Subacute ruminal acidosis and the relationship with the detection of ruminal acidosis in milk based on milk-fat content, other milk production parameters and nutritional aspects in dairy cows

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Abstract

Background: Subacute ruminal acidosis (SARA) is a pathologic condition that is described as impaired ruminal health in which a reversible pH depression occurs. Detection of ruminal acidosis in milk is based on a low milk-fat content. However, clinical symptoms of SARA are important in determining the diagnosis of SARA, because a low ruminal pH or a low milk-fat content does not always mean that a cow is really suffering from SARA. In order to evaluate the detection of ruminal acidosis in milk, clinical examination of 12 early postpartum cows was performed on each of the 26 farms, on the same day as milk test sampling to determine if cows were showing clinical symptoms of SARA. Clinical parameters which were observed were level of consciousness, filling of the rumen, number and strength of ruminal contractions, fecal aspects, ruminating activity and concentrate-intake. Based on this clinical examination, all examined cows were divided in two groups; SARA or no SARA. Expected and actual milk yield, milk fat content, milk protein content, fat/protein and fat-protein were milk recording data that were used and compared between cows with and without SARA to investigate which milk-production parameters are useful for indicating SARA in dairy cows. The relationship between SARA and feeding grass silage with a high digestibility was also investigated. 13 farms were feeding grass silage with a high digestibility, according to Near-Infrared Spectroscopy (NIRS) by a routine lab, as major forage, The other 13 farms were feeding grass silage with an average digestibility. Consequently, the risk of feeding a highly fermentable diet could be evaluated, because feeding this type of diet provides energy precursors needed for high milk production and to restore a possibly present negative energy balance, but may increase the risk of SARA. The goals of this study were to evaluate the risk of feeding grass silage with a high digestibility and to investigate whether milk-production parameters can be useful for detection of SARA in milk of dairy cows.

Results: SARA was not significantly ($P>0.05$) associated with one of the milk production parameters. Significant differences in the prevalence of SARA between both groups of farms based on the digestibility of grass silage, were not found.

Conclusions: Milk-fat content is not reliable for determining SARA. Other milk production parameters are also not indicative for SARA. Feeding a high digestible diet is one of the risks for developing SARA. However, the prevalence of SARA was not significantly higher on farms feeding a high digestible grass silage. On these farms, useful measures were applied to reduce the risk of SARA.

Keywords: (Subacute) ruminal acidosis, ruminal pH, milk test, milk-fat content, digestibility (VCOS).
Abbreviations: SARA = sub acute ruminal acidosis, VCOS = digestibility of organic matter,
VFA = volatile fatty acids, NIRS = Near-Infrared Spectroscopy, DIM = days in milk

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1 Introduction

1.1 Ruminal acidosis.

The ruminal pH fluctuates during the day and is determined by the dynamic balance between the intake of fermentable carbohydrates and fiber, the rate of acid absorption by the rumen and the buffering capacity of the rumen (*Lorenz, 2015*). Ruminal pH decrease after eating and increase during rumination by producing saliva (*Stone, 2004*). The pH can be measured after sampling of ruminal fluid by several techniques, either with a stomach tube or by rumenocentesis, or by placing indwelling pH probes in the rumen of rumen-fistulated cows. Dependent of the technique that has been used, several thresholds of pH indicating SARA are reported by Duffield *et al.*(*Duffield et al. 2004*). Besides the technique that can be used, there is also a difference between in vitro and in vivo measurements. Probably, the difference is caused due to the loss of CO2 by the in vitro samples before recording the pH, which makes the pH higher than the actual in vivo pH (*Stone, 2004*). However, using the different techniques for determining ruminal pH, ruminal acidosis is commonly defined as periods of ruminal pH depression to values lower than 5,5-5,8 for a certain length of time (*Kleen et al. 2009, Kleen et al. 2003, Plaizier et al. 2008, Maekawa et al. 2002, Kleen et al. 2013, van der Berg, 2004 and Stone, 2004*).

The prevalence of SARA has not been investigated widely, but J.L. Kleen *et al.* observed an overall prevalence of 13,8 percent in 18 dairy farms in Dutch province of Friesland. The prevalence on individual farms ranged between 0 and 38 percent (*Kleen et al. 2009).* Another study of Kleen *et al.* in German dairy herds showed that 11 out of 26 farms were likely experiencing SARA. 20 percent of the cows had a ruminal pH of 5,5 or less at time of rumenocentesis (*Kleen et al. 2013*). In a study of van der Berg, the prevalence of SARA was determined at 5,3 percent on cow level with a threshold of 5,5 for ruminal pH. On herd level, on 7 of 17 farms, cows with SARA were observed. He also used rumenocentesis as diagnostic tool (*Van der Berg, 2004).* In Italy, Morgante *et al.* investigated the prevalence of SARA in 10 dairy herds and reported a prevalence of 33% of the cows with a rumen pH of 5,5 or less on three herds, measured with rumenocentesis (*Morgante et al. 2007*). The above mentioned studies all indicate that SARA is present in dairy herds, independent of country or management type, and that the problems of SARA on dairy herds should not be underestimated.

1.2 Causative factors of SARA

The low ruminal pH is caused by excessive accumulation of volatile fatty acids (VFAs). VFAs (acetate, butyrate and propionate) are produced during fermentation and normally absorbed passively across the rumen wall. This passive absorption is enhanced by finger-like papillae, which project away from the rumen wall and increase ruminal surface area. This creates more absorptive capacity (*Lorenz, 2015 and Beauchemin et al. 2009*). In normal situations, ruminal fermentation is stable and ruminal pH can ranges from 5,6-6,5. Reduction of ruminal pH below 5,6 for a brief period occurs often postfeeding and is not uncommon. Penner *et al.* reported that a single mild episode of SARA had no effect on the epithelial barrier function in the short term. He concluded this by measuring the amount of H-labeled mannitol, an indicator of barrier function, while inducing SARA in sheep. He considered a pH nadir of 5,48 and a duration pH< 5,8 of 111 minutes as a mild SARA episode (*Penner et al. 2010*).

