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Can rumination time and some blood biochemical parameters be used as biomarkers for the diagnosis of subclinical acidosis and subclinical ketosis?

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SUMMARY

According to the past reports, the utility value of monitoring rumination time (RT) around the time at which calving takes place and, in particular, during the first week of lactation, is a way of identifying in a timely fashion those cows that are at a greater level of risk when it comes to developing disease in early lactation. Recent reports have focused on the role of minerals in disease resistance in ruminants, but little is known about the concentrations blood parameters in dairy cows with subclinical acidosis and subclinical clinical ketosis. According this we hypothesised that rumination time and some blood biochemical parameters (including cortisol and lactate) can serve as biomarkers for subclinical acidosis (SARA) and subclinical ketosis (SCK). Accordingly, the aim of the current study was to determinate the impact of subclinical acidosis and ketosis on rumination time and some blood biochemical parameters.

For the current study, of a total of 225 fresh dairy cows (between one and sixty days after calving) a general clinical examination produced a selection of 93 cows: ten of these were diagnosed with SARA, thirteen had SCK and seventy were clinical healthy cows. Rumination time (RT), body weight (BW), and milk yield (MY) were registered with the help of Lely Astronaut® A3 milking robots. It was determining the concentrations of blood serum albumin (Alb), total protein levels (TP), glucose (Glu), urea (Urea), calcium (Ca), phosphor (Phos), iron (Fe), alaninaminotranspherase (ALT), aspartataminotranspherase (AST), Gammagliutamyltranspherase (GGT), and creatinine (Cre).

RT decreases and blood lactate rates increase in cases of SARA and SKC, while in cases of SARA the total blood protein levels increased and in the SCK group it decreased.A similar trend of differences between the SARA group and the SCK group in terms of healthy cows could be found in changes in blood urea, glucose, Ca, Mg, P, and Fe. Cows in the SCK group showed statistically higher ALB content levels, while the activity of AST and Crea was at a lower level.

According to this, rumination time, and some blood biochemical parameters can be used as biomarkers in the diagnosis ofsubclinical acidosis and ketosis. Future studies, however, are needed so that these results can be compared across a greater number of animals

1. Introduction

The automatic monitoring of intake and rumination showed some promise for the detection of health problems after calving (Schirmann et al., 2016). Calamari, Soriani, Panella, PetreraF. and Trevisi (2014), the utility value of monitoring RT around the time at which calving takes place and, in particular, during the first week of lactation, is a way of identifying in a timely fashion those cows that are at a greater level of risk when it comes to developing disease in early lactation. According Abdela (2016), sub-acute ruminal acidosis (SARA) is one of the most important metabolic diseases in the modern dairy industry, one which impairs cow health and performance even in well-managed and high-yielding dairy cows. Subacute ruminal acidosis is a metabolic disorder which can mainly be found in high-production dairy cows that are fed highly fermentable diets. One of the consequences of a decreased rumen pH is a shift of the composition of volatile fatty acids (VFA) in the rumen, which may be affected by the rumen flora. In response to the changes in ruminal pH, the bacteria in the rumen shift their pathway in the production of VFA. In case of a decreased ruminal pH, the bacteria reduce the synthesis of acetic acid and increasepropionic and butyric acid. A decreased rumen pH therefore has a negative effect on the acetate to propionate ratio. In general, propionic

