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ORIGINAL ARTICLE

Evaluation of blood adiponectin levels as an index for subacute ruminal acidosis in cows: a preliminary study

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Abstract

The objective of this study was to evaluate blood levels of various hormones and compounds related to energy metabolism in cows with subacute ruminal acidosis (SARA). We investigated 11 lactating cows presumed to have SARA based on duration of ruminal pH <5.6 and reticulum pH <6.3 in 2015–2016. Kraft pulp (KP) was used to supplement feed of 7 of the cows studied in an effort to reduce SARA. We continuously monitored ruminal pH and measured blood concentrations of hormones and metabolites related to energy metabolism. Blood measurements included glucose (GLU), total cholesterol (TC), free fatty acid (FFA), insulin, adiponectin (ADN), malate dehydrogenase (MDH), and lactate dehydrogenase (LDH). Additionally, we analyzed milk data (milk yield, milk fat percentage, milk protein percentage, milk urea nitrogen, and protein fat ratio) and reproduction data. The results demonstrated that ADN levels at 4 weeks post-parturition correlated with the total amount of time that the ruminal or reticulum fluid pH was under the threshold during 1 week post-parturition, as well as the numbers of days the cows were diagnosed with SARA (SARA-positive days) up to 30 days post-parturition. SARA-positive days in 2016 were higher than those in 2015. In both years, numbers of SARA-positive days for cows supplemented with KP were lower than those for cows without KP. Increased ADN levels may be a compensatory reaction to frequent SARA which modulates the inflammatory response against high LPS levels and improves insulin resistance caused by LPS. ADN may serve as an estimative index for SARA.

Keywords Dairy cows · Subacute ruminal acidosis · Ruminal pH · Continuous measurement · Wireless radio transmission · Adiponectin

Introduction

Subacute ruminal acidosis (SARA) is a common health problem in dairy herds globally (Kleen et al. 2003; Enemark 2008). Previous studies showed that point prevalence surveys of SARA at time of rumenocentesis were 11% (O'Grady et al. 2008), 13.8% (Kleen et al. 2009), and 20% (Kleen et al. 2013); prevalence on individual farms was 0–38% (Kleen et al. 2009). SARA is considered to be a herd problem because its clinical signs are manifested in the herd rather than in an individual (Kleen et al. 2003). The clinical signs of SARA are subtle and are often temporally separated from the initiating event; thus, making it difficult to diagnose (Humer et al. 2018). SARA results in severe economic damage to the livestock industry. Financial losses caused by SARA result from liver abscess, laminitis, reduced milk fat percentage, and other factors (Kleen et al. 2003; Plaizier et al. 2008). Monitoring ruminal pH is useful in the diagnosis of SARA because SARA is characterized by repeated occurrences of low ruminal pH (Plaizier et al. 2008). In recent years, continuous monitoring of ruminal pH with a wireless radio transmission pH measurement system has become possible, facilitating easier measurements (Sato et al. 2012b, c).

Furthermore, SARA induces ruminal LPS release and triggers an acute phase inflammatory response (Gozho et al. 2005). Tumor necrosis factor-alpha secreted by macrophages induces insulin resistance (Li et al. 2007; Ohtsuka et al. 2001). We hypothesized that the concentrations of circulating hormones and metabolites related to the metabolism of fatty acids

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may be good indicators of the impact of SARA on inflammatory processes and metabolic diseases.

In this study, ruminal pH was monitored continuously using a wireless radio transmission pH measurement system, and blood concentrations of hormones and metabolites were measured in cows with SARA of varying severity to generate an estimative index for SARA in lactating cows.

Materials and methods

Survey of SARA prevalence

For the past 5 years, we have investigated the prevalence of SARA in certain farms in the Yamagata Prefecture region in Japan. Our current study involved 11 Holstein-Friesian transition cows that were suspected to be affected by SARA at the S farm located in the Yamagata Prefecture. Six cows were investigated in 2015, and the remaining 5 were investigated in 2016 (Table 1). We investigated all delivered cows of the S farm that gave birth in the same season in both years. In an investigation in 2014, SARA was detected in approximately 80% of transition cows at the S farm (Maeda 2016), based on the common criteria for SARA diagnosis (ruminal pH <5.6 or reticulum pH <6.3 for >3 h/day). The rate of SARA in our transition cows was assumed to be equal to the previously reported rate. All experiments were conducted with informed consent from the farmer overseeing the S farm. All procedures and protocols were performed in accordance with the ethical standards of the research animal ethical committee of Nippon Veterinary and Life Science University.

