NON-INVASIVE INDICATORS ASSOCIATED WITH SUBACUTE RUMINAL ACIDOSIS IN DAIRY COWS*

Barbara Stefańska1♦, Jolanta Komisarek2, Włodzimierz Nowak1

1Department of Animal Nutrition, Poznań University of Life Sciences, ul. Wołyńska 33, 60-637 Poznań, Poland
2Department of Animal Breeding and Product Quality Assessment, Poznań University of Life Sciences, Złotniki, ul. Słoneczna 1, 62-002 Suchy Las, Poland
♦Corresponding author: barbara.stefanska@up.poznan.pl

Abstract
The aim of the study was to characterize the interrelationship between decreased ruminal fluid pH during subacute ruminal acidosis (SARA) and concentrations of principal constituents of milk and biochemical indices associated with nitrogen utilizations such as rumen ammonia nitrogen (RAN), blood urea nitrogen (BUN) and milk urea nitrogen (MUN). Ruminal fluid samples were obtained by rumenocentesis from 305 cows representing 13 dairy herds. The cows were divided according to ruminal fluid pH into three groups: low, moderate, and high rumen pH cows. The herds were divided into three groups on the basis of the percentages of cows with an assigned value of ruminal fluid pH: SARA-positive, SARA-risk and SARA-negative. SARA-positive herds were characterized by higher concentrations of RAN (12.6 vs. 6.9 mg/dL), BUN (16.2 vs. 10.1 mg/dL) and MUN (12.4 vs. 9.1 mg/dL) compared to SARA-negative herds. Similarly, low-rumen pH cows had greater concentrations of RAN, BUN and MUN than high-rumen pH cows (11.9 vs. 5.8 mg/dL, 19.9 vs. 14.1 mg/dL, and 12.3 vs. 9.5 mg/dL, respectively). Moreover, SARA-positive herds and low-rumen pH cows had the highest lactose and the lowest fat concentrations in milk. The study demonstrated that the concentration of milk urea nitrogen could be considered one of the indirect and non-invasive indicators of the occurrence of subacute ruminal acidosis in dairy herds.

Key words: dairy cow, rumenocentesis, biomarker, milk urea nitrogen

The increase in the genetic potential of dairy cows for milk production during recent years has led to the use of high-starch diets. However, these diets, which are rich in rapidly fermentable carbohydrates and low in physically effective NDF (peNDF), may lead to the depression of ruminal pH and subacute ruminal acidosis (SARA). Previous studies have indicated that significant variations exist in the extent of the severity of rumen acidosis, which may be due to impaired balance between acid pro-
duction in the rumen and absorption through the rumen epithelial cells, insufficient buffering, and the rate of passage to lower digestive tracts (Gao and Oba, 2014). Additionally, inter-animal variation in rumen pH response to high-concentrate diets has been recently reported in steers (Schlau et al., 2012) and dairy cows (Nasrollahi et al., 2017, 2019; Dewankelle et al., 2019), even when fed the same diet. Part of variation in susceptibility to SARA may be due to the differences among animals in sorting behaviour (Nasrollahi et al., 2017) or in expression of genes that regulate the adaptive function of rumen epithelium (Zhao et al., 2017). Therefore, identifying cows that have a higher or lower risk of SARA and adjusting nutritional management accordingly may reduce the prevalence of this nutritional disorder. Recommendations for diagnosis of SARA on the dairy herd level are mainly based on ruminal pH measured in ruminal fluid sampled by rumenocentesis (Duffield et al., 2004). This time-consuming procedure and cow health problems, such as hematomas and abscess formation at the puncture site, are the reasons that this method is not widely used as a routine monitoring tool on a dairy farm and it is necessary to develop a non-invasive biomarker to identify cows with a high or low risk of SARA.

Milk fat depression is associated with SARA (Stone, 2004; Nasrollahi et al., 2017). However, rumen acidity is an important, but not the only factor affecting milk fat synthesis, which makes milk fat depression an unreliable marker for diagnosing SARA (Lock, 2010). Recent studies have revealed that milk urea nitrogen (MUN) could potentially be a good indicator, but some studies have reported contradictory results – either a decrease (Gao and Oba, 2014, 2015) or an increase in the content of MUN (Kleen et al., 2013) during decreased ruminal fluid pH and SARA occurrence. A better understanding of the role of ruminal pH in regulating ammonia production and absorption to the blood and milk may therefore be important for non-invasive diagnosis of SARA in dairy herds.

The main aim of the study was to characterize the interrelationship between decreased ruminal fluid pH during SARA and concentrations of biochemical indices associated with nitrogen utilizations, such as rumen ammonia nitrogen (RAN), blood urea nitrogen (BUN) and milk urea nitrogen (MUN). We hypothesized that MUN is significantly affected by ruminal fluid pH and it could be a useful tool for non-invasive SARA diagnosis in dairy herds.

**Material and methods**

All procedures were approved for the study by the Local Ethical Committee No. 10 in Poznań, Poland (decision no. 32/2014), and were performed in accordance with the “Act on the Protection of Animals Used for Scientific or Educational Purposes” of the Republic of Poland, which complies with the EU directive (no. 2010/63/EU) for the protection of animals used for scientific purposes.