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acid is the most important substrate for glucose and therefore an important source of energy for the production of milk (Van der Wier, 2018). A prolonged rumen pH level that is below 5.8 can lead to problems in animal health (such as laminitis and liver abscess), and production losses due to milk fat depression and reduced DMI (Nocek, 1997). A reduced dry matter intake (DMI) is commonly seen in SARA. These factors are self-limiting and cause a fluctuating feed intake. On the long term, the low ruminal pH also reduces the fibrolytic bacteria in the rumen and therefore reduces the fiber digestibility in the rumen. Due to the reduced fiber digestibility of the rumen, the total amount of feed intake and the ration efficiency both decrease. Consequently, the uptake of energy and nutrients from the ration are reduced and the cows are more susceptible for a negative energy balance (NEB). The reduced energy and nutrient intake also affect the body condition score negatively (Van der Wier, 2018). In some of the dairy herds, milk production can temporarily appear to be increased by overfeeding with grain and causing SARA to remain an important dairy cow problem. For these reasons, the cattle should be regularly monitored to facilitate the early recognition of the condition and to limit the economic losses that are associated with SARA (Abdela, 2016). Schirmann et al. (2016) observed differences in pre-calving rumination and feeding behaviour. In cows affected by SARA, a fluctuating feeding pattern has been described as the most consistent symptom (E. Humer, Aschenbach, Neubauer, Kröger & Khiaosa-ard, 2018). These cows typically refuse feed intake after eating their initial meal due to a dramatic decline in ruminal pH. Because chewing is closely related to the intake of physically effective fibre, monitoring of chewing activity has also been seen as a more practicable method to identify high-risk cows at an early stage as well as to evaluate structural fibre adequateness of diets. Alternatively, studies have reported lower values (<32 chewing min/kg of DMI) in dairy cows with expectedly healthy rumen fed only 40% concentrates. This also applies when considering the chews per bolus (instead of chewing time) as health indicator. Indeed, measurements of chewing parameters such as chews per bolus are much easier under farm conditions to do, because there is no need to measure DMI of individual cows. Healthy cows are assumed to chew at least 50 times per bolus. In fact, several studies have reported a decreased number of regurgitated bolus as well as ruminating chews per bolus, with increasing concentrate levels. However, several authors observed ≥ 50 chews per bolus in cows fed diets containing 60%–70% concentrates. Interestingly, we recently observed an even higher number of chews per bolus in SARA-susceptible cows compared to tolerant cows without SARA, fed the same diet, which might be due to temporal regulatory mechanisms to counteract the decline in ruminal pH. When looking at other chewing parameters, the SARA-susceptible cows, however, had less ruminating bolus per hour (as an average of the day) and slightly lower eating chews. It has also been suggested that dairy cows after experiencing SARA alter their diet sorting behavior in terms of consuming more forage to attenuate their rumen fermentation disorder. Moreover, cows seem to change their diurnal rumination pattern towards reduced rumination during the day, while they increase rumination during the night when excessive amounts of concentrates are fed (E. Humer et al., 2018). 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 (Paudyal et al., 2018). According Kaufman, LeBlanc, McBride, Duffield, and DeVries (2016), rumination monitoring across the transition period may contribute to the identification of sub-acute ketosis (SCK) and other health problems in multiparous cows. According Pedersen et al. (2010), are sponder that was strapped to the left-hand side of the neck with its microphone positioned so that it picked up the characteristic sounds of mandibular movement during rumination was still unable to identify cows that were at risk of ketosis in early lactation; therefore further work is envisaged in this area. 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 in RT and activity between populations of cows that have already developed periparturient diseases and those that were healthy could be observed, although further experiments are necessary to be able to determine how RT and activity data may be used to precociously diagnose individuals that will develop such periparturient diseases.

Recent reviews have focused on the role of trace minerals in disease resistance in ruminants a number of antioxidants and trace minerals have important roles in immune function and may affect health in transition dairy cows (Spears & Weiss, 2008). Recent reports have focused on the role of minerals in disease resistance in ruminants but little is known about the concentrations blood serum albumin, total protein, glucose, urea, calcium, phosphor, iron, alaninaminotranspherase, aspartataminotranspherase, gammagliutamyltranspherase and creatinine in dairy cows with subclinical acidosis and subclinical clinical ketosis. These blood traits usually reflect cows physiological conditions. And any changes from the norm in these traits show cows health problems. Cortisol is used as an indicator of stress and pain and elevated serum cortisol has been shown in calves castrated without local anaesthesia (Thüer et al., 2007), in surgical stress in dairy cows in endotoxin mastitis and metritis (Kulcsar et al., 2005), in cows suffering from inflammatory foot lesions (Almeida, Weber, Burton, & Zanella, 2008) and in cows with hypocalcemia (Waage, Sjaastad & Blom, 1984). It can be hypothesized that the known relation between stressful periparturient diseases in high yielding dairy cows and SARA and SCK may be mediated through a concurrent increased cortisol secretion leading to hyperglycaemia. As mammalian organisms have no specific mechanism for the metabolization of d-lactate, accumulation of this isomer in blood following absorption from the rumen may give rise to metabolic acidosis (Gentile, Rademacher, Seemann, & Klee, 1998).

We hypothesised that rumination time and some blood biochemical parameters (including cortisol and lactate) can serve as biomarkers for subclinical acidosis (SARA) and subclinical ketosis (SCK). Accordingly, the aim of the current study was to determinate the impact of subclinical acidosis and ketosis on rumination time and some blood biochemical parameters.

