Polish Journal of Veterinary Sciences Vol. 22, No. 4 (2019), 685–693

DOI 10.24425/pjvs.2019.129981

Original article

Biomarkers from automatic milking system as an indicator of subclinical acidosis and subclinical ketosis in fresh dairy cows

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Abstract

The objective of this study was to determine the association between subclinical acidosis (SARA) and subclinical ketosis (SCK) with biomarkers from an automatic milking system (AMS) measuring in relation to rumination time (RT), milk yield (MY), bodyweight (BW), milk temperature, the milk fat-to-protein ratio, and the electrical conductivity of milk at the udder quarters-level which can be read in fresh dairy cows.

During the course of the study, all of the fresh dairy cows (n=711) were examined according to a general clinical investigation plan. The cows were selected for 1-30 days of milk (DIM) and were milked using Lely Astronaut® A3 milking robots with free traffic.

Rumination time shows a statistically significant positive correlation with milk yield (milk temperature) and is negatively correlated with the fat and protein ratio. Healthy cows demonstrated the highest level of rumination time and the lowest milk temperature. The average BW for these cows was 1.64% lower than for the SARA group and the BW kg was 2.10% higher than SCK cows. MY was 14.01% lower in comparison with SARA and 6.42% higher in comparison with SCK. According to these results, some biomarkers from the AMS have an association with SARA and SCK. However, further research with a higher number of cows is needed to confirm this conclusion.

Key words: after calving, diseases, rumination time, milk yield, body weight

Introduction

Subclinical acidosis (SARA) has long-term health and economic consequences. These include feed intake depression, fluctuations in feed intake, reduced diet digestibility, reduced milk yield, reduced milk fat percent, gastrointestinal damage, liver abscesses, and lameness (Abdela 2016). This digestive disorder is the consequence of feeding high grain diets to dairy cows when they are pre-adapted to digesting predominantly forage diets. The current definition and ruminal pH threshold for SARA varies between studies. However, SARA generally occurs when ruminal pH remains within the range of 5.2-6.0 for a prolonged period (Li et al.

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2013). SARA is characterised by daily episodes of low ruminal pH (Krause and Oetzel 2006), during which rumen pH is depressed for several hours a day (Plaizier et al. 2014) due to an accumulation of volatile fatty acids and insufficient rumen buffering.

Subclinical ketosis (SCK) in dairy cows is a metabolic disorder that occurs in the period around calving. In this period, the energy requirements of an individual cow can exceed her energy intake, resulting in a negative energy balance (NEB) (Grummer 1995). An NEB results in an increase of non-esterified fatty acids and beta-hydroxybutyrate levels in the blood. A cow is considered to have SCK when the beta-hydroxybutyrate level is higher than 1.2-1.4 mmol/l blood, but shows no clinical signs of ketosis (Raboisson et al 2014). According to Tatone et al. (2017), automatic milking systems (AMS) have been associated with increased in-herd prevalence diseases, as well as showing increased odds of ketosis in multiparous animals at first test. According to Kaufman et al. (2016), rumination monitoring across the transition period may contribute to the identification of SCK and other health problems in multiparous cows.

As of 2014, over 25,000 farms globally were using AMS (Barkema et al, 2015) and this number continues to grow. Regarding health management, a key advantage of AMS is the availability of daily, cow-level data that can be collected by AMS and associated technologies. As a result, Tse et. al (2017) reported that, after transitioning to AMS, 66% of producers changed their health management strategy and 80% of producers found illness detection to be easier than prior to transitioning due to the AMS and associated health-monitoring software. On the other hand, some of the main barriers which are preventing the adoption of the technology by dairy producers include the claim that such technology is not easy to use, that it provides too much information without any clearly recommended action, and that its performance must be evaluated through independent research (Borchers and Bewley 2015). According to Stangaferro (2017), the overall sensitivity and timing of AMS alerts for cows with metabolic and digestive disorders indicated that AMS which combines rumination and activity could be a useful tool when it comes to identifying cows with metabolic and digestive disorders. The negative effects of health disorders have been well documented for conventional herds, but less is known about these effects in AMS herds (King and DeVries 2018). When functioning optimally, an AMS permits cows voluntarily to visit a robotic milking unit multiple times a day to be milked without requiring human intervention (Tse et al. 2017). The demonstrated benefits of AMS include increased milk production, improved cow comfort, a more flexible lifestyle for producers, less labour required for milking (Hansen 2015), improved cow health, and also more interesting or less routine activities for the producer (Woodford et al. 2015).