**Herds**

The study was performed on 13 commercial Polish dairy herds, located in western and southern Poland (Table 1). Each of these herds had more than 100 lactating dairy
cows, housed in free stalls, under routine milk performance recording conducted by the Polish Federation of Cattle Breeders and Dairy Farmers. The dairy cows were fed total mixed rations (TMR) based on corn, grass and alfalfa silage, corn grain silage and concentrates consisting of barley, wheat, triticale, rapeseed and soybean meals (Table 2). The diets were balanced based on the analysed nutrient contents using the Dairy Max System for dairy cattle software (Cargill, Kiszkowo, Poland) in accordance with the Nutrient Requirements of Dairy Cattle (NRC, 2001). TMR samples were collected for analysis by wet chemistry for dry matter (DM, method no. 6496), crude protein (CP, method no. 976.05), neutral detergent fibre (NDF, method no. 942.05) and starch (method no. 64.785) according to AOAC (2010). The particle size of TMR samples (Table 3) was evaluated using a Penn State Separator, with 3 sieves with holes of different diameters (19, 8, and 1.18 mm) and a solid pan, according to the technique described by Mertens (1997). The content of each sieve and the solid pan was weighed and recorded.

**Animals and ruminal fluid analyses**

The study included 305 dairy cows of the Polish Holstein-Friesian breed. Cows were selected according to their days in milk (DIM; 40 to 150 days), number of lactations (primiparous n = 139, and multiparous n = 166), and health (free of mastitis, metritis, and hoof disease). Cow health was estimated according to their recent medical history and a detailed clinical examination, always by the same veterinarian.

Ruminal fluid samples were collected 4 to 6 h after the morning feeding from the ventral sack of the rumen by rumenocentesis using non-pyrogenic needles (2.0 × 120 mm) and 30 mL syringes (Duffield et al., 2004). Ruminal fluid pH was measured using a CP-104 pH-meter (Elmetron, Poland) according to the methodology presented by Krause and Oetzel (2006). The calibration of the CP-104 pH-meter was performed on each dairy farm before sampling relative to the reference buffer as a standard with value of pH 4, 7 and 9 (Alchem, Poland). Ruminal fluid samples were then transferred into 5 mL plastic probes and kept on dry ice for transport to the laboratory for analysis of RAN, according to the methods described by Novozamsky et al. (1974).

On the basis of ruminal fluid pH, cows were divided into three groups according to the classification of Nordlund and Garrett (1994) on the basis of ruminal fluid pH: high (pH > 5.81, n = 196), moderate (pH 5.8–5.6, n = 51) and low pH (pH < 5.6, n = 58). In these groups, the average ruminal fluid pH of 6.35, 5.71 and 5.45 respectively, were recorded. Moreover, the herds were divided into three groups (Table 3) according to the classification presented by Garrett et al. (1999) on the basis of the percentages of cows with an assigned value of ruminal fluid pH: SARA-positive herd, if at least 25% of the ruminal fluid samples indicated a pH < 5.6; SARA-risk herd, if fewer than 25% of ruminal fluid samples indicated a pH < 5.6, but at least 33% showed a pH ≤ 5.8; and SARA-negative herd, if fewer than 25% of the ruminal fluid samples indicated a pH < 5.6, but fewer than 33% exhibited a pH > 5.8. In these groups, the average ruminal fluid pH of 5.89, 6.05 and 6.22, respectively, were recorded.
Table 1. General information on the observed herds

| Herds | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| No. animals in herds | 330 | 563 | 760 | 210 | 516 | 530 | 630 | 270 | 750 | 310 | 820 | 565 | 400 |
| No. tested animals   | 24  | 25  | 20  | 25  | 21  | 25  | 25  | 24  | 24  | 14  | 24  | 26  | 28  |
| No. primiparous cows/farm | 18  | 63  | 75  | 14  | 66  | 25  | 54  | 12  | 35  | 22  | 65  | 48  | 34  |
| Average lactation number/farm | 3.00 | 2.48 | 2.40 | 3.00 | 2.30 | 3.00 | 2.20 | 3.00 | 3.08 | 2.55 | 2.55 | 2.74 | 2.65 |
| Average 305-d milk production (kg/farm) | 11 986 | 10 081 | 11 573 | 10 271 | 10 620 | 11 064 | 11 215 | 12 041 | 10 383 | 10 987 | 10 070 | 10 339 | 11 620 |
| Average DIM/farm     | 76  | 76  | 79  | 73  | 76  | 75  | 75  | 74  | 73  | 74  | 76  | 79  | 74  |

Data presented in Table 1 were also used in Stefańska et al. (2018).
Table 2. Composition of TMR used in the observed herds

