https://doi.org/10.17221/51/2020-VETMED

Milk urea nitrogen and milk fatty acid compositions in dairy cows with subacute ruminal acidosis

*Kanber Kara**

Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey

**Corresponding author: karakanber@hotmail.com; kanberkara@erciyes.edu.tr*

Citation: Kara K (2020): Milk urea nitrogen and milk fatty acid compositions in dairy cows with subacute ruminal acidosis. Vet Med-Czech 65, 336–345.

Abstract: The present study was aimed at comparing the milk urea nitrogen (MUN) and milk fatty acid (MFA) compositions in Holstein cows with subacute ruminal acidosis (SARA) to those values of Holstein cows that did not have SARA. Also, the correlations among rumen pH value and the compositions of MUN and MFA in milk were determined. Dairy cows (n = 16) with subacute ruminal acidosis (SARA) (pH value 5.60 ± 0.02) and control dairy cows (n = 16) (control) (pH value 6.20 ± 0.04) were studied. The MUN concentrations (578 µg/l) of the dairy cows with SARA was lower than those (1 315 µg/l) of the control dairy cows (P < 0.001). In the milk of the dairy cows with SARA, the unsaturated fatty acids (UFA), thrombogenic index (TI), and hypocholesterolemic fatty acid index (hcFA) decreased; but the saturated fatty acids (SFA), atherogenic (AI) and hypercholesterolemic fatty acid (HcFA) indexes (P < 0.01) increased. The rumen pH value and the concentration of the MUN were positively correlated with the proportions of the monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 fatty acids (n-3), omega-6 fatty acids (n-6), omega-9 fatty acids (n-9), long-chain fatty acids (LCFA) and very-long-chain fatty acids (VLCFA) and the n-3/n-6 ratio of the milk samples (P < 0.05). Consequently, the dairy cow with SARA that are in early-lactation can affect the carbohydrate fermentation, fatty acid hydrogenation and protein degradation. The MUN concentration in the dairy cows with SARA seriously decreased. The SARA changes the milk fatty acid composition and decreases the MUFA, PUFA, n-3, oleic acid and hypocholesterolemic fatty acids and the hypocholesterolemic/hypercholesterolemic ratio (h/H) values of milk. Therefore, the nutritional and functional quality for human nutrition decreases in the milk of dairy cows with SARA.

Keywords: acidosis; milk urea nitrogen; fatty acids; dairy cows

Subacute ruminal acidosis (SARA) is likely to occur when an easily palatable, high-energy diet is presented to a ruminal environment not adapted to this type of substrate. This metabolic disorder can be the result of excess feeding of non-fibrous carbohydrates (NFC), a rapid increase in the dietary content of NFC, or an insufficient rumen buffering (NRC 2001). After calving, a drop in the rumen pH resulting from the accumulation of volatile fatty acids (VFA) can be expected, due to the higher concentrate and a lower effective fibre content of the post-calving diet, resulting in a higher rate of VFA production and the reduction of the absorptive capacity of the rumen mucosa for the VFA during a dry period (Krajcarski-Hunt et al. 2002). However, if the VFA (acetic, butyric, propionic, iso-butyric, etc.) is not metabolised in the reticulorumen epithelium at the same rate, these organic acids, which produced by fermentation of organic matter by enzymes of microorganisms in the rumen, accumulate in the ruminal environment and cause rumen acidosis in case of

Supported by Erciyes University Scientific Research Projects Coordination Unit (Project No. THD-2020-10298).

https://doi.org/10.17221/51/2020-VETMED

sudden excess accumulation (Plaizier et al. 2008). Finally, this may cause to be temporarily below 5.5 of the ruminal pH. Two situations are important in terms of revealing the risk of SARA. First situation, early lactating cows are fed with a diet considerably differing from that in a dry period. A diet change carried out too rapidly, or without proper transition and management will put the animals at risk for fast ruminal fermentation changing. Second situation, in lactation period, an inaccurate calculation of the dry-matter intake leading to the wrong roughage/concentrate ratio, an inadequate content of the structural carbohydrates (neutral detergent fibre – NDF and acid detergent fibre – ADF) within the diet or mistakes in preparing of total mixed rations may produce SARA(Kleen et al. 2003). Dairy cows often receive a high-calorie diet prepartum in order to prepare the ruminal and omasal epithelia to absorb the high postpartum concentrations of the VFA. This high-calorie diet induces the proliferation of the ruminal epithelium, which is essential for the control of the rumen acidosis. The physically adequate neutral detergent fibre (peNDF), particle size, and starch source (i.e., starch type and endosperm structure) in a dairy cow's ration can also affect the ruminal buffer capacity and cause SARA or acidosis (Zebeli et al. 2012). Garret et al. (1995) suggested a cut-off point of the rumen pH of 5.5 for the diagnosis of SARA by rumenocentesis. It has been shown that the *in vitro* fibre digestibility is reduced when the pH reduces below 6.2 (Calsamiglia et al. 1999; Calsamiglia et al. 2002). The data suggest that a period lasting more than 5 h/d to 6 h/d during which the ruminal pH is < 5.8 should be avoided to minimise health disturbances due to SARA (Zebeli et al. 2012). Krajcarski-Hunt et al. (2002) stated that induction of SARA by the excess feeding of wheat/barley pellets reduced the rumen digestion of the neutral detergent fibre (NDF) from grass hay, legume hay, and corn silage.