According to King and DeVries (2018), future studies should (1) incorporate the entire lactating herd whilst accounting for the stage of lactation and parity of each animal; (2) evaluate the deviations that cows exhibit from their own baseline trajectories and relative to healthy contemporaries; (3) combine the use of several variables into health alerts; and (4) differentiate the probable type of health disorder. Soriani et al. (2012) found that automatic measurement of RT is useful when it comes to predicting calving time and to quickly obtaining information on the health status of the animals in a period that is as critical as the transition phase. In fact, Soriani et al. (2013) were able to identify a positive association between milk yield and rumination time. Further, rumination behaviour could be a promising indicator when it comes to tracking metabolic conditions that are associated with a decrease in DMI (Soriani et al. 2012), such as subclinical ketosis (Kaufman et al. 2018). According to our previous research results, the rumination time, subsequent yield, and milk trait changes depend upon the period of lactation and reproductive status of a dairy cow (Antanaitis et al. 2018). However, ability levels were variable when it came to the proposed algorithms that are able to detect each specific disease (Paudyal et al. 2018). Schirmann et al (2016) state that further research is necessary to be able to reach a better level of understanding in relation to the onset of behavioural changes and the relationship between rumination and disease.

We hypothesised that some biomarkers from AMS such as rumination time, milk yield, bodyweight, milk temperature, the milk fat-to-protein ratio, and the electrical conductivity of milk at the udder quarters-level have associations with subclinical acidosis (SARA) and subclinical ketosis (SCK).

Materials and Methods

Location, animals, and experimental design

The experiment was carried out on a dairy farm in the eastern region of Europe at 56 00 N, 24 00 E, between20/01/2018 and 01/12/2018 using Lithuanian Black and White fresh dairy cows (n=711). These were selected according to those which fitted a profile of having had a second or more lactations. The cows were kept in a loose housing system, and were fed total mixed ration (TMR) throughout the year at the same time, balanced according to their physiological needs. Feeds took place every day at 6:00 am and 6:00 pm.

Measurements

The fresh cows (1-30 days of milk (DIM)) were milked with Lely Astronaut® A3 milking robots with free traffic. To motivate the cows to visit the robot, 2 kg/d of concentrates were fed to them by the milking robot. The rations were calculated according to physiological standards. Daily milk yield, rumination time, bodyweight, milk fat milk protein ratio, milk temperature and electric milk conductivity (from all udder quarters) were all collected from the Lely T4C management program for the purposes of analysis.

Determining health status

During the course of the study, all of the fresh dairy cows (n=711) were examined according to the general clinical investigation plan. Blood samples were collected from the coccygeal vein 1×/wk, and blood BHB concentrations were measured in millimoles per litre using a Freestyle Optium H Glucometer. Blood samples were taken each week at the same time relative to feeding (between two and six hours after feeding). Approximately 500 ml of rumen fluid was collected from all animals. The collections of rumen fluid were conducted using a stomach tube a total of three hours after the morning feed. Ruminal pH levels were measured immediately after the sampling, using a handheld pH-meter (Horiba - Twin pH, Spectrum Technologies, Japan). AMS was used to register daily milk F/P.

SCK group (n=132)

Cows were classified as being SCK when at least one BHB reading during the 30 day post-calving period was at ≥1.2mmol/L (McArt et al, 2012). Milk F/P for that group of cows was >1.2. Ruminal pH remained at > 6.