| Ingredient (% DM)                  | Herds |          |          |          |          |          |          |          |          |          |          |          |          |
|-----------------------------------|-------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Corn silage                       | 51.6  | 44.7     | 57.3     | 59.1     | 53.9     | 57.3     | 47.8     | 54.4     | 57.2     | 52.6     | 52.7     | 50.1     | 51.3     |
| Alfalfa silage                    | 16.4  | 15.9     | 5.5      | 16.1     | 14.7     | 13.2     | 18.7     | 18.1     | 11.0     | 11.9     | 12.0     | 9.1      | 9.8      |
| Wilted grass                      | 11.7  | 12.4     | 2.7      | 2.7      | 2.4      | 2.2      | 6.2      | 6.0      | 6.6      | 7.2      | 7.2      | 12.5     | 11.0     |
| Corn grain, ensiled               | 6.6   | 2.5      | 2.7      | 1.3      | 1.2      | 2.2      | 2.1      | 4.0      | 5.5      | 4.8      | 4.8      | 4.6      | 3.7      |
| Barley grain                      | 4.7   | 9.9      | 10.9     | 4.6      | 4.2      | 3.7      | 5.0      | 3.0      | 3.3      | 3.6      | 4.1      | 3.9      | 4.2      |
| Wheat grain                       | 1.2   | 3.7      | 5.5      | 5.4      | 4.9      | 4.4      | 5.0      | 3.4      | 3.7      | 4.8      | 5.3      | 5.0      | 5.4      |
| Triticale grain                   | 1.2   | 1.2      | 1.4      | 1.9      | 7.3      | 6.6      | 5.6      | 4.0      | 3.5      | 4.8      | 4.8      | 4.6      | 4.9      |
| Rapeseed meal                     | 2.8   | 3.0      | 6.8      | 4.0      | 3.7      | 3.3      | 3.1      | 2.6      | 3.3      | 4.1      | 4.1      | 3.9      | 3.4      |
| Soybean meal                      | 3.5   | 6.2      | 6.8      | 4.6      | 7.3      | 6.6      | 6.2      | 4.0      | 5.5      | 6.0      | 4.8      | 6.1      | 6.1      |
| Mineral and vitamin mixa          | 0.35  | 0.37     | 0.41     | 0.40     | 0.37     | 0.33     | 0.31     | 0.30     | 0.33     | 0.36     | 0.34     | 0.37     |          |
| F : C                            | 58:42 | 53:47    | 47:53    | 58:42    | 49:51    | 56:44    | 51:49    | 60:40    | 56:44    | 52:48    | 52:48    | 53:47    | 53:47    |

Nutrients composition (%)

| DM%                                     | 40.8  | 47.2    | 52.4    | 45.2    | 45.4    | 44.6    | 45.5    | 39.6    | 44.7    | 47.6    | 48.5    | 46.6    | 46.3    |
| CP%                                     | 15.6  | 16.4    | 16.5    | 16.0    | 16.8    | 16.6    | 17.1    | 15.6    | 16.2    | 17.1    | 16.3    | 16.9    | 16.4    |
| RDP%, % of CP                           | 65.6  | 64.1    | 65.7    | 64.2    | 64.7    | 65.3    | 65.3    | 66.1    | 64.1    | 64.3    | 64.2    | 64.4    | 65.6    |
| RUP%, % of CP                           | 34.4  | 35.9    | 34.3    | 35.8    | 35.3    | 34.7    | 34.7    | 33.9    | 35.9    | 35.7    | 35.8    | 35.6    | 34.4    |
| NDF%                                    | 35.0  | 30.0    | 27.3    | 32.1    | 30.0    | 31.0    | 30.5    | 34.9    | 33.7    | 29.5    | 30.1    | 29.8    | 28.7    |
| Starch to CP ratio                     | 1.67  | 1.65    | 1.66    | 1.74    | 1.65    | 1.83    | 1.67    | 1.86    | 1.87    | 1.87    | 1.83    | 1.83    | 1.68    |

aData presented in Table 2 were also used in Stefańska et al. (2018).

Mineral and vitamin mix composition – 215 g/kg Ca, 40 g/kg P, 65 g/kg Na, 55 g/kg Mg, 1200 mg/kg Cu, 4000 mg/kg Mn, 15 mg/kg Co, 10 000 mg/kg Zn, 60 mg/kg Se, 1 200 000 IU/kg vitamin A, 180 000 IU/kg vitamin D, 6000 IU/kg vitamin E.

F : C – forage to concentrate ratio.

DM – dry matter.

RDP – rumen degradable protein.

RUP – rumen undegradable protein.

NDF – neutral detergent fibre.

Data presented in Table 2 were also used in Stefańska et al. (2018).
Table 3. Particle size distribution of the TMR, and SARA status of the observed herds

| Herds | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 |
|-------|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Particle size of diets (%) |     |    |    |    |    |    |    |    |    |    |    |    |    |
| Sieve 19 mm | 4.7 | 7.2 | 10.6 | 6.6 | 7.3 | 6.5 | 7.5 | 3.4 | 7.5 | 8.3 | 8.5 | 10.2 | 7.9 |
| Sieve 8 mm   | 46.1 | 35.7 | 35.7 | 42.2 | 35.0 | 64.0 | 39.3 | 59.2 | 46.2 | 36.4 | 34.5 | 36.3 | 35.9 |
| Sieve 1.18 mm| 44.9 | 46.5 | 47.6 | 40.0 | 48.7 | 27.7 | 48.2 | 31.0 | 38.0 | 47.7 | 46.6 | 47.8 | 46.8 |
| Pan           | 4.3 | 10.6 | 6.1 | 11.2 | 9.0 | 1.8 | 5.0 | 6.4 | 8.3 | 7.6 | 10.4 | 5.7 | 9.4 |
| peNDF > 1.18 mm | 33.5 | 26.8 | 25.6 | 28.5 | 27.3 | 30.4 | 29.0 | 32.7 | 30.9 | 27.3 | 27.0 | 28.1 | 26.0 |
| peNDF > 1.18 mm : starch ratio | 1.29 | 0.99 | 0.86 | 1.03 | 0.98 | 1.00 | 0.98 | 1.13 | 1.02 | 0.95 | 0.90 | 0.99 | 0.95 |
| Average rumen pH/farm | 6.30 | 6.05 | 5.80 | 6.24 | 5.91 | 6.06 | 6.05 | 6.25 | 6.23 | 5.85 | 5.81 | 5.92 | 6.06 |
| SARA herd status  | SARA-N | SARA-N | SARA-R | SARA-P | SARA-N | SARA-P | SARA-N | SARA-R | SARA-N | SARA-N | SARA-P | SARA-P | SARA-P |

