Rumination behavior as a predictor for subacute ruminal acidosis (SARA)?

The effect of ruminal pH on rumination behavior

S. R. van der Wier
3753115

17 juni 2018

Supervisor
Drs. R. M. A. Goselink
Wageningen University & Research

Abstract

Objective – The aim of this study was to investigate the effect of ruminal pH on the cows' rumination behavior, in order explore if rumination behavior could be used as a tool to predict subacute ruminal acidosis (SARA).

Animals - 4 multiparous rumen-cannulated Holstein Friesian dairy cows

Procedures – Cows were divided into two groups and the experiment was carried out as a cross-over design, containing two periods of 4 weeks. In weeks 1, 2, 5 and 6 cows were fed the control ration (CON) and in weeks 3 and 7 there was a transition from the control ration to the experimental ration. During the fourth and the last week the cows were fed the experimental ration (SPK of FOR). The SPK ration contains a rapidly fermentable carbohydrate as concentrate and in the FOR ration the total part of forage is reduced. Ruminal pH, DMI and rumination behavior were measured continuously, except for the first and the fifth week.

Results – SARA was induced for more days when feeding the FOR ration compared to feeding the SPK ration. Feeding the FOR ration resulted in a higher DMI (23.0 vs 19.8 kg/day), a lower mean ruminal pH (5.86 vs 6.24) and a longer period of experiencing a ruminal pH lower than 6.0 (926 vs 301 minutes/day) and 5.8 (625 vs 169 minutes/day) compared to the SPK ration ($P<0.05$). However, rumination time (490 vs 503 minutes/day), rumination time per kg DMI (20.3 vs 25.7 minutes) and experiencing a ruminal pH lower than 5.5 (235 vs 77 minutes/day) did not differ significantly between the FOR and the SPK ration ($P>0.05$). The overall correlations between rumination time and mean ruminal pH, time ruminal pH was below 5.8 and 5.5 were low (R^2=0.17, R^2=0.17 and R^2=0.39 respectively). However, the FOR ration had higher correlations compared to the SPK ration (R^2=0.68; R^2=0.74; R^2=0.69 and R^2=0.37; R^2=0.32; R^2=0.27 respectively). All correlations were significant ($P<0.05$).

Contents

Introduction

Sub-acute ruminal acidosis (SARA) is defined as experiencing a status of non-physiological pH values in the rumen for several hours a day (Kleen et al, 2012; Oetzel, 2017). A more specific definition of SARA that can be used is experiencing a ruminal pH below 5.6 for at least 3 hours a day (Plaizier et al, 2008; Oetzel, 2017)). In ruminants, volatile fatty acids (VFA) are produced in the rumen by the fermentation of roughage and concentrates. To maintain a physiological ruminal pH, these VFA need to be absorbed by the ruminal wall, pass through the rumen or need to be buffered (Dijkstra et al, 2012; Plaizier et al, 2008). However, rations have effect on this process. Feeding more highly digestible carbohydrates will increase the production of VFA in the rumen. Feeding a decreased amount of fiber will reduce the time spent on rumination, thereby less rumination activity will reduce the production of saliva, which contains inorganic buffers. Consequently, rumination behavior may also contribute to the process of subacute ruminal acidosis. SARA is mostly caused by an unbalanced ration, which contains a high level of concentrates with rapidly fermentable carbohydrates or less structural fiber or both (Dijkstra et al, 2012; Plaizier et al, 2008).

Several field studies in Europe have investigated the prevalence of SARA in dairy herds. The prevalence ranges from 11% in Irish dairy herds to 33% in Italian dairy herds (O'Grady et al, 2008; Morgante et al, 2007). In 2004 the prevalence of SARA was investigated by a field study in the Netherlands. This study included 197 cows on 18 farms. The prevalence of SARA, which was defined as experiencing a ruminal pH of 5.5 or lower, was almost 14% over all cows. However, ruminal pH was measured only once, in ruminal fluid that was taken by rumenocentesis. The prevalence within the herd ranged between 8% and 38%, however, only 10 of 18 farms had at least one cow experiencing SARA (Kleen, 2004). These prevalences show that SARA is present on a great part of the dairy farms.

Signs of SARA include milk fat depression, reduced DMI, rumenitis, liver abscesses, diarrhea and impaired immune function. These signs will be discussed in the next section.
One of the consequences of a decreased rumen pH is a shift of the composition of volatile fatty acids (VFA) in the rumen, which may be affected by the rumen flora. In response to the changes in ruminal pH, the bacteria in the rumen shift their pathway in the production of VFA. In case of a decreased ruminal pH, the bacteria reduce the synthesis of acetic acid and increase propionic and butyric acid. A decreased rumen pH therefore has a negative effect on the acetate to propionate ratio. In general, propionic acid is the most important substrate for glucose and therefore an important source of energy for the production of milk. Acetic acid on its turn influences the fat content of milk, more specifically, high levels of acetate results in high levels of milk fat. SARA therefore results in a decreasing fat content in milk (Dijkstra et al, 2012; Plaizier et al, 2008).

A reduced dry matter intake (DMI) is commonly seen in SARA. Factors that affect DMI, due to SARA, include decreased ruminal motility (Kleen et al, 2003; Oetzel et al, 2017) and increased osmotic pressure because of increased VFA and lactic acid concentrations (Allen, 2000). These factors are self-limiting and cause a fluctuating feed intake. On the long term, the low ruminal pH also reduces the fibrolytic bacteria in the rumen and therefore reduces the fiber digestibility in the rumen. Due to the reduced fiber digestibility of the rumen, the total amount of feed intake and the ration efficiency both decrease. Consequently, the uptake of energy and nutrients from the ration are reduced and the cows are more susceptible for a negative energy balance (NEB). The reduced energy and nutrient intake also affect the body condition score negatively (Plaizier et al, 2008; Kleen et al, 2012; Oetzel, 2017).