These cows were without any clinical sign of other diseases after calving (metritis, lameness, mastitis, displaced abomasum, indigestion (an average rectal temperature of +38.8°C, rumen motility five-six times per three minutes).

SARA group (n=402).

The milk F/P of this group of cows was F/P<1.2. Cows were classified to this group when all BHB readings during the 30 day post-calving period were at <1.2 mmol/L. SARA occurs when ruminal pH levels remain within the range of 5.2-6.0 for a prolonged period (Li et al. 2013). These cows were without any clinical sign of other diseases after calving (metritis, lameness, mastitis, displaced abomasum, indigestion (an average rectal temperature of +38.8°C, rumen motility five-six times per three minutes).

Healthy group (n=177)

Without any clinical sign of disease after calving. Cows were classified in this group when all BHB readings during the 30 day post-calving period were at <1.2mmol/L. The average milk F/P for this group of cows was F/P=1.2. Ruminal pH remains > 6.

The cows were divided into three groups by Coon et al (2018) according to RT: RT<432 (group 1, n=442), 432≤RT≥479 (group 2, n=228), and RT>479 (group 3, n=41).

Data analysis and statistics

Data from the tested cows were analysed in IBM SPSS statistics Version 20.0 for Windows. Distributions included productivity levels of the cows (MY), milk fat and protein ratios (F/P), bodyweight (BW), milk temperature (MT), and the electrical conductivity of milk (EC) at the udder quarters-level: left front (LF), left right (LR), rear left (RL), and rear right (RR), all of these factors having been assessed according to the Kolmogorov-Smirnov test. The descriptive statistics (mean, standard error, and 95% confidence interval), and linear relationship between normal distributed indicators (Pearson's correlation coefficient) were all evaluated. To compare the evaluated traits between groups, a one-factor analysis of variance (ANOVA) was applied. The differences between the means were evaluated according to Fisher's LSD criterion. Student's T-Test was used to find statistical differences between groups. We also used the $\chi2$ test to determine whether there is a significant relationship between two categorical variables. The results were considered to be reliable at p<0.05.

Results

In our study we established that the average rumination time for the all cows that were included in the evaluation was 417.12±1.66 mins/d body weight was 667.19±3.46 kg, temperature was 38.00±0.04°C, electrical conductivity of milk was 69.82±0.19 µS/cm, and MY was 35.91±0.41 kg. The average milk electrical conductivity for the front quarters of the udder was 69.76±0.193 µS/cm, while the rear quarters were 69.88±0.190 µS/cm.

During our study, the statistically significant RT was positively correlated with MY (r=0.302, p=0.000) and milk temperature (r=0.025, p=0.050), and was negatively correlated with the fat and protein ratio (r=-0.125, p=0.000). A statistically unreliable RT correlation was calculated in terms of body weight (r=0.015, p=0.589). The results that could be taken

Table 1. Milk production, temperature, and bodyweight of cows according to RT group.

Variable	RT	M	SE	95% CI	
				Min	Max
MY	1	33.859 [a]	0.505	32.867	34.850
	2	38.846 [b]	0.686	37.499	40.193
	3	41.265 [b]	1.665	37.996	44.534
BW	1	665.861	4.553	656.920	674.802
	2	663.642	6.183	651.501	675.784
	3	673.892	15.009	644.421	703.363
MT	1	37.969 [a]	0.036	37.899	38.040
	2	38.156 [b]	0.049	38.060	38.251
	3	38.335 [b]	0.118	38.103	38.568