*aPeNDF – physically effective neutral detergent fibre.*

*bSARA herd status – SARA-positive herd (SARA-P), if at least 25% of the ruminal fluid samples indicated a pH < 5.6; SARA-risk herd (SARA-R), if fewer than 25% of ruminal fluid samples indicated a pH < 5.6, but at least 33% showed a pH ≤ 5.8; and SARA-negative herd (SARA-N), if fewer than 25% of the ruminal fluid samples indicated a pH < 5.6, but fewer than 33% exhibited a pH > 5.8.*

Data presented in Table 3 were also used in Stefańska et al. (2018).
Blood sampling and analyses

Blood samples were collected 4 to 6 h after the morning feeding from the tail vein as described by Gozho et al. (2005) to blank 10 mL vacutainers for serum harvesting (KABE, Poznań, Poland, catalog number: KB-0959 0031). The vacutainers with serum were transported to the laboratory in a refrigerated vehicle, and then centrifuged at 3000 × g for 15 min. Subsequently, serum was aspirated and stored at −20°C until analysis. The concentration of BUN (Pointe Scientific, Warszawa, Poland, catalog no. B7552) was analysed with a Pointe Scientific reagent.

Milk sampling and analyses

Milk samples (15 mL) were collected from each cow at the morning and afternoon milkings into special tubes during milk performance evaluation conducted by the Polish Federation of Cattle Breeders and Dairy Farmers. The analysis of fat, protein, lactose, and MUN contents was conducted in the Laboratory of the Polish Federation of Cattle Breeders and Dairy Farmers using a CombiFoss FT 6000 with FT-IR (Fourier Transform Infrared) methods.

Statistical analyses

The obtained data were subjected to analysis by multivariate covariance analysis and Duncan’s multiple range test using the PROC GLM procedure of SAS 9.4 (2014). Before any analysis was carried out, all data were tested for normality using the PROC UNIVARIATE. The RAN, BUN, and MUN concentrations were transformed before statistical analysis by using a logistic transformation function. The model included:

\[
y_{ijklm} = \mu + f_i + l_j + g_k + s_l + \beta_1 d_{lm} + \beta_2 m_n + e_{ijklmn}
\]

where: \(y_{ijklm}\) is the phenotypic value of the trait, \(\mu\) is the overall mean of the trait of the population, \(f_i\) is the fixed effect of the farms (\(i = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13\)), \(l_j\) is the fixed effect of lactations number (\(j = 1, 2, 3, 4, 5, 6, 7, 8\)), \(g_k\) is the fixed effect of the SARA groups (\(k = 1, 2, 3\)), \(s_l\) is the fixed effect of the observation season (\(l = 1, 2, 3, 4\)), \(\beta_1, \beta_2\) are the partial linear regression coefficients, \(d_{lm}\) is the days in milk, \(m_n\) is the milk yield, and \(e_{ijklmn}\) is the random error. Pearson phenotype correlation coefficients were calculated using the PROC CORR procedure. Moreover, the PROC REG procedure was used to determine the relationships between concentrations of RAN, BUN, MUN and lactose versus value of ruminal fluid pH. Statistical significance was declared at \(P \leq 0.05\) and trends were considered when \(0.05 < P \leq 0.10\). The SEM was adopted as a measure of error.

Results

In analysed herds, the mean annual milk production was 10 940 kg, ranging from 10 070 to 12 040 kg of milk containing 3.67% of fat and 3.33% of protein (Table 1). SARA-positive herds were characterized by higher concentrations of RAN (12.6 vs.
6.9 mg/dL), BUN (16.2 vs. 10.1 mg/dL) and MUN (12.4 vs. 9.1 mg/dL) compared to SARA-negative herds (P≤0.009; Table 4). Similarly, low-rumen pH cows had greater concentrations of RAN, BUN and MUN than high-rumen pH cows (respectively, 11.9 vs. 5.8 mg/dL, 19.9 vs. 14.1 mg/dL, and 12.3 vs. 9.5 mg/dL, P≤0.005; Table 5). Decreased ruminal fluid pH was negatively correlated with RAN (r = –0.65, P≤0.006), BUN (r = –0.72, P≤0.008) and MUN (r = –0.84, P≤0.006; Table 6; Figure 1 A–C). Milk produced by both SARA-positive herds and low-rumen pH cows had the highest lactose (P≤0.005) and the lowest fat (P≤0.026) contents (Table 4 and 5). There was a significant, negative correlation between the pH of ruminal fluid and lactose content (r = –0.71, P≤0.009; Table 6; Figure 1 D). The relationships between pH and RAN, BUN, MUN, and lactose concentrations were not entirely consistent across all rumen pH groups, but the greater stability was observed for MUN (Figure 1 A–D).