Rearing method

S farm is a tie-stall barn with 50 Holstein cows that had three separate feeding regimens for dry cows, control lactating cows, and lactating cows whose feed included kraft pulp (KP). Compositions of feeds used at the S farm are presented in Tables 2 and 3. KP is a nutrient-rich feed that is almost pure cellulose fiber. To yield KP from wood chips, lignin was selectively removed, as it is indigestible by cows. The energy value of KP is considered equal to that of corn; it has a slow digestion rate, and is thought to prevent SARA (Nishimura et al. 2019). Cows whose feed was supplemented with KP were randomly chosen from among the 11 cows included in this study.

Measurement of ruminal pH and assessment of pH parameters

Ruminal pH was measured using a wireless radio transmission system reported previously (Sato et al. 2012b, c). The wireless radio transmission system consisted of a pH sensor, a data measurement receiver, a relay unit, and a personal computer with special software (YCOW-S; DKK-Toa Yamagata, Yamagata, Japan). pH sensors were orally administered to cows, and the pH was measured every 10 min. Locations of sensors were checked with a metal detector. One cow had a sensor in the rumen; the other 10 cows had sensors in the reticula. SARA is defined as ruminal fluid pH <5.6 or reticulum fluid pH <6.3 that continues for more than 3 h/day (Gozho et al. 2005; Sato et al. 2012a; Kimura 2013). To manage longitudinal data and control for the effect of time on the measured variables, we calculated the following two pH parameters: low pH time, which is the total amount of time for ruminal pH <5.6 or reticulum pH <6.3 during 1, 4, and 8 weeks after parturition (1w-low pH time, 4w-low pH time and 8w-low pH time); and SARA-positive days, which is the number of days diagnosed with SARA based on the above-described criteria, in the 30 days after parturition. Then, the cows were divided into two groups based on the 4w-low pH time and the numbers of SARA-positive days in the 30 days after parturition: high-value group (n = 4; >25,900 min 4w-low pH time, and > 25

Table 1 Age and reproductive history of 11 cows investigated in this study

Year	Cow number	Birth date	Age	Reproductive history	Administration of kraft pulp
2015	No. 1	1/23/2011	4	2	No
	No. 2	12/23/2010	4	3	No
	No. 3	12/11/2010	4	2	Yes
	No. 4	12/2/2008	6	3	No
	No. 5	9/8/2012	3	1	Yes
	No. 6	10/20/2012	3	1	Yes
2016	No. 7	1/30/2010	6	3	No
	No. 8	4/3/2012	4	1	Yes
	No. 9	1/19/2013	3	1	Yes
	No. 10	1/3/2012	4	2	Yes
	No. 11	1/7/2012	4	2	Yes

Table 2 Feed ingredients used in S farm, 2015 and 2016

Ingredients	Dry cows	Lactating cows	
		Control	With kraft pulp
2015			
Cornflake	1 kg	1 kg	1 kg
Dent corn silage	3–4 kg	8 kg	8 kg
Grass silage		4 kg	4 kg
Rice whole crop silage	6–7 kg	7 kg	7 kg
Oat hay	2 kg	2 kg	
Lucerne hay	3 kg	5 kg	5 kg
Dehydrated brewers grain	5 kg	2 kg	2 kg
Concentrated feed	2 kg	8 kg	8 kg
Vitamin additive		50 g	50 g
Calcium phosphate		150 g	150 g
Sodium bicarbonate pellet		150 g	150 g
Kraft pulp			2 kg
2016			
Dent corn silage		8 kg	8 kg
Grass silage		4 kg	4 kg
Rice whole crop silage	3–5 kg	7 kg	7 kg
Oat hay	2 kg	2 kg	
Lucerne hay	1–2 kg	5 kg	5 kg
Dehydrated brewers grain	2 kg	2 kg	2 kg
Concentrated feed	2 kg (close up)	8 kg	8 kg
Vitamin additive		50 g	50 g
Calcium phosphate		150 g	150 g
Sodium bicarbonate pellet		150 g	150 g
Timothy hay	Full feeding		
Concentrated feed for dry cows	2 kg		
Kraft pulp			2 kg

d SARA-positive), and low-value group (n = 4; ≤25,900 min 4w-low pH time and ≤ 25 d SARA-positive) (Table 4). Three cows that showed inconsistent data with unusual conditions of the pH sensor were excluded.

