DETERMINATION OF SUBCLINICAL METABOLIC DISORDERS IN TRANSITION DAIRY COWS

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Für Hardy, Mama

& Iri

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ADF	Acid detergent fibre
ADFom	Acid detergent fibre expressed exclusive of residual ash
ADL	Acid detergent lignin
AUC	Area under the curve
BEDN	Bacterial and endogenous debris nitrogen
BHBA	β-hydroxybutyrate
BLE	Federal Office for Agriculture and Food
BMELV	Federal Ministry of Food, Agriculture and Consumer Protection
CP	Crude protein
CS	Crude starch
CT	Total C
CV	Coefficient of variation
d	Day
DC	DairyCheck
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
DIM	Days in milk
DP	Dry Period
EMG	Electromyography
GHz	Gigahertz
GmbH	Company with limited liability
h	Hour
HR	Qwes HR sensor
Hz	Hertz
Interleukin-6	IL-6
kg	Kilogram
Lab	Laboratory
LFI	Liver functionality index
Lignin(sa)	Lignin determined by solubilisation of cellulose with sulphuric acid
LP	Lactation Period
M.	Musculus
MJ/kg	Mega joule per kilogram
mmol/l	Millimole per litre
n	Number
NDFom	Neutral detergent fibre not assayed with a heat stable amylase and expressed exclusive of residual ash
NEFA	Non-esterified fatty acids
NEL	Net energy lactation
NFC	Non- fibre carbohydrates
NIRS	Near infrared spectroscopy

NT	Total N
OM	Organic matter
peNDF	Physical effective neutral detergent fibre
pg/ml	Pictograms per millilitre
r	Correlation coefficient
R^2	Coefficient of determination for calibration
ROC- Analysis	Receiver operating characteristic-analysis
RT	Rumination time
RW	RumiWatchSystem
SARA	Sub-acute ruminal acidosis
SCK	Subclinical ketosis
SD	Standard deviation
SEC	Standard error of calibration
SECV	Standard error of cross validation
SEM	Standard error of mean
TBIL	Total bilirubin
TD58	Total daily duration below pH 5.8
TMR	Total mixed ration
UDN	Undigested dietary nitrogen
V	Volt
VFA	Volatile fatty acid
WSN	Water soluble nitrogen
+ve	Positive
-ve	Negative
1-VR	Coefficient of determination of cross validation

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INTRODUCTION

STATE OF THE ART

HERD HEALTH STATUS IN DAIRY FARMS

In the interplay of economics, increasing demands of animal health and welfare and product quality, dairy milk production becomes more and more challenging. The increasing requirements of modern dairy cows represent major challenges for participants such as farmers, feed advisors or veterinarians in global dairy farming. Animal husbandry, feeding and herd management as well as the provision of consulting services for dairy farms need to adapt to meet the needs of high yielding animals. First and foremost, the maintenance of animal health has to be the primary goal in livestock farming in terms of animal wellbeing and productivity. For many years now it has been an acknowledged fact that the presence of a disease reduces performance in dairy cows (Fourichon et al. 1999; Rajala-Schultz et al. 1999). It is, however, also apparent that rising performance levels, which have increased enormously in the past few decades, bring greater susceptibility for diseases such as metabolic or reproduction disorders (Fleischer et al. 2001). The strained economic situation on many farms worldwide means there is an increased need for healthy animals to avoid additional costs due to drops in performance or veterinary treatment and additional labour.

Unfortunately, maintaining the general health status of dairy cattle is a significant challenge for dairy farms including organic farms (Bennedsgaard et al. 2003; Valle et al. 2007; Brinkmann and March 2010). Subclinical diseases are prevalent in dairy herds and are frequently underestimated (Nir 2003; Suthar et al. 2013). Lack of or delayed detection makes them a risk factor for further disorders and more severe disease progressions and leads to reduced chances of recovery and often results in higher treatment costs. Metabolic disorders often occur in a subclinical stage and are

commonly seen in high yielding dairy cows; especially in early lactation (Reinhardt et al. 2011; Kleen and Cannizzo 2012; Berge and Vertenten 2014). Several working groups could demonstrate that animals suffering from metabolic disorders are up to eight times more likely to develop concomitant diseases such as mastitis, retained placenta, metritis or displaced abomasum (LeBlanc et al. 2005; LeBlanc 2010; Ospina et al. 2010; Suthar et al. 2013; Garro et al. 2014) .

TRANSITION PERIOD AS MOST CHALLENGING TIMEFRAME IN LACTATION

The transition period, encompassing three weeks before and after calving, is the most challenging part of lactation and a major determinant for the productivity and profitability of the entire lactation cycle (Drackley 1999). Dairy cows undergo several hormonal, metabolic and physiological changes but also changes in housing, daily routine and nutrition, which often lead to an increased incidence in diseases during this timeframe (Goff and Horst 1997; Martens 2013). Bradford et al. (2015) stated that during this period more than 50% of cows are estimated to suffer from at least one subclinical disorder. Primarily, the initialization of milk synthesis results in increasing demands on the mammary glands with nutritional changes needed in order to cope with the requirements in early lactation (Overton and Waldron 2004). In this context, nutritionists have to face the contrary development of dry matter intake depression and increasing energy demands, which lead to a negative energy balance (Hayirli et al. 2002). Impaired feed intake starts before calving caused by the limited space available due to the growing foetus (Bell et al. 1995) as well as changes in metabolism (Ingvartsen and Andersen 2000). After calving, feed intake increases with insufficient speed and therefore the cows cannot satisfy the rapidly increasing energy needs. The relationship between the energy balance and milk production diseases, especially ketosis, is well described by numerous authors and underlines the high relevance of the right feeding regime in transition cow management (Collard et al. 2000; Mulligan et al. 2006).

Concerning rumen health, the adequate adaptation of the rumen microbial population from dry phase to lactation is the main challenge in the transition phase. The rumen

status during transition is of high significance in enabling productivity and profitability in early lactation. Jami et al. (2014) reported a high correlation between physiological parameters, such as milk yield and composition, with the composition of rumen microbiota. In general, rumen microbiota is highly responsive to changes in diet and consequently the microbiota alters during the transition phase (Kocherginskaya et al. 2001; Pitta et al. 2014). Beginning at dry-off, animals are usually fed high forage diets, which lead to a decrease in amolytic and an increase in cellulolytic microbiota. As a result, bacteria with the ability to convert lactate to acetate, propionate or butyrate, are reduced. As well as the microbiota, the rumen mucosa also changes and therefore the capacity to absorb VFA declines (Goff and Horst 1997). While the reduced amount of lactate-converting bacteria is harmless in the dry phase, it became one of the main problems in early lactation. The abrupt change to starch diets leads to a flood of lactate, which cannot be converted due to a lack of relevant bacteria. The needed bacteria population adapts only slowly over 3 to 4 weeks. Lactate is a stronger acid compared to other VFA in the rumen and is generally absorbed more slowly by the mucosa. Reinforced by the reduced capacity of the mucosa to absorb VFA at the beginning of lactation, lactate accumulation takes place and leads to sub-acute rumen acidosis (SARA) (Block 2010).

There is a growing body of evidence that inflammatory phenomena also play a key role in the transition period (Bertoni et al. 2008; Trevisi et al. 2012; Bradford et al. 2015). Energy deficiency in early lactation is associated with disease susceptibility as a result of immune suppression (Goff and Horst 1997). The increased concentration of fatty acids in plasma caused by lipid mobilization from adipose tissue is known to disrupt several immune and inflammatory functions (Contreras and Sordillo 2011). In this context, authors like Sordillo and Raphael (2013) or Bradford et al. (2015) have described dysfunctional inflammatory responses as the common link between metabolic and infectious diseases around the time of calving. As part of the unspecific immune system, the pro-inflammatory cytokine interleukin-6 is also discussed in the field of subacute, low-grade inflammation during early lactation (Trevisi et al. 2012). The release of interleukin-6 mainly leads to the initialization of early acute phase reaction in the liver but is also involved in lipolysis or impaired insulin sensitivity,

which can be contributed to metabolic disorders such as fatty liver or ketosis (Kushibiki et al. 2003; Mukesh et al. 2010). In a study investigating metabolic and signalling gene networks in the liver, interleukin-6 was highlighted as playing a major role during nutrition-induced ketosis and therefore in general liver function (Loor et al. 2007). The relationship between cytokines and liver function was also described by Trevisi et al. (2012), who reported increased incidence of disease and increased serum interleukin-6 concentrations in dairy cows with low liver functionality index (LFI), which implies a high inflammatory response.

In general, minimizing postpartum disorders has to be the primary objective in transition cow management to ensure the health of dairy cows. Tools for prevention and diagnosis need to be included in the herd management programs of dairy farms.

DETECTION OF SUBCLINICAL KETOSIS AND SUB-ACUTE RUMEN ACIDOSIS

Subclinical ketosis (SCK) and sub-acute rumen acidosis (SARA) seem to be two of the major types of metabolic disorders with a high prevalence on commercial farms. Prevalence rates of up to 39% in the case of subclinical ketosis, and up to 40% in subclinical rumen acidosis are reported in several studies (Reinhardt et al. 2011; Kleen and Cannizzo 2012). SCK as the expression of a long-term energy deficit without clinical signs of ketosis is commonly detectable by means of metabolites, which are associated with energy metabolism such as ketone bodies or NEFAs. They are traceable in blood, milk or urine, while evidence of β-hydroxybutyrate (BHBA) in serum is accepted as the "gold standard" in SCK diagnosis (Iwersen et al. 2009). Numerous cow-side tests are available enabling SCK detection on the farm yet often only deliver semi-quantitative results (Geishauser et al. 2000). In recent years, a handheld device originating from human medicine is commonly used on farms for quantitative BHBA determination in fresh blood. Satisfactory results could be observed compared to laboratory BHBA determination and mean that it is currently the best device available for SCK diagnosis on farms (Iwersen et al. 2009; McArt et al. 2012). The cut-off value described in literature varies between 1.0 to 1.4 mmol of BHBA in blood samples but all thresholds are accepted for the purposes of

distinguishing between cows that have and do not have SCK (Carrier et al. 2004; Ospina et al. 2010).

Unlike SCK, sub-acute rumen acidosis (SARA) is currently difficult to detect at farm level. SARA is defined as a repeated drop in rumen pH below a physiological level provoked by a diet containing high amounts of rapidly fermentable carbohydrates, which causes an accumulation of VFA in the rumen (Kleen and Cannizzo 2012). Analysis of rumen fluid has been accepted as the gold standard, providing direct information about rumen conditions (Kleen et al. 2003). Nevertheless, for direct evidence via rumen fluid analysis, spot samplings such as rumen puncture or stomach tubing still need to be performed by a veterinarian. Furthermore, risk factors for the dairy cows´ health cannot be excluded (Tajik and Nazafi 2011). So far, rumenocentesis is the most commonly used method for rumen fluid analysis in the field. Samples obtained using a stomach tube is, however, often contaminated with saliva leading to a reduced diagnostic value (Enemark 2009). The accepted threshold of rumen pH for identification of SARA using spot sampling methods is pH 5.5 taking into account feeding times and physiological, diurnal pattern of rumen pH (Garrett et al. 1999; Duffield et al. 2004). Other cut-off values such as pH 6.0 and pH 5.8 are also used (Kleen et al. 2003; Enemark 2009). Based on continuous rumen pH measurement, new concepts for the elaboration for thresholds of SARA were carried out, encompassing times below a defined rumen pH value within 24 hours (Gozho et al. 2005; Zebeli et al. 2008). The detection of SARA based on currently available indirect indicators such as milk components or net acid-base excretion in urine seems to be less practical and of less diagnostic value (Seemann and Spohr 2007; Tajik and Nazafi 2011).

DEVELOPMENT OF DISEASE DETECTION DUE TO NEW TECHNIQUES AND KNOWLEDGE

The timely recognition of a disease is still a weak point in herd health management especially in large herds and needs to be improved. On the one hand some existing diagnostic tools have only limited informative value for early disease detection and on the other hand in many cases the utilization of the tools proves too costly, time-consuming or complicated to establish at farm level.

In the course of the technical development in precision livestock farming (Berckmans 2008; Wathes et al. 2008), new and advanced tools have become available, which enable a more detailed control of animal health (Edwards and Tozer 2004; González et al. 2008; Rutten et al. 2013). Whereas some new techniques only facilitate data recording, other innovations generate new data or enable the measurement of parameters at farm level, which was previously only possible under experimental conditions. With the help of new technical innovations, direct and indirect health parameters such as body weight, body temperature, rumination or eating behaviour and long-term rumen pH measurement can be determined easily and implemented in new transition cow management programs (Rutten et al. 2013). In this connection, there is a body of research indicating that change of behaviour can be attributed to illness (Weary et al. 2009). For example, rumination behaviour was recognized by several authors as a suitable indicator in obtaining information on the health status of dairy cattle due to the direct and indirect involvement in physiological processes (Hansen et al. 2003; Soriani et al. 2012; Stangaferro et al. 2016). The long-term measurement of rumen pH, which is the latest method to be used in the detection of SARA had for a long time only been feasible under experimental conditions due to the invasive methods involved (Zebeli et al. 2008). For the first time, innovative sensor-based technology using orally administered rumen sensors and wireless data transmission enable rumen pH measurement at farm level (Gasteiner et al. 2012). The determination of C and N fractions of bovine faeces via NIRS also became more important in recent years (Althaus et al. 2013). Until now the main motivations for faeces fractionating had been gaining information for plant cultivation, and in livestock, reducing losses by emission and enhancing feed efficiency (Sørensen et al. 1999; van Vliet et al. 2007). The method is thus well-established and can also be used to monitor the bovine digestive system in order to detect deviations in faeces provoked by diseases, which affect digestion of the nutrients fed.

Along with developments and innovations in detection techniques, the spectrum of currently implemented tools continues to expand due to a growing awareness of the pathogenesis of diseases. In this regard, blood parameters should also be mentioned as these have for centuries been widely used as a diagnostic tool (Schulze 2009). Newly

gained knowledge about a disease often leads to revelations about further contributing factors, which can sometimes be used in (earlier) disease detection. For example, recent investigations about the relationship between metabolic disorders and inflammatory responses open up the possibility of including immunological mediators such as cytokines in the parameter spectrum for the detection of metabolic disorders (Drackley et al. 2005; Trevisi et al. 2012; Bradford et al. 2015).

Overall, beside disease detection itself, new techniques allow studies to be conducted under commercial as well as under experimental conditions in order to investigate further pathways of disease pathology or epidemiological questioning.

OVERALL AIM AND RESEARCH STRATEGY

The aim of the study is to test new parameters for the early detection of subclinical metabolic disorders at farm level. Two of the most prominent metabolic disorders, subclinical ketosis and sub-acute rumen acidosis (SARA), are to be detected using parameters, which have be revealed as a result of greater knowledge of disease pathology or developments in detection techniques.

The study is divided into two main parts in which the diseases will be investigated separately from one another. In the first part investigations will focus on subclinical ketosis while in the second part sub-acute rumen acidosis will be examined.

DETECTION OF SUBCLINICAL KETOSIS

A trial will be conducted in which the main objective is the detection of cows with subclinical ketosis. The pro-inflammatory cytokine interleukin-6 will be determined in serum of dairy cows suffering subclinical ketosis. Furthermore, total daily rumination time will be measured. The results will be compared with those from recovered and healthy control animals in order to test the diagnostic value of both techniques in terms of SCK detection in the field.

The working hypothesis for the detection of subclinical ketosis is as follows:

- Serum interleukin-6 concentration increases due to the onset of subclinical ketosis. The concentration of serum interleukin-6 is increased in dairy cows with subclinical ketosis compared to recovered and healthy control animals.
- Rumination time is affected by disturbances in energy metabolism before clinical signs occur. Therefore, dairy cows suffering SCK show reduced rumination times compared to recovered or healthy control animals.

To test the working hypothesis, ketosis monitoring will be conducted on a commercial farm to find dairy cows suffering from subclinical ketosis as well as dairy cows without any signs of the disease. Blood analysis will be performed in cooperation with

the Small Animal Clinic, Institute of Veterinary Medicine at the University of Goettingen in Germany. Rumination activity of all tested animal groups will be measured using the DairyCheck system, which was developed within the project "NutriCheck". This project was founded by Federal Office for Agriculture and Food (BLE) and the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) in Germany and is further described by Büchel (2014).

DETECTION OF SUB-ACUTE RUMEN ACIDOSIS

In the second part of the study, the detection of SARA under practical conditions will be tested using newly developed techniques. For this reason we will make use of a rumen pH measurement system, which provides long-term rumen pH values to detect dairy cows suffering SARA on a commercial farm. Furthermore, rumination activity and the fractionation of the dairy cows´ faeces will be tested as parameters for the detection of SARA. Feeding behaviour parameters such as duration of eating and ruminating as well as the number of chews will be measured continuously using a commercial available device. In recent years, the analysis of faecal soluble and insoluble C and N fractions via near infrared reflectance spectroscopy (NIRS) has been successfully established in our working group (Althaus et al. 2013). The analysis provides detailed information about the composition of the faeces. All results will be compared between diseased, recovered and healthy animals in order to test the diagnostic value of the techniques used to detect SARA under commercial conditions.

Thus, the working hypothesis for the detection of SARA is as follows:

- The continuous measurement of reticuloruminal pH values can be used to detect SARA under commercial conditions and can replace invasive spot sampling.
- The presence of SARA alters the feeding behaviour of dairy cows. Dairy cows suffering from SARA ruminate and eat less compared to recovered or healthy

cows. Therefore, continuous measurements of feeding behaviour parameters are useful for detecting SARA in the field.

- Faeces of dairy cows with SARA differ in case of the composition of C and N fraction compared to cows without SARA. Impaired fibre digestibility leads to a higher amount of fibre components in faeces of dairy cows that are suffering from the disease.

The working hypothesis will be tested in trials conducted under practical conditions in transition dairy cows. In contrast to the study focusing on subclinical ketosis, previous disease monitoring will not be carried out due to the difficult nature of SARA detection in the field. Hence, a high-yielding dairy herd receiving a traditional ration containing high amounts of non-structural carbohydrates, which increases the risk of SARA, will be chosen. Such diets are related to an increased risk of SARA and we therefore anticipate a sufficient number of SARA cases in the herd. Reticuloruminal pH will be measured continuously in 24 dairy cows during the transition period beginning week 2 ante partum until week 5 postpartum. Based on the results of reticuloruminal pH, cows were classified as diseased, recovered or healthy control animals. Rumination activity will be measured at different stages of transition according to a defined scheme. Faecal samples will be collected during the rumination measurement periods, additionally opening up the option of comparing the different parameters with one another.