However, continuous reduction in pH below 5,6 has a significant impact on microbial activity, rumen function, animal productivity and health. Therefore, a ruminal pH of 5,6 is considered to be the threshold for ruminal acidosis (*Nagaraja et al. 2007*). A pH range of 5-5,5 is considered as SARA, whereby VFAs concentration increases gradually due to a combination of overproduction and a relatively decreased absorption of VFAs. During SARA, lactic acid does not accumulate, because lactate-fermenting bacteria remain active and metabolize lactic acid into VFAs (Figure 1B). A pH lower than 5,0 is considered to cause acute ruminal acidosis and is the result of increased lactic acid production and decreased lactic acid fermentation into VFAs, because lactate-fermenting bacteria are inhibited due to the low pH. Therefore, lactic acid will accumulate causing a further decline in pH (Figure 1A) (*Lorenz, 2015, Beauchemin et al. 2009 and Nagaraja et al. 2007*). Fortunately, the prevalence of acute ruminal acidosis is very low and therefore in this study, it is more relevant to focus on SARA, which is more common in dairy cows.

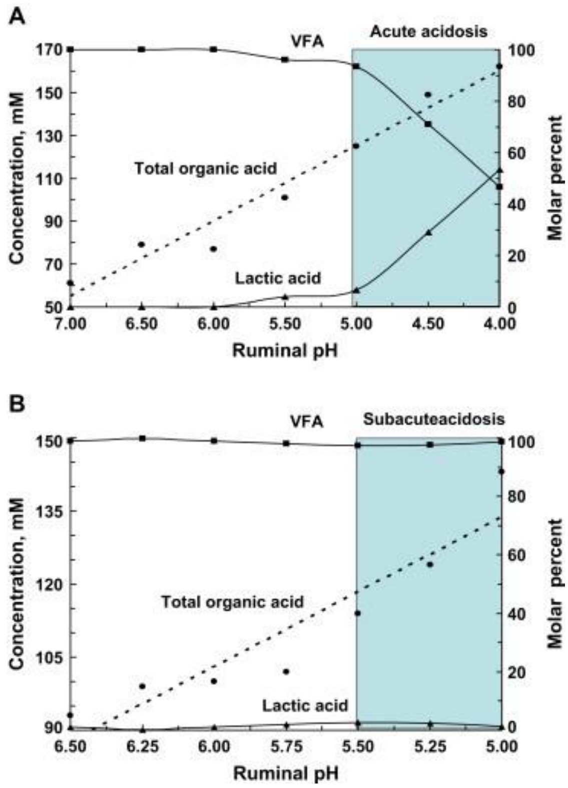


Figure 1: Total VFAs and lactic acid concentration in acute ruminal acidosis (A) and subacute ruminal acidosis (B). *(From Nagaraja TG, Titgemeyer EC. Acidosis in beef cattle. J. Dairy Sci. 2007; 90: E17-38)*

In contrast to acute ruminal acidosis, SARA should be regarded as a herd rather than an individual problem. Groups potentially at risk for SARA have been identified as being early lactating cows and also high-producing cows in mid-lactation. The latter group is sensitive to errors in ration formulation, feed delivery or poor quality of feedstuffs, because in the middle of lactation, feed intake is maximal (*Enemark et al. 2004*). Besides that, high levels of feed intake may also predispose the rumen for SARA because the additional acid production is not compensated anymore by salivary buffer secretion (*Stone, 2004*). Sometimes there is a lack of fiber particles in the ration or the fiber particles are too short. This will not trigger enough rumination and therefore reduces the salivary buffering capacity in the rumen.

After calving, the ruminal function can be disturbed when the difference between the dry cow ration and the lactation ration is too large and/or if the diet change is too abrupt. Raising the concentrate intake after calving too quickly will cause the animals at risk, because their ruminal environment is not yet completely adapted to this type or amount of substrate with high energy density (*Kleen et al. 2003*).

The growth of ruminal papillae will be stimulated by a high energy diet, because the intake of high fermentable carbohydrates will lead to more VFAs production. For absorption of more VFAs, the ruminal wall will adapt by lengthen the ruminal papillae (*Penner et al. 2010).* After calving, cows will be fed a high energy diet, and ruminal wall adaption requires several weeks. Therefore a proper transition management should be advised to reduce the risk of SARA (*Lorenz, 2015 and Beauchemin et al. 2009).*

For an optimal transition, it is recommended to supply more concentrates prepartum to improve the growth of the ruminal papillae, and thereby increasing the VFA absorption surface and capacity, and to change the ruminal flora, such that the rumen is already started to adapt to the high energy diet fed during lactation (*Stone, 2004*). Especially heifers are at risk to develop SARA, because they have not had previous long-term exposure to highly fermentable and energy dense diets (*Enemark et al. 2004*).
In contrast, Penner *et al.* concluded that feeding Holstein heifers additional concentrates prepartum did not reduce postpartum SARA. Heifers fed a high concentrate diet before calving had more, longer and severe daily episodes of SARA postpartum compared with cows fed the control diet. The pH was continuously measured by an indwelling ruminal pH system during several consecutive days in the periparturient period (*Penner et al. 2007*).

Maekawa *et al.* concluded that also the way of feeding can be a risk factor for developing SARA. TMR feeding was compared with feeding a diet with ingredients allocated separately. Feeding TMR avoids feeding large meals of concentrate, that may be beneficial in terms of maintaining a more constant and high ruminal pH. Cows that were fed the non-TMR diet had a lower pH minimum and more pH variables, indicated a greater risk for developing SARA (*Maekawa et al. 2002*).

Furthermore, once cows experience ruminal acidosis, they become more susceptible to subsequent bouts of ruminal acidosis, because the low pH results in a destabilized microflora and damaged rumen epithelium that leads to a decreased absorptive capacity for VFAs (*Beauchemin et al. 2009*). The risk for SARA is not equal for all cows, because there exist some animal variability. Cows in early lactation have a higher incidence of SARA than cows at in mid or end of lactation, because the rumen is not yet adapted to a lot of high energy food. Beside lactation stage, many factors can contribute to development of SARA, like level of feed intake, eating rate, salivation rate, sorting of feed, the individual ruminal microbiological population, previous exposure to SARA, rate of passage of feed from the rumen and other aspects of cow physiology and behavior (*Beauchemin et al. 2007*). Penner *et al.* reported that differences in absorptive capacity also may account for variation in ruminal pH among animals. He used 24 sheep which were randomly assigned to the control or SARA group. The SARA group were orally dosed with a high density glucose solution, and the control group with water. After three hours, sheep were euthanized and ruminal tissue was collected to measure the absorption of VFA's. It was concluded that animals with more absorption had higher ruminal pH (*Penner et al. 2009*). This individual variability among animals in developing SARA makes it very difficult to completely eliminate SARA on herd level in high producing dairy herds, especially when diets are calculated for the average cow.

