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The dynamic of the ruminal content pH change and its relationship to milk composition

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Abstract

The main goal of this study was to evaluate the health condition of dairy cows in relationship to milk production and milk composition using continuous monitoring boluses. In total, seven Holstein cows had boluses implemented for monitoring rumen pH and temperature every 15 min with an accuracy of ± 0.1 pH/ °C. Milk production test day records were noted by Breeding Services of Slovakia, s.e. (state-owned enterprise) 5 × per each cow with a bolus over 27 weeks of lactation. Dairy cows were divided into three groups according to their mean daily pH. After that, the test day records with the selected group were paired. Only two cows had pH values within the normal pH intervals during the control days. Consequently, there was found to be a 6.8% ($P < 0.05$) decrease in daily milk production in cows with lowered pH compared to cows with normal pH. Furthermore, there was found to be a 14.08% ($P < 0.05$) decrease in daily milk production in cows with an increased pH compared to cows with normal pH. Narrower fat to protein ratio and lactose content was found in cows with decreased and increased ruminal pH. The lowest milk fat concentration ($P < 0.05$) but the highest somatic cell count and urea content were determined in cows with decreased pH. These results show that continuous monitoring of rumen environment is a suitable method for nutrition and health management in dairy herds and allows the nutritionist to make nutritional interventions for pH stabilization within normal range in order to keep good milk production and high milk quality.

Rumen pH, rumen fermentation, SARA, rumen alkalosis, milk yield, milk components

Metabolic disorders of dairy cattle are related to disturbances in the metabolic processes in the organism. The transition period, which includes three weeks before and three weeks after parturition is very critical for dairy cows (Ametaj 2010). Subacute ruminal acidosis (SARA) is a common disease in high yielding dairy cows that receive highly digestible diets, and has a high economic impact as it can affect the feed intake and milk production. It can compromise a cow's health by causing diarrhoea, laminitis, liver abscesses, production of bacterial immunogens and inflammation (Plaizier et al. 2008). Gozho et al. (2005) claim that SARA occurs when the rumen pH is between 5.2–5.6 for at least 3 h daily. Subacute ruminal acidosis is defined as periods of moderately depressed ruminal pH (about 5.5–5.0) that are between acute and chronic in duration (Garrett et al. 1999). Plaizier et al. (2008) defined a threshold for SARA time below 6.1 for more than 3 h daily. Clinical and subclinical ketosis is a wide-spread metabolic disease in dairy herds. Ketosis is often caused by a negative energy balance due to high milk production and deficient energy intake, and by excessive body fat mobilization. Intake deficient in energy often occurs after feeding poor quality feeds, insufficient food intake or other metabolic disorders (Correa et al. 1993; Reksen et al. 2002). This disease leads to milk production depression and is often accompanied with depression of reproductive performance (Ospina et al. 2010; Chapinal et al. 2012). Therefore, the impacts of ketosis (clinical or subacute) on the health, reproductive performance, and production can be costly for each affected cow, and

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can affect the profitability of a dairy enterprise (Gohary et al. 2016). Tajik and Nazifi (2011) list the use of rumen fluid, rumen pH, stomach tubing, indwelling electrode, ruminal cannulation, rumenocentesis, rumen microbial composition, rumen fluids temperature, urine pH, faecal sieving, faecal lipopolysaccharide, and blood indices as SARA diagnostic techniques. Clinical and subclinical ketosis can be detected using Fourier Transform Infrared Spectrometry for detection of ketone bodies (acetone, acetoacetate, β-hydroxybutyrate) in milk (De Roos et al. 2007), by the concentration of serum β-hydroxybutyrate (Karimi et al. 2016), or by evaluation of milk fat, milk protein and protein to fat ratio (Negussie et al. 2013). The main goal of this study was to evaluate the health condition of dairy cows in relationship with milk production and milk composition using continuous monitoring boluses.

Materials and Methods

Animals and housing

The experiment was conducted over 27 weeks of lactation in cooperation with the University Experimental Farm in Oponice. Seven selected cows of the Holstein breed (mean age 3.57 years) had an average milk production of 10 175 kg per lactation with 3.94% of fats, 3.10% of crude proteins, and 4.7% of lactose, and each had a similar dry matter intake. Of the seven cows, three were in the 2nd lactation and four were in the 3rd lactation. The experimental cows were loose housed with a laying boxes system and automatic manure scraper in the manure corridor, together with other dairy cows. Daily diet on the feeding table was folded. Two drinkers were available for 20 dairy cows in one section.

