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Susceptibility of dairy cows to subacute ruminal acidosis is reflected in milk fatty acid proportions, with C18:1 *trans*-10 as primary and C15:0 and C18:1 *trans*-11 as secondary indicators

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ABSTRACT

The current study was carried out to assess 2 hypotheses: (1) cows differ in susceptibility to a subacute ruminal acidosis (SARA) challenge, and (2) the milk fatty acid (FA) pattern can be used to differentiate susceptible from nonsusceptible cows. For this, 2 consecutive experiments were performed. During experiment 1, the milk FA pattern was determined on 125 cows fed an increasing amount of concentrate during the first 4 wk in milk (WIM). The coefficient of variation of several SARA indicative milk FA (i.e., C15:0, C18:1 *trans*-10, C18:2 *cis*-9,*trans*-11, and C18:1 *trans*-10 to C18:1 *trans*-11 ratio) increased, indicating that cows reacted differently upon the concentrate build-up. A first grouping was based on the milk fat C18:1 *trans*-10 proportion in the third WIM. Fifteen cows with the highest proportion of the latter FA (HT10) and their counterparts with low C18:1 *trans*-10 and equal parity distribution (LT10) were compared, which revealed that milk fat content and milk fat to protein ratio were lower for the HT10 group. From each of the HT10 and LT10 groups, 5 animals were selected for experiment 2. The subselection of the HT10 group, referred to as HT10s, showed a high proportion of C18:1 *trans*-10 at 3 WIM (>0.31 g/100 g of FA), a high level of C15:0 (on average ≥1.18 g/100 g of FA over the 4 WIM), and a sharp decrease of C18:1 *trans*-11 ($\Delta \geq 0.25$ g/100 g of FA during the 4 WIM). Their counterparts (LT10s) had a low milk fat C18:1 *trans*-10 proportion at 3 WIM (<0.23 g/100 g of FA), an average C15:0 proportion of 0.99 g/100 g of FA or lower, and a rather stable C18:1 *trans*-11 proportion. The HT10s group was hypothesized to be more susceptible to a SARA challenge, achieved by increasing amounts of rapidly fermentable carbohydrates in experiment 2. The HT10s cows had a lower nadir, mean, and maximum reticulo-ruminal pH; longer period of reticulo-ruminal pH below 6.0; and higher daily reticulo-ruminal pH variation compared with LT10s cows. Throughout experiment 2, HT10s and LT10s cows differed in levels of SARA indicative milk FA. Five animals, including one LT10s and 4 HT10s cows, experienced SARA, defined as reticulo-ruminal pH <6.0 for more than 360 min/d. These results indicate that it is possible to distinguish cows with different susceptibility to a SARA challenge within a herd by monitoring the milk FA composition when cows receive the same diet.

Key words: subacute ruminal acidosis, animal variability, milk fatty acid, reticulo-ruminal pH, biomarker

INTRODUCTION

Subacute ruminal acidosis represents one of the most important digestive disorders in intensive dairy farms, with a suggested incidence between 19 and 26% in early and mid lactation (Plaizier et al., 2008). Despite the optimization of diet formulation strategies [e.g., including structure index (De Brabander et al., 1999) or physically effective fiber (Allen, 1997) as dietary characteristics], some cows in a herd still experience SARA, indicating the existence of inter-animal variation to rapidly fermentable carbohydrate (**RFCH**) challenges (Krause and Oetzel, 2006). Indeed, several SARA indicators, such as mean rumen pH and acidosis index (indicator for the severity of SARA and calculated as area under the curve of pH <5.8 divided by DMI; Gao and Oba, 2014), showed a large range within a group of cows receiving the same high-grain treatment (Brown et al., 2000; Schlau et al., 2012). In addition, high standard error of the mean values associated with pH parameters during a ruminal acidosis induction trial further proved the existence of animal variability

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(Penner et al., 2007). Inter-animal variation in SARA susceptibility decreases the effectiveness of herd-based diet formulation strategies and is of concern from an animal welfare perspective. Besides this, it can also mask treatment effects during SARA induction trials. Therefore, it is of importance to identify biomarkers that could distinguish cows with different susceptibility to SARA challenges before the occurrence of ruminal disturbances.

Previous studies from our laboratory indicated that proportions in milk fat of some odd- and branched-chain fatty acids (**OBCFA**) as well as *trans* isomers (e.g., C15:0, C18:1 *trans*-10, and C18:1 *trans*-11) varied with rumen pH (Colman et al., 2010; Fievez et al., 2012). If these milk fatty acids (**FA**) effectively are appropriate SARA indicators, they also should allow to distinguish between cows with different susceptibilities to SARA development when facing the same carbohydrate challenge. Hence, we hypothesized that cows differ in susceptibility to SARA challenges and that this difference can be monitored through the milk FA pattern. To investigate these hypotheses, we carried out 2 consecutive experiments in which first the milk FA pattern was monitored weekly during the first 4 wk of lactation in a herd of 125 cows to identify cows which potentially vary in susceptibility toward SARA development. This was validated in a second experiment, during which a selection of these cows was exposed to a SARA challenge protocol.

MATERIALS AND METHODS

All experimental procedures involving animals were approved by the Central Committee on Animal Experiments (AVD24246002017848) and the Institute for Animal Welfare of the Schothorst Feed Research B.V. (RM17–15-LRA-57), the Netherlands.

Animals, Diets, and Experimental Design

This study consisted of 2 experiments, which both were carried out at the Schothorst Feed Research B.V. (Lelystad, the Netherlands). The first experiment was performed from September 2015 to February 2017, and the second experiment was performed from April to May 2017.

