Institute of Animal Science

Department of Animal Nutrition

Prof. Dr. Markus Rodehutscord

Prediction of ruminal acidosis in dairy cows from milk constituents

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Gero Marc Seyfang

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Dekan:	Prof. Dr. R. Vögele
Leitung des Kolloquiums:	Prof. Dr. J. Bennewitz
Berichterstatter, 1. Prüfer:	Prof. Dr. M. Rodehutscord
Berichterstatter, 2. Prüfer:	Prof. Dr. Dr. S. Dänicke
3. Prüfer:	Prof. Dr. L. Hölzle

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ABBREVIATIONS

aiso	Anteiso-methyl-branched
β0	Slope of curve
β1	Inflection point
c	Cis conjugated
CG	Confinement group
CP	Crude protein
CS	Corn silage
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
FA	Fatty acid
GC	Gas chromatography
GS	Grass silage
IP	Inflection point
iso	Iso-methyl-branched
LDH	Lactic dehydrogenase
MIR	Mid-infrared
peNDF	Physical effective neutral detergent fiber
PFL	Pyruvate formate lyase
pH_{max}	pH maximum of the day
pH_{mean}	pH mean of the day
pH_{min}	pH minimum of the day
pK_a	$-\log_{10}K_a$
RMSE	Root Mean Square error
SARA	Subacute rumen acidosis
SBPS	Pressed sugar beet pulp silage
t	Trans conjugated
TAG	Triacylglycerol
TMR	Total mixed ration
VFA	Volatile fatty acid
VFA_{total}	Sum of: acetate, propionate, butyrate, valerate, isobutyrate and isovalerate

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1 INTRODUCTION AND BACKGROUND

Evolutionary processes through the course of history of grazing have enabled ruminants to digest feedstuff that is mostly indigestible for monogastrics. With their ruminal digestion system, they are highly adapted to grassland-based diets, rich in high amounts of slow fermentable carbohydrates. Their rumen allows ruminants to meet their requirements for growth and physiological maintenance, including milk production, based on a diet consisting solely of fiber and urea (Virtanen, 1966) as long as production is at a moderate but not high level.

Energy density of the feed

Since the milk yield of dairy cows has increased steadily over the past decades, feeding of forages alone does not meet the requirements for milk production any longer. Therefore, the former prevalent feeding of forage mainly based on grassland products and straw, perfectly adapted to the digestive system of cows, had to be changed. More and more concentrate, which is high in starch but low in structure carbohydrates, was added to the feeds of high-yielding cows. With inclusion of concentrate in the ration, the energy density, as well as the utilisable crude protein for cows was increased at the expense of fibrous material. When the dietary forage is reduced the eating rate and the meal size increases as well (Owens et al., 1998), which helps to meet the energy and protein requirements for a higher milk yield. The feed is fermented in the rumen by archaea, bacteria, protozoa, and fungi. While structural carbohydrates are slowly degraded and metabolised to volatile fatty acids (VFAs), releasing their nutrients over a long period, they are not as highly digestible as carbohydrates in form of starch. Starch is degraded very rapidly and is processed by microorganisms to VFAs. If starch is available at a high quantity not all of the intermediates, like glucose, of this degradation process are directly used by microorganisms but are released to the rumen. Due to the availability of these intermediate products a shift in microbial composition in the rumen can occur.

Importance of feed structure

The decrease in fiber concentration in the feed can cause digestion disorders. Owing to a lack of structure in the rations; rumen motility can be reduced and ruminating activity can be impaired (Owens et al., 1998). When rumination activity is reduced, less saliva is produced, which is an

important source of hydrogen carbonate (to be discussed later). Hydrogen carbonate concentration increases when the portion of long particles is higher in the ration (Laporte-Uribe, 2019). In addition, the ruminal fibrous mat is not as properly formed as with enough fiber in the feed. Sutherland (1988) postulated that the fibrous mat helps to increase the retention time of solids in the rumen, by retention of small feed particles, which increases the time for their digestion. As an available model to assess the adequacy of fiber supply to dairy cows the "physically effective Neutral Detergent Fiber" (peNDF) is recommended (Committee for Requirement Standards of the Society of Nutrition Physiology, 2014).

In the past, Mertens (1997) showed that the peNDF which has particle lengths of more than 1.18 mm, the so-called $peNDF_{>1.18mm}$, has to be at least 22% of the ration to maintain a mean pH of 6.0 in the rumen and a $peNDF_{>1.18mm}$ concentration of 20% to maintain the milk fat concentration of Holstein cows at a level of 3.4%. Beauchemin et al. (2003) also stated that a concentration around 22% $peNDF_{>1.18mm}$ is needed to maintain a mean pH of 6.0. To avoid acidosis concentrations of 31.2% $peNDF_{>1.18mm}$ or 18.5% $peNDF_{>8mm}$ are necessary to prevent a critical low pH in the rumen (Zebeli et al., 2012).

The Committee for Requirement Standards of the Society of Nutrition Physiology (2014) stated that to maintain a, physiological harmless, average pH of 6.2 in the rumen the required peNDF is dependent on the dry matter intake (DMI), as well as on the starch content of the ration. For the $peNDF_{>1.18mm}$ with a level of 18 kg DMI a $peNDF_{>1.18mm}$ concentration between 18 and 32% is required if the degradable starch varies between 8 and 20% of DM. For the $peNDF_{>8mm}$ with 18 kg DMI the requirement of $peNDF_{>8mm}$ concentration varies between 12 and 18% if the total starch content of the ration is between 14 and 26%.

Products of ruminal fermentation

The produced VFAs in the rumen are acetate, propionate, butyrate, isobutyrate, valerate and isovalerate with the three main VFAs acetate, propionate, and butyrate. Between 20 and 35% of these VFAs are passed through the reticulo-omasal orifice to the posterior digestive tract (Dijkstra et al., 1993) while the remaining VFAs are absorbed by the rumen wall. Either, they are passively absorbed in their undissociated form, dependent of their lipophilic permeability: butyric acid > propionic acid > acetic acid (Walter and Gutknecht, 1986), or they are actively transported through the rumen epithelium if they are prevalent in their dissociated form, which are the highest proportions of the VFAs. Active transport takes place for VFAs being dissociated as an exchange with hydrogen carbonate (HCO_3) (Owens et al., 1998). Besides production, outflow

and absorption affect the concentration of the VFAs in the rumen; no accumulation of VFAs occurs if the feed provides a balanced mixture of fiber, nitrogen, and other carbohydrates. Another product of ruminal fermentation can be lactate. Depending on the intraruminal circumstances, it can accumulate in the rumen and is a stronger acid than the VFAs.

Interactions of buffers and acids in the rumen

The VFAs in the rumen, as well as lactate cause a pH in an acid range. These acids, being prevalent in the rumen, are buffered by buffers with a pH in a more alkaline range. Therefore, the pH of the rumen is dependent on the concentration of acids and bases in the rumen liquid. As long as the ruminal pH is in a range of around 6 to 7 it is in a physiological range. Therefore, every pK_a higher than 6.0 is a buffer against unphysiological low pH values which means that even weak acids with a pK_a between 6 and 7 can act as buffers for ruminal pH. More detailed definitions of different forms of acidosis will be presented below.

The prevalent buffers are hydrogen carbonates and phosphates contained in saliva. Furthermore the main prevalent inorganic base in the rumen is ammonia (Owens et al., 1998).

Hydrogen carbonate is the main buffer in the rumen and it is supplied by the saliva and in exchange for dissociated VFAs by the rumen wall. It can be found in the saliva at concentrations of about 125 mequiv./L (Bailey and Balch, 1961). The amount of hydrogen carbonate being released from the ruminal wall is at a similar level as from saliva. Hydrogen carbonate is a very potent buffer (pK_a = 10.3). Additionally, saliva provides about 26 mequiv./L phosphate to buffer the rumen liquid (Bailey and Balch, 1961).

The biphosphate (pK_a = 6.1) is additionally an important buffer, although it is not as strong as hydrogen carbonate (Aschenbach et al., 2011). Ammonia is a strong buffering base (pK_a = 9.2) as long as it is available in the rumen, although it does not accumulate to high concentrations in the rumen. Microorganisms metabolise 35 to 65% of the ammonia, and about 10% of the ammonium effluxes to the omasum or is absorbed by the ruminal wall ((McDonald, 1948; Kennedy and Milligan, 1980; Siddons et al., 1985; Obara et al., 1991) cited by Aschenbach et al. 2011). The urea reaching the rumen via the rumino-hepatic cycle is rapidly transferred to ammonia, which furthermore binds one proton that is being removed from the rumen when the ammonia leaves the rumen (Dijkstra et al., 2012).

If too high amounts of VFAs and lactate are produced in a short time they can accumulate in the rumen liquid to an extent, where they can neither be buffered by endogenous buffers nor be

absorbed fast enough by the rumen wall. This results in a decrease of the ruminal pH. A drop in pH to an unphysiological level is called acidosis. If it is a drop to very low pH values it is called a severe acidosis, as long as the drop is in a moderate range, it is called subacute ruminal acidosis (SARA).

Subacute ruminal acidosis (SARA)

The negative effects of acidosis have been in the focus of animal nutritionists for a long time and several negative effects were found to be related with SARA. As Aschenbach et al. (2011) summarised, the ”clinical presentation of the mild form, called subacute ruminal acidosis (SARA), varies and may include mild transient anorexia, intermittent diarrhea, dehydration, poor body condition, depression, decreased rumen motility, laminitis, unexplained abscesses, and decreased milk production (Dirksen, 1970, 1985; Underwood, 1992; Nordlund and Garrett, 1994; Duffield et al., 2004; Krause and Oetzel, 2006)”. Owing to these negative effects, intensive research is conducted on the detection and prevention of this acidotic condition. Since the lack of obvious clinical symptoms of SARA and the fact that the symptoms can show up with several weeks of delay (Nordlund and Garrett, 1994), research has been undertaken in an attempt to develop prediction approaches of acidotic circumstances in the rumen.

SARA can be mainly found in two phases of the lactation. Around parturition, at the early phase of lactation, when the feed is changed to feed high in energy density and the feed intake is increasing, a rapid adaptation is happening. In this phase a drop in pH to unphysiological levels can occur. Also in mid-lactation, when the feed intake is high and therefore high amount of fermentable nutrients are prevalent in the rumen, a drop in pH level can be observed.

Only few research activities were made about the financial costs of SARA. Abdela (2016) has reviewed SARA prevalence of 11 - 29.3% in early-lactation and 18 - 26.4% in mid-lactation cows. The latter author also referred to Donovan (1997) who estimated costs to the US dairy industry at $ 500 million to $ 1 billion, more than 20 years ago.

Measurement of ruminal pH

Ruminal pH measurements are made to detect acidotic circumstances in the rumen liquid. Therefore, samples of rumen liquid via ruminocentesis or oral sampling are taken in practice (Garrett et al., 1999; Duffield et al., 2004). If cannulated animals are available rumen liquid can be

taken through the cannula (Penner et al., 2006). Since these spot samples only reflect the pH conditions at this one moment of spot sampling, the continuous pH measurement becomes increasingly important which can measure the pH value in the rumen continuously.

It was mentioned before that a stratification exists in the rumen, with an increased VFA content in the dorsal part of the rumen with a lower pH than in the ventral sac of the rumen (Tafaj et al., 2004). The pH is higher in the reticulum than in the ventral sac of the rumen (Sato et al., 2012a; Falk et al., 2016) which has to be considered if pH is not measured ventrally.

Samples taken orally tend to be influenced by saliva, which buffers the sample and falsifies the measured pH value. Ruminocentesis and rumen liquid sampling through the cannula must not cope with this contamination. However, if the pH is measured outside the rumen it is slightly higher than the measurement within the rumen, which will be closely analysed in chapter 7.

For cannulated cows, multiple measuring devices are in use that are placed in the ventral sac of the rumen and remain there with the help of attached weights. All these devices consist of a pH electrode attached to an anchor weight. They either use an intern data logger, which can be read when removed from the rumen, an intern data storage device that is capable of transmitting the data without being taken out of the rumen or a cable connection to an external data storage. Different working groups used different approaches to measure ruminal pH (Graf et al., 2005; Duffield et al., 2004; Maekawa et al., 2002b; Rustomo et al., 2006a; Rustomo et al., 2006b; AlZahal et al., 2009; Sato et al., 2012b). Commercial pH recording systems, e.g. DASCOR (Penner et al., 2006) are also available. For a wider application in the field, there are also a couple of sensors available at the market, being able to estimate the pH in the rumen of non-cannulated cows. Either in the reticulum; e.g. smaXtec (Gasteiner et al., 2012), wellCow (Mottram et al., 2008), eCow (Hanušovský et al., 2015; Mensching et al., 2021) or floating in the ruminal mat; Kahne Limited (Lohölter et al., 2013). If inserted via cannula and attached to weights most of these devices can be used in the ventral sac of the rumen.

Definition of SARA based on ruminal pH

Although many working groups directed their research on the rumen and the variation of intraruminal pH value, there is no single and unambiguous definition of SARA. Over the years, a multitude of definitions were proposed. In general, authors agree that a ruminal pH between pH 5.5 and pH 5.8 may indicate conditions of SARA.

Owing to the widespread definitions, Keunen et al. (2002) measured the time and the area under the curve below pH 6.0 and 5.8, without defining how long pH has to be below these thresholds to be considered a case of SARA or how big the area under the curve has to be to identify SARA. Also, Rustomo et al. (2006a) displayed the time and the area under the curve for every 0.2 pH step between 5.0 and 7.0 without selecting thresholds for SARA definition.

Garrett et al. (1999) suggested a pH of 5.5 measured in rumen liquid obtained by rumenocentesis from the caudoventral sac is a useful cut off point for SARA circumstances in the rumen. According to these authors it is a strong indication that more than 30% of the herd suffer from low pH if three or more cows out of 12 cows are detected with a pH of 5.5 or lower while if only two out of 12 cows have a pH of 5.5 or lower the incidence of SARA in the herd is 15% or lower. Nordlund (2003) defined acidosis if 30% of at least 10 cows showed a pH below 5.5 when the pH reaches its approximate minimum, 4 to 8 hours after TMR feeding. Cooper et al. (1997) proposed the average pH threshold of 5.6 because below this pH value a reduced feed intake was observed.

Bevans et al. (2005) considered acidotic circumstances when the pH in the ventral rumen is below 5.6 for more than 12 h and defined an acute acidosis when the pH is < 5.2 for more than 6 hours/day. Martens et al. (2012) defined SARA conditions as pH < 5.6 for longer than 3 hours/day. AlZahal et al. (2007) suggested definitions for SARA for pH thresholds of 5.6 and 5.8. It was proposed to identify SARA when pH < 5.6 for 148 to 283 min/day or pH < 5.8 for 284 to 475 min/day are observed. The latter authors also proposed the mean pH of the day (pH_{mean}) below 6.01 to 6.17 as SARA condition.

Zebeli et al. (2008) stated in their study that a combination of two factors needs to be present for a SARA incidence. The pH_{mean} has to be lower than 6.16 and the time below pH 5.8 has to be longer than 5.24 hours/day.

In the present work the definition of SARA as given by Zebeli et al. (2008) was used, defining the subacute acidosis if the pH in the rumen drops to < 5.8 for more than 5.24 hours/day and the daily pH_{mean} is lower than 6.16.

Since reticular pH measurements are comparably easy to use and ready to use systems are available on the market, many recent publications base their studies on pH measured in the reticular rumen. Sato et al. (2012) compared reticular and ventral rumen pH but were not able to predict a conversion factor. Falk et al. (2016) found a 0.24 pH higher pH_{mean} in the reticulum compared to ventral measurement, but were also unable to define a conversion factor between reticular pH and ventral rumen pH.

Franceiso et al. (2020) did not define a SARA threshold but analysed the times the reticuloruminal pH was below 5.6 and 5.8 in their study. Gao and Oba (2014) measured the ventral pH continuously and introduced an acidosis index which was calculated as the area under curve (pH < 5.8 * min) / DMI (kg). Coon et al. (2019) used this acidosis index from Gao and Oba (2014). The SARA risk threshold was then defined if, in the reticulum, the area under curve (pH < 5.8 * min) / DMI (kg) was 0.5 or greater.

Jonsson et al. (2019) designed a SARA herd status evaluation based on the thresholds of Garrett et al. (1999), as mentioned above, corrected by the factor of 0.24 higher pH which was found by Falk et al. (2016). So, their SARA incidence threshold was a reticuloruminal pH of 6.04. They found a preferable number of 9 boli to estimate the heard reticuloruminal pH_{mean} with a tolerance of 0.5.

Humer et al. (2015) defined cows as SARA susceptible if the duration of reticuloruminal pH < 5.8 exceeded 330 min/day at least during one of the first eight days after parturition.

Denwood et al. (2018) used a different approach to evaluate the ruminal pH circumstances. Instead of focusing on parameters, like pH_{mean} and times below pH thresholds the latter authors put their focus on the pH course of the day. They fitted a model of a sinus wave for the reticuloruminal pH course during the day and counted the residuals for the real observed data. With the help of these differences, they were able to detect animals that had deviations in their pH pattern. However, they did not define a threshold for SARA conditions depending on those residuals.

Parameters under research linked with SARA

Mensching et al. (2021) introduced a SARA risk score, using not only reticular pH_{mean} and the fluctuation of reticular pH, but also the median of the reticular temperature and the rumination parameters, the mean rumination chewing frequency, the daily lying duration, as well as milk fatty acids predicted with mid-infrared (MIR) spectrograph measurement. Combining those parameters from different types of measurements might not be very specific to exclusively SARA incidences. Nevertheless, the addition of a multitude of sub acute signals of animals' health status can help to give a warning if the general health status of a cow becomes critical.

Since the technical and financial effort of pH measurements in the ventral rumen is quite high, other parameters were considered to be potentially helpful to identify SARA. A prediction based on "ME, DM digestibility, OM digestibility, NDF digestibility, ADF, NSC, milk protein percentage, or

the proportion of propionate or butyrate" (Kolver and Veth, 2002) in an analysis of 23 studies failed though.

In a review, Tajik and Nazifi (2011) showed that other attempts are being made to identify the number of SARA incidence days, for example by measuring the concentration of rumen lipopolysaccharides (Gozho et al., 2005; Plaizier et al., 2008) which increases if SARA is induced. The rumen microbial composition was under observation as an indicator for SARA (Plaizier et al., 2008; Nagaraja et al., 1978; Goad et al., 1998; Khafipour et al., 2009). The pH in the urine was also proposed (Enemark et al., 2002) but could not be confirmed (Kleen et al., 2003; Tajik et al., 2009; Gakhar et al., 2008). Blood metabolites were under research for a SARA detection, but it was found that "lactate, non esterified fatty acids, cholesterol, albumin, urea, Na, Cl, K, Ca, P, insulin, triiodothyronine, thyroxine, growth hormone and cortisol as well as blood packed cell volume, gas parameters, white blood cells and plasma glucose […] (Bevans et al., 2005; Brown et al., 2000; Enemark et al., 2002; Gakhar et al., 2008; Goad et al., 1998)" (Tajik and Nazifi, 2011) do not lead to a significant change in the microbiome.

There have been previous attempts to estimate ruminal pH minimum from temperature in the rumen fluid (AlZahal et al., 2008) with prospects of success. Since pH boli can measure temperature reliably, in recent works the ruminal temperature is published. Either as information about the temperature status (Laporte-Uribe, 2019), or with the attempt to correlate reticular pH with reticular or rectal temperature (Humer et al., 2017). Nonetheless, the latter authors could not find a correlation between temperature and reticular pH.

Dijkstra et al. (2020) showed in a recent review that there is the potential of redox potential measurement in the rumen for SARA detection. Since the redox potential in the rumen liquid reflects the intracellular redox balance status (Liu et al., 2013) and significant relations were found between the redox potential, the diet, and rumen characteristics in a meta-analysis (Huang et al., 2018). The pH is also related to the redox potential level, which becomes less negative when the pH decreases. Although some sensors in the market are able to measure the redox potential, the redox potential is not often gathered and published, since the measurements have to be initially corrected for the type of electrode and sensor used. However, since the redox potential in the rumen is negative, the available standards are positive (Dijkstra et al., 2020) increasing the difficulty of calibration.

Milk analyses are used for years in the ruminal acidosis detection. Several authors identified a depressed fat content, e.g. below 2.5% (Griinari et al., 1998; Palmonari et al., 2010) or 2.2% fat (Sutton et al., 2003) in the milk as a strong indicator for a ruminal acidosis.

Milk fatty acids

About 95% of the milk fat consists of triacylglycerols (TAGs) consisting of more than 400 different fatty acids (Bauman and Griinari, 2001). This great amount of different fatty acids found in the milk is based on the fact that milk fat is built with FAs from different origins.

Fatty acids (FAs) can be branched or unbranched and saturated or unsaturated. Unsaturated FAs can be cis (c) or trans (t) conjugated and if there are multiple cis or trans conjugations in one FA it is polyunsaturated. Owing to the possibility of odd- and even-numbered chain length, the number of theoretical possible FAs is extremely high.

One of the origins is FAs being synthesised by *de novo* synthesis in the mammary gland based on VFAs from the rumen. These VFAs reach the mammary gland via the bloodstream after being mainly absorbed in the rumen. Thereby the most common primary carbon source is acetate. Following primary conjunction, the elongation is always happening in cycles of two carbon atoms (Hawke and Taylor, 1995). Other VFAs can be the primary carbon source as well. If butyrate is the primary VFA it leads, like acetate, to an even-numbered FA. Propionate and valerate can be used as primary carbon source, leading to an odd-chain FA.

As another origin, FAs longer than valerate, are transported to the mammary gland via blood circulation as well. These fatty acids are either decarboxylated in the mammary gland or without any further decarboxylation transferred into the milk fat. These longer chain FAs either are available from the feed or are produced by the ruminal microbiome. The microbiome either *de novo* synthesised the FAs or decarboxylated FAs originating from the feed, to the form that is then available in the mammary gland.

In addition, body fat mobilisation is relevant if the cow is in a negative energy balance and supplies FAs from the body fat. With this transformation in the body, FAs from long time consumed feed and its associated microbiome can be found in the milk fat. Therefore, there is a high variability in FAs in the milk but it is very difficult to trace where the FAs in the milk originated. The fatty acids in the chain length range from C4 to C14, found in the milk fat are *de novo* synthesised, while C16 can originate either from the *de novo* synthesis, the feed, the microbiome or the bodyfat mobilisation, as mentioned above. Longer FAs than C16 in the milk cannot come from *de novo* synthesis but from the other origins mentioned above. About 40 to 60% of the milk FAs are long-chain FAs (C17+), of which 88% were derived directly from TAGs of intestinal lipoproteins and 12% were derived from TAGs of endogenous origin (Palmquist and Conrad, 1971).

Microorganisms in the rumen

The FAs, which were not *de novo* synthesized, are highly dependent on the microbiome in the rumen, which itself is highly dependent on the energy source provided by the feed. There are several strains of bacteria predominantly using different carbon sources for their energy requirements. There are two big groups of bacteria that play an important role in the rumen, cellulolytic and amylolytic bacteria. If starch is available to a large extend, amylolytic bacteria utilising starch and intermediates from starch degradation, can build up to 90 - 95% of cultivable bacteria in the rumen (Leedle and Hespell, 1980).

The group of amylolytic bacteria include: *Selenomonas*, *Succinimonas*, *Streptococcus*, *Bifidobacterium*, *Butyrivibrio*, *Eubacterium*, *Lactobacillus*, *Mitsuokella*, *Prevotella*, *Ruminobacter* and *Succinivibrio* (Nagaraja and Titgemeyer, 2007).

The group of cellulolytic bacteria include: *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes, Butyvibrio fibrisolvens*, *Clostridium lochheadii* (Castillo-González et al., 2014; Belanche et al., 2012).

The acetate to propionate ratio in the rumen is affected by these two groups of microorganisms. While the cellulolytic bacteria do not produce propionate as fermentation product, there are some strains among the amylolytic microorganisms—*Selenomonas ruminantium* and *Succinomonas amylolitica*—which produce propionate. Therefore, the acetate to propionate ratio in the rumen liquid can be used as an indicator for the prevalence of amylolytic microorganisms. The higher the proportion of propionate, the greater the number of amylolytic microorganisms in the rumen is.

The amylolytic bacteria *Selenomonas ruminantium* and *Streptococcus bovis* are furthermore lactate producer. As Nagaraja and Titgemeyer (2007) outlined *S. bovis* is a species in the rumen with an abundance of up to 10^{11}/g rumen content during the adaptation phase to a new feed, when easily fermentable carbohydrates are offered. Such a change in feed with an increase of carbohydrates in the rations happens around parturition. It has a short generation time of 12 minutes and a very fast rate of fermentation. *S. bovis* is able to produce acetate, formate and ethanol, but if the pH is low it shifts to lactate production. Therefore, it is one of the main lactate producers in the rumen if the pH is below 5.6. The pathway in *S. bovis* is dependent of the activity of pyruvate formate lyase (PFL) (optimum pH 7.5) and lactic dehydrogenase (LDH) (optimum pH 5.5). This optimum pH for LDH in the organism is reached when the pH in the rumen is 5.0 or lower (Russell and Hino, 1985; Russell, 1991). At a pH value of 6.0, the activity of PFL is reduced to less than 10% (Asanuma and Hino, 2002). Therefore, the production of lactate

increases, the lower the pH in the rumen is. If the ruminal pH drops below 6.0, *S. bovis* is replaced by *Lactobacilli* which are lactate producers and more tolerant to acidotic circumstances in the rumen (Finlayson, 1986; Wells et al., 1997). Since lactate has a low pK_a (3.8), it is a strong acid and, thus, exacerbates the decrease in ruminal pH into an acidosis even further, if not metabolised by other microorganisms.

Other microorganisms, like *Selenomonas lactilytica* or *Megasphera elsdenii* are able to utilise lactate so that lactate does not accumulate under normal circumstances to a concentration above 5 µM (Owens et al., 1998). *Megasphera elsdenii* is known to metabolise up to 60 - 80% of the lactate produced in the rumen (Counotte and Prins, 1981). Since these lactate utilisers are also pH sensitive and cannot withstand acidotic conditions, lactate is not metabolised to the same extend while it is produced more excessively when the ruminal pH drops.

When there is an overwhelming availability of easily fermentable carbohydrates not only the amount of end products in the rumen liquid increases, but intermediates of microbial fermentation, like glucose also increase. The free glucose is used by microbes, which normally are not prevalent and can now compete and grow rapidly. In addition, the glucose boosts opportunistic microbes like coliforms and populations of amino acid decarboxylating microbes that may produce or release endotoxins or amides. Furthermore, it increases the osmolality in the rumen which reduces VFA absorption (Tabaru et al., 1990) and lowers the ruminal pH.

Impact of microorganisms on the fatty acid composition of the milk

The VFAs, which are the products of the microbiotical fermentation, differ between different strains, with some strains being known to mainly produce acetate while others mainly produce propionate. In addition, the microorganisms are producing different types of fatty acids and proteins, e.g. as compounds of their cellular membrane. Therefore, different bacterial species do not only have an impact on the cow through their fermentation products, but also through their cellular composition, which becomes available when the microbiota is digested in the abomasum. Thereby the branched-chain amino acids, valine, leucine, and isoleucine, as well as their corresponding carboxylic acids, isobutyric, isovaleric and 2-methyl butyric acid are released. These are precursors for the branched-chain FAs in the milk fat in the iso-methyl-branched (iso), as well as in the anteiso-methyl-branched (aiso) occurrence. C13:0 iso, C13:0 aiso, C14:0 iso, C15:0 iso, C15:0 aiso, C16:0 iso, C17:0 iso, C17:0 aiso, and C18:0 iso are more prevalent in cellulolytic species than in amylolytic species (Fievez et al., 2012). Vlaeminck et al. (2006b) mentioned that earlier research by Ifkovitz and Ragheb (1968), as well as Minato et al. (1988) showed that “some major

cellulolytic bacteria are enriched in C14:0iso and C15:0iso FAs". The concentrations of these branched-chain FAs in the milk can therefore be used as indicators of the prevalence of cellulolytic microorganisms in the rumen.

Amylolytic bacteria, e.g. *Ruminobacter amylophilus* and *Succinivibrio dextrinosolvens* lead to milk fat that is enriched in linear odd-chain FAs but low in branched-chain FAs (Vlaeminck et al., 2006b). It is also reported that an increased use of propionyl-CoA for the *de novo* synthesis in the mammary gland is leading to odd-chain FAs (Vlaeminck et al., 2006a; Emmanuel and Kennelly, 1985; Rigout et al., 2003). The positive relation between C15:0 in the milk and propionate concentration in the rumen might be explained by these findings (Colman et al., 2010). Colman et al. (2010) reported that C13:0 iso, C16:0 iso and C18:2 c9,t11 were useful in the identification of SARA in their study.

Objectives of own work

It was a point of interest to identify the changes of the pH milieu in the rumen with its coherent change in the milk fat profile. Since the milkfat changes highly in the early stage of lactation, cows in their mid-lactation were chosen, where the milkfat composition is more stable and the body fat mobilisation is low or not happening. This also increased the time window for the trials, so that there was a sufficient adaption phase for every ration. Also, it was possible to choose a Latin square for two of the three trials, enabling to test and statistically analyse more than one ration on every cow under conditions where the rumen is adapted to the feedstuff offered.

In three trials, eight different types of rations were fed to the cows. All of these were potentially capable to induce a pH milieu that is in the threshold area, where either a potential SARA day or a non-acidotic day can occur. The pH was monitored and interpreted as described below. For all three trials, the relevant data were gathered and interpreted in an intertrial evaluation. The objective of this intertrial approach was to investigate whether a feed independent interpretation of FA profiles in the milk is possible, although the feed components do play a very important role for the composition of the milk fat. Furthermore, an initial model was created using in field accessible parameters to predict SARA status, although the dataset was very small.