Diarrhea is commonly seen in cows experiencing SARA. In cows with SARA there is an excessive fermentation in the large intestine, leading to hindgut acidosis (Nordlund et al, 2004; Plaizier et al, 2008; Gressley et al, 2011). This excessive fermentation, related to an increased amount of substrate in the large intestine, is due to the reduced digestibility in the rumen and the increased flow of substrates. By feeding rations with low forage and high concentrates, there is an increased flow of fermentable substrates to the hindgut. This increased flow of fermentable substrate is due to small particle size of the ration and the failure of the rumen to retain these substrates (Gressley et al, 2011) By the hindgut fermentation VFAs are produced, which can be absorbed by the large intestine. However, microbial protein and/or protein deriving from the ration cannot be absorbed, leading to a hypertonic environment in the intestine. Due to the excessive fermentation and an increase of organic acids, the large intestine is becoming more acidic, which affects the mucosal layer and its function. Taken together both the hypertonicity and the impaired mucosa function, there is an increased movement of water into the lumen of the large intestine, resulting in diarrhea probably with mucin and fibrin casts (Plaizier et al, 2008; Oetzel, 2017).

A low ruminal pH may also cause a high level of lipopolysaccharides (LPS) in the rumen (Gozho et al, 2005). LPS is a component of the cell wall from gram negative bacteria. During a period of low ruminal pH, these bacteria can die and release their endotoxins (Oetzel et al, 2017). A decreased ruminal pH and an increased level of LPS in the rumen, due to feeding a high concentrate ration, also contribute to a local inflammation of the rumen epithelium. The local inflammation thereby affects the barrier function of the rumen epithelium (Zhang et al, 2016). Inflammation of the ruminal wall, also called rumenitis, affects the barrier function and allows bacteria in the rumen to translocate to the portal blood and reach the liver. In the liver these bacteria, especially *Fusobacterium necrophorum* and *Trueperella pyogenes*, may cause the formation of an abscess. It is also seen that these bacteria can spread and cause abscesses in other organs (Oetzel et al, 2017; Plaizier et al, 2008). This process has also been described as the parakeratosis-rumenitis-liver abscess complex, which is initiated by a low ruminal pH (Kleen et al, 2003; Plaizier et al, 2012).
The increased levels of LPS in the digestive tract are associated with general inflammation and increasing levels of acute phase proteins in blood serum, such as serum amyloid A and haptoglobin (Gozho et al, 2005; Plaizier et al, 2008). During a grain based ration to induce SARA there is an increased level of LPS in the rumen, caecum and faeces. When SARA is induced by alfalfa pellets, LPS is only increased in the rumen. Moreover, an acute phase response is only seen by a grain induced SARA. This finding suggests that not only the ruminal pH and LPS induce an acute phase response. By a grain induced SARA there is a high acidity of digesta in the large intestine, which affects the mucosal barrier and facilitates the translocation of LPS into the blood where it activates the immune system (Plaizier et al, 2012). Due to this chronic inflammation caused by SARA and the reduced feed intake, it is logical that SARA impairs the cows' immune system and therefore the cow is more susceptible to other diseases. Moreover, laminitis is also frequently seen during SARA and according to some researchers, this may be related to LPS. However, this effect is not proven (Oetzel et al, 2017).

Economic losses due to SARA include decreased milk production, decreased feed efficiency, increased culling and other costs due to the impaired immune system (Enemark, 2008). The economic impact of SARA was estimated at 1.12 US dollars, which is approximately 1 euro, per cow per day (Stone, 1999).

The above-mentioned prevalences and consequences show that SARA is a major problem in the dairy industry in western Europe and it is obvious that SARA has negative effects on the individual welfare, health and production conditions of dairy cattle. To improve animal welfare, health, production conditions and reduce economic losses, an early detection of SARA is of great importance. By early signs of SARA, the feeding management could be adapted to avoid longer periods of low ruminal pH. From a practical point of view, tools are needed that can easily be used on farm by the farmer and can provide daily information.

The definition of SARA is based on the continuous measurement of the ruminal pH. However, rumenocentesis and other methods to collect ruminal fluid are very invasive for the animals and therefore not practical on commercial dairy farms. An indwelling pH logger can measure ruminal pH continuously, however, due to the inability for recalibration and high costs this sensor is not useful as a standard tool (Enemark, 2008; Humer et al, 2018).
Other tools that can indicate signs of SARA include measurements on milk, blood and faeces. Measurements on milk can include fat content, whereas measurement in blood include acute phase proteins. For measuring faeces it is possible to look at particle size and pH. However further research is required to establish thresholds. Moreover, due to the limited specificity of these tools the measurement of more than one of these tools is recommended (Humer et al, 2018). Therefore, in combination with the labor required for these tools it is not useful on common dairy farms to provide daily information.

Feeding behavior is one of the parameters that could predict SARA, since cows with SARA have fluctuating feeding patterns (Enemark, 2008; Humer et al, 2018). However, on commercial dairy farms the individual DMI and feeding behavior is not recorded.

Aim of the study

In modern dairy farms, the use of sensors is upcoming. Sensors can also be used for the continuous monitoring of rumination behavior (Schirmann et al, 2009). Rumination behavior therefore, could be an excellent tool in the early detection of SARA on farm. In literature, rumination behavior is mainly expressed by minutes per kilogram of DMI However, there is a difference in total rumination time and rumination time per kg DMI. To use rumination behavior as a tool to predict SARA, the ruminal pH must correlate with rumination behavior. In case of SARA, the correlation between total time spent on ruminating and ruminal pH has not extensively been investigated (Humer et al, 2018). Therefore, the aim of this study was to investigate the effect of the ruminal pH on the cows' rumination behavior in order to explore if rumination behavior could be used as a tool to predict SARA. It was hypothesized that a reduced ruminal pH also reduced the cows' rumination behavior.