In experiment 1, 125 Holstein-Friesian dairy cows (43 primiparous and 82 multiparous cows with mean parity of 3.5) were monitored during the first 4 wk in milk (**WIM**). All animals were fed a basal diet ad libitum, which mainly consisted of grass silage, maize silage, and soybean meal (Table 1). Concentrate was supplemented to this basal diet (Table 2) to meet the recommendations of early lactating high yielding dairy cows. Concerning the concentrate supplementation, the amount offered was based on the parity and milk production, and the standard protocol applied at the farm was followed to build up the amount of concentrate after calving: the amount of concentrate was gradually increased during the first 4 lactation weeks from 3.0 to 6.0 kg for primiparous cows and from 4.0 to 10.0 kg for multiparous cows. The basal diet was divided into 2 equal meals at 0730 and 1500 h. The concentrate was fed separately 4 times a day.

Table 1. Ingredients (g/kg of DM) and nutritive value of the basal ration fed during experiment 1

Item	Value
Ingredient, g/kg of DM	
Maize silage	354
Grass silage	382
Soybean meal	114
Beet pulp	71
Chopped wheat straw	16
Minerals and trace elements[1]	5.3
Chalk	4.0
Salt	2.7
Magnesium oxide	1.4
Calculated nutritive value	
NE_L,[2] MJ/kg of DM	6.8
DVE,[3] g of DVE/kg of DM	88
Fatty acid composition, g/kg of DM	
C16:0	2.53
C18:0	0.36
C18:1n-9	2.09
C18:2n-6	5.70
C18:3n-3	4.73
Total fatty acids	15.75

[1]Contains: 170 g of Ca, 0 g of P, 14 g of Mg, 7.5 g of Na, 11.6 g of Cl, 0.4 g of S, 2,000 mg of Cu, 4,000 mg of Zn, 2,000 mg of Mn, 50 mg of Se, 50 mg of Co, 120 mg of I, 600,000 IU of vitamin A, 120,000 IU of vitamin D_3, 4,000 IU of vitamin E/kg.

[2]NE_L was calculated based on the Dutch net energy evaluation (VEM) system (Van Es, 1975), 1,000 VEM = 6.9 MJ.

[3]DVE = intestinal digestible protein (Tamminga et al., 1994).

For experiment 2, 10 cows (156 ± 70 DIM) were selected based on the results of experiment 1, aiming at obtaining 2 groups of 5 cows, which were equally stratified for parity and DIM. Cows in these 2 groups differed in proportions in milk fat of milk FA, which have been identified before as biomarkers for SARA. The experimental period lasted for 28 d. The SARA challenge was achieved by replacing concentrate A (low wheat content; Table 3) by concentrate B (high wheat content; Table 3). The first 18 d were considered as the low rapidly fermentable carbohydrate period (**LFC**), during which all cows received the basal diet in combination with concentrate A (676/324, wt/wt on DM basis). During the first 4 d of this LFC period, the concentrate (details not shown) that the cows received before

the experiment was replaced stepwise by concentrate A. From d 19 until d 24, concentrate A was gradually and partly replaced by concentrate B until concentrate B reached 760 g/kg of the total concentrate mixture on d 24 (DM basis), and this period is referred to as the increasing rapidly fermentable carbohydrate (**IFC**) period. On d 25, the proportion of concentrate B in the concentrate mixture was increased to 780 g/kg (DM basis). This composition was maintained for 3 more days [high rapidly fermentable carbohydrate (**HFC**) period]. An overview of this SARA challenge protocol is given in Table 4. However, during the implementation of the SARA challenge protocol, some modifications had to be applied due to (1) the overly pH decrease of some HT10s cows (selection criteria of HT10s cows is shown later) and (2) the lack of reticular pH response of LT10s cows (selection criteria of LT10s cows is shown later). The former (overly pH decrease) refers to the very fast decrease in reticulo-ruminal pH in response to increasing amounts of RFCH (concentrate B). Accordingly, the increase in concentrate B, foreseen in the original protocol, risked seriously impairing the health status of these cows and hence was modified. The latter (lack of reticular pH response) refers to the lack of reticulo-ruminal pH decrease in some LT10s cows as induced by the increase in concentrate B as foreseen in the original protocol. Hence, some individual adjustments were made to avoid dramatic detrimental health effects for some HT10s cows, while attempting to reach SARA conditions in LT10s cows by a somewhat more

Table 2. Ingredients (g/kg of DM) of concentrate used during experiment 1

Item	Value
Ingredient, g/kg of product	
Maize	220
Palm kernel expeller	198
Soybean hulls	131
Rapeseed meal	92
Sugar beet pulp (20–25% sugar)	71
Soybean meal	57
Molasses	36
Vinasses	30
Wheat	26
Sunflower meal	25
Citrus pulp	20
Wheat bran	20
Sugar beet pulp (<10% sugar)	18
Barley	15
Wheat gluten feed	15
Chalk	14
Salt	4.6
Palm oil	3.0
Magnesium oxide	2.9
Chemical composition, g/kg of product	
DM	877
Ash	70
OM	807
CP	158
aNDFom	293
ADF	181
ADL	29
Starch	172
Sugar	66
NE_L,[1] MJ/kg of product	6.49
Fatty acid composition, g/kg of product	
C12:0	5.80
C14:0	1.80
C16:0	2.73
C18:0	0.73
C18:1n-9	6.07
C18:2n-6	7.22
C18:3n-3	1.74
Total fatty acids	26.1

[1]NE_L was calculated based on the Dutch net energy evaluation system (Van Es, 1975).

Table 3. Ingredients (g/kg of DM) and chemical composition of the concentrates of experiment 2

Item	Concentrate A	Concentrate B
Ingredient		
Sugar beet pulp (20–25% sugar)	200	
Wheat	185	705
Rapeseed meal	149	
Maize gluten feed	142	
Maize	132	
Molasses	60	55
Rumen bypass soybean meal	50	
Sugar beet pulp (<10% sugar)	37	
Citrus pulp	22	
Limestone	7	14
Premix[1]	7	7
Salt	3	9
Palm oil	7	
Peas (<22% CP)		200
Urea		11
Chemical composition, g/kg of product		
DM	880	861
Ash	64.5	48.8
OM	814	811
CP	152	149
aNDFom[2]	205	116
ADF	95	40
ADL	12	5
Starch	213	486
NE_L,[3] MJ/kg of DM	6.7	6.7
Fatty acid composition, g/kg of product		
C16:0	2.84	1.20
C18:0	0.66	0.28
C18:1n-9	4.82	0.99
C18:2n-6	9.72	3.67
C18:3n-3	0.97	0.35
Total fatty acids	19.0	6.35

[1]Contains: 140 g of Ca, 0 g of P, 14 g of Mg, 7.5 g of Na, 0.3 g of K, 11.5 g of Cl, 0.3 g of S, 2,000 mg of Cu, 4,025 mg of Zn, 3,050 mg of Mn, 40 mg of Se, 75 mg of Co, 124 mg of I, 600,000 IU of vitamin A, 120,000 IU of vitamin D_3, and 5,000 IU of vitamin E.