METHODOLOGY DEVELOPMENT

The following section describes the development of those research methods used for the present study, which required previous investigation. The first part deals with the timely detection of cows suffering from subclinical ketosis at farm level. Here there was a particular focus on investigating the practicality of available rapid cow-side test devices (blood and urine based) as well as the right period for monitoring under commercial conditions. The second part deals with the continuous measurement of feeding behaviour parameters of dairy cows. Two measurement systems that are already commercially available, as well as one additional device developed by our working group, were taken into account. The latter is the result of the "NutriCheck" project, which was founded by the Federal Office for Agriculture and Food (BLE) and the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) in Germany. Activities related to this project have been comprehensively described by Büchel (2014), including a comparative study involving the above-mentioned three different measuring systems for feeding behaviour parameters. This trial is a point of contact between the current study and Büchel´s study and represents a crucial part of the methodology development. It was the basis for finding the most suitable measurement device for feeding behaviour recording for the different research questions; the study is therefore also described below.

KETOSIS MONITORING OF EARLY LACTATING COWS IN FARM PRACTICE

INTRODUCTION

To determine new parameters for ketosis detection, previous recognition of the disease is essential. Handheld devices are available for the identification of subclinical ketosis in practice, which are able to detect ketone bodies in blood, milk or urine but only provide semi-quantitative results (Geishauser et al. 2000; Iwersen et al. 2009). A device for quantitative BHBA determination in fresh blood developed for human medicine is already used for dairy cows with satisfactory results (Iwersen et al. 2009). Studies report that high correlations could be observed compared to laboratory BHBA determination, which is accepted as the gold standard for ketosis diagnostics. However, only a limited number of studies are available in which this commercial device has been used for ketosis detection.

The aim of this work section is to estimate the SCK prevalence rates to be expected in early lactation in order to find the timeframe with a high number of SCK cases and suitable for monitoring in practice. Furthermore, the practicability of the device will be tested and the correlation between BHBA results from the handheld meter and the laboratory BHBA determination in serum will be examined.

MATERIALS AND METHODS

The test was conducted on the experimental dairy herd of the State Institute for Agriculture, Forestry and Horticulture Saxony-Anhalt in Iden, Germany. Data for this study originated from 78 primiparous and multiparous (25/75) dairy cows with an average milk yield of 11500 kg/year. The dairy cows were milked three times a day in a rotary milking parlour and were housed in a free stall barn with cubicles equipped with single and automatic feed bins, which enables the recording of individual feed intake. Dairy cows received a TMR ad libitum once a day at 10:00 h containing 17% CP, 47% NFC, 39% NDF and 19% ADF on DM basis. The sample collection (urine and blood) took place at day 3 postpartum (no urine, only blood), in week 2

postpartum and week 5 postpartum, respectively. Blood samples were taken in the morning after milking from the vena caudalis mediana. The cow-side test (Precision Xceed®) was conducted using fresh blood directly at the puncture site. For BHBA determination in the laboratory, blood was harvested in a plastic tube; clotted samples were centrifuged and immediately analysed according to standardized procedures. Cow-side urine tests (Ketostix® and Ketur® Test) were used in fresh midstream urine directly in the barn. All results were documented and compared using the statistical program SPSS for Windows (Version 21.0). Pearson correlation was conducted for the cow-side blood test and the BHBA determination in the lab. In the study, dairy cows were considered to have subclinical ketosis if they had a serum BHBA concentration of ≥1.0 mmol/L.

RESULTS

Prevalence of Ketosis cases

In the study, dairy cows were tested for ketosis in three investigation periods. In the first period, immediately after calving on day 3, 14 dairy cows out of 78 were detected. In week 2 postpartum, 11 SCK cases were observed while four of these were already identified in the first period. In the last investigation period (week 5 postpartum), only 4 new cases were detected. The study displays a high volume of ketosis cases within the first 14 days postpartum, while in week 5 the number of dairy cows with ketosis was reduced. This indicates that the most suitable timeframe for ketosis monitoring is in the first weeks of lactation.

Comparison of cow-side tests with results from laboratory analysis

Results originating from the usage of semi-quantitative urine-based tests were compared with the results from the laboratory analysis. A total of 58 probes were available for the analysis. Data from the cow-side blood (88 samples) test were also qualitative compared with the laboratory results (Table 1). The sensitivity and specificity was calculated based on the rate of true positives, true negatives, false negatives and false positives.

Table 1: Comparison between the results of cow-side tests (urine and blood) and the laboratory test as gold standard. Furthermore, the calculated sensitivity and specificity of cow-side urine and blood tests are presented.

Cow-side Test	Laboratory +ve[1]	Laboratory –ve[2]
Cow-side Urine +ve[1]	5	1
Cow-side Urine –ve[2]	4	48
Cow-side Blood +ve[1]	13	4
Cow-side Blood –ve[2]	3	68

	Sensitivity, %	Specificity, %
Cow-side Urine	55	97
Cow-side Blood	81	94

[1] positive; [2] negative

In the case of the cow-side blood test, a comparison of the quantitative results was also conducted to identify the correlation between the two determination procedures. A highly significant (p=0.01) Pearson correlation with r=0.94 was detected and confirmed the results of the sensitivity analysis.

CONCLUSION

Based on the findings of this study, monitoring for dairy cows suffering from ketosis will in subsequent studies take place during the first 14 days postpartum. While the urine test showed only a poor sensitivity for the detection of subclinical ketosis, the cow-side test based on blood gives satisfactory results indicating the practicability of this test.

COMPARISON OF SYSTEMS FOR MEASURING RUMINATION TIME OF DAIRY COWS

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ABSTRACT

The use of automatic systems facilitates the monitoring of the health and nutritional status of individual cows. Measurement of the daily rumination time (RT) constitutes a suitable parameter for individual monitoring of dairy cows with respect to deviations from normal conditions that might indicate disturbances. In the past, different systems for automatic measurement of feeding behavior of ruminants have been developed. Three systems: The Lely Qwes HR (HR) sensor, the DairyCheck (DC) system and the RumiWatchSystem (RW), all of which are based on diverse measurement methods, have achieved market maturity. The aim of this study was to investigate the reliability and validity of the measurement systems and to compare their results. For this study, the RT of nine dairy cows was recorded in two trials. In trial 1, RT was determined by both the DC system and by the HR sensor. In trial 2, RT was determined by both the RW system and by the HR sensor. Results indicated that data generated by both the DC system in trial 1 (total mean of RT per 24h for all cows of 530 ± 60 min) and the RW system in trial 2 (total mean of RT per 24h for all cows of 546 ± 54 min) were clearly different in comparison to those detected by the HR sensor in trial 1 (total mean of RT per 24h about all cows of 399 ± 148 min) and in trial 2 (total mean of RT per

24h for all cows of 413 ± 148 min). RT during one day deviated an average of 131 (DC) and 133 (RW) minutes from those detected by the HR sensor. Results of the measurements with DC and RW demonstrated a high concordance. Thus, these results are clearly more consistent than those detected by the HR sensor and indicate that the DC and RW systems are more common and useful tools for reliably recording rumination behavior. However, all three measurement systems are in need of further development to reduce their individual disadvantages and to achieve a high level of applicability for reliable usage in practice.

Key words: feeding behavior, measurement systems, validation

INTRODUCTION

The use of automated measurement systems to support health management has gained increasing importance in livestock production (Hogeveen et al., 2010; Rutten et al., 2013). Due to larger herd sizes, manual labour is increasingly being replaced by the use of technology. Automated measurement systems enable dairy farmers to manage larger herds with less time needed for surveillance, therefore reducing labour costs (Svennersten-Sjaunja and Pettersson, 2008; de Koning, 2010). Thus, automatic systems within Precision Dairy Farming will become of greater relevance in the future.

The development of automatic systems to measure feeding behaviour of ruminants has engaged many scientists for several decades (Nagel et al., 1975; Penning, 1983; Rutter et al., 1997; Kononoff et al., 2002). Further technical developments have led to innovative and new options for the automatic recording and interpretation of chewing and rumination activity of dairy cows to facilitate farm management (Rutten et al., 2013). These measurements are now a high priority and are considered to be a reasonable and helpful indicator in gaining relevant information about the individual animal and its ability to cope with farm-specific feeding situations (Owens et al., 1998; DeVries et al., 2009). For example, the daily observation of individual rumination time (RT) provides information about the fibre content and composition of the ration and its

benefit for rumen health function (Murphy et al., 1983; Leonardi et al., 2005) and is strongly associated with dry matter intake (Metz, 1975; Yang and Beauchemin, 2006). Data concerning rumination time are suitable for the early detection of deviations from normal conditions of feeding behaviour and diseases in dairy cows deriving thereof (Hansen et al., 2003; Krause and Oetzel, 2006).

Many different devices for recording rumination behaviour with automatic measurement systems have been developed. Some of these devices are in use in practice or in research work and are commercially available or will be available in the near future. However, the reliability of such devices has not been proven in a comparative analysis, until now. The overall objective of the current study was to test three different measurement systems in regard to their reliability and to the validity of generated data when measuring RT of dairy cows. The objectives were (1) to assess the concordance of measuring RT of a measurement system already implemented in practice compared to two systems so far used primarily in research work and (2) to determine the extent to which generated RT of both research systems are comparable to each other.

MATERIALS AND METHODS

Measuring Techniques

The measurement of RT was conducted by using three different systems which varied in terms of their area of application and use of technology. They corresponded to acoustic biotelemetry, pressure transducer and electromyography systems. For each system one representative device was chosen. For the acoustic biotelemetry system, the Lely Qwes HR (HR) sensor was selected, for the pressure transducer, the RumiWatchSystem (RW) was used, and for electromyography, the chosen device was the DairyCheck (DC) system. These three devices were selected for their stage of development and applicability. The HR sensor has already been applied in practical use, while both of the other systems have been primarily used in research.

Lely Qwes HR. The first prototype of the acoustic biotelemetry system was developed by Israeli scientists and is labeled as Vocal Tag, RuminAct™ or HR-Tag (SCR

Engineers Ltd., Netanya, Israel used by Lely Ltd., Maassluis, the Netherlands). The HR sensor consists of a neck collar with a tag which incorporates a touch-sensitive microphone and data logger. Rumination activity is recorded through the sound of regurgitation of boluses during a rumination phase. To ensure the right position of this tag, a counterweight is fixed to the collar ventrally. Rumination is recorded with a resolution of two minutes, which results in 60 possible agreements or disagreements per two-hour interval (Burfeind et al., 2011). The technology is set up for the collection of data from eleven two-hour intervals. Consequently, a maximum of 22 hours can be recorded, whereupon the first interval is overwritten and the results of data are lost if they are not downloaded in time. Data are infrared transferred and downloaded either by antennas, positioned at highly frequented positions within the barn, such as above the water trough or in the milking parlour, or by a handheld reader to a receiver and transmitted by wire connection to a computer (Lindgren, 2009; Schirmann et al., 2009; Burfeind et al., 2011). The battery lifetime is three to four years (VocalTag, 2008). RT is analysed by an algorithm which considers rumination events if successive regurgitations were separated. The different automatic analysis software program generates graphs which reflect the rumination activity during the course of a day of each documented cow.

RumiWatchSystem. The RW system is a pressure transducer for recording feeding behaviour of dairy cows. The technique consists of a halter with a noseband sensor comprising a vegetable oil-filled silicon tube with a built-in pressure sensor, a data logger, power supply, and the corresponding evaluation software (Itin + Hoch GmbH, Liestal, Switzerland). The data logger registers the pressure during chewing and ruminating at a frequency of 10 Hz, saves the raw data to an SD Memory Card and stores them for up to four months. Under laboratory conditions the battery lifetime is up to three years. Data are transmitted wirelessly or via an SD Memory Card to a computer (Zehner et al., 2012). For automatic measurement, a generic algorithm without animal specific learning data divides individual jaw movements of different amplitudes and chewing bout pauses into ruminating, eating, drinking or other activities (Zehner et al., 2012).

DairyCheck. The DC system is a sensor-based system for monitoring rumination and chewing behaviour of dairy cows by electromyography (Büchel and Sundrum, 2013a, unpublished data). The system comprises a measurement halter with two incorporated electrodes, a data logger, a power supply, and evaluation software (BITSz engineering GmbH, Zwickau, Germany). The myo-electrodes are closely attached to the skin of the cow for measuring electrical impulses of the M. masseter. They are connected to a data logger, which registers the electrical impulses with a resolution of 600 measuring points per minute and saves them to a mobile central data processing unit for up to 11 hours, after which the first recorded minute is overwritten. If the connection between the data logger and the computer is disconnected, data can be saved for up to 11 hours. Direct data transmission takes place via bi-directional radio transmission at a frequency of 2.4 GHz. Thereby, live observation and constant monitoring of feeding behaviour in real time is possible. The system is powered by a rechargeable 3.7 V, 2.7 Ah lithium-ion battery, which allows up to three weeks of uninterrupted recordings. Data are evaluated in terms of graphs. Data analysis is based on an algorithm with animal specific values. Differentiation of generated data into active feeding and rumination phases and into non-active dormant phases is possible. Since the analysis software for achieving an automatic classification of feeding behaviour is still under development, these data are processed manually by the researchers themselves.

Study Design

The study was carried out to test different measurement systems for measuring RT of dairy cows. The experiment was divided into two trials. They were conducted on a commercial dairy farm under practical conditions in April 2013. A total of nine randomly selected lactating Holstein Friesians were used: four primiparous (mean milk performance of 30.0 ± 2.6 kg) and five multiparous cows (mean milk performance of 42.2 ± 4.8 kg). The cows were kept in free-stall barns with a free cow traffic routine that meant that all parts of the stable areas, such as the cubicles, the feed alley, and the automatic milking system could be adjusted in every situation at any time. The dairy cows were fed a total mixed ration once a day (at approximately 1800 h) comprising 42.1% grass silage, 17.0% corn silage, and 40.9% concentrate and mineral mix on a

basis of 44.5% dry matter. Feed was pushed up at 0730, 1000, 1400 and 2230 h. The study consisted of two weeks during which the RT was recorded. In trial 1, nine cows were used. The RT of each cow was recorded by both the DC system and the HR sensor simultaneously over a time period of six days. Trial 2 used the same nine cows. The RT of each cow was recorded by both the RW system and the HR sensor simultaneously over six days, too. The DC system and the RW system were integrated into a halter; the HR sensor was incorporated into a head collar, which enabled the comparison of this combination. With regard to RT, a direct comparison of both halter-fitted systems was not practicable because it was not possible to guarantee that the functionality of one halter would not be influenced by the other halter.

Statistical Analysis

Statistical data analysis was achieved with the SPSS program (Version 20.0.0, IBM Company Inc., USA). The program calculated values for the Cohen's kappa coefficient (Cohen, 1960) and the asymptotic standard deviation and Pearson's correlation coefficient (Pearson, 1920), together with the coefficient of determination. Data were summarized for all nine measured cows and for all of the experimental days. The kappa coefficient was used because it reveals agreement for nominal scales and assumes that the events are independent (Alexopoulos, et al., 1988; Viera et al., 2005). The Mann Whitney U test was used to determine whether two sampled groups were from a single population with no specific distribution (Wilcoxon, 1945; Mann and Whitney, 1947).

RESULTS

The DC system provided estimates of RT that were very different to those detected by the HR sensor. Moreover, the RW system delivered estimates of RT that were dissimilar to those measured by the HR sensor. Rumination activity per day for all nine cows ranged from 410 to 666 minutes with a mean of 530 ± 60 minutes for the DC system, from 382 to 643 minutes with a mean of 546 ± 54 minutes for the RW system and from 75 to 635 minutes with a mean of 413 ± 148 minutes for the HR sensor. Base data of rumination activity recorded by the three different systems were summarized

for each cow and for each day of the study and resulted in n = 51 data sets for trial 1 and in n = 54 for trial 2. Mean deviation of recorded RT in trial 1 was -131 minutes when comparing the DC to the HR. In trial 2, mean deviation of recorded RT was -133 minutes when comparing RW to HR. Discrepancies of measuring RT were distinctly underestimated by the HR sensor in comparison to both of the other systems. Mean and standard deviation of recorded RT for each of the nine cows measured for both trials is given in Table 2.

For trial 1, correlation coefficients (r) and coefficients of determination (R2) between rumination times measured with the DC system and with the HR sensor were low ($r=0.30$, $R2=0.09$, $n=14$, $P<0.05$). For trial 2, correlation coefficients and coefficients of determination between rumination times measured with the RW system and with the HR sensor were also low ($r=0.14$, $R2=0.02$, $P<0.10$). Values of the Cohen's kappa coefficient (κ) and asymptotic standard deviation (σ) were correspondingly very low for trial 1 ($\kappa=-0.001$, $\sigma=0.001$, $P<0.10$) and for trial 2 ($\kappa=-0.004$, $\sigma= 0.002$, $P<0.10$).

Within the two trials, no significant differences in individual rumination activity and no significant effect between cows wearing halters or wearing the head collar were detected. The Mann-Whitney U test confirmed that the rumination times measured in trial 1 in the first week by the DC halter and the HR head collar were equal with respect to trial 2 in the second experimental week, in which the cows wore the RW halter and the HR head collar. Asymptotic significance was 0.401 by comparing RT measured by both halters, each used in one trial. By comparing RT measured by the HR head collar in both trials, asymptotic significance was 0.508. Thus, generated data were similar for the same cows over the two weeks in which both halter-related systems (DC, RW) were worn consecutively, and the neck collar system (HR) was worn continuously.

Table 2 Mean and standard deviation of recorded rumination time in minutes per cow when comparing the DC system to the HR sensor (trial 1) and the RW system to the HR sensor (trial 2).

Cow	DairyCheck	Lely Qwes HR	RumiWatch	LelyQwes HR
	trial 1		trial 2	
1	471 ± 32	413 ± 53	567 ± 26	469 ± 47
2	499 ± 31	132 ± 43	555 ± 33	181 ± 39
3	461 ± 48	223 ± 38	541 ± 27	218 ± 18
4	572 ± 38	367 ± 73	568 ± 63	545 ± 29
5	590 ± 65	453 ± 31	538 ± 83	475 ± 34
6	511 ± 32	524 ± 51	537 ± 39	539 ± 39
7	529 ±27	501 ± 15	555 ± 47	517 ± 36
8	546 ± 43	198 ± 59	533 ± 63	264 ± 91
9	561 ± 45	590 ± 51	522 ± 57	506 ± 76
Total mean[1]	530 ± 60	399 ± 148	546 ± 54	413 ± 148
Mean. dev.[2]	-131		-133	

[1] **Mean and standard deviation of all data recorded for all cows for each comparison.**

[2] **Mean deviation between DC and HR, and between RW and HR.**

DISCUSSION

The data presented represent the results of a comparative study of three different measurement systems. Due to technical reasons, it was not possible to assess feeding behavior simultaneously by visual observation, which is generally accepted as the gold standard for validating the classification of feeding behavior into feeding, ruminating or idling (Büchel and Sundrum, 2013b, unpublished data). Moreover, recording feeding behavior during a whole day over several consecutive days is not possible using this method. However, every single one of the three measurement systems has been independently validated through visual observance. The HR sensor was validated by external scientists in several trials under various conditions (Lindgren, 2009; Schirmann et al., 2009; Burfeind et al., 2011). The other two systems were validated by the developing scientists themselves (Nydegger et al., 2011; Zehner et al., 2012; Büchel and Sundrum, 2013a, unpublished data). All previous validation trials were realized under different practical conditions, varying also in sample sizes and lengths of monitoring duration. The current trials were accomplished under equal practical

conditions for all three systems, enabling a direct comparison, instead of a comparison of different validation results.