2. Materials and methods

2.1. 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 01/01/2019 using Lithuanian Black and White fresh dairy cows ($n = 225$). 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. Cows were fed a TMR consisting of 30% corn silage, 10% grass silage, 4% grass hay, and 50% grain concentrate mash. Diets were formulated according to the NRC (2001)) to meet or exceed the requirements of a 550 kg Holstein cow producing 35 kg/d. Total mixed ration was fed to the cows twice per day at 10:00 am and 08:00 pm.

2.2. Determining health status

For the current study, of a total of 225 fresh dairy cows a general clinical examination produced a selection of 93 cows: ten of these were diagnosed with subclinical acidosis (SARA), thirteen had subclinical ketosis (SCK), and seventy were clinical healthy cows. AMS was used to register daily milk F/P. After calving, cows health was monitored for 1 d to 60 days by researchers.

2.2.1. ***SCK group*** ($n = 13$)

Cows were classified as being SCK when at least one BHB reading during the 30 day post-calving period was at ≥1.2 mmol/L Milk F/P for that group of cows was >1.2. Without any clinical sign of another 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).

2.2.2. ***SARA group*** *(n = 10)*

The milk F/P of that group of cows was $F/P<1.2$. Cows were classified in this group when all BHB readings during the 30 day post-calving period were at <1.2 mmol/L. Without any clinical sign of another diseases after calving (metritis, lameness, mastitis, displaced abomasum, indigestion (an average rectal temperature of +38.8 °C, rumen motility less 3 times per three minutes).

2.2.3. ***Health group*** *(n = 70)*

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 <1.2 mmol/L. The average milk F/P for this group of cows was at $F/P = 1.2$.

We differentiate the clinical status of cows as healthy and sick in all investigated time. The SCK and SARA were diagnosed in different parts of time, but this time was from 1 to sixty days after calving. All cows were sick or healthy in all investigated period.

The cows were milked with Lely Astronaut® A3 milking robots with free traffic. To be able to motivate the cows to visit the robot, a total of 2 kg/d of concentrates were fed to them by the milking robot. Rumination time (RT), body weight (BW), and milk yield (MY) were registered with the help of Lely Astronaut® A3 milking robots.

Blood samples for measuring biochemical indices were taken from the coccygeal vessels in four stages. Blood samples were taken at 10:00 am, prior to feeding taking place. The blood samples were collected into vacuum test tubes (BD Vacutiner, Great Britain). The samples were delivered for examination to the Large Animal Clinic's Laboratory of Clinical Tests at the Veterinary Academy of the Lithuanian University of Health Sciences, and were centrifuged for five minutes at a speed of 3000 rpm. The blood serum that was obtained was examined using the Hitachi 705 analyser (Hitachi, Japan), and DiaSys reagents (Diagnostic Systems GmbH, Germany), determining the concentrations of blood serum albumin (Alb), total protein levels (TP), glucose (Glu), urea (Urea), calcium (Ca), phosphor (Phos), iron (Fe), alaninaminotranspherase (ALT), aspartataminotranspherase (AST), Gammagliutamyltranspherase (GGT), and creatinine (Cre).

Plasma ketone body levels were found by using the Medi Sense and Free Style Optium H systems (Abbott, Great Britain) by taking a sample of capillary blood at the ear. Blood examples were taken and checking BHB concentration one time a day, 7 days after calving. All examples were taken during clinical examination.

The blood cortisol concentration levels were tested with a Tosh Corporation AIA-360, using the fluorescence enzyme immunoassay method for cortisol analysis. Lactate concentrations were tested with a Lactate Pro2 ® using the enzyme electrode method. Compatible reagents were Lactate Pro 2 Test Strips (sensors with an electrode for measuring lactates in the blood).

2.3. *Statistical analysis*

The data set that was obtained was statistically evaluated by SPSS 20.0 for Windows. The distributions for quantitative data (milk yield, body weight, RT, and blood parameters in the cows) were tested by means of the Kolmogorov-Smirnovtest. All of the results were stated as mean (M) ± standard deviation (SD). For the analysis of the influence of RT on the investigated traits, the cows were selected according to the RT level sand were placed in the following classes: 1) <400mins per day, 2) 400–450mins per day, 3) 450–500mins per day, and 4) >500 mins per day.A one-way ANOVA test was used to define the influence of health status and RT class on the evaluated traits, plus a $\chi 2$ test for an evaluation of the relationship between the health status of cows and their RT class. The significance of any differences in the samples according to the health status of the cows was something that was evaluated using the *t*-test. The results were considered to be statistically significant at $P < 0.05$.