2 EVALUATION OF CONTINUOUS pH MEASUREMENTS

When the pH in the rumen is measured continuously by an inserted pH measuring device in freely chosen intervals, a lot of data can be generated in the course of the day. Owing the large number of measurements, an interpretation is difficult, as evident from the example given in Figure 2.1, where the ventral ruminal pH data from two cows for one day are graphically shown.

Generally, the pH value in cow 1 between 06:00 and 11:00 was ~0.3 pH values higher than for cow 2, but then the pH value in the rumen of cow 1 decreased more than the pH value of cow 2. While the pH of cow 1 stayed quite constantly low until midnight, the pH value of cow 2 increased in the evening for a short period and then dropped again.

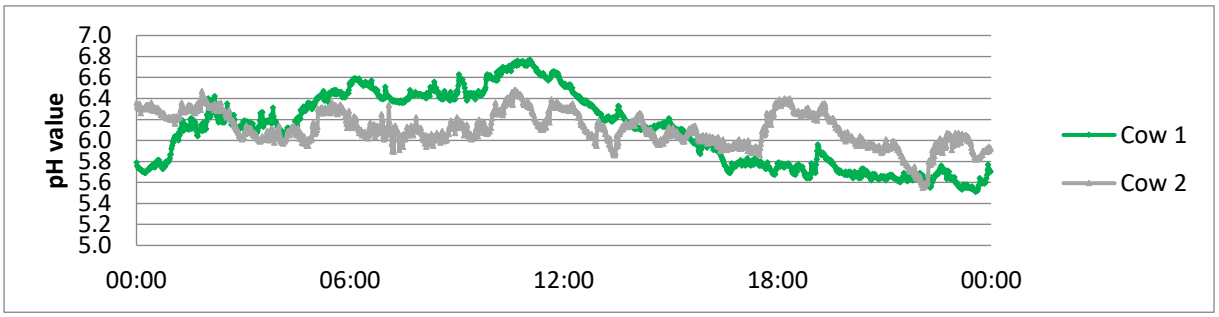

Figure 2.1 Example for two continuous pH courses during a 24 h measurement interval (data taken from one own experiment)

When attempting to analyse the data from Figure 2.1 some main characteristics can be gained quite easily. The pH minimum of the day (pH_{min}) and the pH maximum of the day (pH_{max}) values are easy to access, and enable a first impression whether the pH drops to a critical point during the day and if the ruminal pH alternates massively. However, the information about the extremes is not sufficient because it contains no information whether the extremes in ruminal pH value lasted for a longer period or only for a short time. As in the given example, the pH value of cow 2 reached its minimum (pH = 5.6) at ~ 23:00 for one short time period, while for cow 1 the pH_{min} (pH = 5.5) was for a quite long period in a very close range to pH_{min}.

The mean pH (pH_{mean}) is more resistant against short-term extremes in ruminal pH conditions than pH_{min} and pH_{max}. Both the cows in the given example had a pH_{mean} of 6.1 (Table 2.1).

Table 2.1 Main characteristics from the 24 h measurement shown in Figure 2.1

pH characteristics	Cow 1	Cow 2
mean pH of the day	6.1	6.1
pH minimum of the day	5.5	5.6
pH maximum of the day	6.8	6.5

With the data from Table 2.1, the information about the diurnal pH variation remains unconsidered. To get a better description of the pH conditions in the rumen the pH data gained from the continuous pH measurement have to be used and reduced to more simple parameters. For this purpose, the data from the continuous pH measurement have to be transformed. For every pH value between pH_{min} and pH_{max}, the time spent below every specific pH value is counted and summarised as shown in Table 2.2 for the given example.

Table 2.2 Minutes per day below a certain pH threshold during the 24 h measurement shown in Figure 2.1

pH threshold	Cow 1 min/day	Cow 2 min/day
5.5	0	0
5.6	48	8
5.7	241	24
5.8	464	46
5.9	518	95
6.0	567	332
6.1	634	733
6.2	846	976
6.3	937	1204
6.4	1038	1410
6.5	1237	1440
6.6	1332	1440
6.7	1398	1440
6.8	1440	1440

With the help of this transformation, the subjective information that the pH of cow 1 was longer close to the pH_{min} than the pH of cow 2, as described above, can now be specified, e.g. that cow 1 had a ruminal pH lower than pH 5.8 for 464 minutes while cow 2 only had 46 minutes below pH 5.8.

These differences become more apparent while showing the transformed data from Table 2.2 in a graph with the cumulated time spent (y-axis) below a certain pH value (x-axis) as illustrated in Figure 2.2.

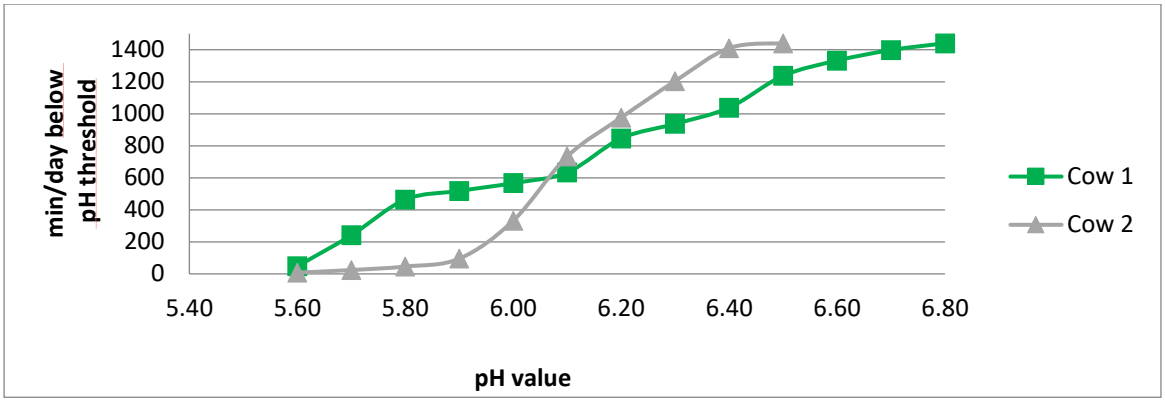

Figure 2.2 Cumulated time ruminal pH was below pH thresholds during the 24 h measurement shown in Figure 2.1

AlZahal et al. (2007) suggested that fitting a logistic curve to the data is a proper way to describe continuous pH measurements, by reducing the dataset from the continuous measurement to two factors, the Inflection Point (IP) of the logistic curve and the slope in the IP. Colman et al. (2012) continued using this mathematical approach of AlZahal et al. and also used a logistic curve for their studies with Equation 2.1:

$$y = \frac{\beta 2}{1 + \exp[-\beta 0 * (x - \beta 1)]}$$

Y = minutes/day
β0 = Slope of curve
β1 = Inflection Point (IP)
β2 = upper limit = 1440 minutes
x = pH level

The parameters β0 and β1 are predicted for every measuring day individually by using transformed data in the form of the example shown in Figure 2.2. The code to run the Equation 2.1 in SAS is shown in Equation 2.2 in the appendix. Using the logistic curve, for the given example, the graph with the accumulated time spent (y-axis) below a certain pH value (x-axis) is shown in Figure 2.3.

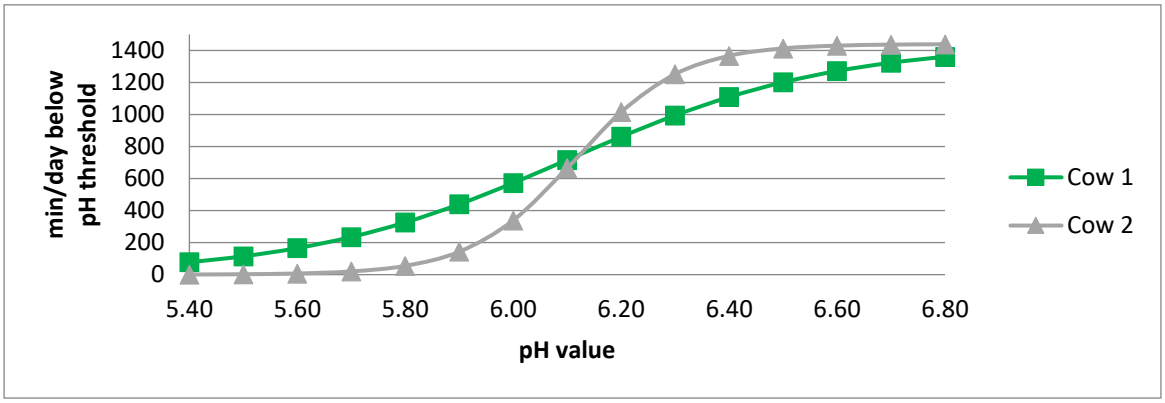

Figure 2.3 Time below pH threshold estimated using Equation 2.1 during the 24 h measurement shown in Figure 2.1

For both cows in this example, the IP β1 was 6.1. For cow 1, the slope β0 was 4.06 and for cow 2 the slope was 10.25. The pH value of the IP is the pH_{mean} and is always at the 720-minute mark on the y-axis. The bigger β0 is, the smaller is the amplitude between pH_{min} and pH_{max}. A small amplitude can be interpreted, as a robust ruminal pH where the pH range around the mean is relatively small. A low value for β0 indicates a broad variation of pH during the measurement period and a long time closer to pH_{min} and pH_{max}.

With the parameters β0 and β1, it is possible to estimate the time below any threshold from the literature defining an acidosis. As mentioned in the introduction various definitions are available. Figure 2.4 shows the two days of measurement of the given example and various SARA definition thresholds. Every measurement that is on the left side of the cut-off line is defined as an acidotic day. With the definitions given by Bevans et al. (2005) and Martens et al. (2012) none of the cows showed acidotic circumstances. Both the cows showed acidotic circumstances if the threshold $pH_{mean} < 6.17$ is used, but not if the benchmark 6.01 is used, according to AlZahal et al. (2007). For the definitions of AlZahal et al. (2007), the span of time the pH is below 5.6, as well as pH < 5.8, it is depending whether cow 1 showed SARA or not from the definition used. With the definition given by Zebeli et al. (2008) cow 1 revealed no SARA condition, while cow 2 was defined to be in an acidotic condition with pH_{mean} = 6.1 and 326 min at a pH below 5.8.

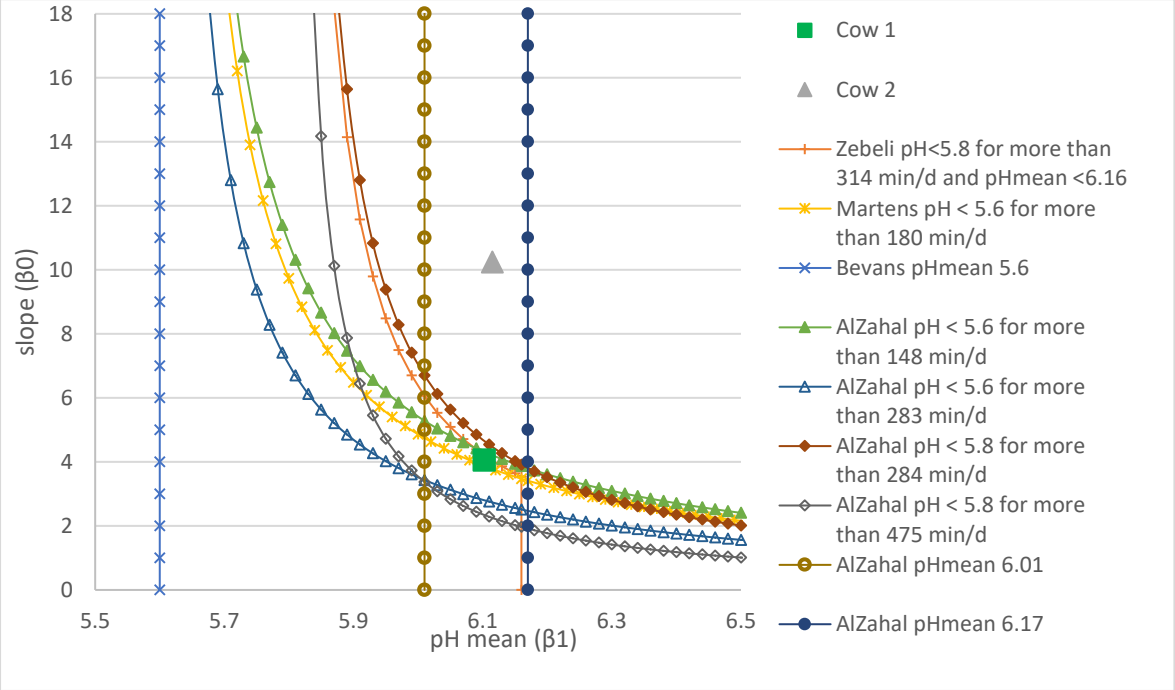

Figure 2.4 SARA incidence determination during the 24 h measurement shown in Figure 2.1. Every threshold line stands for a different SARA definition. If the point of measurement (β1 x β0) is on the left side of the threshold line, the measured day is acidotic, if it is on the right side, non-acidotic defined

For the two cows in the previous example with a β1 = 6.1 the acidotic slope of β0 is 4.24 or lower. As mentioned above the definition of Zebeli et al. (2008) is used as benchmark of SARA in the present work. The ruminal pH is predicted with a logistic curve as supposed by AlZahal et al. (2007). A day is defined as acidotic when the ruminal pH spent longer than 5.24 h (> 314 min) below 5.8 and the β1 is below 6.16. For every β1, an individual β0 exists as cut off point for acidosis detection. For every β1, the minimal slope of SARA incidence can be predicted by $\beta 0 = -\frac{ln\left(\frac{1440}{315}-1\right)}{5.8-\beta 1}$. Figure 2.5 illustrates that the closer β1 lies to 5.8, the more stable the pH value has to be and therefore β0 has to be bigger to avoid SARA.

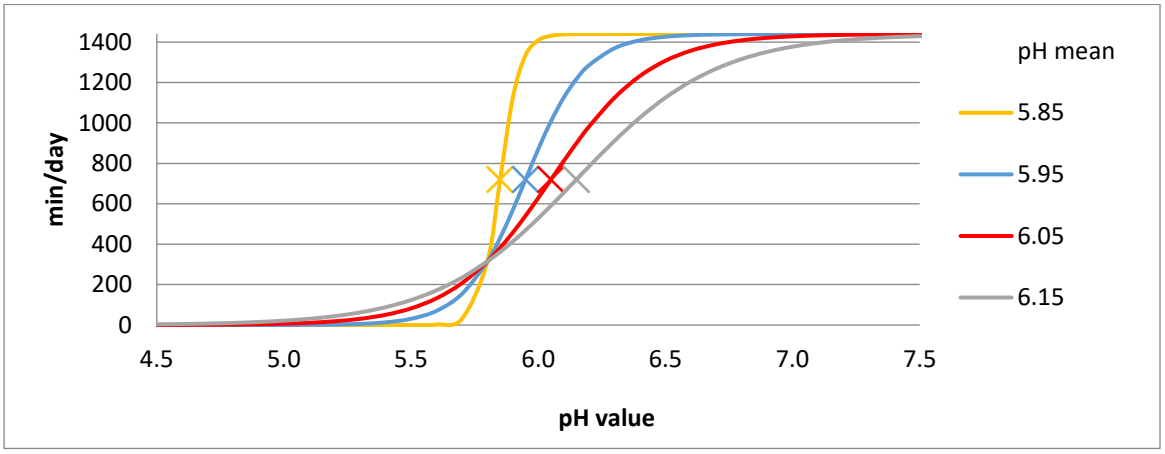

Figure 2.5 Exemplary SARA days that fulfill the minimum requirements of Zebeli et al. (2008) exactly

For the highest pH_{mean} (6.15) where SARA still could occur, according to the definition used, a slope for β0 of 3.64 or smaller would lead to a SARA while for a β1 of 5.85 SARA occurs if the slope is higher than 25.5. A slope of 25.5 and a pH_{mean} of 5.85 would imply that the pH in the rumen does only vary between 5.7 and 6.0 for almost the whole day, 1380 minutes per day. A slope of 3.5 with a pH_{mean} of 6.15 would imply that the ruminal pH varies between 5.1 and 7.2 within 1380 minutes of the day. Both pH courses are physically highly doubtful to be seen *in vivo*.

This interaction between β1and β0 can be seen in Figure 2.4. The higher the pH_{mean} is, the lower the slope has to be, if the definition includes a time spent below pH threshold.

3 TRIAL 1

The objective of this trial was to gain knowledge about the influence of two types of silages, corn silage (CS), and pressed sugar beet pulp silage (SBPS), as well as two levels of concentrates, 20% and 60% of the ration, when SBPS comprised the forage, on ruminal fermentation characteristics and milk composition of dairy cows.

3.1 Materials and Methods

3.1.1 Animals and animal housing / Experimental design

The trial was conducted at the Friedrich-Loeffler-Institute in Brunswick, Germany in accordance with the German Animal Welfare Act approved by the Lower Saxony State Office for Consumer Protection and Food Safety.

Three cannulated Holstein cows with an average of 160 days in milk when the first pH measurements were taken, were randomly allocated to three groups of 15 animals each. Each group was fed with a different ration. The cannulated cows changed the groups following the design of a 3x3 Latin square after 21 days, while the rest of the feeding group remained on the same ration all the time. For the adaptation to the new ration, the cannulated cows were separated from the group and received a mixture of their previous and their new ration with 25% increase per day of the new ration (mixed on fresh matter basis). After three days of adaptation, the cows were brought to the main barn and were integrated in the respective group.

3.1.2 Rations

The rations were mixed each morning between 9:00 and 11:00, the feed residues were removed from the troughs and the new feed was distributed. The feed was available ad libitum and an oversupply of 5% was calculated.

3.1.2.1 Ration components

All rations consisted of 20% grass silage (GS) and the two levels of concentrate were 20 and 60% of dry matter (DM) of the rations. The remaining 60 or 20% of DM of the rations consisted of CS or SBPS as shown in Table 3.1.

Table 3.1 Composition of the rations used in Trial 1 (percent, on DM basis)

	Ration		
	CS60[1]	SBPS60[2]	SBPS20[3]
Grass silage	20	20	20
Sugar beet pulp silage	-	20	60
Corn silage	20	-	-
Concentrate20[4]	-	-	20
Concentrate60[5]	60	60	-

[1] CS60 = CS ration with 60% concentrate
[2] SBPS60 = SBPS ration with 60% concentrate
[3] SBPS20 = SBPS ration with 20% concentrate
[4,5] composition shown in Table 3.2

For the 20% concentrate treatment the composition of the concentrate had to be changed to meet the nutritional requirements of the animals. The concentration of crude protein (CP) provided by solvent-extracted rapeseed meal in the concentrate was almost tripled as well as the concentration of minerals and urea and replaced the cereal grains as shown in Table 3.2.

Table 3.2 Composition of the concentrates used in Trial 1 (percent, on DM basis)

	Concentrate60	Concentrate20
Rapeseed meal	12.7	38.0
Wheat	28.0	20.0
Barley	13.0	10.0
Corn	27.0	15.0
Dried beet pulp	16.6	10.0
Dicalciumphosphate	0.6	2.5
Limestone	0.5	-
Mineral feed (BASU 243401)[1]	0.9	2.5
Urea	0.7	2.0

[1] Composition (per kg): 140 g Ca, 120 g Na, 70 g P, 40 g Mg, 6000 mg Zn, 5400 mg Mn, 1000 mg Cu, 100 mg I, 40 mg Se, 25 mg Co, 1000000 IU Vitamin A, 100000 IU Vitamin D_3, 15000 mg Vitamin E

The resulting chemical composition of the rations used in Trial 1 is shown in Table 3.3. The SBPS20 ration had a higher proportion of crude fiber and a higher proportion of crude ash than the rations with more concentrate, while lower concentrations of CP and ether extract.

Table 3.3 Calculated chemical composition of experimental diets used in Trial 1 (g/kg, on DM basis)

	CS60[1]	SBPS60[2]	SBPS20[3]
Crude ash	59	63	75
Crude protein	141	145	133
Ether extract	36	32	21
Crude fiber	137	137	189
Neutral detergent fiber[4]	357	364	508
Acid detergent fiber[5]	163	165	230

[1] CS60 = CS ration with 60% concentrate
[2] SBPS60 = SBPS ration with 60% concentrate
[3] SBPS20 = SBPS ration with 20% concentrate
[4] Neutral detergent fiber = aNDFom
[5] Acid detergent fiber = ADFom

3.1.2.2 peNDF

The content of physically effective NDF (peNDF) was analysed for each ration every day in duplicate, according to Kononoff et al. (2003) and the Committee for Requirement Standards of the Society of Nutrition Physiology (2014).

Table 3.4 peNDF content of rations used in Trial 1 (n = 57, Least Squares Means, *Standard Deviation, Minimum and Maximum)*

peNDF (% of DM)	CS60 >8mm	CS60 >1.18mm	SBPS60 >8mm	SBPS60 >1.18mm	SBPS20 >8mm	SBPS20 >1.18mm
Mean	14	23	14	24	29	46
Std. dev.	2	2	1	1	2	1
Min	10	19	11	21	25	43
Max	19	26	17	27	32	48

With concentrations of 14% $peNDF_{>8mm}$ and 23%, or 24% $peNDF_{>1.18mm}$ the rations, including 60% concentrate were very similar to each other, while the SBPS20 ration had higher peNDF concentrations (29% $peNDF_{>8mm}$ and 46% $peNDF_{>1.18mm}$).

3.1.3 Samplings

Samplings took place following the sampling overview in Table 3.5. Detailed information about samplings can be found in chapters 3.1.3.1, 3.1.3.2 , 3.1.3.3 and 3.1.3.4.

Table 3.5 Overview of sampling procedure in Trial 1

	Treatment											
	CS60				SBPS60				SBPS20			
Date	pH	MIR-Milk	GC-Milk	Rumen liquid	pH	MIR-Milk	GC-Milk	Rumen liquid	pH	MIR-Milk	GC-Milk	Rumen liquid
12.5.2013					x	x			x	x		
13.5.2013	x	x							x	x		
14.5.2013	x	x			x	x						
15.5.2013					x	x			x	x		
16.5.2013	x	x							x	x		
17.5.2013	x	x			x	x						
18.5.2013					x	x			x	x		
19.5.2013	x	x							x	x		
20.5.2013	x	x			x	x						
21.5.2013					*	*			x	x		
22.5.2013	x	x							x	x		
23.5.2013	x	x			x	x						
24.5.2013					x	x			x	x		
25.5.2013	x	x							x	x		
26.5.2013	x	x			x	x						
27.5.2013					x	x	x		x	x	x	
28.5.2013	x	x	x	x					x	x	x	x
29.5.2013	x	x	x		x	x	x					
30.5.2013					x	x	x	x	x	x	x	x
31.5.2013	x	x	x						x	x	x	
01.6.2013	x	x	x	x	x	x	x	x				
02.6.2013	Feed adaption											
03.6.2013												
04.6.2013												
05.6.2013	x	x							x	x		
06.6.2013	x	x			*	*						
07.6.2013					*	*			x	x		
08.6.2013	x	x							x	x		
09.6.2013	x	x			x	x						
10.6.2013					x	x			x	x		
11.6.2013	x	x							x	x		
12.6.2013	x	x			*	*						
13.6.2013					*	*			x	x		
14.6.2013	x	x							x	x		
15.6.2013	x	x			x	x						
16.6.2013					x	x			x	x		
17.6.2013	x	x	x						x	x	x	
18.6.2013	x	x	x	x	x	x	x	x				
19.6.2013					x	x	x		x	x	x	
20.6.2013	x	x	x	x					x	x	x	x
21.6.2013	x	x	x		x	x	x					
22.6.2013					x	x	x	x	x	x	x	x
23.6.2013	Feed adaption											
24.6.2013												
25.6.2013												
26.6.2013	x	x			x	x						
27.6.2013					x	x			x	x		
28.6.2013	x	x							x	x		
29.6.2013	x	x			x	x						
30.6.2013					x	x			x	x		
01.7.2013	x	x							x	x		
02.7.2013	x	x			x	x						
03.7.2013					x	x			x	x		
04.7.2013	x	x							x	x		
05.7.2013	x	x			x	x						
06.7.2013					x	x			x	x		
07.7.2013	x	x							x	x		
08.7.2013	x	x	x		x	x						
09.7.2013					x	x	x	x	x	x	x	x
10.7.2013	x	x	x						x	x	x	
11.7.2013	x	x	x	x	x	x	x	x				
12.7.2013					x	x	x		x	x	x	
13.7.2013	x	x	x	x					x	x	x	x

x Data collected *Data not available due to sampling problems

3.1.3.1 Continuous rumen pH measurement

Ruminal pH was measured with two indwelling pH data loggers for cannulated ruminants (Large Ruminant Logger M5-T7, Dascor Inc., Oceanside, USA). Every five minutes the pH and temperature was measured and stored in the internal memory of the data logger. The logger was attached to stainless steel weights, weighing 1.5 kg in total, to anchor the logger in the ventral sac of the rumen. A two-point calibration was made using pH 4 and pH 7 buffer solutions (pH 7.00 ± 0.02 and pH 4.00 ± 0.02, Carl Roth, Karlsruhe, Germany) every 48 h. For this purpose, the logger was removed and the stored data were transferred to a notebook. After the logger was recalibrated, it was introduced to the ventral sac of the rumen according to the time schedule given in Table 3.5. For the interpretation of the values, data from the adaptation phase, day 1 to day 7 of each period, were ignored. One day of measurement in the SBP60 treatment (21.05.2013) was not considered, due to implausibility of the measured data. Four days of measurement (06.06., 07.06., 12.06. and 13.06.) are not available due to a breakage of the pH electrode during the measurement intervals.

The measuring interval in every cow started after the morning milking and lasted almost 48 h, with a lack of the time needed for recalibration of the device, before an interval of 24 h followed where no measurements in this cow were performed, since only two datalogger were available but three cannulated cows were included in this trial. From the measuring interval, the first 24 hours and the last 24 hours were evaluated as measuring days. This led to about half an hour of measurement which basically was only measured once, but used in the dataset of two consecutive days. With this overlapping, it was ensured that every measuring day had a span of 24 hours/1440 minutes and every measuring interval was composed of two measured days. Otherwise, a systematic error would have been in the data. Leaving out the time after morning milking, every second measuring day when the loggers were recalibrated, would tamper the results, because the pH in the morning was generally higher, as will be shown in Figure 3.1.

3.1.3.2 Performance data

The information from the animal house: milk yield, feed intake, water intake, body weight, were collected daily and stored in a central server.

The access to the feeding troughs was regulated individually for each cow by using a responder. The fresh matter feed intake was measured by a scale in the weighing trough (Insentec, B.V., Markenesse, the Netherlands) for every individual visit and summarised for daily consumption.

For the calculation of DMI the feed intake was multiplied with its DM content. Water access in a modified through (Insentec, B.V., Markenesse, the Netherlands) was possible all the time and quantified the same way as feed intake.

The milk yield was ascertained individually for each cow. The amount from morning and evening milking was summarised for a daily milk yield of every cow. Animal body weight was measured on a balance, which the cows had to pass after milking. From two measurements per day, an average daily weight was calculated for each cow.

3.1.3.3 Milk sampling

Representative milk samples were taken at every milking time in the periods when the continuous pH measurements were performed. The milk samples were collected into the standard flasks for the official milk control, where the sample is preserved with sodium azide. The samples were stored in the fridge at +5 °C and the collected samples were sent twice a week to the Landeskontrollverband Nordrhein-Westfalen in Krefeld, for mid-infrared (MIR) analysis (Foss FT+, FT6000; Hillerød, Denmark). Samples were analysed for fat, protein, lactose, urea contents, pH value, and cell count. On days when problems with the pH measurement occurred as mentioned above, the corresponding milk samples were discarded.

In the last week of each period, milk samples from the morning and the evening milking were taken for gaschromatographical analysis on four days when continuous pH measurements took place. From the evening and morning milking, 50 ml-samples were taken into polyethylene flasks and stored at -20 °C.

In the laboratory, the samples were defrosted and aliquots were made in the ratio of the milk yield during the morning and the evening milking. For every analysed day one sample of 30 ml was created. It was centrifuged using 16743 *g* at 4 °C for 30 minutes. 600 mg of the lipid fraction was transferred to a micro tube and mixed with 10.8 ml of a 3:2 hexane:isopropanol mixture for one minute on a whirlmix. Then 7.2 ml of a 6.7% sodium sulfate solution was added and whirlmixed for another minute. The supernatant was transferred to another tube and 1 g water free sodium sulfate was added, whirlmixed and centrifuged for 10 min using 1942 *g* at room temperature. Then the hexane was purged by exposure to ammonium at maximum 40 °C. The methyl esthers of the FAs were separated in a gas chromatograph (Hewlett-Packard 6890, Agilent, Waldbronn, Germany) with a capillary column (Sulpeco SP-2380, 30 m x 0.25 mm, 0.25 micrometer nominal film thickness, Sigma-Aldrich Co. LLC, Munich, Germany) and detected with a flame ionization detector.

With the GC setup it was not possible to separate the FAs C14:1 c and C15:1 aiso. Also, C18:1 t6, C18:1 t9, C18:1 t7 and C18:1 t11 could not be detected separately, as well as C18:2 c9,12 and C19:1 t7.

These FAs were therefore categorised as sums of C14:1 c + C15:1 aiso, C18:1 t6 + t9 + c7 + t11 and C18:2 c9,12 + C19:1t7. This furthermore led to the inclusion of C14:1 c in the sum of odd-chain FAs and to the sum of aiso-chain FAs, as well as C15:1 aiso was included in the sum of even-numbered chain FAs. C18:2 c9,12 was added to the sum of monounsaturated FAs and C19:1 t7 to the sum of double unsaturated FAs.

3.1.3.4 Rumen liquid spot sampling

Rumen liquid was sampled on two days between day 15 and 21 of each period. On the sampling days, rumen liquid was collected six times in 4-hour intervals, starting at 08:30.