Feeding

The animals were fed once daily with the Total Mix Ratio (Table 1) *ad libitum* between 4:00 and 5:00 h and milked × 3 per day at 6:00, 12:00 and 18:00 h. Corn silage acidity (pH 3.85) and alfalfa silage acidity (pH 4.85) were neutralised with sodium bicarbonate (550 g·head^{-1} daily) and magnesium oxide (51 g·head^{-1} daily) (Table 2). Nutritional composition of the diet was determined by standard laboratory methods and procedures (AOAC, 2000).

Table 1. Composition of daily diet.

Feed	DM kg	NEL MJ	CP %	NDF %	Starch %
Corn silage	7.60	49.52	14.83	50.26	47.48
Alfalfa silage	5.80	25.88	29.09	39.94	1.08
Feed mixture	7.65	43.81	44.17	0.00	13.09
HMC	3.67	27.90	7.62	4.71	38.12
Cotton seed	0.74	6.76	4.29	5.08	0.23
Total	25.45	153.86	15.74	24.35	25.39

DM - dry matter, NEL - netto energy of lactation, CP - crude protein, NDF - neutral detergent fibre, HMC - high moisture corn

Data measuring, data collecting and statistical evaluation

Every dairy cow had a farm bolus for continual data measuring implemented orally through the oesophagus with the use of a special balling gun. Ruminal pH and temperature values were measured every 15 min (96 data points per day) with a ± 0.1 accuracy for pH. The boluses used (eCowDevon, Ltd., Great Britain) are characteristic for their small dimensions (135 × 27 mm) and low weight 207 g. Data were downloaded in the milking parlour using a handset with an antenna and a dongle connected with a USB dongle connector with a radio frequency of 434 MHz. Milk production test-day values (milk yield, milk fat, milk proteins, lactose, somatic cells and urea) were recorded over the lactation period (5 months) by the Breeding Services of Slovakia, s.e., 5 times per each cow with a bolus during the second milking at 12:00 h. Collected data were summarized using HathorHBClient v. 1.8.1 and statistically evaluated using IBM SPSS v. 20.0 (One-way ANOVA for description statistics, Tukey test for significance of differences). Dynamics and changes in the pH in % between the previous hour were calculated. After statistical evaluation, 3 levels of pH were created according to the mean daily pH using filters. Dairy cows with a mean daily pH under 5.8 were designated as cows with a decreased pH; from 5.8 to 6.8 as cows with a normal pH; and over 6.8 as cows with an increased pH. During the monitored period, the pH of

Table 2. Composition of feed mixture.

Ingredient	%
ESM	25.2
ERM	25.2
Corn meal	18.5
DDGS	12.3
Oat meal	9.9
BF	4.2
Sodium bicarbonate	3.9
Magnesium oxide	0.7

ESM - extracted soya meal, ERM - extracted rapeseed meal, DDGS - dried distillers grains with solubles, BF - by-pass fat

dairy cows fluctuated, so the pH of one dairy cow could have occurred in each different level of pH during test-day recordings during the whole lactation period (Table 3). Then, 35 results from milk production test days were paired to the cows within filtered groups according to the pH level during the test day.

Results

The difference between dairy cows during the monitored period in relation to the test day is shown in Table 4. Only in two dairy cows (cows no. 1 and 6), ruminal pH within the normal pH interval was found on the test days during the whole monitored period. In this case, the mean pH for 5 test days was 6.59 ± 0.12 and 6.54 ± 0.13. However, in the first cow a decreasing trend, and in the second cow an increasing trend of pH was observed during lactation. Similarly, a rising trend of ruminal pH in three other cows was found (cow no. 2, 4 and 7). However, in the case of cow no. 2, pH was within the normal interval only on the second test day. In contrast, ruminal pH in cow no. 4 was between 6.2 and 6.8 during the first 3 test days, after which increased pH was determined in this cow during the last 2 test days. Overall, the mean pH from all the test days was in these two animals (no. 2 and 4) the highest (6.82 ± 0.58 and 6.75 ± 0.40). However, animal no. 7 was constantly in the condition of decreased pH during the first 3 test days. Fortunately, at the end of the lactation period, its ruminal pH was normal during the last two test days, but the total average in this animal compared to other animals was the lowest on all test days (6.03 ± 0.35). The last two animals (no. 3 and 5) had similar mean pH on all test days (6.20 ± 0.17 and 6.23 ± 0.24) and the ruminal pH was fluctuating during the monitored period. However, ruminal pH was in the normal interval except for two test days in dairy cow no. 3, and one test day in dairy cow no. 5, where decreased pH was found. The mean

Table 3. Level of pH during the test-day records for milk yield and composition.