The results of this study demonstrate that the recorded RT varied significantly between the HR sensor and the DC system within trial 1 and between the HR sensor and the RW system in trial 2. On average, RT during one day was 131 and 133 minutes lower than that detected by the HR sensor (Table 2). RT was therefore obviously underestimated by the HR sensor. The underestimation of RT was similar to those results obtained by Pahl et al. (2012). They compared the ART-MSR pressure transducer, which was the preceding model of the RW system (Nydegger et al., 2011), to RuminAct™, which also includes the tag of the HR sensor. RT determined by this RuminAct™ sensor was underestimated compared to direct observation and correlated only moderately with the ART-MSR pressure transducer ($r=0.58$, $p<0.01$, $n=527$). Thus, the HR sensor does not fulfil the demands of precision and reliability required during measurements. Technical deficiencies of the HR sensor must be further improved so that the significance of results of the automatic analysis software can be trusted. In combination with other parameters which can be recorded by this device (Lely, 2013; SCR, 2013), the HR sensor seems to be more suitable for practical use. Its restrictions in terms of the valid and suitable recording of RT are compensated by measurements of physical activity or lying time and other measurement parameters. When more information is achievable, better concrete statements about the condition of individual animals can be made. This additional information provides suitable opportunities for successful usage in practical trials, which have been reported by Lindgren (2009) and Bar and Solomon (2010). But due to the lack of precise measurement results generated within this study, the HR sensor is only of limited use for research trials and casts doubt on the significance of RT data already generated during practical trials.

Data recorded by the DC system are comparable to those detected by the RW system, which is confirmed by the asymptotic significances of the Mann Whitney U test. In the represented trials a comparison between the halter-fitted systems, DC and RW, to HR, which is fitted to a neck collar, were viable. The reason for the considerable deviation of the HR sensor to both of the other systems might be due to the positioning of the

neck collar with its incorporated touch-sensitive microphone. Considering that cows are different sizes, weights and shapes, it was not possible to maintain the correct position of the neck collar for every cow. That's why the individual cow had a crucial influence on the absolute difference of RT of the HR sensor compared to the other research systems (Pahl et al., 2012). Burfeind et al. (2011) also stated the significance of the exact positioning of the neck collar and the data logger fixed behind the left ear, which should be ensured by a counterweight, because incorrect positioning of the microphone might impair the recording of rumination activity. Ungar and Rutter (2006) recognized the problem that the microphone attached to one cow might pick up grazing sounds from another cow. During this study an increase of measured rumination minutes could not be observed by the HR sensor in comparison to the DC system and the RW system. Burfeind et al. (2011) highlighted the slight resolution of two minutes within one two-hour interval, which can affect precise recordings and might be another explanation for the significant deviation. A further disadvantage could be the indirect measurement of the neck collar by acoustic biotelemetry of the HR sensor (Soriani et al., 2012), instead of direct measurement using halters with the other two systems. Direct measurement of rumination activity by electromyography and by a built-in pressure sensor, which are both attached closely to the area of jaw movements to be analysed, offers the possibility of more exact recordings and reduces the amount of disturbing external influences. This advantage is reflected in the results presented. On the other hand, using a neck collar is an advantage because many dairy farmers make use of neck collars for identifying their cows with fixed number tags or for identifying them by feeding stations. Thus, unlike with halters, the use of neck collars requires no changeover for the cows.

However, both halter-fitted systems are not free from certain problems. During this study, the application of the RW halter caused injuries to the area of the muzzle of some cows. The material used for the halter should be improved to ensure animal health and welfare. Two crucial disadvantages of the DC system are the missing automatic analysis software, which is still under development, and the short battery lifetime compared to both of the other systems.

CONCLUSION

It is apparent that the DC system and the RW system are useful tools for the recording of RT of dairy cows. Rumination time recorded by the tag of the HR sensor is of limited use for measuring reliable and convincing data due to restricted accuracy and reproducibility. This study has demonstrated that the DC system and the RW system are clearly more consistent with regard to measurement of rumination behaviour than the HR sensor. Their measured data are reliable and usable for the surveillance of feeding behaviour. However, to achieve most of the demands required for reliable usage in practice, further research and development of all three systems is necessary. Thus, the implementation of analysis software for automatic data interpretation should be one of the next steps of the DC system. To increase the comfort of the RW halter for the cows, the material used should be adapted. For the HR sensor, the major challenge is to ensure optimal positioning of the acoustic tag on the neck collar.

The recent development of three new measurement systems provides evidence that the recording of rumination time is a particularly suitable method for monitoring the feeding behaviour of dairy cows. Each of the systems has different drawbacks which should be overcome, thus enabling them to develop into appropriate and helpful management tools in the field of Precision Dairy Farming.

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CHAPTER 1 Serum interleukin-6 levels in transition dairy cows with subclinical ketosis

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SUMMARY

This research communication aimed to describe the concentrations of pro-inflammatory interleukin-6 (IL-6) in subclinical ketotic and non-ketotic fresh lactating dairy cows. The question whether ketotic dairy cows show high levels of IL-6 compared to non-affected cows was based on already reported relationships between metabolic disorders and inflammatory mediators. Blood samples from 24 diseased, recovered and healthy dairy cows was available and revealed differences between the groups but these results proved contrary to what had been expected. Non-ketotic dairy

cows (recovered as well as the control group) showed the highest IL-6 concentration compared to cows suffering subclinical ketosis in this study. Nonetheless, the general IL-6 level for all probes was on a low level averaging 27.2 pg/ml ± 10.2 and within a close range. The nonappearance of a higher degree of pro-inflammatory mediators in cows suffering subclinical ketosis as well as poor relationships between IL-6 and serum BHBA (β-hydroxybutyrate), NEFA (non-esterified fatty acids), and total Bilirubin suggest that IL-6 concentration is a poor indicator to indicate possible inflammatory conditions during subclinical ketosis in an early stage of lactation.

Keywords: interleukin 6, subclinical ketosis, transition cows, inflammation

INTRODUCTION

The transition period of dairy cows is characterized in particular through increased risk for diseases in different stages. Many dairy cows in the transition period are overstressed in their capacity to adapt to nutritional challenges and are thus linked to an increased risk for health issues (Sundrum, 2015). With respect to this, the role of inflammatory pathways during transition period of dairy cows was recently the topic of several studies but is still not fully understood (Trevisi et al. 2012; Kasimanickam et al. 2013; Sordillo and Raphael 2013). In a comprehensive review, Bradford et al. (2015) highlighted the relevance of inflammation in newly lactating dairy cows and stated that almost every fresh cow suffers from some degree of systemic inflammation. They distinguished between the classical acute inflammation and a sub-acute form, which causes mild increases in inflammatory mediators that contribute to chronic low-grade systemic inflammation. These inflammatory (dysfunctional) responses have been proposed as a missing link in the pathology of metabolic disorders around calving (Sordillo and Raphael, 2013). Additionally, a relationship between a negative energy balance and an upregulation of inflammatory factors has been reported (Loor et al. 2007; Kasimanickam et al. 2013; Esposito et al. 2014). In this context, it is worth highlighting the pro-inflammatory cytokine IL-6, which is able to promote lipolysis or

impaired insulin sensitivity (García-Escobar et al. 2010; Mukesh et al. 2010; Saremi 2013). In a study investigating metabolic and signaling gene networks in the liver, IL-6 was highlighted as playing a major role during nutrition-induced clinical ketosis and therefore in general liver function (Loor et al., 2007). The purpose of the current study was to investigate whether differences in IL-6 levels in subclinical ketotic and non-ketotic early lactating dairy cows exist and to gain greater insights into the role of pro-inflammatory mediators in an early stage of ketosis.

MATERIALS AND METHODS

The study was conducted on a commercial farm with a herd size of 140 dairy cows in Lower Saxony, Germany and complied with the guidelines prescribed by German animal welfare legislation. Data for this investigation originated from 24 cows (primiparous and multiparous) including 14 ketotic cows (diseased and recovered) and 10 non-ketotic control cows, with an average milk yield of 9520 kg/a. All dairy cows were housed in a tie stall barn for the first 7 days postpartum and were milked twice a day. Beginning on day 8 postpartum, dairy cows were kept in a free stall with cubicles and were milked using an automatic milking system (AMS). A partial TMR was offered twice a day with additional concentrate given manually as well as automatically during milking using the AMS. In order to identify diseased and healthy (control) dairy cows, every dairy cow in the herd was monitored at 2-day intervals during the first 3 weeks postpartum using an electronic β-hydroxybutyrate (BHBA) hand-held meter (Iwersen et al. 2009). Dairy cows were considered to have subclinical ketosis if they had serum BHBA concentrations ≥1.0 mmol/L, while BHBA concentrations <1.0 mmol/L indicated a healthy status. After this pre-selection, a clinical observation was conducted according to a protocol for examination of fresh cows including the testing parameters posture, rectal temperature, vaginal discharge, rumen fill and milk secretion (Bijmholt 2013). All conspicuous animals were excluded from the study. Additionally, the described procedure was conducted daily on every selected cow during the trial to detect any clinical disease also occuring during the investigation. Blood samples of the cows were collected from the vena caudalis mediana in the morning and harvested in a plastic serum tube (S-Monovette, Sarstedt,

Germany). Clotted samples were centrifuged; supernatants were aliquoted and stored at -80°C until analysis. Besides BHBA (used for evaluation of health status, also determined in laboratory to confirm the results of the onfarm device), additional parameters were analyzed to describe the extent of the energy shortage in detail: non-esterified fatty acids (NEFA) and total bilirubin (TBIL). The assays were performed according to standardized procedures using an analyzer (Konelab, Thermo Fisher Scientific) and commercial kits. For IL-6 determination, the highly sensitive ELISA kit for IL-6 (catalogue number HEA079Bo, Wuhan USCN Business Co., Ltd.) with an intra-assay CV of <10% and inter-assay CV of <12% was used. Plate preparation and assay procedure were performed in accordance with the manufacturer´s instructions. Immediately afterwards the optical density was determined using the microtiter reader (SLT Spectra, Tecan) at 450 mm. Statistical analysis and calculations were conducted using either the statistical program SPSS for Windows (Version 21.0) or the calculation program Excel for Windows (Microsoft Office 2010). In all calculations, differences were considered to be significant at $p \leq 0.05$.

Based BHBA levels, dairy cows were divided into a ketotic group with the two subsets "ketotic" and "recovered" and the non-ketotic group (control). Observed values of all tested parameters were tested for normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variance using Levene´s test. The mean value including SD of all tested parameters was computed for each group. Calculations of variations between the groups were conducted using Friedman´s non-parametric one-way repeated measures analysis of variance by ranks and post hoc pairwise analysis (Wilcoxon test). Pearson correlations were calculated for the interrelation between BHBA, NEFA, TBIL and IL-6.

RESULTS AND DISCUSSION

The present study includes IL-6 values as well as data from other serum parameters characterizing the energetic metabolic status of the 24 dairy cows. The characteristic energy shortage in ketotic animals has been illustrated by an increase of serum BHBA, NEFA and TBIL. Clinical signs of ketosis or other diseases were not observed in any of the investigated animals. BHBA concentration levels in diseased animals differed

significantly from non-diseased cows (1.38 ± 0.27 mmol/l *versus* 0.51 ± 0.13 mmol/l). Thus all were considered to be subclinical. NEFA and TBIL were also increased in diseased animals compared to the recovered and the control group. There were significant correlations between all three parameters, confirming the close relationship they have with one another (Table 3). Overall, the findings concerning energy status are in line with the observed severity grade of SCK. IL-6 levels of dairy cows during SCK and after recovery as well as a control group were compared with each other and revealed differences between the groups (Table 3). In general, the serum concentrations of IL-6 were on a low level of 7.9 pg/ml up to 57.9 pg/ml compared to other studies (Trevisi et al., 2012; Kasimanickam et al., 2013). Recovered dairy cows showed higher IL-6 levels than in SCK (p=0.019). While diseased cows had an average level of 22.46 pg/ml ± 6.79, recovered cows had an IL-6 concentration of 28.18 pg/ml ± 7.5. The control group, consisting of 10 consistently healthy dairy cows, surprisingly showed the highest average IL-6 concentration but also with a two-fold higher standard deviation compared to the SCK group (32.43 pg/ml ± 14.4). As previously mentioned, all investigated animals were clinically checked before and during the trial to exclude obvious diseases. Data comparison with the values of SCK cows revealed the same tendency as the comparison with the recovered dairy cows (p=0.042). This fact supports the previous observation in the SCK group that cows suffering from an abnormal energy deficit have lower IL-6 levels than non-affected cows. In addition, no significant differences between recovered dairy cows and completely healthy (control) cows were observed (p=0.23). A negative Pearson correlation between the parameters BHBA, NEFA and TBIL to IL-6 were on a low level and not significant. The results regarding IL-6 contrasted with our assumption that dairy cows suffering ketosis have increased serum IL-6 levels. In the study carried out by Loor et al. (2007), a three-fold increased gene expression of IL-6 mRNA during a nutrition-induced clinical ketosis in the liver was observed. It is to be noted that in our study IL-6 serum levels instead of mRNA in the liver were examined. Furthermore, in the present study focused on subclinical cases so it was not possible to detect a possible increase of serum IL-6. However, our findings allow us to assume that potential changes in IL-6 occur only locally and are not reflected in blood. In this

context, Saremi (2013) detected a decrease of IL-6 in adipose tissue while at the same time IL-6 was increased in the liver.

Table 3 Serum concentrations of IL-6, BHBA, NEFA and TBIL, as well as the Pearson correlations between the investigated parameters.

Blood Parameter	Ketosis	Recovered	Control	Correlations Pearson R			
	Mean±SD	Mean±SD	Mean±SD	IL-6	BHBA	NEFA	TBIL
IL-6 (pg/ml)	22.46±6.8	28.18±7.5	32.43±14.4	1	-0.3	-0.15	-0.29
BHBA (mmol/l)	1.38±0.27	0.62±0.16	0.51±0.13		1	0.55**	0.55**
NEFA (mmol/l)	0.77±0.23	0.49±0.35	0.41±0.19			1	0.64**
TBIL (mg/dl)	0.4±0.22	0.22±0.08	0.2±0.05				1

**** indicate significant differences with $p = 0.05$**

Again, with regard to the observed results, it is worth highlighting that all samples values did not differ profoundly, although the trend showing that healthy animals have higher IL-6 levels in serum is still interesting. Previously reported low-grade inflammation in healthy fresh lactation dairy cows fit with our observations (Bradford et al. 2015) and thus the question arises if higher pro-inflammatory factors could be helpful in preventing diseases in periparturient phase. Nevertheless, there is evidence that inflammation interferes with normal metabolism of dairy cows and leads to an increased risk of disease (Galvão et al. 2012; Trevisi et al. 2012; Bradford et al. 2015). In conclusion, this is the first study reporting serum IL-6 concentrations in dairy cows suffering SCK to have shown that the meaningfulness of the parameter during SCK is apparently limited. However, the unexpected low IL-6 levels in ketotic cows compared to the healthy group require further investigation, first and foremost to ascertain if this trend can be confirmed in dairy cows suffering from clinical ketosis.

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CHAPTER 2 *Additional Results:* Detection of Subclinical Ketosis with Measurement of Rumination Activity

BRIEF SUMMARY

Subclinical ketosis (SCK) is one of the most common diseases in the early stage of lactation and leads to economic losses due both to the disease itself as well as sequelae. Efforts aimed at finding new practical ways of detecting SCK in time in commercial herds seem to be beneficial. In the following section, additional results from the study described in Chapter 1 are presented. Rumination activity was measured continuously in order to investigate whether rumination activity is affected by disturbances in energy metabolism before clinical signs occur and if it can be used to detect SCK. In total, 25 animals with different health status (diseased, recovered and healthy) were monitored to address these questions. The daily average rumination time differed only slightly between the groups at 475 min/d ± 56 (SCK cows), 497 min/d ± 48 (recovered) and 521 min/d ± 76 in the healthy control group respectively. A huge variability in values across the groups impedes a clear discrimination between the different health statuses in this small test herd. Nevertheless, the tendency that this metabolic disease affects rumination activity is observed in the current study.

MATERIALS AND METHODS

The study was conducted on a commercial farm in Lower Saxony, Germany with 150 dairy cows. The animals, housing conditions and the selection procedure for ketotic and non-ketotic control cows were previously described in detail in Chapter 1. In order to investigate the impact of subclinical ketosis on daily rumination time (RT), 15 diseased ketotic transition dairy cows between 3 DIM and 21 DIM were selected as well as a group of 10 healthy control cows of the same lactation status. An electromyography-based (EMG) halter device with electrodes positioned at the M.

masseter was used to record characteristic muscle contractions during rumination. While the RT of healthy control cows was measured once a day for at least 5 days, the RT of ketotic cows was determined twice a day for 5 days respectively during ketosis and after recovery. The data was edited and analysed using the statistical program SPSS for Windows (Version 21.0) or the calculation program Excel for Windows (Microsoft Office 2010). Observed values were tested for normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variance using Levene´s test. Values were averaged on a daily basis for each dairy cow. Variation in rumination activity between diseased and recovered dairy cows was conducted using the Wilcoxon test, differences between dairy cows suffering SCK and the control group was tested using Man-Whitney U-Test. Pearson correlations between rumination time and the level of BHBA were also investigated.

RESULTS AND DISCUSSION

Continuous measurement of rumination activity is already established in practice. Although commercial devices are available, supporting studies investigating the importance of rumination activity level and influences of this parameter are still needed. In addition to the usefulness of rumination activity measurement in terms of oestrus detection and prediction of calving (Reith und Hoy 2012; Schirmann et al. 2013; Pahl et al. 2014), several studies have also proven a relationship between general health status and rumination activity (Soriani et al. 2012; Soriani et al. 2013). This part of the study describes the determination of rumination activity in ketotic and non-ketotic fresh lactating dairy cows to test if SCK has an impact on rumination behaviour. In total, 90 fresh cows were examined, while 24 dairy cows were classified as ketotic due to increased BHBA levels of ≥1.0 mmol/l, which represents a prevalence of 27% in the test herd. This rate is in line with other studies such as Suthar et al. (2013) and reflects a typical situation on commercial farms. Rumination activity recordings of 15 SCK cows and 10 healthy control cows were able to be used after data screening and analysed in terms of a possible impact of SCK. With a focus on the whole data, rumination activity varied between 371 min/d and 839 min/d in the test

herd. The observed values are on a comparable level to others; for instance, Soriani et al. (2012) reported a range of rumination times in fresh lactating cows of 400 min/d to 685 min/d. The group consisting of animals suffering from SCR showed an average rumination time of 475 min/d ± 56 while after recovery the average rumination time was 497 min/d ± 48. A comparison between rumination activity of diseased cows and the recovered cows revealed no significant differences. Also a comparison between ketotic cows (diseased and recovered) and the control group did not show any significant difference although the control group tends to have higher rumination times of 521 min/d ± 76. The rumination activity times of all investigated SCK cows is set out in Figure 1. As shown, the observed values across all dairy cows were in a close range and also showed high variability with coefficients of variation (CV) at 11.8% (diseased dairy cows), 9.6% (recovered) and 14.4% in the control group (not displayed in the figure). Under these circumstances, it was not possible to use rumination activity monitoring to differentiate between healthy and ketotic cows, particularly as the sample size was limited. The relationship between rumination activity and the serum BHBA revealed a negative correlation of -0.21, mainly caused by the values of the control group.