[2]aNDFom = ash-free aNDF organic matter.

[3]NE_L was calculated based on the Dutch net energy evaluation system (Van Es, 1975).

severe challenge (Supplemental Table S1 for individual details; https://doi.org/10.3168/jds.2018-14903).

In both trials, cows were housed in a freestall barn and had continuous access to fresh water. Cows were fed at 0700 and 1400 h, and milked at 0500 and 1600 h daily. Milk production was recorded daily.

Unfortunately, one HT10s cow got stuck by the manger and died at the beginning of the IFC period. Accordingly, the removal of the cow from the experiment was not linked to dietary treatments.

Chemical Composition of Feeds

Analysis of chemical composition of concentrates consisted of determination of DM (European Economic Community 1971a), crude ash by incineration (550°C, 2 h; European Economic Community 1971b), and CP according to the Kjeldahl method (European Community 1993).

Neutral detergent fiber: the measurement of ash-free aNDF organic matter (aNDFom) and ADF were as described by Fustini et al. (2017), with ADF expressed inclusive of residual ash. Acid detergent lignin (sulfuric acid) was determined after ADF analysis by solubilization of cellulose with sulfuric acid. Starch was determined according to ISO 14914–2004 (ISO, 2004). Sugars were determined as the total reducing sugars content, expressed as glucose equivalents, following the procedure reported in AOAC method 942.15 (AOAC International, 1995).

Milk Fatty Acid Composition

In experiment 1, milk samples for FA analysis were collected once every week on Wednesday evening. In experiment 2, milk samples were collected twice a day from d 11 until d 28.

Milk fat was extracted based on the mini Röse-Gottlieb method (adapted from Chouinard et al., 1997), after which methylation was performed according to Stefanov et al. (2010). Analysis of FAME was carried out using a gas chromatograph (HP 7890A, Agilent Technologies, Diegem, Belgium) equipped with a SP-2560 capillary column (75 m × 0.18 mm i.d. × 0.14 μm thickness; Supelco Analytical, Bellefonte, PA) and a flame ionization detector. A combination of 2 oven temperature programs was used in this study to achieve separation of most *cis* and *trans* 16:1 isomers, branched-chain FA, and 18:1 isomers (Kramer et al., 2008). For the first GC run, the temperature program was as follows: at the time of sample injection, the column temperature was 70°C for 2 min, then gradually increased at 15°C/min to 150°C, followed by a second increase at 1°C/min to 165°C and maintained for 12 min, followed by a third increase at 2°C/min to 170°C, maintained for 5 min, and a final increase at 5°C/min to 215°C, which was maintained for 20 min. Injection volume was 1 μL with a split ratio of 50:1. Inlet and detector temperatures were 250 and 255°C, respectively. The flow rate for hydrogen carrier gas was 1 mL/min. For the second GC run, the temperature program was as follows: at the time of sample injection the column temperature was 70°C, then gradually increased at 50°C/min to 175°C, which was maintained for 13 min, followed by a final increase at 5°C/min to 215°C, which was maintained for 20 min. Injection volume was 0.5 μL with a split ratio of 100:1. The flow rate of the carrier gas and inlet and detector temperatures were similar as in the first GC run. Most FA peaks were identified using quantitative mixtures of methyl ester standards (GLC463, Nu-Chek Prep, Elysian, MN; *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA, Larodan 279, Fine Chemicals AB, Malmö, Sweden).

Milk Fat and Protein

The milk fat and protein content were determined by means of Fourier transform infrared spectrometry (Milkoscan FT6000, Foss Electric, Hillerød, Denmark) at Qlip for experiment 1. During experiment 2, milk fat content was calculated based on the method of Ackman and Sipos (1964) and Wolff et al. (1995), assessed as the sum of FAME. For this, an internal standard (TAG-13) was added before milk fat extraction, which allowed

Table 4. Experimental design and intended ratios (g/kg of DM) of basal diet, concentrate A, and concentrate B during experiment 2

Day[1]	Period	Basal diet	Concentrate A	Concentrate B
5–18	LFC period	676	324	0
24	IFC period	678	77	245
25–28	HFC period	659	75	266

[1]Mean values are reported for the low rapidly fermentable carbohydrate (LFC) and high rapidly fermentable carbohydrate (HFC) period. The value of the last day is reported for the increasing rapidly fermentable carbohydrate (IFC) period.

Table 5. Information of the milk fatty acid (FA)-based criteria to select 5 HT10s cows and 5 LT10s cows for the SARA challenge trial (experiment 2)

Parameter, g/100 g of FA		HT10s[1]	LT10s[1]
C18:1 *trans*-10	Proportion at third week in lactation	≥0.31	≤0.23
C15:0	Average proportion during first 4 wk in lactation	≥1.18	≤0.99[2]
Δ C18:1 *trans*-11	Maximum decrease in proportion during first 4 wk in lactation	≥0.25[3]	≤0.18

[1]HT10s and LT10s cows: 5 cows selected from the HT10 group and 5 cows selected from LT10 group, respectively, based on milk FA data obtained during experiment 1.

[2]Two LT10s cows had a more elevated average C15:0 proportion (1.16 and 1.27 g/100 g of FA).

[3]One HT10s cow showed a smaller decrease in C18:1 *trans*-11 (Δ = 0.15 g/100 g of FA).

assessment of the milk fat content based on the exact ratio of internal standard weight to milk sample weight.