The sub-groups labelled [ab] refer to significant differences at p <0.05

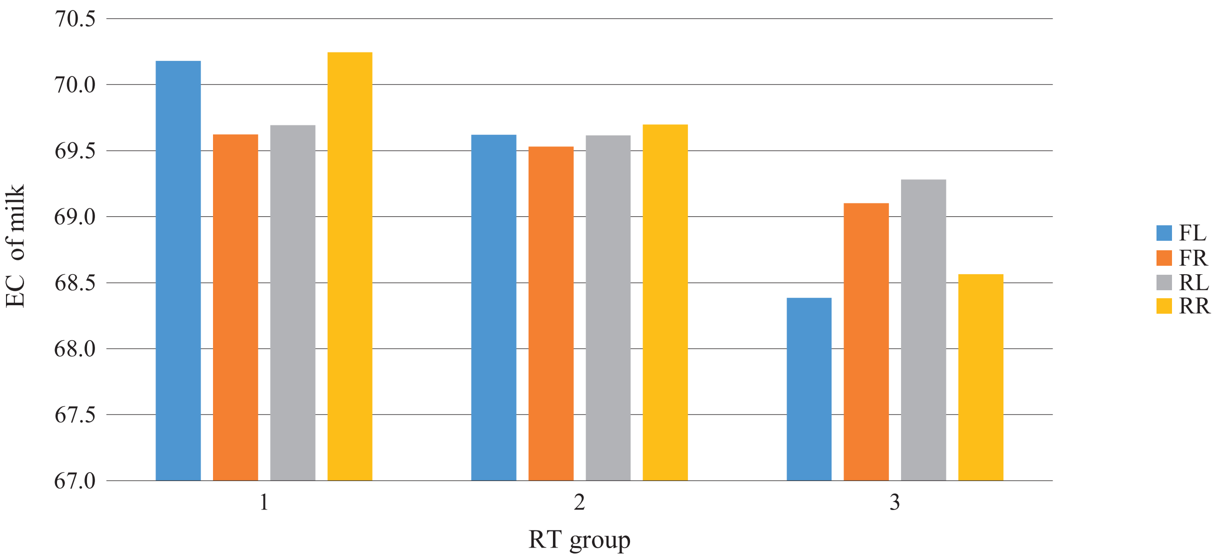

Fig. 1. Milk EC (µS/cm) at the udder quarter level by group of cows.

from the descriptive statistics for those traits that were investigated are presented in Table 1 and Fig. 1. The cows of group 1 accounted for 62.2% of the total, while group 2 made up 32.1% and group 3 just 5.8%. According to the one-way ANOVA test, the RT group had a statistically significant impact on the milk production of cows (p=0.000) and the temperature of the milk (p=0.010). We did not find a statistically significant influence for any specific RT group in terms of weight changes in cows (p=0.863). The cows of group 3 were estimated to have the highest milk yield (21.87% higher when compared with group 1, p=0.000) and milk temperature (1.21% higher than group 1, (p=0.003), and also the greatest weight of the animals themselves (0.96% higher than group 1, p=0.609).

The cows of group 3 (according to RT) (see Fig. 1) demonstrated the lowest electrical conductivity of milk at the udder quarter level- ranging from 68.4µS/cm (FL quarter) to 69.3µS/cm (RL quarter), while the cows of group 1 had the highest level of electrical conductivity in their milk - between 69.6 µS/cm (FR quarter) to 70.2 µS/cm (FL and RR quarters). However, the difference between quarters in terms of EC was statistically unreliable. In 8.9% of udder quarters we estimated EC>80 µS/cm (in 9.7 % of the udder quarters in the cows of group1, in 7.9 % in group 2, and in 4.9 % in group 3).

A statistically significant RT could be related to F/P (p=0.004). F/P=1.2 was found in 43.9% of the cows of group 3, and in 21.5% of the cows of group 1 (Table 2). The cows of the HLT group demonstrated the highest levels of RT and the lowest MT (Table 3). The average BW for these cows was 10.81kg lower (1.64%) when compared to the SARA group (p=0.021) and 13.88 kg greater (2.10%) when compared to those of the SCK group (p=0.319); MY was 4.733 kg lower (14.01%) when compared to those of SARA (p=0.0001) and 2.17kg higher (6.42%) in comparison with those of SCK (p=0.965).

Table 2. The relationship between RT and F/P.