Table 4. Ruminal ammonia, blood urea nitrogen, milk urea nitrogen, and milk composition indices classed by herd SARA status

| Item          | Treatmenta | SEM  |
|---------------|------------|------|
|               | SARA-negative | SARA-risk | SARA-positive |     |
| RANb (mg/dL)  | 6.9 A       | 7.1 AB     | 12.6 B       | 0.44|
| BUNc (mg/dL)  | 10.1 A      | 12.4 AB    | 16.2 B       | 0.64|
| MUNd (mg/dL)  | 9.1 A       | 9.8 AB     | 12.4 B       | 0.08|
| Fat (g/kg)    | 32 a        | 30 ab      | 29 b         | 0.11|
| Protein (g/kg)| 29          | 29         | 30           | 0.06|
| Lactose (g/kg)| 42 A        | 43 AB      | 45 B         | 0.08|

a, b – means within a row with different letters differ at P≤0.05.
A, B – means within a row with different letters differ at P≤0.01.

Treatment – SARA-positive herd, if at least 25% of the ruminal fluid samples indicated a pH<5.6; SARA-risk herd, if fewer than 25% of ruminal fluid samples indicated a pH<5.6, but at least 33% showed a pH≤5.8; and SARA-negative herd, if fewer than 25% of the ruminal fluid samples indicated a pH<5.6, but fewer than 33% exhibited a pH>5.8.

bRAN – rumen ammonia nitrogen.
cBUN – blood urea nitrogen.
dMUN – milk urea nitrogen.

Table 5. Ruminal ammonia, blood urea nitrogen, milk urea nitrogen, and milk composition in group of cows differing in ruminal average fluid pH

| Item          | Treatmentb | SEM  |
|---------------|------------|------|
|               | high-rumen pH | moderate-rumen pH | low-rumen pH |     |
| RANb (mg/dL)  | 5.8 A       | 6.9 AB     | 11.9 B       | 0.57|
| BUNc (mg/dL)  | 14.1A       | 15.5 AB    | 19.9 B       | 0.94|
| MUNd (mg/dL)  | 9.5 A       | 10.8 AB    | 12.3 B       | 0.03|
| Fat (g/kg)    | 31 a        | 31 ab      | 29 b         | 0.09|
| Protein (g/kg)| 29          | 29         | 29           | 0.06|
| Lactose (g/kg)| 41 A        | 44 B       | 45 B         | 0.09|

a, b – means within a row with different letters differ at P≤0.05.
A, B – means within a row with different letters differ at P≤0.01.

Treatment – high rumen pH (pH>5.8); moderate rumen pH (pH 5.8–5.6); low rumen pH (pH<5.6).
bRAN – rumen ammonia nitrogen.
cBUN – blood urea nitrogen.
dMUN – milk urea nitrogen.
Table 6. Correlation coefficient (r) between ruminal fluid pH and indices associated with nitrogen metabolism and milk composition indices

| Item            | Ruminal fluid pH | P       |
|-----------------|------------------|---------|
| RAN^a (mg/dL)   | –0.65            | **      |
| BUN^b (mg/dL)   | –0.72            | **      |
| MUN^c (mg/dL)   | –0.84            | **      |
| Fat (g/kg)      | 0.03             | NS      |
| Protein (g/kg)  | 0.01             | NS      |
| Lactose (g/kg)  | –0.71            | **      |

P – **correlation significant at P≤0.01.
NS – non-significant correlation.
^aRAN – rumen ammonia nitrogen.
^bBUN – blood urea nitrogen.
^cMUN – milk urea nitrogen.

Figure 1. Relationship between ruminal fluid pH and concentration of A: rumen ammonia nitrogen (RAN), B: blood urea nitrogen (BUN), C: milk urea nitrogen (MUN), and D: milk lactose. Individual data points are shown for low pH (circles), moderate pH (triangles), and high pH (squares)

Discussion

In our previous research, we analysed SARA effects on ruminal microbiota composition, fermentation indices, biochemical blood indices, and expression of genes
involved in systemic immune response (Stefańska et al., 2016, 2017, 2018), whereas this part of the study focused mostly on indices associated with nitrogen utilizations. In terms of both cow rumen pH and herd SARA status classification, decreased ruminal fluid pH was associated with increased concentrations of RAN, BUN, and MUN. Also, there was a negative correlation between the pH of ruminal fluid and all above biochemical indices in our study characterizing the nitrogen metabolism of lactating dairy cows.

Ruminal ammonia nitrogen is an important nutrient supporting efficient rumen fermentation. Its level is associated with the rate of rumen protein degradation, the concentration of ruminal degradable protein, and the amount of dietary energy available for rumen microorganisms. The extent to which ammonia is used to synthesize microbial protein is largely dependent upon the availability of energy generated by the fermentation of carbohydrates. However, in the current study all cows were fed diets with similar levels of CP, rumen degradable protein (RDP), rumen undegradable protein (RUP) and similar starch to CP ratios.