Selection of SARA Susceptible and Unsusceptible Cows for Experiment 2

Ample of literature reports the close relationship of increased milk fat C18:1 *trans*-10 proportions and low rumen pH during SARA induction trials (e.g., Enjalbert et al., 2008; Colman et al., 2013). Accordingly, herd data of experiment 1 were at first instance explored based on the milk C18:1 *trans*-10 proportions. The latter FA peaked at 3 WIM in experiment 1 and was used to make a first classification. Thus, 15 cows with the highest milk fat C18:1 *trans*-10 proportion at 3 WIM (minimum milk fat C18:1 *trans*-10 proportion of these 15 cows was 0.31 g/100 g of FA) were classified as the high C18:1 *trans*-10 group (**HT10**). Additionally, a second group was created including the same number of animals. These animals were selected from the herd with the lowest proportions of C18:1 *trans*-10 at 3 WIM. To avoid an unintended and confounding parity effect when comparing both groups, LT10 cows were selected to ensure an equal parity distribution as for HT10 cows. This finally resulted in the selection of 15 cows with a maximum milk fat C18:1 *trans*-10 proportion of 0.25 g/100 g of FA (**LT10**).

Further, the selection of 2 subgroups for the SARA challenge trial (experiment 2) was based on the milk fat C18:1 *trans*-10 proportion in the third WIM, the average C15:0 proportion during the first 4 WIM, and the decrease in C18:1 *trans*-11 over the first 4 WIM. These milk FA were chosen based on previously published research (Colman et al., 2012): the biohydrogenation intermediates C18:1 *trans*-10 and C18:1 *trans*-11 in milk showed the highest correlation with either the absolute rumen pH level and the rumen pH range, respectively, whereas the C15:0 was the OBCFA, which showed the largest correlation with both the rumen pH levels as well as the rumen pH range. Five cows were selected from the HT10 group and were expected to be most SARA susceptible (**HT10s** cows). They were characterized by a proportion of C18:1 *trans*-10 of 0.31 g/100 g of FA or higher at 3 WIM, mean proportion of C15:0 over the first 4 WIM higher than 1.18 g/100 g of FA, and a sharp decrease of the proportion of C18:1 *trans*-11 during the first 4 WIM ($\Delta \geq 0.25$ g/100 g of FA). Additionally, 5 LT10s cows were selected from the LT10 group (**LT10s** cows). They showed C18:1 *trans*-10 proportions below 0.23 g/100 g of FA at 3 WIM, a mean proportion of C15:0 over the 4 WIM below 0.99 g/100 g of FA and a stable proportion of C18:1 *trans*-11 during the first 4 WIM. Three cows only fulfilled 2 of the 3 criteria mentioned above; however, they were still selected to match parity and expected calving date over the LT10s and HT10s groups (Table 5).

Measurement of Reticulo-Ruminal pH During Experiment 2

In experiment 2, the reticulo-ruminal pH was continuously measured with a wireless SmaXtec Premium Bolus (105 mm long and 35 mm i.d.; SmaXtec GmbH, Graz, Austria). A 50-d accurate data recording was guaranteed by the manufacturer, which should be sufficient given the 28-d measurement period during the current experiment. Also, others did not report pH drifts during a period of 28 d (Villot et al., 2017). Reticulo-ruminal pH and temperature were recorded every 10 min. After calibration (pH 4 and 7), each bolus was introduced with an oral balling gun following the manufacturer's instructions (https://www.smaxtec.com/en/smaxtec-premium-bolus/; SmaXtec GmbH, Graz, Austria). It is assumed that the bolus ends up at the bottom of the reticulum due to its weight and the rumen's motility (Gasteiner et al., 2009; Schneider et al., 2010). Data were recorded for a period of 28 d. A mobile reader (SmaXtec Mobile reader, SmaXtec GmbH, Austria) was positioned near the cows and the recorded data were transmitted to the mobile reader via radio transmission every half hour and then uploaded on the internet server. The data were downloaded and

exported to a digital spreadsheet (Microsoft Excel, Microsoft, Redmond, WA) for further analysis. Subacute ruminal acidosis was defined as reticulo-ruminal pH under 6.0 for more than 6.0 h per day (AlZahal et al., 2007). As compared with the rumen pH threshold of 5.8, proposed by AlZahal et al. (2007), a threshold of 6.0 was considered in the current study as the reticulo-ruminal pH is generally 0.2 higher than the rumen pH (Beauchemin et al., 2003; Neubauer et al., 2017).

Statistical Analysis

Experiment 1. Data were analyzed using the general linear model of SPSS 20.0 software (SPSS Inc., Chicago, IL). Data of milk yield, milk content, and milk FA composition of all the cows in the first experiment were analyzed using the repeated statement, as variables were measured at different weeks of this period. This statistical model included the fixed effect of week and cow as random factor. The fixed effect of sampling week was evaluated as a repeated measure using the first-order autoregressive as covariance structure based on the Akaike's information criterion with cow as subject.

Additionally, data of experiment 1 were used to compare HT10 and LT10 groups. This comparison was made using the univariate statement with week (first 4 wk after calving) and group (HT10 vs. LT10) as fixed factors.

Finally, data of experiment 1 were subjected to a principal component analysis (**PCA**) in SPSS to determine components that account for most of the total variation within the SARA-indicative milk components considered in the current study. Variables included were milk fat and protein content, the milk fat to protein ratio, the milk FA proportions of C15:0, C18:1 *trans*-10, and C18:1 *trans*-11 as well as the C18:1 *trans*-10 to C18:1 *trans*-11 ratio in the third WIM. Results are shown in a biplot that contains a score plot to find similarities and contrasts between samples, whereas correlations among the 7 parameters can be identified in the loading plot.

Experiment 2. Because multiple measurements per animal cannot be regarded as independent units of observation, in experiment 2, observations were averaged per animal and per period before statistical analysis. The HT10s and LT10s groups were then compared using the univariate statement with period (LFC, IFC, and HFC period) and group (HT10s vs. LT10s) as fixed factors.