RT	Health status		
	SARA (F/P<1.2)	HLT (F=/P 1.2)	SCK (F/P >1.2)
1 (RT<432)	64.0%	21.5%	14.5%
2 (RT432-479)	54.3%	24.9%	20.8%
3 (RT>479)	39.0%	43.9%	17.1%
$\chi 2$= 15.204, df=4, p=0.004			

Table 3. Rumination time, milk production levels, temperature, and the bodyweight of cows according to health status.

Variable	Health status	M	SE	95% CI	
				Min	Max
BW	SARA	671.704 [a]	4.727	662.421	680.986
	HLT	660.891 [b]	7.290	646.576	675.206
	SCK	647.010 [b]	8.885	629.561	664.458
MY	SARA	38.504 [a]	0.516	37.490	39.517
	HLT	33.771 [b]	0.796	32.208	35.334
	SCK	31.604 [b]	0.970	29.698	33.509
RT	SARA	419.919 [a]	2.247	415.506	424.332
	HLT	422.103 [a]	3.465	415.298	428.908
	SCK	405.990 [b]	4.224	397.696	414.285
MT	SARA	38.138 [a]	0.035	38.070	38.206
	HLT	37.955 [b]	0.053	37.850	38.060
	SCK	38.033 [b]	0.065	37.906	38.161
EC_FL	SARA	69.652 [a]	0.367	68.932	70.373
	HLT	69.750 [a]	0.566	68.639	70.861
	SCK	70.057 [a]	0.690	68.703	71.412
EC_FR	SARA	69.771 [a]	0.272	69.237	70.305
	HLT	69.346 [a]	0.419	68.523	70.169
	SCK	68.743 [a]	0.511	67.740	69.746
EC_RL	SARA	69.871 [a]	0.258	69.364	70.377
	HLT	69.487 [a]	0.398	68.706	70.268
	SCK	69.086 [a]	0.485	68.134	70.038
EC_RR	SARA	69.825 [a]	0.375	69.089	70.561
	HLT	69.744 [a]	0.578	68.608	70.879
	SCK	70.390 [a]	0.705	69.007	71.774

The sub-groups labelled [ab] refer to significant differences at $p<0.05$

Discussion

The automatic monitoring of intake and rumination has shown some promise in terms of the detection of health problems after calving (Schirmann et al. 2016). According to Calamari et al. (2014), the utility value in monitoring RT around the time at which calving takes place and, in particular, during the first week of lactation, is a way in which identification can be made in a timely fashion for those cows that are at a greater level of risk when it comes to developing diseases in early lactation. White et al. (2017) reported that the mean ruminating time was 436 mins/d (n = 179), ranging between 236 and 610 mins/d, and Zebeli et al. (2006) reported that mean ruminating time was 434 mins/d, ranging between 151 and 630 mins/d. The duration of individual rumination can be developed so that it automatically detects post-calving health problems which include ketosis and metritis (Steensels et al. 2017). According to Liboreiro et al. (2015), although differences could be observed in RT and activity levels between populations of cows that had already developed periparturient diseases and those that were healthy, further experiments are necessary to be able to determine how RT and activity data may be used to specifically diagnose individuals that will develop such periparturient diseases.

Rumination behaviour therefore, could be an excellent tool in the early detection of SARA. In the literature, rumination behaviour is mainly expressed by minutes per kilogram of dry matter intake (DMI). However, there is a difference in total rumination time and rumination time per kg DMI. To use rumination behaviour as a tool to predict SARA, the ruminal pH must correlate with rumination behaviour. In the case of SARA, the correlation between total time spent on ruminating and ruminal pH has not been extensively investigated (Humer et al. 2018).