Material and Methods

Animals and housing

This study was carried out at the Dairy Campus in Lelystad, the Netherlands. All experimental protocols and interventions were approved by the Ethics Committee on Animal Experiments of the Animal Sciences Group of Wageningen University and Research Centre, the Netherlands. In this study, 4 multiparous rumen-cannulated Holstein Friesian cows were divided into two groups of 2 animals. One cow was available as a substitute in the case that one of the cows ruled out of the trial. The cows had a parity of at least 3 and were between 80 and 267 days in milk (DIM), information of the animals is summarized in table 1. Cows were housed in one group in a free stall barn and had free access to drinking water. Feed is provided *ad libitum* as a total mixed ration (TMR) and is provided in RIC bins (Roughage Intake Control, Hokofarm Group, Marknesse, the Netherlands), automatically recording individual feed intake. The cows were milked twice daily at set times, between 5 and 6 AM and between 4 and 5 PM.

Table 1. Characteristics of the rumen-cannulated cows enrolled in the trial

Group	Cow	Parity	Days in milk at start
1	5349	5	80
1	6014	3	243
2	5347	5	135
2	6175	3	185
substitute	5961	3	267

Experimental design

The experiment was carried out as cross-over study for a total period of 8 weeks. Every experimental week started on Friday (day 1). During the trial each group is exposed to one of two dietary treatments. Both treatments start with the control ration (CON) for a period of two weeks followed by the experimental ration (SPK or FOR) for a period of two weeks. During the fifth and the sixth week of the trial, animals received the CON ration for a second time. In the last two weeks of the trial, animals received the remaining experimental ration (SPK or FOR). The experimental design is summarized in table 2.

Table 2. Experimental design of the trial

Experimental week	Notification	Group 1	Group 2
1	Control diet	CON	CON
2	Control diet	CON	CON
3	Changing diet	CON > SPK	CON > FOR
4	Experimental diet	SPK	FOR
5	Control diet	CON	CON
6	Control diet	CON	CON
7	Changing diet	CON > FOR	CON > SPK
8	Experimental diet	FOR	SPK

CON = control ration; SPK = treatment ration with different concentrate composition; FOR = treatment ration with different roughage to concentrate ratio

Rations

During the experiment rations are fed as a total mixed ration (TMR) containing forage and concentrates. To provide a constant ration, a mixture of grass silage and maize silage was made and wrapped in bales before starting the trial. This mixed silage contains 50% grass and 50% maize, based on dry matter. The control diet is based on almost 60% dry matter of mixed silage completed with 40% of concentrates, composed as a "save" ration and common used on Dutch dairy farms in practice and therefore used as the adaptation ration in this trial. The SPK ration is almost the same as the control ration, however, a part of the normal concentrates (3.9 kilograms per cow) is replaced for a concentrate with rapidly fermentable carbohydrates. In the FOR ration the total amount of concentrates is increased, whereby the proportion of forage in the ration is reduced. In the FOR ration, the proportion of mixed silage is decreased to 35.2% of dry matter. The composition of the rations on dry matter base is described in table 3 During the third and seventh week of the trial, the transition from the control ration to the experimental rations was carried out in four stages. However, transition from the experimental ration to the control ration in week 5 of the trial was carried out directly.

Table 3. Relative ration composition on a dry matter base (%) and feeding value of the different rations

	CON	SPK	FOR
Grass silage	29.3	29.3	17.6
Maize silage	29.3	29.3	17.6
Wheat, rape and soy bean meal (1:1:1)	13.5	13.5	8.1
Control concentrate	27.9	-	56.6
High fermentable concentrate	-	27.9	-
TOTAL	100	100	100
Net energy for lactation (VEM / kg DM)	1028	1031	1056
Intestinal digestible protein (DVE, g/kg DM)	97	90	109
Rumen digestible protein balance (OEB, g/kg DM)	9	-8	8

CON = control ration; SPK = treatment ration with different concentrate composition; FOR = treatment ration with different roughage to concentrate ratio; VEM = voedereenheid melkvee, 1000 VEM = 6.9 MJ

Recording rumination time

To determine the time of rumination the animals were stored with a SCR HR tag (SCR, Netanya, Israel) on a neck collar, which automatically record the duration of rumination. The sounds produced by regurgitation and rumination are recorded by a microphone in the logger, which is placed on the left side of the cow's neck. Sounds produced by regurgitation and rumination are processed and converted to output data. Output data consists of the time, in minutes, spent on rumination during a period of 2 hours. These automatically recorded data are highly correlated with visually observed rumination ($r^2 = 0,93$) (Schirmann et al, 2009).

Rumen pH registration

Rumen pH was measured continuously by a DASCOR pH logger in the rumen and the ruminal pH was registered every two minutes. The pH logger was placed in the rumen through the rumen fistula. During the experimental week, the pH logger was replaced twice, on the fifth and on the seventh day of the experimental week the pH logger was retrieved from the rumen and recalibrated. All data from the pH loggers was transferred to an Excel file for further calculations, which include the mean pH and total time of a pH lower than 5.5, 5.8 and 6.0 per cow per day.

Dry matter intake

Feed was provided in RIC (Roughage Intake Control) bins, which automatically recorded feed intake of the individual cow. Each cow had a responder which gave her access to a RIC bin with the assigned diet. During every meal of a cow the begin and end weight of the bin was registered and the amount of feed intake was calculated.

To calculate the dry matter intake, each of the ration components was sampled weekly and dry matter (DM) contents were determined per component, by calculating the weight difference before and after oven drying at 104°C during 36 hours. Every day the relative contribution of each of the ration components to the mixture was registered (kg product of each component mixed) and total DM content of the mixture was calculated. Dry matter intake per individual cow was calculated by multiplying dry matter content of the mixture with daily intake of fresh product.

Statistical analysis

The ruminal pH data consists of a pH value of every two minutes for every cow. To compare these data, the mean ruminal pH and the total time experiencing a ruminal pH lower than 6.0, 5.8 and 5.5 were calculated per cow per 24h. Due to the removal of the pH logger in the early morning of the last day in the experimental week, pH data of 6 days was available for statistical analysis.

The total daily feed intake was registered by the RIC bins for the individual cow. The DMI thereafter is calculated by multiplying feed intake with the percentage of dry matter of the corresponding ration. The daily individual DMI from each cow of 7 days was available for statistical analysis.