Both for experiment 1 as well as for experiment 2, significances were declared at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$.

RESULTS

Mean Values and Coefficients of Variation of Milk Fat and Protein Content as well as Milk Fatty Acids (Experiment 1)

During the first 4 wk in lactation, primiparous cows had a mean concentrate intake of 4.5 kg of DM/d and a milk production of 26.4 kg/d, whereas these amounts were 7.0 kg of DM/d and 39.0 kg/d for multiparous cows, respectively. Table 6 presents the average values as well as the coefficients of variation of milk yield, milk fat and protein content, and proportions of specific milk FA during the first 4 WIM, upon the build-up of concentrate intake. Reported milk FA were limited to those identified before as potential SARA biomarkers (Enjalbert et al., 2008; Colman et al., 2013). The changes in the coefficient of variation over the 4 wk reflect differences in inter-animal variability in response to the concentrate rise. Milk fat C15:0 increased continuously from 0.97 to 1.21 g/100 g of FA from wk 1 to 4 ($P < 0.001$), accompanied by an increasing coefficient of variation from 19 to 25%. Proportion of milk fat *anteiso* C15:0 ($P < 0.001$) and the ratio of milk fat to milk protein ($P < 0.001$) gradually rose with a decreasing or fluctuating coefficient of variation value during these 4 wk. An increase of milk fat C18:1 *trans*-10 (0.18 to 0.27 g/100 g of FA) and a decrease of C18:1 *trans*-11 (0.90 to 0.67 g/100 g of FA) occurred from wk 1 to 3 and wk 2 to 4, respectively. Meanwhile, coefficients of variation of these 2 *trans* isomers reached their peak at wk 2. The C18:1 *trans*-10 to C18:1 *trans*-11 ratio as well as its coefficient of variation showed a continuous increase over the 4-wk period.

Comparison of Concentrate Intake, Milk Yield, Milk Fat, and Protein Content and Milk Fatty Acid Composition (Experiment 1)

No differences were observed in concentrate intake ($P_{group} = 0.776$), milk yield ($P_{group} = 0.414$), or milk protein content ($P_{group} = 0.934$) between the 2 groups of animals (Table 7). Cows in the HT10 group tended to have a lower milk fat content than LT10 cows over the 4 wk (4.61 vs. 4.87 g/100 g of milk, $P_{group} = 0.075$). Furthermore, the milk fat to protein ratio was lower for HT10 cows compared with LT10 cows ($P_{group} = 0.001$). The HT10 and LT10 groups have not been statistically compared in terms of milk FA as both groups were selected to differ in milk fat C18:1 *trans*-10 proportions. As other SARA-indicative milk FA that are considered in the current paper are likely (at least moderately) correlated with C18:1 *trans*-10, the validity of any sta-

Table 6. Average milk yield (kg/d), milk fat and protein content (g/100 g of milk), proportions of specific milk fatty acids (g/100 g of fatty acids; FA), and CV of 125 cows during the first 4 wk of lactation during which increasing amounts of concentrate were fed (experiment 1)

	Week in milk						CV, %			
Item	1	2	3	4	SEM	*P*-value	Wk 1	Wk 2	Wk 3	Wk 4
Milk yield, kg/d	29.8	35.3	38.4	40.2	0.492	<0.001	28	24	25	24
Milk fat content, g/100 g	5.05	4.71	4.65	4.64	0.043	0.025	20	15	16	19
Milk protein content, g/100 g	4.00	3.56	3.36	3.29	0.027	<0.001	19	9	7	7
Milk fat:protein	1.29	1.33	1.39	1.42	0.014	<0.001	24	17	17	20
Fatty acid, g/100 g of FA										
iso C14:0	0.029	0.029	0.029	0.030	0.001	0.301	21	21	24	26
C15:0	0.97	1.06	1.17	1.21	0.013	<0.001	19	23	24	25
iso C15:0	0.20	0.19	0.20	0.21	0.001	0.107	21	17	17	17
anteiso C15:0	0.38	0.40	0.42	0.43	0.004	<0.001	24	20	19	17
iso C16:0	0.19	0.17	0.16	0.16	0.002	0.005	23	21	25	22
C17:0	0.63	0.60	0.55	0.54	0.004	<0.001	18	15	14	14
iso C17:0	0.36	0.36	0.33	0.34	0.002	0.108	14	11	13	14
anteiso C17:0	0.41	0.42	0.42	0.42	0.003	0.411	17	16	17	16
C18:1 *trans*-10	0.18	0.23	0.27	0.26	0.004	0.027	26	33	32	30
C18:1 *trans*-11	0.87	0.90	0.77	0.67	0.014	0.070	32	38	37	33
C18:2 *cis*-9,*trans*-11	0.31	0.34	0.33	0.30	0.005	0.210	29	32	34	29
trans-10:*trans*-11[1]	0.21	0.28	0.39	0.45	0.017	0.015	26	45	53	61

[1]C18:1 *trans*-10 to C18:1 *trans*-11 ratio.

tistical analysis on these milk FA is impaired. Nevertheless, mean values are reported (Supplemental Table S2; https://doi.org/10.3168/jds.2018-14903).

In addition, these data (concentrate intake, milk yield, milk fat and protein content, and milk FA proportions) were also reported for the selected cows of experiment 2 (HT10s and LT10s; Supplemental Tables S3 and S4; https://doi.org/10.3168/jds.2018-14903).