Ruminal acidosis is a nutritional disorder of high-producing dairy cows, particularly in early lactation, when the energy density of diets is increased to meet their high nutritional requirements, and feeding excessively fermentable diets increases the risk of depressed rumen pH. Plaizier et al. 2008 stated that ruminal pH affects the growth of microbial populations and the physiological functions of the rumen, and it is also possible that greater rumen acidity would be related to different RT. In fact, differences in RT can affect saliva secretion, which seems to be greater during eating than during resting and helps to stabilize rumen pH by buffering the organic acids produced during the fermentation of carbohydrates (Sudeb Saha et al. 2019). In the current research, we found that the average daily RT seems to be unaffected by differences in rumen acidity. In a subclinical acidosis challenge trial, Khiaosa-ard et al. (2018) observed that cows susceptible to SARA had longer ruminating and total chewing times compared with tolerant cows (Sudeb Saha et al. 2019). Increased RT could, therefore, be interpreted as a sign of a cow's resilience and a way to counterbalance the decrease in rumen acidity with increased saliva production (Sudeb Saha et al. 2019).

Depressed rumen pH has been linked to lower feed intake and milk yield and an increased risk of liver abscesses and lameness. Depressed ruminal pH has frequently been associated with lower milk fat content. Experimentally-induced subclinical acidosis reduced the milk fat percentage in some studies, but not in others.

Numerous studies have been carried out to evaluate the accuracy of EC for predicting infection status, and several authors have concluded that it has some potential in terms of detecting mastitis (Norberg et al. 2004). According to Norberg (2005) EC has been used solely for the purpose of monitoring the mastitis status of dairy cows. On a positive note, EC has been proven to show genetic variation. A few studies indicate that the genetic correlation between EC and mastitis is high, and therefore the trait may be suitable for use in the genetic evaluation of mastitis. Postpartum disorders such as SARA and subclinical ketosis have a negative effect not only on milk yield and reproduction, but also on cow immunity. Elevated somatic cell count (SCC) (over 300000 cells/ml) after calving could increase milk conductivity. It is extremely important to detect postpartum disorders as early as possible and to use only trustworthy techniques. Timely assessment of cow condition prevents complications that occur in the postpartum period. It is absolutely necessary to detect diseases effectively as the majority of postpartum complications occur in the subclinical form (Antanaitis et al. 2015). According to Antanaitis et al. (2010), walking activities and milk conductivity, depending upon season and lactation stage, could be used as early predictors of reproductive disorders in lactation.

