 mmol/l, and cortisol levels of 0.93 ± 0.086 ug/dl. Comparing the first and third groups, RT levels could be seen to have increased by 6.18%, while lactate levels increased by 66.49%, and cortisol concentrations increased by 5.87% (Table 1).

The RT increased during all exploratory periods (between 1.12-4.90%). This investigation showed a decrease in lactate levels (by 1.10 times) and cortisol levels (by 1.98 times, $p < 0.05$), whereby the value obtained in the second group (8-14dpp) could be compared with the average value for the first group (1-7dpp) in the previous study period (group 3 (15-30dpp) having a higher value). A similar comparison was done in terms of lactate levels (1.84 times, $p < 0.05$), and cortisol levels (2.09 times, $p < 0.05$) regarding concentrations which increased when compared with the average value (Fig. 1) for the second group.

The RT during the entire study period positively, correlated with lactate concentration levels, but was negatively significant ($p < 0.01$) for cortisol levels. Within 1-14 days, a negative correlation with lactate levels was detected along with a 15-30 dpp-positive correlation coefficient. The correlation coefficient between the RT and cortisol concentrations in cow

Table 1. A description covering the statistics for the investigated traits.

Traits	Group	M	SEM	95% CI	
				Lower Bound	Upper Bound
Lactate concentration levels (mmol/l)	1. (1-7dpp)	0.79 ^a	0.206	0.376	1.199
	2. (8-14dpp)	0.71 ^{ab}	0.221	0.274	1.154
	3. (15-30dpp)	1.31 ^{ac}	0.194	0.923	1.699
Cortisol levels ug/dl	1. (1-7dpp)	1.06 ^a	0.144	0.773	1.347
	2. (8-14dpp)	0.54 ^b	0.154	0.229	0.842
	3. (15-30dpp)	1.12 ^{bc}	0.136	0.852	1.393
Rumination time, mins a day	1. (1-7dpp)	424.13	14.121	395.954	452.296
	2. (8-14dpp)	429.29	15.096	399.169	459.402
	3. (15-30dpp)	450.33	13.314	423.773	476.894

Explanations: M - those means which have different superscript letters differ significantly at $p < 0.05$.

The average rumination time for the animals being investigated was 435.46 ± 7.277 minutes, with a mean lactate level of 0.96 ± 0.099 mmol/l, and cortisol levels of 0.93 ± 0.086 ug/dl. Comparing the group 1 (1-7 days after calving) and group 3 (15-30 days after calving), RT levels could be seen to have increased by 6.18%, while lactate levels increased by 66.49%, and cortisol concentrations increased by 5.87%.

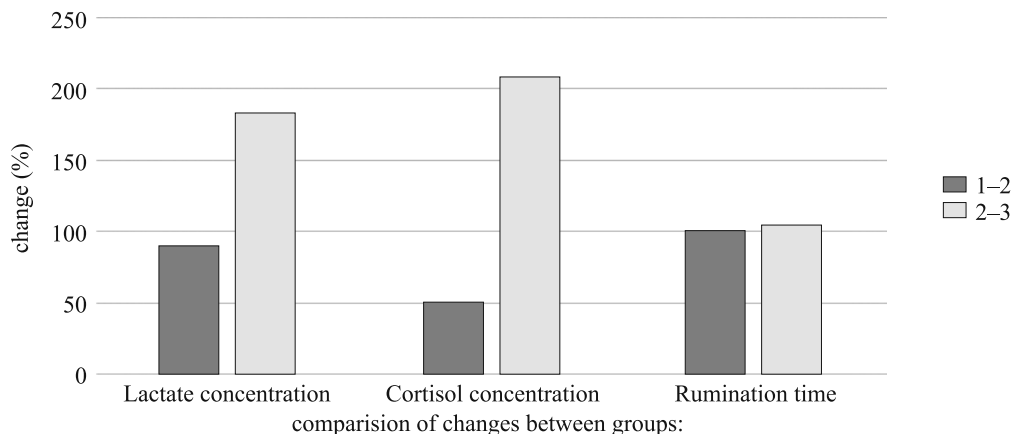


Fig. 1 A comparison of changes between groups.

The RT increased during all exploratory periods (between 1.12- 4.90%). A decrease in lactate levels (by 1.10 times) and cortisol levels (by 1.98 times, $p < 0.05$). A similar comparison was done in terms of lactate levels (1.84 times, $p < 0.05$), and cortisol levels (2.09 times, $p < 0.05$) regarding concentrations which increased when compared with the average value for the group 2 (8-14 days after calving).

groups ranged from $r = -0.407$; $p < 0.05$ (group 1) to $r = -0.532$; $p < 0.01$ (group 2). The increase in lactate concentration levels between 1-7dpp was associated with an increase in cortisol levels ($p < 0.01$), and the value obtained for 8-14dpp was associated with a decrease in cortisol levels ($p < 0.01$). The results are summarised in Table 2.