The cannulated cows were fixed in a box with a cord. With a manual vacuum pump, 230 ml of rumen liquid were pumped from the ventral sac of the rumen and filled in a 250 ml polyethylene flask. The pH of the rumen liquid was measured immediately in the animal house using a portable pH meter (PCE-228, PCE Deutschland GmbH) with a pH electrode (inPro3100/120/Pt100 combination, Mettler Toledo, Columbus, Ohio, USA) and samples were immediately stored on ice. Samples were later stored at -20 °C until further analysis.

Lactic acid was analysed using a test kit for L-lactate. Only if a concentration of 0.25 mmol/l L-lactate was measured, D-lactate also was analysed, because the lactate tests for D-lactate were very expensive and the lactate concentration was still in a concentration that is so low that its not a suitable parameter for SARA detection (D-Lactate/L-Lactate UV-Test, Boehringer Mannheim/R-Biopharm, Darmstadt, Germany).

The NH_3 concentration was analysed in duplicates by end point titration (Titrator TR154, Schott AG, Mainz, Germany) of a distilled (Vapodest, C. Gerhardt GmbH and Co. KG, Königswinter, Germany) subsample of the rumen liquid. Around 30 g of rumen liquid was used and the exact weight was recorded. It was then brought to an alkaline pH by mixing it with 15 ml of a 0.25 mol/l phosphate buffer with a pH of 11.0 and 90 g Na_2HPO_4 H_2O/l. The distilled NH_3 was caught in 3% boric acid and titrated with 0.05 mol/l HCl.

The samples for the VFAs analysis were prepared by vacuum distillation as described by Zijlstra et al. (1977) with modifications described by Wischer (2013). The samples were defrosted, and centrifuged for 20 min with 5000 U/min at 4 °C. From the decanted rumen liquid, three

subsamples with 4.5 ml each were taken while stirring. Exactly 0.1 ml of internal standard solution (80 mmol/l 2-methylvaleric acid in 50% formic acid) was added. Under continuous rotation the sample was frozen in a -20 °C alcohol bath and thereafter distilled in a three-way distilling receiver, which was connected to a vacuum pump (RZ 16, Vacuubrand, Wertheim, Germany). The requested part of the sample was caught by engulfing the distilling receiver in a Dewar vessel with liquid nitrogen. When the initial flask was dry, the vacuum was set off and the distilling receiver was defrosted by room temperature. Afterwards, the VFAs were analysed in duplicate with a gas chromatograph (Hewlett-Packard 6890, Agilent, Waldbronn, Germany) with a capillary column (HP-FFAP silica glass column, 25m x 0.32mm, 0.5 micrometer nominal film thickness, HP 7683, Agilent) and a flame ionization detector.

3.1.4 Statistical analysis

Continuous pH measurements were subjected to regression analysis as described in chapter 2. For continuous pH measurements, milk, and performance data the following mixed model was used in SAS 9.2:

$$y_{ijkl} = \mu + R_i + P_j + C_k + (P\ x\ C)_{jk} + (P\ x\ D)_{jl} + \varepsilon_{ijkl}$$

With R_i = ration (CS60, SBPS60, SBPS20), P_j = period (1,2,3) and C_k = Cow (1,2,3) as fix effects and $(P \times C)_{jk}$ = period *x* cow, and $(P \times D)_{jl}$ = period *x* day as random effects and ε_{ijkl} as error.

For the rumen liquid the model was extended by the fix effects time (T_m) and ration *x* time $(R\ x\ T)_{im}$.

$$y_{ijklm} = \mu + R_i + P_j + C_k + T_m + (R\ x\ T)_{im} + (P\ x\ C)_{jk} + (P\ x\ D)_{jl} + \varepsilon_{ijklm}$$

Rumen liquid was analysed using ration, period, cow, time, and ration *x* time as fix effects and period *x* cow, as well as period *x* day as random effects with ε_{ijklm} as error.

3.2 Results

3.2.1 Continuous rumen pH measurement

For the interpretation, pH_{mean}, pH_{min}, and pH_{max} was used from the measurements, while β1 and β0 were gained as described in chapter 2. The time spent below several cut-off points were calculated by using values of β1 and β0.

The results of the continuous measurements of ruminal pH are shown in Table 3.6. For all three rations, the pH values of rumen fluid were very low. With a pH_{min} of 5.1, the treatments CS60 and SBPS60 were in an unphysiological low pH range. While SBPS20 tended (p=0.06) to have a higher pH_{min} with 5.4. The pH_{max} estimate was not that different between the three treatments with 6.5 and 6.6, respectively. Although not being statistically significant, pH_{mean} was 5.9 for SBPS20 while the treatments with 60% concentrate had a pH_{mean} of 5.7.

No differences were found for the estimated time below pH thresholds between the treatments, but the slope of the logistic curve β0, which is a value for the stability in the rumen, was significantly higher in the SBPS20 treatment (p = 0.05).

Although the time spent below the cut off points 5.2, 5.6, and 6.0 are not relevant for SARA detection following the definition of Zebeli et al. (2008) they are presented because these values were used for acidosis detection by other authors (AlZahal et al., 2007; Bevans et al., 2005; Keunen et al., 2002; Martens et al., 2012) as mentioned in the introduction. For all three treatments, the coincidence of SARA occurred with a time pH < 5.8 that was in every treatment long enough in average for SARA. The SARA incidence, expressed as percentage of acidotic measurement days per total measurement days, was highest in the SBPS60 treatment with 100% SARA incidence, followed by 89% in the CS60 treatment. The lowest rate of SARA incidence was observed in the SBPS20 treatment, where 61% of the measured days were SARA days.

Table 3.6 pH data based on continuous measurements in Trial 1, gained as described in chapter 2 (CS60 n = 27; SPBS60 n = 24; SBPS20 n = 27; Least Squares Means and *Standard Error)*

Parameter	CS60	SBPS60	SBPS20	Ration p-Value
pH Mean	5.73 *0.07*	5.69 *0.08*	5.89 *0.08*	0.34
pH Minimum	5.11 *0.04*	5.05 *0.04*	5.36 *0.04*	0.06
pH Maximum	6.53 *0.05*	6.57 *0.05*	6.47 *0.05*	0.50
β0	4.81[b] *0.31*	4.02[b] *0.33*	6.74[a] *0.31*	0.05
β1	5.72 *0.07*	5.68 *0.07*	5.92 *0.07*	0.36
Minutes per day pH < 5.2	188 *48.1*	222 *48.5*	26 *48.1*	0.15
Minutes per day pH < 5.6	599 *87.7*	630 *89.0*	248 *87.8*	0.12
Minutes per day pH < 5.8	854 *86.5*	880 *88.4*	528 *86.6*	0.17
Minutes per day pH < 6.0	1066 *81.2*	1096 *83.4*	839 *81.4*	0.25
SARA incidence[1]	25/28 89%	26/26 100%	17/28 61%	-

[a,b] Values in a row with different superscript letters differ ($p \leq 0.05$) between rations

[1] SARA incidence for every measurement day evaluated as described in chapter 2. Days defined as SARA positive if the time pH < 5.8 was 5.24 hours (315 min) or longer and β1 < 6.16.

In Figure 3.1, the average pH course during the day is shown for the three treatments.

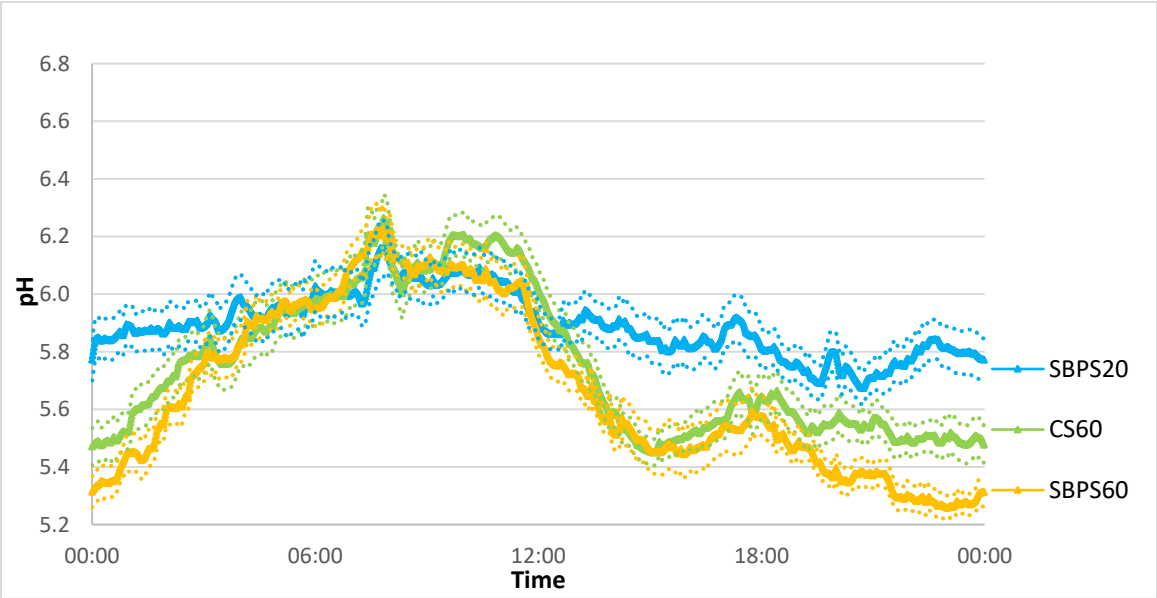

Figure 3.1 Means of 24 h pH measurements ± *Standard Error* of the treatments SBPS20, SBPS60 and CS60 in Trial 1 (CS60 n = 27; SBPS60 n = 24; SBPS20 n = 27)

From midnight until ~7:50 a steady increase in ruminal pH was detected. When the new feed was offered, between 11:00 and 12:00, a decrease in ruminal pH for all three treatments was observed, but it was fastest and deepest for the rations containing high amounts of concentrate. The significant more stable pH course during the day, expressed as β0, can be seen there as well. The pH in cows with high amounts of concentrate in the ration started to recover from 15:30 on until about 18:00 and from 18:00 going down in pH again until midnight.

3.2.2 Performance data

The data shown in Table 3.7 demonstrate that no significant differences in feed intake or body weight between the treatments existed.

Table 3.7 Feed and water intake and body weight in Trial 1 (n = 40, Least Squares Means and *Standard Error)*

	CS60	SBPS60	SBPS20	*SE*	Ration p-Value
Body weight (kg)	664.2	641.4	661.9	*5.60*	0.16
Water intake (kg/day)	96.1	93.6	80.0	*3.58*	0.13
Feed intake (kg DM/day)	19.2	15.4	17.6	*1.76*	0.46
Feed intake per kg $BW^{0.75}$ (g)	0.147	0.120	0.135	*0.0130*	0.48

In Table 3.8 the milk data for the different treatments are shown. The milk yield was not significantly affected by different treatments. Although the fat content in the milk for the SBPS20 treatment (4.3%) was higher than in the SBPS60 and CS60 treatment (2.4 and 2.5%), no significant effect was observed. Only the protein content in the milk of the CS60 treatment was significantly higher than in the SBPS treatments, although with 3.3% only being slightly higher than in the SBPS treatments. A tendency (p=0.06) of a lower lactose content in the SBPS20 treatment (4.4%) than in the treatments with 60% concentrate (4.6%) was observed.

Table 3.8 Milk data in Trial 1 (n = 30, Least Squares Means and *Standard Error)*

Milk	CS60	SBPS60	SBPS20	*SE*	Ration p-Value
Yield (kg/d)	26.7	28.2	23.4	*1.94*	0.38
Fat (%)	2.4	2.5	4.3	*0.33*	0.12
Protein (%)	3.3^{a}	3.2^{b}	3.2^{b}	*0.02*	<.0001
Lactose (%)	4.6	4.6	4.5	*0.01*	0.06
pH value	6.6	6.6	6.7	*0.02*	0.28

3.2.3 Rumen liquid spot samples

The main VFA detected in the rumen samples was acetate. No differences were shown between the SBPS treatments with 70.0 and 73.2 mmol/l, but the concentration of acetate was significantly lower for the CS60 treatment with 59.6 mmol/l. The concentration of butyrate also did not differ between the SBPS treatments with 13.3 and 12.8 mmol/l, but was lower for the CS60 treatment with a concentration of 11.5 mmol/l.

For propionate, no difference between the treatments CS60 and SBPS60 were found, which were 42.0 and 38.1 mmol/l, but a significant lower concentration for the SBPS20 treatment was measured with 25.4 mmol/l.

The ratio of acetate to propionate was significantly widest for the SBPS20 treatment, with 2.91, followed by SBPS60 treatment, with 1.92. The closest acetate to propionate ratio was found for CS60 treatment with 1.47. The closer acetate to propionate ratio in the treatments with 60% concentrate is an indicator that a shift in the microbial community to amylolytic microorganisms took place, which is linked to the low ruminal pH when more concentrate was included in the ration.

Table 3.9 VFAs, pH, NH_3 and lactate in the rumen liquid in Trial 1 (n = 36, Least Squares Means and *Standard Error)*

	CS60	SBPS60	SBPS20	*SE*	Treatment p-Value
VFA_{total} (mmol/l)	119.3	126.9	115.8	*4.69*	0.50
C2 (mmol/l)	59.9^{b}	70.0^{a}	73.2^{a}	*2.68*	<0.0001
C3 (mmol/l)	42.0^{a}	38.1^{a}	25.4^{b}	*1.66*	0.04
C4iso (mmol/l)	0.5	0.5	0.5	*0.06*	0.78
C4 (mmol/l)	11.5^{b}	13.3^{a}	12.8^{a}	*0.60*	<0.01
C5iso (mmol/l)	1.0	1.1	1.5	*0.25*	0.40
C5 (mmol/l)	4.5^{a}	4.0^{ab}	2.3^{b}	*0.38*	0.05
Acetate/Propionate	1.47^{c}	1.92^{b}	2.91^{a}	*0.12*	0.01
pH Value	5.83^{b}	5.89^{b}	6.13^{a}	*0.07*	<0.01
NH_3-N (mmol/l)	4.4	3.6	4.5	*0.58*	0.37
Lactate[1] (mmol/l)	1.78^{a}	1.52^{a}	1.26^{b}	*0.54*	0.03
C2 (% of VFA_{total})	50.5^{c}	55.5^{b}	63.2^{a}	*0.38*	<0.01
C3 (% of VFA_{total})	34.9^{a}	29.7^{b}	21.9^{c}	*0.69*	0.01
C4iso (% of VFA_{total})	0.4	0.4	0.5	*0.06*	0.71
C4 (% of VFA_{total})	9.6	10.4	11.1	*0.37*	0.18
C5iso (% of VFA_{total})	0.8	0.9	1.3	*0.22*	0.47
C5 (% of VFA_{total})	3.7^{a}	3.0^{ab}	2.0^{b}	*0.21*	0.05

C2 = acetate, C3 = propionate, C4iso = isobutyrate, C4 = butyrate, C5iso = isovalerate, C5 = valerate

[1]D-lactate was only analysed in samples with concentrations of more than 0.25 mmol/l L-lactate. Otherwise, only L-lactate concentrations were measured.

Lactate was highest for CS60 with no significant difference to SBPS60, but the concentration of lactate was lower in SBPS20. In general, lactate concentration was not as high as expected in an acute acidosis. An acute acidosis can lead to peak concentration of 100 mmol lactate/l rumen liquid, but SARA can also be achieved without an accumulation above 5 mmol/l, according to a review by Nagaraja and Titgemeyer (2007).

The lactate concentration in the rumen was correlated with the feed intake before the rumen liquid sample was taken. It showed that the strongest correlation was found between the feed intake within the last 1:15 h before the sampling and the lactate concentration for the CS60 treatment (R^2=0.73), for the feed intake 1:30 h before the sampling and the lactate concentration for the SBPS60 treatment (R^2=0.71), and 0:30 h before the sampling and the lactate concentration for the SBPS20 treatment (R^2=0.62) as shown in Table 3.10.

When the data were adjusted to samplings, where a feed intake took place in the time before the sampling, the number of observations decreased. For the SBPS20 treatment the correlation became weaker, the CS60 treatment remained at a comparable level and increased for SBPS60 as shown in Table 3.10.

Table 3.10 Coefficients of determination between feed intake and lactate concentration in rumen liquid for the last 4:00 hours until last 15 minutes before rumen liquid sampling. Once for all measurements (n = 36), as well as for measurements only when feed intake took place in the corresponding time before rumen liquid sampling

Timespan before spot-sampling	CS60 N=36 R^2=	CS60[1]		SBPS60 N=36 R^2=	SBPS60[1]		SBPS20 N=36 R^2=	SBPS20[1]	
		R^2=	N=		R^2=	N=		R^2=	N=
4:00	0.16	0.15	34	0.41	0.43	31	0.36	0.35	34
3:00	0.23	0.22	33	0.50	0.61	27	0.49	0.47	28
2:00	0.49	0.48	26	0.65	0.79	21	0.44	0.40	28
1:45	0.64	0.65	25	0.71	0.81	19	0.39	0.34	27
1:30	0.70	0.71	25	0.71	0.79	16	0.39	0.34	27
1:15	0.73	0.75	22	0.63	0.67	14	0.41	0.35	26
1:00	0.62	0.62	21	0.53	0.57	14	0.48	0.42	24
0:45	0.41	0.31	20	0.40	0.33	12	0.55	0.47	21
0:30	0.21	0.13	15	0.12	0.01	11	0.62	0.51	17
0:15	0.01	0.01	10	0.12	0.02	8	0.30	0.03	14

[1] dataset only consisting of measurements if a feed intake was registered in the according span of time

This difference in the point of time of the highest correlation may indicate that the lactate in the CS60 and SBPS60 treatments originated from lactate production in the rumen. For the

SBPS20 treatment, the higher correlation when a feed intake took place in a shorter timespan before the sampling may indicate that the lactate measured is not primarily from the intra-ruminal fermentation of the feedstuff, but was already ingested as lactate with the SBPS. Unfortunately the lactate content was not analysed in the feedstuff, but it is reported in literature that, depending on the silaging conditions, 2 - 11% lactate can be prevalent in beet pulp silage (Leupp et al., 2006).

3.2.4 Milk fatty acids

Although high numerical differences between the treatments were observed for some fatty acids, only for a rare number of fatty acids significant differences in milk fat were detected. The biggest numerical difference between the treatments was for the sum of C18:1 t6 + t9 + c7 + t11, where the CS60 treatment had a concentration of 12.9% in the analysed milk fat, while the concentrations in the treatments SBPS60 (6.29%) and SBPS20 (1.41%) were lower, but no significant difference was observed ($p = 0.20$).

Other fatty acids, which are less concentrated in the milk fat, were statistically significantly different, due to a very low *SE* within the measurements, as shown in Table 3.11. C17:0 iso was significant highest in SBPS20 (0.35%), followed by SBPS60 (0.21%) and lowest in CS60 treatment (0.28%).

The C18:2 t9,12 content in milk fat was about twice as high, and statistically significant higher, in CS60 (0.58%) and SBPS60 (0.47%) than in SBPS20 treatment (0.28%). Also C18:2 c9,t11 which was highest concentrated in CS60 (0.89%), followed by SBPS60 (0.69%), and lowest in SBPS20 (0.39%) was significant different between all three treatments.

Besides the fatty acids shown in Table 3.11, the following fatty acids were analysed but had concentrations below the detection limit (< 0.02%): C13:0 iso, C14:1 t, C15:1 c + C16:0 aiso, C17:1 c, C17:1 t, C18:2 t10,c12, C18:1 c6, C18:3 c6,9,12 and C20:1 t11.

Table 3.11 Milk fatty acids in Trial 1 (n = 12, Least Squares Means and *Standard Error*)

Concentration in percent of analysed milk fat	CS60	SBPS60	SBPS20	*SE*	Ration p-Value
C4:0	2.80	3.59	4.21	*0.287*	0.14
C6:0	1.73	2.42	2.83	*0.223*	0.14
C8:0	0.78	1.06	1.20	*0.106*	0.20
C10:0	2.06	2.71	2.85	*0.248*	0.26
C11:0	0.17	0.11	0.09	*0.034*	0.45
C12:0	3.12	3.64	3.60	*0.280*	0.48
C13:0	0.28	0.19	0.13	*0.038*	0.22
C14:0	10.47	11.52	10.99	*0.458*	0.43
C14:0 iso	0.03	0.03	0.04	*0.005*	0.77
C14:1 c + C15:1 aiso	2.45	2.35	1.98	*0.113*	0.16
C15:0	2.21	1.78	1.88	*0.164*	0.35
C15:0 iso	0.10	0.13	0.17	*0.026*	0.40
C15:1 t	0.21	0.26	0.26	*0.019*	0.27
C16:0	24.40	28.26	35.37	*1.857*	0.10
C16:1 t	0.11	0.10	0.10	*0.004*	0.50
C16:1 c	3.03	2.56	2.63	*0.239*	0.46
C17:0	0.80	0.77	0.87	*0.041*	0.40
C17:0 iso	0.28[c]	0.31[b]	0.35[a]	*0.010*	<0.001
C17:0 aiso	0.50	0.62	0.73	*0.051*	0.16
C18:0	5.28	5.95	5.58	*0.720*	0.82
C18:0 iso	0.47	0.38	0.42	*0.063*	0.67
C18:1 t6 + c7 + t9 + t11	12.91	6.29	1.41	*2.900*	0.20
C18:1 c9	18.31	17.82	17.93	*2.211*	0.99
C18:1 c11	1.97	1.31	0.92	*0.135*	0.06
C18:1 c12	0.28	0.31	0.16	*0.046*	0.25
C18:2 t9,12	0.58[a]	0.47[a]	0.28[b]	*0.031*	0.04
C18:2 c9,12 + C19:1 t7	2.88	3.10	1.93	*0.407*	0.30
C18:2 t9,c11	0.14	0.06	0.00	*0.041*	0.25
C18:2 c9,t11	0.89[a]	0.69[b]	0.39[c]	*0.029*	<.0001
C18:3 c9,12,15	0.47	0.58	0.48	*0.045*	0.34
C19:1 t10	0.04	0.07	0.07	*0.030*	0.75
C20:0	0.10	0.10	0.10	*0.000*	0.39
C20:1 c11	0.13	0.09	0.06	*0.017*	0.17
C20:2 c11,14	0.03	0.03	0.03	*0.003*	0.50
C20:3 c11,14,17	0.09	0.10	0.10	*0.007*	0.50
C22:0	0.02	0.06	0.06	*0.010*	0.15
Sum even-numbered chain +C15:1 aiso	95.52	95.56	95.63	*0.159*	0.87
Sum odd-chain + C14:1 c	6.07	5.72	5.57	*0.178*	0.32
Sum iso-chain	0.88	0.86	0.97	*0.089*	0.69
Sum aiso-chain + C14:1 c	2.95[a]	2.97[a]	2.71[b]	*0.074*	0.02
Sum monounsaturated + C18:2 c9,12	42.32	34.28	27.44	*3.231*	0.16
Sum 2x unsaturated + C19:1 t7	4.52	4.35	2.63	*0.430*	0.14
Sum 3x unsaturated	0.56	0.68	0.58	*0.038*	0.24
Sum polyunsaturated + C19:1 t7	5.08	5.03	3.22	*0.468*	0.16
Sum short-chain (C4 to C13)	8.13	10.13	10.71	*0.891*	0.30
Sum medium-chain (C14 to C16)	43.01	46.99	53.40	*2.250*	0.15
Sum long-chain (C17+)	46.16	39.10	31.87	*3.239*	0.17

[a,b,c] Values in a row with different superscript letters differ ($p \leq 0.05$) between rations

3.3 Conclusions

An inclusion of 60% concentrate in the ration caused measurable negative effects on the animal. In the rumen, there were acidotic conditions, shown by a low pH, a peNDF content, which is not sufficient to meet the requirements, low content of milk fat, and a close ratio of acetate to propionate. Also, the SBPS inclusion did not prevent a low pH, since beet pulp as a by-product of sugar production has some characteristics that do not make it a suitable replacement for forages, but it is reported to be more often used to replace grains than forage (Münnich et al., 2017).

Münnich et al. (2017) reviewed that including beet pulp in rations does not affect the milk yield, as well as the rumen parameters NH_3 and total VFA concentration but it causes a heavy shift to a higher acetate concentration in the rumen. Since it consists of high levels of pectin, which is fermented at a lower rate, when the pH sinks below 6.0, it is beneficial to the rumen health.

The pH value of rumen fluid in the SBPS20 treatment was significantly highest of all three treatments during the spot samplings. This was slightly higher than the pH_{mean} of the continuous pH measurements, but this cannot be compared properly, because in the data of the continuous pH measurements the data of more days (n = 78) were used than the data from the rumen liquid samplings (n = 18 days). In addition, the spot samplings were only repeated six times a day, while the continuous pH measurement had 288 measurements to build up a pH_{mean}. The higher ruminal pH in the ration with highest inclusion of SBPS is in congruence with the beneficial effects, reported by Münnich et al. (2017).

In addition, an inclusion of 60% SBPS in the TMR of the SBPS20 treatment caused a high SARA incidence although the milk fat, as well as the peNDF are in a moderate concentration. The 24 h measurements showed that the pH in the rumen when fed SBPS20 ration was more stable and in the time after the feeding the decrease was not as big as in the other treatments with the 60% concentrate, being rapidly fermentable (compare Table 3.6 and Figure 3.1). In addition, the acetate to propionate ratio did not indicate a shift of microbial community in the rumen in the SBPS20 treatment. The milk fat content in the SBPS20 treatment (4.3%) was very high compared to the other treatments (2.4 and 2.5%). Since SBPS is known to be very low in pH itself, a pH below 4 is not uncommon in the SBPS component of the feed, according to the Verein der Zuckerindustrie (2012). The acidity of the SBPS together with 20% concentrate in the ration could have led to the low pH_{mean} but lactate from the SBPS does not seem to be a reason for a low pH due to lactate accumulation, because only low concentration of lactate was found. Although the peNDF content indicates a sufficient supply of structure in the feed, it has to be mentioned that

the peNDF system is developed for mixed rations including forages and is not necessarily adequate to determine the effectiveness of NDF in mixed rations, including by-products such as SBPS. This is in accordance with Teimouri Yansari (2014) who found that in rations with 12% beet pulp grinding of the beet pulp even increased ruminating time.

The peNDF content in the rations SBPS60 and CS60 were in the tolerance range assumed by Mertens and Beauchemin et al. (2003), while the benchmark for SARA prevention given by Zebeli et al. (2012) was not achieved. For the definition given by the Committee for Requirement Standards of the Society of Nutrition Physiology (2014) the starch content or its degradability would be needed. Since neither starch content in the rations nor starch degradability was measured, no definite conclusions can be made about the peNDF contents of the rations with 60% concentrate. However, if the average starch content of the ingredients of the concentrate are summarised, according to The Swiss Feed Database, they lead to a starch content of 28% in the ration, only based on the concentrate. The starch content of CS increases this concentration for the CS60 ration even further. The $peNDF_{>8mm}$ concentration for the SBPS60 treatment is able to maintain an average pH of 6.17, for CS60 a pH_{mean} of 6.05 and for SBPS20 a pH_{mean} of 6.45 according to the Committee for Requirement Standards of the Society of Nutrition Physiology (2014). The fact that SARA was induced for at least 60% of the measured days, in the SBPS20 treatment shows that the peNDF concentration is not a suitable parameter for rations based on SBPS. The structural properties of SBPS are not comparable to other forage sources such as hay or grass and corn silage although the particle size is sufficient.

Generally, prevalent fatty acids were C14:0, C16:0, C18:1 c9, and for the CS treatment also the sum of C18:1 t6 + t9 + c7 + t11. There is a significant difference for the sum of aiso-chain FAs + C14:1 c, but the pooled group C14:1 c + C15:1 aiso is included in these data and provides the biggest part (more than 70%) of the “aiso” chained FAs. Since C14:1 c and C15:1 aiso cannot be split, no statement can be given to what extend C14:1 c or C15:1 aiso is in the milk. Therefore, statements about significant differences of aiso fatty acids between the treatments cannot be made properly.

It is evident, that 60% concentrate in the ration in combination with grass silage and SBPS or CS is too high concentrated, so the animal’s health will probably be negatively affected when these rations are fed for longer time. In addition, an inclusion of 60% SBPS showed a high number of SARA incidences, so it can be concluded that 60% SBPS in combination with 20% concentrate is also not recommendable as TMR for dairy cows.

4 TRIAL 2

This trial was planned with a lower concentration of concentrate in the TMR than Trial 1. In Trial 2, three treatments were under research with rations consisting of 48% forage and 52% concentrate in every treatment. Three different types of forages were used; hay, corn silage, and grass silage.

The concentrate was mixed in the same composition for all treatments, so that an influence from the source or level of concentrate could be excluded. Only an adaptation for the corn silage treatment in urea and limestone supplementation was necessary to meet the needs of the cows for calcium and nitrogen.

4.1 Material and methods

4.1.1 Animals and animal housing / Experimental design

The trial was conducted at the Meiereihof, Stuttgart Hohenheim, from 25.02.2014 until 25.04.2014. It was approved by the Provincial Government of Baden-Württemberg, Germany.Three cannulated lactating Jersey cows with an average of 203 days in milk (DIM) at the start of the trial were housed in a group of five cows. The cows were milked twice a day. The experimental design was a 3x3 Latin square with a trough to cow ratio of 1:1 for the cows included in the trial. Each period lasted for 20 days, sampling took place from day 11 to 20 of each period. On day 1 of each period, the previous ration was mixed with the following ration in equal parts (mixed on fresh matter basis).

4.1.2 Rations

The rations were mixed each morning between 7:00 and 8:00, the feed residues were removed from the troughs and the new feed was distributed. The feed was available ad libitum and an oversupply of 5% was calculated. The rations consisted of 48% forage and 52% concentrate as shown in Table 4.1.