Table 3 Dietary ingredient composition at S farm

Ingredient	2015		2016	
	Dry cow	Lactating cow	Dry cow	Lactating cow
Water (%)	46.5	38.1	32.2	38.8
TDN/DM (%)	63.5	65.3	61.7	64.4
CP/DM (%)	15.3	15.2	14.8	15.4
ADF/DM (%)	27.0	23.6	30.1	24.4
NDF/DM (%)	40.6	34.4	51.6	35.4
NFC (NSC)/DM (%)	31.6	37.4	23.1	35.9
NDF/NFC	1.3	0.9	2.2	1.0
eNDF/DM (%)	39.5	32.7	51.0	33.7
Roughage DM/BW (%)	1.1	2.2	1.2	2.2
Starch/DM(%)	16.8	21.1	9.6	19.1

TDN: total digestible nutrients; DM: dry matter; CP: crude protein; ADF: acid detergent fiber; NDF: neutral detergent fiber; NFC: non-fiber carbohydrate; eNDF: effective neutral detergent fiber; BW: body weight

Table 4 Two groups divided based on the total amount of time for ruminal fluid pH <5.6 or reticulum fluid pH <6.3 during 4 weeks after parturition (4w-low pH time) and the number of SARA-positive days in 30 days after parturition

Group	4w-low pH time (min)	Number of SARA-positive days	Number of heads	Breakdown	
				Control	With kraft pulp
High-value group	>25,900	>25	4	3	1
Low-value group	≤25,900	≤25	4	1	3
Median	25,900	25.5			

8 cows were divided based on two pH parameters, total amount of time for ruminal pH <5.6 or reticulum pH <6.3 during 4 weeks after parturition and the number of SARA days in 30 days after parturition. 3 cows that showed error data with unusual condition of pH sensor were eliminated

4w-low pH: total amount of time for ruminal fluid pH < 5.6 or reticulum fluid pH < 6.3 during 4 weeks after parturition; SARA-positive days: days defined as SARA by duration time for ruminal pH <5.6 or reticulum pH <6.3

Measurement of blood parameters related to energy metabolism

Blood samples were obtained from the tail veins of 11 cows at 1, 4, and 8 weeks after parturition. All samples were collected between 9:00 AM and 5:00 PM. Serum was harvested by centrifugation at 800×g for 10 min at 4 °C and stored at −80 °C until use. Glucose (GLU), total cholesterol (TC), and free fatty acid (FFA) were measured using an autoanalyzer (AU680, Access 2; Beckman Coulter, Inc., Brea, CA, USA). Insulin and adiponectin (ADN) concentrations were determined with a commercial kit (Insulin ELISA kit; Shibayagi Co., Ltd., Gunma, Japan, and Mouse/Rat Adiponectin ELISA kit; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan, respectively). Serum malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) activities were measured using the methods described by Kaloustian et al. (1969) and Bergmeyer and Bernt (1974), respectively. One unit was defined as 1 μmol of substrate degraded per min. The MDH activity/LDH activity (M/L ratio) was calculated to indicate energy usage (Arai et al. 2003; Li et al. 2012). Revised quantitative insulin sensitivity check index (RQUICKI) is considered a useful indicator of insulin sensitivity (Holtenius and Holtenius 2007) and was calculated using the following formula.

$$\mathrm{RQUICKI} = 1/\left(\log\left(\mathrm{Gb}\right) + \log\left(\mathrm{Ib}\right) + \log\left(\mathrm{FFAb}\right)\right)$$

Where

Gb plasma glucose (mg/dL)
Ib plasma insulin (μU/mL)
FFAb plasma FFA (mmol/L)

Assessment of milk parameters and reproduction performance

Individual milk yield, milk fat percentage, milk protein percentage, milk urea nitrogen (MUN), and protein:fat ratio (P/F) were recorded at a single point between 50 and 80 days in milk (DIM) from data of the herd tests published by the Livestock Improvement Association of Japan. Adjusted milk yields in the entire milking period were also recorded. Individual delivery intervals were obtained from a breeding ledger.

Statistical analysis

Normality tests for ADN concentration and the number of SARA-positive days were assessed using the Shapiro-Wilk test and the Kolmogorov-Smirnov test along with a histogram and a Q-Q plot. Statistical analysis of the relationships between low pH time, GLU, TC, FFA, M/L ratio, and ADN was conducted by multiple regression analysis. Variables were chosen by stepwise selection using AIC. Multicollinearity of the model was assessed by VIF. Spearman's rank correlation coefficient was calculated between the ADN concentration and the number of SARA-positive days. The Friedman test was used to determine the differences in blood parameters at 1, 4, and 8 weeks after parturition. Milk production and

Table 5 Multiple regression analysis of blood parameters and pH parameters

Independent variables	β	t
1w-low pH	0.747*	2.95
F	8.71*	
R^2	0.521	
Adjusted R^2	0.461	
Degrees of freedom	8	
N	10	

Multiple regression analysis was conducted (dependent variable: ADN concentration at 4 weeks after parturition; independent variable: glucose, total cholesterol, free fatty acid, malate dehydrogenase activity/lactate dehydrogenase activity at 4 weeks after parturition and 1w-low pH time)

β indicates standard partial regression coefficient

1w-low pH time: total amount of time for ruminal fluid pH <5.6 or reticulum fluid pH <6.3 during 1 week after parturition