Time spent on ruminating was recorded by a SCR HR tag on the neck collar. The output data consists of time (minutes) spent on ruminating during a period of two hours. To compare these data an average time spent on ruminating during a period of two hours was calculated per 24 hours for every cow. Not all sensor registrations were successful. The degree of success was expressed in the rumination mark. A rumination mark of 100 therefore means that all registrations were successful. However, it was assumed that the rumination mark should be at least 60 to get enough reliable data. All data with a rumination mark below 60 were excluded. To calculate an average time, it was defined that there were at least ten (out of twelve) periods in 24 hours to get a reliable average. If there were fewer periods with a rumination mark of at least 60, the 24 hours average was discarded from the analysis. Due to a lack of data, there were only 5 days of data used for statistical analysis. The time spent ruminating for one kilogram of dry matter was calculated by the total time spent on ruminating and the total daily DMI. To calculate the total time spent on ruminating, the average time spent on ruminating during the period of 2 hours was used.

All obtained data were statistically analysed with a ANOVA model for repeated measures by the computer program SPSS. In this model ruminal pH, dry matter intake and rumination behavior were categorised as ‘within subjects’. Ration and time were included as ‘between subjects’. The experimental period, which was defined as the first four weeks or the second four weeks of the trial, had no significant effect on ruminal pH, DMI or rumination behavior, and was therefore released from the model.

The relationship between ruminating and the ruminal pH was analysed by the total time spend on ruminating and the mean ruminal pH and time experiencing a ruminal pH below 5.5 and 5.8. All data were pooled and analysed by the computer program SPSS using the model of linear regression.

Results

Because of illness one cow was excluded from the experiment in the fourth week and replaced for another cow. However, rumination behaviour of this cow was not recorded and data of only three cows was available. Therefore, the data of the replaced cow was excluded.

Ruminal pH

It was assumed that cows were experiencing SARA if ruminal pH was lower than 5.5 for at least 180 minutes a day. During the FOR ration two cows experienced SARA for three and five days. The SPK ration has induced SARA in one cow, however, SARA was only induced for two days.
There was an overall effect of the ration on ruminal pH. On average, the FOR ration induced a lower ruminal pH compared to the SPK ration. During the FOR ration the mean ruminal pH was 5.86, whereas during the SPK ration the mean ruminal pH was 6.24. This effect on mean ruminal pH was significant (P=0.04). During the FOR ration cows experienced on average 926 minutes a day a pH that was lower than 6.0. Whereas, during the SPK ration cows experienced only 301 minutes a day a pH lower than 6.0. This effect of ration was significant (P=0.024). There was also a significant effect of the ration on the time that cows were experiencing a ruminal pH lower than 5.8 (P=0.046). Feeding the FOR ration resulted in experiencing a longer period with a ruminal pH below 5.8 than it did for the SPK ration. Cows experienced a ruminal pH lower than 5.8 for 625 minutes and 169 minutes respectively. In case of a ruminal pH lower than 5.5 the FOR ration caused a period of 235 minutes and the SPK ration caused a period of 77 minutes with a ruminal pH lower than 5.5. However, there was no significant effect between the rations (P=0.297). The effects of ration are described in table 4.

Table 4. Effects of ration on dry matter intake, rumination behavior and ruminal pH

	Ration	Time							SEM	P-value		
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		Ration	Time	T x R
DMI	FOR	22.9	24.6	25.1	24.3	22.3	24.2	23.4	0.851	0.028	0.068	0.015
	SPK	19.1	17.7	21.7	19.6	19.0	20.4	21.0				
RB	FOR	-	42.3	41.2	36.4	42.2	41.7	-	5.438	0.887	0.342	0.298
	SPK	-	42.5	40.4	39.4	43.5	43.8	-				
RB/DMI	FOR	-	20.6	19.9	18.0	22.7	20.7	-	2.473	0.204	0.681	0.412
	SPK	-	28.8	22.4	24.2	27.5	25.8	-				
pH mean	FOR	5.90	5.84	5.86	5.87	5.83	5.87	-	0.089	0.040	0.814	0.986
	SPK	6.30	6.31	6.15	6.13	6.24	6.31	-				
pH < 6.0	FOR	867	1021	956	820	989	901	-	124.9	0.024	0.812	0.856
	SPK	321	151	431	485	247	168	-				
pH < 5.8	FOR	570	669	633	613	637	629	-	112.8	0.046	0.959	0.847
	SPK	136	60	257	321	135	65	-				
pH < 5.5	FOR	290	239	215	252	226	189	-	93.15	0.297	0.634	0.503
	SPK	9	28	169	191	66	0	-				

SPK = treatment ration with different concentrate composition; FOR = treatment ration with different roughage to concentrate ratio; DMI = dry matter intake in kilograms per 24 hours; RB = rumination behavior in minutes per 2 hours; RB/DMI = ruminating per kilogram dry matter in minutes; pH mean = average pH during 24 hours; pH < 6.0, 5.8, 5.5 = time pH was lower than 6.0, 5.8 and 5.5 in minutes per 24 hours

Dry matter intake

The total daily DMI was significantly higher for the FOR ration compared to the SPK ration (P=0.028). Feeding the FOR ration resulted in a DMI of 23.8 kilograms per day, whereas the SPK ration resulted in a DMI of 19.8 kilograms per day. For the SPK ration there was also a significant effect of time for DMI (P<0.05). During the week the daily DMI increased for the SPK ration, whereas it did not for the FOR ration. The effects of ration are described in table 4.

Rumination behavior

Time spent on ruminating within a period of 2 hours did not differ significantly between the FOR and SPK ration (P=0.887). On average, cows were spending 40.8 minutes and 41.9 minutes ruminating per 2 hours respectively. During the FOR ration total rumination time was 490 minutes and during the SPK ration the tot rumination time was 503 minutes per day. For the SPK ration cows were ruminating more minutes per kilogram of dry matter compared to the FOR ration, 25.7 and 20.3 minutes respectively. However, this effect was not significant (P=0.204). The effects of ration are described in table 4.