Table 1
The dependence of rumination time in dairy cows on their health status.

Health status	Statistic M	SD	Distribution of cows (%) by RT: 400<	400–450	450–500	>500
SARA	429.67 [a]	30.278	0.0	66.7	33.3	0.0
Health	448.91 [a]	62.648	26.1	17.4	30.4	26.1
SCK	391.75 [b]	95.188	50.0	0.0	50.0	0.0
Statistic	ANOVA; $P = 0.023$		$\chi 2=23.946$, df=6, $P = 0.001$			

Explanations: ‘M'readings with various superscript letters show significant differences in terms of $P < 0.05$.

3. Results and discussion

3.1. *The impact of sara and sck on rumination time (RT)*

We found that the healthy cows showed the longest RT when compared to the SARA group (being longer by 4.29%) and the SCK group (longer by 12.73%, $P<0.05$). The relationship between the health status of the cows and RT was established as being statistically significant ($P = 0.001$). These results are summarised in Table 1. According Schirmann et al. (2016), the automatic monitoring of intake and rumination showed some promise when it comes to the detection of health problems after calving.. Compared to healthy cows, those with SCK and metritis +SCK had lower dry matter intake during the precalving period and continued to eat less until days fourteen and twenty postpartum, respectively. On a herd level, numerous observations of the proportion of cows that were ruminating at any one time would need to be taken in order to accurately be able to detect an acute bout of acidosis using changes in rumination behaviour. Overall, these results suggest that the risk of acidosis may have little overall effect on general behaviour, with the exception of rumination (DeVries, Beauchemin, Dohme, & Schwartzkopf-Genswein, 2009). A substantial variation exists in the severity of SARA among lactating dairy cows that have been fed with the same high-grain diet, and that 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 & Oba, 2014). Feed restriction and long particles (≥19 mm) have a greater effect on eating time, whereas the intake of forage-neutral detergent fibre and medium particles (between 4–19 mm) affects rumination time. 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, and milk yield (Steensels et al., 2017). Kaufman et al. (2016) found that the largest differences in rumination time between healthyand SCK cows could be seen during weeks-1, +1, and +2, when HYK+ cows ruminated 48 ± 17.2, 73 ± 16.0, and 65 ± 19.4 mins per day less than HLT cows, respectively.

3.2. *The impact of sara and sck on rumination time, and rt on blood cortisol and lactate concentrations and biochemical blood parameters*

This investigation has shown (in Table 2) the highest concentrations of cortisol in the blood of healthy cows - being 2.44% higher when it is compared to the SARA group and 66.83% higher when compared to the

Table 2
An analysis of blood cortisol and lactate concentration.

Trait	Health status	M	SD	Influence of health status	Influence of RT
Lactate (mmol/l)	SARA	1.001 [a]	0.055	$P = 0.920$	$P = 0.028$
	Health	0.945 [a]	1.127		
	SCK	1.083 [a]	0.278		
Cortisol (ug/dl)	SARA	0.340 [a]	0.055	$P = 0.099$	$P = 0.002$
	Health	1.025 [b]	0.787		
	SCK	1.000 [ab]	0.521		

Explanations: 'M'readings with various superscript letters show significant differences in terms of $P < 0.05$.

SCK group ($P<0.05$). Remnant, Tremlett, and Huxley, (2017) state that assisted parturition due to dystocia in cattle is considered both by farmers and veterinary surgeons to be a painful and stressful event. According Viitasaari, Raekallio, and Heinonen (2014), biochemical markers are, in addition, potentially rather crude indicators of stress and pain in animals. Milk cortisol levels are at their highest during the first week of lactation, and this remains at comparable levels thereafter (Gellrich, Sigl, Heinrich, & Wiedemann, 2015). Hernandez et al. (2014) found that social separation and unfamiliar surroundings are stressful situations which can result in an increase in cortisol secretion in cattle. According our results the SARA and SCK had no effect on cortisol levels in the cows blood.