*$p < 0.05$

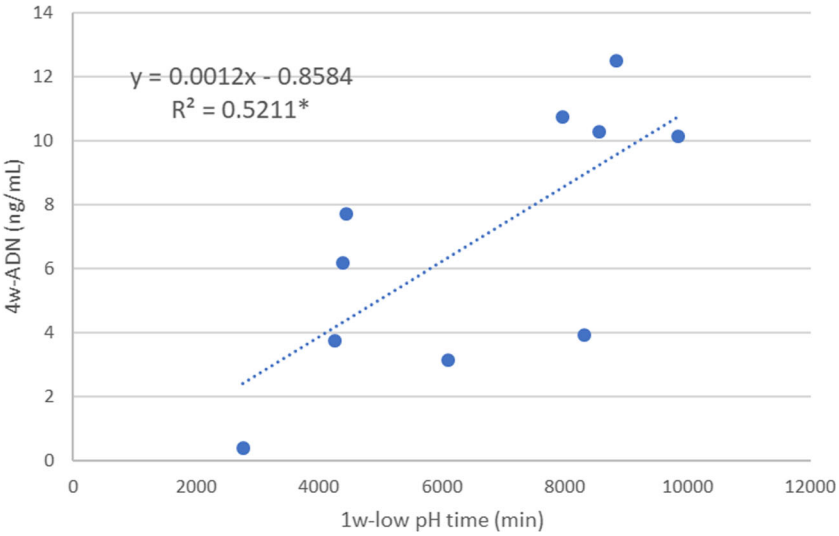


Fig. 1 Relationship between 1w-low pH time and ADN concentrations at 4 weeks after parturition. Values are presented as regression analysis. 1w-low pH time was identified as a factor associated with the ADN concentration at 4 weeks after parturition. ADN: adiponectin; 1w-low pH time: total amount of time for ruminal pH <5.6 or reticulum pH <6.3 during 1 week after parturition; 4w-ADN: ADN concentration at 4 weeks after parturition. *Statistical significance of the model (F test, $p < 0.05$)

reproduction performance in high-value versus low-value groups were compared using the Mann-Whitney U test. Statistical significance was set at $p < 0.05$. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

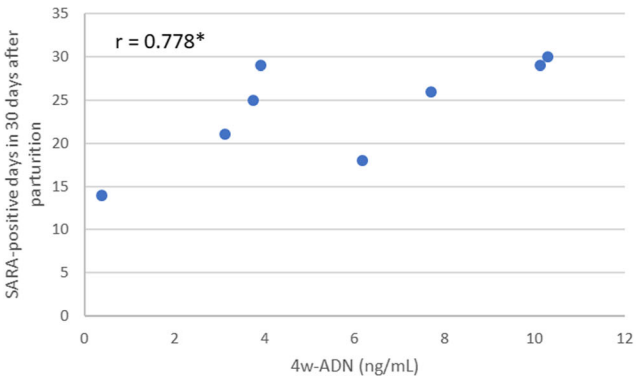
Results

Relationships between ADN and pH parameters

Table 5 and Fig. 1 show the results of multiple regression analysis (dependent variable: ADN concentration at 4 weeks after parturition; independent variable: other blood parameters and 1w-low pH time). The ADN concentration followed a normal distribution. Adjusted R-square in this model using stepwise selection was 0.461 ($p < 0.05$). 1w-low pH time was identified as a factor associated with the ADN concentration at 4 weeks after parturition ($p < 0.05$). Figure 2 presents the relationships between the number of SARA-positive days and the ADN concentration at 4 weeks or the average of ADN concentrations at 1 and 4 weeks after parturition. The number of SARA-positive days did not follow a normal distribution, based on the asymmetry of the histogram. Spearman's rank correlation coefficient between the number of SARA-positive days and the ADN concentration at 4 weeks was 0.778 ($p < 0.05$), whereas it was 0.731 ($p < 0.05$) for the average of ADN concentrations at 1 and 4 weeks after parturition. This indicated a strong correlation between ADN levels and the number of SARA-positive days.

Relationships between the frequency of SARA and blood parameters

Groups based on low pH time and the frequency of SARA were compared using the Friedman test (Fig. 3). TC was