Discussion

According to an earlier investigation, RT, subsequent yield, and milk trait changes depend on the lactation period and the reproductive status of the specific dairy cow (Antanaitis et al. 2018). Ruminant time in the electronic system correlated very well with that of direct observations, indicating that the electronic system was an accurate tool for monitoring this behaviour in dairy cows (Schirmann et al. 2012). We also observed

an increase in RT during all exploratory periods. A decrease in lactate levels by 1.10 times and cortisol levels by 1.98 times was observed in this study. Soriani et al. (2012) reported that the decrease in RT during the first few days of lactation was observed in cows which suffered from subclinical diseases or health disorders, for example, cows which were being affected by clinical mastitis during the trial period showed a reduction in RT and a change in its variability as early as a few days before the drug treatment. In fact, a reduction in rumination time was associated with increased cortisol levels in cattle (Trevor et al. 2016). According to Bristow et al. (2007), cows with high levels of cortisol spent less time ruminating and also vocalised more than subjects which had low levels of cortisol. The RT during the entire study period was positively correlated in statistical terms with lactate concentration levels and negatively correlated with cortisol levels. Within 1-14

Table 2. Correlations between traits.

Relationship between traits	All groups	Group 1	Group 2	Group 3
Rumination time-lactate concentration	0.133	-0.055	-0.135	0.328
Rumination time-Cortisol levels	-0.339**	-0.407*	-0.532**	-0.504**
Lactate concentrations-Cortisol levels	-0.154	0.498**	-0.686**	-0.310

Explanations: The correlation coefficient is statistically significant at the level of * $p < 0.05$, and ** at the level of $p < 0.001$.

The RT positively, correlated with lactate concentration levels, but was negatively significant ($p < 0.01$) for cortisol levels. The correlation coefficient between the RT and cortisol concentrations in cow groups ranged from $r = -0.407$; $p < 0.05$ (group 1) to $r = -0.532$; $p < 0.01$ (group 2).

days, a negative correlation with lactate levels was detected along with a 15-30 dpp-positive correlation coefficient. According to the available literature, lactating is not only an anaerobic metabolic end product -it also signals the unfavourable situation of the animal which causes it to move to a more stable environment. In healthy individuals, there is a continuous cycle of lactate production and metabolism, which ensures that normal blood lactate concentrations are low (Isehunwa et al. 2017). Cortisol, a steroid hormone, causes hyperglycaemia through gluconeogenesis. This may lead to a rise in glucose levels through the use of gluconeogenic substrates such as lactate, amino acids, and fatty acids (Isehunwa et al. 2017). The decrease in lactate levels which was observed following a cortisol injection is possibly due to the fact that lactate was used partially for glucose production when the glycogen store was depleted. Jacobs (1981) reported that a diet that was low in carbohydrates may lead to a depletion of glycogen stores and could ultimately lead to a decrease in lactate levels (Isehunwa et al. 2017). Cortisol-induced hyperglycaemia, at the same time, caused an increase in blood lactate levels when compared to cortisol levels, this confirms that the beta adrenergic receptors are involved in and play a dominant role in cortisol-induced hyperglycaemia (Isehunwa et al. 2017). Previous studies in humans showed that cortisol levels can increase hepatic glucose production and blood glucose levels through gluconeogenesis (Khani and Tayek. 2001). Glucocorticoids help to enhance beta receptor-mediated responses, along with myocardial contractility, hepatic and muscle glucose metabolism responses, myocardial contractility, and hepatic and muscle glucose metabolism. Soriani et al. (2012) suggested that the automatic measurement of RT is useful in predicting calving time and in quickly obtaining information on the health status of the animals within a period which is as critical as the transition phase. Milk cortisol levels are at the peak during the first week of lactation and this has remained at comparable levels thereafter (Gerlich et al. 2015). According to Beerd et al. (2004), the assessment of plasma cortisol

concentrations has been widely used as an indicator of the activation of the hypothalamic pituitary adrenal axis in response to stressful situations in cattle. Hernandez et al. (2014) found that social separation and unfamiliar surroundings are stressful situations which can result to an increase in cortisol secretion in cattle. Changes in housing conditions by tethering the animals in a separate area of a barn was unknown to all the animals, particularly during the initial period of lactation. According to Isehunwa et al. (2017), lactate levels decreased significantly in response to a cortisol increase, suggesting that lactate levels could serve as a gluconeogenic substrate. Beta-adrenergic receptors are also involved in the use of lactates as a gluconeogenic substrate in order to induce cortisol hyperglycaemia.

Based on the results of the present study, it can be seen that RT increased during all exploratory periods, along with a decrease in lactate levels by 1.10 times and cortisol levels by 1.98 times. Furthermore, RT during the entire study period was positively correlated in statistical terms with lactate concentration levels, but it has a negatively statistically-significant figure in terms of cortisol levels. Within 1-14 days, a negative correlation with lactate levels was detected, along with a 15-30 dpp-positive correlation coefficient. Based on these results, we can conclude that RT can be used as a form of stress indicator for cows in the first thirty days after calving. However, further research is required to ascertain this conclusion.

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