Healthy cows showed the lowest levels of lactate concentration in the blood. The cows in the SCK group showed the highest levels (6.98% higher when compared to the SARA group and 12.23% higher with healthy cows). We estimated a significantly influence by RT on blood lactate and cortisol concentrations (Table 2). Figueiredo, Nydam, Perkins, Mitchell, and Divers, (2006) suggest that plasma L-lactate concentrations may be a useful predictor of productive outcomes in cows which have right-sided abomasal disorders. Harmon, Britton, Prior, and Stock (1985) detected that, when found to have low rumen pH levels and high blood D-lactate levels (with a peak of 4.8 mM, at 26 h), G-animals experienced only a mild acid-base disturbance which was accompanied by a six-fold increase in the rate of D-lactate absorption, whereas L-lactate absorption increased by only 70% despite higher rumen levels.

SARA increased the total blood total protein by 1.70%, while SCK decreased by 6.63% ($P<0.05$).A similar trend in terms of differences could be seen between the SARA group and the SCK group, with healthy cows being found to have changes in blood urea ($P<0.05$), Mg ($P<0.05$), and Fe ($P<0.05$). Health status and RT had a significant effect on Mg and Fe content. We estimated that the Mg content in healthy cows was higher by 4.18%, while Fe content was up by 20.86% when compared to the SARA group. The cows in the SCK group showed 16.03% lower levels of Mg and 23.66% of Fe (Table 3). Total protein concentration increased with parity, mainly because of an increase in globulin concentration (Bobbo et al., 2017). The periparturient or transition period of four weeks before and four weeks after calving is characterised by a greatly increased risk of disease (DeGaris et al., 2008). Studies of the effect of any exposure to well-designed pre-calving diets have shown that substantial improvements in production, reproduction, and animal health can in fact be made (DeGaris et al., 2008). The mean plasma iron concentrations showed a gradual increasing trend from day fourteen prepartum to days 28–42 postpartum; however, the group difference was not significant overall or in any of the intervals (Theodore, Panchal, Dhami, Patel, & Ramani, 2016).

The cows in the SCK group showed statistically higher levels of ALB content (104.33%), the activity of AST (24.66%) and Crea (22.41%), and lower levels of ALT (55.85%) ($P<0.05$) in comparison with the healthy cow group.

We detected that there were statistically reliable differences ($P<0.05$) for ALT between SARA (35.09% higher) and healthy cows.

Table 3
An analysis of blood protein, glucose, and mineral contents.

Trait	Health status	M	SD	Influence of health status	Influence of RT
Total protein	SARA	55.600 [a]	1.064	$P = 0.016$	$P = 0.204$
	Health	54.673 [a]	4.629		
	SCK	51.050 [b]	1.595		
UREA	SARA	5.220 [a]	0.479	$P = 0.026$	$P = 0.981$
	Health	4.633 [a]	0.562		
	SCK	4.733 [b]	1.025		
Glu	SARA	4.540 [a]	0.369	$P = 0.599$	$P = 0.474$
	Health	4.453 [a]	0.224		
	SCK	4.438 [a]	0.311		
Ca	SARA	2.570 [a]	0.052	$P = 0.598$	$P = 0.092$
	Health	2.533 [a]	0.156		
	SCK	2.505 [a]	0.137		
Mg	SARA	0.897 [a]	0.087	$P = 0.001$	$P = 0.003$
	Health	0.861 [a]	0.122		
	SCK	0.723 [b]	0.107		
Phos	SARA	2.173 [a]	0.377	$P = 0.176$	$P = 0.076$
	Health	1.981 [a]	0.302		
	SCK	1.943 [a]	0.206		
Fe	SARA	27.667 [a]	2.901	$P = 0.000$	$P = 0.021$
	Health	22.891 [b]	5.422		
	SCK	17.475 [c]	5.040		

Explanations: 'M'readings with various superscript letters show significant differences in terms of $P < 0.05$.

Table 4
An analysis of blood enzyme activity levels and albumin content.