The concentration of the proteins in the milk is given genetically and is significantly influenced by the nutrition and the level of rumen fermentation. Due to the imbalanced nutrition and frequent occurrence of indigestion, various changes in the milk's composition are encountered. The syndrome of low-fat milk is often diagnosed, being predominantly the result of rumen acidosis (Illek 1995). Moreover, the milk fat content and milk fat to milk protein ratio decrease in SARA affected cows (Danscher et al. 2015). The optimal pH of rumen proteolytic enzymes ranges from 5.5 to 7.0. However, the protein degradation is reduced at the lower end of the ruminal pH environment (Bach et al. 2005). Also, Lana et al. (1998) reported that a decrease in ruminal pH from 6.5 to 5.7 reduced the ruminal ammonia concentration only when bacteria were obtained from cows fed with a 100% forage ration, whereas bacteria from cows fed with a 90% concentrate had a lower ammonia N concentration regardless of the pH value. The overproduction of ammonia in the rumen is the leading cause of urea transfer in the blood (Roy et al. 2011).

Furthermore, the catabolism of the amino acids and excess peptides in different parts of the body contribute to increasing the urea flow into the portal blood (Huntington and Archibeque 2000; Roy et al. 2011). The synthesised urea passes into the hepatic sinuses to join the circulatory system and is filtered from the blood by the kidney before being excreted from the body in the urine (Swenson and Reece 1993). By simple diffusion, urea moves into the mammary gland, where it is an integral part of the non-protein nitrogen (NPN) components of the milk. Due to the physiological process of the urea cycle in mammals, the MUN concentration equilibrates with the body fluids and is proportional to the concentration of the blood urea (Roseler et al. 1993). The urea concentration in milk could be used as an indicator of the protein/energy balance of lactating cows (Jonker et al. 1998; Godden et al. 2001). As a result of SARA, which causes a low pH in the rumen, the feed digestion is also altered by the degraded rumen fermentation. We hypothesise that the milk fatty acid composition and milk urea nitrogen concentration, which may be indicative of the carbohydrate and protein digestion in the rumen, may be affected by a rumen pH change.

This study aims at determining the relationship between the urea and fatty acids in the milk of cows with an ideal rumen pH and dairy cows with a SARA problem.

MATERIAL AND METHODS

This study was approved by the Local Ethics Committee for Animal Experiments of Erciyes University, Province of Kayseri, Turkey (No. 20/049).

https://doi.org/10.17221/51/2020-VETMED

The nutrition and pH values of dairy cows

In the present study, dairy cows (n = 16) with subacute ruminal acidosis (SARA) (an average rumen pH value of 5.60 ± 0.02) and control dairy cows (n = 16) (control group) (an average rumen pH value of 6.20 ± 0.04) were investigated.

Dairy cows from commercial farms in the Cappadocia region (Nevsehir province, Turkey) were used. The investigated dairy cows were in the first 100 days of lactation and were multiparous. The milk production information of the dairy cows' farms was obtained from the Turkey Breeding Cows Breeders Association's e-Breeding Database. The rumen pH values of the dairy cows were detected with a digital pH meter (S220 pH/ion meter; Mettler Toledo, Ohio, USA) in the rumen fluids taken via a rumen probe 2–3 h after feeding.

The Holstein dairy cows in the SARA group had an average 620 ± 12 kg live weight and a 25.2 l/day milk yield. The Holstein dairy cows in the control group had an average 618 ± 9 kg live weight and a 26.5 l/day milk yield. The SARA group consumed a total mix ration (TMR) (total 20.56 kg DM/day; 29.75 Mcal net energy lactation (NEL)/day), which included corn silage, feed mixture concentrate, cornflake, lucerne hay, wheat straw, barley grain, salt and a vitamin-mineral premix (Table 1). The control group consumed TMR (total 20.74 kg DM/day; 29.29 Mcal NEL/day), which included corn silage, concentrate feed mixture, cornflake, lucerne herbage, wheat straw, salt and a vitamin-mineral premix (Table 1) (NRC 2001). The TMR taken by these dairy cows' farms were collected and analysed (Table 1). The milk samples of the non-pregnant cows were collected individually.

Table 1. The ingredients and chemical compositions of the total mix rations of the dairy cows

Ingredients	Farm 1 (Control group)		Farm 2 (SARA group)	
	Feed basis (kg/day)	As DM basis (kg/day)	Feed basis (kg/day)	As DM basis (kg/day)
Corn silage*	15.0	4.95	16.0	4.8
Concentrate feed mixture**	8.0	7.36	8.0	7.2
Corn flake	2.0	1.8	1.0	0.97
Lucerne herbage***	4.0	3.56	5.0	4.5
Wheat straw	3.0	2.76	3.0	2.7
Barley grain	–	–	0.5	0.47
Salt	0.03	0.03	0.02	0.02
Vitamin-mineral premix	0.10	0.10	0.08	0.08
Total DM consumption (kg/day)	32.13	20.56	33.6	20.74
Energy and nutrient matter compositions				
ME (Mcal/kg DM)	2.47		2.47	
NEL (Mcal/kg DM)	1.43		1.44	
NEL (Mcal/day)	29.75		29.29	
CP (%)	12.4		13.7	
CP (g/day)	2 578		2 781	
NFC (%)	40.4		44.9	
ADF (%)	24.3		24.1	
NDF (%)	39.4		39.3	
ADIN[&] (%)	2.65		2.47	
EE (%)	3.74		3.65	
Ash (%)	7.72		8.18	
Urea (%)	0.54		0.61	