Table 4.1 Composition of rations used in Trial 2 (percent, on DM basis)

	Ration		
	Corn silage	Grass silage	Hay
Corn silage	47.6		
Grass silage		48.1	
Hay			48.1
Concentrate	46.4	45.9	
Molasses	6.0	6.0	

The concentrate was mixed for the trial for all three rations, once before the trial started. For the CS ration, urea and limestone were added to the concentrate to adjust to the animals' requirements. The concentrates were based on barley, wheat, soybean meal, and minerals, detailed shown in Table 4.2.

Table 4.2 Composition of the concentrates used in Trial 2 (percent, on DM basis)

	Corn silage ration	Grass silage ration	Hay ration
Barley	39.6	40.3	
Wheat	39.6	40.3	
Soybean extraction meal	16.0	16.4	
Urea	1.3	-	
Limestone	1.5	1.0	
NaCl	0.5	0.5	
Mineral and vitamin premix[1]	1.5	1.5	

[1]Ingredients per kg: 137 g Ca, 60 g Mg, 43 g Na, 2429 mg zinc chelate, 3857 mg zinc oxide, 2000 mg manganese chelate, 2571 mg manganese oxid, 600 mg copper, 343 mg copper chelate, 17 mg selenite, 13 mg selenite yeast, 51 mg iodine, 17 mg cobalt, 103571 mcg biotin, 571428 I.E. vitamin A, 55714 mg vitamin D, 6143 mg vitamin E, 43 mg vitamin B_1, 21 mg vitamin B_2, 21 mg vitamin B_6, 48 mg calcium-D-panthothenate, 215 mg niacinamide, 161 mcg vitamin B_{12}, 3 mg folate, 857 mg beta-carotene

The hay, CS, and GS used during the trial was harvested in 2013. To maintain a constant quality of the forage during the trial hay and grass silage batches were selected not only because of their quality, but also because of the homogeneity of the harvest. The corn silage was ensiled in a silo and was used in the rations of the other cows of the Meiereihof as well as to maintain the needed emptying speed rate in the silo. Because of operational reasons, the silo was changed after the second period.

The hay was chopped to a theoretical cutting length of 4.4 cm (Botsch-Häcksler 28, Bad Rappau, Germany). The grass silage was chopped to a length of approximately 15 cm.

Molasses was included in the ration to improve the mixing quality of the rations. For a better homogeneity in the grass silage and hay TMR, 310 g water per kilogram DM was added together

with the molasses. The corn silage particles were wet enough so no water was needed for a good homogeneity in the corn silage TMR.

The concentrates were analysed in duplicate once, the forages for every period from pooled samples. Based on these samples the chemical composition of the rations was calculated by using the means of analysis and ingredient composition of the diet. The chemical composition of the rations is shown in Table 4.3. Analyses were made according to the methods published by VDLUFA Methodenbuch III in the paragraphs: 3.1 (DM), 8.1 (XA), 4.1.1 (XP), 5.2 (EE), 6.1.1 (XF), 6.5.1 (NDF), 6.5.2 (ADF). For the analyses the pooled feed samples were ground in a mill (SM1, Retsch GmbH, Haan, Germany), using a sieve with a 1 mm mesh width.

Table 4.3 Calculated chemical composition of experimental diets used in Trial 2 (g/kg, on DM basis)

	Corn silage ration	Grass silage ration	Hay ration
DM (g/kg)	634	606	714
Crude ash	53	89	74
Ether extract	28	32	26
Crude protein	142	153	154
Crude fiber	104	166	169
aNDFom	272	332	360
ADFom	133	188	196
ADL	10	14	16

Table 4.3 shows that the rations with GS and hay were very similar in their chemical composition while CS ration had a lower content of crude ash, crude protein, and crude fiber. The aNDFom was also lower for CS than for the other rations, which was one of the reasons for the low peNDF content in the CS ration, shown in Table 4.4.

While the peNDF$_{>8mm}$ content in the rations GS and Hay was sufficient according to Zebeli et al. (2008), the peNDF content in the CS ration was not. With only 23.6% peNDF$_{>1.18mm}$ it was far below the minimum of 31.2% stated by Zebeli et al. (2008) for peNDF$_{>1.18mm}$. A peNDF$_{>8mm}$ of 8.3 in the CS ration was also extremely low. For a determination based on the Committee for Requirement Standards of the Society of Nutrition Physiology (2014) the starch content was needed which has not been analysed.

Table 4.4 peNDF content of rations used in Trial 2 (n: CS = 45; GS = 48; Hay = 48, Least Squares Means, *Standard Deviation,* Minimum and Maximum*)*

	CS ration		GS ration		Hay ration	
peNDF (% of DM)	>8mm	>1.18mm	>8mm	>1.18mm	>8mm	>1.18mm
Mean	8.3	23.6	22.7	30.2	19.4	31.8
Std. dev.	0.9	1.9	1.6	0.6	1.2	0.5
Min	7.1	21.6	19.3	29.0	17.2	30.6
Max	11.0	27.9	27.6	31.5	23.1	33.1

Some measurements had to be removed from the dataset due to incorrect weighing results.

4.1.3 Samplings

The samplings followed the timesheet given in Table 4.5. Detailed information about the samplings can be found in 4.1.3.1, 4.1.3.2, 4.1.3.3 and 4.1.3.4.

Table 4.5 Overview of sampling procedure in Trial 2

	Treatment											
	Corn silage				Grass silage				Hay			
Date	pH	MIR-Milk	GC-Milk	Rumen liquid	pH	MIR-Milk	GC-Milk	Rumen liquid	pH	MIR-Milk	GC-Milk	Rumen liquid
25.2.2014	Feed change											
26.2.2014	Adaptation											
27.2.2014												
28.2.2014												
01.3.2014												
02.3.2014												
03.3.2014												
04.3.2014												
05.3.2014												
06.3.2014												
07.3.2014	x	x	x		x	x	x		x	x	x	
08.3.2014	x	x	x		x	x	x		x	x	x	
09.3.2014	x	x	x		x	x	x		x	x	x	
10.3.2014	x	x	x		x	x	x		x	x	x	
11.3.2014	x	x	x		x	x	x		x	x	x	
12.3.2014	x	x	x		x	x	x		x	x	x	
13.3.2014	x	x	x		x	x	x		x	x	x	
14.3.2014	x	x	x	x	x	x	x	x	x	x	x	X
15.3.2014	x	x	x		x	x	x		x	x	x	
16.3.2014	x	x	x	x	x	x	x	x	x	x	x	X
17.3.2014	Feed change											
18.3.2014	Adaptation											
19.3.2014												
20.3.2014												
21.3.2014												
22.3.2014												
23.3.2014												
24.3.2014												
25.3.2014												
26.3.2014												
27.3.2014	x	x	x		x	x	x		x	x	x	
28.3.2014	x	x	x		x	x	x		x	x	x	
29.3.2014	x	x	x		x	x	x		x	x	x	
30.3.2014	x	x	x		x	x	x		x	x	x	
31.3.2014	x	x	x		x	x	x		x	x	x	
01.4.2014	x	x	x		x	x	x		x	x	x	
02.4.2014	x	x	x		x	x	x		x	x	x	
03.4.2014	x	x	x	x	x	x	x	x	x	x	x	X
04.4.2014	x	x	x		x	x	x		x	x	x	
05.4.2014	x	x	x	x	x	x	x	x	x	x	x	X
06.4.2014	Feed change											
07.4.2014	Adaptation											
08.4.2014												
09.4.2014												
10.4.2014												
11.4.2014												
12.4.2014												
13.4.2014												
14.4.2014												
15.4.2014												
16.4.2014	x	x	x		x	x	x		x	x	x	
17.4.2014	x	x	x		x	x	x		x	x	x	
18.4.2014	x	x	x		x	x	x		x	x	x	
19.4.2014	x	x	x		x	x	x		x	x	x	
20.4.2014	x	x	x		x	x	x		x	x	x	
21.4.2014	x	x	x		x	x	x		x	x	x	
22.4.2014	x	x	x		x	x	x		x	x	x	
23.4.2014	x	x	x	x	x	x	x	x	x	x	x	X
24.4.2014	x	x	x		x	x	x		x	x	x	
25.4.2014	x	x	x	x	x	x	x	x	x	x	x	X

4.1.3.1 Continuous rumen pH measurement

Ruminal pH was measured continuously with an indwelling pH datalogger for cannulated ruminants (Large Ruminant Logger M5-T7, Dascor Inc., Oceanside, USA) between day 10 and 20. Every minute the pH and temperature was measured and stored in the internal memory of the datalogger. The logger was attached to stainless steel weights, weighing 1.5 kg in total, to anchor the logger in the ventral sac of the rumen.

A two-point calibration was made in pH 4 and pH 7 buffer solutions every 3 to 4 day. The logger was removed, stored data were transferred to a notebook, the logger was recalibrated and through the cannula reintroduced to the rumen.

For the time when the datalogger was outside the rumen for recalibration, the pH in the rumen was estimated assuming a linear course with the last measured pH value before the recalibration as start point and the first pH value after calibration as the end point.

4.1.3.2 Performance data

The information from the animal house: milk yield, fresh matter intake, and body weight, were collected daily and stored on a central server.

The milk yield was ascertained individually for each cow. The amount from morning and evening milking was summarised for a daily milk yield of every cow. Animal weight was measured on a balance, which the cows had to pass after milking. From two measurements per day, an average daily weight was calculated for each cow. Water access was possible all the time ad libitum but not quantified. The ad libitum access to the trough was regulated individually for each cow with the help of a responder, the feed intake was measured by a scale in the weighing through for every visit individually and summarized for daily consumption. For the calculation of DMI the feed intake from the fresh material was multiplied with the DM content of the feed.

4.1.3.3 Milk sampling

Representative milk samples were taken at every milking time from day 10 to day 20 in every period. At every milking time, three milk samples were taken per cow. One of them was used for gaschromatographical analysis. Therefore, a 50 ml-sample was given into a polyethylene flask. To gain representative milk samples of the day the milk was pooled in the same ratio of milk yield from the morning and evening milking. After pooling, the samples were stored at -20° C until further analysis in the same way as described in chapter 3.1.3.3.

The other two milk samples were collected into the standard flasks for the official milk control, where the samples are preserved with sodium azide and stored in the fridge at + 5 °C. Twice a week the collected samples of every milking were shipped in a temperature-isolated box for milk analysis. One set of samples was sent to the "Landeskontrollverband Nordrhein-Westfalen" in Krefeld (MIR analysis with Foss FT+, FT6000; Hillerød, Denmark) and the other was sent to the Zentrallabor of the Milchprüfring Baden-Württemberg in Kirchheim unter Teck (MIR analysis with Bentley FTS) for a comparison between the two MIR techniques. This comparison was not part of this work and the data used in this work for MIR data are those from Krefeld, analysed by Foss spectrograph. Samples were analysed by the laboratories for fat, protein, lactose, urea, pH value and cell count.

4.1.3.4 Rumen liquid spot sampling

Rumen liquid was sampled for 24 hours at day 18 and 20 of each period in intervals of 4 hours starting at 08:30 am. Rumen liquid was pumped with an electric vacuum pump from the ventral sac of the rumen. The pH of the rumen liquid was measured immediately in the animal house (pH meter: Type CG 842, pH electrode: Blueline 14 pH, Schott Instruments, Germany) and a sample was stored on ice. Samples were stored at -20 °C until further analysis for VFAs and NH_3 as described in chapter 3.1.3.4.

4.1.4 Statistical analysis

Continuous pH measurements were subjected to regression analysis as described in chapter 2. For continuous pH measurements, milk, and performance data the following mixed model was used in SAS 9.2:

$$y_{ijkl} = \mu + R_i + P_j + C_k + (P\ x\ C)_{jk} + (P\ x\ D)_{jl} + \varepsilon_{ijkl}$$

With R_i = ration (CS, GS, Hay), P_j = period (1,2,3) and C_k = cow (1,2,3) as fix effects and $(P \times C)_{jk}$ = period *x* cow, and $(P \times D)_{jl}$ = period *x* day as random effects and ε_{ijkl} as error.

For the rumen liquid the model was extended by the fix effects time (T_m) and ration *x* time $(R\ x\ T)_{im}$.

$$y_{ijklm} = \mu + R_i + P_j + C_k + T_m + (R\ x\ T)_{im} + (P\ x\ C)_{jk} + (P\ x\ D)_{jl} + \varepsilon_{ijklm}$$

Rumen liquid was analysed using ration, period, cow, time, and ration *x* time as fix effects and period *x* cow, as well as period *x* day as random effects with ε_{ijklm} as error.

4.2 Results

4.2.1 Continuous rumen pH measurement

For interpretation, pH_{mean}, pH_{min}, and pH_{max} were used from the measurements, while β1 and β0 were obtained as described in chapter 2. The time spent below several cut off points was calculated using estimated values of β1 and β0.

The results of the continuous measurements of ruminal pH are shown in Table 4.6. The pH_{mean}, as well as the minutes per day pH < 5.8 were not indicative for SARA, following the definition of Zebeli et al. (2008). No influence of treatment was found for β1, although the CS treatment tended to cause a lower pH_{mean} with 6.2 compared to 6.4 in the GS and Hay treatment. The daily variation of pH (β0) seems to have been wider for the Hay treatment, but no significant difference of β0 between Hay (5.4) and GS (6.6) or CS (6.8) treatment was found.

Table 4.6 pH data of the three treatments based on continuous measurements in Trial 2, gained as described in chapter 2 (n = 30, Least Squares Means and *Standard Error)*

Parameter	Corn silage	Grass silage	Hay	*SE*	Ration p-Value
pH Mean	6.21	6.39	6.35	*0.06*	0.27
pH Minimum	5.60	5.83	5.64	*0.07*	0.25
pH Maximum	6.78	6.91	6.93	*0.07*	0.39
β0	6.77	6.55	5.38	*0.54*	0.45
β1	6.22	6.38	6.36	*0.06*	0.28
Minutes per day pH < 5.2	16	2	6	*8.4*	0.55
Minutes per day pH < 5.6	74	15	35	*29.6*	0.49
Minutes per day pH < 5.8	160	47	87	*48.2*	0.41
Minutes per day pH < 6.0	339	142	206	*67.7*	0.31
SARA incidence[1]	7/30	0/30	0/30	-	-

[1] SARA incidence for every measurement day evaluated as described in the introduction. Days defined as SARA positive if the time pH < 5.8 was 5.24 hours (315 min) or longer and β1 < 6.16.

The average 24-hour pH course of the treatments is shown in Figure 4.1. After the morning feeding, a very fast decrease of the pH value in the treatments GS and Hay was observed.

While from about 15:00 onwards the pH value in the Hay and GS treatments recovered, the pH value of the CS treatment decreased until ~21:00 o clock. The pH decline in the CS treatment was more constant, but also to the lowest extent and its pH_{max} was roundabout 0.15 pH values lower than the Hay treatment.

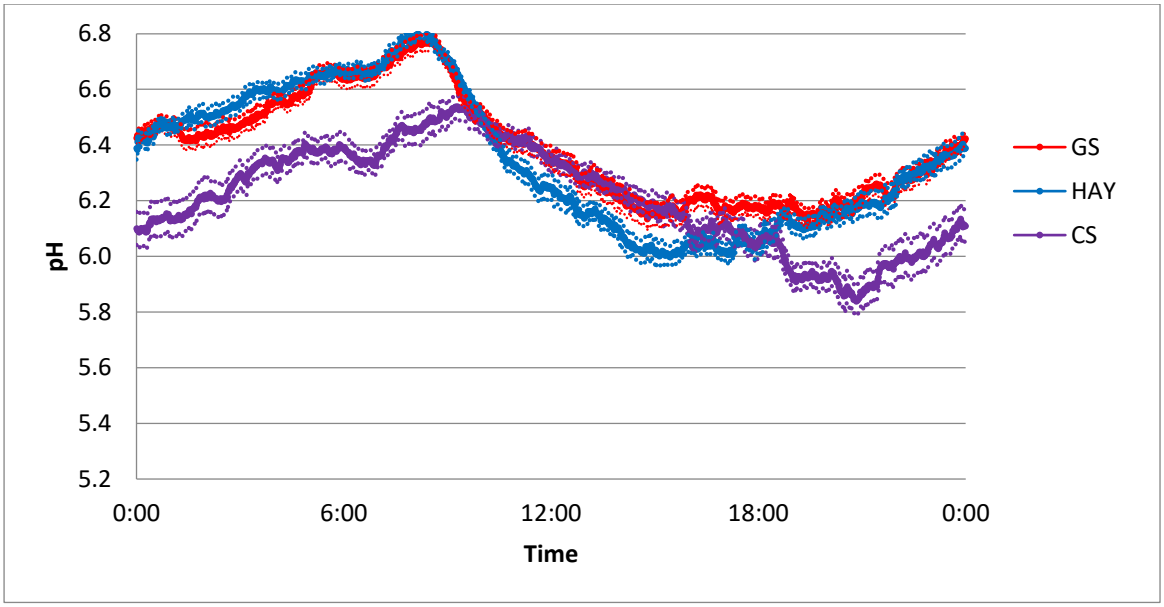

Figure 4.1 Means of 24 h pH measurements ± *standard error* for the treatments GS, Hay and CS in Trial 2 (n = 30 days)

4.2.2 Performance data

The feed intake, as well as the milk yield was numerically highest in the CS treatment, but no statistically significant differences were found. In addition, the content of the milk fat, protein, lactose, and the milk pH only differed in a range of 0.1% between the treatments. The fat content was very high with 5.3% fat in the milk, but this is in the normal range for the Jersey breed.

Table 4.7 Body weight, feed intake and milk data in Trial 2 (n = 30, Least Squares Means and *Standard Error)*

	Corn silage	Grass silage	Hay	*SE*	Ration p-Value
Body weight (kg)	550.8	540.9	545.0	*4.61*	0.45
Feed intake (kg DM/day)	21.4	19.3	19.1	*1.53*	0.58
Feed intake per kg $BW^{0.75}$	0.19	0.17	0.17	*0.013*	0.64
Milk data					
Yield (kg)	17.9	16.0	14.6	*0.63*	0.21
Fat (%)	5.4	5.3	5.3	*0.13*	0.70
Protein (%)	4.3	4.3	4.3	*0.06*	0.79
Lactose (%)	4.5	4.5	4.4	*0.03*	0.26
pH value	6.7	6.8	6.7	*0.02*	0.35

4.2.3 Rumen liquid spot samples

As shown in Table 4.9, the VFA_{total} concentration in the CS treatment was significantly highest with 114 mmol/l. The differences to GS (104.5 mmol/l) and Hay (99.0 mmol/l) were based on the significant higher concentrations of propionate in the CS treatment (22.4 mmol/l), compared to GS (20.3 mmol/l) and Hay treatments (17.5 mmol/l), and an increased concentration of butyrate (CS: 19.9 mmol/l, GS: 14.6 mmol/l, Hay: 15.3 mmol/l). No significant differences were found for the concentrations of acetate, isobutyrate, valerate, and isovalerate.

The acetate to propionate ratio differed significantly between CS and Hay treatments. It was closest for CS (3.1) and widest for Hay (3.7) while the GS treatment showed a ratio in between the other treatments (3.4).

Significant differences in the composition of total VFAs were found for acetate and butyrate. The proportion of butyrate was significantly lower in the GS treatment with 13.6% of VFA_{total} than in the CS treatment with 17.3% of VFA_{total}. The butyrate concentration of Hay treatment with 15.1% of VFA_{total} was not significantly different from the other treatments. No differences were found between treatments for the proportion of propionate, isobutyrate, valerate, and isovalerate.

Table 4.8 Rumen liquid spot sample data for the three treatments in Trial 2 (n = 36, Least Squares Means and *Standard Error)*

	Corn silage	Grass silage	Hay	*SE*	Ration p-Value
VFA_{total} (mmol/l)	114.1[a]	104.5[b]	99.0[b]	*2.58*	<0.01
C2 (mmol/l)	67.1	66.4	62.9	*2.11*	0.45
C3 (mmol/l)	22.4[a]	20.3[b]	17.5[c]	*0.61*	<.0001
C4iso (mmol/l)	0.8	0.8	0.7	*0.06*	0.43
C4 (mmol/l)	19.9[a]	14.6[b]	15.3[b]	*1.23*	0.03
C5iso (mmol/l)	1.3	0.9	0.9	*0.16*	0.32
C5 (mmol/l)	2.5	1.5	1.6	*0.14*	0.06
Acetate/Propionate	3.08[b]	3.37[ab]	3.68[a]	*0.12*	0.02
pH Value	6.25	6.34	6.47	*0.06*	0.10
NH_3-N (mmol/L)	9.3	6.9	6.8	*1.05*	0.35
C2 (% of VFA_{total})	59.1[b]	64.0[a]	64.0[a]	*0.95*	0.01
C3 (% of VFA_{total})	19.5	19.3	17.6	*0.50*	0.14
C4iso (% of VFA_{total})	0.8	0.8	0.8	*0.05*	0.94
C4 (% of VFA_{total})	17.3[a]	13.6[b]	15.1[ab]	*0.79*	0.01
C5iso (% of VFA_{total})	1.2	0.9	1.0	*0.14*	0.53
C5 (% of VFA_{total})	2.2	1.4	1.6	*0.12*	0.09

[a,b] Values in a row with different superscript letters differ ($p \leq 0.05$) between rations

4.2.4 Milk fatty acids

As shown in Table 4.9, the concentration of the minor FA C20:1 c11 was significantly different between the three treatments, with its highest concentration in the CS treatment (0.07%) followed by Hay treatment (0.04%), while the GS treatment had a concentration below the limit of detection. Between the CS and the GS treatment a difference in the concentration of C18:2 c9,12 + C19:1 t7 was found (2.45% to 1.62%). While the sum of short-chain fatty acids (C4 to C13) did not differ significantly between the three treatments, the sum of medium-chain fatty acids (C14 to C16) was significant highest for the CS (51.92%) and lowest for GS (50.56%) and Hay treatment (50.75%). The minor FA C15:1 t was also significant lower in the Hay treatment.

Besides the fatty acids shown in Table 4.9, the following fatty acids were analysed but had concentrations below the detection limit (< 0.02%): C13:0 iso, C14:1 t, C15:1 c + C16:0 aiso, C17:1 c, C17:1 t, C18:1 c6, C18:2 t9,c11, C18:2 t10,c12, C18:3 c6,9,12, C20:1 t11 and C20:2 c11,14.

Table 4.9 Milk fatty acids in Trial 2 (n = 30; Least Squares Means and *Standard Error)*

Concentration in percent of analysed milk fat	Corn silage	Grass silage	Hay	*SE*	Ration p-Value
C4:0	4.33	4.26	4.35	*0.051*	0.55
C6:0	3.33[a]	3.03[b]	3.14[b]	*0.027*	0.03
C8:0	1.64	1.44	1.50	*0.052*	0.19
C10:0	4.47	3.68	3.89	*0.269*	0.30
C11:0	0.19	0.12	0.12	*0.025*	0.30
C12:0	6.13	4.90	5.18	*0.441*	0.32
C13:0	0.23	0.15	0.14	*0.036*	0.33
C14:0	12.86	12.00	12.19	*0.368*	0.40
C14:0 iso	0.10	0.10	0.10	*0.004*	0.79
C14:1 c + C15:1 aiso	1.55	1.59	1.64	*0.031*	0.30
C15:0	1.51	1.29	1.22	*0.144*	0.48
C15:0 iso	0.17	0.20	0.20	*0.011*	0.25
C15:1 t	0.32[a]	0.30[a]	0.27[b]	*0.010*	<0.01
C16:0	33.82	33.13	33.13	*0.410*	0.51
C16:1 t	0.05	0.09	0.00	*0.015*	0.11
C16:1 c	1.53	1.87	1.97	*0.177*	0.37
C17:0	0.51	0.58	0.53	*0.029*	0.40
C17:0 iso	0.23	0.27	0.29	*0.012*	0.14
C17:0 aiso	0.36	0.39	0.41	*0.017*	0.25
C18:0	7.04	7.68	6.75	*0.193*	0.14
C18:0 iso	0.17	0.21	0.22	*0.020*	0.37
C18:1 t6 + c7 + t9 + t11	1.46	1.18	0.86	*0.259*	0.43
C18:1 c9	13.69	17.65	17.77	*1.370*	0.26
C18:1 c11	0.70	0.72	0.81	*0.058*	0.50
C18:1 c12	0.22	0.13	0.12	*0.021*	0.11
C18:2 t9,12	0.20	0.20	0.16	*0.016*	0.30
C18:2 c9,12 + C19:1 t7	2.45[a]	1.62[b]	1.90[ab]	*0.100*	0.05
C18:2 c9,t11	0.30	0.40	0.29	*0.030*	0.19
C18:3 c9,12,15	0.27	0.57	0.58	*0.074*	0.14
C19:1 t10	< DL	0.03	< DL	*0.021*	0.72
C20:0	0.10	0.10	0.10	*0.002*	0.37
C20:1 c11	0.07[a]	< DL[c]	0.04[b]	*0.006*	<.0001
C20:3 c11,14,17	0.12	0.10	0.10	*0.010*	0.50
C22:0	0.02	0.07	0.02	*0.014*	0.21
Sum even-numbered chain + C15:1 aiso	96.68	96.81	96.90	*0.254*	0.84
Sum odd-chain + C14:1 c	5.10	4.95	4.87	*0.243*	0.81
Sum iso-chain	0.67[c]	0.79[b]	0.82[a]	*0.011*	<.0001
Sum aiso-chain + C14:1 c	1.91	1.99	2.06	*0.035*	0.17
Sum monounsaturated + C18:2 c9,12	22.10	25.23	25.45	*1.271*	0.31
Sum 2x unsaturated + C19:1 t7	2.98	2.25	2.37	*0.115*	0.08
Sum 3x unsaturated	0.40	0.69	0.70	*0.067*	0.13
Sum polyunsaturated + C19:1 t7	3.38	2.93	3.07	*0.182*	0.38
Sum short-chain (C4 to C13)	20.31	17.60	18.34	*0.793*	0.24
Sum medium-chain (C14 to C16)	51.92[a]	50.56[b]	50.75[b]	*0.324*	<0.01
Sum long-chain (C17+)	27.99	32.00	31.03	*0.836*	0.14

[a,b,c] Values in a row with different superscript letters differ ($p \leq 0.05$) between rations

< DL: below detection limit (0.02%)

4.3 Conclusions

Overall, the milk FAs did not differ remarkably between the three treatments. For some FAs the differences were statistically significant. The concentration of C15:1 t was significantly lowest in the Hay treatment, C18:2 c9,12 + C19:1 t7 was lowest in the GS treatment and C20:1 c11 was highest in the CS and lowest in the GS treatment. For the sums of iso-chain FAs, the CS treatment showed the lowest and the Hay treatment the highest concentrations, while for FAs with a medium-chain length the CS treatment showed the highest concentrations.

The prevalence of 7 out of 30 measurement days with SARA incidence in the CS treatment was in accordance with the predicted pH_{mean} of 5.83, when assuming the starch content in the ration according to The Swiss Feed Database and using the DMI (Table 4.7) for the equation of the Committee for Requirement Standards of the Society of Nutrition Physiology (2014). The actual pH_{mean} was not as low as predicted, which was probably caused either by the fact that the slow fermentation rate of CS starch was not considered adequately, or by the fact that the particle structure of CS was adequate to form the fibrous mat in the rumen.

The peNDF content in the rations, including GS and hay were above the upper limits for peNDF in rations recommended by the Committee for Requirement Standards of the Society of Nutrition Physiology (2014). If 32% $peNDF_{>1.18mm}$ or 22% $peNDF_{>8mm}$ are in the ration, the DMI is expected to be reduced, but only a numerical, not a statistical low DMI was recorded.

Nonetheless, no negative effects on performance data, such as feed intake or milk yield were detected in the CS treatment. Since adverse effects of SARA can occur several weeks after the actual SARA, a Latin square design is not adequate to get information about the long-term effects. To gain such information an experimental design has to be chosen where long-term observations can be related to a previous SARA challenge.

5 TRIAL 3

This trial focused on the differences between a barn and a pasture-based husbandry for cows, with a focus on animal data of ruminal fermentation and milk constituents, indicating if SARA occurs in grazing cows when only small amounts of concentrate are added in the grazing treatment.

5.1 Material and methods

The experimental design will be explained in 5.1.1, data shown in this work are only shown for data from the cannulated cows from week 5 to 10, when the pasture group was on full time pasture and not in the transition period anymore. Data of the whole trial from all animals were published by Schären et al. (2016).

5.1.1 Animals and animal housing / Experimental design

The trial took place at the Friedrich-Loeffler-Institute in Brunswick, Germany in accordance with the German Animal Welfare Act approved by the Lower Saxony State Office for Consumer Protection and Food Safety, Germany from the 21.04.2014 to 29.06.2014.

Five cannulated cows were allocated to both, the confinement group (CG) in the barn, as well as to the pasture group (PG). The cannulated cows had an average of 185 days in milk (DIM) at the start of the trial. The PG had a size of 29, the CG of 31 cows in total. The trial lasted 10 weeks. The PG was adapted to the new environment stepwise, starting from week two for 3 hours/day on pasture, week 3 and 4 12 hours/day on pasture, and from week 5 onwards pasture only + 1.75 kg concentrate per day. In the first week and in the hours of the day when they were not on pasture in the weeks 2 to 4 the cows were in the barn. Except during the milking time, the cows had ad libitum access to the same TMR mixture as the CG while being in the barn. During the adaption time to pasture the feed intake in barn remained very high, which replaced the grass intake, which is consistent with literature (Graf et al., 2005).

5.1.2 Rations

The TMR for the CG was provided once per day at approximately 11:00, the feed residuals were removed from the troughs and the new feed was distributed. The feed was available ad libitum and an oversupply of 5% was calculated.