Trait	Health status	M	SD	Influence of health status	Influence of RT
ALB	SARA	33.367 [a]	3.073	$P = 0.125$	$P = 0.620$
	Health	33.063 [a]	2.265		
	SCK	34.495 [b]	0.682		
ALT	SARA	25.667 [a]	4.093	$P = 0.000$	$P = 0.038$
	Health	19.000 [b]	4.426		
	SCK	21.000 [c]	1.954		
AST	SARA	97.667 [a]	16.800	$P = 0.001$	$P = 0.393$
	Health	113.913 [a]	28.677		
	SCK	142.000 [b]	31.723		
Crea	SARA	78.130 [a]	9.208	$P = 0.000$	$P = 0.558$
	Health	76.840 [a]	8.448		
	SCK	94.060 [b]	10.118		
GGT	SARA	19.420 [a]	11.668	$P = 0.007$	$P = 0.587$
	Health	12.358 [b]	7.412		
	SCK	8.468 [b]	5.343		
ALK Phos	SARA	165.000 [a]	75.974	$P = 0.817$	$P = 0.005$
	Health	153.652 [a]	59.680		
	SCK	148.250 [a]	53.459		

Explanations: 'M'readings with various superscript letters show significant differences in terms of $P < 0.05$.

Likewise there were differences for GGT (in terms of the SARA group, being 57.15% higher). A statistically significant effect of RT was found in the activity of ALT and ALK Phos (Table 4). Farid, Honkawa, Fath, Nonaka, and Horii (2013) indicated that the serum AST activity in cows with a fatty liver was significantly higher than in normal cases, but ALP and GGT activities did not significantly increase, while Sevinc, Basoglu, Birdane, and Boydak (2001) reported that the serum activities of AST, GGT, and CPK were increased significantly in cows with a fatty liver. The length of the dry period also affected plasma concentrations for urea, cholesterol, aspartate transaminase, and glutamate dehydrogenase (Weber et al., 2015).Albumin can be considered as being a negative acute-phase protein (Fleck, 1989), with subnormal concentrations indicating impaired liver function, following a diverted synthesis to positive acute-phase proteins (Bertoni, Trevisi, & Han, 2008). Accordingly, the lower concentrations of albumin (and the albumin-to-globulin ratio) may indicate impaired liver function in cows.

Table 5
An analysis of productivity levels and bodyweight in cows.

Trait	Health status	M	SD	Influence of health status	Influence of RT
Milk yield (kg)	SARA	22.83[a]	7.802	$P = 0.008$	$P = 0.000$
	Health	33.26 [b]	8.932		
	SCK	34.25 [b]	12.914		
Body weight (kg)	SARA	730.00 [a]	74.282	$P = 0.012$	$P = 0.107$
	Health	665.57 [b]	77.785		
	SCK	718.00 [a]	66.004		

Explanations: 'M'readings with various superscript letters show significant differences in terms of $P < 0.05$.

3.3. The impact of SARA, sck and rt on productivity and body weight

The lowest productivity levels in cows was estimated for the SARA group (amounting to 31.36% lower when compared to healthy cows, $P < 0.05$), with the highest rating going to the SCK group (2.98% higher when compared to healthy cows, $P < 0.05$). On the other hand, the cows in the SARA group had the greatest bodyweight, amounting to 9.68% greater when compared to healthy cows ($P < 0.05$), and 1.64%greater when compared to the SCK group. Consequently, we estimated a statistically reliable impact for the health status of cows in terms of productivity levels ($P = 0.008$) and bodyweight ($P = 0.012$). A statistically significant effect in relation to RT ($P = 0.000$) was observed in the milk yield of cows (Table 5). Similar dynamics have been described in other studies which looked at commercial herds, where a greater loss in the BCS score was encountered in hypocalcemic cows (Martinez et al., 2012). According to Antanaitis, Žilaitis, Juozaitienė, and Žiogas (2010), walking activity and milk conductivity, depending upon season and lactation stage, could be used as early predictors of reproductive disorders in lactation. According to our previous research, in the results that were shown for cows which were suffering with 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, Žilaitis, Kučinskas, Juozaitienė, & Leonauskaitė, 2015). 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 a consensus that a negative association exists between hyperketonemia and milk production (Antanaitis et al., 2010).

On the basis of our results we can conclude that rumination time decreases and blood lactate rates increase in cases of SARA and SKC, while in cases of SARA the total blood protein levels increased and in the SCK group it decreased.A similar trend of differences between the SARA group and the SCK group in terms of healthy cows could be found in changes in blood urea, glucose, Ca, Mg, P, and Fe. Cows in the SCK group showed statistically higher ALB content levels, while the activity of AST and Crea was at a lower level.

According to this, rumination time, and some blood biochemical parameters can be used as biomarkers in the diagnosis ofsubclinical acidosis and ketosis.Futurestudies, however,are needed so that these results can be compared across a greater number of animals.