ADF = acid detergent fibre; ADIN = acid detergent nitrogen; CP = crude protein; DM = dry matter; EE = diethyl ether extract; ME = metabolizable energy; NDF = neutral detergent fibre; NEL = net energy lactation; NFC = non-fibrous carbohydrate
*For Farm 1: 33% DM, for Farm 2: 29% DM; **For Farm 1: 16% CP, 2 500 ME kcal/kg DM, for Farm 2: 18 %CP, 2 600 ME kcal/kg DM; ***For Farm 1: 16.1 CP %, for Farm 2: 16.4% CP; [&]The ADIN value is given as % CP in the ADF residue

https://doi.org/10.17221/51/2020-VETMED

The chemical analyses of dairy cows' total mix rations

The dry matter (DM), ash, crude protein (CP) (nitrogen × 6.25), and diethyl ether extract (EE) levels were determined according to the method reported by the Association of Agricultural Chemists (AOAC 1995). The neutral detergent fibre (NDF) (using 0.5 g of sodium sulfite and 200 µl of heat stable amylase), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were analysed according to methods of Van-Soest et al. (1991). After the determination of the ADF content in the TMR, this residue was analysed with the Kjeldahl method for the nitrogen level. Then the acid detergent insoluble nitrogen (ADIN) content was calculated (nitrogen × 6.25). The urea concentrations of the concentrated feeds were determined using the spectrophotometric method, which uses 4-dimethyl amino benzaldehyde (FAO 2011). All the analyses were carried out in triplicate. The non-fibrous carbohydrate (NFC) values of the TMRs were calculated according to National Research Council (NRC 2001). The metabolizable energy (ME) and net energy lactation (NEL) were calculated using the formulas by Donker (1989) – Equation 2 and Weiss and Tebbe (2019) – Equation 1:

$$\text{ME (Mcal/kg DM)} = \text{DE (Mcal/kg DM)} \times 86/100 \quad (1)$$

$$\text{NEL (Mcal/kg DM)} = 1.64 - 0.001 \times (\text{g ADF/kg DM}) \quad (2)$$

where:
ME – metabolizable energy;
DM – dry matter;
DE – digestible energy;
NEL – net energy lactation;
ADF – acid detergent fibre.

The determination of milk urea nitrogen

10 ml milk samples were collected from the individual dairy cows January 2020. The control and acidosis groups at the different dairy cows' farms were collected (16 + 16 = 32 samples), and the samples were stored at –20 °C. The milk samples were analysed for the milk urea nitrogen concentration (MUN) using commercial kits (cdR FoodLab Urea, Latte-Milk, catalogue No.: 181610, Italy) in a MUN analyses device (cdR FoodLab Junior MUN, Italy).

The determination of fatty acid compositions in TMR's and milk samples

For the fatty acid analyses, the fat samples of the TMRs and milk samples were methylated with the three-stage modified procedure of Wang et al. (2015). According to this procedure, 40 µl of fats in falcon tubes with 15 ml volumes were mixed with 0.7 ml of potassium hydroxide (10 M) and 5.3 ml of methanol and it was vortexed.

The mixture was incubated for 45 min at 55 °C in an incubator (Nüve, Turkey) and cooled to 21 °C. The mixture was combined with 0.58 ml of H_2SO_4 (10 M) and was vortexed. After this mixture was incubated for 45 min at 55 °C, 3 ml of n-hexane was added. The tubes were centrifuged for 5 min at 1 600 *g*. 1.5 ml of the supernatants were taken in a vial with blue Polytetraflorethylene (PTFE) screw and white silicone septa caps and analysed in a gas chromatograph (Thermo Scientific, USA) with automatic sampling (Thermo AI 1310).

A Fatty Acid Methyl Esters (FAME) column (Length 60 m, I.D: 0.25 mm, film: 0.25 µm and maximum temperature 250–260 °C) with an injection split temperature of 255 °C, a colon of 140 °C and a flow rate of 30 ml/min was used for the processing method for 42 minutes.

The fatty acid identification was performed by comparing the peaks in the chromatogram with the retention times by the standard (Kramer et al. 1997). Saturated fatty acids (SFA), unsaturated fatty acids (UFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA) and very-long-chain fatty acids (VLCFA) were detected.

The atherogenic index (AI) of the milk samples, which is a marker of the atherosclerosis risk, was calculated (Ulbricht and Southgate 1991):

$$\text{AI} = (\text{C12:0} + 4 \times \text{C14:0} + \text{C16:0})/\text{UFA} \quad (3)$$

where:
AI – atherogenic index;
UFA – unsaturated fatty acids.

The thrombogenic index (TI), indicative of the potential accumulation of blood flakes in the blood vessels, was calculated by the following formula (Ulbricht and Southgate 1991):

https://doi.org/10.17221/51/2020-VETMED

$$TI = (C14:0 + C16:0 + C18:0)/[(0.5 \times MUFA) + (3 \times n\text{-}3) + (0.5 \times n\text{-}6) + (n\text{-}3/n\text{-}6)] \quad (4)$$

where:
TI – thrombogenic index;
MUFA – monounsaturated fatty acids.