Correlation between ruminal pH and rumination time

To investigate the relation between ruminal pH and rumination behavior, the total time spent on ruminating was plotted against data from ruminal pH. Thereafter the correlation between rumination time and the ruminal pH was calculated. Three indicators of ruminal pH were used in these calculations. The mean ruminal pH during 24 hours and the total time of a ruminal pH below 5.8 and 5.5. On average, these data showed that cows were more ruminating when ruminal pH decreased. The relationship between the mean ruminal pH during the day and the total time spent on ruminating was slightly negatively correlated (R^2=0.17, P=0.022), see figure 1.

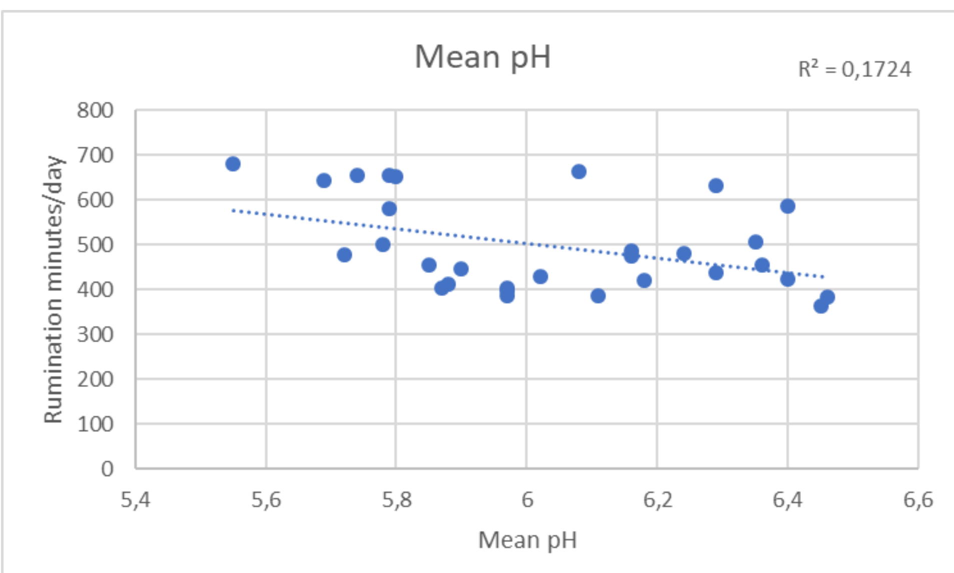

Figure 1. Linear regression model describing the relationship between mean ruminal pH (mean pH/day) and ruminating (min/day) (P=0.022)

The time of experiencing a ruminal pH lower than 5.5 and 5.8 had a slightly positive effect on the cows' rumination behavior. When cows were experiencing a longer period of a pH below 5.5 or 5.8, they were ruminating more. However, the correlation for time below 5.5 was stronger than time below 5.8: R^2=0.39 (P<0.01) and R^2=0.19 (P=0.012) respectively. See figures 2 and 3.

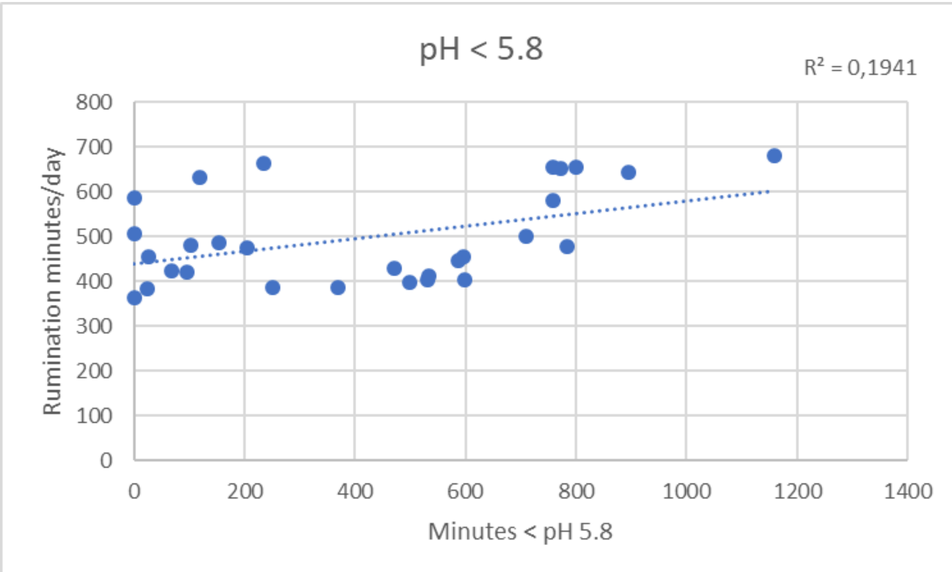

Figure 2. Linear regression model describing the relationship between experiencing a ruminal pH below 5.8(min/day) and rumination behavior (min/day) (P<0.001)