1.3 Clinical symptoms of SARA

Mostly, periods of low ruminal pH resolve without treatment and are therefore rarely diagnosed. One mechanism for cows to resolve ruminal acidosis and return their ruminal pH to a normal pH range is by selecting feeds that are high in structure, like hay and other long forage particles. Intake of feed with a longer particle size will result in more saliva production and therefore increase rumen buffering capacity to prevent or treat SARA (*Radostits et al. 2007*). Another mechanism is reducing overall feed intake during some days, especially concentrates, but dry matter feed intake will also be decreased during SARA (*Radostits et al. 2007*). Reduced intake of concentrates can be monitored on most farms by using a computer program. These mechanisms are therefore symptoms which can be observed if a cow is suffering from SARA.

In the subacute form of ruminal acidosis, the symptoms that can be observed are mild depression and abdominal discomfort, evidenced by kicking at the belly an lying with the head in their flank. Affected cattle are often anorexic and do not ruminate for a few days. Rumen contractions are reduced but not entirely absent and the power of the contractions is less strong. Alterations in fecal aspects can also be observed, like a lower consistency, a lower pH, a sweet-sour smell, and the size of ingesta particles are often being too large. Inspection of the feces will usually show soft to watery feces with foamy aspects. A change in feces color is also described, like a gray and yellow green color. Because the feces color depends on the diet, interpretation of a change in feces color will be difficult (*Radostits et al. 2007, Smith, 2015*). In some cases, on the long term, subacute or chronic laminitis is described. The affected animals are lame in all four feet, shuffle while they walk slowly and may be reluctant to stand. Laminitis is often accompanied by other claw horn lesions (*Kleen et al. 2003 and Radostits et al. 2007*). Other long term symptoms are considered to be a decreased milk production, reduced milk-fat and a poor body condition score (*Lorenz, 2015*). These symptoms are also noticed on herd level if ruminal acidosis is present, often in combination with diarrhea and at a later stage lameness.

The percentage of cows ruminating at any given time is also used to monitor rumen health at herd level. Maekawa *et al.* reported that at least 40% of the cows must be ruminating at any given time, except when they are eating or being milked, for considering a dairy herd to have a healthy rumen function when TMR is applied. When farms are not feeding TMR, but ingredients are allocated separately, at least 35% of the cows should be ruminating at any given time during the day (*Maekawa et al. 2002*).

1.4 Association between SARA and low milk-fat content

Several studies concluded that a low milk-fat content is not specific for ruminal acidosis, but in general can be seen as a result of changes in ruminal fermentation pattern, that possibly is caused by SARA (*Kleen et al. 2003 and Radostits et al. 2007*). Changes in ruminal fermentation pattern are associated with changes in ruminal flora. And the ruminal microflora is related to ruminal pH and type of nutrients. When the fermentation pattern changes by a change in microflora, the ratio between the VFA's will change. Acetate, the precursor of milk-fat, is more produced when the ration contains a lot of silage and fiber. The amount of propionate and butyrate will increase when the intake of concentrates is high (*Beauchemin et al. 2007, Smith, 2015 and Steele et al. 2009*).

During SARA, propionate will be high and is used for glucose metabolism of a cow, while acetate will be decreased and possibly can cause a drop in milk-fat content (*Van der Berg, 2004*). Danscher *et al*. found that milk-fat content was decreased in cows during the SARA challenge, compared to the control group, but this finding was not significant (P=0,06) (*Danscher et al. 2015*).

In a study of Kleen *et al.* using Dutch dairy farms, the correlation between milk-fat content and ruminal pH was also not appeared to be statistically significant (*Kleen et al. 2009*). Enemark *et al.* did not found a

correlation between milk-fat percentage and rumen pH, except when grouping the cows according to lactational state and analyzing the relationship for every group separately. They found an association between rumen pH and milk-fat percentage in cows, which were more than 30 days in milk (DIM) (*Enemark et al. 2004*).
Gao and Oba suggested that other unidentified factors, besides rumen pH, affect milk-fat content, and that milk-fat content alone is not a sensitive indicator to identify cows with low or high risk of SARA. They also measured milk urea nitrogen concentration (MUN). Together with MUN, milk-fat content can be used as a predictive tool to determine the risk of SARA for an individual cow (*Gao and Oba, 2015).*

Van der Berg observed in a study for his master thesis that there was a significant relationship between ruminal pH and milk-fat content. In this study, 171 cows were used for rumenocentesis. 5,3% of all these cows were considered to suffer from SARA, because a ruminal pH of 5,5 or lower was found. When a milk-fat content of 4% was the limit and the threshold pH was 5,5, the milk recording test for ruminal acidosis was not appeared to be reliable, because the sensitivity and specificity was 0,33 and 0,74 respectively. The test was becoming more reliable when higher pH values and higher limits of milk-fat content were used. The test shows the best results when a pH threshold of 5,7 and a milk-fat content of 4,2 was used, but these values are corresponding less with SARA (*Van der Berg, 2004*).

However, ruminal acidosis is reported as an attention in milk-recording data when milk-fat is lower than milk-protein and whereby milk-fat is lower than 4% (*MPR Voeding, CRV*). When this criterion is met, a mark is shown indicating that a cow is suffering from ruminal acidosis. However, this mark should be considered as an indication and not a definitive diagnosis. To perform a more reliable diagnosis, other aspects must be also examined, like filling of the rumen, the number and strength of rumen contractions, feacal aspects, feed intake and ruminating activity.

1.5 Association between SARA and high digestibility in grass silage

After calving, milk energy output increases rapidly and exceeds energy intake from feed, causing a negative energy balance. To meet the energy requirements of high-yielding cows and to counteract the negative energy balance in early lactation, their ration should have a high energy density, often achieved by feeding a large quantity of concentrates. As a consequence, diet fiber content may be marginal. As a result of marginal fiber intake, early lactation cows are at risk of SARA due to reduced rumination and salivary buffering capacity. SARA can further reduce dry matter intake, because rumen motility is reduced and most cows are not feeling comfortable (*Naylor et al. 1991*).
Additionally, feeding forages having a high digestibility can also meet the energy requirements. The digestibility of organic matter (VCOS) is one of the parameters which can be measured in forages and is the proportion (%) of consumed organic nutrients that is digested and absorbed by the animal in its alimentary tract. The digestibility can be measured in vivo by feeding animals for a period of two weeks and monitoring feed intake and excretal output. Nowadays, NIRS is used to estimate the VCOS in vitro as routine analysis (*Laidlaw et al. 2013*). NIRS calculate the contents of the Weende analysis, and make an estimation of the VOS, the amount of digestible organic matter. Subsequently, the VCOS will be calculated according to the following formula (*CVB, 2006*).