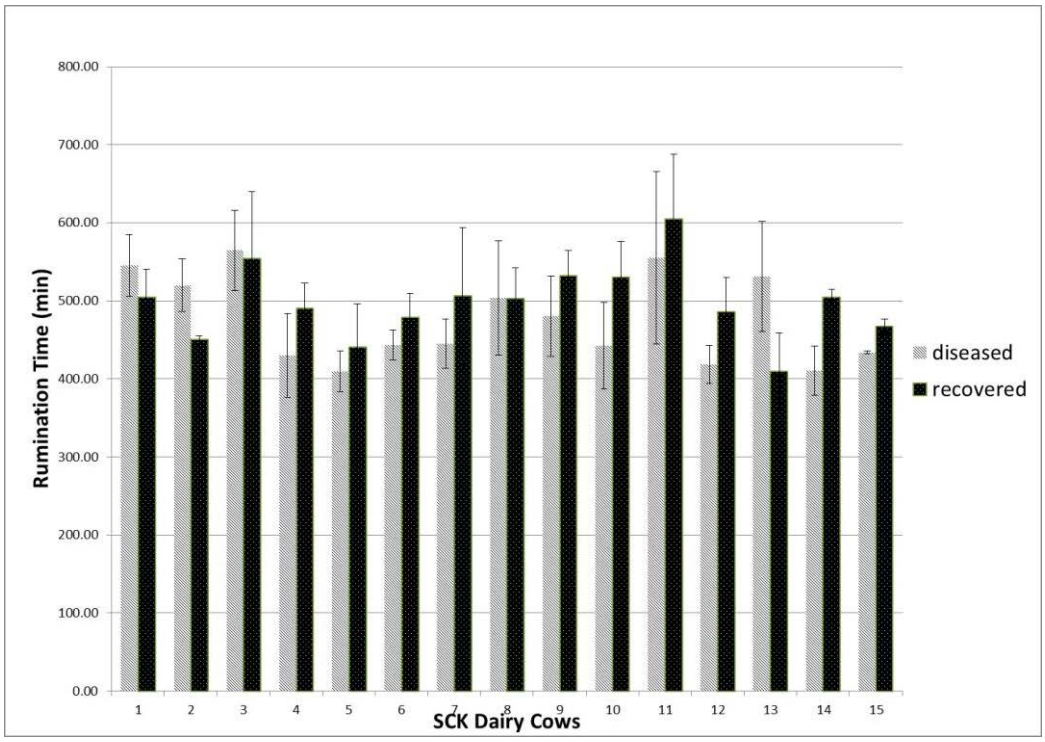

Figure 1 Averaged rumination time (min per day) of each investigated SCK dairy cow during the disease and after recovery.

The results indicate that the ability to use rumination activity for the purposes of disease detection was only limited although we detected similar tendencies to those revealed by other studies. In a study carried out by Kaufman (2015), average rumination time of cows with SCK is 25 min/d less; for cows suffering from an additional disease rumination time was shown to be 44 min/d less than for healthy ones. These results are on the same level as our own but a higher sample size (n=339) was used. Interestingly, the author did not provide a sensitivity and specificity rate in order to detect SCR in the field with rumination time. The development of SKC as result of an enduring lack of energy supply, which is often caused by impaired feed intake, is less closely associated with rumination activity than expected. There is evidence that rumination activity is affected by the particle size of the diet (Beauchemin und Yang 2005). In our study, all animals received the same diet; however, there is no information available about individual feed intake because the study was conducted in practice without the opportunity to record this. On the other hand, according to studies conducted by Schirmann et al. (2012) and Clément et al.

(2014), dry matter intake and rumination time are not closely related to each other. Individual variability also has a marked impact on this correlation. Again, it also becomes apparent in this study that the individuality of the cows plays a major role in rumination behaviour. The general ability to cope with external influences such as health status but also rations, social circumstances, heat stress and other triggers varies between the animals, as is comprehensively described by Sundrum (2015). The approach to finding absolute reference values that indicate abnormalities seem to be the wrong way to use rumination activity as an indicator for health issues. It is indeed more the variation in individual behaviour itself, which can be used as an approach for detecting health issues. In this context, we also tried to find differences based on one dairy cow absolute value but did not take the daily rumination pattern into account. There is reason to assume that it is possibly daily rumination behaviour that changes rather than the absolute rumination time. New technologies enable such aspects to be investigated. It is worth noting that consideration of the daily pattern of rumination activity can only be beneficial for commercial farms with numerous dairy cows worldwide if decision-supporting tools are developed and made accessible for commercial farms.

In conclusion, the presented studies showed that SCK might only be one influencing factor for rumination activity, which limits the usefulness of rumination activity in terms of SCK detection. Nevertheless, like the authors of other studies, we recommend also including individual rumination behaviour as a factor that should be routinely observed as part of dairy cow health monitoring and herd management.

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CHAPTER 3 Detection of SARA in the Transition Period of Dairy Cows by a Wireless Indwelling Technique

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SUMMARY

Recently conducted scientific studies suggest a general increase in the occurrence of sub-acute rumen acidosis (SARA) in dairy cows. However, the detection of SARA in the field is still facing various difficulties, preventing a timely and valid diagnosis in practice. The standard method for SARA diagnosis still consists of taking single measurements of the ruminal pH-value, mainly by rumenocentesis. However, results do not typically cover the variation in pH-values over time. A newly developed and commercially available technique for long-term pH measurements using a wireless indwelling system seems an appropriate way of overcoming this issue in the detection of SARA. A study was conducted to compare values obtained by the new technique with the standard method in a SARA affected commercial dairy herd in the transition

period. Milk yields and milk ingredients were also assessed, along with the chewing activity of the dairy cows. Results for the SARA detection, based on single pH-values, revealed a sensitivity-specificity ratio of 61% and 97% after morning feeding and 79% and 93% after evening feeding, respectively. The sensitivity and specificity analysis of the indicators (milk yield, protein and fat content, fat:protein ratio, feeding and rumination time, and chews per bolus) showed a comparably poor accuracy in relation to SARA cases. It is concluded that SARA diagnosis based on wireless indwelling pH-measurements is a reliable method for the detection in the field, while standard methods for rumen fluid analysis based on single pH-values, as well as long-term measurements of milk parameter and chewing behavior, provide only insufficient information with respect to SARA detection.

Keywords sub-acute rumen acidosis, reticuloruminal pH, milk records, chewing behavior, diagnostics

INTRODUCTION

The transition period, defined as the period encompassing the three weeks before to three weeks after parturition (Drackley, 1999), is the most challenging and critical period in a dairy cow's life. The risk of developing sub-acute rumen acidosis (SARA) is particularly increased due to physiological and dietary changes within a short period of time (Goff and Horst, 1997; Block, 2010). High dietary amounts of rapidly fermentable carbohydrates, combined with a reduced absorption capacity, can lead to an accumulation of organic acids in the rumen, causing SARA in the early lactation stage (Krause and Oetzel, 2006; Kleen and Cannizzo, 2012).

The occurrence of SARA is often accompanied by further disturbances like reduced feed intake, decreased milk yield, low fat:protein ratio in the milk and low milk fat content. Secondary diseases, such as diarrhea, liver abscesses, rumenitis, and lameness/laminitis, may also occur (Kleen et al., 2003; Enemark, 2009; Plaizier et al., 2009). While numerous reviews have described such symptoms, the diagnosis of the

disease in farm practice is complicated by the fact that SARA often creates diverse and contrary symptom pictures among the dairy cows (Mulligan et al., 2006).

Analysis of rumen fluid has been generally accepted as the gold standard for the provision of direct information on conditions of the rumen (Kleen et al., 2003). Rumen fluid can either be collected by spot sampling methods like stomach tubing and rumen puncture or by using indwelling systems (Tajik and Nazafi, 2011). While the employment of indwelling systems is not yet established in practice, rumenocentesis is the most popular method for rumen fluid collection in the field, and is comprehensively described by Garrett et al. (1999). Because this method can only be conducted by veterinarians, rumen fluid collection is often limited to clinical cases with a tentative SARA diagnosis. A severe disadvantage of spot sampling methods like rumen puncture and stomach tubing is the limited applicability of single values as they don´t cover the diurnal variation. In contrast, a recently developed wireless indwelling system enables the display of the diurnal daily pH pattern. While such devices are costly, they can be used in many animals during and before the occurrence of clinical symptoms without consulting the vet. In the past, the definition of SARA was based on a single pH-value falling below a threshold, which varied between 5.5 and 5.8 in several studies (Plaizier et al., 2009). Current definitions also focus on the duration of the dip in pH, e.g. <pH 5.6 for 3 h/day (Gozho et al., 2005) or 310 min/day below pH 5.8 (Zebeli et al., 2008).

It is not just absence of diurnal variation and thus limited potential for application which bring the rumenocentesis technique into question, its possible impacts on animal welfare and productivity also bring this method into doubt (Kamphues, 2009). While several authors reported the ease use of the rumenocentesis technique, others highlighted abscess formation and hematomas on the puncture side as well as dips in milk yield (Enemark, 2009; Mialon et al., 2012). Recent developments of wireless, orally-administered indwelling systems enable continuous determination of the reticuloruminal pH under commercial conditions (Mottram et al., 2008; Gasteiner et al., 2012; Sato et al., 2012). They facilitate continuous measurements and allow the diurnal pH variation in the rumen to be recorded, contrary to spot sampling measurements (Gozho et al., 2005; Zebeli et al., 2008; AlZahal et al., 2009; Khiaosa-

ard and Zebeli, 2014). Most of the sensors are located in the reticulum as it is a part of the reticulorumen. pH-values differ slightly between the different areas of the bovine reticulorumen but are reported to have highly positive correlation values (Bryant, 1964; Duffield et al., 2004), indicating that measuring with indwelling systems is consistent with reticuloruminal pH conditions. Mottram (2014) determined an average pH level in the reticulum of approximately 0.25 pH units above the ventral sac.

There is a growing body of research stating that changes in animal behavior can be attributed to illness (Sowell et al., 1999; Weary et al., 2009). The rumination time was recognized by Soriani et al. (2012) as a good way of obtaining information on health status in the transition phase, especially in relation to metabolic disorders. Given that SARA might also affect the feeding and rumination time; there is reason to assume that chewing behaviour might be affected by SARA and that measuring this could be helpful for the detection of SARA (Kamphues, 2009; Tajik and Nazafi, 2011).

The aim of the study was to examine whether a wireless indwelling system, providing continuous data on reticuloruminal pH, as well as data on the feeding and rumination time, could improve and facilitate the diagnosis of SARA in the field and thus replace the established method of rumenocentesis.

MATERIALS AND METHODS

ANIMALS, MANAGEMENT AND DIETS

The study was conducted on a commercial farm with a herd size of 300 cows in the Free State of Thuringia/ Germany from August to November 2013. All procedures were approved by the Thuringia Office for Customer Protection and were in accordance with the guidelines from the German laws for animal welfare. In total, 12 primiparous and 12 multiparous (not more than three lactations) dairy cows were allocated to a study group. These were close-up cows (approx. 14 d ante partum), which were visually healthy and fed the same diet. The close-up cows were housed in a straw bedded pen, and moved to a free stall with cubicles after parturition. The cows

were milked twice a day (at 0500 and at 1600) in a rotary milking parlour. Herd milk yield averaged 10,022 kg/cow/year. The daily milk yield of each dairy cow was monitored during the trial with the measurement device provided with the milking equipment. In addition, milk samples were collected biweekly and sent to the Milk Control Station Thuringia for further analysis. A total mixed ration was offered once daily in the dry period (0900) and three times a day in lactation (0600, 1200, and 1600), respectively. The ingredients and composition of the diets are set out in Table 4 Ingredient composition and nutrient contents of the diets fed during the experiment (DM basis).. Diets were calculated based on a DMI of 11 kg in the dry period, an average DMI of 17 kg between d+1 and d+14 and an average DMI of 23 kg between d +15 and d +45. Daily reweighing of feed residuals during the trial confirmed the previously calculated DMI on herd level. It was not possible to implement an individual DMI recording on the commercial farm. An automatic feed pusher was used fifteen times in 24h to ensure continuous forage availability. Water was available ad libitum throughout the experiment.

Table 4 Ingredient composition and nutrient contents of the diets fed during the experiment (DM basis).

Ration composition, % of DM	Period[1] DP	LP
Forages		
Corn silage	39	44
Ryegrass haylage	14	10
Alfalfa hay		4
Barley straw	28	
Concentrates		
Barley	3	16
Rapeseed meal extraction	12	8
Rapeseed cake		6
Soy meal		4
Sugar beet pulp		5
Protein premix	2	
Mineral premix dry	2	
Mineral premix lactation		2
Rumen-stable fat		1
Nutrient content[2,3]		
DM, %	68.2	62.6
Chemical composition, g/kg of DM		
CP	121	156
NFC	271	349
NDF	461	399
Hemicellulose	209	191
Cellulose	216	174
ADF	252	208
ADL	35.9	34.3
Energy, MJ/kg		
NE_L	5.1	6.9

[1] Investigation periods: DP= contents of diet in dry period fed between d -15 to d 0; LP= contents of diet in lactation between d +1 to d +45

[2] Averaged values based on weekly conducted feed analysis; diet was offered as TMR.

[3] Analysis methods: modified Weender analysis and Van Soest´s detergent fibre method

MEASUREMENT OF RETICULORUMINAL PH, MILK PARAMETERS AND CHEWING BEHAVIOUR

Beginning two weeks before estimated calving, reticuloruminal pH was measured with an indwelling and wireless data transmitting system (smaXtec animal care sales GmbH, Graz, Austria). Measuring units were activated followed by calibration using a buffer solution provided by the manufacturer. The units were given to each cow orally, and expected to end up in the reticulum at least 24h after their administration (Gasteiner et al., 2012; Gasteiner et al., 2015). Reticuloruminal pH-values were recorded 144 times per day in 10 min intervals during the whole transition phase and were transmitted to an external receiver placed in the barn via radio. The receiver was connected to a computer for further data transmission and analysis.

Biweekly milk records comprised the milk yield per day (kg), milk fat percentage, milk protein percentage, and milk fat:protein ratio. The data, including the daily milk yields which were routinely measured by the device on the milking equipment, was assessed with the herd management software (HERDE®, dsp Agrosoft, Ketzin, Germany) and linked with the results of SARA detection for the corresponding test day. The average milk yield, milk fat percentage, milk protein percentage, and milk fat:protein ratio were calculated both for the whole herd and for all cows which exhibited SARA during the period of investigation.

Eating and rumination behaviour was recorded two times in early lactation for 6 d per cow respectively. The timeframes were: d +10 to d +25 and d +30 to d +45. A sensor-based system incorporating a vegetable oil filled noseband sensor, a data logger, a power supply and evaluation software (RumiWatch, Itin + Hoch GmbH, Liestal, Switzerland) was used. The noseband-sensor was located in the casing of a halter over the bridge of the cow's nose and was altered by the cow's jaw movement. The change in pressure on the noseband was thus recorded. The deflections were assigned via an integrated algorithm to the characteristic chewing activities of "rumination", "eating", and "drinking" or other activities. Furthermore, analysis enabled quantification of total rumination and eating time per day, number of ruminated boli per day, chews per bolus while ruminating as well as chews whilst eating on both an hourly and a daily basis.

Validation of the system revealed high correlations (r^2=0.95) between the measurement unit and visual observation (Selje-Aßmann et al., 2015). The data logger saved the raw data at a frequency of 10 Hz on a SD Memory Card for up to 4 months and transmitted the data wirelessly to a computer (Zehner et al., 2012).

SARA DEFINITION AND IDENTIFICATION

The definition of Zebeli et al. (2008) is employed for SARA detection based on continuous pH measurement. According to the definition, SARA could occur when the total daily duration of the ruminal pH-value was below pH 5.8 (TD5.8) for ≥310 min/d. This threshold-value is the result of the analysis of 17 published studies, which revealed that pH 5.8 is considered as the critical point to ensure adequate fibre digestion. This is due to suboptimal conditions for digestive microorganisms below this value. The same threshold-value was applied in the case of SARA detection and was analogous to spot sampling (Plaizier et al., 2009).

STATISTICAL ANALYSIS

Statistical analysis and calculations were either conducted using the statistical program SPSS for Windows (Version 21.0) or the calculation program Excel for Windows (Microsoft Office 2010). In all calculations, differences were considered to be significant at $p<0.05$.

Observed pH-values were tested for normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variance with Levene´s test. Values were averaged on both an hourly and a daily basis for each dairy cow. Data on SARA detection were calculated for each cow, based on either the long-term pH values or single pH values at a given time on a daily basis. For SARA detection with spot sampling, single 10 min interval pH-values, observed via the monitoring system in the reticulum, were used. The first pH data point in each hour (five to eight hours after feeding) was considered for the analysis. The prevalence of each SARA diagnosis (long-term and single pH) was calculated for d 5 antepartum as well as for d 5, d 10, d 20, d 30 and d 40 postpartum. The sensitivity and specificity of SARA detection

based on spot sampling values of pH was conducted, based on this data. In total, 7360 intervals/ spot sampling values were available over the recommended timeframe for rumenocentesis (Garrett et al., 1999). They were used to compute the proportion of true positive values, false positive values plus true and false negative values. Sensitivity, expressed as the ratio of true positives and the sum of true positives and false negatives (all animals with SARA), as well as the ratio of true negatives and the sum of true negatives and false positives (all animals without SARA), defined as specificity, were evaluated for the monitoring system.

Sensitivity and specificity of the milk recording data were evaluated using Receiver Operating Characteristic-Analysis (ROC-Analysis) using the statistical program SPSS for Windows (Version 21.0). The ROC curve is the true positive rate (sensitivity) as a function of the false positive rate (1-specificity) for the range of cut-off values being considered. The Youden´s Index was determined in order to assess the optimal cut-off value with the best sensitivity to specificity ratio, defined as the difference between the true positive rate and the false positive rate. The index enables the identification of an optimal cut-off point from the ROC curve, independently of the prevalence. As a quality parameter of the individual ROC curves, the area under the curve (AUC) was also calculated.