The PG was held on a continuous grazing system with two pasture areas with a size of 6 ha each, directly next to the barn. Depending on pasture management criteria, the cows had access to one or the other pasture. Detailed information about the pasture composition, soil, and fertilisation were described by Schären et al. (2016).

The TMR consisted of 35% corn silage, 35% grass silage, and 30% concentrate, as shown in Table 5.1. In the weeks 5 to 10, when the PG was grazing, a fix relation between concentrate and forage was not possible, all cows in the PG got 1.75 kg (DM) concentrate and had ad libitum access to grass.

A similar density of energy and nutrients for both treatments was achieved with both rations, as shown in Table 5.3.

Table 5.1 Composition of rations used in Trial 3 (percent, on DM basis)

Treatment	Confinement group	Pasture group (week 5 – 10)
Corn silage	35	-
Grass silage	35	-
Pasture	-	Ad libitum
Concentrate	30	1.75 kg DM/d

The concentrates of the treatments differed in composition. As shown in Table 5.2, the concentration of wheat, corn, and barley were higher in the PG concentrate than in the CG and Mg-oxide was included only in the PG concentrate. On the other hand, the CG concentrate included soybean, rapeseed meal, dried sugar beet, and limestone, and had a slightly higher concentration of mineral feed.

Table 5.2 Concentrate ingredients used in Trial 3 (percent, on DM basis)

Ingredient	Concentrate TMR CG	Concentrate PG
Soybean meal	15.8	-
Rapeseed meal	11.0	-
Wheat	21.4	29.6
Corn	21.5	29.6
Barley	21.5	29.6
Dried sugar beet pulp	5.0	-
Limestone	1.5	-
Soybean oil	1.0	1.0
Mg-oxide	-	1.2
Mineral feed[1]	1.2	9.0

[1] Per kilogram of mineral feed: 104 g Ca, 120 g Na, 70 g P, 40 g Mg, 6 g Zn, 5.4 g Mn, 1 g Cu, 100 mg I, 40 mg Se,
25 mg Co, 1,000,000 IU Vitamin A, 100,000 IU Vitamin D_3, 1,500 mg Vitamin E

5.1.2.1 Chemical composition of experimental diets

Pasture intake could not be measured. Therefore, the chemical composition for grass and concentrate are shown separately in Table 5.3.

Table 5.3 Chemical composition of experimental diets[1] and feeds used in Trial 3 (g/kg, on DM basis)

	TMR CG[1]	Concentrate PG[2]	Grass PG[2]
DM (g/kg)	342	876	183
Crude ash	71	108	97
Ether extract	33	27	39
Crude protein	124	96	189
uCP	143	148	151
Crude fiber	207	32	218
aNDFom	403	141	547
ADFom	231	67	268
NEL (MJ/kg DM)	6.7	7.6	6.7
Sugar	14	24	124
Starch	259	582	0
RNB	-3.1	-8.3	4.2

[1]CG = confinement group; [2]PG = pasture group; uCP = utilizable crude protein; RNB = ruminal nitrogen balance; aNDFom = neutral detergent fiber; ADFom = acid detergent fiber. NDF and ADF were expressed without residual ash and therefore referred to as NDFom and ADFom.
[2]Chemical composition in weeks 5 to 10, the pasture samples were collected twice a week and the means of week 5 to 10 are shown.

5.1.3 Samplings

Although pH data and MIR milk analysis started earlier, all data presented in this work are from weeks 5 to 10. This period of data collection was used for both treatments because this was the period when the PG was on pasture only. At the start of week 5, the cannulated cows were on average on DIM 203 (CG) and DIM 220 (PG), respectively.

Every Tuesday continuous pH measurement was conducted in the PG. Rumen liquid was also collected from the ventral sac of the rumen on Tuesdays after every milking and milk samples from every milking were taken, for MIR analyses and GC analysis of an aliquot of the daily milk. The same samples were taken on Thursdays from the CG.

5.1.3.1 Continuous rumen pH measurement

Ruminal pH was measured with indwelling pH datalogger for cannulated ruminants (Large Ruminant Logger M5-T7, Dascor Inc., Oceanside, USA), following the timesheet shown in Table 5.4. Every minute the pH and temperature were measured and stored in the intern memory of the datalogger. The logger was attached to stainless steel weights, weighing 1.5 kg in total, to anchor the logger in the ventral sac of the rumen.

A two-point calibration was made using pH 4 and pH 7 buffer solution after every period of measurement. Therefore, the logger was removed after milking, stored data were transferred to a notebook, and the logger was recalibrated. After the next milking, the loggers were reintroduced to the rumen of the other experimental group.

5.1.3.2 Performance data

Milk yield, animal body weight, feed-, and water intake were recorded as described in chapter 3.1.3.2, with the limitation that the PG had no quantified feed and water intake.

5.1.3.3 Milk sampling

Representative milk samples were taken at every milking time when the continuous pH measurements were performed. The milk samples were collected into the standard flasks for the official milk control, where the sample is preserved with sodium azide. The samples were stored in the fridge at +5 °C and the collected samples were sent twice a week to the Landeskontrollverband Nordrhein-Westfalen in Krefeld, for MIR analysis (Foss FT+, FT6000;

Hillerød, Denmark). Samples were analysed for fat, protein, lactose, urea content, pH value, and cell count.

On the weekly measurement days (PG = Tuesday, CG = Thursday) (compare Table 5.4), milk samples from the morning and the evening milking were taken for gaschromatographical analysis and immediately stored at -20 °C until further analysis following the protocol mentioned in chapter 3.1.3.3.

5.1.3.4 Rumen liquid spot sampling

Rumen liquid was sampled on one day per week from week 5 to 10. On sampling days, rumen liquid was collected twice, after milking. With a manual vacuum pump, 230 ml of rumen liquid were pumped from the ventral sac of the rumen and filled in a 250 ml polyethylene flask. The pH in the rumen liquid was measured in the barn with a glass electrode (digital pH measurement devise, pH 525, WTW, Weilheim, Germany) and immediately stored at -20 °C until further analysis.

Table 5.4 Overview of sampling procedure in Trial 3

	Treatment							
	Confinement				Pasture			
Date	pH	MIR-Milk	GC-Milk	Rumen liquid	pH	MIR-Milk	GC-Milk	Rumen liquid
19.5.2014					X	X		
20.5.2014					X	X	X	X
21.5.2014	X	X						
22.5.2014	X	X	X	X				
23.5.2014		X						
24.5.2014					X	X		
25.5.2014					X	X		
26.5.2014					X	X		
27.5.2014					X	X	X	X
28.5.2014	X					X		
29.5.2014	X	X	X	X				
30.5.2014	X	X						
31.5.2014	X	X						
01.6.2014		X						
02.6.2014					X	X		
03.6.2014					X	X	X	X
04.6.2014	X					X		
05.6.2014	X	X	X	X				
06.6.2014	X	X						
07.6.2014		X						
08.6.2014						X		
09.6.2014					X	X		
10.6.2014					X	X	X	X
11.6.2014	X					X		
12.6.2014	X	X	X	X				
13.6.2014	X	X						
14.6.2014	X	X						
15.6.2014						X		
16.6.2014					X	X		
17.6.2014					X	X	X	X
18.6.2014						X		
19.6.2014	X	X	X	X				
20.6.2014	X	X						
21.6.2014	X	X						
22.6.2014		X						
23.6.2014					X	X		
24.6.2014					X	X	X	X
25.6.2014	X					X		
26.6.2014	X	X	X	X				
27.6.2014	X	X						
28.6.2014	X	X						
29.6.2014		X						

5.1.4 Statistical analysis

Continuous pH measurements were subjected to regression analysis as described in chapter 2. For continuous pH measurements, milk, and performance data the following mixed model was used in SAS 9.2:

$$y_{ijk} = \mu + R_i + W_j + C_k + (R\ x\ W)_{ij} + \varepsilon_{ijk}$$

With R_i = ration (CG, PG) as fix effect and W_j = week (5,6,….10) and C_k = Cow (1,2,…10) and ration *x* week as random effects with ε_{ijk} as error.

Rumen liquid was analyszed using the following mixed model in SAS 9.2:

$$y_{ijkl} = \mu + R_i + W_j + T_l + (R\ x\ W)_{ij} + C_k + (W\ x\ T)_{jl} + (W\ x\ C)_{jk} + (T\ x\ C)_{lk} + \varepsilon_{ijkl}$$

With R_i = ration (CG, PG), W_j = week (5,6,…10), T_l = time and ration *x* week as fix effects and cow, week *x* time, week *x* cow and time *x* cow as random effects.

5.2 Results

5.2.1 Continuous rumen pH measurement

The rumen pH measurement showed, as shown in Table 5.5, that the span of pH values was significantly higher in the CG with a significantly lower β0 of 7.96, compared to 9.51 in the PG. In addition, a significantly higher pH_{max} of 6.70 was detected for the CG compared to 6.57 in the PG.

Since the lower pH_{mean} combined with the higher β0, the minute per day pH < 6.0 was significantly higher in the PG (439 min/day) than in the CG (290 min/day). Nevertheless, because the pH varied less around the pH_{mean} in the PG, the time the pH was below 5.8 was 141 minutes in the PG while it was 105 minutes per day in the CG. Therefore, the risk of a SARA is not given for the average of all measurements, but the single day measurements showed that in 7% of the measured days SARA was prevalent in the CG and in 8% of the measured days in the PG.

Table 5.5 pH data of the two treatments on continuous measurements in Trial 3, gained as described in chapter 2 (CG; n = 99 and PG; n = 62, Least Squares Means, *Standard Error*)

Parameter	CG	PG	Ration p-Value
pH Mean	6.22[a] *0.018*	6.14[b] *0.023*	<0.01
pH Minimum	5.66 *0.024*	5.71 *0.031*	0.27
pH Maximum	6.70[a] *0.016*	6.57[b] *0.021*	<.0001
β0	7.96[b] *0.218*	9.51[a] *0.285*	<.0001
β1	6.23[a] *0.018*	6.14[b] *0.023*	<0.01
Minutes per day pH < 5.2	4 *0.6*	2 *0.8*	0.06
Minutes per day pH < 5.6	35 *5.2*	34 *6.8*	0.92
Minutes per day pH < 5.8	105 *14.2*	141 *18.6*	0.13
Minutes per day pH < 6.0	290[b] *29.8*	439[a] *39.0*	<0.01
SARA incidence[1]	7/99 7%	5/62 8%	-

[a,b] Values in a row with different superscript letters differ (p ≤ 0.05) between rations

[1] SARA incidence for every measurement day evaluated as described in the introduction. Days defined as SARA positive if the time pH < 5.8 was 5.24 hours (315 min) or longer and β1 < 6.16.

The pH in the PG reached its maximum in the morning at 5:30, when the cows finished the morning milking. In the consecutive hours, a slight but steady decrease in pH was found as shown in Figure 5.1. The constant decrease indicates that the 0.875 kg concentrate/cow that was fed to the PG after each milking did not have a substantial impact on the pH in the rumen. After the pH reached its minimum shortly before midnight, it steadily increased until the next morning milking. In the CG the pH reached its maximum level at ~7:00 in the morning. These cows were milked after the pasture group due herd management reasons around that time. The pH dropped from ~12:30, after the distribution of fresh feedstuff, until its pH_{min} at ~21:30 with a following recovery until morning. The average daily pH courses of the treatments, shown in Figure 5.1, are in a pH region where no risk of SARA for the herd can be detected. The pH is lower for the longest time of the day in the PG, which concurs with the significantly lower β1 in the PG of pH 6.14 compared to 6.23 in the CG and with a more stable pH.

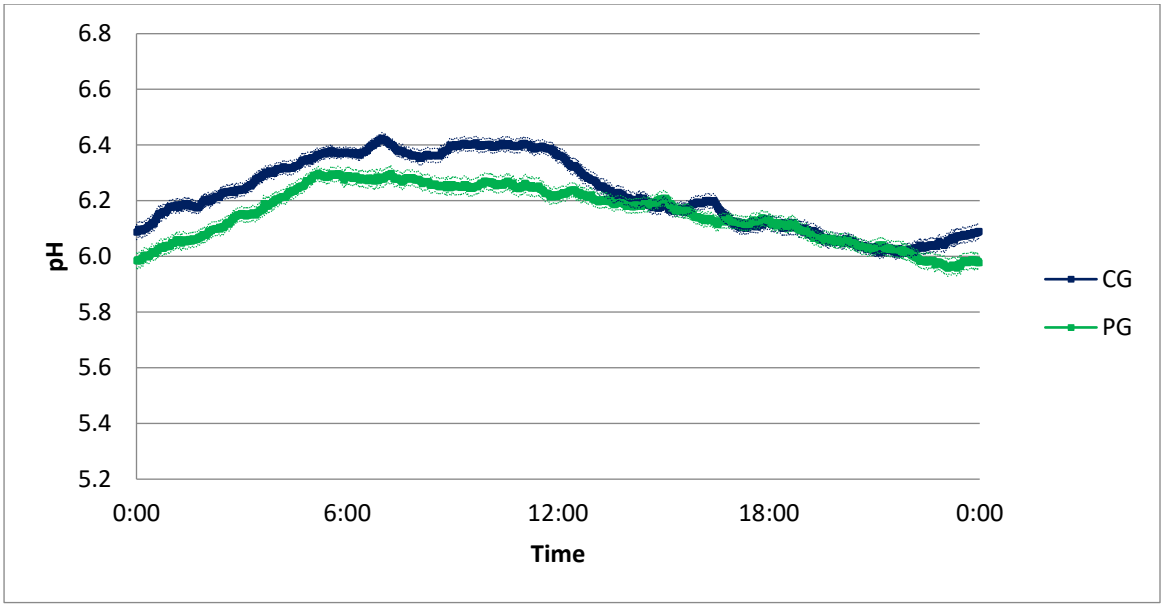

Figure 5.1 Means of 24 h pH measurements ± *Standard Error* for the treatments CG and PG in Trial 3 (CG; n = 99, PG; n = 62)

5.2.2 Performance data

Although for the PG no feed intake was quantified, it subjectively seemed that the rumen in the PG were not as filled with rumen content as in the CG and therefore in tendency had a reduced feed intake compared to the CG. Concurrent with these subjective observations the estimation of feed intake with a marker for the whole group, including not cannulated cows, showed for week 7 12.7 kg DMI in the PG compared to 20 kg DMI in the CG and in week 9 15.0 kg DMI in the PG compared to 19 kg DMI in the CG (Schären et al., 2016).

The CG had a significantly higher milk yield and fat content in the milk while its urea concentration and pH value of the milk was lower than in the PG.

Table 5.6 Performance data week 5 to 10 (CG; n = 79, PG; n = 70, Least Squares Means and *Standard Error*)

Milk	Confinement	*SE CG*	Pasture	*SE PG*	Ration p-Value
Yield (kg)	20.8[a]	*0.33*	18.8[b]	*0.34*	<.0001
Fat (%)	4.5[a]	*0.08*	3.9[b]	*0.08*	<.0001
Protein (%)	3.1	*0.02*	3.1	*0.02*	0.22
Lactose (%)	4.6	*0.03*	4.6	*0.03*	0.35
pH value	6.68[b]	*0.01*	6.72[a]	*0.01*	<.0001
Urea (ppm)	13.6[b]	*0.52*	19.9[a]	*0.53*	<.0001

5.2.3 Rumen liquid spot samples

Rumen liquid was sampled once a week, after the morning and evening milking.

As shown in Table 5.7, the concentration of VFA_{total}, as well as the concentration of the main VFAs, acetate, propionate, and butyrate did not differ significantly between the PG and CG. The acetate to propionate ratio was 3.22 in the CG and 3.32 in the PG. Therefore, the ratios were in a range where no acidotic pH is expected, which is in accordance with the pH_{mean} measured in spot samples of 6.45 in the CG and 6.48 in the PG. As mentioned above, and shown in Table 5.5, also the continuous pH measurements were predominantly non-SARA days, in accordance to the spot samples.

Isobutyrate and isovalerate, as well as valerate were significantly higher in the CG, with a maximum difference for isovalerate with 2.11 mmol/l in the CG and 1.19 mmol/l in the PG.

The significantly higher NH_3 concentration in the PG with a concentration of 7.92 mmol/l compared to the CG with 6.22 mmol/l might be caused by the higher CP content of the pasture-based diet compared to the TMR in the barn.

Table 5.7 Rumen liquid spot sample data (n = 60, Least Squares Means and *Standard Error*)

	Confinement	*SE CG*	Pasture	*SE PG*	Ration p-Value
VFA_{total} (mmol/l)	113.1	*2.33*	111.1	*2.273*	0.51
C2 (mmol/l)	70.6	*1.35*	70.9	*1.32*	0.88
C3 (mmol/l)	22.3	*1.13*	21.7	*1.05*	0.67
C4iso (mmol/l)	1.1^{a}	*0.03*	0.9^{b}	*0.03*	<0.01
C4 (mmol/l)	15.2	*0.36*	15.0	*0.35*	0.69
C5iso (mmol/l)	2.1^{a}	*0.15*	1.2^{b}	*0.14*	<0.01
C5 (mmol/l)	1.8^{a}	*0.07*	1.2^{b}	*0.07*	<0.001
Acetate/Propionate	3.22	*0.152*	3.32	*0.141*	0.66
pH Value	6.45	*0.07*	6.5	*0.07*	0.12
NH_3-N (mmol/l)	6.2^{b}	*0.52*	7.9^{a}	*0.50*	0.04
C2 (% of VFA_{total})	62.5	*0.63*	64.0	*0.59*	0.12
C3 (% of VFA_{total})	19.7	*0.70*	19.5	*0.65*	0.88
C4iso (% of VFA_{total})	1.0^{a}	*0.03*	0.8^{b}	*0.03*	0.01
C4 (% of VFA_{total})	13.4	*0.22*	13.5	*0.21*	0.78
C5iso (% of VFA_{total})	1.9^{a}	*0.11*	1.1^{b}	*0.10*	<0.001
C5 (% of VFA_{total})	1.6^{a}	*0.04*	1.1^{b}	*0.04*	<0.0001

^{a,b} Values in a row with different superscript letters differ ($p \leq 0.05$) between rations

5.2.4 Milk fatty acids

The composition of the milk differed significantly between the rations. While in the CG the medium-chain fatty acids were the prevalent type of FAs in the milk fat, in the PG the long-chain FAs were prevalent. This may indicate that in the PG a considerable body-fat mobilisation occurred. Besides the subjectively lower filling level in the rumen and the reported lower DMI for the pasture group, this is another indicator that the feed intake was not sufficient for adequate energy supply. Differences between the treatments in milk fat composition are mainly based on different intake, and therefore the energy supply, levels. A conclusion about ruminal status of the cows' based on the milk fatty acid concentrations is not effective, owing to the expected overlay from body fat mobilisation.

Besides the fatty acids shown in Table 5.8, the following fatty acids were analysed but had concentrations below the detection limit (< 0.02%): C11:0, C13:0 iso, C14:1 t, C15:1 c + C16:0 aiso, C17:1 c, C17:1 t, C18:1 c6, C18:2 t9,c11, C18:2 t10,c12, C18:3 c6,9,12, C20:1 t11 and C20:2 c11,14.

Table 5.8 Milk fatty acids from GC analysis (n = 30, Least Squares Means and *Standard Error*)

Concentration in percent of analysed milk fat	CG	*SE CG*	PG	*SE PG*	Ration p-Value
C4:0	5.03	*0.134*	4.95	*0.126*	0.67
C6:0	2.93	*0.089*	2.70	*0.084*	0.09
C8:0	1.17	*0.048*	1.04	*0.045*	0.08
C10:0	2.63[a]	*0.125*	2.19[b]	*0.118*	0.03
C12:0	3.16[a]	*0.138*	2.51[b]	*0.130*	<0.01
C13:0	0.10[a]	*0.007*	0.06[b]	*0.006*	<0.01
C14:0	10.80[a]	*0.247*	8.63[b]	*0.237*	<.0001
C14:0 iso	0.07[b]	*0.005*	0.09[a]	*0.005*	0.05
C14:1 c + C15:1 aiso	1.20	*0.060*	1.37	*0.057*	0.07
C15:0	1.17	*0.061*	1.03	*0.057*	0.13
C15:0 iso	0.20[b]	*0.010*	0.24[a]	*0.010*	0.02
C15:1 t	0.27	*0.014*	0.27	*0.013*	0.98
C16:0	35.32[a]	*0.938*	24.75[b]	*0.879*	<.0001
C16:1 t	0.04[b]	*0.004*	0.09[a]	*0.004*	<.0001
C16:1 c	1.67	*0.162*	2.13	*0.150*	0.06
C17:0	0.63[b]	*0.017*	0.83[a]	*0.016*	<.0001
C17:0 iso	0.25[b]	*0.016*	0.41[a]	*0.015*	<.0001
C17:0 aiso	0.37[b]	*0.027*	0.52[a]	*0.025*	<0.01
C18:0	10.11[b]	*0.421*	11.59[a]	*0.400*	0.03
C18:0 iso	0.19[b]	*0.024*	0.36[a]	*0.023*	<0.001
C18:1 t6 + c7 + t9 + t11	0.56[a]	*0.073*	< DL[b]	*0.073*	<.0001
C18:1 c9	18.69[b]	*1.169*	29.68[a]	*1.095*	<.0001
C18:1 c11	0.73[b]	*0.055*	1.03[a]	*0.051*	<0.01
C18:1 c12	0.28[a]	*0.010*	0.16[b]	*0.009*	<.0001
C18:2 t9,12	0.27[b]	*0.016*	0.39[a]	*0.016*	<0.001
C18:2 c9,12 + C19:1 t7	1.12	*0.051*	1.24	*0.048*	0.13
C18:2 c9,t11	0.35[b]	*0.027*	0.56[a]	*0.026*	<0.001
C18:3 c9,12,15	0.30[b]	*0.031*	0.75[a]	*0.029*	<.0001
C19:1 t10	0.07[b]	*0.004*	0.08[a]	*0.004*	<0.01
C20:0	0.14	*0.004*	0.14	*0.004*	0.54
C20:1 c11	0.03[b]	*0.004*	0.05[a]	*0.004*	<0.01
C20:3 c11,14,17	0.07	*0.006*	0.06	*0.006*	0.20
C22:0	0.03[b]	*0.002*	0.06[a]	*0.002*	<.0001
Sum even-numbered chain + C15:1 aiso	96.91[a]	*0.081*	96.57[b]	*0.079*	0.01
Sum odd-chain + C14:1 c	3.63[b]	*0.131*	4.02[a]	*0.123*	0.05
Sum iso-chain	0.72[b]	*0.037*	1.13[a]	*0.035*	<.0001
Sum aiso-chain + C14:1 c	1.57[b]	*0.087*	1.90[a]	*0.081*	0.02
Sum monounsaturated + C18:2 c9,12	24.67[b]	*1.419*	36.07[a]	*1.323*	<0.001
Sum 2x unsaturated + C19:1 t7	1.74[b]	*0.081*	2.18[a]	*0.076*	<0.01
Sum 3x unsaturated	0.37[b]	*0.032*	0.81[a]	*0.030*	<.0001
Sum polyunsaturated + C19:1 t7	2.11[b]	*0.105*	2.99[a]	*0.098*	<.0001
Sum short-chain (C4 to C13)	10.01[a]	*0.391*	8.54[b]	*0.369*	0.02
Sum medium-chain (C14 to C16)	50.75[a]	*0.963*	38.61[b]	*0.912*	<.0001
Sum long-chain (C17+)	34.20[b]	*1.222*	47.98[a]	*1.159*	<.0001

[a,b] Values in a row with different superscript letters differ ($p \leq 0.05$) between rations

< DL: below detection limit (0.02%)

5.3 Conclusions

During this trial, SARA occurred in rare abundance in both groups. It can be concluded that 30% concentrate in TMR was not critical as in 93% of the days SARA did not occur in the CG. In the PG, 92% of the measured days were without SARA incidence, so both groups are equally low in acidosis risk. Nonetheless, it has to be stated that the feed intake in the pasture group was low and the amount of feed intake has a high impact on SARA incidence. Therefore, a general deduction whether SARA is a major problem for pasture fed cows cannot be given based on this trial.

6 INTERTRIAL ANALYSIS OF TRIALS 1 – 3

The three trials were conducted to identify if it is possible to determine the ruminal conditions by analysing the milk. With the three trials, it was possible to induce different pH profiles, as shown in Figure 6.1, including fast changes in the pH profile and with very stable pH profiles, as well as acidotic and non-acidotic circumstances.

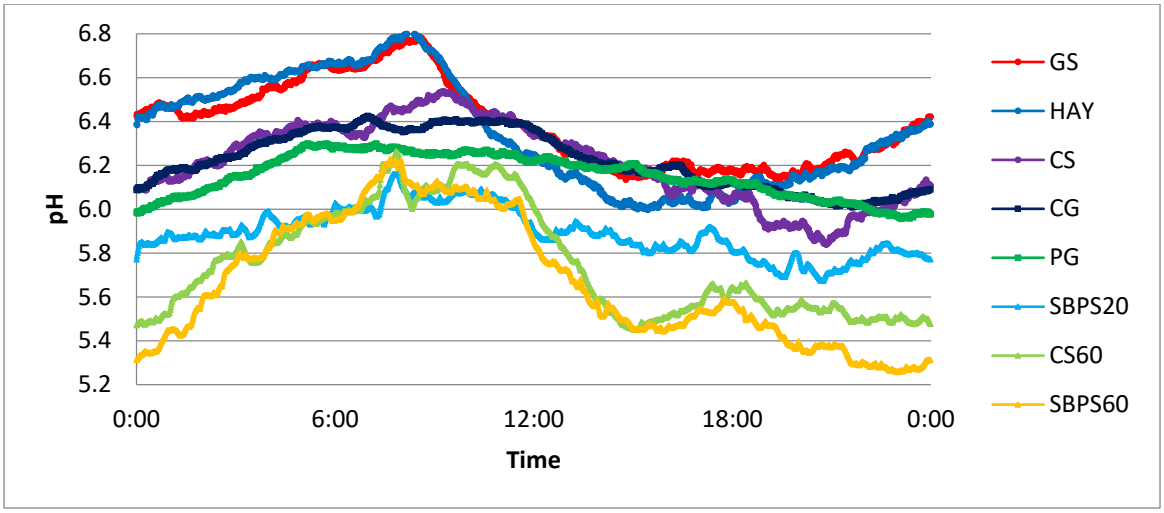

Figure 6.1 Means of 24 h pH measurements of all treatments of the three trials (Trial 1: CS60 (n = 27), SBPS60 (n = 24), SBPS20 (n = 27); Trial 2: GS (n = 30), Hay (n = 30), CS (n = 30); Trial 3: CG (n = 99), PG (n = 62))

The lowest pH values over the course of a day were reached in Trial 1, especially in the treatments CS60 and SBPS60. The treatments that induced the highest pH values were the GS and Hay treatment in Trial 2. The highest variations within the pH course of the day were found in the treatments GS and Hay in Trial 2, and CS60 and SBPS60 in Trial 1.

For the days when milk samples were taken for gaschromatographical analysis, the ruminal pH conditions are shown in Figure 6.1. For every measurement day the pH course of the day was characterised by β1 and β0 as described in chapter 2. In Figure 6.2, β1 is shown on the x-axis, and β0 on the y-axis. Every measurement point on the left side of the SARA threshold line, defined by Zebeli et al. (2008), and transformed as shown in Figure 2.4, shows a SARA incidence, while measurements on the right side of the threshold are non-SARA days.

Figure 6.2 shows that in Trial 1 most of the analysed days were SARA days, while in the other trials the non-acidotic days were prevalent, but also SARA days were measured.

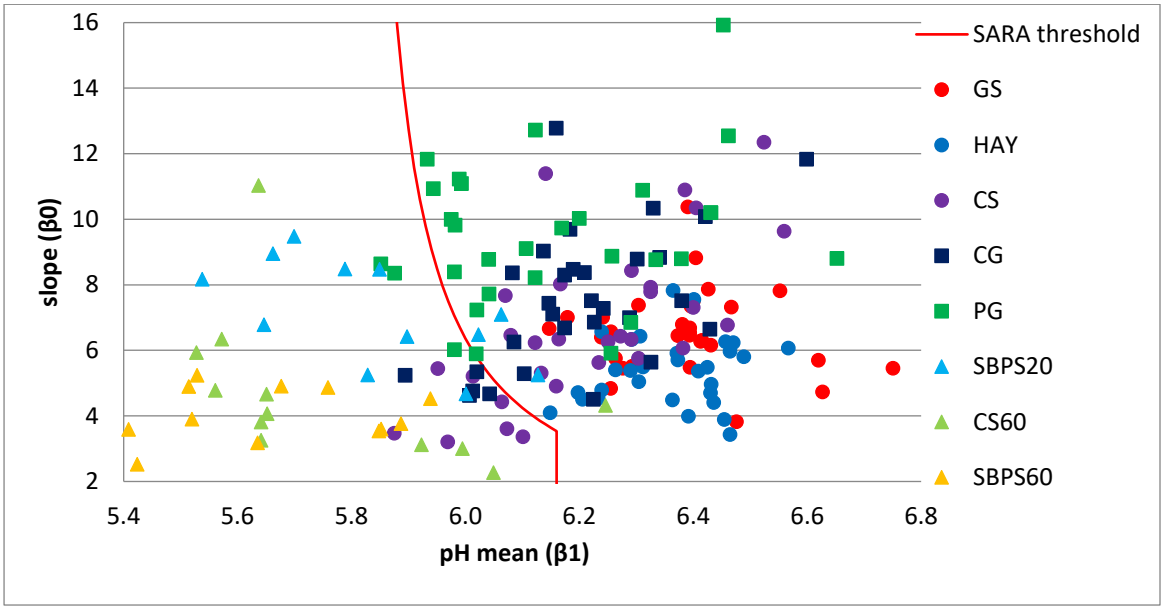

Figure 6.2 Distribution of pH measurements of the eight treatments of the three experiments on the days of milk sampling. SARA incidence following the definition of Zebeli et al. (2008), if pH_{mean} < 6.16 and pH < 5.8 for more than 314 minutes/day. Measurements represent SARA days if the measurement lies on the left side of the SARA threshold line. The SARA threshold in dependency of β1 was calculated as described in chapter 2, according to Figure 2.4.