VOS = (1000-RAS) x VCOS in vitro/100

The VCOS is an estimation of the degree of digestibility. In general, highly digestible grass silage results in rapid production of VFAs. The carbohydrate fractions differ in their rate of digestion, with sugars and starches digested faster than fiber. Therefore, a high quality forage with a high VCOS may increase the risk on SARA (*Beauchemin et al. 2007*).

1.6 Objectives of this study

The objectives of this study were to determine if detection of ruminal acidosis in milk, based on low milk fat content, can be considered as a reliable indicator for SARA. This study will also give an answer on the question of SARA can be determined by making use of clinical symptoms or other milk production parameters, like milk protein of the ration between fat and protein.

This study will also evaluate the risk of feeding a high digestible grass silage on SARA, compared with feeding grass silage with an average digestibility under farm conditions.

2 *Materials and methods*

2.1 Herds and husbandry

Twenty-six dairy farms in the Dutch provinces of Groningen and Drenthe were selected to participate in this study. All of them were housing Holstein-Friesian cows, with some crossbreeds with Fleckvieh, Blister head or Swedish/Norwegian roan cows. The farms were served by veterinarians of Arts en Dier and Veterinary Practice Noord-West Groningen, from which farms were selected on the basis of willingness to cooperate in the study. Farms were also selected on having documentation of grass silage analysis and connection with PirDAP to obtain the milk test results. Cow records such as calving date and milk recording data were obtained from both veterinary practices by use of PirDAP. Thirteen farms were selected based on the criterion of feeding grass silage with a high VCOS, that was 79 or higher (Group H). The other thirteen farms were feeding grass silage with an average VCOS, namely 76-78 (Group M). If farmers fed different grass silages based on the moment of harvesting, the average VCOS of the grass silage was used for dividing the farms into group H or M. Farms with TMR feeding were à priori excluded because individual concentrate intake cannot be monitored when using this feeding system. Farms which had the cows at pasture were also excluded when it was possible, because grass intake and composition of the pasture is expected to fluctuate and VCOS is not measured. All farms were visited in the in-house period from 12 October to 5 December.

2.2 Animals and clinical examination

Based on the number of cows used in other studies (*Kleen et al. 2009, Kleen et al. 2013, Van der Berg, 2004 and Colman et al. 2010*), on every herd that participating in this study, twelve fresh cows were selected (DIM<60 days). Taking samples for the milk recording test is possible from 4 days in lactation, because colostrum often contains higher contents of fat and protein. From 4 days in lactation, the twelve most freshly cows were selected and were clinically examined according to a protocol. First, the level of consciousness was scored using a 5-point-scale, whereby score 1 is bright and alert and 5 is moribund. In the mild form of SARA, cows are still fairly bright and alert, but in the severe form, most affected cows are apathetic and depressed (*Radostits et al. 2007*). The degree of rumen filling was also scored by means of a 4-point-scale, whereby 1 is completely filled, and 4 is poorly filled. During 5 minutes, the number of rumen contractions was counted in combination with auscultation

of the rumen. The strength of the contractions was scored as -, +/- or +. The number of contractions must be 10-12 in 5 minutes in a healthy cow. When the number of contractions was 9, but with enough strength, it was considered as a good rumen function (*Kuiper et al. 2008*). After clinical exam of the rumen, some aspects of the feces were observed. Fecal samples were obtained directly from the rectum. According to two cards (*Zaaijer et al. 2001*), the consistency of the feces and the presence and size of ingesta particles are scored using a 5-point-scale. When the cow was ruminating during clinical examination, the number of chewing cycles was counted.
Concentrates were fed individually to cows in a feed station and were registered automatically by a computer. Concentrate intake was checked in the computer for the last 4 days.
Based on the abovementioned clinical parameters, an individual score was given for each examined cow. Because the clinical symptoms of SARA are partly aspecific, a combination of three of more symptoms related to SARA had to be found before a cow would get score 2 'SARA'. When there were no or less than three clinical symptoms, score 1 'No SARA' was given, because the symptoms were not present prominently enough to consider it as SARA. For example, when a cow was only showed diarrhea as the only clinical symptom, score 2 was not given, because diarrhea can have more causes, like paratuberculosis, Bovine Virus Diarrhea (BVD) or an excess of potassium in grass of the pasture.

Clinical examination of the cows was performed on the morning of milk sampling. Milk sampling was carried out by the farmer himself. When the milk test results were available, mostly 2 days later, the milk-test results were noted for each individual cow.

Expected milk yield, actual milk yield, milk fat content, milk protein content, fat/protein, fat-protein are the milk production parameters that were related to the clinical symptoms, that divide the cows into SARA or no SARA. Also days in milk and parity are included. Besides the clinical exam of the cows and the milk test results, farmers were also asked to take part in a survey with some questions about the composition of the lactation ration and feeding methods.

At last, the number of cows ruminating was counted on herd level once during each farm visit on a quiet moment.

2.3 Data analysis and statistical methods

In this study, two hypotheses were tested. The first hypothesis is made to investigate if a low milk-fat content is discriminating for cows with SARA. In the same way, also other milk production parameters will be investigated to see if there is a significant association with SARA.

H0 = There is no significant difference between the presence of clinical SARA and attentions of SARA in current milk-recording data.
H1= There is a significant difference between the presence of clinical SARA and attentions of SARA in current milk recording data.

In addition, the risk of feeding grass silage with a high digestibility (Group H), compared with grass silage with an average digestibility (group M) will be evaluated, considering the prevalence of SARA on herd level.

H0= There is no significant difference in prevalence of SARA between group H and M.
H1= There is a significant difference in prevalence of SARA between group H and M.

For the statistical analysis, the computer program SPSS was used. For this study, descriptive statistics were mostly used to obtain the frequencies and make some graphs. To calculate the difference in prevalence of SARA between both groups (H and M), Independent Sample T-test was used. For determining which milk production parameters are useful to diagnose SARA, multivariate analysis of the general linear model was applied, with SARA as fixed factor and the milk production parameters as dependent variables. P values <0,05 were considered significant.

3 Results

3.1 Use of clinical parameters for diagnosing SARA

To determine if cows were suffering from SARA, several clinical symptoms were checked during clinical examination, which could be important and might contribute to making the correct diagnosis SARA in combination with using milk production parameters. In total, 312 cows were used for clinical examination, but 303 were used for statistical analysis. 9 cows were excluded from the database, because some milk-test results were unknown or because of health reasons, like an abomasal displacement the day before.

Of 2 clinical parameters, there were a lot of missing values, namely residual feed of concentrate intake (16 missing values), and ruminating chewing cycles (243 missing values). Those parameters were tested separately using the univariate analysis.