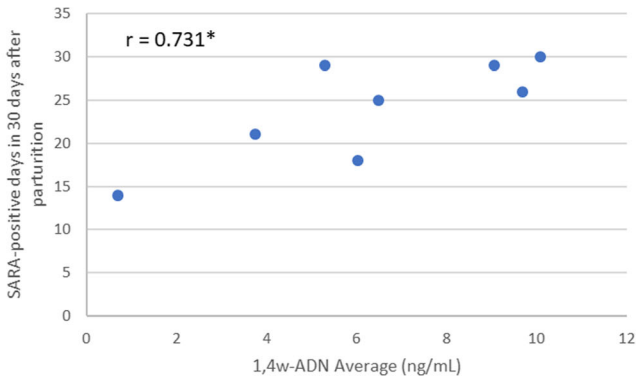


Fig. 2 Relationship between the number of SARA-positive days in 30 days after parturition and ADN concentration. Values are presented as correlation analysis. Spearman's rank correlation coefficient between the number of SARA-positive days and the ADN concentration indicated a strong correlation between the two. SARA-positive days: days defined as SARA by duration time for ruminal pH <5.6 or reticulum pH <6.3; 1-4w ADN average: average of ADN concentration at 1, 4 weeks after parturition; 4w-ADN; ADN concentration at 4 weeks after parturition. *Significant correlation based on the Spearman's rank correlation coefficient ($p < 0.05$)

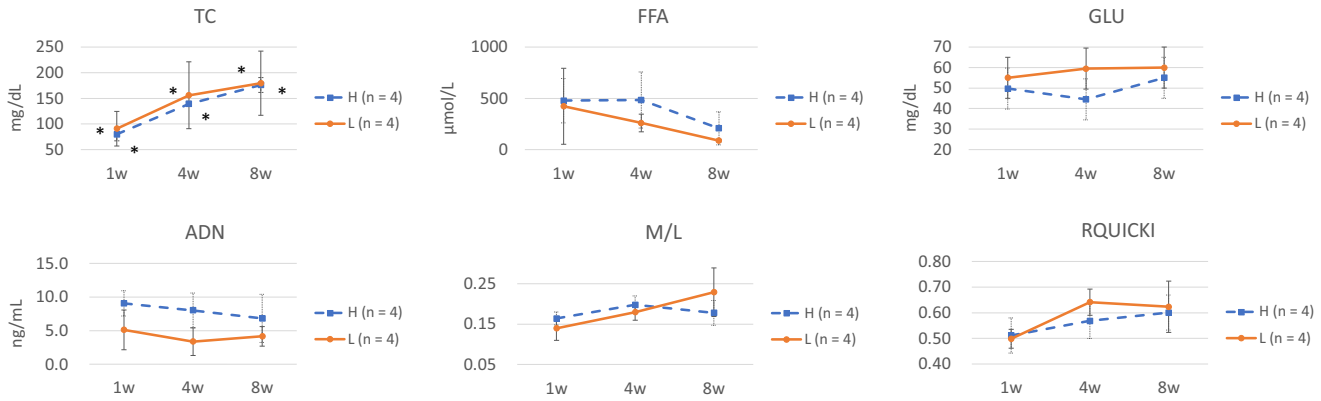


Fig. 3 Comparison of blood parameters with 2 groups divided based on pH parameters. Two groups were compared using the Friedman test, and TC was significantly different among the 2 groups at 1, 4 and 8 weeks. H: High-value group (n = 4; >25,900 min 4w-low pH time and > 25 days SARA-positive); L: Low-value group (n = 4; ≤25,900 min 4w-low pH time and ≤ 25 days SARA-positive); 1w, 4w, 8w: 1, 4, 8 weeks after parturition; ADN: adiponectin; FFA: free fatty acid; GLU: glucose; M/L: ratio of malate dehydrogenase activity to lactate dehydrogenase activity; RQUICKI: revised quantitative insulin sensitivity check index; TC: total cholesterol. Bars indicate mean ± standard deviation. *Significant difference among 1w, 4w, 8w (Friedman test, $p < 0.05$)

significantly different among the 2 groups at 1, 4 and 8 weeks ($p < 0.05$). The high-value group with more frequent SARA tended to exhibit higher ADN concentrations than the low-value group with less-frequent SARA at 4 weeks, although this difference was not statistically significant ($p = 0.057$). There were no significant differences in other blood parameters.

Relationships between the frequency of SARA, milk production, and reproduction performance

High-value and low-value groups were compared using the Mann-Whitney U test (Fig. 4), and no statistically significant differences were observed in milk production or delivery intervals between the 2 groups.

Effects of KP supplementation on pH parameters

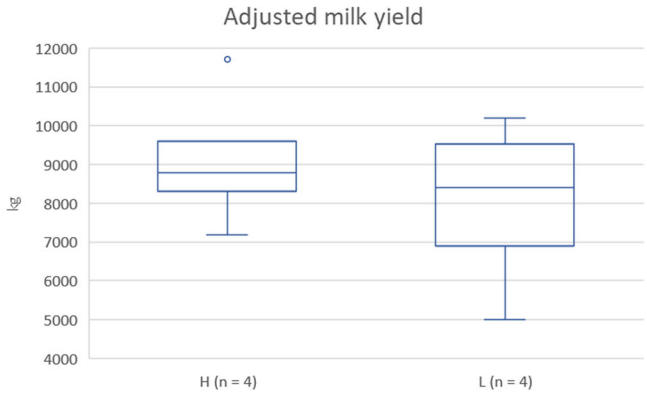
Effects of KP supplementation on the 4w-low pH time and SARA occurrence in 30 days after parturition are presented in Table 6. Overall, the 4w-low pH time and the number of SARA-positive days in 2016 increased from 2015. In 2015, the 4w-low pH time for cows supplemented with KP was lower than for cows without KP (21,623 min vs. 27,090 min). In both years, the numbers of SARA-positive days for cows supplemented with KP were lower than for cows without KP (21.3 days vs. 23 days in 2015, 29 days vs. 30 days in 2016). Statistical analysis could not be conducted because of the small sample size.