The hypocholesterolemic fatty acid index (hcFA), hypercholesterolemic fatty acid index (HcFA) and hypocholesterolemic/hypercholesterolemic ratio (h/H) were calculated using following formulas (Pilarcyzk et al. 2015):

$$hcFA = C18:2n6t + PUFA \quad (5)$$

$$HcFA = C14:0 + C16:0 \quad (6)$$

$$h/H = (C18:2n6t + PUFA)/(C14:0 + C16:0) \quad (7)$$

where:
hcFA – hypocholesterolemic fatty acid index;
PUFA – polyunsaturated fatty acids;
HcFA – hypercholesterolemic fatty acid index;
h/H – hypocholesterolemic/hypercholesterolemic ratio.

Statistical analysis

SPSS v17.0 software was used for the statistical analysis of the data obtained from the studies. The data were analysed with a *t*-test. The statistical significance was taken below 0.05 ($P < 0.05$). The relationship between the investigated variables was determined by Pearson's Correlation (r) and the SPSS v17.0 package program.

RESULTS

The energy and nutrient compositions of the dairy cows TMRs are given in Table 1. The SFA, UFA, MUFA, PUFA, n-3, n-6, n-3/n-6, MCFA, LCFA, and VLCFA proportions of the TMRs were similar for the control and acidosis groups ($P > 0.05$) (Table 2).

The MUN concentrations of the dairy cows with SARA was lower than those of the control dairy cow group ($P < 0.001$) (Table 3).

The myristic acid, myristoleic acid, palmitic acid and palmitoleic acid proportions in the milk samples of the dairy cows with SARA increased compared to those of the control dairy cows ($P < 0.05$) (Tables 4 and 5). However, the linolelaidic acid, linoleic acid , α-linolenic acid (ALA), γ-linolenic acid, *cis*-11-eicoenioic acid, erucic acid, *cis*-11,14,17-eicosatrienoic acid, *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and nervonic acid proportions in the milk of the dairy cows with SARA were lower than those of the control dairy cows ($P < 0.05$) (Table 4).

The SFA, AI values and HcFA compositions in the milk samples of the dairy cows with SARA were higher than those of the control dairy cows ($P < 0.05$; Table 5). The concentrations of UFA, MUFA, PUFA, n-3, n-6, n-9, and VLCFA fatty acids, TI, hcFA and h/H rate in the milk samples of

Table 2. Compositions of the fatty acids (g/100 g fat) in the dairy cows TMR's ($n = 6$)

	Control	Acidosis	SD	SEM	*P*-value
SFA	69.75	66.33	2.98	1.72	0.137
UFA	30.25	33.65	2.97	1.71	0.138
MUFA	5.46	5.94	4.81	2.78	0.120
PUFA	29.79	27.71	1.84	1.06	0.171
n-3	1.72	1.44	0.22	0.12	0.093
n-6	28.06	26.26	1.97	1.13	0.243
n-9	0.21	5.75	4.81	2.78	0.117
n-3/n-6	0.06	0.05	0.01	0.01	0.419
MCFA	0.08	0.07	0.03	0.01	0.624
LCFA	98.12	94.91	3.92	2.26	0.230
VLCFA	1.80	4.99	3.87	2.23	0.226

LCFA = long chain fatty acids; MCFA = medium chain fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SD = standard deviation of means; SEM = standard error of means; SFA = saturated fatty acids; UFA = unsaturated fatty acids; VLCFA = very long chain fatty acids

Table 3. Milk urea nitrogen concentrations (μg/l) of milk samples

	n	MUN	SD	SEM	Minimum	Maximum
Acidosis	16	578.10	231.0	57.0	240.0	980.0
Control	16	1 315.20	284.0	73.0	940.0	1 710.0
Total	32	934.30	452.0	81.0	240.0	1 710.0
P-value	–	< 0.001	–	–	–	–

MUN = milk urea nitrogen; SD = standard deviation of means; SEM = standard error of means

https://doi.org/10.17221/51/2020-VETMED

Table 4. Compositions of the individual fatty acids (as g/100 g milk fatty acids) in the milk samples (n = 16)