In an attempt to better understand relationships between ruminal conditions and milk constituents correlations between the ruminal parameters β0 (slope), β1 (pH_{mean}), time pH < 5.8, and the milk constituents, milk yield, fatty acids from gaschromatographical analysis and milk components from MIR analysis, were calculated. These correlations are discussed in chapter 6.1.

With the data, also a regression model was built to estimate the influencing factors of greatest importance for ruminal pH from a dataset, including data from the official milk control, gained by MIR analysis, as well as fatty acids analysed by gaschromatographical analysis. Since gas chromatography is very expensive and time-consuming, a simpler model was also calculated only using constituents given by the official milk control and fatty acids which can be estimated by MIR spectral data. Only fatty acids with a high accuracy of estimation ($R^2 \geq 0.95$) were used (Grelet et al., 2014). Chapter 6.2 will cover both models in detail.

6.1 Correlations

For the three important pH parameters β1 (pH_{mean}), β0 (slope), and time pH < 5.8 correlations with the milk fatty acids analysed by GC, as well as data from the official milk control, gained by MIR analysis, were calculated. In addition, the correlations between the different rumen pH parameters were calculated. The significant correlations with $\alpha \leq 0.05$ are shown in the appendix in Table 13.1, Table 13.2 and Table 13.3.

Spearman correlations were calculated using the "proc corr spearman" command in SAS 9.2. The correlations were made for each trial individually and for the complete dataset, including all three trials. The sample size was for Trial 1 = 36; Trial 2 = 90; Trial 3 = 60 (59 for official milk control data) and for all trials together; Trial 1-3 = 186 (185 for official milk control data).

6.1.1 Milk constituents

A multitude of significant correlations was found between the parameters β1, β0, time pH < 5.8 and the milk constituents, as shown in Table 13.1, Table 13.2 and Table 13.3 in the appendix. Nevertheless, significant correlations in every single trial as well as in all trials were very rare.

Many of the milk fatty acids did correlate significantly, but the correlation within the single trials did not necessarily correlate the same way as for the complete dataset. They showed changeable types of correlations. Either significant correlations appeared only if the complete dataset, but not when data from only one trial, were used, or it also happened that within one trial the direction of the correlation was significantly negative, while in another trial the correlation was significantly positive.

For β0 only with one FA, C17:0 (r = 0.24 to 0.39), a significant correlation was found in every single trial, as well as in all three trials, as shown in Table 6.1. For the pH in milk a significant correlation (r = 0.26 to 0.61) was also found with β0, in every single trial, as well as in all three trials, as shown in Table 6.3. All other FAs did not correlate significantly to β0 in at least one of the trials, or did correlate significantly, but not in every trial in the same direction.

Between β1, as well as for the time pH < 5.8, and the milk constituents no significant correlation was found in every single trial as well as in all three trials (Table 13.2 and Table 13.3 in the appendix). Nonetheless, between β1 (pH_{mean}) and two FAs a significant correlation was found in every single trial as well as in all three trials. With β1 only C16:0 correlated significantly positive (r = 0.30 to 0.35) and C18:1 c11 significantly negative (r = -0.29 to -0.51), as shown in Table 6.1. All

other FAs were not significantly correlated to β1 in at least one of the trials, or did correlate significantly, but not in every trial in the same direction.

Between the time pH < 5.8 and the FAs no significant correlation was found in every single trial, as well as in all three trials (Table 13.3 in the appendix).

Table 6.1 Significant (α ≤ 0.05) spearman correlations, in every trial, as well as in all trials, between β0 (slope) and β1 (pH_{mean}) and fatty acids and milk constituents

	Parameter	Trial 1	Trial 2	Trial 3	All trials
Sample size n =		36	90	60[1]	186[1]
C16:0	β1	0.35	0.35	0.30	0.35
C17:0	β0	0.39	0.35	0.38	0.24
C18:1 c11	β1	-0.36	-0.29	-0.31	-0.51
pH value in milk	β0	0.61	0.36	0.26	0.35

[1] For milk constituents n = 59 in Trial 3 and n = 185 in all trials

6.1.2 Ruminal parameters

As shown in Table 6.2, there was only a weak correlation between β1 and β0 which only became significant if all three trials were considered together (r = 0.21), but not within the respective trials. This shows that the pH variation is very weak correlated with the pH_{mean} but the rumen pH tends to be more stable during the day, the higher the pH_{mean} is.

For the whole dataset the time pH < 5.8 was highly negatively correlated with β1 (r = -0.89). Therefore, the relationship between a long time pH < 5.8 in the rumen and a low daily average pH is very high. The correlation (r = -0.54) between the time pH < 5.8 and β0 suggests that the time pH < 5.8 is not as much dependent from β0 as it is from β1 and that the more stable the pH is the shorter the time the pH remains below 5.8. This is only accurate as long as the pH_{mean} is higher than 5.8. For example, in Trial 1, the pH_{mean} were below the pH 6.0 benchmark, which leads to a positive correlation between the time pH < 6.0 and β0, because then the more stable the pH was around the pH_{mean} the longer the pH remained below 6.0. In all other trials, it was negatively correlated because there the pH_{mean} was above 6.0. Therefore, it can be conducted, that, as expected, if the pH_{mean} was higher than the threshold the time below the threshold was shorter the more stable (higher β0) the pH was.

Table 6.2 Significant (α ≤ 0.05) spearman correlations between β0 and β1 (pH_{mean}) and the time spent below several pH thresholds

	Parameter	Trial 1	Trial 2	Trial 3	All trials
Sample size n=		36	90	60	186
β0	β1				0.21
Min/day pH < 5.2	β0	-0.70	-0.85	-0.81	-0.79
	β1	-0.67	-0.56	-0.54	-0.66
Min/day pH < 5.6	β0		-0.80	-0.68	-0.66
	β1	-0.94	-0.67	-0.82	-0.81
Min/day pH < 5.8	β0		-0.71	-0.55	-0.54
	β1	-0.91	-0.76	-0.91	-0.89
Min/day pH < 6.0	β0	0.45	-0.57	-0.33	-0.38
	β1	-0.83	-0.87	-0.97	-0.95

6.2 Regression model

For the development of a regression, the data set from all three trials was used. Overall, the data originated from cows fed with eight different rations, which were tested in the three trials. The detailed feed information is available in chapters 3.1.2, 4.1.2, and 5.1.2.

6.2.1 Whole dataset

The Whole dataset had 63 variables and 185 observations. It contained information from the animal house, the official milk control, calculated data, milk fatty acids (analysed by GC), and sums of fatty acid groups as listed in detail in Table 6.3.

Table 6.3 Whole dataset (63 offered variables)

Variable Origin	Number of variables	Variables
Animals' house data	2	Days in milk, milk yield
Milk data from official milk control	6	Milk-, -fat, -protein, -lactose, -pH value, number of cells, -urea
Calculated data	3	Energy corrected milk, fat/protein ratio, fat produced per day
GC analysed FAs	37	C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:0 iso, C14:1 c + C15:1 aiso, C15:0, C15:0 iso, C15:1 t, C16:0, C16:1 t, C16:1 c, C17:0, C17:0 iso, C17:0 aiso, C18:0, C18:0 iso, C18:1 t6 + c7 + t9 + t11, C18:1 c9, C18:1 c11, C18:1 c12, C18:2 t9,12, C18:2 t9,c11, C18:2 c9,12 + C19:1 t7, C18:2 c9,t11, C18:3 c6,9,12, C18:3 c9,12,15, C19:1 t10, C20:0, C20:1 c11, C20:2 c11,14, C20:3 c11,14,17, C22:0
Sums built from analysed FAs	15	∑ C18:1, ∑ C18:1 c, ∑ even-chain, ∑ odd-chain (+ C14:1 c), ∑ iso, ∑ aiso (+ C14:1 c), ∑ saturated, ∑ unsaturated, ∑ monounsaturated (+ C18:2 c9,12), ∑ 2x unsaturated (+ C19:1 t7), ∑ 3x unsaturated, ∑ polyunsaturated, ∑ short-chain (C4 to C13), ∑ medium-chain (C14 to C16), ∑ long-chain (C17+)

6.2.2 Cross validation

The data gained from seven treatments were used for the calculation of regression models, one model for β1 and one for time pH < 5.8, using SAS 9.2. Proc reg command and a stepwise selection were used with a significance level for entry and stay of 0.10. The regression model was applied to data from the eighth treatment. For this treatment the estimated parameter β1 and time pH < 5.8 were correlated with the calculated β1 and time pH < 5.8. Data for β0 are not further discussed because the R^2 was low for all equations (correlation between β0 and Whole dataset, α = 10%: R^2 = 0.53; Whole dataset, α = 5%: R^2 = 0.43; Reduced dataset, α = 10%: R^2 = 0.47 ; Reduced dataset, α = 5%: R^2 = 0.40).

The regression calculation was repeated for every treatment. So, every treatment was once used to determine the correlations between the data gained by the regression model that was built using seven other treatments and the data calculated on the basis of the pH measurements. The regression had the highest correlation with the calculated data when the treatment CS60 from Trial 1 was used to evaluate the model. The data for every ration, when left out of the model formulation and used for validation, are shown in Table 6.4. The 173 samples from the seven treatments, except CS60, were used for generating the regression model which was chosen to be the best fitting model and the 12 observations of CS60 were used for the validation of this model.

Table 6.4 Correlations (r) between estimated and calculated pH parameters for every treatment using the seven other treatments to model the regression and the left-out treatment as validation treatment

$Treatment_{validation}$[1]	β1	Time pH < 5.8	n_{model}[2]	$n_{validation}$[3]
CS60	0.91	0.88	173	12
SBPS60	0.72	0.03	173	12
SBPS20	0.30	-0.38	173	12
CS	0.16	0.69	155	30
GS	-0.12	0.56	155	30
Hay	0.37	0.52	155	30
CG	-0.14	-0.13	156	29
PG	0.32	0.35	155	30

[1] $Treatment_{validation}$ = Treatment, left out of the regression formulation and used for validation purpose instead

[2] n_{model} = Number of observations used to formulate the regression

[3] $n_{validation}$ = Number of observations within this treatment used for validation

The accessed regression model used 15 variables for time pH < 5.8 and 16 variables for β1, as shown in Table 6.5. A detailed list of variables used will be given later, in Table 6.9.

Table 6.5 Regression models for time pH < 5.8 and β1 with the Whole dataset (63 variables) and validation data from Trial 1 Group CS60

Estimate	pH < 5.8	β1
Dataset	Whole[1]	Whole[1]
Sig. level	10%	10%
Variables used[2]	15	16
Dependent Mean	205.50	6.19
Root MSE	120.45	0.12
Coeff Var[3]	58.61	1.90
R^2	0.84	0.81
Adj. R^2	0.82	0.79
Validation:		
Mean	851.8	5.76
Root MSE	162.46	0.096
Coeff Var	19.1	1.7
R^2	0.78	0.84

[1] Whole dataset consisting of 63 variables as listed in Table 6.3

[2] Variables used are listed in detail in Table 6.9

[3] Coeff var $= \frac{Root\ MSE}{Dependent\ Mean} * 100$

Nevertheless, with 16 (β1), and 15 (pH < 5.8) variables used, the risk of an over-fitting model is given. No statistical analysis were made to define a level of overfitting, but to reduce the risk the number of variables was reduced by reducing the significance level for entry and stay in 1% steps from 10% to 1%. Information about the number of variables used, R^2 and coefficients of variation are given in the appendix in Table 13.4. Based on the information gained by these reduction steps, a level of significance of 5% was additionally used and is shown in Table 6.6. With a reduced level of significance to 5%, the number of variables in the model was reduced from 15 to 6, and from 16 to 9 variables, respectively. This led to a decrease of R^2 from 0.84 to 0.73 for the time pH < 5.8, respectively from 0.81 to 0.75 for β1.

Table 6.6 Regression models for time pH < 5.8 and β1 with the Whole dataset (63 variables from the three trials) and validation data from Trial 1 Group CS60

Estimate	pH < 5.8	pH < 5.8	β1	β1
Dataset	Whole[1]	Whole[1]	Whole[1]	Whole[1]
Sig. level	10%	5%	10%	5%
Variables used[2]	15	6	16	9
Dependent Mean	205.5	205.5	6.19	6.19
Root MSE	120.45	152.19	0.12	0.13
Coeff Var[3]	58.61	74.06	1.9	2.11
R^2	0.84	0.73	0.81	0.75
Adj. R^2	0.82	0.72	0.79	0.74
Validation:				
Mean	851.8	851.8	5.76	5.76
Root MSE	162.46	151.53	0.096	0.101
Coeff Var	19.1	17.8	1.7	1.8
R^2	0.78	0.81	0.84	0.83

[1] Whole dataset consisting of 63 variables as listed in Table 6.3

[2] Variables used are listed in detail in Table 6.9

[3] Coeff var $= \frac{Root\ MSE}{Dependent\ Mean} * 100$

6.2.3 Reduced dataset

Since many of the milk fatty acids are only available, if a gaschromatographical analysis is made, an additional model based on less, but easy to access, variables was also tested.

Therefore, the variables for animals' house data, milk data from official milk control, and calculated data were available variables to formulate the regression, like for the Whole dataset. However, for the fatty acids and sums of fatty acids only FAs and their sums were offered that are predictable by MIR analysis with an accuracy of $R^2 \geq 0.95$ according to Grelet et al. (2014). These data are shown in Table 6.7. Regression models were calculated using SAS 9.2. In the same way as for the Whole dataset the proc reg command and a stepwise selection were used with a significance level for entry and stay of 0.10 and in a second step of 0.05.

Table 6.7 Reduced dataset (20 offered variables)

Variable Origin	Number of variables	Variables
Animals' house data	2	Days in milk, milk yield
Milk data from official milk control	6	Milk-, -fat, -protein, -lactose, -pH value, number of cells, -urea
Calculated data	3	Energy corrected milk, fat/protein ratio, fat produced per day
GC analysed FAs[1]	2	C16:0, C18:1 c9
Sums built from analysed FAs[1]	7	∑ C18:1, ∑ C18:1 c, ∑ saturated, ∑ unsaturated, ∑ monounsaturated (+ C18:2 c9,12), ∑ medium-chain (C14 to C16), ∑ long-chain (C17+)

[1] being estimable by MIR (R^2 ≥0.95) according to Grelet et al. (2014)

6.2.4 Best fitting models

The quality of the models mentioned above is shown in Table 6.8. With the Reduced dataset a R^2 of 0.79 and 8 variables used for time pH < 5.8 compared to 0.73 and 6 variables used for time pH < 5.8 in the Whole dataset were achieved with a level of significance of 10%. For β1 a R^2 of 0.71 and 9 variables used in the Reduced dataset were achieved while a R^2 of 0.75 and 9 variables were used for β1 in the Whole dataset.

The models of the Reduced dataset were very similar to those of the Whole dataset when both are on a significance level of 5%. For time pH < 5.8 a R^2 of 0.79 and 8 used variables in the Reduced dataset, compared to a R^2 of 0.73 and 6 used variables in the Whole dataset with a level of significance 5% were achieved. For β1 a R^2 0.71 and 9 used variables in the Reduced dataset, compared to a R^2 of 0.75 and 9 used variables in the Whole dataset were achieved.

The coefficient of variation is in an acceptable range for the estimation of β1, but is very high for the time pH < 5.8. In addition, the RMSE lies at a level of more than two hours per day for the time pH < 5.8 while the β1 has a RMSE of 0.14.

The validation with the CS60 treatment from Trial 1 shows that for β1 the estimations based on the Whole dataset are both working very well with a R^2 of 0.84 and 0.83 and with a very low coefficient of variation. With the Reduced dataset such high accuracy cannot be achieved.

For the estimation of time below pH 5.8, the model with the 10% significance level and Whole dataset tends to be overfitting. Therefore, the R^2 is with 0.81 slightly higher in the Whole dataset with a level of significance of 10% than 0.78 for the 5% level of significance. In addition, the validation coefficient of variation is lower when the level of significance for the model is at 5% and

only 6 variables instead of 15 are used. For the Reduced dataset the level of significance did not have an impact. The R^2 is a little bit lower with 0.75 and the coefficient of variation is a little higher than in the models with the Whole dataset.

Table 6.8 Regression models (n = 173) for time pH < 5.8 and β1 with the Whole dataset (all variables from the three trials) and with a Reduced dataset (not all fatty acids, but only those being precisely estimable by MIR) (n = 173) and validation data from Trial 1 CS60 (n = 12)

Estimate	pH < 5.8	pH < 5.8	pH < 5.8	pH < 5.8	β1	β1	β1	β1
Dataset	Whole[1]	Whole[1]	Red.[2]	Red.[2]	Whole[1]	Whole[1]	Red.[2]	Red.[2]
Sig. level	10%	5%	10%	5%	10%	5%	10%	5%
Variables used	15	6	8	8	16	9	10	9
Dependent Mean	205.5	205.5	205.5	205.5	6.19	6.19	6.19	6.19
Root MSE	120.45	152.19	136.51	136.51	0.12	0.13	0.14	0.14
Coeff Var[4]	58.6	74.1	66.4	66.4	1.9	2.1	2.2	2.3
R^2	0.84	0.73	0.79	0.79	0.81	0.75	0.72	0.71
Adj. R^2	0.82	0.72	0.77	0.77	0.79	0.74	0.71	0.69
Validation:								
Mean	851.8	851.8	851.8	851.8	5.76	5.76	5.76	5.76
Root MSE	162.46	151.53	174.25	174.25	0.096	0.101	0.140	0.163
Coeff Var[4]	19.1	17.8	20.5	20.5	1.7	1.8	2.4	2.8
R^2	0.78	0.81	0.75	0.75	0.84	0.83	0.67	0.56

[1] Whole dataset consisting of 63 variables as listed in Table 6.3
[2] Reduced dataset consisting of 20 variables as listed in Table 6.7
[3] Variables used are listed in detail in Table 6.9

[4] Coeff var $= \frac{Root\ MSE}{Dependent\ Mean} * 100$

The variables that were used, are shown for every model in Table 6.9. For the Reduced dataset in total 13 out of the 22 variables offered were selected in at least one of the models. For the Whole dataset, 27 out of the 63 variables were selected in at least one of the models.

It became apparent that the milk variables yield, protein, lactose, and number of cells were chosen in almost every model, while the milk FAs were less consistently included. Some of the milk fat data were included in the models as the single analysed FA, as well as summed up together with other FAs. For example, C18:3 c9,12,15 was included in the model for the time pH < 5.8 for the Whole dataset (10%) as the analysed FA, as well as in the ∑ 3x unsaturated and ∑ polyunsaturated FAs.

The multiple inclusion leads to almost a complete override with +973.2*C18:3 c9,12,15 - 939*∑ 3x unsataturated + 172.9*∑ polyunsaturated, although C18:3 c9,12,15 is by far the main 3x unsaturated FA in the milk FA.

Table 6.9 Regression models for time pH < 5.8 and β1 with the Whole dataset[1] (Table 6.3) and with a Reduced dataset[2] (Table 6.7)

Estimate	pH <5.8	pH <5.8	pH <5.8	pH <5.8	β1	β1	β1	β1
Dataset	Whole	Whole	Red.	Red.	Whole	Whole	Red.	Red.
Significance level	10%	5%	10%	5%	10%	5%	10%	5%
Variables used	15	6	8	8	16	9	10	9
Intercept	14680.0	13265.0	-267.1	-267.1	7.8759	7.0576	15.5374	5.8715
C6:0*	178.3							
C8:0*					0.5042			
C12:0*						-0.0941		
C13:0*	-906.4				0.6792	0.9462		
C15:0*					-0.4057	-0.4368		
C15:0 iso*					0.5890			
C16:0							0.0502	0.0801
C16:1 c*	220.5							
C17:0*					0.6095	0.3403		
C17:0 aiso*					-0.5791			
C18:1 t6 + c7 + t9 + t11*					-0.0231			
C18:1 c9			-508.9	-508.9				
C18:1 c11*	646.0	754.0			-0.2918	-0.5717		
C18:1 c12*	745.7							
∑ C18:1			-235.8	-235.8			0.1104	0.1154
∑ C18:1 c	-39.6		521.1	521.1				
C18:2 t9,t12*		738.6						
C18:2 t9,c11*	-4462.5							
C18:3 c9,12,15*	973.2				-1.6448			
C20:1 c11*		756.6						
∑ even-chain*	-160.1	-159.7						
∑ saturated							-0.1216	
∑ unsaturated			180.7	180.7	-0.0156		-0.2347	
∑monounsaturat-ed+ C18:2 c9,12								-0.0956
∑ 3x unsaturated*	-939.0				1.6295			
∑ polyunsaturat-ed+ C19:1 t7*	172.9					-0.0585		
∑ short-chain (C4-C13)*					-0.1086			
∑ medium-chain (C14-C16)		31.5						-0.0505
∑ long-chain (C17+)							0.0446	
Milk yield	-14.8		-17.2	-17.2			0.0109	0.0080
Protein	-195.3		-328.8	-328.8	0.2228	0.3098	0.4168	0.3252
Lactose	195.3		266.8	266.8	-0.1588	-0.1713	-0.3173	-0.2598
Number of cells	-0.1	-0.1	-0.1	-0.1	0.0001	0.0001	0.0001	0.0001

[1] Whole dataset consisting of 63 variables as listed in Table 6.3

[2] Reduced dataset consisting of 20 variables as listed in Table 6.7

* Variables only available for the Whole dataset

In Table 6.10, the data estimated and calculated are shown for every treatment individually. These data show that the correlations between measured and estimated β1 and time pH < 5.8 is not always fitting, so that the accuracy of the model was not equally good for every treatment. The correlation between β1 and min/day pH < 5.8 and the calculated β1 and min/day pH < 5.8 were made for every treatment individually as well. It showed that the correlation between the model and the treatments were sometimes higher and sometimes lower.

Table 6.10 Correlation (r) between estimated by regression models and calculated[1] from measured data β1 and min/day pH < 5.8 for the Whole[2] datasets, as well as for the Reduced[2] (Red.) datasets

Estimate	pH < 5.8	pH < 5.8	pH < 5.8	pH < 5.8	β1	β1	β1	β1
Dataset	Whole	Whole	Red.	Red.	Whole	Whole	Red.	Red.
Significance Level	10%	5%	10%	5%	10%	5%	10%	5%
Trial 1 CS60	0.88	0.90	0.86	0.86	0.92	0.91	0.82	0.75
Trial 1 SBPS60	0.84	0.50	0.82	0.82	0.67	0.69	0.81	0.78
Trial 1 SBPS20	0.74	0.66	0.71	0.71	0.75	0.58	0.69	0.73
Trial 2 GS	0.49	-0.28	0.28	0.28	0.79	0.43	0.61	0.69
Trial 2 Hay	0.58	0.28	0.58	0.58	0.51	0.52	0.49	0.52
Trial 2 CS	0.77	0.57	0.75	0.75	0.78	0.83	0.78	0.79
Trial 3 CG	0.54	0.30	0.27	0.27	0.68	0.54	0.25	0.29
Trial 3 PG	0.67	0.57	0.56	0.56	0.71	0.62	0.48	0.33

[1] Calculation included all treatments from all trials, except Trial 1 CS60 treatment
[2] Whole and Reduced models shown in Table 6.9

6.2.5 Test of regression model

The regression models with the criteria Whole dataset and Reduced dataset were used with levels of significance 5 and 10% respectively on the data of all experiments and diets (n = 185 samples). To test to which extent SARA can be predicted with the combination of the two estimated ruminal parameters β1 and time pH < 5.8, days that may be SARA days were classified as potential SARA day by checking if β1 is lower than 6.16. In addition, the time spent below 5.8 was analysed and if the time was longer than 314 minutes, the day was defined as potential SARA day. In a third step the combination of the potential SARA days, detected with β1 and time pH < 5.8, was used to identify the accuracy of SARA detection with the help of the regression model.

Table 6.11 Test for correct definition of critical β1 point (< 6.16) by using the Whole or the Reduced dataset on levels of significance of 5% and 10% compared to the measured days with a critical β1 (n = 185)

β1 < 6.16	Measured	Whole dataset 10%	Whole dataset 5%	Reduced dataset 10%	Reduced dataset 5%
Detected	78	97	66	112	117
Correct detected		74	59	74	77
Not detected		4	19	4	1
False positive detected		23	7	38	40

For every model and every of the 185 measurements in the datapool the number of days with an estimated β1 < 6.16 was determined and listed as “detected”. Then, it was checked how many of these days where a β1 < 6.16 was predicted the β1 was also measured as below 6.16 and classified as ”correct detected”. Every day where the measurement of β1 was below 6.16 but not the prediction, was classified as “not detected” while every day where the prediction resulted in a β1 < 6.16, but not the measurement, the day was classified as “false positive detected”.

For β1, where originally 78 days were potential SARA days, the prediction tended to increase the number of potential SARA days to up to 117 days. Only the Whole dataset with the significance level of 5% predicted, with 66 SARA days, less potential SARA days than measured. These misinterpretations of the β1 are still in an acceptable range, taking into consideration that many of the observed pH_{mean} values were in the region of pH 6.2. In the second step, for all days the times pH < 5.8 were used in the same way as described for β1 and potential SARA days were determined if pH < 5.8 for longer than 314 minutes occurred.

Table 6.12 Test for correct critical time pH < 5.8 (>314min/day), by using the Whole or the Reduced dataset on levels of significance 5% and 10% compared to the measured days with a critical time pH < 5.8 (n = 185)

pH < 5.8 for longer than 314 min/day	Measured	Whole dataset 10%	Whole dataset 5%	Reduced dataset 10%	Reduced dataset 5%
Detected	47	38	43	39	39
Correct detected		35	34	34	34
Not detected		12	13	13	13
False positive detected		3	9	5	5

While from the measured days (n = 185) 47 days (25.4%) with potential SARA incidence can be identified, for the estimated Whole dataset only 38 (20.5%) at 10% level of significance, respectively 43 (23.2%) at 5% level of significance, and for the Reduced dataset 39 (21.1%), with 10% and 5% level of significance, of measured days were detected as SARA days.

In the third step, the SARA days identified by the critical pH β1 and time pH < 5.8 were compared. It showed that in the three trials all days that were identified to be potentially SARA days by the time spent below a pH of 5.8 also had a β1 smaller than 6.16. Therefore, the accuracy of the determination of SARA days, is the same as the determination of, potential SARA causing, time pH < 5.8, shown in Table 6.12.

To predict a SARA incidence in one cow on a specific day it has to be handled with care. In total the “not detected” and the “false positive detected” measurements reduce the accuracy of the sum of SARA detections. There are several SARA days that were “not detected” or “false positive detected” as SARA days (Whole dataset 10% = 15 days (8.1%); Whole dataset 5% = 22 days (11.9%); Reduced dataset = 18 days (9.7%) of SARA incidence). Nevertheless, this information and accuracy is pretty strong to identify SARA problems in a herd.

6.3 Conclusions

It was possible with the three trials to provoke various pH conditions in the rumen. With these data it was possible to develop a model using milk constituents from the official milk control and milk yield data to estimate the risk of SARA with a high rate of SARA detection. Not only it was possible to develop a model with the very time-, labor-, and cost-intense gaschromatographical analysis of milkfat, but also with data available from MIR analysis. From originally 47 SARA days out of a total of 185 measurement days it was possible to predict 43 respective 38 SARA days, depending on the model used, with the help of the combination of GC analysis and MIR analysis. When no GC analysis was used, the MIR data-based model had a prediction of 39 SARA days. Nonetheless, several wrong detections lowered the accuracy of the test for exemplary days, so these models should be used only for SARA predictions on herd basis. It also has to be considered, that the dataset for the modulation only includes a small number of animals (n = 16), a small sample size (n = 185), with two different breeds on eight various feeding rations, from three different trials. Therefore, the obtained regression models may not be precise enough for a use in field at this time, but have to be developed further with a bigger sample size, including more individual cows and more repetitions. Nonetheless, it seems that a SARA prediction model can be made up independent of the feed, if a bigger dataset is used. Owing to the labor intensity of pH measuring with the available pH data loggers no big sampling size will be achieved in near future. The limiting factor up till now is the missing availability of a reliable, easy to handle, long life, indwelling pH measuring device for cows without cannula. Owing to those difficulties, latest research activities go in the direction of measuring with indwelling boli that measure pH in the reticulo-rumen and deduce rumen health status from those data. Additionally, they have to deal with inaccuracy of measurements, depending from the device used of 0.2 to 0.3 pH (Jonsson et al., 2019) and a drift in the pH sensor that is not controllable at the moment. However, when there is a technical solution for the problem of inaccurate measurements and a solution for the interpretation of reticular measurements, the dataset of pH measurements can increase to a high number for a model, which then can also be reliable in field.

7 DISCUSSION

Possible sources of error

There is a cow individual susceptibility to SARA (Penner et al., 2009) which may also have an impact on the inclusion of concentrate required to induce SARA. Recent studies focus on this different susceptibility to SARA (Oba and Gao, 2014; Humer et al., 2015; Jing et al., 2018; Coon et al., 2019). Since for a pH measurement in the ventral part of the rumen a cannula is necessary, not many cannulated cows are available. Especially, if a comparable phase of lactation is required. Therefore, the overall number of cows used for the trials herein remained low and differences in SARA susceptibility was not addressed in these trials.

Like every other pH meter, the pH meter that was used in the logger had to be calibrated regularly. This calibration was made by measuring analog digital counts from known buffer solutions (pH 7 and pH 4). Depending on the age of the sensor the pH meter did take some time until the analog digital count measurement reached a constant level. When this level was reached the calibration measurement was conducted. The same inertia has to be expected when measuring in the rumen, so that sharp and short peak changes in ruminal pH might not be outlined properly, which might lead to inaccurate pH_{min} and pH_{max} measurements.