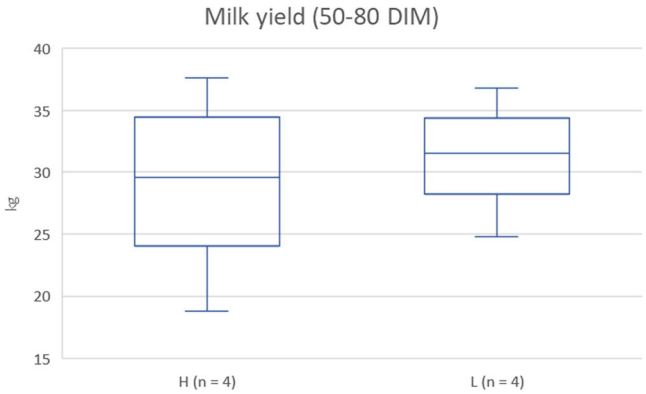
Discussion

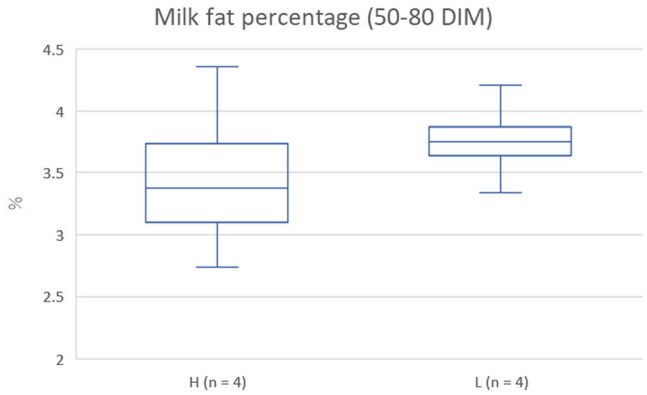
Our previous study suggested that ruminal pH values in herds are influenced by the feeding system (total mix ration or separated), non-fiber carbohydrate (NFC), and starch concentrations in feed; diet changes for cows during transition; and other factors (Maeda 2016). Conversely, abnormal ruminal fermentation is not necessarily reflected by changes in plasma biomarkers and the occurrence of periparturient disease. Changes in ordinary plasma biomarkers are not always useful for detecting abnormal ruminal fermentation. Therefore, routine blood biochemical examination is not necessarily effective for evaluating SARA in lactating cows.

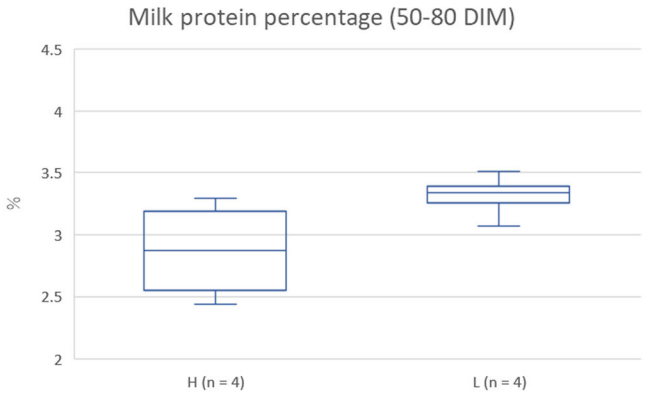
SARA induces ruminal LPS release and triggers an acute phase inflammatory response (Gozho et al. 2005). However, its effects on metabolism are poorly understood. Analysis of correlations between ruminal pH values and blood parameters for energy metabolism is important to understand the influence of SARA. This study demonstrated the effectiveness of ADN as an estimative index for SARA as a metabolic disease.