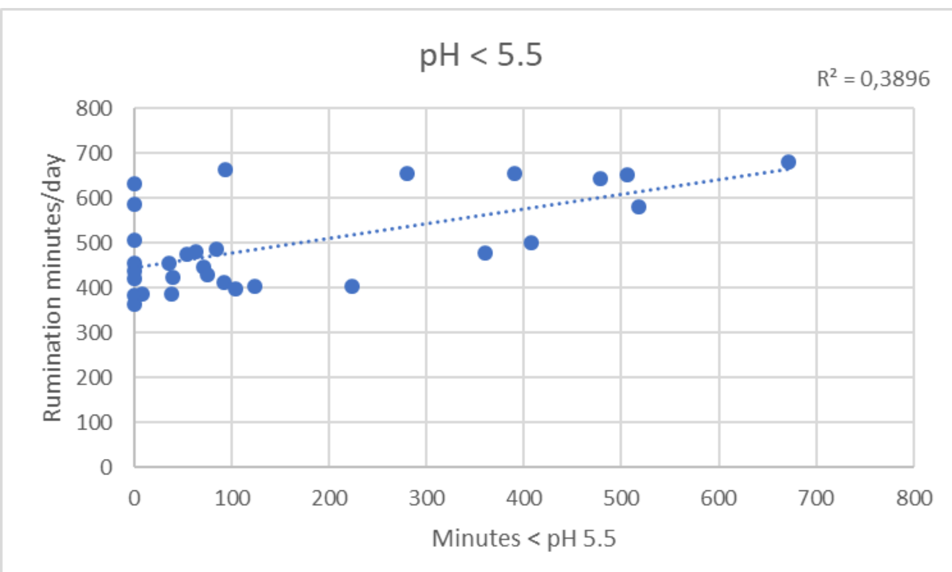

Figure 3. Linear regression model describing the relationship between experiencing a ruminal pH below 5.5 (min/day) and rumination behavior (min/day) (P=0.012)

There was also an effect of the ration on the correlations between ruminal pH and total time spent on ruminating. The FOR ration had a stronger correlation compared to the SPK ration. These data are summarized in table 5.

Table 5. Relationship ruminating and ruminal pH for the different rations

Ration	Ruminating	pH indicator	Correlation (R^2)	Line Coefficient	P-value
FOR	Minutes per day	Mean pH	0.68	-627x	<0.001
FOR	Minutes per day	pH <5.8	0.74	0.43x	<0.001
FOR	Minutes per day	pH <5.5	0.69	0.46x	<0.001
SPK	Minutes per day	Mean pH	0.37	-284x	0.015
SPK	Minutes per day	pH <5.8	0.32	0.22x	0.049
SPK	Minutes per day	pH <5.5	0.27	0.29x	0.028

SPK = treatment ration with different concentrate composition; FOR = treatment ration with different roughage to concentrate ratio; pH < 5.8 and 5.5 = time pH was lower than 5.8 and 5.5 in minutes per 24 hours; R^2 = correlation between ruminating and pH indicator; - = negative correlation; + positive correlation

Discussion

Correlation between ruminal pH and rumination time

The aim of this experiment was to investigate if rumination time could be used as a tool to predict SARA in dairy cows. The hypothesis therefore was that rumination time decreases when ruminal pH decreases. However, the results of the current study do not support the hypothesis of this study. The results of this study suggest that rumination time increased when the mean ruminal pH decreased, however, this correlation was very weak R^2=0.17 (P=0.022). This study showed also a difference in the correlation between ruminal pH and rumination time for the FOR and SPK ration. In case of the FOR ration, the correlations were higher compared to the SPK ration. The positive correlation between rumination time and time below pH 5.8 was high (R^2=0.74) for the FOR ration. The time spend on ruminating increased when the total time of a ruminal pH below 5.8 increased.

These findings agree with those of DeVries et al, who found a strong positive correlation (R^2=0.91) between rumination time and the total time ruminal pH was below 5.8. The longer the period of ruminal pH below 5.8, the longer the cows were ruminating. In this trial 4 cows were fed as high-risk cows with a TMR containing a forage to concentrate ratio of 45:55. The cows had an acidosis challenge in each of the three experimental periods of 14 days. The TMR was fed ad libitum all days, except for the fourth and the fifth day. On the fourth day the feed was restricted and on the fifth day the cows were challenged with 4 kg ground barley and wheat in de morning. After the challenge TMR was fed again during the rest of the period. The time ruminal pH <5.8 (hours per day) and the total time spent on ruminating (minutes per day) on the day after challenge were measured and averaged across the three periods per cow. These data were analyzed by a linear regression model. There was a high positive correlation R^2=0.91 (P = 0.045) (DeVries et al, 2009).

This finding may be explained by an effect of the ruminal pH on the fiber digestibility in the rumen. Fibrolytic bacteria in the rumen are sensitive to an acidic environment, which means that they can hardly adapt to a decreasing environmental pH. A ruminal pH below 5.8 is harmful to these bacteria and therefore a ruminal pH below 5.8 reduces their capacity of fiber digestion (Russell and Wilson, 1996; Dijkstra et al, 2012). A study by Krajcarski-Hunt et al showed that the fiber digestibility could be reduced with more than 20% within a few days of experiencing SARA (Krajcarski-Hunt et al, 2002). The digestibility of fiber may contribute to rumination behavior, however, the effects of fiber digestibility on rumination are not consistent in literature (Zebeli et al, 2012). Taylor and Allen reported that a ration with a high fiber digestibility may be less effective in stimulating rumination behavior (Taylor and Allen, 2005).

The difference in positive correlation between the FOR (R^2=0.74) and SPK (R^2=0.32) ration may be explained by the time that ruminal pH was lower than 5.8, which was significantly lower for the SPK ration compared to the FOR ration (P=0.046). In case of the FOR ration it may be acceptable that cellulolytic bacteria were more affected by the periods where ruminal pH was below 5.8 compared to the SPK ration. Because the cellulolytic bacteria in the rumen were probably less affected by the SPK ration, the fiber digestibility was less affected. The reduced fiber digestibility in the rumen therefore may have increased rumination behavior for the FOR ration. As a result, rumination time increased when ruminal pH below 5.8 increased during the FOR ration and not for the SPK ration.

A shortcoming in the analysis of the correlation between ruminal pH and rumination time that should be discussed is the statistical analysis. The data in this study were pooled and analyzed by a linear regression model. However, this model does not take the repeated measurement of the cows into account and therefore all the regression models were significant. To take these repeated measurements into account for the FOR ration, the data of the ruminal pH below 5.8 and rumination time were averaged per cow across the 5 experimental days. These data were analyzed again by SPSS in a linear regression model. This model showed also a high positive correlation (R^2=0.94) between time of ruminal pH was below 5.8 and rumination time, however, this regression model was not significant (P=0.158), see figure 4. These results suggest that the above mentions correlations were a good indicator, but they may be not significant when repeated measurements are considered.