Order of test day	Level of pH		
control	Lowered	Normal	Increased
1	2	5	0
2	1	6	0
3	2	4	1
4	2	3	2
5	0	5	2

Table 4. Rumen pH of dairy cows on test days.

Order of test day	Number of cow							SEM	*P*
control	1	2	3	4	5	6	7		
1	6.73[a]	5.85[b]	6.28[c]	6.41[c]	6.28[c]	6.39[c]	5.60[d]	0.14	< 0.05
2	6.69[a]	6.75[a]	6.30[b]	6.35[b]	6.37[b]	6.46[b]	5.95[c]	0.10	< 0.05
3	6.55[a]	6.99[b]	6.10[c]	6.74[d]	6.40[a]	6.50[a]	5.89[bd]	0.14	< 0.01
4	6.54[a]	7.11[b]	5.95[c]	6.88[d]	5.80[c]	6.65[ad]	6.22[e]	0.18	< 0.01
5	6.43[ae]	7.38[b]	6.35[a]	7.35[b]	6.29[c]	6.71[d]	6.51[e]	0.17	< 0.01
$\bar{x}$	6.60[a]	6.82[b]	6.24[c]	6.73[ab]	6.21[cd]	6.57[a]	6.13[d]	0.00	< 0.01

Different letters in the columns indicate significant differences. The mean difference is significant at the 0.05 level (Tukey Test).
SEM – standard error of mean, $\bar{x}$ – whole monitored period mean, *P* – effect on ruminal pH (ANOVA)

daily pH on test days was in relationship with the pH for every day in whole monitored period. Thus, the same tendencies and differences in pH levels were found between animals on the test days. For 189 of the monitored days, only cow no. 1 was not in the condition of lowered pH under 6.2, and only 24 days with increased pH were detected. On the other hand, cow no. 2 had the highest number of increased pH days for 115 days of lactation, but only 44 days with decreased pH were observed. However, in dairy cow no. 7, decreased pH for 105 days was found and there were no days with lowered pH. In total, 5 animals had pH within normal interval at least for 100 days. These results show that dairy cows under the same feeding regime had different pH values during the whole lactation period.