The chewing behaviour parameter set including rumination time per day, rumination chews per day, chews per bolus, chews per minute, eating time, and chews during eating was tested for normal distribution with the Kolmogorov- Smirnov test and for homogeneity of variance using Levene´s test. The archived data set was linked with the SARA detection results from the corresponding test day and reviewed with respect to the cows' health status. Furthermore, the sensitivity and specificity of all feeding behaviour parameters was calculated following the same procedure used for milk recording parameters using the ROC analysis.

RESULTS

In the current study, different methods for the detection of SARA under practical conditions were suggested, examined and compared with one another. The indwelling wireless data transmitting system provided a very comprehensive data set of pH-

values. The prevalence of SARA was computed and compared with the results obtained from spot sampling values (Table 5), based on long-term pH measurements from 23 dairy cows. Single pH-values were used either after morning or evening feeding, in line with the recommended time frame for sample collection (Garrett et al., 1999). It turned out that more cases were detected after evening feeding than after morning feeding. However, the differences were only moderate, except for test day 5 ante partum where no SARA detection occurred after the morning feeding whereas 14% was detected after evening feeding. In contrast, the long-term pH values revealed a much higher number of SARA cows in comparison to the spot sampling over the total length of the investigation. Overall, spot sampling revealed only 70% of the cases which were identified with the indwelling technique.

Table 5 Prevalence (%) of SARA based on single pH-values (spot sampling, SpotS) as well as the SARA prevalence based on long-term measurement of reticuloruminal pH (LTM)

Time	SpotS mor[1]	SpotS eve[1]	LTM[2]
d 5 antepartum	0	14	18
d 5 postpartum	41	49	55
d 10 postpartum	55	59	82
d 20 postpartum	34	39	50
d 30 postpartum	31	40	55
d 40 postpartum	36	47	64

[1] SARA detection was based on single pH-values after the feeding times in the morning (SpotS mor) and the evening (SpotS eve) (Garrett et al., 1999). The threshold of reticuloruminal pH was pH 5.8.

[2] Cut-off for SARA detection based on long-term measurements: TD5.8≥310min/d (Zebeli et al., 2008)

Based on these findings, sensitivity and specificity analysis were conducted to ascertain the diagnostic value of the SARA detection methods examined. Sensitivity and specificity were calculated based on the proportion of true and false positive values as well as true and false negative values, for every hour investigated. The five to eight hours after morning and evening feeding time were studied. Compared to the results from long-term measurements, the average sensitivity of spot sampling after morning feeding was 61%; the average specificity was 97%. Observed values were slightly higher after evening feeding with a mean sensitivity of 79% and a specificity of 93%. The sensitivity and specificity values for each hour are displayed at Table 6.

Table 6 Sensitivity and specificity of SARA detection based on single pH-values compared to the detection based on long-term measurements. SARA detection was based on spot sampling five to eight hours after feeding with a threshold of pH 5.8. [1]

Morning feeding[2]	Sensitivity, %	Specificity, %
5 h	63	97
6 h	60	96
7 h	60	98
8 h	62	98
MEAN morning feeding	**61**	**97**

Evening feeding[2]	Sensitivity, %	Specificity, %
5 h	71	90
6 h	85	85
7 h	81	85
8 h	77	92
MEAN evening feeding	**79**	**88**

[1]Cut-off for SARA detection based on long-term measurements: of Zebeli et al., 2008 (TD5.8≥310min/d)

[2]sampling after the feeding times

DO MILK RECORDING PARAMETERS HAVE AN INFORMATIVE VALUE FOR SARA DETECTION IN THE FIELD?

Apart from SARA detection based on single pH-values, the use of single milk recording data as an indirect way of alerting to the presence of SARA has been suggested. In the present study, milk data were also recorded. In the first 25 d of lactation, an average daily milk yield of 30.2 kg (SEM= 1.4), a fat percentage of 4.7% (0.2), protein content of 3.3% (0.1) and a fat:protein ratio of 1.5 (0.1) were determined throughout the whole herd. Dairy cows suffering from SARA during the full experimental period (all-time SARA), showed a below-average milk yield of 26 kg (1.4) in this timeframe. The sensitivity and specificity of the milk recording parameters milk yield, milk fat, milk protein and fat:protein ratio in relation to the occurrence of SARA were assessed in the study. The ROC analysis only revealed poor or insignificant sensitivity and specificity ratios (table 4).

DOES THE CONTINUOUS MEASUREMENT OF CHEWING BEHAVIOUR HELP TO DETECT SARA UNDER COMMERCIAL CONDITIONS?

While the continuous measurement of chewing activity was not possible in the past, new techniques have also been developed in this field, increasing the options for simplified data collection from individual animals. In this study, feeding behaviour parameters like eating time, chews during eating, ruminating time, chews per bolus, and chews per minute were measured and tested in terms of SARA detection. Calculations based on the data revealed the tendency that cows which were either affected by SARA throughout the study period or on many of the days, showed a higher ruminating time than cows unaffected by SARA (530 min vs. 490 min per day). SARA-cows showed the longest eating times (400 min per day) of the group. It was not easy to distinguish dairy cows both with SARA from those without based on the values recorded for chewing behaviour. The trends observed are reflected in the results of the ROC analysis conducted to calculate the sensitivity and specificity of chewing behaviour and relate this to the detection and presence of SARA. Youden Indices were calculated to detect the best cut-off value with the highest sensitivity to specificity ratio. As shown in table 4, the sensitivity was between 52% and 96%, whereas the specificity revealed values between 12% and 61%. The highest area under the ROC curve (AUC) was achieved for rumination time. The AUC shows how well a parameter can be used to distinguish between the two groups. While 0.5 represented the worst and 1.0 the best value, the AUC value for rumination time was 0.62 ($P<0.005$). Rumination time had a sensitivity and specificity of 65% and 58% respectively, with a cut-off value of 494 minutes. At the cut-off point of 250 minutes, eating time delivered a very high sensitivity of 96% but a low specificity of 12%. This led to an AUC value of 0.51. The remaining parameters characterizing chewing behaviour showed only poor sensitivity and specificity values with respect to AUC (Table 7).

Table 7 Results of ROC analysis of milking parameters and chewing behaviour parameters.

	Cut-off value	Sensitivity, %	Specificity, %	AUC[1]	SE[3]
Milk yield, kg/d	> 38.6	32	90	0.563	0.08
Milk fat, %	< 4.1	56	58	0.59	0.079
Milk protein, %	> 3.25	35	85	0.463	0.079
Fat:protein ratio	< 1.22	74	30	0.569	0.082
Rumination, min/d	> 494	65	58	0.616*	0.029
Chews of rumination, n/d	> 34036	52	61	0.572*	0.029
Chews per bolus, n	> 52.64	66	39	0.517	0.029
Chews per minute, n	> 45.7	76	30	0.477	0.029
Eating, min/d	> 250	96	12	0.512	0.029
Chews of eating, n/d	> 25909	54	57	0.54	0.03

[1]AUC= Area under the curve

[2]ROC analysis= Receiver Operating Characteristic-Analysis

[3]SE= Standard Error

DISCUSSION

According to various studies, SARA is widespread in high producing dairy herds and is attributed to other metabolic disorders and various production diseases (Tajik and Nazafi, 2011; Kleen and Cannizzo, 2012). This metabolic disorder is characterized by a more or less persistent low rumen pH, mainly caused by rations with high sugar and starch content or abrupt dietary changes in terms of easily degradable carbohydrates (Kleen et al., 2003). This is also confirmed by our study results. The dietary change from dry period to lactation period with a forage:concentrate change from 80:20 to 40:60, combined with an increase of easily degradable carbohydrates from 19.3% to 27%, led to the high rate of SARA in early lactation and enabled the comparison of the different methods for the detection of SARA under field conditions (table 1). Based on long-term pH measurements, the investigation revealed a low prevalence of SARA in the dry period and a high incidence at the beginning of the lactation period (table 2). Kleen et al. (2009) found an overall prevalence during lactation of 13.8% in 18 Dutch commercial herds ranging from 0% to 40%. The prevalence was not affected by the stage of lactation (first 25 days of lactation versus day 26-180 postpartum). Garrett et al. (1999) assessed the occurrence of SARA in the United States and observed lower

rates of 19% during early lactation and up to 26% during mid-lactation. Both studies used rumenocentesis for the detection of SARA. The results of the current study suggest that the prevalence of SARA is underestimated when using spot sampling, due to a much higher detection rate when devices for long-term pH measurements are used.

Although SARA is of increasing importance in modern dairy herds, diagnosis in the field is still challenging. In the past, the standard method for the detection of SARA was based on single values. With the development of new techniques which allow the continuous measurement of reticuloruminal pH-values, revised definitions of SARA can be applied in practice, which are based on long-term measurements in cannulated cows.

The diverse picture of various SARA definitions combined with several diagnostic techniques has been comprehensively studied by Plaizier et al. (2009). The authors see the cause of different interpretations of this metabolic disorder as a result of differences in definition and differences in method of detection. Starting with SARA detection based on a certain threshold (Kleen et al., 2003) with spot sampling methods like stomach tubing or rumen puncture as standard practice, the knowledge on SARA has increased over past years. Scientific work, conducted mainly on cannulated animals, has enabled long-term measurements and has clearly increased our understanding of SARA, the background for development, and the possibilities for the detection of SARA in the field. Diurnal pH patterns have been identified and have been tested as a means of finding the right time for spot sampling methods (Garrett et al., 1999). Nevertheless, we observed higher prevalence of SARA in single pH-values when measurements were taken after the evening feeding instead of after morning feeding. It was confirmed by a higher sensitivity of 79% after evening feeding compared to 61% after morning feeding with less changes in specificity. We assume that this circumstance can be explained by the diurnal variation of rumen pH, resulting from the temporary drop of rumen pH due to the degradation of nutrients and release of VFA (Yang et al., 2000; Oetzel, 2003; Beauchemin and Yang, 2005; Block, 2010). The pH level decreases during the day with a pH nadir in the evening. This phenomenon was also observed in our study (data not displayed). Thus, pH-values below the threshold selected occurred more often in the evening. The diurnal pH

variation is (amongst others) influenced by diet composition, as well as feeding and resting times. Dynamics of ruminal fermentation are influenced by the interactions between the rate of passage of feed through the rumen and the rate of digestion of feed in the rumen. Passage of feed from the rumen involves mixing, disruption, and comminution of various components. The rumen operates as a continuous-flow reactor (with partial stirring action) and different sub-compartments with different flow characteristics may be distinguished: liquid, escaping particles, and retained particles (Dijkstra et al., 2007). The production and absorption of volatile fatty acids (VFA) involves dynamic processes resulting in large postprandial variations in ruminal concentrations. The fractional ruminal VFA absorption rates increase with chain length and decrease with pH (Dijkstra et al., 1993; Nozière and Hoch, 2006). Diets higher in non-fibre carbohydrates (NFC) promote the development of ruminal papillae for adequate absorption of volatile fatty acids produced during ruminal fermentation (Rabelo et al., 2003). Effects of pH on ruminal VFA concentrations and the kinetics of VFA absorption by the rumen wall differ considerably (Nozière and Hoch, 2006). pH-values can thus vary considerably amongst individual herd animals and between different farms or test days. In definitions based on single threshold values, this fact is not properly considered. Disregarding dynamics of pH-values can lead to an underestimation of SARA, as our study showed. The monitoring of reticuloruminal pH with additional tools for data processing is required, to assess changes and gain more information on the fluctuation of ruminal pH on commercial farms. Real data from commercial herds would be beneficial for a better understanding of how SARA occurs in the heterogeneous nutritional conditions on the farms.

Due to the difficulties of rumen fluid sampling in practice so far, many practitioners have used indirect parameters, thought to provide information on SARA. Unfortunately, most of them are only single recordings with limited informative value. In the current study, the milk recording data revealed no clear relationships between SARA and milk yield and therefore only poor sensitivity or specificity values were observed for milk parameters. In a study by Seemann and Spohr (2007), the sensitivity and specificity of milk fat percentage and fat:protein ratio were evaluated. They found comparable results to our study with a sensitivity/specificity of 48%/ 78% for milk fat

and 57%/ 78% for fat:protein ratio. Both milk yield depression and a positive relationship between milk yield and rumen pH-values have been observed in practice (Kolver and Veth, 2002; Kleen et al., 2003). In the current study, the fat:protein ratio gave no proper hint for SARA in individual cows. We assume that the higher milk fat content in the first days of lactation (on physiological grounds) is a confounding factor which influences the results. The results reflect the various factors and their interrelationships involved in the development of SARA. These make it nearly impossible to detect, let alone predict the occurrence of SARA by means of single parameters such as milk yield or milk composition.

Regarding the indicators of chewing behaviour, only rumination time showed a sensitivity and specificity ratio with a significant area under the curve (AUC). Rumination time of more than 494 min indicated a SARA event with a sensitivity of 65% and specificity of 58% across the study. This is contrary to the general understanding that high rumination time is an indicator of rumen health (Krause and Oetzel, 2006; DeVries et al., 2009). Findings of Maekawa et al. (2002) demonstrated that chewing time does not necessarily increase the total daily saliva secretion, which is the main supplier of the bicarbonate buffer in the rumen (Owens et al., 1998). The production of saliva is stimulated by chewing but secretion also takes place during resting. There is reason to assume that the buffering capacity of rumination is overestimated and that the acidity of the diet may have a higher impact on the pH-values in the rumen. Given that pH <5.8 is harmful to cellulolytic bacteria and detrimental to fibre degradation, the affected cows spent more time ruminating to digest a unit of NDF (Beauchemin, 1991). Our findings confirm the results of DeVries et al. (2009), who found a positive correlation between the duration of pH <5.8 (h/d) in the rumen and the rumination time on d 1 after an acidosis challenge followed by an increase on the second day. Rumination on days without acidosis (recovered days) was the same as prior to the acidosis challenge. This corresponds with our results and leads to the conclusion that the sensitivity and specificity ratio achieved is too poor to detect SARA. This confirms the statement of Mulligan et al. (2006) that SARA often exhibits diverse and contrary pictures amongst the dairy cows, which complicates the diagnosis of the disease with indirect parameters. In addition, the understanding of variations in

reticuloruminal pH amongst individual cows, which also affects SARA prevention and detection, increased in the few last years (Palmonari et al., 2010; Penner and Beauchemin, 2010; Steinwidder et al., 2015). In the present study, SARA could only be reliably detected using on long-term reticulorumen pH values. Furthermore, continuous measurements of pH-values can be used for feeding management related to monitoring and optimization; a further positive side-effect of long-term rumen pH measurement.

It is concluded that the use of wireless indwelling systems for continuous reticulouminal pH measurement provides valid data for the detection of SARA in the field and is certainly an improvement upon the results gained from rumenocentesis. Due to the poor results, both milk recording data and long-term measurements of chewing behaviour cannot be recommended for SARA detection.

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CHAPTER 4 Effects of Dietary Changes and different Rumen pH Levels on Faecal Fractions of Transition Dairy Cows

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ABSTRACT

In the light of increasing milk yields and the increasing addition of easily fermentable carbohydrates in the diet to meet the consequent nutritional requirements, sub-acute ruminal acidosis (SARA) has developed into a significant metabolic disorder in modern dairy herds. Besides the negative effects on animal health and welfare, ruminal acidotic conditions during SARA are known to reduce fibre degradation and lead to an impaired digestibility of the cell wall components of feed material. Due to constraints in the validity of diagnostic tools, there are considerable difficulties exist in the identification of dairy cows suffering from SARA in actual farm practice. The aim of

our study was to examine the diagnostic value for SARA detection of the fractionation of faecal matter. Long-term measurements of reticuloruminal pH-values were conducted on 24 cows with an indwelling and wireless monitoring system during the transition phase. Faecal samples were taken repeatedly both during the dry phase and in early lactation. A total number of 180 samples were analysed using near-infrared spectroscopy for various fractions; particularly soluble and insoluble carbohydrates. Comparison between faeces samples from both the dry and the lactation periods originating from cows without SARA exhibited significant differences in their content of starch, hemicellulose, ADFom and lignin (sa). Dairy cows with no signs of SARA, permanent SARA and a mixed SARA status revealed no significant differences in their lactation period, but dairy cows with SARA had a clear tendency towards impaired fibre degradability. Cows with a mixed SARA status showed a significant increase in faecal hemicellulose and NDFom during SARA in comparison to the time when they did not have SARA. A ROC-analysis confirmed that hemicellulose was the most sensitive parameter linking with different rumen pH levels. With a cut-off score of 237 g/kg DM of faecal hemicellulose, a sensitivity of 69% and specificity of 70%, the identification of animals with SARA revealed promising results and this method thus has the potential to be a feasible tool for monitoring variation in the fermentation processes in dairy cows on the farm level.

Keywords: NIRS, Dairy Cows, SARA, Faeces

INTRODUCTION

Sub-acute ruminal acidosis (SARA) is defined as a repeated drop of rumen pH below a physiological level caused by a diet containing high amounts of rapidly fermentable carbohydrates, which can cause an accumulation of volatile fatty acids (VFA) in the rumen (Kleen et al., 2003; Kleen and Cannizzo, 2012). The occurrence of SARA mirrors the conflict between needing to supply high levels of energy to high yielding animals and the increasing risk of negative side effects due to dairy cows' limited ability to adapt to nutritional changes (Sundrum, 2015). Acid overload can compromise rumen epithelium cells with potential consequences such as liver

abscesses and lameness (Kleen et al., 2003; Nordlund et al., 2004; Enemark, 2009; Plaizier et al., 2009). A time period of more than 310 min/d with ruminal pH values below pH 5.8 was defined by Zebeli et al. (2008) as the reticuloruminal condition that describes SARA. While the main factors involved in the development of SARA are obvious, the detection of SARA cows in farm practice is still a challenge. This is mainly due to the lack of appropriate diagnostic tools and the complexity of the dynamic processes which depend on interactions between various factors, thus causing great variability in the reactions between individual animals. There is evidence that both the susceptibility to SARA and the intensity of SARA varies between animals, due to the ability to cope with the dietary and environmental factors differing between individuals (Stein and Sundrum, 2016). Factors involved are (inter alia): lactation number, previous SARA challenges, feed intake, an individual's ruminal microbiom, passage rate and the capacity to absorb fatty acids via rumen villi (Dohme et al., 2008; Li et al., 2009; Penner et al., 2009; Jami et al., 2014).

Using rumen fluid in spot sampling methods in the field such as rumenocentesis and stomach tubing is generally accepted as a good way of directly detecting cows suffering from SARA. However, the possible negative impacts of the sampling procedure on the cows and a low sensitivity ratio are still of general concern (Garrett et al., 1999; Tajik and Nazafi, 2011). Indwelling devices enable long-term rumen pH measurement and have provided proven valid results (Sato et al., 2012; Gasteiner et al., 2015; Stein and Sundrum, 2016). Unfortunately, these tools are still rather expensive for routine dairy application on all cows in early lactation. Other recommendations like the prediction of ruminal pH and the occurrence of SARA using feed ration analysis, urine pH, fat to protein ration in milk, faecal sieving or blood analysis have only achieved limited success so far and poor sensitivity to specificity ratios (Seemann and Spohr, 2007; Tajik and Nazafi, 2011). Further approaches for early detection of SARA in practice are therefore required.