Declaration of Competing Interest

None.

References

Abdela, N. (2016). Sub-acute ruminal acidosis (SARA) and its consequence in dairy cattle: A review of past and recent research at global prospective. *Achievements in the Life Sciences, 10*(2), 187–196.

Almeida, P. E., Weber, P. S., Burton, J. L., & Zanella, A. J. (2008). Depressed dhea and increased sickness response behaviors in lame dairy cows with inflammatory foot lesions. *Domest Anim Endocrinol, 34*, 89–99 10.1016.

Antanaitis, R., Žilaitis, V., Juozaitienė, V., & Žiogas, V. (2010). Sveikatos būklės, sezono ir laktacijos įtaka karvių judrumo, masės, produkcijos ir pieno elektrinio laidumo pokyčiams. *Veterinarija ir zootechnika, 49*(71), 3–7.

Antanaitis, R., Žilaitis, V., Kučinskas, A., Juozaitienė, V., & Leonauskaitė, K. (2015). Changes in cow activity, milk yield, and milk conductivity before clinical diagnosis of ketosis, and acidosis. *Veterinarija ir Zootechnika, 70*(92), 3–9.

Beauchemin, K. A. (2018). Invited review: Current perspectives on eating and rumination activity in dairy cows. *Journal of dairy science, 101*(6), 4762–4784.

Bertoni, G. E., Trevisi, X., & Han, Bionaz M. (2008). Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. *Journalofdairyscience, 91*(9), 3300–3310.

Bobbo, T. E., Fiore, M., Gianesella, M., Morgante, L., Gallo, P. L., Ruegg, G., et al. (2017). Variation in blood serum proteins and association with somatic cell count in dairy cattle from multi-breed herds. *Animal : an International Journal of Animal Bioscience, 11*(12), 2309–2319.

Calamari, L. U. I. G. I., Soriani, N., Panella, G., PetreraF., Minuti A., & Trevisi, E. (2014). Rumination time around calving: An early signal to detect cows at greater risk of disease. *Journal of dairy science, 97*(6), 3635–3647.

DeGaris, P. J., & Lean, I. J. (2008). Milk fever in dairy cows: A review of pathophysiology and control principles. *The Veterinary Journal, 176*(1), 58–69.

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

Farid, A. S., Honkawa, K., Fath, E. M., Nonaka, N., & Horii, Y. (2013). Serum paraoxonase-1 as biomarker for improved diagnosis of fatty liver in dairy cows. *BMC Vet Res, 9*(1), 73.

Figueiredo, M. D., Nydam, D. V., Perkins, G. A., Mitchell, H. M., & Divers, T. J. (2006). Prognostic value of plasma llactate concentration measured cow side with a portable clinical analyzer in holstein dairy cattle with abomasal disorders. *Journal of veterinary internal medicine, 20*(6), 1463–1470.

Fleck, A. (1989). Clinical and nutritional aspects of changes in acutephase proteins during inflammation. *Proc. Nutr. Soc. 48*(3), 347–354.

Gao, X., & Oba, M. (2014). Relationship of severity of subacute ruminal acidosis to rumen fermentation, chewing activities, sorting behavior, and milk production in lactating dairy cows fed a high-grain diet. *Journal of dairy science, 97*(5), 3006–3016.

Gellrich, K., Sigl, T., Heinrich, H. D., & Wiedemann, S. (2015). Cortisol levels in skimmed milk during the first 22 weeks of lactation and response to short-term metabolic stress and lameness in dairy cows. *J Animal Sci and Biotech, 31*(1), 1–7.

Gentile, A., Rademacher, G., Seemann, G., & Klee, W. (1998). Systemische auswirkungen der pansenazidose im gefolge von pansentrinken beim milchkalb – Retrospektive Analyse von 293 fällen. *Tierärztl. Praxis, 26*, 205–209.

Harmon, D. L., Britton, R. A., Prior, R. L., & Stock, R. A. (1985). Net portal absorption of lactate and volatile fatty acids in steers experiencing glucose-induced acidosis or fed a 70% concentrate diet ad libitum. *Journal of Animal Science, 60*(2), 560–569.

Hernandez, C. E., Thierfelder, T., Svennersten-Sjaunja, K., Berg, C., Orihuela, A., & Lidfors, L. (2014). Time lag between peak concentrations of plasma and salivary cortisol following a stressful procedure in dairy cattle. *Acta Veterinaria Scandinavica, 56*(1), 61.