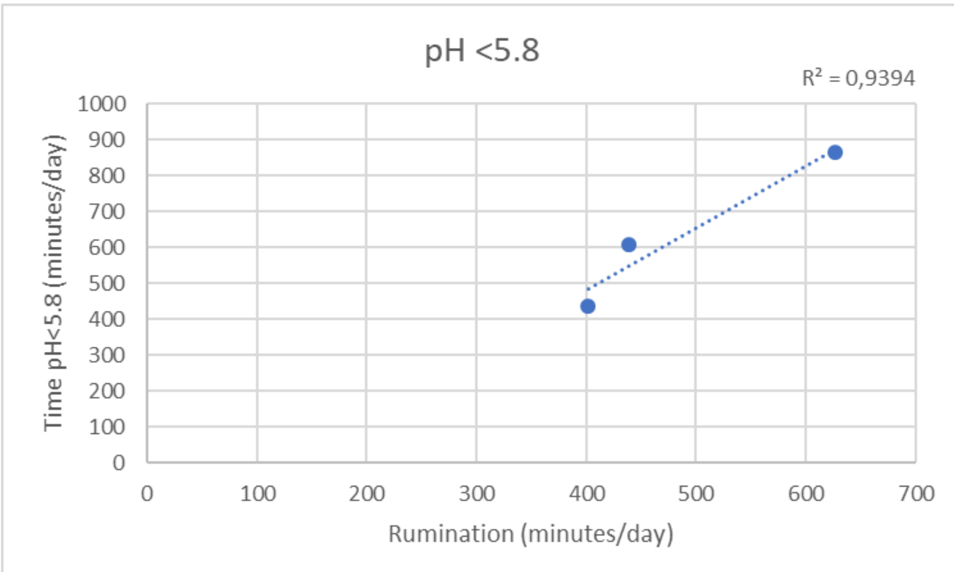

Figure 4. Relationship between experiencing a ruminal pH below 5.8 (min/day) and rumination behavior (min/day) of the averaged data per cow across the experimental days for the FOR ration (P=0.158)

Rumination behavior

There was no significant difference in rumination behavior between the SPK and the FOR ration. However, the FOR ration had a decreased forage content compared to the SPK ration and therefore it was assumed that during the SPK ration, cows should spend more time ruminating compared to the FOR ration. It was proven that an increasing forage to concentrate ratio increases the amount of chewing (Yang and Beauchemin, 2007). Maekawa et al increased the amount of forage in their TMR and concluded that ruminating increased when the content of forage increased (Maekawa et al, 2002). The findings of the current study do not agree with those of Maekawa et al. However, this study showed an increased DMI when decreasing the forage to concentrate ration in FOR ration. Maeakawa et al did not find this difference in DMI between the different forage contents in the ration. This difference in DMI therefore may explain why total rumination time was not decreased in the FOR ration compared to the SPK ration.

Rumination time per kilogram of dry matter differed numerically between rations. During the SPK ration cows were ruminating 25.7 minutes for one kilogram of dry matter, whereas during the FOR ration cows were ruminating 20.3 minutes per kilogram of dry matter. However, this effect was not significant (P=0.204). The same effect was observed by studies of Jiang et al and Kröger et al who found a decreased time of ruminating per kg of DMI when the forage to concentrate ratio decreased (Jiang et al, 2017; Kröger et al, 2017). This effect may be due to the

increased forage content per kg of dry matter, which increases rumination time (Maekawa et al, 2002)
The measured rumination behavior may be affected by the rumination recording system. An increased roughage content can be associated with an increased eating time (Jiang et al, 2017). If the SCR HR tag (SCR, Netanya, Israel) does not distinguish between eating and ruminating, ruminating may be increased due to an increased eating time. However, the SCR HR tag claims to record only rumination time (Schirmann et al, 2009).

Dry matter intake

Feeding the FOR ration resulted in a higher DMI compared to the SPK ration. Because the FOR ration contained a higher concentrate content, this effect was expected. It is proven that decreasing the roughage to concentrate ration of the ration increased the DMI for the ration (Allen, 2000). Under normal conditions, the DMI is limited by the fill of the rumen and the amount of NDF. When the content of NDF in the ration is reduced, the DMI will increase. DMI will increase until rumen fill is no longer the limiting factor and an excess of metabolic products will limit the DMI (Allen, 2000). Hence, decreasing the forage to concentrate ratio of the ration will increase the DMI (Allen, 2000). Therefore, the findings of the current study are in agreement with literature.
One of the signs of SARA include a fluctuating or reduced DMI. In this study, SARA was more often induced in the FOR ration compared to the SPK ration. However, the FOR ration had a higher DMI compared to the SPK ration. This effect is not in agreement with the expected signs of SARA. This effect may be explained by the difference in forage to concentrate ratio between the FOR and SPK rations. The lower forage to concentrate ratio in the FOR ration can explain the higher DMI compared to the SPK ration, as mentioned above (Allen, 2000). The difference in ration may help explain the findings of dry matter intake.

Conclusion, critical note and recommendations for further research

To counteract SARA, practical on farm tools are needed to predict SARA. Since rumination behavior can easily be measured on farm by a sensor on a neck collar, rumination behavior could be a usable tool. This study hypothesized that a reduced ruminal pH also reduces rumination behavior. However, this study was not able to proof this hypothesis.
This study showed a significant difference in ruminal pH parameters between the SPK and FOR ration. However, there was no significant difference between total rumination time and rumination per kg of DMI.
The overall correlations between ruminal pH and rumination time were low. These correlations suggest that rumination time increases when the time of a ruminal pH below 5.8 increases. The correlation between time of a ruminal pH below 5.8 and rumination time for the FOR ration was high, but was not significant when repeated measurements were considered.
The fact that relations between ruminal pH and rumination behavior are not significant may be due to the size of the study. Data was only available for three cows during one week for every ration. Therefore, the individual cow effect may affect the outcome of the study. More research is required to investigate the relation between ruminal pH and rumination behavior. A follow-up study should focus on the effect of ruminal pH on total rumination time and rumination time per kg DMI. Furthermore, the next study should use also more animals, to minimize the individual cow effects of the results in the study.