Ruminants harbour a very complex set of bacteria, protozoa and fungi, which process through degradation and make use of the nutrients in the diet. The bacterial digestion of the cell wall components cellulose and hemicellulose, originating mainly from forage, is essential for the provision of energy for deconstruction, degradation and

synthesis of nutrients (Jung and Allen, 1995). Several studies have shown that a shift of ruminal conditions alters the composition of the microbiota and has an impact on the quality and quantity of the digestion rates (Plaizier et al., 2001; Sung et al., 2007; Palladino et al., 2010; Mao et al., 2012; Mao et al., 2013). A reduced NDF degradability when SARA was induced was demonstrated by Krajcarski-Hunt et al. (2002). Others reported a higher number of undigested particles in the faeces of animals with SARA due to a lack of fibre digestion and changes in faecal consistency such as diarrhoea and in faecal pH (Kleen et al., 2003; Grove-White, 2004).

In general, optimal fibre digestion occurs at rumen pH values above 6.1; otherwise fibrolytic bacteria are strongly reduced or disappear and the amylolytic population increases (Palladino et al., 2010; Mao et al., 2012). Zebeli et al. (2008) evaluated the response of ruminal pH on different fibre or starch levels based on a large set of quantitative research data. They revealed reduced fibre digestion as well as lower dry matter intake and recommended a minimum of 30 to 33% of physical effective neutral detergent fibre (peNDF) in the feed ration to ensure optimal conditions for fibre degradation in the rumen.

A high variability of the faecal composition due to different feeding regimes is described by van Vliet et al. (2007). The analysis of faecal soluble and insoluble C and N fractions with near infrared reflectance spectroscopy (NIRS) could aid the detection of disturbances taking place in the preceding sections of the gastrointestinal tract (Althaus et al., 2013). The aim of our study was to analyse nitrogen and carbon fractions and fibre in the faeces of transition cows to assess the effect of reduced rumen pH levels on faeces composition and to evaluate whether studying the modified composition of faeces could help identify SARA cows in the herd.

MATERIALS AND METHODS

ANIMALS, MANAGEMENT AND DIETS

The study was conducted on a commercial farm with a herd of 300 cows in the Free State of Thuringia/ Germany from August to November 2013. All procedures were approved by the Thuringia Office for Customer Protection and were in accordance with the German legal guidelines for animal welfare. In total, 12 primiparous and 12 multiparous (not more than three lactations) dairy cows were allocated to a study group. These were close-up cows (approx. 14 d ante partum) and visually healthy while being fed the same diet. The cows were housed in a pen with straw bedding. Housing was altered to a free stall with cubicles after parturition. The cows were milked twice a day (at 0500 and at 1600) in a rotary milking parlour. Herd milk yield averaged 10,022 kg/cow/year. A total mixed ration was offered once daily in the dry period (0900) and three times a day (0600, 1200, and 1600) in lactation, respectively. Diet ingredients and composition are set out in Table 8. Diets were calculated based on a DMI of 11 kg in the dry period, an average DMI of 17 kg between d +1 and d +14 and an average DMI of 23 kg between d +15 and d +45. Daily reweighing of feed residuals within the trial confirmed the DMI calculated previously for the herd level. It was not possible to implement an individual DMI recording on the commercial farm. An automatic feed pusher was used fifteen times in 24h to ensure continuous forage availability. Water was available ad libitum throughout the experiment.

Table 8 Ingredient composition and nutrient contents of the diets fed during the experiment (DM basis).

Ration composition, % of DM	Period[1] DP	LP
Forages		
Corn silage	39	44
Ryegrass haylage	14	10
Alfalfa hay		4
Barley straw	28	
Concentrates		
Barley	3	16
Rapeseed meal extraction	12	8
Rapeseed cake		6
Soy meal		4
Sugar beet pulp		5
Protein premix	2	
Mineral premix dry	2	
Mineral premix lactation		2
Rumen-stable fat		1
Nutrient content[2,3]		
DM, %	68.2	62.6
Chemical composition, g/kg of DM		
CP	121	156
NFC	271	349
NDF	461	399
Hemicellulose	209	191
Cellulose	216	174
ADF	252	208
ADL	35.9	34.3
Energy, MJ/kg		
NE_L	5.1	6.9

[1] Investigation periods: DP= contents of diet in dry period fed between d -15 to d 0; LP= contents of diet in lactation between d +1 to d +45

[2] Averaged values based on weekly conducted feed analysis; diet was offered as TMR.

[3] Analysis methods: modified Weender analysis and Van Soest´s detergent fibre method

DETECTION OF PH LEVELS

Beginning in the dry period, reticuloruminal pH was measured continuously for 50 days with an indwelling and wireless data transmitting system (smaXtec animal care sales GmbH, Graz, Austria). Measuring units (boli) were activated after calibration using buffer solution provided by the manufacturer. The boli were given orally to each cow, which ended up in the reticulum at least 24h after administration (Gasteiner et al., 2012; Gasteiner et al., 2015). Reticuloruminal pH-values were recorded 144 times per day in 10 min intervals over the whole transition phase and were transmitted via radio to an external receiver placed in the barn. The receiver was connected to a computer for further data transmission and analysis. The definition from Zebeli et al. (2008) was used for SARA detection based on continuous pH measurement. SARA was considered to occur when the total daily duration below pH 5.8 (TD5.8) was ≥310 min/d. This threshold is the result of the analysis of 17 published studies, which revealed that the threshold of pH 5.8 was the critical point for ensuring adequate fibre digestion as the conditions for digestive microorganisms are suboptimal below this value. During the experimental period, no signs of clinical acidosis or other diseases were observed in the dairy cows from this study.

FAECES ANALYSIS

Faeces samples were taken at three different stages during the transition period. These were in the dry period (abbreviated form "DP"; d -15 to d -1), lactation period 1 (LP1) from d +9 to d +20, and lactation period 2 (L2, d +26 to d +45). In each stage, three rectal faeces samples were taken at an interval of two days in the two hours between 0900 and 1100. Taking into account the passage rate of the feed, the SARA classification of the day before faeces sampling was considered in order to classify the faeces samples. Reflecting the SARA status over the investigation period, 21 out of 24 cows were evenly distributed in three groups during the lactation period. Two animals were excluded due to culling; the amount of faeces samples from one cow was insufficient for analysis. Dairy cows not exhibiting SARA ("SARA negative") were distinguished from cows permanently fulfilling the SARA criteria ("SARA positive").

The third group included animals that had days with and days without SARA over the period of investigation ("mixed SARA").

For the determination of dry matter (DM) content, all faeces samples were first dried at 60°C for 48h and ground to 1 mm particle size, followed by a second drying at 105°C for 3h until they had a constant weight prior to analysis. To estimate the organic matter, OM samples were reduced to ashes overnight at 550°C. Faeces samples were analysed using Near Infrared reflectance Spectroscopy (NIRS) for various C and N fractions: total C, total N, crude starch (CS), neutral detergent fibre (NDFom), acid detergent fiber (ADFom) and lignin (sa) were all determined. For NDF analysis, a pre-treatment of the samples with heat stable amylase was not conducted due to the previous contact with the enzyme inside the intestine. The cell wall components hemicellulose and cellulose were calculated as the difference between NDFom and ADFom or ADFom and lignin (sa) for each sample, respectively. Validation and external validation of calibration regressions, coefficient of determination between laboratory analysis and predicted values, and standard errors of prediction for all determined parameters except for starch have been described by Althaus et al. (2013). To enable the determination of starch via NIRS, a set of 54 randomly chosen samples was used to carry out a starch calibration using the partial least squares regression method based on a one-out validation (Tillmann, 1996). First, samples were analysed in a wet chemical procedure according to Ewers´ Polarimetric method (Mitchell, 1990). The near infrared spectroscopy was carried out using the apparatus from FOSS (FOSS 6500, Rellingen, Germany). The values were divided into a set for calibration (41 samples) and one for validation (13 samples). The results of the reference analysis were transmitted from NIRS to the WINISI software program (version 1.4, FOSS) and further edited. The correlation coefficient between the values obtained by wet chemical analysis and NIRS analysis (R^2) was 0.99; showing a very good accordance. The correlation coefficient in the cross validation (1-VR), indicated a satisfactory degree of prediction accuracy of 0.61. The standard error of calibration (SEC) was 0.075 and standard error of cross validation (SECV) was observed to be 0.45. The slope, which should be close to 1, was found to be exactly by 1. The quality parameter for calibration, the SEC to SD ratio, was estimated at 0.11. Prepared samples were packed

into rectangular sample containers with a quartz window, closed with a lid and then scanned between 408 and 2493 nm, containing visible (408–1108 nm) and near-infrared (1108–2493) wavelengths.

STATISTICAL ANALYSIS

Variables of C and N factions were tested for normal distribution with the Kolmogorov- Smirnov test and for homogeneity of variance with Levene´s test; mean value, standard deviation and coefficient of variation were determined. The variation of the single variables between the investigation periods, as well as the variation between the SARA groups during lactation was calculated using the non- parametric Friedman´s test for one-way repeated measures analysis of variance by ranks. For post hoc pairwise analysis, the Wilcoxon test was used. Pearson correlations were calculated for the interrelation between daily rumen pH, hemicellulose and cellulose. Sensitivity and specificity were evaluated with Receiver Operating Characteristic-Analysis (ROC-Analysis) using the statistical program SPSS for Windows (Version 21.0). The ROC curve is the true positive rate (sensitivity) as a function of the false positive rate (1-specificity) for the range of cut-off values considered. The Youden´s Index was determined to assess the optimal cut-off value with the best sensitivity to specificity ratio, defined as the difference between the true positive rate and the false positive rate. The index enables the identification of an optimal cut-off point from the ROC curve, independently from the prevalence. The area under the curve (AUC) was also calculated as a quality parameter of the individual ROC curves. Statistical analysis and calculations were conducted using either the statistical program SPSS for Windows (Version 21.0) or the calculation program Excel for Windows (Microsoft Office 2010). In all calculations, differences were considered to be significant at $P<0.05$.

RESULTS

In the current study evaluating methods of SARA detection for the best practical use, the value of analyzing the fractionated faecal composition of cows under different

rumen pH levels was investigated. SARA status was determined based on continuous reticuloruminal pH. While all feces samples derived from non-affected SARA cows in the dry period, during lactation (LP1+LP2) 60% of the feces samples were classified as "SARA". This was also reflected in the reticuloruminal pH levels with a mean pH of pH 6.19 ± 0.26 in DP, pH 5.89 ± 0.35 in LP1, and pH 5.90 ± 0.37 in LP2.

FAECAL COMPOSITION OF DAIRY COWS IN THE TRANSITION PERIOD

The transfer from the dry period to lactation was accompanied by a dietary change from dry to lactation period with a forage:concentrate change from 80:20 to 40:60, combined with an increase of easily degradable carbohydrates from 19.3% to 27%. To assess the differences between faecal C and N factions in the dry and lactation periods, only data originating from dairy cows without SARA were taken into account.

With pH-values around 6.2, rumen pH averaged at the same level in the dry and lactation period. A faecal dry matter percentage also exhibited no significant difference between the periods. In contrast, C fractions did vary between dry and lactation periods. Particularly the concentration of starch (as a rapidly fermentable and soluble carbohydrate) increased significantly from 6.2 g/kg DM in DP to 10.5 g/kg DM in LP1 and 12.5 g/kg in LP2. Although the values were on a low level, a high coefficient of variation (CV) of 48% to 73% was detected, indicating a high variability in faecal starch.

The content of structural carbohydrates (fibre) also differed between the dry and lactation periods (Table 9). Whilst the faecal NDFom content (encompassing all cell wall components except pectin) did not differ significantly between the periods, the composition of this parameter varied between dry and lactation: Faecal hemicellulose, a partially digestible and soluble fibre fraction of NDFom, significantly increased from 198 g/kg DM in the dry phase to 243 g/kg DM in LP1 and 239 g/kg in LP2 ($P<0.05$). While the cellulose content at an average level of 260 g/kg DM varied little over the periods of investigation, faecal content of lignin (sa) was significantly reduced from 108 g/kg in DP to 74.4 g/kg DM in LP1 and 65.5 g/kg DM in LP2. Faecal cell wall components had a CV from 6 to 34% and were almost stable over the periods

investigated. Total N and total C of faeces were almost on the same level within the periods investigated with a CV of approx. 13% and 2%, respectively. With the exception of starch, no differences in the N and C content of the faeces were detected between LP1 and LP2.

Table 9: Fecal C and N fractions (g/kg of DM) of dairy cows without SARA, presented by mean, standard derivation (SD), and pooled coefficient of variation in three periods (%).

	DP		LP1		LP2	
	Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV
Dairy Cows (n)	23		12		12	
pH mean	6.20 ± 0.2	3	6.18 ± 0.16	3	6.22 ± 0.17	3
DM (%)	13.2 ± 2	15	13.1 ± 2.5	19	12.5 ± 1.9	15
CS (g/kg)	6.2^{a} ± 4.5	73	10.5^{b} ± 7	63	12.5^{c} ± 6	48
NDF (g/kg)	571 ± 38	7	578.1 ± 47	8	568.3 ± 27	5
Hemicellulose (g/kg)	198^{a} ± 28	14	243^{b} ± 38	16	239^{b} ± 18	8
Cellulose (g/kg)	265 ± 19	7	261 ± 20	8	264 ± 20	8
ADF (g/kg)	373^{a} ± 22	6	335^{b} ± 27	8	329^{b} ± 22	7
Lignin (sa) (g/kg)	108^{a} ± 19	17	73.4^{b} ± 25	34	65.5^{b} ± 16	24
CT(g/kg)	476 ± 9	2	483 ± 9	2	485 ± 7	1
NT (g/kg)	25.1 ± 3.1	13	27 ± 3	11	27.5 ± 4	15

$^{a\text{-}b\text{-}c}$ Vertical means within a row with different superscripts differ (p<0.005)

CHANGES IN FAECAL COMPOSITION IN RELATION TO DIFFERENT RUMEN PH LEVELS

Parameters were investigated for their predictive value with respect to SARA in individual cows based on faecal samples collected in the early lactation period (LP1+ LP2). The results are set out in Table 10. Mean reticuloruminal pH levels ranged from pH 5.56 (SARA positive group) to pH 6.34 (SARA negative group). In the mixed SARA status group, the animals' mean rumen pH level averaged at pH 5.88 (days with SARA: pH 5.7 and days without SARA: pH 6.0). Comparisons between the different groups revealed differences in faecal dry matter percentage, where the lowest values occurred in dairy cows without SARA (12.5% versus 13.5% and 14.2% respectively).

NDFom and ADFom tended to be higher in the SARA positive group than in the SARA negative group (p=0.097) and meaning an increase of hemicellulose and lignin (sa) while cellulose levels remained unaffected. CV was generally on a low level, usually below 10%. Starch was an exception, for which a CV of (respectively) 42% and 27% was detected. A similar pattern was seen in the comparison between the SARA negative group and the mixed group, where there were virtually no significant differences but NDFom and ADFom tended to be higher in the mixed SARA group (p=0.16). No significant differences were detected between the SARA positive group and the mixed SARA group.

Table 10 Mean and coefficient of variation (CV) with Standard Deviation (SD) of pH-value and DM (%), as well as different C fractions from faeces samples. Samples came from cows without SARA (SARA negative), cows with permanent SARA (SARA positive) and cows with mixed SARA status during one investigation period at lactation time.

Independent samples							**Paired samples**	
	SARA negative		**SARA positive**		**Mixed Status -all samples-**		**Mixed Status -SARA-**	**Mixed Status – no SARA-**
	Mean ± SD	CV ± SD	Mean ± SD	CV ± SD	Mean ± SD	CV ± SD	Mean ± SD	Mean ± SD
Dairy Cows (n)	7		7		7			
pH mean	6.24± 0.17	1 ± 1	5.56 ± 0.28	2 ± 1	5.88 ± 0.09	3 ± 1	5.72^{a} ± 0.11	6.03^{b} ± 0.06
DM (%)	12.49 ± 2.11	16 ± 10	13.7 ± 2.1	8 ± 7	14.17 ± 0.69	7 ± 4	14.24 ± 0.77	13.96 ± 1.44
CS (g/kg)	10.7 ± 2.6	42 ± 26	13 ± 4.3	27 ± 17	8.8 ± 3	44 ± 31	7.9 ± 2.9	8.8 ± 4.8
NDFom (g/kg)	575 ± 32	4 ± 3	604 ± 39	4 ± 3	601 ± 48	6 ± 5	631^{a} ± 30	583^{b} ± 37
Hemicellulose (g/kg)	244 ± 13	9 ± 5	256 ± 20	7 ± 2	253 ± 30	13 ± 4	275^{a} ± 20	238^{b} ± 24
ADFom (g/kg)	331 ± 18	3 ± 3	349 ± 22	4 ± 3	348 ± 17	5 ± 7	356 ± 12	344 ± 30
Cellulose (g/kg)	268 ± 20	6 ± 4	270 ± 20	5 ± 5	263 ± 11	8 ± 4	272 ± 11	257 ± 22
Lignin (sa) (g/kg)	63^{a} ± 15	16 ± 7	80^{b} ± 14	14 ± 12	85^{b} ± 8	27 ± 16	84 ± 15	87 ± 23

a, b indicate significant differences within the paired samples with $p = 0.018$

In a further step, paired samples (2 samples from each cow, one with SARA and one without SARA) from the mixed SARA group were compared. Faeces samples from days with SARA showed a significant increase of hemicellulose compared to faeces samples from days without SARA. The mean hemicellulose differed significantly on days with SARA with 275 g/kg DM compared to 238 g/kg DM on days without SARA (P=0.018). NDFom increased significantly from 631 g/kg DM on days with SARA compared to 583 g/kg of DM on days without SARA (P=0.018). When only faecal samples from days with SARA were considered, a comparison between cows in the mixed SARA group on days with SARA and the SARA negative group revealed that NDFom was significantly higher in the mixed SARA group compared to the SARA negative group (631 g/kg DM to 575 g/kg DM; P=0.029). Faecal hemicellulose was significantly higher in the mixed group (only looking at samples from days with SARA) at 275 g/kg DM compared to the SARA negative group at 244 g/kg DM (P =0.024). Correlation between the daily mean rumen pH and faecal hemicellulose revealed a coefficient of r=-0.39 (p=0.01) and r =-0.16 (p=0.01) between rumen pH and faecal cellulose, taking into account every investigation day and every animal.

In additionally, a ROC analysis was carried out to detect cut-off scores of the faecal fibre fractions with the best sensitivity and specificity. The ROC analysis was conducted with a data set of 21 samples from seven animals, including days with and days without SARA as well as all estimated values from the test herd. The results are presented in Table 11.