The recommended concentration of RAN for efficient digestion has been variously estimated as 50 to 70 mg/L (Satter and Styler, 1974) and 45 to 120 mg/L (Boniace et al., 1986). In this study, in both low-rumen pH cows, and SARA-positive herds, levels of RAN exceeded reference values, which could indicate that decreased ruminal fluid pH modified microbial populations and decreased the organic matter fermentation rate, thus lowering the amount of energy available for microbial protein synthesis. Furthermore, a change in the pH of ruminal fluid could alter the rumen bacterial composition leading to increased concentrations of ammonia in the rumen. According to Endres and Stern (1993), a decrease in ruminal fluid pH from 6.3 to 5.9 during SARA decreased cellulolytic activity by 50% without reducing proteolytic bacteria. The same authors suggested that amylolytic bacteria, which predominate during the occurrence of SARA, tend to be more often proteolytic than cellulolytic bacteria and are able to produce most of the ruminal ammonia. Starch-fermenting bacteria such as *Prevotella ruminicola* are highly pH-resistant, can grow at pH values as low as 5.1 and are able to cause microbial protein degradation, which could affect an increase in ammonia concentrations (Russell and Dombrowski, 1980). Recently, Nagata et al. (2018) subjected Holstein bulls to four repeated SARA challenges and found increased ammonia concentrations in periods when animals were fed high-grain diets and the pH of their ruminal fluid was decreased. A more remarkable increase in ammonia level was observed during the latter challenges compared to the former ones, possibly as a result of changes in the composition and diversity of ruminal bacterial communities.

After absorption of urea to the blood, the liver has a central role in the integration of the body’s N metabolism. Ammonia in the liver is detoxified by conversion to urea, which can re-enter the rumen via saliva or directly across the rumen wall, or be excreted in urine, milk or sweat (Powell et al., 2014). Little research has focused on the influence of ruminal acidification on concentrations of BUN. Lu et al. (2015) found higher levels of RAN with lower ruminal pH in goats consuming diets rich in non-fibre carbohydrates. The effect of decreased pH on BUN depended, however, on dietary CP supply, and in animals receiving low-protein diets increased RAN did not
lead to a higher BUN, probably as a result of better utilization of recycled N by rumen bacteria. As cows in the herds analysed in this study were fed high-protein diets, our results are in agreement with those obtained by Lu et al. (2015). Recently, higher BUN concentrations in cows with low value of ruminal fluid pH (< 5.8; rumenocentesis) were also reported by Nasrollahi et al. (2019).

Urea in the blood is the major end product of N metabolism in ruminants, and its high concentrations suggest the inefficient utilization of dietary N. However, this cannot be measured routinely on a farm (Huhtanen et al., 2015). Milk urea nitrogen concentration seems to be a better indicator of N status than does BUN, because milk samples represent the nitrogen metabolism during an entire 24 h period, while BUN levels represent the N metabolism occurring for a short period immediately after feeding. Moreover, MUN is widely used in Europe (mostly as milk urea; MU = MUN × 21.4) and North America (as milk urea nitrogen, MUN) as a tool for monitoring diets and its measurements are routinely available to Holstein cow breeders (Siachos et al., 2017). In our study, MUN increased significantly with lower ruminal fluid pH in both cow and herd SARA status classifications. A similar tendency was reported for German dairy herds by Kleen et al. (2013). Moreover, the correlations between pH and MUN were more consistent across low, moderate, and high-rumen pH groups compared to other indices analysed in this study. These results suggest that increased milk urea nitrogen concentration in mid-lactating dairy cows fed high-grain diets may be used to identify cows with a greater risk of SARA. Conversely, Gao and Oba (2014, 2015) showed lower MUN levels in cows with a higher risk of SARA. In our opinion, the variation between studies may be associated with the fact that the research by Gao and Oba (2014, 2015) was performed on a smaller number of animals (16 and 35 cows), which were at a different stage of lactation (late lactation, 282 and 250 DIM). Moreover, SARA in those studies was induced experimentally, which might not be representative of the occurrence of this disorder on dairy farms. As discussed in previous papers (Aguilar et al., 2012; Gao and Oba, 2014), a variety of factors are correlated with MUN concentration, including extensive protein consumption, the extent of CP degradation in the rumen, and the ratio of dietary CP to energy. The results of this study suggest that MUN could be one of the indirect indicators of SARA occurrence, although its usefulness in dairy herds needs further clarification.

The chemical composition of milk is one of the important and useful indicators for evaluating the metabolic status of high-yielding dairy cows. We found a higher lactose content in both low-rumen pH cows and SARA-positive herds. Also, ruminal fluid pH was negatively correlated with the content of lactose in milk. These results are similar to those obtained in the studies of Plaizier et al. (2008), Guo et al. (2013) and Zhao et al. (2016). High-starch diets, which can lead to SARA, are known to result in changes to the fermentation pattern in the rumen, with possible changes in mammary production. Milk lactose content and production are increased as the starch concentrate increases in the diet, resulting in a higher hepatic supply of propionate for glucose and then milk lactose synthesis (Li et al., 2012). A greater availability of glucose could increase the capacity of nutrient absorption and promote milk lactose synthesis in the mammary gland (Liu et al., 2013). Nasrollahi et al. (2017)
and Coon et al. (2019) showed differences in sorting activity between cows susceptible and tolerant to SARA fed the same diet, which led to differences in composition of feed actually consumed by animals. Sorting behaviour was not analysed in herds included in this study. However, selecting fine and avoiding long particles by low-rumen pH cows could be one of reasons for the higher lactose content in their milk.