12 out of 303 cows were showing 3 or more clinical symptoms which are described above, indicating that they were suffering from SARA. In table 1 it is shown which clinical symptoms were mostly found in the 12 cows with SARA. These 12 cows were all DIM <60 days. 4 out of 26 farms had one or more cows which were in a later stage of lactation than 60 days, because the number of cows on these farms were smaller. Consequently, 14 out of 303 cows were between 60 and 120 DIM, but these cows did not show clinical signs of SARA (see figure 1).The prevalence of SARA on cow-level is 4%. The 12 cows with SARA were housed on 10 farms (see figure 2).

Most of the clinically examined cows were between 10 and 20 days postpartum. In Figure 1 it is shown that SARA is most prevalent in cows between 50 and 60 days postpartum. SARA was not found in cows after 60 days postpartum.

Based on the literature, it would be expected that especially primiparous cows are at risk for SARA. On the contrary, in this study, cows exhibited SARA also in later numbers of lactation, while SARA likely plays no major role in lactation number 2 and 3 (see table 2).

All 26 dairy farms were not feeding TMR, so concentrates were allocated separately. According to Maekawa *et al.* at the moment of counting the number of ruminating cows, 35% of the cows should be ruminating (*Maekawa et al. 2002*).

In total, 2 of 26 farms had cows with access to pasture at the time of observation and on 2 farms counting rumination was not reliable, because cows just had received fresh food. Therefore, ruminating activity on herd level could not be determined on these farms. On the other 22 farms, the proportion of ruminating cows was determined once to gain a first impression of ruminal health on herd level. The mean proportion of ruminating cows was 39,3%. There were 7 farms with a ruminating activity below 35% at the moment of counting (18-33,7%). On 3 of these 7 farms cows with SARA were found.

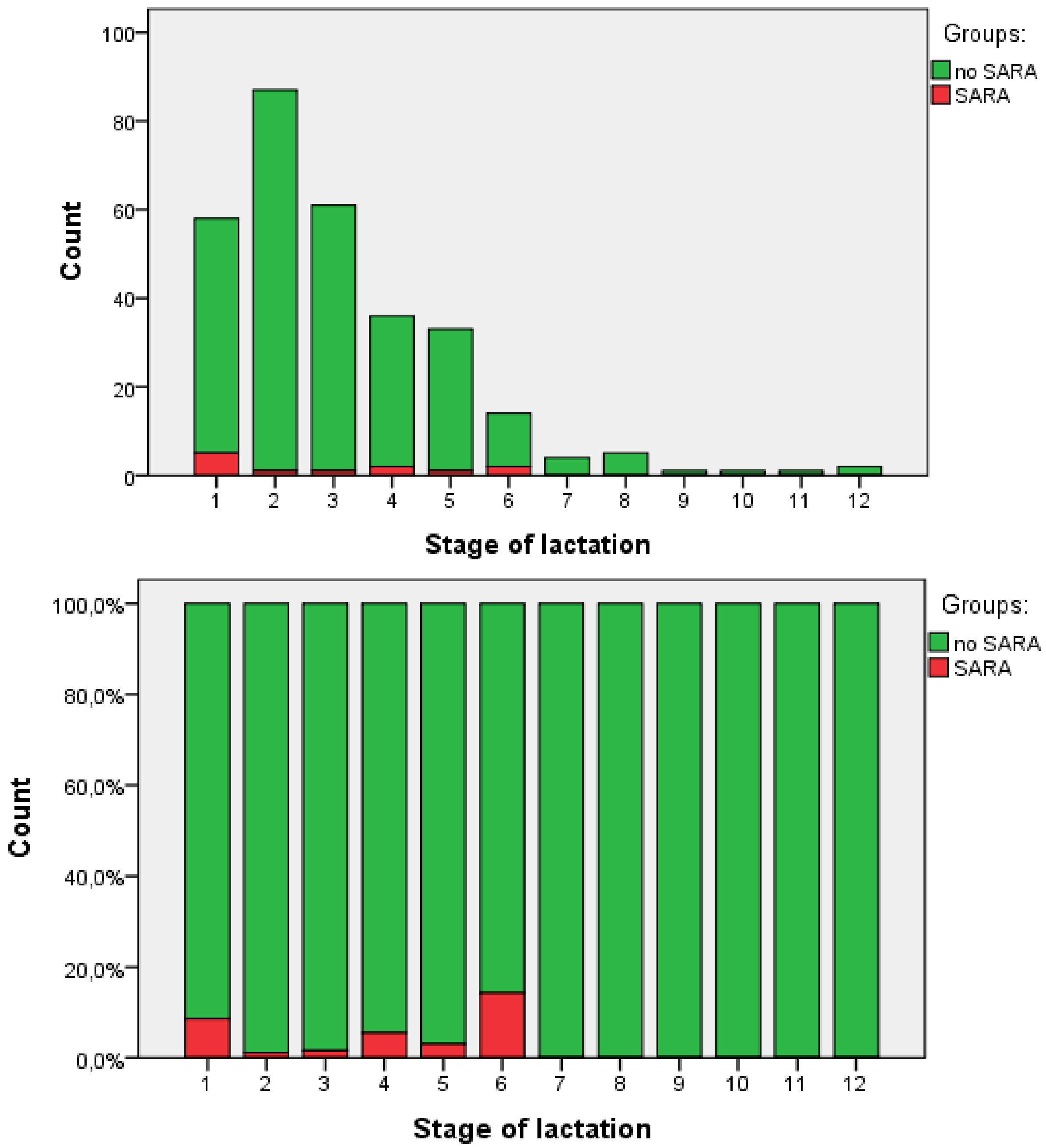
Table 1: Clinical parameters and their frequencies in 12 cows with SARA.

Clinical parameter	Number of cows (n=12)
General demeanor – depression	0
Rumen filling score 3 and 4	5
Decreased ruminal contractions (<9/5 minuten)	5
Weak ruminal contractions (+ and +/-)	5
Feacal consistency score 1	5
Ingesta particles faeces score 4 and 5	4
More as 10% rest of concentraties	9

Table 2: Prevalence of SARA per number of lactation.

Number of lactation	Score 1	Score 2	Total
1	88	6	94
2	64	1	65
3	59	0	59
4	27	1	28
5	31	2	33
6	12	1	13
7	6	0	6
8	3	1	4
10	1	0	1
Total	291	12	303

Figure 2: Number and percent of cows distributed over several lactational stages and the prevalence of SARA in every lactational stage (stage 1 is 0-10 days in milk, stage 2 is 11-20 days in milk etc.).