The area under the ROC curve (AUC) is used to assess on how well the test separates the group being tested into those with and without the SARA, while 0.5 represents the worst and 1.0 the best accuracy. When only data from SARA and SARA free animals was used for the ROC, a value of 248 g/kg DM of hemicellulose in faeces was the best cut-off score seen with a sensitivity of 100% and specificity of 80% and an AUC of 0.9. Based on the data calculations from every animal in the study, the ROC analysis indicated a hemicellulose threshold value of 237 g/kg DM in faeces with a sensitivity of 69% and specificity of 70% (AUC =0.718). For NDFom, in both cases a cut-off score of 595 g/kg DM was obtained, with a sensitivity and specificity of 100% and 70% based on samples from seven animals (AUC =0.838) and 55% and 76% based on

all samples (AUC =0.646). The AUC values obtained were significant for both parameters (P<0.016). The results indicate that hemicellulose and NDFom emerge as the most promising parameters for indicating the presence of SARA in dairy cows over the lactation period. The remaining parameters only exhibited a poor sensitivity to specificity ratio.

Table 11 ROC analysis of different fibre fractions of faeces factions based on a) all available samples and b) samples of cows which exhibited SARA and some with no SARA within one period of investigation.

	Cut-off value (g/kg of DM)		Sensitivity,%		Specificity,%		AUC	
	Total	7 cows	Total	7 cows	Total	7 cows	Total	7 cows
ADL	>59	>73	81	88	23	30	0.379	0.450
ADF	>315	>342	90	88	14	40	0.422	0.600
NDF	>594	>595	55	100	76	70	0.646*	0.838*
Cellulose	>264	>260	66	100	48	60	0.570	0.688
Hemicellulose	>237	>249	71	100	68	80	0.718*	0.9*

Hemicellulose: p = 0.000 (total), p = 0.004 (7 cows); NDF: p = 0.000 (total), p = 0.016 (7 cows)

DISCUSSION

In the present study changes in the faecal composition were observed and were seen to be related to dietary changes as well as to different reticuloruminal pH levels. The study took place in the transition period, which encompasses the three weeks before and after calving, and is known to be the most challenging part of the entire lactation cycle (Drackley, 1999). The changes in the feeding regime and alterations in metabolic and physiological patterns from the dry period to lactation are characteristic for this period and often lead to an increase in metabolic disorders in early lactation, which is also reflected in the faecal composition (Goff and Horst, 1997; Martens, 2013). Continuous measurement of reticuloruminal pH was conducted to determine the influence of the rumen pH-levels on the faecal composition. In a study from Stein and Sundrum (2016), SARA detection based on long-term measurement was identified as reliable in the field. Faecal analysis of C and N fractions, representing the result of the digestive processes in the gastrointestinal tract, provides some insight in the effects of

the feeding regime and possible impacts of disturbances in the degradation process. While distinguishing different C and N fractions in feed has greatly encouraged and is now widely established (Tylutki et al., 2008), the fractionation of faecal C and N has only been conducted so far on the experimental level and has not yet been implemented in farm management. The impact of dietary changes in the faecal composition from dairy cows without SARA could be clearly seen in the present study. The ration for dry cows had a lower energy density with straw as the main fibre source, while the content of NFC and protein was increased in the lactating cows' ration to cover their energy requirements, and NDF and ADF content decreased. NDF and ADF levels were comparable to those found in other studies (Powell et al., 2009). Feed sources of NDF were altered from straw to alfalfa hay, which was expected to improve the digestibility of fibre components (Jung and Allen, 1995). The share of hemicellulose, cellulose and lignin remained almost stable in the diet with a slight increase of hemicellulose. Contrary to this, lignin (sa) decreased significantly in faeces from lactating cows. The results suggest that lactating dairy cows frequently selected more indigestible feedstuff. The with a variation among the cows, reflected by a high CV of lignin (sa).

The decrease in dietary structural carbohydrates was not confirmed in the C fractions of faeces; a higher faecal content of cell wall components was actually observed. This phenomenon had also been previously detected in a study by our working group (unpublished data), indicating that lactating dairy cows were less able to digest fibre than non-lactating cows, although the lignin content had not been increased in the diet offered. Lignin is known as an essentially indigestible compound in the cell wall of plants which reduces ability of rumen microbes to digest cellulose and hemicellulose. Taking the calculated DMI into account, it can be assumed that the dry cows' lower feed intake caused a lower rumen passage rate, which may have improved the ability to digest cell wall components during the dry period, while increase in DMI accelerated the ruminal retention time and therefore the time for fibre degradation.

In a study by Fredin et al. (2014), the faecal starch concentration and the total-tract digestibility of starch were both closely and linearly correlated (r^2=0.94). Faecal starch is thus suitable for evaluating dairy cows' capacity to digest starch. In the current

study, the faeces' starch content was low and comparable to results from other studies. Fredin et al. (2014) reported faecal starch levels of 0-5%, we observed a level of less than 1%. The presence of starch in faeces indicates that starch is not completely digested. One reason for this might be that the ruminal microbiota is limited in their ability to adapt which may lead to an inefficient utilization of starch. Interestingly, the large variations in faecal starch composition between cows indicate individual differences in capacity to exploit the starch content of the diet. The dry matter content did not differ between dry period and lactation, which is in accordance with the results from other studies, indicating that different feeding regimes have either little or no influence on faecal DM (van Vliet et al., 2007).

Besides the analysis of faeces samples from different stages of the transition period, the significance of faeces analysis for SARA detection using NIRS under field conditions was tested. So far, the use of faecal matter is almost restricted to screening the consistency and the content of undigested particles by sieving and a visual inspection of the residues (Mgbeahuruike, 2007). Clinicians assume that when the in dry matter content drops and larger ingested particles are found in the faeces, typically SARA is present (Grove-White, 2004). By contrast, our study revealed that faeces from cows without SARA had the lowest dry matter content compared to faeces of cows affected with SARA. For that reason, we disagree with Grove-White (2004) and only partly agree with Tajik and Nazafi (2011), who found no differences in faecal consistency of animals experiencing SARA. Differences in the results of various studies emphasize that faecal dry matter is not a valid indicator for the detection of metabolic disturbance. Looking at the results on the easily degradable carbohydrates, the use of faecal starch also seems to be unsuitable for detecting SARA due to the high variability in the calculated coefficients of variation.

Structural carbohydrates are first digested by the ruminal microbiota, while the microbial digestion downstream is marginal. As a general rule, the digestion of roughage is influenced by several factors such as forage quality and source, retention time in the rumen as well as DMI and the availability of energy. On the other hand, the impact of SARA on the digestion is proven (Plaizier et al., 2001; Zebeli et al., 2008; Palladino et al., 2010; Mao et al., 2012). In the present study, we found significant

differences in faecal fibre content between dairy cows with SARA and those without SARA. However, the differences were less pronounced between the groups than expected. This might primarily have been due to the fact that the number of animals in each group (7) was small. In the mixed status group, an increase of NDF and hemicellulose on days with SARA was detected, while the increase of NDF was mainly caused by the increase in hemicellulose. The observed rumen pH levels in cows with SARA can be characterized as inadequate conditions for fibre digestion in the rumen. Zebeli et al. (2008) stated that the daily average pH should not drop below 6.16 in order to reduce the risk of SARA. The increase of hemicellulose and NDF on days with SARA is in line with the findings of Plaizier et al. (2001), who observed reduced digestion levels of NDF during SARA. To complete the picture, the group of animals that did not suffer from SARA (SARA negative) exhibited the lowest fibre fraction content of all groups.

The ROC analysis confirmed hemicellulose as the most promising parameter for SARA detection using fractional faecal analysis. We observed the best sensitivity and specificity ratio with 69% and 70% respectively, with a cut-off score of 237 g/kg DM for hemicellulose. The sensitivity and specificity of NDF was similar but rather worse for sensitivity (55%). Hence, hemicellulose seems to be a good indicator for the onset of fermentation disorders in the rumen. However, it must be noted that the thresholds observed are context-dependent and related to the farm-specific feeding regimes. Nevertheless, faecal analysis enables the detection of differences in the fermentation process between individuals in the herd and helps to identify animals at risk without necessitating further investigation. Repeated analysis under different farm conditions may provide more general thresholds for faecal hemicellulose with respect to the detection of SARA.

The observed sensitivity to specificity ratio of faecal analysis for the detection of SARA is comparable to other indirect parameters related to SARA detection in the field. For instance, Seemann and Spohr (2007) calculated the sensitivity and specificity of commonly used diagnostic tools for SARA, including dietary factors like crude fibre (sensitivity of 62% and specificity of 69%) or easily degradable carbohydrates (62%/ 73%). Although the results are on the same level as ours, dietary factors are on

herd level, and can be only used for an assessment of the SARA risk in the herd. The analysis of faeces samples provides information for the individual level; hence it is can be employed for SARA diagnosis of individual dairy cows. Recent studies by Stein and Sundrum (2016) revealed a sensitivity of 65% and specificity of 58% for rumination time in terms of SARA identification, but very poor results for milk components. Although the parameters mentioned can be used for individual SARA diagnosis, it should be noted that milk is only routinely analysed on a 4-week cycle on commercial farms, which also restricts how helpful milk recording data could be for SARA detection. By contrast, faecal samples can be easily collected by the farmer and analytical NIRS results from the lab can be promptly returned. Furthermore, as was also proved in the current study, faeces analysis provides a range of detailed information that can be further used by the management to ascertain the efficiency of the feeding regime and the digestive fermentation processes in dairy cows on the farm level.

CONCLUSION

The results indicate that the analysis of C fractions in faeces has the potential to be used as a management tool to monitor rumen fermentation on the herd level. Further research on a higher number of dairy cows from various farms and external validation is needed in order to understand the relationship between faecal matter and fermentation processes better.

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CHAPTER 5 *Additional results*: Individual Variation of Reticuloruminal pH in Transition Dairy Cows

BRIEF SUMMARY

The current section describes further results of the study, which are described in detail in Chapter 3 and Chapter 4. The reticuloruminal pH was measured long-term in 24 transition dairy cows in order to detect SARA under commercial conditions. Continuous recording of the parameter meant that it could be also demonstrated that the change from dry phase to lactation period resulted in significant changes in reticuloruminal pH levels. Closer investigations of the development of this parameter during the first 12 DIM revealed major differences between the dairy cows. While individual animals showed the same pH level as before parturition, a larger part of the group experienced a decline in pH of varying intensities and progressions. In general, different pH levels were detected within the dairy cows although they were managed and fed the same. A SARA prevalence of 60% was detected during the lactation period. The test herd included animals without any SARA challenge, animals that had SARA for several days, but also dairy cows, which suffered from SARA throughout the whole measurement period. The investigation showed that the severity of SARA is variable and depends on the number of previous SARA challenges. Overall, the findings indicate that there is a huge variability within a dairy herd in terms of ability to maintain reticuloruminal pH on a physiological level and thus to avoid SARA.

MATERIALS AND METHODS

The study was conducted on a German commercial farm with 300 dairy cows, of which 24 primiparous and multiparous (50/50) transition dairy cows were investigated. The housing conditions, performance level, as well as the milking and feeding times were already described in further detail in Chapter 3. The offered total mixed rations represent traditional European rations based on maize silage with high NDF levels in the dry period, mainly originating from straw, and a high energy diet for lactating cows

with less fibre and an increased amount of NFC. The ingredients and composition of both diets are set out in Table 8 of Chapter 4.

Throughout the whole investigation, reticuloruminal pH was measured using an indwelling and wireless data transmitting system (smaXtec animal care sales GmbH, Graz, Austria) as previously described in Chapter 3. In total, 144 data points were used to display the daily pattern of reticuloruminal pH of each animal on every test day. Measured pH-values were tested for normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variance using Levene´s test and were averaged on an hourly and daily basis for each dairy cow. Daily pH values between d +1 to d +12 were used as factors to conduct a k-means cluster analysis (MacQueen, 1967). Based on the analysis, cows were clustered in 4 groups with characteristic courses of reticuloruminal pH in this timeframe.

In a second part, the total duration of pH-values <5.8 (TD5.8), as well the time below pH 5.8, 5.5 and 5.2 were calculated for each cow and each test day. Furthermore, the severity of SARA on days with SARA was calculated by measuring the absolute value of the negative deviation of reticuloruminal pH from the threshold value of 5.8 (i.e. 0.6 for pH 5.2), multiplied by the corresponding 10-minute interval. Pearson correlations were calculated for the interrelation of SARA frequency and TD5.8 and the severity of SARA. The averaged results from dairy cows, which were long-term healthy, healthy with some days with SARA (within a shorter timeframe of 7 days), and dairy cows, which suffered from SARA throughout the whole measurement period were compared with one another. The variation among the groups within one investigation period was tested using the Kruskal-Wallis test with pairwise analysis (Man-Whitney U-Test). Differences between dry period and lactation period were tested using the non-parametric Friedman´s test for one-way repeated measures analysis of variance by ranks. The Wilcoxon test was used for post hoc pairwise analysis.

RESULTS AND DISCUSSION

CLUSTER ANALYSIS

In the current study, the reticuloruminal pH curve was characterized by a massive drop from dry phase to lactation, resulting in a high incidence of SARA at approx. 60% (see Chapter 3). The reticuloruminal pH altered significantly from the dry period with a daily mean of pH 6.19 ± 0.26 to a daily pH mean of 5.89 ± 0.36 in lactation ($p<0.05$). The increase in standard derivation from dry period to lactation shows a higher variability between the animals in lactation, reflecting the different health status. In all three periods, no significant differences were detected in the prevalence of pH between primiparous and multiparous animals. A cluster analysis based on daily pH values of d +1 to d +12 revealed 4 different groups showing different developments in reticuloruminal pH (Figure 2). In the first group, represented by four cows, the reticuloruminal pH level remained almost stable at around pH 6.20 (6.12 to 6.27) during the timeframe. The second group, consisting of nine cows, showed a decrease between d +1 and d +9 from pH 6.09 to pH 5.77 followed by an increase up to pH 5.85. In the third group of five cows, reticuloruminal pH increased from 5.71 on d +1 to pH 5.94 on d +9 and decreased to base level until d +12. The last group comprising six cows, showed a sharp fall until d +6 from pH 5.68 to pH 5.4 while the pH level increased in the subsequent days up to pH 5.73. Accordingly, 15 out of 24 animals showed a decrease in reticuloruminal pH during the first nine days of lactation. Heifers and multiparous cows were distributed almost equally within the groups. The results clearly show the varying ability of the animals to deal with characteristic physiological and dietary changes within a short period of time (Goff and Horst, 1997; Block, 2010). Kleen et al. (2003) described the change in diet for the dry period to the lactational diet as a major risk factor for SARA in early lactation. Dairy cows adapted to digest and metabolize predominantly forage diets in the dry period are restricted in their ability to digest a modified diet in lactation. The issue of feed change is also highlighted by Kamphues (2009) as a factor influencing the absorption and passage process in the rumen. In our study, the previously mentioned

abrupt change in diet between the dry period and the lactation period occurred with a change in the forage-concentrate ratio from 80:20 to 40:60 and an increase in easily degradable carbohydrates from 19.3% to 27% in the diet. It turned out that the traditional commercial diet used in the study resulted in an enhanced risk of cows developing SARA. According to Kamphues (2009), this might primarily be due to the large amount of highly fermentable carbohydrates. It is worth mentioning that the differences in reticuloruminal pH development observed between the individual animals between d +1 and d +12 provide evidence that reticuloruminal pH values are not exclusively influenced by diet. Several dairy cows were able to maintain stabilized conditions in their rumen throughout the investigation period.

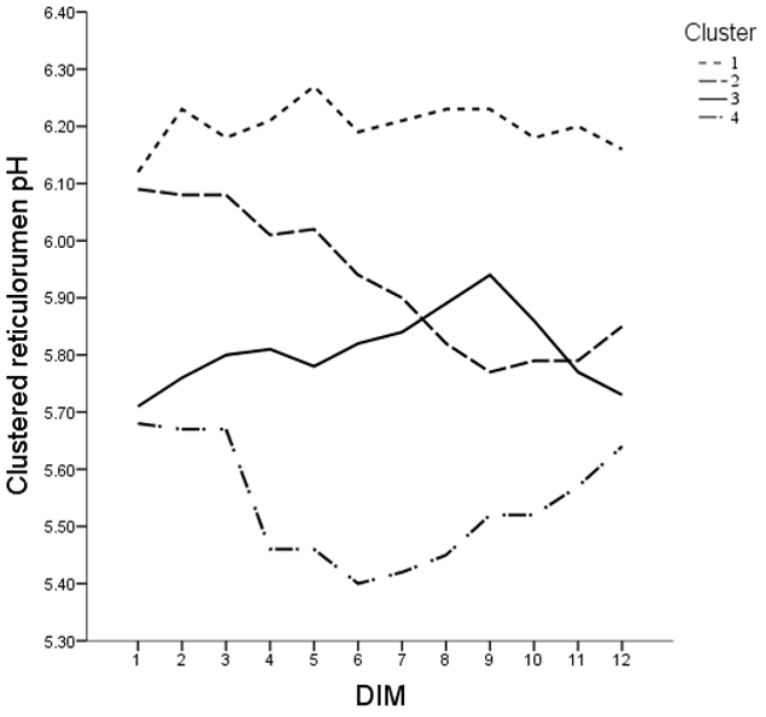

Figure 2 Reticuloruminal pH of the first 12 DIM as a result of a k-means cluster analysis based on 24 cows. Cluster 1 (4 cows), Cluster 2 (9 cows), Cluster 3 (5 cows), and Cluster 4 (6 cows).

INTENSITIES OF PH DROPS IN TRANSITION DAIRY COWS

With the focus on all dry and lactating dairy cows, the total duration of pH <5.8 (TD58), the duration of pH below 5.8, 5.5 and 5.2, the severity of SARA as well as the mean and nadir pH were calculated and presented in Table 12. In the presentation of the results, we distinguished between animals which did not suffer from SARA on any day, animals with equally distributed days suffering from SARA and days not suffering from SARA, as well as dairy cows, which suffered from SARA throughout the entire investigation period. The prevalence of SARA changed dramatically from the dry period to lactation, which was also confirmed by the fact that only around 10% of the dairy cows were classified as healthy without any SARA challenges in the lactation period compared to 60% in the dry period. The proportion of dairy cows suffering permanent SARA was around 25% in lactation; the remaining cows showed a mixed picture with days suffering from SARA as well as healthy days. No dairy cow suffered SARA permanently in the dry period.

Table 12 Reticuloruminal pH (mean and nadir), characteristic parameters of SARA during each investigation period. Numbers in brackets are the SEM of the mean; numbers after ± standard derivation.