Fatty acids		Control	Acidosis	SD	SEM	*P*-value
Butyric acid	C4:0	1.17	1.14	0.10	0.04	0.590
Caproic acid	C6:0	1.33	1.42	0.06	0.02	0.171
Caprylic acid	C8:0	1.07	1.16	0.02	0.01	0.197
Capric acid	C10:0	2.80	3.18	0.02	0.01	0.156
Undecanoic acid	C11:0	0.04	0.05	0.01	0.002	0.114
Lauric acid	C12:0	3.43	3.97	0.04	0.02	0.102
Tridecanoic acid	C13:0	0.12	0.14	0.01	0.003	0.072
Myristic acid	C14:0	12.20	14.28	0.14	0.07	0.008
Myristoleic acid	C14:1	1.29	1.40	0.02	0.01	0.022
Pentadecanoic acid	C15:0	1.42	1.47	0.07	0.03	0.300
cis-10-Pentadecenoic acid	C15:1	0.45	0.51	0.02	0.01	0.076
Palmitic acid	C16:0	25.93	31.15	1.42	0.71	0.008
Palmitoleic acid	C16:1	1.65	1.96	0.17	0.08	0.059
Heptadecanoic acid	C17:0	0.01	0.00	0.01	0.002	0.356
cis-10-Heptadecenoic acid	C17:1	0.73	0.62	0.05	0.03	0.012
Stearic acid	C18:0	0.24	0.28	0.01	0.01	0.131
Elaidic acid	C18:1n9*t*	11.46	8.96	0.27	0.14	< 0.001
Oleic acid	C18:1n9*c*	26.92	23.36	0.62	0.31	< 0.001
Linolelaidic acid	C18:2n6*t*	0.31	0.23	0.05	0.03	0.040
Linoleic acid	C18:2n6*c*	5.28	3.16	0.29	0.14	< 0.001
α-Linolenic acid	C18:3n3	0.22	0.16	0.03	0.01	0.006
γ-Linolenic acid	C18:3n6	0.48	0.40	0.02	0.01	0.012
Arachidic acid	C20:0	0.02	0.01	0.01	0.01	0.114
cis-11-Eicoenioic acid	C20:1	0.23	0.14	0.03	0.01	0.004
cis-11,14,17-Eicosadienoic acid	C20:2	0.02	0.03	0.01	0.01	0.228
cis-8,11,14-Eicosatrienoic acid	C20:3n6	0.06	0.17	0.12	0.06	0.104
cis-11,14,17-Eicosatrienoic acid	C20:3n3	0.22	0.04	0.11	0.06	0.019
Arachidonic acid	C20:4n6	0.05	0.04	0.01	0.004	0.094
cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5n3	0.05	0.04	0.01	0.003	0.040
Heneicosanoic acid	C21:0	0.05	0.04	0.01	0.003	0.253
Behenic acid	C22:0	0.18	0.11	0.02	0.01	0.002
Erucic acid	C22:1n9	0.26	0.17	0.02	0.01	< 0.001
cis-13,16-Docosadienoic acid	C22:2	0.02	0.02	0.02	0.01	0.809
cis-4,7,10,13,16,19-Docosahexaenoic acid	C22:6n3	0.06	0.06	0.01	0.01	0.488
Tricosanoic acid	C23:0	0.05	0.06	0.01	0.005	0.387
Lignoceric acid	C24:0	0.02	0.00	0.02	0.01	0.028
Nervonic acid	C24:1	0.02	0.01	0.01	0.003	0.005

SD = standard deviation of means; SEM = standard error of means

the dairy cows with SARA were lower than those of the control dairy cow group ($P < 0.05$; Table 5).

In the present study, the MUN concentration of the milk was positively correlated with the rumen pH value ($r = 0.700$; $P < 0.001$). The SFA proportions of the milk was negatively correlated with the MUN concentration ($r = -0.830$) and the rumen pH values ($r = -0.921$; $P < 0.001$).

In addition, the UFA proportion of the milk was positively correlated with the MUN concentration ($r = 0.835$) and the rumen pH values ($r = 0.923$; $P < 0.001$). The rumen pH value and the concen-

https://doi.org/10.17221/51/2020-VETMED

Table 5. Compositions of the fatty acids (as g/100 g milk fatty acids) in the milk samples (n = 16)

	Control	Acidosis	SD	SEM	*P*-value
SFA	51.47	59.91	0.94	0.47	< 0.001
UFA	49.86	41.50	1.01	0.50	< 0.001
MUFA	43.04	37.14	0.63	0.32	< 0.001
PUFA	6.81	4.36	0.36	0.18	< 0.001
MCFA	8.69	9.79	0.09	0.04	0.126
LCFA	89.30	88.43	0.26	0.13	0.198
VLCFA	0.71	0.48	0.08	0.04	0.004
n-3	0.57	0.30	0.14	0.07	0.011
n-6	6.24	4.06	0.22	0.11	< 0.001
n-9	40.17	34.04	0.41	0.20	< 0.001
n-3/n-6	0.09	0.07	0.02	0.01	0.255
AI	1.56	2.22	0.35	0.12	< 0.001
TI	6.70	5.48	0.73	0.25	0.003
hcFA	33.73	27.72	0.36	0.19	< 0.001
HcFA	38.13	45.43	0.41	0.24	< 0.001
h/H	0.88	0.61	0.02	0.01	< 0.001

AI = atherogenic index; h/H = hypocholesterolemic/hypercholesterolemic ratio = (C18:1n9c + PUFA)/(C14:0 + C16:0); HcFA = hypercholesterolemic fatty acids index = C14:0 + C16:0; hcFA = hypocholesterolemic fatty acids index = C18:2n6t + PUFA; LCFA = long chain fatty acids; MCFA = medium chain fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SD = standard deviation of means; SEM = standard error of means; SFA = saturated fatty acids; TI = thrombogenic index; UFA = unsaturated fatty acids; VLCFA = very long chain fatty acids

Table 6. Pearson's correlations among the compositions of the milk fatty acids and the milk urea nitrogen and the rumen pH value in the dairy cows