3.2 Use of milk-production parameters for determining SARA

According to the milk-test, a low milk-fat content is an important parameter to determine if a cow is suffering from SARA (*MPR Voeding, CRV*).

In this study, significant differences in milk-production parameters between cows with and without SARA were not found. Of three cows, expected milk-yield was unknown. Therefore these three cows were excluded from the multivariate analysis of milk-production parameters.

Table 3 shows the milk-production parameters and their p-values, which were all not significant. The p-value of milk-fat content was not significant (p=0,118) between cows with and without SARA. When both groups were compared, milk-fat content was just higher in cows experiencing SARA than in cows without SARA (4,84% vs. 4,48%, p=0,22), while there was no significant difference in the stage of lactation (2,92 vs. 3,08, p= 0,38).

Totally, 5 out of 26 farms received attentions in the milk recording results for ruminal acidosis of 1 or more of the examined cows. However, none of the attentions corresponds with the findings found with clinical examination of the cows. The 12 cows with SARA did not have any attentions for ruminal acidosis according to the milk test result. And on the other hand, cows which were examinated and received an attention on the milk recording results had not showed clinical signs of SARA on the day of milk sampling.

Table 3: Milk-production parameters and their p-values when they were related to SARA.

Milk production parameter	P- value
Milk-fat content (%)	0,118
Milk- protein content (%)	0,081
Fat/protein	0,459
Fat - protein	0,445
Difference kg milk expected – kg milk actual	0,539
Days in milk	0,847
Number of lactation	0,676

3.3 Prevalence of SARA on different farms

One of the objectives of this study is to investigate whether the prevalence of SARA is higher on farms feeding a high digestible grass silage compared to farms feeding a grass silage with an average digestibility. 3 of the 13 farms of group M had attentions of ruminal acidosis according to milk-test results for one or more of the examinated cows. In group H were 2 of 13 farms with attentions for ruminal acidosis of the examinated cows.

In this study, cows were divided in two groups after clinical examination (1= no SARA, 2=SARA,). Figure 3 shows the number of examinated cows per farm and the scores of these cows. In total, on 10 of 26 farms SARA was present according to clinical examination of the cows. In group M (farm 1-13) were 4 farms with clinical symptoms of SARA, while group H (farm 14-26) contained 6 farms where SARA played an obviously role. Based on this observation, it may be concluded that SARA is probably more prevalent on farms where a high digestible ration is fed. However, statistical analysis showed that there is no significant difference between cows of group M or H in the prevalence of SARA (p=0,269). On herd level, there is also no significant between both groups (p=0,443).

The farmers of this study all answered the questions of the survey about the composition of the lactation ration and the way of feeding. One of the questions of the survey was if they have taken any measures to prevent SARA, and which measures they applied. Figure 4 shows the measures and their percentages per group. More answers were possible. Farmers in group H were feeding more brewers spent grain and more bicarbonate buffer in the diet. The farmers of group M were more often applying adapted concentrates in terms to achieve a slower digestibility.

Figure 3: The presence of SARA on herd level for all farms of group M (farm 1-13) and group H (farm 14-26), and the number of cows used for clinical examination and milk-recording data per farm.

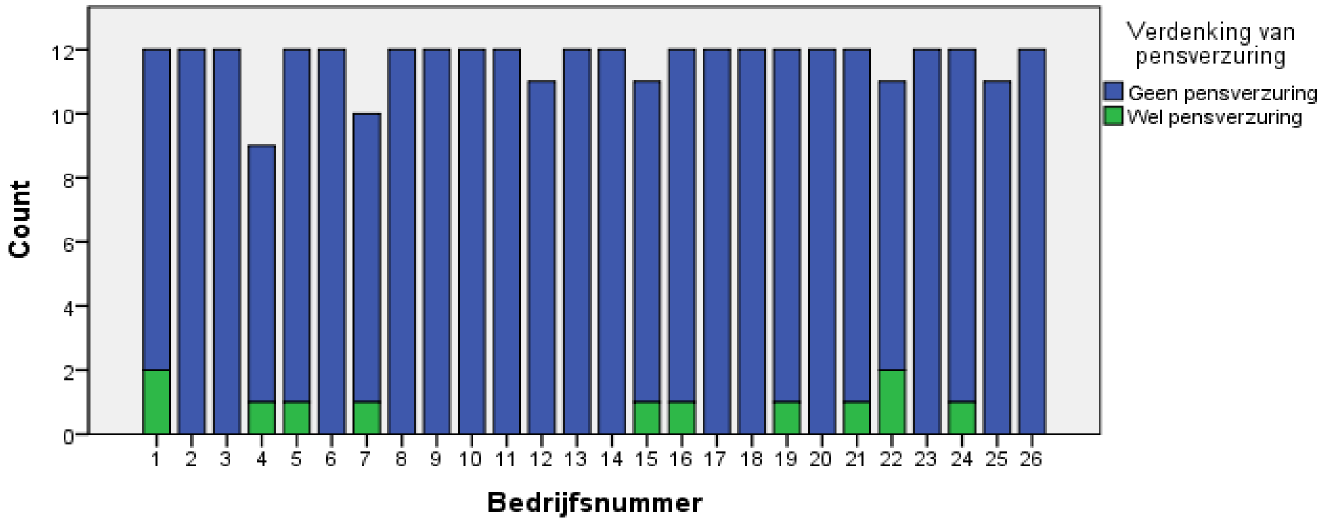
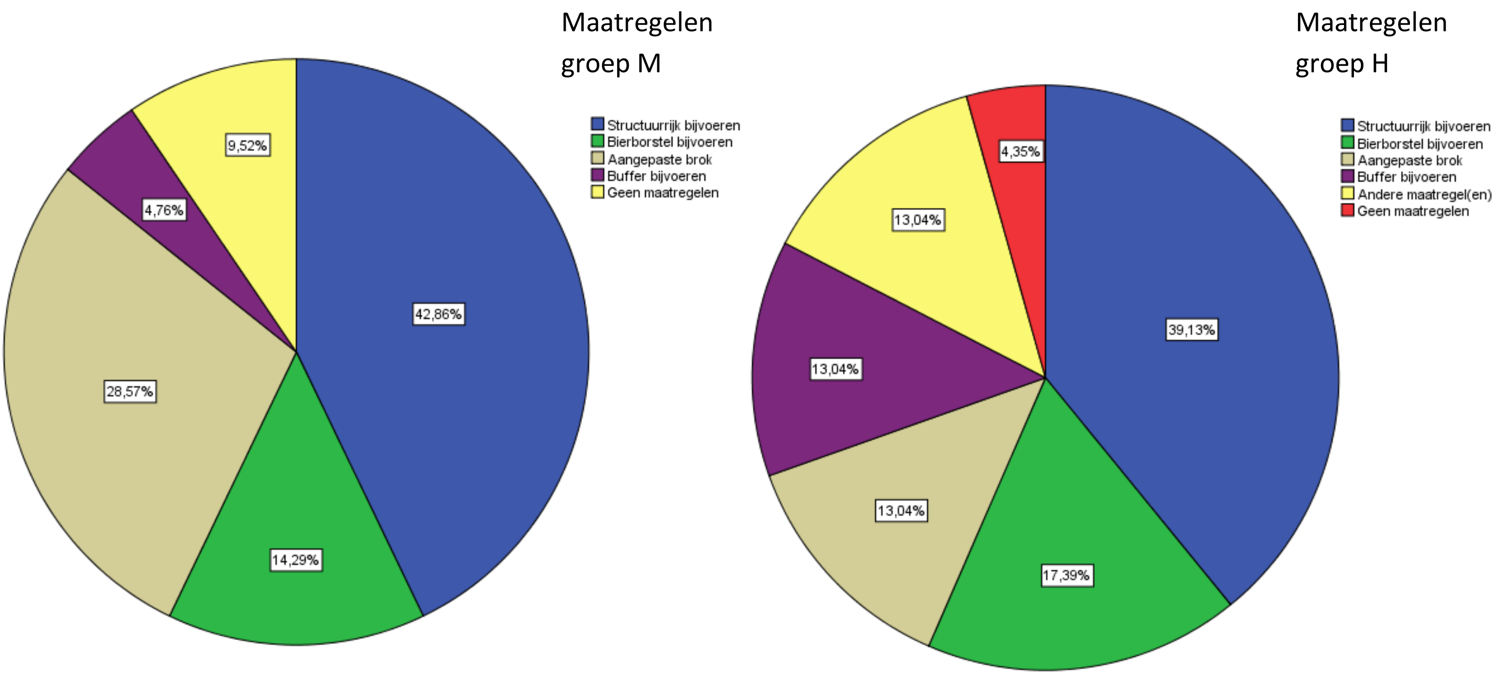