The available literature has firmly established that promoting chewing increases salivary secretion in dairy cows, which in turn helps to reduce the risk of acidosis (Beauchemin 2018). The greatest significant differences occurred three days before any diagnosis in rumination duration, and one day before any diagnosis in terms of activity and milk yield. These results indicate that a model can be developed to automatically detect post-calving health problems including ketosis and metritis, based on rumination duration, activity levels, and milk yield (Steensels et al. 2017). Kaufman et al. (2016) found that the largest differences in rumination time between healthy and SCK cows could be seen during cows which were at weeks -1, +1, and +2 which ruminated for 48 ± 17.2, 73 ± 16.0, and 65 ± 19.4 mins per day less than healthy cows, respectively. Kaufman et al. (2016) recently demonstrated that cows which were diagnosed with SCK after calving tend to ruminate less than healthy cows during the week before and the week after calving. Related to this is increased milk fat: protein (FP) ratios which are associated with a negative energy balance and may be indicative of SCK (Jenkins et al. 2015). Kaufman et al (2018) found that for every thirty minute decrease in daily RT, the FP ratio increased by 0.026 units. Increased FP ratios have been associated with SCK (Jenkins et al. 2015). Therefore this current association may reflect the findings by Kaufman et al. (2016) that multiparous cows with SCK tended to ruminate less than did healthy cows. According to Duffield (2000), higher milk fat content could be found in cows with SCK. Kaufman et al. (2018) found that cows with SCK in second and ≥third lactations produced, on average, 0.2% more fat than HLT cows, but this difference was not statistically significant ($p \geq 0.2$), possibly because of a lack of energy as described by Kaufman et al. (2016). Kaufman et al. (2018) also found no association between milk protein content and RT. Rumination time was found to be positively associated with milk yield in early-lactation dairy cows, across all parities (Kaufman el al. 2018). An overall increase in reticulorumen pH in week four is expected given the fact that the greatest risk of SARA occurring is immediately after calving (Penner et al. 2007). According to Coon et al. (2019), the risk of developing SARA had no effect on DMI or DMI as a percentage of BW, which is consistent with the findings by Gao and Oba (2016) who found no difference in DMI between higher and lower risk mid-lactation cows which were being fed a high-grain diet. Cows which were experiencing rumen acidosis were seen to ruminate less than healthy cows. DeVries et al. (2009) reported that rumination time in dairy cows was decreased on day one following an acidosis challenge by more than ninety minutes when compared with the baseline figure despite increased feeding activity and similar DMI. Schirmann et al. (2016) observed differences in pre-calving rumination and feeding behaviour. Consistent reductions in rumination activity, both within the cow and relative to healthy mate cohorts, were observed for each health disorder on the day of diagnosis. To be able to detect the lower proportion of high-risk cows that were ruminating subsequent to an acidosis challenge, DeVries et al. (2009) noted that the percentage of cows that were ruminating would have had to be observed on, and would have had to have averaged over, 48 individual minutes before a determination could be made on whether the percentage truly varied from the baseline figure. Milk fat depression could be seen in cows that were experiencing low rumen pH levels (Alzahal et al. 2010). Consequently, milk and milk fat production usually decreased in those cows that were suffering from SARA (Krause and Oetzel 2006). Rumen acidity can alter the rumen microorganism composition, which affects the ruminal fermentation pattern and the acetate to propionate ratio, leading to alterations in milk components, particularly the milk fat content. The association between ruminal pH, VFA concentration, and milk composition is controversial, as some authors found that rumen pH depression is related to a reduction in milk fat content, while others did not. Rumen volatile fatty acids (VFAs) are the basis of the de novo synthesis of fatty acids in the udder, and are also on the basis for changes in the milk fatty acids profile due to alterations in rumen biohydrogenation of polyunsaturated fatty acids, which occurs in the presence of low ruminal pH and may depress milk fat synthesis (Sudeb Saha et al 2019). A substantial variation exists in the severity of SARA being experienced in lactating dairy cows that have been fed with the same high-grain diet, and cows which are tolerant to a high-grain diet may be characterised by less sorting behaviour but less chewing time, and higher milk urea nitrogen concentration levels (Gao and Oba 2014). Weber et al. (2013) showed that high liver fat levels and high concentrations of NEFA post-partum were correlated to a BW loss. Similar dynamics have been described in other studies which looked at commercial herds, where a greater loss in the BCS score was encountered in hypocalcaemic cows (Martinez et al. 2012). The length of the dry period affects energy metabolism levels around the calving period in dairy cows, as well as milk production levels in subsequent lactation (Weber et al. 2015).

According to our previous research, in those results that were shown for cows which were suffering from ketosis, the milk yield had a tendency to decrease by its most significant amount for the last eight days of their clinical symptoms, and in cows which were suffering with acidosis the milk yield tended to decrease by its most significant amount for six days before the emergence of their clinical symptoms (Antanaitis et al. 2015). In the current study, greater rumen acidity was related to a significant reduction in milk yield of cows with SARA to nearly 3 kg/d lower than that of healthy cows (Sudeb Saha et al. 2019). According to the available data, each case of ketosis results in a milk loss of up to 10 kg per day. In general, there is consensus that a negative association exists between hyperketonemia and milk production (Antanaitis et al. 2010). Cows which were subjected to thermoneutral conditions (18°C, 60% RH) and hot conditions (32°C, 50% RH) in climatic chambers tended to exhibit sharp increases in rectal temperature when exposed to the hot environment, and increases were at their greatest for early lactation cows which were at high production levels when compared with lower yielding mid and late-lactation cows (West et al. 2003).

Conclusions

According to the results of the present study we can conclude that biomarkers from AMS as indicators of SARA can be shorter rumination time before clinical signs, milk conductivity and lower than 1.2 F/P ratio. Indicators of subclinical ketosis can be bodyweight, RT, milk yield and higher than 1.2 F/P ration.

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