	UFA	MUFA	PUFA	n-3	n-6	n-9	n-3/n-6	MCFA	LCFA	VLCFA	MUN	Rumen pH
SFA	–0.992**	–0.980**	–0.997**	–0.882**	–0.997**	–0.973**	–0.522**	0.612**	–0.471**	–0.907**	–0.830**	–0.921**
UFA	1	0.997**	0.983**	0.898**	0.979**	0.993**	0.576**	–0.549**	0.476**	0.935**	0.835**	0.923**
MUFA	–	1	0.966**	0.891**	0.961**	0.998**	0.586**	–0.517**	0.489**	0.937**	0.833**	0.920**
PUFA	–	–	1	0.895**	0.998**	0.956**	0.538**	–0.612**	0.434*	0.908**	0.820**	0.910**
n-3	–	–	–	1	0.865**	0.868**	0.853**	–0.331	0.211	0.973**	0.726**	0.792**
n-6	–	–	–	–	1	0.952**	0.484**	–0.642**	0.458**	0.883**	0.820**	0.912**
n-9	–	–	–	–	–	1	0.560**	–0.502**	0.492**	0.926**	0.834**	0.920**
n-3/n-6	–	–	–	–	–	–	1	0.145	–0.138	0.811**	0.444*	0.464**
MCFA	–	–	–	–	–	–	–	1	–0.817**	–0.281	–0.435*	–0.524**
LCFA	–	–	–	–	–	–	–	–	1	0.221	0.372*	0.445*
VLCFA	–	–	–	–	–	–	–	–	–	1	0.763**	0.847**
MUN	–	–	–	–	–	–	–	–	–	–	1	0.700**

LCFA = long chain fatty acids; MCFA = medium chain fatty acids; MUFA = monounsaturated fatty acids; MUN = milk urea nitrogen; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; UFA = unsaturated fatty acids; VLCFA = very long chain fatty acids

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

tration of MUN were positively correlated with the MUFA, PUFA, n-3, n-6, n-9, LCFA, and VLCFA proportions and the n-3/n-6 ratio in the milk samples (P < 0.05) (Table 6).

https://doi.org/10.17221/51/2020-VETMED

DISCUSSION

Dairy cows often receive a high-calorie TMR in the last 2–3 weeks of prepartum in order to prepare the ruminal epithelial and rumen environment to absorb the high postpartum concentrations of VFA. This high-starch diet (easily digestible carbohydrates) induces the profile of the microorganisms in the rumen and the proliferation of the ruminal epithelium, which is vital for the control of the acidosis in rumen. The sudden shift to a high carbohydrate diet can cause the ruminal pH to decrease and may lead to SARA or acidosis. Besides, the levels of peNDF, the particle size, and the starch source (i.e., the starch type and endosperm structure) and the fermentation capacities of the fibrous feedstuffs in dairy cow's ration can affect the buffer capacity in the rumen and cause high ruminal acidity (Calsamiglia et al. 1999; Zebeli et al. 2012). The cut-off point of the rumen fluid for the diagnosis of SARA by rumenocentesis is a pH of 5.5 (Garret et al. 1995). The 5.6 ruminal pH value in the present study demonstrates that SARA was present. Also, a period lasting more than 5 h/d to 6 h/d during which the ruminal pH is < 5.8 should be avoided to minimise the health disturbances due to SARA (Zebeli et al. 2012). The reason for SARA in the present study can be the high NFC or the prepartum diet and forage particle size, peNDF. Krajcarski-Hunt et al. (2002) stated that the induction of SARA by excess feeding of wheat/barley pellets reduced the rumen NDF digestion from grass hay, legume hay, and corn silage. The TMRs (control and SARA) had the same fatty acid concentration. However, the proportions of the UFA, MUFA, PUFA, n-3, n-6, n-9, and VLCFA and TI values in the milk samples of the dairy cows with SARA were lower than those of the control dairy cows, which can be related to decreasing the fibre digestion in SARA (Krajcarski-Hunt et al. 2002). The MFA profile is also seen as an essential factor in the technological quality of the raw milk and its effect on human health (Hanus et al. 2018).

The rising SFA content in animal and human diets may bring the risk of cardiovascular and other metabolism diseases. Previous researchers demonstrated that the n-3 PUFA, MUFA, oleic acid and ALA contents had in preventing heart disease, improving the immune response, decreasing the low-density lipoproteins effects, anticancer and anti-atherogenic properties (Williams 2000; Haug et al. 2007; Muchenje et al. 2009). In general, in the present study, it was seen that the milk quality of cows with SARA was negatively affected and the nutritional quality of the milk taken from the cows with SARA decreased in terms of its effect on human health, especially in the UFA, PUFA, n-3, n-9, hcFA and h/H values. Previous researchers showed that SARA can negatively impact various milk production parameters, especially the milk fat content (Danscher et al. 2015).

In the present study, the predominant fatty acids were palmitic acid, oleic acid, myristic acid and elaidic acid. The decreasing at UFA (49.9% *vs* 41.5%), MUFA (43.0% *vs* 37.1%), PUFA (6.8% *vs* 4.4%), n-3 (0.57% *vs* 0.30%), n-6 (6.2% *vs* 4.1%) and n-9 (40.2% *vs* 34.0%) fatty acids in the milk fat in the present study were positively correlated with the rumen pH value that demonstrated the importance of the ruminal acidity factor. The milk fat of cow milk in conventional herds included 2.74% C18:2n6 (linoleic, *cis* and linolelaidic acids, trans), 0.51% C18:3n3 (α-linolenic acid), 68.2% SFA, 26.8% MUFA, 4.39% PUFA, 2.54% PUFA n-6 and 0.76% PUFA n-3 fatty acids as stated by Hanus et al. (2018). In the study, the change in the milk fatty acid profile of the dairy cows with SARA changed the milk AI and TI values. The milk AI values in the study were similar to results of Santillo et al. (2016) and Pilarczyk et al. (2015). But, the milk TI values of both the control and SARA dairy cows in the present study were lower than the results of Santillo et al. (2016) and Pilarczyk et al. (2015). The differences in the milk TI values may be due to the diet differences, environmental conditions, lactation period or breed difference. The hypocholesterolemic fatty acids and h/H rates of the milk in present study decreased in the dairy cows with SARA in relation to the high content of myristic and palmitic acids and low content of PUFA and oleic acids. It is thought that changes in the carbohydrate fermentation (VFA concentration) and fatty acid hydrogenation in the rumen environment due to the decrease in the rumen pH in SARA cows may affect the milk's fatty acid composition.