Although the datalogger was designed to measure in rumen it happened that particles were prevalent in the cage around the sensor when taking the datalogger out of the rumen. Although they were easy to remove, it is not possible to consider how long these particles already remained there without being removed by rumen motility or if they had an influence on measurements. A visual test of the sensor led to the conclusion that there was no threat of a microbial population attached to the sensor, which might have caused an error in the measurements.

On the days when the spot samplings were made in Trial 1 and 2, the cows were caught in intervals of 4 hours. Although all cows in the trials were used to get handled by men, this might had an influence on their behaviour, regarding feed intake, resting time or rumination time.

When taking the spot samples the probe sampler had to be placed in the ventral rumen. By introducing the sampler to the ventral rumen this leads automatically to a tunnel through the ruminal content from the cannula to the ventral sac of the rumen. This might have induced a flow of rumen liquid to the ventral part of the rumen, which is known to be lower in pH and higher in VFA concentration in the middle of the rumen content.

The Trial 2 was conducted with Jersey cows, which might lead to results that are not one-to-one comparable to results from Holstein cows. The Jersey breed is known to be smaller but has very

high milk yield, with a high concentration of fat in the milk. In our trial, they showed the highest fat content in the milk and although not the overall highest feed intake, they showed a higher feed intake per kg metabolic body weight than the Holstein cows in Trial 1, while we did not have data for feed intake for all cows in Trial 3. Although the feed intake was higher per kg metabolic body weight no increased concentrations of VFAs in the rumen were detected, which might have been induced by a higher absorption rate of VFAs from the rumen or an increased water intake or passage rate, which can dilute VFA concentrations in the rumen.

SARA incidences

The rations of the presented trials were planned to be in a shape of constituents where an acidosis threat in the rumen is predominant. Nevertheless, with those rations also days without SARA incidences, using the SARA definition of Zebeli et al. (2008), were expected. With this abundance of acidotic and non-acidotic conditions in the rumen, the influence of acidotic circumstances in the rumen on the milk parameters were researched.

While in earlier studies, with heifers, concentrations of 82% (Cooper et al., 1997) or up to 90% concentrate (Bevans et al., 2005) were used, the concentration of concentrate was more moderate when dairy cows were used in acidosis studies. Keunen et al. (2002) induced SARA with an amount of 37% concentrate in the ration, Rustomo et al. (2006a) used 42.6% concentrate in their ration, AlZahal et al. (2009) used 44% concentrate in their ration and Enemark et al. (2004) reported SARA in a herd receiving 8.4 Scandinavian feed units of concentrate and 9.5 Scandinavian feed units of forage per day which also resembled a ratio of approximately 45% concentrate to 55% forage.

It was observed in Trial 1 that the amount of 60% concentrate in the ration was inducing SARA conditions in 51 out of 54 measured days in cows while SARA occurred at 17 out of 28 days for the treatment with 20% concentrate plus 60% SBPS in the ration. With this information from Trial 1 the second trial was planned and conducted with a concentration of 50% concentrate in the rations, which showed up that only on 7 out of 90 days SARA was evoked. This was a lower ratio than expected because other authors achieved SARA with lower concentrate concentrations, as mentioned above.

In Trial 3, the concentration in the ration of the confinement group was 30% concentrate, a little bit lower than in the rations in the literature, but still higher than in the SBPS20 ration of Trial 1. In Trial 3, SARA was detected on 7 out of 99 measurement days in the confinement group. For the

cows on pasture a pH in the range of SARA threshold was expected because grazing cows are in a risk of SARA as well, as reported earlier (O'Grady et al., 2008) but only 5 out of 62 days with SARA conditions were recorded.

While the SARA incidence in Trial 1 was a little bit higher than expected, the numbers of SARA incidences in Trial 2 was surprisingly low. This might have to do with the fact that in Trial 2, with the Jersey cows, a different breed was used than in Trials 1 and 3.

Acid and buffer load in the ventral rumen

The main source of acidity in the rumen are the VFAs being prevalent in the rumen. The concentrations of VFA_{total} measured in the three trials were lowest for the Hay treatment of Trial 2 with 99 mmol/l and highest for the SBPS60 treatment of Trial 1 with 127 mmol/l VFA_{total}. The range of VFA concentrations fit to VFA concentrations reported by Dijkstra et al. (2012). They collected 104 data pairs of ruminal pH and VFA concentration in the rumen from 13 studies. All samples had a VFA concentration between 70 and 150 mmol/l and a pH_{mean} between 5.5 and 6.6. Another source of acidity in the rumen is lactic acid, which is produced in the rumen by some strains of microbes, e.g., *Selenomonas ruminantium*, *Streptococcus bovis*, and *Lactobacilli*, but also utilized by other microbes, like *Selenomonas lactolytica* or *Megaspheri elsdenii*. Although lactic acid is a strong acid it is very dependent on the amount of feed and of the components of the feed (Counotte and Prins, 1981). Only the concentration of lactate in Trial 1 was investigated. The concentrations were at a low level although the pH dropped in this trial to a pH threshold where the lactate production should be increased already, but no physiological relevant accumulation was detected. While 40 mmol/l lactate would indicate severe acidosis (Owens et al., 1998), the highest average lactate concentration in Trial 1, found in the CS60 treatment, was 1.78 mmol/l with a multitude of measurements that did not even reach 0.1 mmol/l lactate so, lactate was not a suitable parameter for SARA identification. Therefore, lactate was only analysed in Trial 1.

Although VFAs are the main source of acidity in the rumen, it is not possible to determine pH in the rumen with the VFA_{total} concentration only. Dijkstra et al. (2012) found only a weak negative relationship between ruminal pH and VFA concentration in the rumen (R^2=0.24) which the authors explained with the differences in the neutralisation, buffering and absorption between the different diets. With ammonia as a buffer, it leads to the assumption that feeds with high degradable protein can buffer the decline of pH which is evoked by VFA release of their digestion to some extend by the release of ammonia in the same time (Dijkstra et al., 2012). The

NH_3 concentration varied between the three trials with drastically lower concentrations (4 to 5 mmol/l) in Trial 1, which showed the lowest pH values, than in the other trials (6 to 9 mmol/l) that had higher pH values. Highest intraruminal NH_3 was measured in the CS treatment in Trial 2 (9.3 mmol/l), which showed a more stable ruminal pH than the other two treatments in Trial 2. The NH_3 concentrations found in the rumen matched the prediction that a lower ruminal pH was less buffered by NH_3 but are in contrast to the findings of Stefańska et al. (2020) who reported higher ruminal ammonia levels when low ruminal pH was measured. A downside of ammonia as buffer is though that ammonia is not captured when the protein synthesis is inhibited by low pH, which increases the urine N (Dijkstra et al., 2012). The high presence of ammonia in the CS treatment in Trial 2 might also explain why there is no significant effect on the pH in Trial 2 although its VFA_{total} concentrations were significant highest.

Besides the acid load, the VFAs in the rumen are suggested to have an impact on the lipometabolism of the cow. More energy went to the body tissues if propionate was infused to the rumen while the milk fat content increased when acetate was infused (Ørskov et al., 1969). These correlations between propionate and the fat in body tissues could not be researched because of the lack of body fat measurements. With the three trials, it was also not possible to confirm that the acetate concentration in the rumen has an impact on the milk fat content. In Trial 1, no statistical difference in milk fat was found although the CS60 treatment induced lowest acetate concentration in the rumen. In Trial 2, neither acetate nor milk fat concentration was significantly different between the treatments. In Trial 3, the confinement group treatment showed a significantly higher fat content although the acetate concentration in the rumen was on the same level.

Differences between spot samplings and continuous measurements

The continuous measurement of the pH in the rumen provides an insight into the rumen function, which cannot be achieved by single spot measurements. The single spot measurement only reveals the rumen condition at one point in time during the day, while the continuous pH measurement is achieving information over the day continuously.

Nonetheless, single spot samples can help with an approximation of the general level of pH in the rumen. This can be seen from a comparison of data taken from the present experiments, on the one hand collected by continuous measurement and on the other hand collected by spot samplings. With a spot sampling measurement every 4 hours it was possible in the trials to gain

information about the average pH in the rumen quite well with a slightly higher average pH in the samples that have been measured in spot samples instead of the continuously pH_{mean} measurement. As shown in Table 7.1, the pH_{mean} during the experimental phases was, when continuously measured, in average 0.15 pH points lower than the pH_{mean} gained by the spot samples.

Table 7.1 Average pH_{mean} gained with continuous or spot sampling measurement and its discrepancy in the present thesis

Ration		pH_{mean} spot sampling	pH_{mean} continuous	Δ pH cont. - pH spot.
Trial 1	CS60	5.83	5.73	-0.10
	SBPS60	5.89	5.69	-0.20
	SBPS20	6.13	5.89	-0.24
Trial 2	CS	6.25	6.21	-0.04
	GS	6.34	6.39	0.05
	Hay	6.47	6.35	-0.12
Trial 3	CG	6.45	6.22	-0.23
	Pasture	6.48	6.14	-0.34
Mean of all treatments		6.23	6.08	-0.15

The dataset of continuous pH measurement and spot sampling pH measurement do differ. The number of measured days for the continuous pH measurement were: Trial 1: SBPS20 (n = 22), SBPS60 (n = 22), CS60 (n = 26); Trial 2: GS (n = 30), Hay (n = 30), CS (n = 30), Trial 3: CG (n = 108), PG (n = 84)

The number of measured days for spot sampling pH measurement were: Trial 1: SBPS20 (n = 6), SBPS60 (n = 6), CS60 (n = 6), Trial 2: CS (n = 6), GS (n = 6), Hay (n = 6), Trial 3: CG (n = 60), PG (n = 60)

Only for the grass silage treatment in Trial 2, the pH_{mean} of the day was higher in the continuous measurement than in the spot samplings. This does not necessarily mean that there has been an error in pH measurement in this treatment, because it is also possible that the pH in the grass silage treatment on the measurement days of the spot sampling was generally higher than in the average continuous pH measurement phase.

In Trial 3, when only two measurements per day were made, after the milkings, the discrepancy between continuous pH measurement and spot sampling was higher with a Δ of -0.23 and -0.36. This tends to show that the accuracy of an estimation of a pH_{mean} is lowered when the number of measurements, building the mean, is reduced.

According to the recent observations, it was reported in literature that the pH measured extraruminally is slightly higher than intraruminal measured pH as shown in Table 7.2.

Table 7.2 Discrepancy between extra- and intraruminal pH measurements in literature and present thesis

Authors	Δ pH_{mean} extraruminal - pH_{mean} intraruminal
Smith (1941)	0.28
McArthur and Miltimore (1968)	0.1
Dado and Allen (1993)	0.11
Nordlund (2003)[1]	0.1
Graf et al. (2005)	0.25
Present thesis Trial 1	0.18
Present thesis Trial 2	0.04
Present thesis Trial 3	0.30

[1] referring to unpublished data

The difference between intra- and extraruminal pH measurements might be explained by a loss of CO_2 to the atmosphere. CO_2 is built by the reaction from $HCO_3^- \leftrightarrow H_2O + CO_2$ where the chemical equilibrium is on the side of HCO_3^-. Therefore, the loss of CO_2 keeps the reaction going and when pH is measured outside of the rumen a higher pH has to be expected (Dado and Allen, 1993; McArthur and Miltimore, 1968; Smith, 1941). These differences between extra- and intraruminal measurements are, besides the difference number of observations building the pH_{mean}, suitable for the discrepancy between the continuous and spot sampling measurements in the present trials.

This deviation between intra- and extra-ruminal measurements has to be considered as a very important detail when discussing ruminal pH values. It is not only a slight shift in the direct comparison of measurements between intra- and extraruminal measurement, it also has to be recognised when pH values of rumen content are presented in any context; be it reduced feed intake, lowered fat content in milk, microbiota composition in the rumen or fatty acids in milk fat. Therefore, the measurement technique, if intra- or extra-ruminal, has to be considered when interpreting own results and when comparing them with literature.

Opportunities and restrictions of spot samplings during the day

When taking the deviation of 0.1 to 0.3 pH points, between extraruminal and intraruminal pH measurements, into account, it was possible to get a good estimation of the pH_{mean} in the rumen, as shown in Table 7.1. The mean of eight spot sample measurements of a day, in Trial 1 and 2, and two spot samples in Trial 3 averaged as pH_{mean} was very similar to the pH_{mean} of the continuous pH measurement.

It is generally known that the pH drops in the course of the day from a maximum before the morning feeding (Dragomir et al., 2008) to a minimum in the evening, which can also be seen in the data of the three trials, shown in Figure 6.1. In Trial 2, the pH starts to drop earlier than in Trial 1 and 3 which is related to the feeding time at ~ 8 in Trial 2 compared to ~ 11 o'clock in Trial 1 and 3. The pasture group in Trial 3 did not show a fast decrease in the pH value which might be related to the fact that these cows had access to the pasture all day and there was no stimulus of fresh feed being available each morning. In Trial 1, it became also obvious that during the time of morning milking an extra peak to high maximum pH was observed. This might be induced by feed restriction during milking or because the cows might spend their time in the waiting area before milking with an increased rate of rumination and therefore with an increased buffer secretion (Maekawa et al., 2002a). Therefore, if the intension is to detect the maximum pH during the day, samplings before the morning feeding can provide an assumption of the pH_{max}.

For an assumption of pH_{min} Bevans et al. (2005) supposed a measurement 8 hours after feeding, Nordlund (2003) expects the pH_{min} of the day 4 to 8 hours after feeding and in the present trials a pH_{min} was observed 7 to 10 hours after the morning feeding. The time between feeding and pH_{min} is dependent on various factors like the actual start of feed intake, the amount of feed intake, the content of nutrients and their fermentability, the prevalence of structural carbohydrates, as well as the mixture homogeneity and the according selection possibility of the feed. In addition, animal dependent resting and ruminating times have to be considered. The variance in the times of a ruminal pH minimum makes it obvious that a pH_{min} is difficult to assume in a single spot sample, even if taken anytime between 4 and 10 hours after feeding. For a reliable pH_{min}, the pH has to be measured constantly.

Measuring the pH_{mean} with one single spot sampling is barely possible. In the present trials, it became obvious that the pH_{mean} is only prevalent for a short period between 2.5 and 6 hours after morning feeding. Since the pH level rapidly decreases in this phase of the day, a pH_{mean} measurement seems inappropriate with a single spot measurement. However, as mentioned

earlier, a pH_{mean} can be assumed precisely by repeated pH measurements during the day, with a higher accuracy if the pH was measured every 4 hours than only twice per day.

SARA detection with the help of spot samplings

When it comes to a point where it is important to know how long the pH remains below a threshold like, when using the SARA definition of Zebeli et al. (2008), more than 314 minutes per day a ruminal pH lower than pH 5.8, it shows that the pH_{mean} is only the most important factor.
If a pH_{mean} smaller than 5.91 and lower was detected, all days were SARA days, while no SARA days were detected when the pH_{mean} was higher than 6.10. Between these two pH_{mean} thresholds, the diversification of intraruminal pH, modelled by β0, comes as second important factor into play if the level of pH_{mean} does not clarify SARA circumstances by itself as shown in Table 7.3.
Within the dataset from all three trials of all days with continuous pH measurements, 369 days were recorded. This included additional data not contained in the results from the adaptation phases, where no other samples were taken.

Table 7.3 SARA incidence, defined as > 314 min/day pH < 5.8, and its dependency from pH_{mean} over all 369 continuous measurements

pH_{mean}	SARA days	Non acidotic days
< 5.91	90	0
5.91 - 6.00	20	11
6.01 - 6.10	7	29
> 6.10	0	212

Based on these data it can be concluded that for levels of pH_{mean} below 5.9 and for pH_{mean} higher than 6.1 the pH_{mean} itself was a sufficient parameter for SARA detection in the present trials.
Nonetheless, for days with a pH_{mean} between 5.9 and 6.1 it was inevitable to know the diversification in ruminal pH during the day because it mainly influenced the risk of SARA in this range of pH_{mean}. This importance is also illustrated by the example given in Figure 2.3 where both cows had a pH_{mean} of 6.1 but only one of the two cows was in SARA condition, caused by lower β0. While it is possible with repeated spot samples during the day to estimate the pH_{mean} of the day, it is not possible to gain the information about the diversification of the ruminal pH during the day. When following the SARA definition of Zebeli et al. (2008), the concerning pH_{mean} range is 5.9 to 6.1 where an intraruminal pH_{mean} determination did not clarify SARA incidence in the present trials. In this pH range a continuous measurement is needed.

Coherence of feed and milk FAs

As explained in the introduction, milk FAs are influenced by various factors, one of them is the feed offered. This is on the one hand from the FAs that are coming to the cow from the feed, either as the FA that can be found in the milk or as a precursor for the respective milk FA. On the other hand, the FAs, that are built and released by the microbiome of the rumen, is dependent on the feed. In addition, the feed and the feed intake are important to maintain the energetic requirements of the cows. If the cow does not have enough energy, body fat is mobilised into the milk, which leads to a higher concentration of long-chain FAs in the milk fat.

There are some milk FAs that are reported to be linked with the protein content in the feed. C16:0 is known to be a plasma lipid in animal, as well as plant oil (MacGibbon and Taylor, 1995). Cabrita et al. (2007) found a reduced C16:0 content if the CP concentration in the feed was increased. In the present trials, the same negative relation with a significant lower C16:0 content for Trial 3, as well with a C16:0 concentration of 35.32% in the CG, with 12.4% CP in the feed compared to 24.75% in the PG, where 18.9% CP were in the grass, was observed. In Trial 2, with about 15% CP in all rations, about 33% C16:0 FAs were prevalent in the milk. In Trial 1, the SBPS60 treatment with 28.26% C16:0, with 14.5% CP in the feed, and the SBPS20 treatment with 35.37% C16:0, with 13.3% CP in the feed, also showed the same coherence but not statistically significant. However, the CS60 treatment with 24.40% C16:0, with 14.1% CP in the feed, did not fit in this row because the CP content was lower than in the SBPS60 treatment but the C16:0 concentration was lowest in the CS60 treatment.

Linoleic acid (C18:2 c9,12) is one of the main feed FAs which is isomerisated to conjugated linoleic acid (C18:2 c9,t11) and further reduced to vaccenic acid (C18:1 t11) (MacGibbon and Taylor, 1995). C18:2 c9,t11, as well as C18:1 t11 was increased when protein and starch concentration was increased (Cabrita et al., 2007). It became apparent, that in the present trials the conjugated linoleic acid C18:2 c9,t11 was in Trial 3 higher in the PG, with 0.56%, than in the CG, with 0.30% in milk fat, where the grass also had a higher supply in protein, though not in starch concentration. While Cabrita et al. (2007) found a positive correlation between C18:1 t11 and the protein content in the ration, Colman et al. (2010) described a negative correlation between C18:1 t11 and the amount of concentrate in the ration. These statements could not be clarified because C18:1 t11 was analysed in a pool together with C18:1 t6 + t9 + c7. This pooling might have induced the not prevalent differences between the different rations for this pool of FAs, although C18:1 t11 was reported to be the main source of C18:1 trans FAs, with a mean of 43% of total trans 18:1 FAs (Precht and Molkentin, 1996).

Cabrita et al. (2007) found a positive coherence between protein and C22:0 which was also found in Trial 3 with a significant higher concentration of 0.06% in PG treatment compared to CG treatment with 0.03% C22:0 in milk fat. In Trial 2, only the GS treatment with 0.07% was slightly higher than the treatments CS and Hay, which both showed a concentration of 0.02%, which was also the limit of detection. In Trial 1, the concentration in the CS treatment was 0.2% as well while the SBPS treatments showed both a concentration of 0.06%. So no trend could be found for this minor FA, except for Trial 3 where the observations were in accordance with Cabrita et al. (2007). Other FAs are reported to be linked with an increased starch content in the ration. For example, an increased odd-chain FA concentration in milk was reported when high concentrate amounts in the feed influenced the concentration of odd-chain fatty acids in a medium-chain length (Apper-Bossard et al., 2006; Gaynor et al., 1995). Lowest concentrations of C11-C15 FAs were found in Trial 3, where considerably less concentrate was contained in the ration.

Loor et al. (2005) and Colman et al. (2010) described a positive correlation of C18:1 t10 when the concentrate in the ration was increased, but with the gaschromatographical setup used it was not possible to identify C18:1 t10.

C14:0 iso was reported in literature to be significant higher in high concentrate treatments (Loor et al., 2005). In the present trials, a high concentration of C14:0 iso could not be confirmed. The concentration of C14:0 iso was in Trial 1 with 0.03% in the CS60 and SBPS60 treatments, and 0.04% in the SBPS20 treatment lower than in Trial 2. In Trial 2, with 52% concentrate the concentration of C14:0 iso was at 0.10% in the milk fat. Trial 3 showed C14:0 iso concentrations of 0.07% in the CG, which had 30% concentrate in the ration, and 0.09% in the PG, where a fix amount of 1.75 kg/day concentrate was fed.

Vlaeminck et al. (2006a) reported a reduction in C14:0 iso and C15:0 iso when grass silage was replaced by corn silage. No change was measured in the concentration of C14:0 iso in Trial 2, when grass silage was replaced by corn silage, while C15:0 iso was slightly lower in the CS treatment, with 0.17% compared to 0.20% in the milk fat of the GS treatment, although this shift was not significant. Vlaeminck et al. (2006a) also reported an increase of C10:0 iso and C17:0 aiso when CS was included which was not confirmed by the present trials. A decrease of C17:0 iso from 0.27% in the GS treatment to 0.23% in the CS treatment was found. Also, C17:0 aiso showed lower levels in the CS treatment with 0.36% compared to the GS treatment with 0.39%.

Coherence of ruminal parameters and milk FAs

An increased propionate concentration in the rumen, which is a primary FA for the *de novo* synthesis of odd-chain milk FAs, increased the amount of odd-chain FAs (Vlaeminck et al., 2006b; Enjalbert et al., 2008) in the milk. In Trial 1 and Trial 2, numerically the sum of odd-chain FAs in the milk was increased when propionate concentration in the rumen was higher, but not in Trial 3. Odd-, branched-chain FAs in the milk are mainly based on ruminal bacterial membrane lipids that are digested, while only a small proportion of odd-, branched-chain FAs comes from the feed (Vlaeminck et al., 2006a).

The level of C11:0, C13:0, and C15:0 FAs showed highest concentrations in the CS60 treatment in Trial 1 (C11:0 0.17%; C13:0 0.28%; C15:0 2.21%) where the acetate to propionate ratio was 1.47 and marked lower concentrations in the Hay treatment of Trial 2 (C11:0 0.12%; C13:0 0.14%; C15:0 1.22%) where the acetate to propionate ratio was 3.68. Lowest concentrations of C11:0, C13:0, and C15:0 FAs were found in Trial 3, CG (C11:0 < 0.02%; C13:0 0.10% C15:0 1.17%) and PG (C11:0 < 0.02%; C13:0 0.06%; C15:0 1.03%) though, where considerably less concentrate was in the ration although the acetate to propionate ratio was not wider than in Trial 2 with 3.22 in the CG and 3.32 in the PG as shown in Table 7.4.

Table 7.4 The ruminal parameters, pH_{mean}, acetate, propionate, acetate to propionate ratio and the milk constituents milk odd-chain FAs and milk fat

Ration		pH_{mean}	Acetate	Propionate	Acetate to propionate ratio	Odd- chain + C14:1	Milk fat percent
Trial 1	CS60	5.73	59.9^{b}	42.0^{a}	1.47^{c}	6.07	2.4
	SBPS60	5.69	70.0^{a}	38.1^{a}	1.92^{b}	5.72	2.5
	SBPS20	5.89	73.2^{a}	25.4^{b}	2.91^{a}	5.57	4.3
Trial 2	CS	6.21	67.1	22.4^{a}	3.08^{b}	5.10	5.4
	GS	6.39	66.4	20.3^{b}	3.37^{ab}	4.95	5.3
	Hay	6.35	62.9	17.5^{c}	3.68^{a}	4.87	5.3
Trial 3	CG	6.22	70.6	22.3	3.22	3.63^{b}	4.5^{a}
	PG	6.14	70.9	21.7	3.32	4.02^{a}	3.9^{b}

^{abc} Different superscripts do resemble statistical differences within the column and within the trial. No statistics were made across the trials.

Bauman et al. (1971) reported an acetate to propionate ratio in the rumen of 1 to 1 with 15% hay and 85% grain in the ration and an acetate to propionate ratio of 3 to 1 in the rumen if 55% hay and 45% grain were in the ration. In accordance to this, the SBPS60 treatment in Trial 1 showed with 1.92 a closer ratio than the SBPS20 treatment with 2.91 between acetate and propionate.

The PG treatment in Trial 3 had FAs in the range of concentration reviewed by Chilliard et al. (2001) with a concentration of C14:0 of 8.63% and a described range between 7 and 12%, concentration of C16:0 24.75% and a described range between 23 and 28% and a concentration of C18:0 of 11.59% and a described range of 9 - 13%. A slightly higher concentration of monounsaturated FAs + C18:2 c9,12 in the milk was measured with 36.07%, compared to the described concentration of 23 - 32%. This concentration of 36.07% was higher than the reported values which is not exclusively based on the fact that C18:2 c9,12 was detected together with C19:1 t7 because the concentration of this sum of these FAs was only 1.24%. Like in literature (Chilliard et al., 2001), in the present trials C18:1 c9 hold the biggest proportion of the monounsaturated FAs, which already provides 29.68% of these 36.07% monounsaturated FAs in the PG of Trial 3.

Coherence of ruminal pH and milk FAs

The feed highly influenced the microbiota and the concentration and composition of acids and buffers in the rumen, which has a direct impact on ruminal pH milieu, as discussed above. Therefore, there is a coherence between feed components and milk FAs. Nonetheless, the microbiota in the rumen and the corresponding ruminal factors, which react to the feed, are, besides the FAs directly from the feed, responsible for the composition of FAs in the milk.

Between the milk FA C16:0, not only a positive correlation with the protein content in the feed, but also a positive correlation to β1, the pH_{mean}, (r=0.35) within every and over all three trials was found.

The FA C18:1 t10, where Colman et al. (2010) found a correlation to the concentrate concentration in the ration, is known to be responding to acidotic circumstances in the rumen as reported by Craninx et al. (2008). Unfortunately, the gaschromatographical setup used was unable to identify C18:1 t10.

Enjalbert et al. (2008) detected a reduction of the FA C18:1 t11 when pH_{mean} was reduced, which is in accordance to the increased concentration if concentrate concentration is increased, reported by Colman et al. (2010). In the gaschromatographical setup used for the present trials it was not possible to separate C18:1 t11 from the other C18:1 FAs t6, t9 and c7, but this sum of C18:1 t6,t9,c7,t11 was negatively correlated with β1, with a r = -0.41.

Overall, the correlation between ruminal pH parameters and milk FAs were inconclusive. Some FAs were correlated to ruminal parameters β1, β0, or time pH < 5.8, but those correlations have not necessarily been significant in every trial. Additionally, the correlation was not consistently in the same direction in every trial.

The only correlation that was significant positive in all three trials was found between β0 and C17:0 with a weak overall correlation of r = 0.24. The FA C18:1 c11 is constantly negatively correlated to β1 over all trials with an overall correlation of r = 0.51.

With the time spent with a pH < 5.8 no significant correlations were found that were consistent for every trial and in the overall dataset.

C18:2 t10,c12 is said to be closely related to milk fat reduction (Veth et al., 2004), which is an indicator of ruminal acidosis, but in the present trials C18:2 t10,c12 remained in its concentration below the limit of detection of 0.02%. Even in Trial 1, where a low milk fat content was detected, the concentration of C18:2 t10,c12 did not reach the detection limit.

Furthermore Colman et al. (2010) identified C18:2 c9,t11, C16:0 iso and C13:0 iso as best predictors for low ruminal pH in their study. The fatty acid C16:0 iso was not analysed in the gaschromatographical setup used. Furthermore, no C13:0 iso was detected in any treatment, it always stayed below the limit of detection of 0.02%. Over the three trials it showed that the C18:2 c9,t11 concentration was significantly correlated to β1 with r = -0.46, although no significant correlations were found within the respective trials.

C18:1 c9 was decreased when acidosis was induced (Enjalbert et al., 2008) and also, in Trial 3, a significantly lower C18:1 c9 content was observed in the CG with 18.69%, compared to 29.68% in the PG treatment, as shown in Table 7.5. However, this reduction was not significantly correlated to a lower pH_{mean} or the time pH < 5.8 in Trial 3.

Table 7.5 Concentrations of the milk FAs C18:1 c9 and C18:2 c9,t11 and the pH_{mean} in every treatment

Treatment		pH_{mean} continuous	C18:2 c9,t11	C18:1 c9
Trial 1	CS60	5.73	0.89[a]	18.31
	SBPS60	5.69	0.69[b]	17.82
	SBPS20	5.89	0.39[c]	17.93
Trial 2	CS	6.21	0.30	13.69
	GS	6.39	0.40	17.65
	Hay	6.35	0.29	17.77
Trial 3	CG	6.22	0.35[b]	18.69
	PG	6.14	0.56[a]	29.68

[abc] Different superscripts do resemble statistical differences within the column and within the trial. No statistics were made across the trials.

8 CONCLUSIONS AND PERSPECTIVES FOR FUTURE RESEARCH

With the trials, it was possible to provoke SARA conditions in lactating cows. The level of concentrate played an important role for the prevalence of SARA days, as well as the roughage compartments of the feed. The physically effective NDF determination might be a useful tool to guarantee the structure in the feed, but for the rations with SBPS the prediction of a sufficient peNDF content in the feed failed. It was possible to evoke differences in the pH levels and to show differences in the ruminal parameters as well, with significant differences in the VFA and NH_3 concentrations in the three trials. The lactate content was not in a relevant concentration in Trial 1. Therefore, lactate was identified as a weak indicator for SARA and only analysed in the first trial. Since there is still no unambiguous definition of SARA in future research the temperature of the rumen and the redox potential should both be considered as potentially relevant in future SARA research activities. Since the dataloggers used were measuring these data, it can be tried to interpret those, together with other data. The temperature is easily available, for the redox potential a standardisation within anaerobic atmosphere in a negative potential has to be established though.