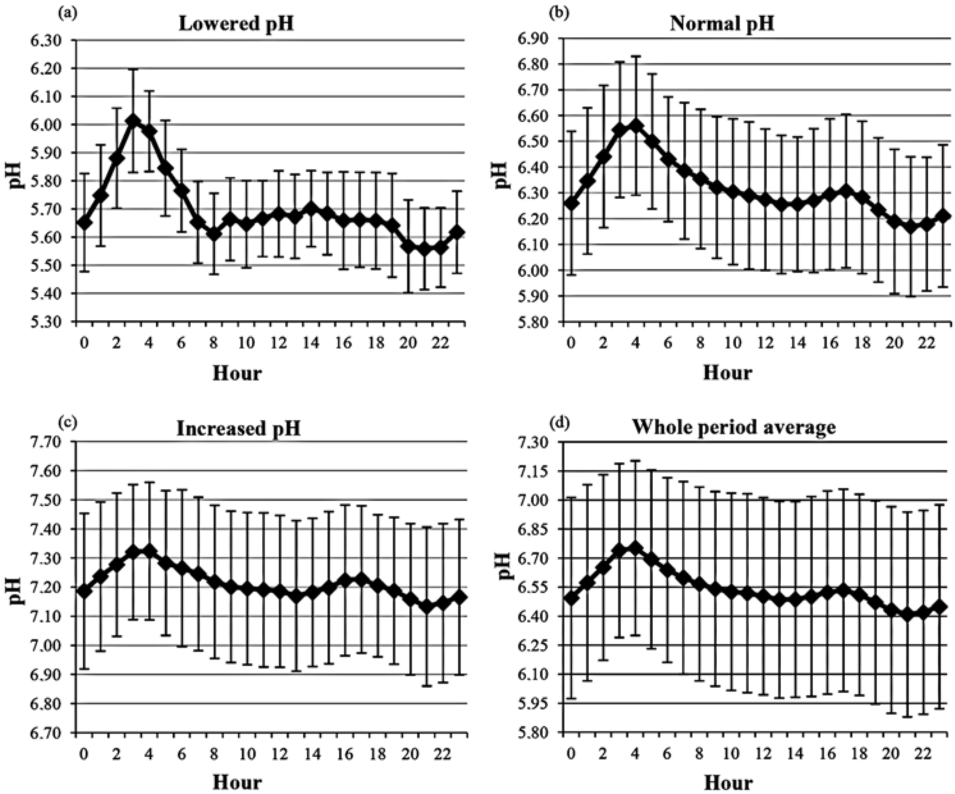

Fig. 1 (a, b, c, d). Average daily courses of ruminal pH during the whole monitored period (d) and according to pH level (a, b, c)

After the evaluation of measured pH results, the dairy cows were divided into 3 levels of pH (Fig. 1). First, animals with the daily pH values under the threshold 5.8 were marked as cows with lowered pH. In dairy cows with lowered pH, the mean daily pH of 5.70 ± 0.20 was determined. Then, a group of dairy cows with the mean daily pH of 5.8 to 6.8 was formed. These cows were identified as the normal pH group. Compared to the cows with lowered pH, significantly higher pH by 9.81% ($P < 0.01$) with the daily mean of 6.32 ± 0.29 was found in the cows with normal pH. Furthermore, dairy cows with the mean daily pH over 6.8 were designated as cows with increased pH. In contrast to the normal pH cows, significantly higher ($P < 0.01$) daily mean pH by 14.16% (7.21 ± 0.26) was found in cows

Table 5. Frequency of pH values in the selected intervals according to pH level.

Interval	Lowered	Normal pH frequency in %	Increased pH
under 5.8	71.66	4.39	-
5.8-6.2	27.30	29.09	-
6.2-6.8	1.04	61.67	5.31
over 6.8	-	4.85	94.69

with increased pH. Afterwards, all cows with similar circadian changes were characterized because of the same feeding regime of dairy cows. Nevertheless, large differences were found in all cows in terms of lowered, normal or increased pH. Firstly, a contrast between minimal and maximal values was found in dairy cows. The lower the daily pH was, the lower the variance between maximal and minimal pH was determined. In cows with lowered pH difference between maximal pH at 3:00 h and minimal circadian pH 7.56% at 21:00 h was found. In cows with normal pH it was 5.96% and in cows with increased pH only 2.60%. Moreover, 5 h after the morning feeding, cows with lowered pH showed a faster decrease of rumen pH in comparison with normal pH cows and cows with increased pH. An average decrease of 1.37 ± 0.71% was found in cows with lowered pH during 5 h after the first feeding (from 6.01 ± 0.18 to 5.61 ± 0.14). In cows with normal pH, a slower decrease of 0.74 ± 0.25% (from 6.56 ± 0.27 to 6.31 ± 0.28) and in cows with increased pH of only 0.34 ± 0.14% (from 7.32 ± 0.24 to 7.20 ± 0.26) was determined. In contrast, in cows with lowered pH the best recovery of 1.57 ± 0.75% of pH (from 5.56 ± 0.15 to 5.58 ± 0.18) was detected 5 h before the first feeding compared to cows with normal pH (1.10 ± 0.57%; from 6.21 ± 0.28 to 6.56 ± 0.27) and cows with increased pH (0.44 ± 0.27%; from 7.15 ± 0.27 to 7.32 ± 0.23).

Furthermore, frequency of pH intervals under 5.8, from 5.8 to 6.2, from 6.2 to 6.8 and over 6.8 were monitored (Table 5). For rumen environment and cellulolytic bacteria, a pH range from 6.2 to 6.8 is considered optimal. On average, cows with normal pH spent 14 h and 48

Table 6. Milk yield and composition according to pH level.