The optimal pH of the rumen proteolytic enzymes ranges from 5.5 to 7.0. However, protein degradation is reduced at the lower end of the ruminal pH environment (Bach et al. 2005). Lana et al. (1998) reported that a decrease in the ruminal pH from 6.5 to 5.7 reduced the ruminal ammonia concentration. The ruminal ammonia concentration

https://doi.org/10.17221/51/2020-VETMED

can be increased due to high ruminal degradable proteins in the diet or those not used for the microbial protein production by the microorganisms. The ammonia in the rumen environment is the leading cause of urea transfer in the blood (Roy et al. 2011). In addition, the catabolism of the amino acids and excess peptides in the different parts of the body contribute to increasing the urea flow into the portal blood (Huntington and Archibeque 2000; Roy et al. 2011). The excess degradable protein in the rumen or energy deficiency in the diet increases the blood urea levels, and, thus, the level of the milk urea (Arunvipas et al. 2003).

A low level of urea in the milk results from a high proportion of fermentable carbohydrates compared to the degradable protein content in the diet, which decreases the ammonia production, reflecting an inadequate synthesis of microbial proteins in the rumen (Bruning-Fannand and Kaneene 1993). Urea, which could be used as an indicator of the protein/energy balance of lactating cows, is a water-soluble molecule and, when present in the circulatory system, diffuses into the body's aqueous organs such as the udder and other genital glands (Butler 1998). Urea is the main product of nitrogen metabolism synthesised in the liver from an excess ammonia product derived from rumen-degraded proteins, digestible proteins in the small intestine, and amino acids catabolised in different parts of the body or during the glycogenesis processes in the liver (Schepers and Meijer 1998). The low MUN value in the cows with SARA symptoms in the study may be due to the decreased protein breakdown due to acidity in the rumen, and consequently the decreased ruminal ammonia and decreased overall circulation urea level. Similar to the results of the present study, Gao and Oba (2015) stated that a low MUN concentration and milk fat in mid-lactating cows fed a high-grain diet may be used to identify cows that have a higher risk of SARA. Besides, the MUN value in the milk of the control dairy cows used in the study was in the range of normal MUN reference values (Gao and Oba 2015; Munyaneza et al. 2017).

As a result, the increased acidity in the rumen of dairy cow in early-lactation can affect the carbohydrate fermentation, fatty acid hydrogenation and protein degradation. The MUN concentration in dairy cows with SARA seriously decreased. The SARA changes the fatty acid proportion in the milk and decreases the MUFA, PUFA, n-3, oleic acid and hypocholesterolemic fatty acids and the h/H values of the milk. Therefore, the nutritional and functional quality of the milk in the dairy cows with SARA decreases for human nutrition.

Acknowledgement

I thank to Osman Semih Cavdar for his valuable help on data collect of study.

Conflict of interest

The author declares no conflict of interest.

REFERENCES

AOAC – Association of Official Analytical Chemists. Official methods of analysis of AOAC international. Washington, DC: Association of Official Analytical Chemists; 1995.

Arunvipas P, Dohoo IR, VanLeeuwen JA, Keefe GP. The effect of non-nutritional factors on milk urea nitrogen levels in dairy cows in Prince Edward Island, Canada. Prev Vet Med. 2003 May 30;59(1-2):83-93.

Bach A, Calsamiglia S, Stern MD. Nitrogen metabolism in the rumen. J Dairy Sci. 2005 May;88(Suppl 1):E9-21.

Bruning-Fann CS, Kaneene JB. The effects of nitrate, nitrite and N-nitroso compounds on animal health. Vet Hum Toxicol. 1993 Jun;35(3):521-38.

Butler WR. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J Dairy Sci. 1998 Sep;81(9):2533-9.

Calsamiglia S, Ferret A, Plaixats J, Devant M. Effect of pH and pH fluctuations on microbial fermentation in a continuous culture system. J Dairy Sci. 1999;82(Suppl 1):38.

Calsamiglia S, Ferret A, Devant M. Effects of pH and pH fluctuations on microbial fermentation and nutrient flow from a dual-flow continuous culture system. J Dairy Sci. 2002 Mar;85(3):574-9.

NRC – National Research Council. Nutrient requirements of dairy cattle. 7th ed. Washington, DC, USA: National Academy Press; 2001.

Danscher AM, Li S, Andersen PH, Khafipour E, Kristensen NB, Plaizier JC. Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. Acta Vet Scand. 2015 Jul 17;57(1):39.