The analysis of milk samples gave a good insight to the milk fatty acids and their different compositions with the different treatments. It was possible to develop a model, which included easy accessible data from the barn, official milk control and gaschromatographical analysis of the milk FAs to predict SARA conditions in the rumen. In addition, it was even possible to get a proper model when no gaschromatographical analysis but MIR spectroscopies of the milk samples were available.

Nonetheless, several difficulties have to be fixed before the model can be used in field. Owing to the low number of cannulated cows, only a low number of cows were used to develop the model. There could be differences between the individual cows regarding their behavior, like number of feed intakes, quantity of feed intakes, feed selection, and rumination time, or even their physiological abilities like the activity of rumen motility, quantity and composition of saliva, or absorption of VFAs out of the rumen.

All cows were in their mid-lactation, because the mid-lactation is, besides the early-lactation, the phase where SARA incidences are prevalent. Since the milk composition differ between the early-mid- and late- lactation state, a model to predict SARA in early-lactation could include other indicating fatty acids than the recent, mid-lactation based, model.

Owing to the small quantity of available data, all three trials were used for model development, although two different breeds of cows were used. On the one hand this might have weakened the prediction model for the particular breeds, on the other hand there are more than two dairy breeds in Germany and every other breed could have a breed specific composition of milk, so that the breed might be needed as additional information in the model.

To adapt the model to meet the requirements for on-field SARA detections, the dataset has to be enlarged, not only in numbers of repetitions of measurements but also with a larger number of cows, being fed in different barns and a larger variety of feed and in all stages of lactation.

While the milk data are easy to access from samples of the official milk control and also the milk yield is known for every cow individually, gaschromatographical analysis are quite time and cost intensive. Further development of the model should be based on the easily available data from the Reduced dataset model. The pH measurement is and will be the main problem that has to be solved before the model can be further developed. To get big datasets it is inevitable to use pH measurements of boli, which can be used in every dairy cow, instead of pH measurements with big dataloggers in cannulated cows. Therefore, the development of pH boli has to reach the next milestone, where a continuous pH measurement, that is taken by a pH bolus, that is either remaining in the reticulorumen or is floating in the ruminal mat can be transformed to pH in the ventral part of the rumen can be used. Alternatively, a new definition of SARA has to be found, taking the pH in the reticulum respectively in the ruminal mat as reference pH. When this milestone is reached, the technical difficulties, like the short span of life of such boli due to the unpredictable drift of the pH measurement, which cannot be equalised with the help of recalibrations in the cow, are only moneywise important. For the development of the model with the actual pH measurement with dataloggers in cannulated cows it is not able to get these many data from individual cows for a model development.

9 SUMMARY

Subacute rumen acidosis (SARA) is a common, but hardly assumable disease in modern dairy cows' herds. SARA incidences are prevalent in two circumstances. The first, when the cows have to adapt fast to a ration high in carbohydrates after parturition. Since the feed composition has to be changed fast, to meet the cows' requirements energy- and nutrients wise, the rumen microbiota climate has to adapt fast, which can cause unbeneficial rumen circumstances. The second, when the lactating cows have, beside high milk yield also a high feed intake in mid-lactation, when feed high in energy but low in structural carbohydrates is fed. This can lead to high density of VFAs in

the rumen, if the outflow and absorption through the ruminal wall, as well as the buffer capacity in the rumen is not sufficient for the high production of those acids. Then the ruminal milieu becomes more acid, which can negatively affect the cow's health. The cows suffering SARA, if at all, show mild symptoms like reduced water and feed intake, depression, diarrhea, reduced rumen motility, laminitis or reduced milk yield and milk fat depression. Since those symptoms can also show up with a delay in time and can be caused by several other factors, monitoring SARA in herds can be difficult. An unambiguous definition of SARA circumstances in the rumen cannot be found in literature, although it is under research for decades. The definitions differ in regards if pH measurements were taken as spot samples or continuously, from which part of the rumen site, and what pH conditions are defined as unphysiological condition. We use the definition from Zebeli et al. (2008) who defined a SARA incidence if the pH mean is below 6.16 and the time spent below a pH of 5.8 is longer than 5.24 hours (314 minutes) in the ventral sac of the rumen.

Since SARA can influence the milk yield and can lead to a milkfat depression and a change in composition of milkfat, we focused on milk parameters and milkfat composition in particular with the aim of correlating those with pH conditions in the rumen.

Three trials were made with feeding rations that were predictably capable of inducing SARA conditions in mid-lactation. During the trials, besides performance and ruminal parameters, as well as continuous pH measurement, milk samples were taken. Milk samples were taken and analysed following the methods of the official milk control with MIR spectrograph, as well as milk samples for gaschromatographical analysis of the milk fat composition were taken.

The cows used were all rumen cannulated. Therefore, datalogger with integrated pH meter (Large Ruminant Logger M5-T7, Dascor Inc., Oceanside, USA) were placed in the ventral sac of the rumen to measure reliably and continuously. The structure of the feed was analysed with the help of a Penn State Particle Separator and the physically effective NDF was determined ($peNDF_{>8mm}$ and $peNDF_{>1.18mm}$). In Trial 1, three feeding rations with constant 20% grass silage were used. One ration consisted of additionally 20% corn silage and 60% concentrate (CS60 treatment), the other two rations included 20 respectively 60% pressed sugar beet pulp silage and 60 respectively 20% concentrate (treatments SBPS60 respectively SBPS20). With those rations, low pH values were induced in the rumen, leading to SARA incidences of 89% in the measured days in the CS60, 100% in the SBPS60, and 61% in the SBPS20 treatment. In Trial 2, for all three rations a fix concentration of 52% concentrate was used. The remaining 48% consisted of corn silage (treatment CS), grass silage (treatment GS) or hay (treatment Hay). In the CS treatment, SARA incidence was 23%, while the GS and Hay treatments did not show SARA incidence. While the first two trials were designed as a 3x3 Latin square, in Trial 3 the cows remained in their

respective treatment. One group stayed in the barn with a TMR, including 30% concentrate (treatment CG), while the other group was full time grazing and got additional 1.75 kg concentrate per day (treatment PG). SARA incidences were 7% in the CG and 8% in the PG. All three trials were individually statistical analysed.

Additionally, in an intertrial approach, regression models for SARA detection were developed. Therefore, easily accessible performance data from the barn and milk parameters from the official milk control and milk fatty acids were used to estimate the rumen parameters pH mean and the time spent below pH 5.8. With seven of the treatments (n = 173) the models were established and one treatment (n = 12) was used to validate the models.

One model (Whole dataset) was designed to include 63 variables. Besides 11 parameters gained in the barn or from the official milk control, also 52 parameters that were gaschromatographically detected fatty acids and sums of these fatty acids. The other model was designed to be useable if no gaschromatographical milkfat analysis was available. Therefore, only those FAs were included that can be estimated in a good quality with MIR spectroscopy (Reduced dataset = 20 variables). With a 5% level of significance the Whole dataset model used 9 variables to estimate the pH mean, and 6 variables to estimate the time pH < 5.8. The Reduced dataset model used also 9 variables to estimate the pH mean, and 8 variables to estimate the time pH < 5.8.

With those regression models the SARA days from the 185 measurement days were calculated to test the accuracy of the models. From the original 47 SARA days the model Whole dataset was able to detect 43 days and the Reduced dataset model detected 39 SARA days. The inaccuracy of the models is higher than the total SARA days detected might pretend, since there are also “not detected” and “false positive detected” days. Although the accuracy of SARA prediction based on these models might be too inaccurate for a decision if a single day was SARA prevalent or not, an information on herd basis seems assessable. Still the small number of cows and measured days, as well as the fact that two breeds of cows and only cows in the later lactation phase were integrated in the model establishment has to be considered and further developed before it becomes a useful tool in field use for SARA detection.

10 ZUSAMMENFASSUNG

Subakute Pansenazidose (SARA) ist eine weit verbreitete, aber schwer erfassbare Krankheit in der modernen Milchkuhhaltung. SARA tritt meist unter zwei Bedingungen auf. Zum einen, wenn die Kühe sich schnell an eine kohlenhydratreiche Ration nach der Kalbung anpassen müssen. Da die Futterzusammensetzung schnell geändert werden muss um den Energie- und Nährstoffbedarf der Kuh zu decken muss sich das Pansenmilieu ebenfalls schnell an das neue Futter anpassen, was zu ungünstigen Pansenbedingungen führen kann. Zum anderen, wenn die laktierenden Kühe parallel zu einer hohen Milchleistung auch eine hohe Futteraufnahme haben und das Futter energiereich, aber strukturarm ist. Das kann zu hohen Konzentrationen von kurzkettigen Fettsäuren im Pansen führen, falls der Abfluss aus dem Pansen und die Absorption durch die Pansenwand, sowie die Pufferkapazität im Pansen nicht ausreichend für die hohe Produktionsrate dieser Säuren ist. Dadurch kann es zu einer Versauerung des Pansens kommen, welche die Tiergesundheit negativ beeinflussen kann. Die Kühe zeigen unter solchen SARA Bedingungen im Pansen, wenn überhaupt, milde Symptome in Form von verringerter Wasser- und Futteraufnahme, geringerer Aktivität, Durchfall, reduzierter Pansenaktivität, Laminitis oder eine verminderte Milchleistung und einen reduzierten Milchfettgehalt. Da die Symptome auch erst mit zeitlicher Verzögerung zu den Missständen im Pansen auftreten können ist ein Erkennen von SARA in der Herde schwierig, da diesen Symptomen auch andere Ursachen zugrunde liegen können. Auch nach Jahrzehnten der Forschung ist keine einheitliche Definition der Pansenbedingungen, die eine subakute Azidose darstellen, gegeben. Definitionen für SARA unterscheiden sich, ob die pH-Werte stichprobenhaft, oder kontinuierlich gemessen werden, aus welchem Pansenbereich sie gezogen werden und ab welchem pH Zustand im Pansen ein unphysiologisch saures Milieu festgelegt wird. Wir nutzen die Definition von Zebeli et al. (2008), die eine kontinuierliche pH Messung im ventralen Pansen voraussetzt. Ein Tag wird dabei als Tag mit SARA definiert, wenn der pH Mittelwert kleiner als 6,16 und ein pH Grenzwert von 5,8 für länger als 5,24 Stunden (314 Minuten) unterschritten wird. Da SARA einen Einfluss auf die Milchleistung, den Milchfettgehalt und die Milchfettzusammensetzung haben kann, lag unser Fokus darauf Änderungen der Milchfettzusammensetzung zu erfassen und in Zusammenhang mit dem pH im ventralen Pansen zu bringen.

Dafür wurden drei Versuche mit Kühen in fortgeschrittenem Laktationsstadium und unterschiedlichen Rationen durchgeführt, die das Pansenmilieu erwartbar in den Grenzbereich einer SARA bringen können. Tierindividuell wurden während der Versuche nicht nur Leistungs- und Pansenparametern erfasst sowie kontinuierlich pH Daten gemessen, sondern auch

Milchproben für Analysen der Milchleistungsprüfung mittels mittlerem Infrarot Spektrograph und Milchproben für die gaschromatographische Analyse des Milchfetts genommen. Die Versuchskühe waren alle pansenfistuliert, wodurch Datenlogger mit integriertem pH Meter (Large Ruminant Logger M5-T7, Dascor Inc., Oceanside, USA) in den ventralen Pansen eingebracht werden konnten um die pH Verläufe zuverlässig und kontinuierlich zu messen. Die Futterstruktur wurde mit Hilfe eines Penn State Partikel Separators ermittelt und die peNDF ($peNDF_{>8mm}$ und $peNDF_{>1,18mm}$) bestimmt. In Versuch 1 kamen drei Rationen mit konstant 20 % Grassilage zum Einsatz. Eine Ration bestand aus zusätzlich 20 % Maissilage und 60 % Kraftfutter (CS60), die anderen beiden Rationen hatten 20 beziehungsweise 60 % Pressschnitzelsilage und 60 beziehungsweise 20 % Kraftfutteranteil (SBPS60 beziehungsweise SBPS20). Mit diesen Rationen wurden niedrige pH-Werte im Pansen provoziert, mit SARA Inzidenzen von 89 % der gemessenen Tage in der CS60 Behandlung, 100 % in der SBPS60 und 61 % in der SBPS20 Behandlung. In Versuch 2 wurde ein fixer Kraftfutteranteil von 52 % eingesetzt, die übrigen 48 % der Ration bestanden aus Maissilage (CS), Grassilage (GS) oder Heu (Hay). In der CS Behandlung lag die SARA Inzidenz bei 23 %, wohingegen in den beiden anderen Behandlungen keine SARA vorkam. Während die ersten beiden Versuche als 3x3 Lateinisches Quadrat angelegt waren, blieben in Versuch 3 die Kühe in einer Behandlung. Eine Gruppe im Stall (CG) bekam 30 % Kraftfutter in der TMR vorgelegt, die zweite Gruppe hatte ganztägig Weidegang (PG) mit 1.75 kg Kraftfutter Zufütterung. Die beobachteten SARA Inzidenzen lagen bei 7 % (CG) bzw. 8 % (PG). Die drei Versuche wurden jeweils versuchsintern statistisch ausgewertet.

Darüber hinaus wurden, in einem versuchsübergreifenden Ansatz, Regressionsmodelle zur Feststellung von SARA entwickelt. Dafür wurden einfach erfassbare Leistungsparameter aus dem Stall, sowie Milchparameter aus der Milchleistungsprüfung sowie Milchfettsäuren verwendet, um den pH Mittelwert und die Zeit, die der Pansen einen pH-Wert von weniger als 5,8 aufweist, zu schätzen. Mit sieben Behandlungen (173 Datenpaaren) wurden die Modelle zur Abschätzung entwickelt und eine Behandlung (12 Datenpaare) zur Validierung der Modelle genutzt.

Für ein Modell (Whole dataset) wurden 63 Variablen zur Verfügung gestellt. Neben 11 Parametern aus der Milchleistungsuntersuchung und Leistungsdaten der Kuh, auch 52 Parameter aus der gaschromatographischen Milchfettanalyse und Summen der analysierten Fettsäuren.

Für ein reduziertes Modell (Reduced dataset) wurden statt der kompletten 52 gaschromatographischen Milchfettparameter nur 9 Milchettsäurenparameter zur Verfügung gestellt, die zuverlässig aus MIR Daten abgeleitet werden können, sodass insgesamt 20 Parameter für das Reduced dataset zur Verfügung standen.

Bei einem Signifikanzniveau von 5 % wurden für das Whole dataset 9 Variablen gewählt um den pH Mittelwert zu schätzen, bzw. 6 Variablen um die Zeit pH < 5,8 zu schätzen.
Bei einem Signifikanzniveau von 5 % wurden für das Reduced dataset 9 Variablen gewählt um den pH Mittelwert zu schätzen, bzw. 8 Variablen um die Zeit pH < 5,8 zu schätzen.
Anschließend wurden mithilfe der Modelle aus den gewählten Parametern SARA Inzidenzen berechnet um die Genauigkeit der Modelle zu testen. An den 185 Messtagen, mit 47 gemessenen SARA Tagen, konnte das Whole dataset Modell 43 Tage und das Reduced dataset Modell 39 SARA Tage identifizieren. Die Ungenauigkeit der Modellvorhersagen ist höher als die Abweichung der identifizierten SARA Tage zu den tatsächlich gemessenen Tagen, da einige Tage auch nicht erfasst, beziehungsweise falsch positive erfasst wurden. Obwohl die Genauigkeit der Vorhersage auf Grundlage dieser Modelle zu ungenau für eine Einzeltagbeobachtung ist, scheint eine Einschätzung, ob SARA ein Problem in einer Herde ist, möglich zu sein. Dennoch muss die geringe Tierzahl und der kleine Stichprobenumfang, sowie die Tatsache, dass zwei Kuhrassen und nur ein späterer Laktationsabschnitt für die Modellierung verwendet werden konnten berücksichtigt, und die Modelle weiterentwickelt werden, bevor sie in der Praxis zur SARA Identifikation angewendet werden können.

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13 APPENDIX

Equation 2.2 Model used for estimation of β0 and β1 in SAS 9.2

```
Proc nlmixed data= Dataset;
parameters beta0=6.0 beta1=5.8;
est=1440/(1+exp(-beta0*(pH-beta1)));
model Cow_1_07_05_2014 ~ normal(est,var);
run;
```

Beta0 (β0) and beta1 (β1) with their assumed start point, somewhere between 5.5 and 6.2 for β1 and between 4 and 8 for β0 have to be defined. In this example 6.0 for β1 and 5.8 for β0 were chosen for the dataset of cow 1 for one day (Cow_1_07_05_2014). The dataset consists of data in a form shown in Table 2.2. If the gap between the assumed and the actual value does vary too much no results are given by SAS. Then the calculation has to be repeated with other estimations of β0 or β1 that fit better to the actual value of that day.

Table 13.1 Significant (α ≤ 0.05) spearman correlations between β0 (slope) and fatty acids, sums of fatty acids, milk constituents, and pH parameters

Trial	1	2	3	All trials
Parameter	β0	β0	β0	β0
Sample size	n = 36	n = 90	n = 60[1]	n = 186[1]
Milk FAs:				
C4:0	0.48			0.44
C6:0				
C8:0			-0.27	-0.21
C10:0			-0.27	-0.25
C11:0		0.27		-0.36
C12:0			-0.33	-0.29
C13:0	-0.46	0.25	-0.36	-0.39
C14:0			-0.36	-0.40
C14:0 iso				
C14:1 c + C15:1 aiso				-0.39
C15:0		0.37	-0.33	-0.28
C15:0 iso				0.25
C15:1 t				
C16:0	0.37	0.35	-0.32	
C16:1 t			0.28	
C16:1 c			0.27	
C17:0	0.39	0.35	0.38	0.24
C17:0 iso		-0.32	0.27	
C17:0 aiso	0.39		0.30	
C18:0				0.44
C18:0 iso			0.36	
C18:1 t6 + c7 + t9 + t11	-0.45		-0.41	-0.51
C18:1 c9		-0.22	0.40	0.23
C18:1 c11		-0.42		-0.18
C18:1 c12	-0.46		-0.39	
C18:2 t9,12	-0.35			
C18:2 c9,12 + C19:1 t7	-0.34			-0.45
C18:2 t9,c11				-0.32
C18:2 c9,t11	-0.42			
C18:3 c9,12,15				
C19:1 t10				0.18
C20:0				0.44
C20:1 c11		-0.25	0.30	-0.34
C20:2 c11,14				-0.33
C20:3 c11,14,17				-0.36
C22:0		0.21		0.23

Continued Table 13.1 Significant (α ≤ 0.05) spearman correlations between β0 (slope) and fatty acids, sums of fatty acids, milk constituents, and pH parameters

Trial	1	2	3	All trials
Parameter	β0	β0	β0	β0
Sample size	n = 36	n = 90	n = 601	n = 1861
Milk FAs:				
Sum even-numbered chain + C15:1 aiso		-0.27		
Sum odd-chain + C14:1 c		0.34		-0.23
Sum iso-chain	0.37	-0.22	0.31	
Sum aiso-chain + C14:1 c				-0.30
Sum monounsaturated + C18:2 c9,12		-0.29	0.37	
Sum 2x unsaturated + C19:1 t7	-0.41			-0.39
Sum 3x unsaturated			0.27	
Sum polyunsaturated + C19:1 t7	-0.40			-0.38
Sum short-chain (C4 - C13)			-0.28	-0.17
Sum medium-chain (C14 to C16)		0.32	-0.34	
Sum long-chain (C17+)		-0.26	0.34	
Milk parameters:				
Yield (kg)	-0.55	0.34		
Fat (%)	0.38	-0.23		
Protein (%)				-0.26
Lactose (%)	-0.42			0.17
pH value	0.61	0.36	0.26	0.35
Number of cells	-0.51	0.31		
Urea		-0.44	0.33	
ECM		0.30		
Fat per day (g)		0.23		
Rumen pH parameters:				
β1				0.21
Min/day pH < 5.2	-0.70	-0.85	-0.81	-0.79
Min/day pH < 5.6		-0.80	-0.68	-0.66
Min/day pH < 5.8		-0.71	-0.55	-0.54
Min/day pH < 6.0	0.45	-0.57	-0.33	-0.38

[1] For data from official milk control n = 59 in Trial 3 and n = 185 in all trials

Table 13.2 Significant (α ≤ 0.05) spearman correlations between β1 (pH mean) and fatty acids, sums of fatty acids, milk constituents, and pH parameters

Trial	1	2	3	All trials
Parameter	β1	β1	β1	β1
Sample size	n = 36	n = 90	n = 60[1]	n = 186[1]
Milk FAs:				
C4:0	0.37			0.20
C6:0	0.40	-0.21		0.41
C8:0	0.59	-0.40		0.41
C10:0	0.48	-0.51		0.36
C11:0		-0.30		
C12:0	0.41	-0.48		0.33
C13:0		-0.28		
C14:0	0.36	-0.60		
C14:0 iso				0.53
C14:1 c + C15:1 aiso	-0.69	0.24	-0.32	-0.30
C15:0				-0.37
C15:0 iso				0.24
C15:1 t	-0.43			0.16
C16:0	0.35	0.35	0.30	0.35
C16:1 t		-0.22		-0.43
C16:1 c	-0.61	0.35		-0.20
C17:0		0.34		-0.46
C17:0 iso	0.40		-0.35	-0.22
C17:0 aiso		0.45	-0.38	-0.37
C18:0	0.62			
C18:0 iso	-0.44	0.42	-0.29	-0.42
C18:1 t6 + c7 + t9 + t11	-0.53	-0.55		-0.41
C18:1 c9		0.31		
C18:1 c11	-0.36	-0.29	-0.31	-0.51
C18:1 c12		-0.23		-0.34
C18:2 t9,12		-0.24	-0.32	-0.62
C18:2 c9,12 + C19:1 t7	-0.72	-0.27		-0.21
C18:2 t9,c11	-0.59			-0.46
C18:2 c9,t11				-0.46
C18:3 c9,12,15	-0.66		-0.26	
C19:1 t10				-0.18
C20:0				
C20:1 c11		-0.48		-0.39
C20:2 c11,14				
C20:3 c11,14,17	0.42		0.37	0.22
C22:0				

Continued Table 13.2 Significant (α ≤ 0.05) spearman correlations between β1 (pH mean) and fatty acids, sums of fatty acids, milk constituents, and pH parameters

Trial	1	2	3	All trials
Parameter	β1	β1	β1	β1
Sample size	n = 36	n = 90	n = 60[1]	n = 186[1]
Milk FAs:				
Sum even-numbered chain + C15:1 aiso			0.26	0.45
Sum odd-chain + C14:1 c	-0.37		-0.40	-0.41
Sum iso-chain		0.23	-0.34	-0.23
Sum aiso-chain + C14:1 c	-0.69	0.36	-0.39	-0.33
Sum monounsaturated + C18:2 c9,12	-0.49	0.24		-0.32
Sum 2x unsaturated + C19:1 t7	-0.63	-0.27		-0.37
Sum 3x unsaturated	-0.66	0.27		
Sum polyunsaturated + C19:1 t7	-0.63	-0.28		-0.38
Sum short-chain (C4 to C13)	0.52	-0.46		0.40
Sum medium-chain (C14 to C16)		0.22		0.29
Sum long-chain (C17+)				-0.32
Milk parameters:				
Yield (kg)				-0.44
Fat (%)	0.56	-0.28	0.27	0.48
Protein (%)				0.38
Lactose (%)				
pH value		0.41		0.39
Number of cells		0.58		0.18
Urea				0.49
ECM	0.55			
Fat per day (g)	0.65		0.26	0.19
Rumen pH parameters:				
β0				0.21
Min/day pH < 5.2	-0.67	-0.56	-0.54	-0.66
Min/day pH < 5.6	-0.94	-0.67	-0.82	-0.81
Min/day pH < 5.8	-0.91	-0.76	-0.91	-0.89
Min/day pH < 6.0	-0.83	-0.87	-0.97	-0.95

[1] For data from official milk control n = 59 in Trial 3 and n = 185 in all trials

Table 13.3 Significant (α ≤ 0.05) spearman correlations between the time the ruminal pH was < 5.8 and fatty acids, sums of fatty acids, milk constituents, and pH parameters

Trial	1	2	3	All trials
Parameter	Time pH < 5.8	Time pH < 5.8	Time pH < 5.8	Time pH < 5.8
Sample size	n = 36	n = 90	n = 60[1]	n = 186[1]
Milk FAs:				
C4:0				-0.33
C6:0	-0.35			-0.33
C8:0	-0.52	0.33		-0.25
C10:0	-0.49	0.35		-0.21
C11:0				
C12:0	-0.42	0.30		-0.17
C13:0				0.20
C14:0	-0.41	0.53		
C14:0 iso				-0.45
C14:1 c + C15:1 aiso	0.62		0.27	0.45
C15:0				0.39
C15:0 iso				-0.27
C15:1 t	0.40			-0.18
C16:0		-0.45		-0.30
C16:1 t				0.40
C16:1 c	0.56			0.28
C17:0		-0.47		0.29
C17:0 iso	-0.38			0.18
C17:0 aiso		-0.27		0.36
C18:0	-0.54			-0.33
C18:0 iso	0.48	-0.25		0.39
C18:1 t6 + c7 + t9 + t11	0.48	0.39		0.51
C18:1 c9				
C18:1 c11	0.37	0.44		0.50
C18:1 c12				0.25
C18:2 t9,12		0.21		0.45
C18:2 c9,12 + C19:1 t7	0.56	0.21		0.35
C18:2 t9,c11	0.48			0.46
C18:2 c9,t11				0.37
C18:3 c9,12,15	0.53			
C19:1 t10				
C20:0				-0.19
C20:1 c11		0.45		0.43
C20:2 c11,14				0.20
C20:3 c11,14,17	-0.36		-0.31	
C22:0				

Continued Table 13.3 Significant (α ≤ 0.05) spearman correlations between the time the ruminal pH was < 5.8 and fatty acids, sums of fatty acids, milk constituents, and pH parameters

Trial	1	2	3	All trials
Parameter	Time pH < 5.8	Time pH < 5.8	Time pH < 5.8	Time pH < 5.8
Sample size	n = 36	n = 90	n = 60[1]	n = 186[1]
Milk FAs:				
Sum even-numbered chain + C15:1 aiso		0.29		-0.41
Sum odd-chain + C14:1 c	0.44	-0.28	0.33	0.45
Sum iso-chain				0.21
Sum aiso-chain + C14:1 c	0.63	-0.25	0.30	0.45
Sum monounsaturated + C18:2 c9,12	0.46			0.29
Sum 2x unsaturated + C19:1 t7	0.53			0.47
Sum 3x unsaturated	0.53			
Sum polyunsaturated + C19:1 t7	0.52	0.28		0.47
Sum short-chain (C4 to C13)	-0.48	0.29		-0.27
Sum medium-chain (C14 to C16)		-0.33		-0.21
Sum long-chain (C17+)				0.21
Milk parameters:				
Yield (kg)	-0.33	-0.26		0.32
Fat (%)	-0.49	0.29		-0.34
Protein (%)				-0.19
Lactose (%)				
pH value		-0.52		-0.43
Number of cells	-0.42	-0.62		-0.15
Urea		0.31		-0.38
ECM	-0.53			
Fat per day (g)	-0.59			-0.20
Rumen pH parameters:				
β0		-0.71	-0.55	-0.54
β1	-0.91	-0.76	-0.91	-0.89
Min/day pH < 5.2	0.47	0.94	0.79	0.89
Min/day pH < 5.6	0.88	0.99	0.98	0.98
Min/day pH < 6.0	0.95	0.97	0.96	0.98

[1] For data from official milk control n = 59 in Trial 3 and n = 185 in all trials

Table 13.4 Influence of level of significance on R^2, number of used variables, and coefficient of variation

	pH < 5.8 Whole dataset	pH < 5.8 Reduced dataset	β1 Whole dataset	β1 Reduced dataset
Level of significance	R^2 (Variables used/Coeff Var)	R^2 (Variables used/Coeff Var)	R^2 (Variables used/Coeff Var)	R^2 (Variables used/Coeff Var)
10%	0.84 (15 / 58.61)	0.79 (8 / 66.43)	0.81 (16 / 1.90)	0.72 (10 / 2.23)
9%	0.83 (14 / 59.65)	0.79 (8 / 66.43)	0.77 (12 / 2.06)	0.71 (9 / 2.29)
8%	0.83 (14 / 59.49)	0.79 (8 / 66.43)	0.77 (13 / 2.05)	0.71 (9 / 2.29)
7%	0.74 (8 / 72.68)	0.79 (8 / 66.43)	0.77 (13 / 2.05)	0.71 (9 / 2.29)
6%	0.73 (6 / 74.06)	0.79 (8 / 66.43)	0.80 (14 / 1.94)	0.71 (9 / 2.29)
5%	0.73 (6 / 74.06)	0.79 (8 / 66.43)	0.75 (9 / 2.11)	0.71 (9 / 2.29)
4%	0.73 (6 / 74.06)	0.79 (8 / 66.43)	0.75 (9 / 2.11)	0.71 (9 / 2.29)
3%	0.73 (6 / 74.06)	0.79 (8 / 66.43)	0.75 (9 / 2.11)	0.71 (9 / 2.29)
2%	0.73 (6 / 74.06)	0.79 (8 / 66.43)	0.71 (7 / 2.26)	0.66 (6 / 2.43)
1%	0.72 (5 / 75.19)	0.79 (8 / 66.43)	0.74 (8 / 2.16)	0.66 (6 / 2.43)