References

Allen, M. S. (2000). Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *Journal of Dairy Science*, *83*(7), 1598-1624.

DeVries, T. J., Beauchemin, K. A., Dohme, F., & Schwartzkopf-Genswein, K. S. (2009). Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feeding, ruminating, and lying behavior. *Journal of dairy science*, *92*(10), 5067-5078.

Dijkstra, J., Ellis, J. L., Kebreab, E., Strathe, A. B., López, S., France, J., & Bannink, A. (2012). Ruminal pH regulation and nutritional consequences of low pH. *Animal Feed Science and Technology*, *172*(1-2), 22-33.

Enemark, J. M. (2008). The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *The Veterinary Journal*, *176*(1), 32-43.

Gozho, G. N., Plaizier, J. C., Krause, D. O., Kennedy, A. D., & Wittenberg, K. M. (2005). Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *Journal of Dairy Science, 88*(4), 1399-1403.

Gressley, T. F., Hall, M. B., & Armentano, L. E. (2011). RUMINANT NUTRITION SYMPOSIUM: Productivity, digestion, and health responses to hindgut acidosis in ruminants1. *Journal of animal science*, *89*(4), 1120-1130.

Humer, E., Aschenbach, J. R., Neubauer, V., Kröger, I., Khiaosa- Ard, R., Baumgartner, W., & Zebeli, Q. (2018). Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. *Journal of animal physiology and animal nutrition*, *102*(2), 380-392.

Jiang, F. G., Lin, X. Y., Yan, Z. G., Hu, Z. Y., Liu, G. M., Sun, Y. D., ... & Wang, Z. H. (2017). Effect of dietary roughage level on chewing activity, ruminal pH, and saliva secretion in lactating Holstein cows. *Journal of dairy science, 100*(4), 2660-2671.

Kleen, J. L., Hooijer, G. A., Rehage, J., & Noordhuizen, J. P. T. M. (2003). Subacute ruminal acidosis (SARA): a review. *Transboundary and Emerging Diseases, 50*(8), 406-414.

Kleen, J. L. (2004). Prevalence of subacute ruminal acidosis in Dutch dairy herds–A field study. *Inaugural Dissertation, Tierärztliche Hochschule. Hannover*.

Kleen, J. L., & Cannizzo, C. (2012). Incidence, prevalence and impact of SARA in dairy herds. *Animal Feed Science and Technology*, *172*(1-2), 4-8.

Krajcarski-Hunt, H., Plaizier, J. C., Walton, J. P., Spratt, R., & McBride, B. W. (2002). Effect of subacute ruminal acidosis on in situ fiber digestion in lactating dairy cows. *Journal of dairy science*, *85*(3), 570-573.

Kröger, I., Humer, E., Neubauer, V., Reisinger, N., Aditya, S., & Zebeli, Q. (2017). Modulation of chewing behavior and reticular pH in nonlactating cows challenged with concentrate-rich diets supplemented with phytogenic compounds and autolyzed yeast. *Journal of dairy science*, *100*

Maekawa, M., Beauchemin, K. A., & Christensen, D. A. (2002). Effect of Concentrate Level and Feeding Management on Chewing Activities, Saliva Production, and Ruminal pH of Lactating Dairy Cows1. *Journal of Dairy Science*, *85*(5), 1165-1175.

Morgante, M., Stelletta, C., Berzaghi, P., Gianesella, M., & Andrighetto, I. (2007). Subacute rumen acidosis in lactating cows: an investigation in intensive Italian dairy herds. *Journal of animal physiology and animal nutrition*, *91*(5- 6), 226-234.

Nordlund, K. V., Cook, N. B., & Oetzel, G. R. (2004). Investigation strategies for laminitis problem herds. *Journal of Dairy Science*, *87*, E27-E35.

O'Grady, L., Doherty, M. L., & Mulligan, F. J. (2008). Subacute ruminal acidosis (SARA) in grazing Irish dairy cows. *The Veterinary Journal*, *176*(1), 44-49.

Oetzel, G. R. (2017). Diagnosis and management of subacute ruminal acidosis in dairy herds. *Veterinary Clinics: Food Animal Practice*, *33*(3), 463-480.

Plaizier, J. C., Krause, D. O., Gozho, G. N., & McBride, B. W. (2008). Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *The Veterinary Journal*, *176*(1), 21-31.

Plaizier, J. C., Khafipour, E., Li, S., Gozho, G. N., & Krause, D. O. (2012). Subacute ruminal acidosis (SARA), endotoxins and health consequences. *Animal Feed Science and Technology*, *172*(1-2), 9-21.

Russell, J. B., & Wilson, D. B. (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH?. *Journal of dairy science*, *79*(8), 1503-1509.

Schirmann, K., von Keyserlingk, M. A., Weary, D. M., Veira, D. M., & Heuwieser, W. (2009). Validation of a system for monitoring rumination in dairy cows. *Journal of Dairy Science*, *92*(12), 6052-6055.

Stone, W. C. (1999). effect of subclinical rumen acidosis on milk components. In *Proceedings*.

Taylor, C. C., & Allen, M. S. (2005). Corn grain endosperm type and brown midrib 3 corn silage: Feeding behavior and milk yield of lactating cows. *Journal of dairy science*, *88*(4), 1425-1433.

Yang, W. Z., & Beauchemin, K. A. (2007). Altering physically effective fiber intake through forage proportion and particle length: Chewing and ruminal pH. *Journal of Dairy Science*, *90*(6), 2826-2838.

Zebeli, Q., Aschenbach, J. R., Tafaj, M., Boguhn, J., Ametaj, B. N., & Drochner, W. (2012). Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. *Journal of Dairy Science*, *95*(3), 1041-1056.

Zhang, R., Zhu, W., & Mao, S. (2016). High-concentrate feeding upregulates the expression of inflammation-related genes in the ruminal epithelium of dairy cattle. *Journal of animal science and biotechnology*, *7*(1), 42.