In the current study, SARA-positive herds and low-rumen pH cows produced milk with 0.27% and 0.23% lower concentration of fat than SARA-negative herds and high-rumen pH cows, respectively. However, we found no significant correlation between the ruminal fluid pH and milk fat content. Similarly, Stone (1999) found that SARA reduced the average milk fat content by 0.3%. Gao and Oba (2014) and Nasrollahi et al. (2017) suggested that milk fat depression is commonly associated with SARA as a result of the formation of trans-fatty acids in the rumen. Low ruminal pH leads to alterations in rumen biohydrogenation patterns and production of trans-octadecenoic acids isomers, which inhibit milk fat synthesis (Bauman and Grinarri, 2003). Rumen acidity is an important, but not the only factor affecting milk fat synthesis, which makes this parameter an unreliable marker for diagnosing SARA (Lock, 2010). The experiment conducted by Enemark et al. (2004) demonstrated that the correlation between milk fat percentage and ruminal fluid pH was negative ($r = –0.06$) for cows in early lactation (under 30 DIM).

In summary, this study demonstrated that a decrease in ruminal fluid pH (<5.6) affected indices associated with nitrogen utilizations of lactating dairy cows by increasing RAN, BUN and MUN. The results suggest that concentrations of milk urea nitrogen could be considered one of the indirect indicators of SARA occurrence in dairy cows. However, additional extensive experiments are needed to clarify its usefulness as a SARA biomarker.

References

Aguilar M., Hanigan M.D., Tucker H.A., Jones B.L., Garbade S.K., McGilliciard M.L., Stalling C.C., Knowlton K.F., James R.E. (2012). Cow and herd variation in milk urea nitrogen concentrations in lactating dairy cattle. J. Dairy Sci., 95: 7261–7268.

AOAC (2010). Association of Official Analytical Chemists. Official methods of analysis, vol. 2, 18th edition, Arlington, VA, USA.

Bauman D.E., Grijnari J.M. (2003). Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr., 23: 203–227.

Boniface A.N., Murray R.M., Hogan J.P. (1986). Optimum level of ammonia in the rumen liquor of cattle fed tropical pasture hay. Proc. Aust. Soc. Anim. Prod., 16: 151–154.

Coon R.E., Duffield T.F., DeVries T.J. (2019). Short communication: Risk of subacute ruminal acidosis affects the feed sorting behavior and milk production of early lactation cows. J. Dairy Sci., 102: 652–659.

Dewanccele L., Jing L., Stefańska B., Vlaeminck B., Jeyanathan J., Van Straalen W.M., Koopmans A., Fievez V. (2019). Distinct blood and milk 18-carbon fatty acid proportions and buccal bacterial populations in dairy cows differing in reticulorumen pH response to dietary supplementation of rapidly fermentable carbohydrates. J. Dairy Sci., 102: 4025–4040.

Duffield T., Plaizier J.C., Fairfield A., Bagg R., Vessie G., Dick P., Wilson J., Aramini J., McBride B.W. (2004). Comparison of techniques for measurement of rumen pH in lactating dairy cows. J. Dairy Sci., 87: 59–66.
Non-invasive indicators of subacute ruminal acidosis in cows

Endres M.I., Stern M.D. (1993). Effects of pH and diets containing various levels of lignosulfonate-treated soybean meal on microbial fermentation in continuous culture. J. Dairy Sci., 76: 177.

Enemark J.M.D, Jørgensen R.J., Kristensen N.B. (2004). An evaluation of parameters for the detection of subclinical rumen acidosis in dairy herds. Vet. Res. Commun., 28: 687–709.

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. J. Dairy Sci., 97: 3006–3016.

Gao X., Oba M. (2015). Short communication: Noninvasive indicators to identify lactating dairy cows with a greater risk of subacute rumen acidosis. J. Dairy Sci., 98: 5735–5739.

Garrett E.F., Pereira M.N., Nordlund K.V., Armentano L.E., Goodger W.J., Oetzel G.R. (1999). Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. J. Dairy Sci., 82: 1170–1178.

Gozho G.N., Plaizier J.C., Krause D.O., Kennedy A.D., Wittenberg K.M. (2005). Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J. Dairy Sci., 88: 1399–1403.

Guo Y., Wang L., Zou Y., Xu X., Li S., Cao Z. (2013). Changes in ruminal fermentation, milk performance and milk fatty acid profile in dairy cows with subacute ruminal acidosis and its regulation with pelleted beet pulp. Arch. Anim. Nutr., 67: 433–447.

Huhtanen P., Cabezas-Garcia E.H., Krause D.O., Shingfield K.J. (2015). Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows. J. Dairy Sci., 98: 3182–3196.

Kleen J.L., Upegang L., Rehage J. (2013). Prevalence and consequences of subacute ruminal acidosis in German dairy cows. Acta Vet. Scand., 55: 48.

Krause M.K., Oetzel G.R. (2006). Understanding and preventing subacute ruminal acidosis in dairy herds. Anim. Feed Sci. Technol., 126: 215–236.

Li S., Gozho G.N., Gakhar N., Khaipour E., Krause D.O., Plaizier J.C. (2012). Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. Can. J. Anim. Sci., 92: 353–364.