PCA Based on the Milk Fat and Protein Content as well as Proportions in Milk Fat of Selected Milk Fatty Acids (Experiment 1)

Parameters used in the PCA analysis were milk fat, milk protein, milk fat to protein ratio, C15:0, C18:1 *trans*-10, C18:1 *trans*-11, and C18:1 *trans*-10 to C18:1 *trans*-11 ratio in the third WIM. Although other OB-CFA and *trans* isomers were also related to SARA development, the factors we selected were considered as primary biomarkers of SARA (Čejna and Chladek, 2006; Enjalbert et al., 2008; Colman et al., 2013). The PCA analysis successfully reduced the dimensionality of the original 7 parameters into 2 main parameters while explaining 66.2% of the variation in the original 7 parameters (Figure 1), with the first and second principal components (**PC1** and **PC2**, respectively) describing 40.5 and 25.7% of the total variation, respectively. The biplot as presented here combines both the loading as well as the score plot. The score plot is used to find similarities and contrasts between samples,

Table 7. Concentrate intake (kg/d), milk yield (kg/d), milk fat and milk protein content (g/100 g of milk), and milk fatty acid composition (g/100 g of fatty acids; FA) of HT10 and LT10 cows during the first 4 wk of lactation (experiment 1)

		Week in milk					*P*-value		
Item	Group[1]	1	2	3	4	SEM	Group	Week	Group × week
Concentrate intake	HT10	4.16	5.75	6.55	7.13	0.226	0.776	<0.001	0.959
	LT10	4.11	5.69	6.67	7.41	0.253			
Milk yield	HT10	28.5	34.6	35.2	37.0	1.02	0.414	<0.001	0.851
	LT10	28.4	34.9	38.1	40.3	1.34			
Milk fat	HT10	4.97	4.60	4.45	4.42	0.110	0.075	0.190	0.931
	LT10	5.09	4.80	4.81	4.77	0.096			
Milk protein	HT10	4.02	3.70	3.50	3.40	0.060	0.934	<0.001	0.884
	LT10	3.99	3.64	3.50	3.53	0.082			
Milk fat:protein ratio	HT10	1.24	1.25	1.27	1.30	0.030	0.001	0.314	0.864
	LT10	1.30	1.34	1.42	1.43	0.030			

[1]HT10: 15 cows with the highest milk fat C18:1 *trans*-10 concentration in the third week of lactation (>0.31 g/100 g of FA) of the cohort of 125 animals. LT10: 15 cows with milk fat C18:1 *trans*-10 concentration in the third week of lactation below 0.24 g/100 g of FA and stratified with the HT10 group for parity.

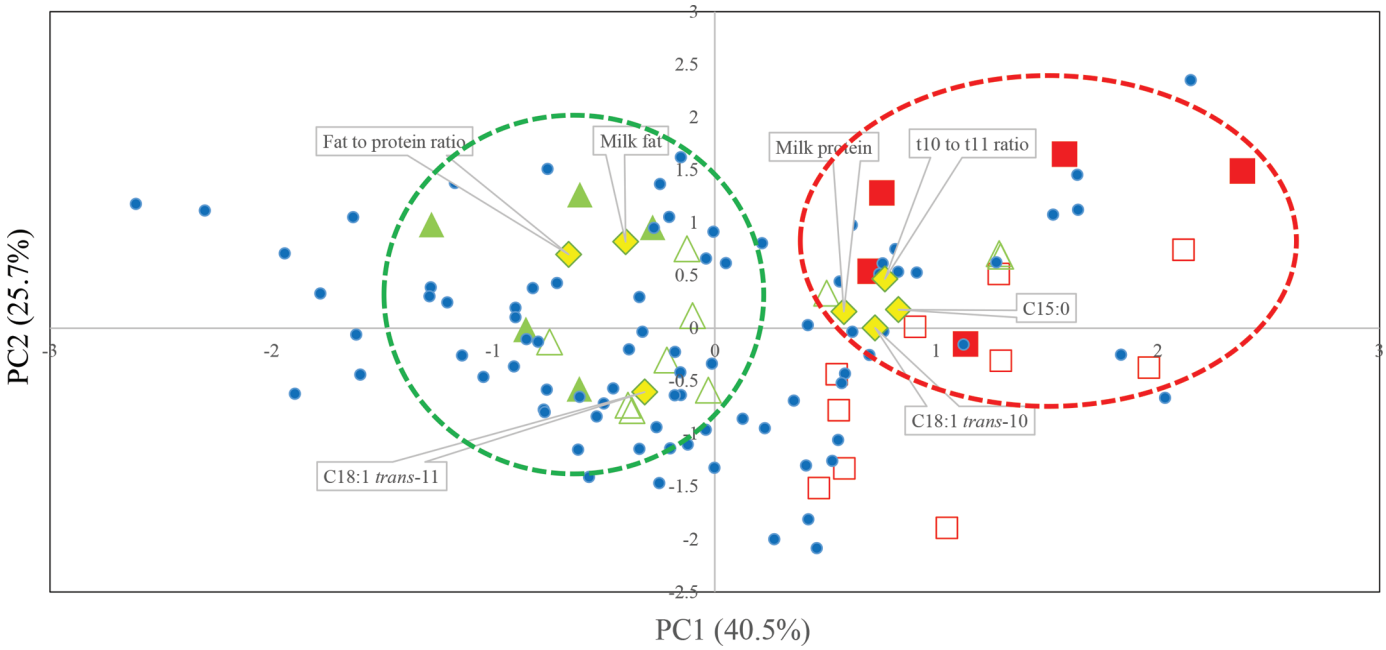

Figure 1. Biplot of the principal component (PC) analysis based on milk fatty acid parameters (concentrations in milk fat of C15:0, C18:1 *trans*-10, C18:1 *trans*-11, as well as C18:1 *trans*-10 to C18:1 *trans*-11 ratio) and milk content parameters (milk fat, milk protein, and milk fat to protein ratio) in the third week of lactation (†) during experiment 1. This biplot combines the loading plot and the score plot. Solid triangle = LT10 cows chosen for the second trial (LT10s); open triangle = LT10 cows that were not chosen for the second trial; solid square = HT10 cows chosen for the second trial (HT10s); open square = HT10 cows that were not chosen for the second trial; closed circle = cows that did not belong to HT10 or LT10 group. HT10: 15 cows with the highest milk fat C18:1 *trans*-10 concentration in the third week of lactation (>0.31 g/100 g of FA) of the cohort of 125 animals. LT10: 15 cows with milk fat C18:1 *trans*-10 concentration in the third week of lactation below 0.24 g/100 g of FA and stratified with the HT10 group for parity. Color version available online.

whereas correlations among variables can be identified in the loading plot. From the loading plot, 2 groups could be distinguished within the parameters: milk protein content, C15:0, C18:1 *trans*-10, and C18:1 *trans*-10 to C18:1 *trans*-11 ratio were positively correlated with PC1, whereas milk fat content, fat to protein ratio, and C18:1 *trans*-11 were negatively correlated with PC1. From the score plot, it could be observed that all HT10s cows were grouping and showed a positive PC1-score ($\geq$0.70), whereas LT10s cows had a negative PC1-score (≤ -0.32). Hence, LT10s and HT10s cows were mirrored across the y-axis.