Level of pH		M kg·day^{-1}	F %	P %	F/P ratio	L %	SC 1000·1 ml^{-1}	U mg·100 ml^{-1}
Lowered (n = 7)	x̄	43.02^{a}	2.88^{a}	2.67^{a}	1.10^{a}	4.77^{a}	1723.83^{a}	24.77^{a}
	SD	7.45	0.78	0.26	0.34	0.23	3451.32	5.22
	Min	33.90	1.42	2.39	0.51	4.35	24.00	20.10
	Max	55.20	3.63	3.15	1.52	5.02	8752.00	33.70
Normal (n = 23)	x̄	46.15^{b}	3.64^{b}	2.77^{a}	1.31^{b}	4.91^{a}	265.87^{a}	22.61^{a}
	SD	5.78	0.57	0.28	0.14	0.12	390.25	5.00
	Min	37.40	2.94	2.33	1.16	4.69	0.00	15.20
	Max	61.40	5.04	3.30	1.73	5.07	1239.00	31.30
Increased (n = 5)	x̄	30.45^{c}	3.73^{b}	2.91^{b}	1.28^{b}	4.81^{a}	54.00^{a}	24.63^{a}
	SD	10.82	0.35	0.15	0.08	0.20	25.83	8.00
	Min	14.60	3.41	2.81	1.21	4.53	25.00	18.50
	Max	39.00	4.14	3.13	1.38	4.99	80.00	36.30
Total	x̄	42.89	3.47	2.77	1.26	4.86	581.88	23.45
	SD	8.84	0.67	0.26	0.21	0.17	1733.60	5.42
	Mini	14.60	1.42	2.33	0.51	4.35	0.00	15.20
	Max	61.40	5.04	3.30	1.73	5.07	8752.00	36.30

x̄ – mean, SD – standard deviation, Min – minimal value, Max – maximal value, M – milk yield, F – milk fat, P – milk proteins, F/P ratio – milk fat to milk protein ratio, L – lactose, SC – somatic cells count, U – urea min per day in this interval. In contrast, in cows with lowered pH it was only 14 min and 56 s on average per day. For another comparison, the time spent in the optimal range in the case of cows with increased pH was 1 h and 16 min. The pH interval of 5.8 to 6.2 is potentially a risk for rumen environment and its bacteria. Dairy cows with lowered pH were in this interval for 6 h 33 min. Unfortunately, in cows with normal pH the time spent in this interval was 6 h and 58 min. Under the pH of 5.8 the growth of cellulolytic bacteria is inhibited. Dairy cows with lowered pH spent on average 17 h and 11 min daily under this threshold. For comparison, cows with normal pH spent in this time on average only 1 h and 3 min. Finally, pH frequencies over 6.8 in cows with normal and increased pH were detected as follows: cows with normal pH only 1 h and 9 min and cows with increased pH 22 h and 43 min.

Changes in rumen pH lead to changes in the milk production and milk content (Table 6). In cows with lowered pH, a lower daily milk production by 6.80% ($P < 0.05$) was found in comparison with cows with normal pH. Moreover, cows with increased pH were producing daily less than 14.08% ($P < 0.05$) of milk in comparison with cows with normal pH. Furthermore, rumen pH also affected the content of milk fat. In the case of cows with lowered pH, lower milk fat by 12.87% ($P < 0.05$) was determined in comparison with normal pH cows. On the other hand, cows with increased pH had a higher fat content by 2.54% in comparison with normal pH cows. Moreover, the content of milk proteins in cows with lowered pH in comparison with normal pH cows was lower by 4.74%. On the contrary, in cows with increased pH, higher milk protein content by 5.13% ($P < 0.05$) was detected. Next, narrower fat to protein ratio in cows with lowered and increased pH was found. In the case of cows with lowered pH it was narrower by 7.53% ($P < 0.05$) and increased pH by 2.59%. Afterwards, the lactose content in the milk of cows with lowered (-1.16%) and increased pH (-2.07%) in comparison with normal pH cows was lower. On the contrary, the number of somatic cells in cows with lowered pH increased by 548.37% in comparison with cows with normal pH. On the other hand, in cows with increased pH the number of somatic cells was lower by 79.69%. Finally, differences between groups in the urea content were found. In cows with lowered (9.52%) and increased pH (8.90%), higher concentration of urea was determined in comparison with normal pH cows.