Figure 4: Measures for compensate grass silage with a average or high digestibility and their percentages per group.



4. Discussion

4.1 Prevalence of SARA

The results of this study give an estimation of the prevalence of SARA in Dutch dairy herds in the provinces Groningen and Drenthe. 12 of 303 cows (4%) were experiencing SARA based on clinical examination. This prevalence is comparable with a study of van der Berg, who determines SARA with rumenocentesis and found a prevalence of 5,3% (*Van der Berg, 2004*).

Cows which had one or two symptoms of SARA, were classified as 'doubtful', but in the statistical data included as score 1 (no SARA). So the real prevalence is probably higher than 4%, in accordance with Kleen *et al.* who found an overall prevalence of 13,8% in dairy herds in the province of Friesland (*Kleen et al. 2009*) and 20% in German dairy herds (*Kleen et al. 2013*) and with Morgante *et al.* who found an prevalence of 33% in intensive Italian dairy herds (*Morgante et al. 2007*).

In contrast with Beauchemin who described that it is difficult to indentify animals suffering from SARA (*Beauchemin et al. 2009*), those 12 cows were likely suffering from SARA based on a combination of 3 of more clinical symptoms. However, the real prevalence could be higher because not all the cows who are suffering from SARA clearly show clinical signs, and clinical signs which can be seen are very aspecific.

The 12 cows with SARA were housed on 10 out of 26 farms. This observation is consistent with the study of Kleen *et al.* which showed 11 out of 26 herds likely experiencing SARA (*Kleen et al. 2013*).

4.2 Clinical examination

There are several ways to measure the rumen pH. The most reliable techniques are rumen cannulation or using an indwelling pH meter. Both can only be used in fistulated cows. There are currently only two techniques for measuring rumen pH under field conditions: rumenocentesis and oral stomach tube. Rumenocentesis is more preferred than an oral-ruminal probe, because oral-ruminal probes are often contaminated with variable

quantities of saliva, resulting in higher pH values what makes this method less accurate than rumenocentesis. Besides that, oral ruminal probes are difficult to clean which makes them a potential transport of infectious agents form farm to farm (*Duffield et al. 2004 and Nordlund, 2003*).
However, rumenocentesis is a more invasive technique and suffers from a risk of localized abscesses or peritonitis (*Duffield et al. 2004*). Van der Berg also reported a high probability of abscesses in cows when using rumenocentesis for determining ruminal pH. He reported a chance of 5% for development of abscesses after taken samples with rumenocentesis and 16,6% of the samples were not useful (*Van der Berg, 2004*). It can be done with relatively little risk to the animal, when it is performed by a trained veterinary practitioner. Although, its routine use raises ethical questions due to its invasiveness as compared to its diagnostic value (*Enemark et al. 2004*). Due to this reason, clinical examination was chosen over measuring ruminal pH. The diagnosis of SARA will be benefit when simple clinical diagnostic tools that can be used under field conditions, can contribute to recognize cows with SARA.

In this study, level of consciousness was not affected in cows with SARA in accordance with Danscher *et al.* who also performed clinical examination and evaluate general demeanor with the same 5-point scale. They concluded that general demeanor was generally not affected, apart from a few hours of mild depression related to the phases of lowest ruminal pH. However, Danscher *et al.* also found decreased ruminal contractions and reduced feacal consistency in cows with induced SARA (*Danscher et al. 2015*), which corresponds with the results of this study. However Kleen *et al.* found no significant relationship between ruminal pH and the consistency of cows' feaces (*Kleen et al. 2009*). Van der Berg found a significant correlation between ruminal pH and body condition score, number of ruminal contractions and feaces consistency (*Van der Berg, 2004*).
In this study, the most prominent clinical symptom of the SARA cows was a lower concentrate intake. 83,3 percent of the cows with SARA had 10 percent of more concentrate residues. In combination with the other clinical parameters, concentrate intake may be an important parameter to detect SARA. This is in agreement with Keunen *et al.* who examined the effects of SARA on the diet selection of dairy cows. He induced SARA in 4 mid-late lactation cows and let them make a choice between TMR, wheat barley pellets, alfalfa hay or alfalfa pellets. The cows did not consume the wheat barley pellets anymore and less of the TMR and alfalfa pellets. As expected, they eat more of the alfalfa hay to stimulate their rumination (*Keunen et al. 2002*).