Donker JD. Improved energy prediction equations for dairy cattle rations. J Dairy Sci. 1989 Nov;72(11):2942-8.

FAO – Food and Agriculture Organization of the United Nations. Quality assurance for animal feed analysis labo-

https://doi.org/10.17221/51/2020-VETMED

ratories. FAO Animal Production and Health Manual No. 14. Rome: Food and Agriculture Organization of the United Nations; 2011.

Gao X, Oba M. Short communication: Noninvasive indicators to identify lactating dairy cows with a greater risk of subacute rumen acidosis. J Dairy Sci. 2015 Aug;98(8):5735-9.

Garrett EF, Pereira MN, Armentano LE, Nordlund KV, Oetzel GR. Comparison of pH and VFA concentration of rumen fluid from dairy cows collected through a rumen canal vs. rumenocentesis. J Dairy Sci. 1995;78 (Suppl 1):299.

Godden SM, Kelton DF, Lissemore KD, Walton JS, Leslie KE, Lumsden JH. Milk urea testing as a tool to monitor reproductive performance in Ontario dairy herds. J Dairy Sci. 2001 Jun;84(6):1397-406.

Hanus O, Samkova E, Krizova L, Hasonova L, Kala R. Role of fatty acids in milk fat and the influence of selected factors on their variability – A review. Molecules. 2018 Jul 4; 23(7):1636.

Haug A, Hostmark AT, Harstad OM. Bovine milk in human nutrition – A review. Lipids Health Dis. 2007 Sep 25;6:25.

Huntington GB, Archibeque SL. Practical aspects of urea and ammonia metabolism in ruminants. J Anim Sci. 2000; 77(Suppl E):1-11.

Illek J. A tehenek alacsony tejfehérje-szindrómája [Syndrome of low-protein cow milk]. Magyar Allatorvosok Lapja. 1995 Oct 30;50(10):738-9. Hungarian.

Jonker JS, Kohn RA, Erdman RA. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J Dairy Sci. 1998 Oct;81(10): 2681-92.

Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP. Subacute ruminal acidosis (SARA): A review. J Vet Med A Physiol Pathol Clin Med. 2003 Oct;50(8):406-14.

Krajcarski-Hunt H, Plaizier JC, Walton JP, Spratt R, McBride BW. Short communication: Effect of subacute ruminal acidosis on in situ fiber digestion in lactating dairy cows. J Dairy Sci. 2002 Mar;85(3):570-3.

Kramer JK, Fellner V, Dugan ME, Sauer FD, Mossoba MM, Yurawecz MP. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids. 1997 Nov;32(11):1219-28.

Lana RP, Russell JB, Van Amburgh ME. The role of pH in regulating ruminal methane and ammonia production. J Anim Sci. 1998 Aug;76(8):2190-6.

Munyaneza N, Niyukuri J, Hachimi YE. Milk urea nitrogen as an indicator of nitrogen metabolism efficiency in dairy cows: A review. Theriogenology Insight. 2017;7(3):145-59.

Muchenje V, Dzama K, Chimonyo M, Strydom PE, Hugo A, Raats JG. Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Food Chem. 2009 Jan 15;112(2):279-89.

Pilarczyk R, Wojcik J, Sablik P, Czerniak P. Fatty acid profile and health lipid indices in the raw milk of Simmental and Holstein-Friesian cows from an organic farm. S Afr J Anim Sci. 2015;45(1):30-8.

Plaizier JC, Krause DO, Gozho GN, McBride BW. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet J. 2008 Apr;176(1):21-31.

Roseler DK, Ferguson JD, Sniffen CJ, Herrema J. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J Dairy Sci. 1993 Feb 1;76(2):525-34.

Roy B, Brahma B, Ghosh S, Pankaj PK, Madal G. Evaluation of milk urea concentration as useful indicator for dairy herd management: A review. Asian J Anim Vet Adv. 2011 Jan 1;6(1):1-19.

Santillo A, Caroprese M, Marino R, d'Angelo F, Sevi A, Albenzio M. Fatty acid profile of milk and Cacioricotta cheese from Italian Simmental cows as affected by dietary flaxseed supplementation. J Dairy Sci. 2016 Apr;99(4):2545-51.

Schepers AJ, Meijer RG. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. J Dairy Sci. 1998 Feb;81(2):579-84.

Swenson MJ, Reece WO. Water balance and excretion. In: Swenson MJ, Reece WO, editors. Duke`s physiology of domestic animals. 11th ed. Ithaca, New York: Cornell University Press; 1993. p. 573-604.

Ulbricht TL, Southgate DA. Coronary heart disease: Seven dietary factors. Lancet. 1991 Oct 19;338(8773):985-92.

Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991 Oct; 74(10):3583-97.

Wang J, Wu W, Wang X, Wang M, Wu F. An affective GC method for the determination of the fatty acid composition in silkworm pupae oil using a two-step methylation process. J Serb Chem Soc. 2015;80(1):9-20.

Weiss WP, Tebbe AW. Estimating digestible energy values of feeds and diets and integrating those values into net energy systems. Transl Anim Sci. 2019 Nov 5;3(3):953-61.

Williams CM. Dietary fatty acids and human health. Ann Zootech. 2000 May 1;49(3):165-80.

Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN, Drochner W. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. J Dairy Sci. 2012 Mar;95(3):1041-56.

Received: February 27, 2020
Accepted: June 2, 2020