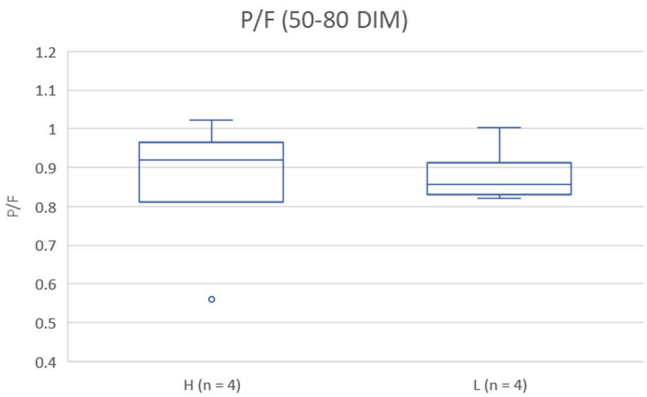
Fig. 4 Comparison of milk production and reproduction performance between high-value and low-value groups based on pH parameters. Values represented as box plots. Two groups were compared using the Mann-Whitney U test, and no statistically significant differences were observed. H: High-value group (n = 4; >25,900 min 4w-low pH time and > 25 days SARA-positive); L: Low-value group (n = 4; ≤25,900 min 4w-low pH time and ≤ 25 days SARA-positive); 50–80 DIM: at a single point between 50 and 80 days in milk; MUN: milk urea nitrogen; P/F: protein:fat ratio

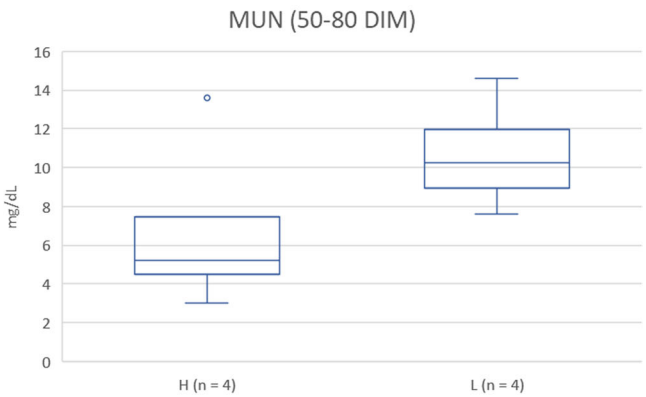

Adjusted milk yield
kg
12000
11000
10000
9000
8000
7000
6000
5000
4000
H (n = 4)
L (n = 4)


Milk yield (50-80 DIM)
kg
40
35
30
25
20
15
H (n = 4)
L (n = 4)


Milk fat percentage (50-80 DIM)
%
4.5
4
3.5
3
2.5
2
H (n = 4)
L (n = 4)


Milk protein percentage (50-80 DIM)
%
4.5
4
3.5
3
2.5
2
H (n = 4)
L (n = 4)


P/F (50-80 DIM)
P/F
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
H (n = 4)
L (n = 4)


MUN (50-80 DIM)
mg/dL
16
14
12
10
8
6
4
2
0
H (n = 4)
L (n = 4)

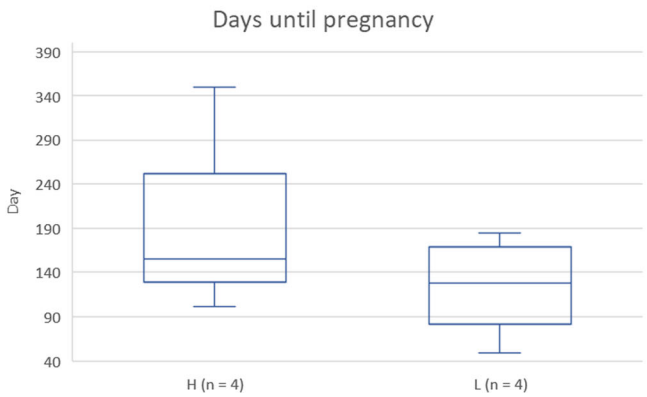

Days until pregnancy
Day
390
340
290
240
190
140
90
40
H (n = 4)
L (n = 4)

Table 6 Effects of kraft pulp supplementation on pH parameters

	2015		2016		Average	
	4w-low ph time (min)	Number of SARA-positive days	4w-low ph time (min)	Number of SARA-positive days	4w-low ph time (min)	Number of SARA-positive days
Control	27,090	23.0 ($n = 3$)	32,930	30.0 ($n = 1$)	28,550	24.8 ($n = 4$)
With KP	21,623	21.3 (n = 3)	33,550	29.0 (n = 1)	24,605	23.3 (n = 4)
Average	24,357	22.2 ($n = 6$)	33,240	29.5 ($n = 2$)	26,578	24 ($n = 8$)

3 cows that showed error data with unusual condition of pH sensor were eliminated

KP: kraft pulp; 4w-low pH: total amount of time for ruminal fluid pH < 5.6 or reticulum fluid pH < 6.3 during 4 weeks after parturition; SARA-positive days: days defined as SARA by duration time for ruminal pH <5.6 or reticulum pH <6.3