Liu H., Zhao K., Liu J. (2013). Effects of glucose availability on expression of the key genes involved in synthesis of milk fat, lactose and glucose metabolism in bovine mammary epithelial cells. PLoS One, 8: 66092.

Lock A.L. (2010). Update on dietary and management effects on milk fat. Proc. 19th Annual Tri-State Dairy Nutrition Conference, 15: 26.

Lu Z., Gui H., Yao L., Van L., Martens H., Aschenbach J.R., Shen Z. (2015). Short-chain fatty acids and acidic pH upregulate UT-B, GPR41, and GPR4 in rumen epithelial cells of goats. Am. J. Physiol. Regul. Integr. Comp. Physiol., 308: R283–R293.

Mertens D.R. (1997). Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci., 80: 1463–1481.

Nagata R., Kim Y.H., Ohkubo A., Kushibiki S., Ichijo T., Sato S. (2018). Effects of repeated subacute ruminal acidosis challenges on the adaptation of the rumen bacterial community in Holstein bulls. J. Dairy Sci., 101: 4424–4436.

Nasrollahi S.M., Zali A., Ghorbani G.R., Moradi Shahrabak M., Heydari Soltan Abadi M. (2017). Variability in the susceptibility to acidosis among high producing mid-lactation dairy cows is associated with rumen pH, fermentation, feed intake, sorting activity, and milk fat percentage. Anim. Feed Sci. Technol., 228: 72–82.

Nasrollahi S.M., Zali A., Ghorbani G.R., Kahyani A., Beaucourn A.K. (2019). Blood metabolites, body reserves, and feed efficiency of high-producing dairy cows that varied in ruminal pH when fed a high-concentrate diet. J. Dairy Sci., 102: 672–677.

Nordlund K.V., Garrett E.F. (1994). Rumenocentesis: A technique for collecting rumen fluid for the diagnosis of subacute rumen acidosis in dairy herds. Bovine Pr., 28: 109–112.

Novozamsky I., van Eck R., Showenburger J.C.H., Waltinga F. (1974). Total nitrogen determination in plant material by means of the indole-phenol blue method. Neth. J. Agr. Sci., 22: 3–5.

NRC (2001). Nutrient Requirements of Dairy Cattle: Seventh Revised Edition.

Plaizier J.C., Krause D.O., Gozho G.N., McBride B.M. (2008). Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet. J., 176: 21–31.
Powell J.M., Rotz C.A., Wattiaux M.A. (2014). Potential use of milk urea nitrogen to abate atmospheric nitrogen emissions from Wisconsin dairy farms. J. Environ. Qual., 43: 1169–1175.

Russell J.B., Dombrowski D. (1980). Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Appl. Environ. Microbiol., 39: 604–610.

Satter L.D., Slyter L.L. (1974). Effect of ammonia concentration of rumen microbial protein production in vitro. Br. J. Nutr., 32: 199–208.

Schlau N., Guan L.L., Oba M. (2012). The relationship between rumen acidosis resistance expression of genes involved in regulation of intracellular pH butyrate metabolism of ruminal epithelial cells in steers. J. Dairy Sci., 95: 5866–5875.

Siatos N., Panousis N., Arsenos G., Valergakis G. (2017). Investigation of milk urea nitrogen concentration and factors affecting its variation in Greek Holstein herds. J. Hellenic Vet. Med. Soc., 68: 423–432.

Stefańska B., Nowak W., Komisarek J., Taciak M., Barszcz M., Skomial J. (2016). Prevalence and consequence of subacute ruminal acidosis in Polish dairy herds. J. Anim. Physiol. Anim. Nutr., 101: 694–702.

Stefańska B., Pruszyńska-Oszmałek E., Szczepankiewicz D., Stajek K., Stefaniński P., Gehrke M., Nowak W. (2017). Relationship between pH of ruminal fluid during subacute ruminal acidosis and physiological response of the Polish Holstein-Friesian dairy cows. Pol. J. Vet. Sci., 20: 551–558.

Stefańska B., Czlapa W., Pruszyńska-Oszmałek E., Szczepankiewicz D., Fievez V., Komisarek J., Stajek K., Nowak W. (2018). Subacute ruminal acidosis affects fermentation and endotoxin concentration in the rumen, and relative expression of the CD14/TLR4/MD2 genes involved in LPS systemic immune response in dairy cows. J. Dairy Sci., 101: 1297–1310.

Stone W.C. (1999). The effect of subclinical acidosis on milk components. Proc. Cornell Nutrition Conference for Feed Manufacturers, Cornell Univ., Ithaca, NY, pp. 40–46.

Stone W.C. (2004). Nutritional approaches to minimize subacute ruminal acidosis and laminitis in dairy cattle. J. Dairy Sci., 87: E13–E26.

Zhao M., Bu D., Wang J., Zhou X., Zhu D., Zhang T., Niu J., Ma L. (2016). Milk production and composition responds to dietary neutral detergent fiber and starch ratio in dairy cows. Anim. Sci. J., 87: 756–766.

Zhao K., Chen Y.H., Penner G.B., Oba M., Guan L.L. (2017). Transcriptome analysis of ruminal epithelia revealed potential regulatory mechanisms involved in host adaptation to gradual high fermentable dietary transition in beef cattle. BMC Genomics, 18: 976.

Received: 3 IX 2019
Accepted: 19 III 2020