DMI, Reticulo-Ruminal pH Values, and Acidosis Index of HT10s Cows and LT10s Cows During a SARA Challenge (Experiment 2)

No difference was observed in DMI between the HT10s cows and LT10s cows, irrespective of period (P_{group} = 0.634; Table 8). Duration of pH below 6.0 was longer for HT10s cows compared with LT10s cows (P_{group} = 0.014). Meanwhile, HT10s cows had lower values of nadir, mean, and maximum reticulo-ruminal pH during the whole experiment ($P_{group} < 0.001$, P_{group} = 0.002, and P_{group} = 0.007, respectively). However, nadir and maximum reticulo-ruminal pH were not directly affected by increasing intake of concentrate (P_{period} = 0.851 and P_{period} = 0.542). Diurnal reticulo-ruminal pH range was greater for HT10s cows compared with LT10s cows (P_{group} = 0.003). Furthermore, the area under the curve of pH below 6.0 as well as the acidosis index tended to be greater for HT10s cows (P_{group} = 0.069 and P_{group} = 0.086, respectively).

A group × period interaction effect was not observed for any of the reticulo-ruminal pH parameters, indicating that HT10s cows differed from LT10s cows throughout experiment 2, even during the LFC period, when a low amount of RFCH was supplied.

Despite the higher intake of RFCH in both groups during the third period (HFC period), reticulo-ruminal pH values increased and acidosis index decreased for both LT10s and HT10s cows.

Besides the mean values of reticulo-ruminal pH parameters of the LT10s and HT10s groups, the acidosis index of individual cows is presented for the 3 experimental periods in Figure 2. Four LT10s cows showed a very low acidosis index throughout the trial, whereas one LT10s cow had an acidosis index that was more

Table 8. Dry matter intake and reticulo-ruminal pH parameters of HT10s and LT10s cows during the 3 periods of the SARA challenge trial (experiment 2)

Item	HT10s[1]			LT10s[1]			SEM[2]	*P*-value		
	LFC[3]	IFC[3]	HFC[3]	LFC[3]	IFC[3]	HFC[3]		Group	Period	Group × period
DMI, kg/d	22.4	22.7	21.3	22.8	23.4	21.9	1.535	0.634	0.609	0.997
Reticulo-ruminal pH										
Nadir	5.73	5.58	5.68	5.99	6.09	6.14	0.139	<0.001	0.851	0.610
Mean	6.21	6.07	6.19	6.40	6.47	6.54	0.119	0.002	0.683	0.615
Maximum	6.68	6.64	6.69	6.78	6.90	6.92	0.087	0.007	0.542	0.572
Range of pH[4]	0.92	1.05	1.01	0.76	0.81	0.78	0.084	0.003	0.506	0.859
Duration pH <5.6, h/d	0.86	1.78	0.45	0.21	0.43	0.01	0.782	0.191	0.490	0.808
Duration pH <5.8, h/d	2.68	4.67	2.37	0.84	1.52	0.65	1.82	0.199	0.612	0.901
Duration pH <6.0, h/d	6.05	10.3	6.56	1.88	2.73	1.75	2.73	0.014	0.537	0.783
Area pH <6.0, pH × min/d	77.2	138	70.5	22.2	39.6	17.8	47.4	0.069	0.555	0.843
Acidosis index,[5] pH × min/kg, DM	3.19	6.09	3.11	1.15	1.96	0.95	2.03	0.086	0.517	0.827

[1]HT10s and LT10s cows: 5 cows selected from the HT10 group and 5 cows selected from LT10 group, respectively, based on milk fatty acid data obtained during experiment 1. HT10: 15 cows with the highest milk fat C18:1 *trans*-10 concentration in the third week of lactation (>0.31 g/100 g of FA) of the cohort of 125 animals. LT10: 15 cows with milk fat C18:1 *trans*-10 concentration in the third week of lactation below 0.24 g/100 g of FA and stratified with the HT10 group for parity.

[2]Due to the death of one animal, the experimental design was unbalanced and SEM differs between groups; the largest SEM is presented.

[3]LFC = low rapidly fermentable carbohydrate period; IFC = increasing rapidly fermentable carbohydrate period; HFC = high rapidly fermentable carbohydrate period.

[4]The maximum reticulo-ruminal pH minus the nadir reticulo-ruminal pH for 1 d.

[5]Acidosis index was calculated as the area under pH 6.0 divided by DMI.

comparable with the HT10s cows. One HT10s cow had a low acidosis index during the LFC period; however, this cow died due to an accident. The other 4 cows showed a higher acidosis index as compared with the 4 LT10s cows throughout the trial.

Milk Fatty Acid Composition of HT10s and LT10s Cows During 3 Experimental Periods (Experiment 2)

Throughout the 3 subperiods of experiment 2, LT10s cows had higher milk fat proportions of *iso* C14:0 and *iso* C16:0 (P_{group} = 0.002 and P_{group} = 0.002, respectively), whereas the proportions in milk fat of C15:0, C18:1 *trans*-10 as well as the C18:1 *trans*-10 to C18:1 *trans*-11 ratio were lower compared with the HT10s cows (P_{group} = 0.054, P_{group} = 0.017, and P_{group} = 0.012, respectively; Table 9). These results were consistent with the ones of experiment 1. Furthermore, increasing amounts of RFCH in the diet decreased the proportion of milk fat C18:1 *trans*-11 in both groups (P_{period} < 0.001). An interaction effect was not observed for any of the milk FA.