	HEALTHY (no SARA challenges at all)	**HEALTHY** (with SARA challenges in between)	**SARA** (With healthy days in between)	**SARA** (No healthy days at all)
Dry period				
Total duration pH <5.8, min/d	21	94	507	-
pH < 5.8, min/d	18	87	423	-
pH < 5.5, min/d	2	6	79	-
pH < 5.2, min/d	0	1	5	-
SARA severity, pH x min	-	-	68.9	-
Mean pH	6.33 ± 0.12	6.12 ± 0.14	5.94 ± 0.08	-
Nadir pH	5.8	5.6	5.34	-
Lactation period				
Total duration pH <5.8, min/d	23	86	753	1157
pH < 5.8, min/d	23	83	586	558
pH < 5.5, min/d	0	3	143	463
pH < 5.2, min/d	0	0	24	136
SARA severity, pH x min	-	-	115.3	261.7
Mean pH	6.25 ± 0.24	6.13 ± 0.14	5.8 ± 0.18	5.56 ± 0.05
Nadir pH	5.8	5.7	5.4	5.2

Interestingly, cows not suffering from SARA at all showed a similar daily mean pH of 6.33 in the dry period and pH 6.22 in lactation. Furthermore, cows suffering from SARA on some days and also having healthy days showed the same mean pH of 6.13 on the healthy days. As displayed, healthy dairy cows with single SARA challenges in between have a lower mean pH than absolute healthy cows. The other way round, the TD58 was approx. 4 fold higher in healthy cows with single SARA days compared to the healthy ones without any SARA challenges. TD5.8 and the severity of SARA differed significantly between cows, which exhibited SARA on several days and those showing SARA permanently. There was also a difference between SARA cows with healthy days between the dry period and the lactation period with SARA not as intensive in the dry period compared to in the lactation period. Furthermore, repeated and enduring occurrence of SARA obviously increased the severity of SARA, demonstrated by the fact that the most severe cases of SARA were observed in animals suffering from SARA throughout the entire investigation period. These findings are in line with those of Penner et al. (2007) as well as Dohme et al. (2008), reporting an increased severity of SARA due to repeated rumen acidosis challenges. In general, all SARA cows greatly exceeded Zebeli´s threshold of 310 min/d with 507 min/d, 753 min/d, and 1157 min/d, which indicates that the problem of SARA was clearly reflected in the current test herd. With regard to TD5.8, our results showed the same tendency as those of Penner et al. (2007), who studied the occurrence of rumen acidosis during early lactation, as well as the effect of additional concentrate feeding antepartum. In their study, they demonstrated that additional concentrate antepartum does not reduce rumen acidosis in early lactation, whereas rumen acidosis increased immediately postpartum. In our study, the TD5.8 in the dry period was on the same level as for the cows in the high concentrate group in Penner's study (97 minutes and 90 minutes). Dairy cows in Penner's control group showed TD5.8 for only 42 minutes. In both studies, there was a clear drop in reticuloruminal pH in early lactation and documented by a general increase of TD5.8 with values between 347 minutes and 498 minutes. With regard to correlations calculated by Pearson, the TD5.8 and the number of days with SARA showed a strong and significant correlation, revealing a coefficient factor of $r=0.93$ ($p<0.001$). Furthermore, the severity of SARA and the

number of days with SARA were positively correlated and showed a coefficient of $r=0.76$ ($p<0.001$) and reflect the observations (Table 1).

Overall, a huge variability in the diurnal course of reticuloruminal pH as well as the day-to-day variation between the cows was detected in the current study. The results concerning individual variation are in accordance with those of Penner and Beauchemin (2010). Additionally, Khiaosa-Ard and Zebeli (2014) presented in detail the variation of animals in terms of rumen ecology and metabolism and discussed the contributions to feed efficiency. Clearly displayed in Figure 2, the reticuloruminal pH during d +1 and d +12 developed individually for the dairy cows in our study, regardless of what were apparently identical conditions. Although there was a high incidence of SARA in the test group, individual dairy cows were able to cope with the environmental conditions and remained healthy or only developed SARA at a low rate. These findings are in line with Mohammed et al. (2012) who showed varied susceptibility for and severity of rumen acidosis independent of feed intake, total VFA concentrations and the bacterial community detected with PCR. Much of the variability in the diurnal pattern of reticuloruminal pH and thus, the level of reticuloruminal pH can be attributed to the individual microbiome in the rumen and previous SARA challenges (Dohme et al., 2008; Li et al., 2009; Penner et al., 2009; Jami et al., 2014). Furthermore, Penner and Beauchemin (2010) stated that the majority of the variation with regard to susceptibility to ruminal acidosis is explained by differences in the absorptive capacity of the rumen wall, based on the estimation that absorption of SCFA accounts for approximately 53% of acid removal from the rumen (Allen, 1997).

In summary, this part of the study emphasized the relevance of individual variance in dairy herds and confirms a need for the monitoring of individual animals in order to ensure adequate health and feeding monitoring.

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CONCLUSION

There is no doubt that the health of our dairy cows must come first in herd management. As already described, awareness of the importance of herd health has grown considerably in recent years, not least due to increasing pressure from society, but also due to increasing economic pressures. Sick cows are expensive. This has been proved in several studies in the past but calculating the actual and overall cost of specific diseases proves to be more complex. One needs to take into account the costs of secondary diseases, medication and veterinary treatment as well as additional costs for labour and animal replacement. The percentage of diseased animals on farms is often very high, which has been also illustrated in the present study. The reasons for this can of course vary but it should be emphasised that one of the main reasons for the high incidence of disease is the increase in animal production performance levels with high-yield cows being more and more susceptible to health disorders. It is also known from other disciplines that such high performance systems require more intensive monitoring. If we challenge our cows, for instance with a very low fibre content in the diet or hot ambient conditions, then closer monitoring of the animals is essential in order to counteract at an early stage any health problems that may arise. Established monitoring methods, such as monthly milk recording, continue to make a valid contribution but are no longer sufficient in certain situations as indeed we demonstrated in the study focusing on SARA detection. In addition to increasing demands on the animals, farming conditions have been also changed in recent years and require new methods and approaches in herd management. Although a significant number of dairy cows are still kept in smaller herds, the general trend is towards larger dairy farming operations. In these larger scale operations, individual animal observation is simply not possible. In particular as untrained staff is frequently used on commercial farms and are not able to identify symptoms of certain diseases. This leads to an increased demand for other means of detecting problems as early as possible and which are suitable for use under the relevant working conditions. For this reason, various approaches have been adopted in recent years. One widespread and

increasingly popular approach is the use of technologies for automatic, continuous monitoring of dairy cows. Long-term measurement of various parameters provides important information about the animals and the environmental conditions and can be used as a decision-making support tool. This thesis also described the use of new sensor technologies, which have been developed to enable the monitoring of animals for disease detection. It has been shown that the devices deliver reliable data about the dairy cows and provide closer insights into individual variability. Inter-individual variation, both in the severity and expression of disease as well as in the measured health parameters, highlights the need for this to be considered in herd and health management in the future. There is indeed an increasing need to include farm-specific information in the decision-making process in order to take into account dairy cows' individual ability to cope with their particular living conditions. This also means that the use of fixed and absolute reference values can in some cases be replaced by individual, farm-specific thresholds or data patterns, which are tailored to and thus more specific to the animal as is the practice in precision dairy farming. In the current study, the data was used to detect dairy cows already suffering from a disease. This new animal observation method should be used to adjust farming conditions to meet animals' needs in order to reduce stress levels and avoid diseases. It would be conceivable to group a herd not only according to the dairy cows´ performance level or lactation stage but rather based on additional data, e.g. energy status or individual pH level. Furthermore, recordings about healthy animals also deliver beneficial data in terms of disease recognition and understanding.

Overall, new sensor technologies as well as other detection tests enable fast and easy comprehensive data collection. In future, we should make use of such possibilities and use the data to understand and treat our dairy cows better and thus improve their health and wellbeing.

SUMMARY

Health management of dairy cows has become increasingly important at farm level. Alongside preventative measures aimed at maintaining animal health, the main focus here is on early and systematic detection of health disorders. It is increasingly evident that particularly cows in the transition period are more susceptible to metabolic disorders in both clinical and subclinical forms. The latter constitute a high risk on the one hand because subclinical disorders are often difficult to detect or go unnoticed and on the other hand because in many cases they can lead to serious secondary diseases. This thesis examines the issue of early detection of subclinical ketosis and sub-acute rumen acidosis. Different methods were tested under practical trial conditions to establish their suitability in detecting health disorders.

In an initial study, ketosis monitoring was conducted on a commercial dairy farm among early lactating cows from day 3 postpartum. A total of 14 animals were found to be suffering from subclinical ketosis, which shows a prevalence rate of 26% in the tested. Blood samples were taken from 14 sick animals and from a healthy control group of 10 animals for analysis of interleukin-6 (IL-6) concentration. Interleukin-6 was selected as other studies have indicated that the pro-inflammatory cytokine IL-6 is regarded as playing a role in ketosis. Contrary to expectations, there was found to be no increased concentration of IL-6 in animals suffering from subclinical ketosis and in fact the lowest values were recorded and were in fact very close to the values recorded in the healthy animals. Overall, the IL-6 concentrations were shown to be at a low level at 27.2 pg/ml ±10.2. It was shown that determination of serum IL-6 is of only limited applicability with regard to diagnosis of subclinical ketosis. It was only possible to detect a weak negative correlation between serum beta- hydroxybutyrate (BHBA, accepted as the “gold standard” in subclinical ketosis diagnosis) and IL-6. In addition to the blood analyses, daily rumination activity was measured using the DairyCheck system, which continuously records the characteristic contractions of the chewing muscles thus enabling calculation of rumination. An investigation was conducted to establish if there were differences between diseased animals suffering

from subclinical ketosis and nonketotic animals with regard to length of daily rumination periods. The daily average rumination time in dairy cows suffering from subclinical ketosis was 475 min/d ± 56 and 497 min/d ± 48 for cows that had recovered from the disorder. The average for these animals was thus always below that of cows in the healthy control group with an average rumination time of 521 min/d ± 76. Only a very weak negative correlation between rumination time and serum BHBA was established, largely due to the fact that the animals showed high variability in rumination activity.

A further study, also conducted under commercial conditions, focused on the detection of subacute rumen acidosis (SARA). In the investigations, an indwelling, wireless, commercially available bolus system was used, which continuously measures reticuloruminal pH. The system enables SARA to be detected under practical working conditions without the need for invasive methods such as the lancet technique. The bolus system was orally administered to 24 dairy cows shortly before calving to enable measurement and monitoring of reticuloruminal pH values throughout the entire transition phase. Whereas only a few cases of SARA occurred in the dry period, a large proportion of the animals suffered from SARA during early lactation. Based on pH data from lactating dairy cows, a sensitivity analysis was also conducted using different, commonly used diagnosis methods in order to investigate the practicality of the various methods in SARA diagnostics. This involved detecting SARA based on individual values, eating and rumination times as well as specific milk components and milk yield. The analysis showed that in comparison with long-term measurement methods almost all diagnostic methods have only limited practicability with regard to the diagnosis of SARA. In another part of the study, faeces fractionation was carried out for the same animals in order to establish the extent to which bovine faeces analysis can also be used to detect SARA. It was shown on the one hand that the ration influences faecal composition (dry period ration versus ration for lactating cows), while on the other hand there are differences in the composition of faeces from cows suffering from SARA. Thus only faecal samples from lactating cows were analysed in order to exclude the influence of the ration in this respect. An increased amount of fibre components in the faeces of dairy cows with SARA indicated impaired

digestibility. Hemicellulose in particular was shown to be a useful parameter in the detection of SARA.

The trial conditions also enabled the reticuloruminal pH curves of the animals to be monitored in early lactation. A cluster analysis of pH values during the first 12 days postpartum showed that despite identical herd management and feeding conditions the monitored animals developed different pH curves. Thus there was one group of dairy cows, which was able to maintain stable pH values whereas the others showed drops in pH of varying duration and intensity. It was also shown that animals in the test herd suffered from SARA to varying degrees. As indicated in other studies, this study also clearly showed that individual animals in a herd may differ significantly in their abilities to react to their environment and/or to counteract or adapt to suboptimal conditions.

To summarize, a range of different diagnostic methods for the detection of subclinical ketosis and SARA were tested of which only a few can be recommended as being suitable for routine use. Inter-individual variation among animals both with regard to the severity and expression of diseases as well as in the measured health parameters clearly highlights the need for a stronger focus to be placed on these areas within herd and animal health management in the future.

ZUSAMMENFASSUNG

Das Gesundheitsmanagement von Milchkühen hat in den vergangenen Jahren auf den landwirtschaftlichen Betrieben an Bedeutung gewonnen. Neben Präventionsmaßnahmen zur Gesunderhaltung der Tiere ist die frühzeitige und systematische Erkennung von Erkrankungen hierbei der Hauptbestandteil. Es zeigt sich vermehrt, dass vor allem Transitkühe verstärkt an Stoffwechselerkrankungen in sowohl klinischer als auch subklinischer Form erkranken. Letztere stellen ein hohes Risiko dar, zum einen weil subklinische Erkrankungen oftmals nur schwer oder gar nicht erkannt werden und zum anderen, weil sie in vielen Fällen die Grundlage für meist schwerwiegendere Folgeerkrankungen sind. In der vorliegenden Studie wird das Thema der Früherkennung von subklinischen Ketosen und der subakuten Pansenazidose behandelt. Verschiedene Methoden wurden unter praktischen Versuchsbedingungen auf ihre Tauglichkeit zur Krankheitserkennung hin geprüft.

In einer ersten Studie wurde auf einem konventionellen Milchviehbetrieb ein Ketose-Monitoring bei frischlaktierenden Kühen ab Tag 3 postpartum durchgeführt. Insgesamt 15 Tiere waren an einer subklinischen Ketose erkrankt, was eine Aufkommensrate von 26% in den untersuchten Tieren bedeutete. Die Blutproben von insgesamt 24 Tieren wurden auf ihren IL-6-Gehalt untersucht. Von den untersuchten Tieren waren 14 Tiere erkrankt, 10 Tiere bildeten die gesunde Kontrollgruppe. Interleukin-6 wurde bestimmt, da dem Zytokin IL-6 in anderen Studien in Bezug auf Ketosen eine Rolle zugesprochen wurde. Die erwartete Erhöhung von IL-6 bei erkrankten Tieren konnte nicht festgestellt werden; die erkrankten Kühe zeigten vielmehr die niedrigsten IL-6 Werte der Studiengruppe. Insgesamt waren die IL-6 Konzentrationen auf einem niedrigen Niveau mit 27.2 pg/m l± 10.2. Es zeigte sich, dass die IL-6 Bestimmung im Blut hinsichtlich der Erkennung von subklinischen Ketosen nur eingeschränkt nutzbar ist. Es konnte ausschließlich eine schwache negative Korrelation zwischen Beta-Hydroxybutyrat (BHBA, Goldstandard für den Nachweis einer Ketose) und IL-6 detektiert werden. Zusätzlich zu den Blutanalysen wurde ebenfalls die tägliche Wiederkauaktivität mit dem „DairyCheck“ System bestimmt, welches kontinuierlich

die charakteristischen Kaumuskelkontraktionen aufzeichnet und somit die Dauer des Wiederkäuens bestimmt werden kann. Es wurde geprüft, ob sich ketotische Tiere von nicht ketotischen Tieren hinsichtlich der täglichen Wiederkäuzeit unterscheiden. Milchkühe mit einer Ketose kauten im Schnitt 475 min/d ± 56 wieder, nach Genesung 497 min/d ± 48. Sie befanden sich somit im Durchschnitt immer unterhalb der gesunden Kontrollgruppe, welche 521 min/d ± 76 wiederkaute. Eine Korrelation zwischen der Wiederkauzeit und dem BHBA- Gehalt im Blut war nur sehr schwach ausgeprägt, nicht zuletzt da die Tiere generell eine hohe Variabilität in der Wiederkauaktivität zeigten.

Bei einer weiteren Studie, ebenfalls auf einem Praxisbetrieb durchgeführt, wurde auf die Erkennung der subakuten Pansensazidose (SARA) fokussiert. Hierbei kam ein drahtloses, kommerziell verfügbares Bolussystem zum Einsatz, welches den pH Wert kontinuierlich im Retikulorumen misst. Es macht die Erkennung einer SARA auch unter Praxisbedingungen ohne invasive Methoden wie der Punktion möglich. Das Bolussystem wurde 24 Milchkühen kurz vor der Abkalbung oral eingegeben, um den pH-Wert während der gesamten Transitphase messen und überwachen zu können. Während in der Trockenstehphase nur vereinzelte SARA Fälle auftraten, erlitt ein Großteil der untersuchten Tiere in der Frühlaktation eine SARA. Auf Grundlage von pH-Werten von laktierenden Milchkühen, wurde zusätzlich eine Sensitivitätsanalyse von verschieden, bereits eingesetzten Nachweismethoden durchgeführt, um die Tauglichkeit für die SARA-Diagnostik zu untersuchen. Es handelte sich hierbei zum einen um einen SARA-Nachweis unter Heranziehung von Einzelwerten, Fress- und Wiederkäuzeiten, sowie ausgewählten Milchinhaltsstoffen und der Milchmenge. Die Analyse ergab, dass nahezu alle Nachweismethoden im Vergleich zur Langzeitmessung nur eingeschränkt zur SARA-Diagnostik nutzbar sind. In einem weiteren Teil der Studie wurde eine Kotfraktionierung bei den gleichen Tieren durchgeführt, um damit SARA-Tiere auch mittels der Kotanalyse erkennen kann. Es konnte gezeigt werden, dass zum einen die Ration einen Einfluss auf die Kotzusammensetzung hat (Trockensteherration versus Ration für Laktierende) zum anderen aber auch, dass eine SARA die Zusammensetzung des Kotes verändert. Hierfür wurden Kotproben ausschließlich von laktierenden Kühen untersucht, sodass

der Einfluss der Ration ausgeschlossen werden konnte. Erhöhte Faseranteile im Kot von SARA - Kühen gaben Hinweis auf eine verminderte Verdaulichkeit. Dabei erwies sich vor allem die Hemizellulose als guter Parameter, um auf eine SARA schließen zu können.

Die Versuchsbedingungen ließen es ebenfalls zu, die pH-Verläufe der Tiere in der Frühlaktation zu untersuchen. Eine Clusteranalyse von pH-Werten der ersten 12 Tage postpartum zeigte, dass die untersuchten Tiere trotz gleicher Haltungs- und Fütterungsbedingungen unterschiedliche pH-Wert Verläufe entwickelten. So gab es eine Gruppe von Milchkühen, die den pH-Wert stabil halten konnte, während die restlichen pH-Abfälle in verschiedenen Verläufen und Intensitäten aufzeigten. Es konnte ebenfalls aufgezeigt werden, dass Tiere innerhalb der Testherde unterschiedliche Schweregrade der SARA entwickelten. Auch in dieser Studie wurde deutlich, dass Tiere scheinbar unterschiedliche Möglichkeiten haben, auf ihre Umwelt zu reagieren, bzw. suboptimalen Bedingungen entgegenwirken zu können.

Zusammengefasst wurden verschiedene Methoden zur Ketose- und SARA- Erkennung geprüft, von denen nur einzelne für die Praxis zu empfehlen sind. Die Variabilität der Tiere, sowohl bei der Ausprägung der Erkrankungen als auch bei den gemessenen Parametern verdeutlicht die Notwendigkeit, diese im Herden- und Gesundheitsmanagement in Zukunft stärker zu berücksichtigen.

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