Discussion

Similar circadian changes in pH values were found by Kimura et al. (2012). Average rumen pH (6.82) decreased after the morning feeding and hit a low 11 h later (6.46) and hit a peak after pH recovery by the next morning (6.91). From the results listed in their research, the average decrease of pH 5 h after feeding by 1.17% and recovery of pH before feeding 0.88% can be calculated. During the first 3 h after feeding, a similar drop in pH development was found in all dairy cows (Křížová et al. 2011). From their results, the fact that on average rumen pH decreased 5 h after feeding by 1.56% (from 6.4 to 5.9) and increased by 1.51% (from 6.10 to 6.60) 5 hours before feeding can be calculated. The best range of rumen pH for rumen bacteria is between 6.2 and 7.0 (Barber et al. 2010). Luan et al. (2016) found the mean pH in the rumen within 6.24 to 6.45 according to different grain challenges with an average decrease of 1.42% to 2.00% after feeding. Furthermore, the lowest pH of 5.28 to 5.59 and the highest of 6.69 to 6.95 were found (Maulfair et al. 2013). Similar results were found by Mottram (2015). An interval of measured pH from 5.32 to 7.25 was found. An average time under 6.1 during lactation from 1 h and 13 min to 5 h 42 min and under 5.8 from 18 min to 1 h and 24 min was found (Hasunuma et al. 2016). Luan et al. (2016) determined the time spent under 5.8 from 2 h daily to 4 h and 19 min daily. Moreover, depending on the lactation number, dairy cows spent from 5 h and 24 min to 6 h and 48 min under the threshold of 5.8 (Bowman et al. 2003). Danscher et al. (2015) found milk yield depression and milk fat decrease from 5.08% to

4.14% in the group of dairy cows with lowered pH. Furthermore, in their study, the fat to protein ratio was narrower in cows with lowered pH (1.37 vs. 1.21) and a higher milk protein content was found in the control group (3.65% vs. 3.45%). In the study of Sulzberger et al. (2016) lower milk yield (27.72 $kg\cdot d^{-1}$ vs. 30.58 $kg\cdot d^{-1}$), milk protein content (3.05% vs. 3.01%) and number of somatic cells (633.47 $1000\cdot ml^{-1}$ vs 675.78 $1000\cdot ml^{-1}$) was determined in the group with low daily pH. In contrast, lower milk fat content (3.86% vs. 3.63%), lactose concentration (4.66% vs. 4.46%) and urea concentration (12.43 $mg\cdot 100\ ml^{-1}$ vs. 11.69 $mg\cdot 100\ ml^{-1}$) were found in the control group. Krause and Oetzel (2005) found a drop from 35.2 $kg\cdot d^{-1}$ to 31.7 $kg\cdot d^{-1}$ in the milk production during the SARA challenge. On the other hand, higher milk fat (3.73% vs. 4.29%) and milk protein content (2.86% vs. 2.95%) were determined. In the study of De Roos et al. (2007) ketosis was detected by ketone bodies in milk. In cows with increased pH, lower milk yield (36.6 $kg\cdot d^{-1}$ vs. 39.9 $kg\cdot d^{-1}$), milk protein percentage (3.17% vs. 3.30%), lactose percentage (4.44% vs 4.64%), urea concentration (18.0 $mg\cdot 100\ ml^{-1}$ vs. 22.4 $mg\cdot 100\ ml^{-1}$) and higher content of milk fat (4.49% vs. 5.90%) were found.

To conclude, these results showed that dairy cows under the same feeding regime had different pH values during the whole lactation. Thus, only 2 cows had ruminal pH within the normal pH interval on test days. Cows with lowered pH had a faster decrease of rumen pH in comparison with normal pH and increased pH cows 5 h after morning feeding. However, in the cows with lowered pH, better recovery ability of pH during 5 h before feeding was found. Afterwards, in dairy cows with lowered and increased pH group, lower milk production, narrower fat to protein ratio and lactose content in comparison with normal pH cows was found. Finally, in cows with lowered pH the lowest concentration of milk fat but the highest count of somatic cells and urea were determined. These results show that continuous monitoring of rumen environment is a suitable method for nutrition and health management in dairy herds, which can help in the avoidance of financial losses caused by metabolic diseases. Furthermore, on the basis of rumen monitoring, it is possible to make nutritional interventions, which can lead to the stabilization of rumen pH into optimal interval, correlating the relationship with milk quality and its production.

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