Humer, E., Aschenbach, J. R., Neubauer, V., Kröger, I., & Khiaosa-ard, R. (2018). Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. *J Anim Physiol Anim Nutr, 2018*(102), 380–392.

Kaufman, E. I., LeBlanc, S. J., McBride, B. W., Duffield, T. F., & DeVries, T. J. (2016). Association of rumination time with subclinical ketosis in transition dairy cows. *Journal of Dairy Science, 99*(7), 5604–5618.

Kulcsar, M., Janosi, S., Lehtolainen, T., Kátai, L., Delavaud, C., Balogh, O., et al. (2005). Feeding-unrelated factors influencing the plasma leptin level in ruminants. *Domest Anim Endocrinol, 29*(1), 214–226.

Liboreiro, D. N., Karine, S. M., Paula, R. B. S., Milton, M., Maturana, T. K., & Nishimura, A. P. (2015). Characterization of peripartum rumination and activity of cows diagnosed with metabolic and uterine diseases. *Journal of Dairy Science, 98*(10), 6812–6827.

Martinez, N., Risco, C. A., Lima, F. S., Bisinotto, R. S., Greco, L. F., & Ribeiro, E. S. (2012). Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. *Journal of Dairy Science. 95*(12), 7158–7172 1.

Nocek, J. E. (1997). Bovine acidosis: Implications on laminitis. *Journal of Dairy Science, 80*, 005–1028.

Paudyal, S., Maunsell, F. P., Richeson, J. T., Risco, C. A., Donovan, D. A., & Pinedo, P. J. (2018). Rumination time and monitoring of health disorders during early lactation. *Animal : an international Journal of Animal Bioscience, 12*(7), 1484–1492.

Pedersen, A. (2010). Rumination measurements and ketosis in early lactation. *Dansk Veterinaertidsskrift, 93*(14), 28–32.

Remnant, J. G., Tremlett, A., & Huxley, J. N. (2017). Clinician attitudes to pain and use of analgesia in cattle: Where are we 10 years on? *Vet Rec, 181*(15), 400.

Schirmann, K., Weary, D. M., Heuwieser, W., Chapinal, N., Cerri, R. L. A., & vonKeyserlingk, M. A. G. (2016). Rumination and feeding behaviors differ between healthy and sick dairy cows during the transition period. *Journal of Dairy Science, 99*(12), 9917–9924.

Sevinc, M., Basoglu, A., Birdane, F., & Boydak, M. (2001). Liver function in dairy cows with fatty liver. *Revue Med Vet, 152*, 297–300.

Spears, J. W., & Weiss, W. P. (2008). Role of antioxidants and trace elements in health and immunity of transition dairy cows. *Vet J, 176*, 70–76.

Steensels, M., Maltz, E., Bahr, C., Berckmans, D., Antler, A., & Halachmi, I. (2017). Towards practical application of sensors for monitoring animal health: The effect of post-calving health problems on rumination duration, activity and milk yield. *Journal of Dairy Research, 84*(2), 132–138.

Theodore, V. K., Panchal, M. T., Dhami, A. J., Patel, J. A., & Ramani, V. P. (2016). Influence of peripartum nutritional supplementation on plasma macro-micro minerals profile and postpartum fertility in crossbred cows. *International J. of Sci. Environ. and Techno, 5*(6), 4052–4060.

Thüer, S., Mellema, S., Doherr, M. G., Wechsler, B., Nuss, K., & Steiner, A. (2007). Effect of local anaesthesia on short- and long-term pain induced by two bloodless castration methods in calves. *Vet J, 173*(2), 333–342.

Viitasaari, E., Raekallio, M., & Heinonen, M. (2014). The effect of ketoprofen on postpartum behaviour in sows. *Appl Anim Behav Sci, 158*, 16–22.

Waage, S., Sjaastad, O. V., & Blom, A. K. (1984). Plasma concentrations of cortisol in cows with hypocalcaemia in relation to their responses to treatment with calcium. *Res Vet Sci, 36*(2), 164–168.

Weber, C., Losand, B., Tuchscherer, A., Rehbock, F., Blum, E., Yang, W., et al. (2015). Effects of dry period length on milk production, body condition, metabolites, and hepatic glucose metabolism in dairy cows. *Journal of Dairy Science, 98*(3), 1772–1785.

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