ADN is an adipocytokine secreted by adipose tissue (Hu et al. 1996) and is involved in the regulation of glucose and fatty acid metabolism (Yamauchi et al. 2001). ADN improves insulin resistance by reducing gluconeogenesis in the liver and promoting sugar uptake in skeletal muscles (Yamauchi et al. 2002; Maeda and Shimomura 2011). ADN also affects bovine monocyte inflammatory responses by reducing their responsiveness to LPS during metabolic stress in cows (Kabara et al. 2014). ADN may reflect ruminal fermentation conditions in lactating cows, as its concentrations at 4 weeks after parturition correlated with 1w-low pH time. Additionally, there was a strong correlation between the number of SARA-positive days and the ADN concentrations at 4 weeks or the average of ADN concentrations at 1 and 4 weeks. When we divided the cows into 2 groups based on low pH time and the frequency of SARA in 30 days after parturition, the high-value group tended to exhibit higher ADN levels than the low-value group at 4 weeks, although the difference was not statistically significant ($p < 0.1$). Plasma ADN concentrations are reportedly reduced during the first week after parturition, compared to the levels measured during the dry period (Kabara et al. 2014; these levels then recover after parturition in Holstein cows (Ohtani et al. 2012). Reduction of plasma ADN after parturition is partially driven by the negative energy balance, and may enhance lipolysis in adipose tissue (Krumm et al. 2017; Kabara et al. 2014). Consistent with previous reports, we found that ADN concentrations were reduced at 1 week after parturition compared to the preparturient levels (data not shown). However, we did not observe recovery after parturition. ADN concentrations in the high-value group did not recover until 8 weeks after parturition, although they remained higher than those of the low-value group. We speculate that cause of this was persistent increased FFA, reduced GLU, and increased insulin resistance in these individuals. We hypothesized that frequent SARA would depress energy production. To test this assumption, we measured the M/L ratio, which is reportedly an index of energy metabolism (Arai et al. 2003; Li et al. 2012). However, no correlation was found between M/L ratio and SARA frequency.

SARA is reported to increase endotoxin levels in the rumen and trigger nonspecific and systemic inflammation (Gozho et al. 2007). Tumor necrosis factor-alpha secreted by macrophages induces insulin resistance (Li et al. 2007; Ohtsuka et al. 2001). Therefore, SARA may indirectly induce insulin resistance. We confirmed a similar trend with RQUICKI in this study at 4 weeks. Cows with more frequent SARA tended to exhibit lower RQUICKI values than cows with less frequent SARA, although the difference was not statistically significant ($p > 0.05$). ADN concentrations may increase as a compensatory action to ameliorate insulin resistance by reducing inflammatory responses to LPS and promoting insulin sensitivity with more frequent SARA. Although we demonstrated that ADN correlated with pH parameters, we could not distinguish whether there was a temporal relationship between ADN levels and pH parameters, or if a compensatory increase in ADN levels occurred in real time or with a delay. This was likely because it was necessary to analyze ADN at a single instance and pH parameters over time. Further studies comparing all combinations of ADN concentrations and pH parameters, including preparturient levels, may reveal a temporal relationship.

Although we were unable to reduce the occurrence of SARA by supplementation with KP, the frequency of SARA in the same individual slightly decreased. KP, which contains nutritious fiber, is thought to increase the neutral detergent fiber (NDF) content and stabilize the ruminal pH (Nishimura et al. 2019). In this study, administration of KP in 2015 was considered to make improvements in the ruminal pH when cows were given feeds with low levels of NDF, high levels of NFC, and high levels of starch—feeds that can decrease ruminal pH (Table 3). In contrast, administration of KP in 2016 led to less improvement in ruminal pH when cows were given feeds with an increased gap between NDF level and starch concentrations (feeds which can cause disruption of ruminal pH) before and after parturition (Table 3). Therefore, the difference in constituent concentrations of feeds is considered to cause the difference in KP effects. We replaced KP with roughage, but a greater effect in reducing SARA would be expected if we replaced it with concentrated diet.

The limitations of this study were the insufficient sample size and the inability to compare cows with SARA to cows without SARA. Further studies with larger sample sizes, which include comparisons between cows with SARA and cows without SARA may permit SARA to be diagnosed by measuring ADN levels. Additionally, associations with rumen microorganisms should be investigated.

In this study, we continuously monitored ruminal pH and measured blood concentrations of hormones and metabolites related to energy metabolism. We also analyzed information regarding milk production and reproduction. This study suggests that ADN may serve as an estimative index for SARA as a metabolic disease.

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Compliance with ethical standards

Conflict of interests The authors declare no conflict of interest.

Statement on the welfare of animals Ethical approval: All procedures performed in this study involving animals were in accordance with the ethical standards of the research animal ethical committee of Nippon Veterinary and Life Science University and the current laws of Japan, wherein this study was conducted.

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