The objective of this study was to make clear if there is an association between clinical signs of SARA and milk-production parameters, especially milk-fat content, which is used in the milk recording data by CRV to determine cows with SARA. However, there was no significant relationship found between milk-fat content and the presence of SARA on individual cow level. Cows with attentions of SARA in the milk recording results were not showing clinical signs of SARA, and on the other hand cows with SARA had no attentions of SARA based on milk-fat content. An explanation could be that the decrease in milk- fat content and showing clinical signs are not visible on the same time, or even on the same day. Further research is needed to find out whether this can be the case.

4.3 Milk-production parameters

According to Nordlund, the average milk-fat content of tank milk is definitely not a suitable

parameter for determining the presence of SARA in a herd. Many herds with normal average milk-fat content have problems of ruminal acidosis, while cows with SARA that produce low fat milk will not always be found, because their milk is pooled with the rest of the herd which may mask the effect (*Nordlund et al. 2003*).
In this study, individual milk fat content was used but none of the milk-production parameters were significantly different between cows with or without SARA, in accordance with Maekawa *et al.* who observed no difference in milk components between diets with different proportions of barley silage (*Maekawa et al. 2002*). Krause and Oetzel did not observe a decrease in milk fat content while ruminal pH was decreased significantly. They suggest that a single bout of SARA will not induce a decrease in milk fat percentage, only repeated bouts of SARA (*Krause et al. 2005*). Kleen *et al.* also found that an association between ruminal pH and milk production parameters had no noteworthy result. He only observed a significantly higher urea level in cows with SARA, and the fat-protein ratio was considered to be more narrow in animals with a ruminal pH of 5,5 or less (*Kleen et al. 2013*). The latter result is consistent with Danscher *et al.* and Gohzo *et al.* who also observed a decreased milk-fat to milk-protein ratio in induced SARA cows, cause to a decreased milk-fat and a small increase of protein-content (*Danscher et al. 2015 and Gohzo et al. 2012*).
Some studies considered a decreased milk-fat content to be specific for SARA (*Danscher et al. 2015, Gao et al. 2015 and Khafipour et al. 2009*). In this study is been concluded that milk-fat content was not significantly different between cows with or without SARA. Surprising was that milk fat content was just a little bit higher in cows experiencing SARA. An explanation could be that those fresh cows were also suffering from a negative energy balance and therefore have mobilized their body fat reserves. This result in a higher milk fat content which may be counteract the lower milk fat content caused by SARA. Anyway, a higher milk-fat content in cows experiencing SARA is just another argument that milk-fat content is not a useful and reliable parameter for determining SARA in milk.

4.4 Nutritional aspects

One of the objectives of this study was to investigate whether the prevalence of SARA is higher on farms feeding a high digestible grass silage compared to farms feeding a grass silage with an average digestibility. At the moment of visiting the farms, most of the farms were feeding the first silage of previous season. Some farmers were combinating the first silage with the second or third silage. The average VCOS of the grass silage was used for dividing the farms into group H or M. 5 out of 26 farms were feeding only grass silage and some concentrates. The other farms were feeding a lot of byproducts like maize, pulp, potatoes, hay, straw, brewer grains etc.

In this study, a significant difference in the prevalence of SARA between group H and M was not found. Feeding a high digestible grass silage is not per se associated with a higher prevalence of ruminal acidosis on herd or cow level, compared with farms feeding a ration with an average VCOS. Feeding a high digestible reason is one of the risks for having more cases of SARA, but there are lot of ways to avoid this. A lot of farmers were feeding byproducts to make the overall digestibility of the ration more slowly, like hay, straw, brewers spent grain, sugar beet pulp and a slower digestible type of concentrates. A part of the farmers were feeding additional bicarbonate as buffer. Applying this kind of measures to prevent SARA is really good, but may have interfered with the results of the prevalence between group M and H. Besides that, only the VCOS of the grass silage has been taken, and not the VCOS of the byproducts or maize silage. The overall VCOS of the complete diet will determine the finally degree of the risk of SARA. There it would be better to control the diet for each of the two groups, instead of investigation under different farm conditions.

4.5 Future possibilities

Difficulties with the diagnosis of SARA are caused by a lack of specific pathognomonic symptoms, diurnal fluctuations in ruminal pH, and problems in obtaining representative rumen fluid samples for rumen pH measurement. Rumenocentesis has been associated with development of abscesses and peritonitis (*Duffield et al. 2004, Van der Berg, 2004*). In addition, this technique is invasive and requires surgical preparation, makes it less suitable for routine use.
Although the oral stomach tube does not require surgical preparation, pH determination by this method is less accurate than rumenocentesis because samples can be contaminated with saliva. Because of the diurnal fluctuation in rumen fermentation and variation in ruminal pH, one sample is not enough to diagnose SARA. A low milk-fat content is also not reliable to detect SARA in milk, as well as other milk production parameters. Therefore, it is necessary to search for new methods for detecting SARA.

Colman *et al.* observed that milk-fat content showed a weaker link with SARA than specific milk-fatty acids. Some milk fatty acids (C18:2 *cis*-9, *trans*-11, *iso* C16:0 and *iso* C13:0) were considered to be the most effective predictors in SARA in milk and thus have potential value in identifying cows suffering from SARA or are at risk of SARA (*Colman et al. 2010*).

Another way of detecting SARA, is by measuring ruminal pH by a sensor. This sensor is made by Smaxtec Animal Care and is designed to provide continuously measurement of ruminal pH and temperature during 50 days. The sensor is measuring every ten minutes and recorded data are transmitted wireless to a base station or a mobile reader, which gives you up to date information. In this way, rumen health can be monitored and maintained easily (*Smaxtec website*).

5. Conclusions

Feeding grass silage with a high VCOS is considered to be a risk for developing SARA on herd level, but the prevalence of SARA on farms feeding grass silage with a high digestibility of organic matter was not higher compared with farms feeding grass silage with an average digestibility. Furthermore, none of the milk-production parameters are significantly different between cows with and without SARA. Milk-fat content, the parameter that is regularly used for detection of ruminal acidosis, don't seem to be reliable as a tool to diagnose SARA in milk.
Clinical signs that were often found in cows with SARA were decreased concentrate intake and lower fecal consistency. Also the filling of the rumen, the number of ruminal contractions and their strength, and ruminating activity was significantly reduced. Results of this study emphasize the importance of developing new testing strategies for detection of ruminal acidosis in milk. At this moment, clinical examination of the cows and the presence of several clinical signs can be used as a diagnostic tool to give an indication if SARA is present on cow or herd level.

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