DISCUSSION

In the current study, we attempted to identify susceptible and unsusceptible cows to a SARA challenge based on milk OBCFA and *trans* isomers that had been identified before as potential biomarkers of SARA (Colman et al., 2010; Fievez et al., 2012). For this purpose, the first 4 WIM were targeted, when cows were exposed to a concentrate build-up scheme. Although the concentrate build-up during this early lactation period creates the possibility to monitor inter-animal differences in response to a concentrate challenge, this period also poses challenges to the milk FA biomarkers, as milk FA proportions have been reported to be particularly influenced by mobilization of FA from body reserves during the first 10 WIM (Craninx et al., 2008). Accordingly, progressive lactation might be a confounding factor. In this respect, proportions of SARA indicative OBCFA were reported to follow the lactation curve during the first 10 WIM, with increasing proportions of FA with a chain length of 14 or 15 carbon atoms, whereas proportions decreased for the FA with chain lengths of 17 carbon atoms (Craninx et al., 2008). The same pattern was observed in experiment 1. On the other hand, proportions of C15:0, *anteiso* C15:0, C18:1 *trans*-10 as well as the C18:1 *trans*-10 to C18:1 *trans*-11 ratio were enhanced when feeding diets with high levels of concentrate and during SARA challenge trials, whereas *iso* even-chain FA were reported to decrease (Craninx et al., 2008; Enjalbert et al., 2008; Zened et al., 2013). This is in agreement with changes in these parameters upon build-up of concentrate during the first 4 WIM in experiment 1. Furthermore, increased coefficients of variation of some of these indicators (particularly C15:0, C18:1 *trans*-10, C18:1 *trans*-11,

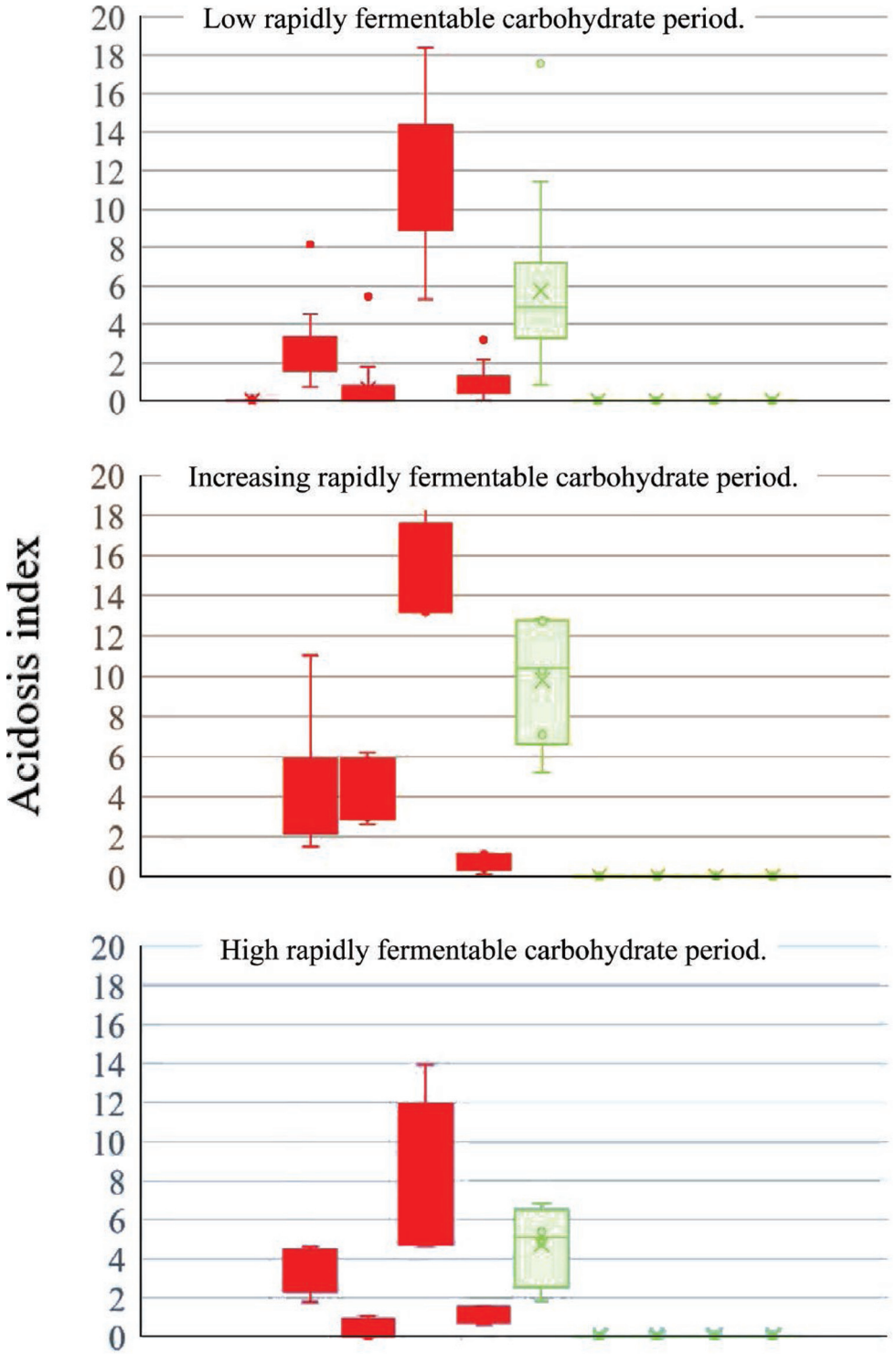

Figure 2. Acidosis index of individual cows during 3 experimental periods in experiment 2 (average of 18, 6, and 4 measurements for each cow during the low, increasing, and high rapidly fermentable carbohydrate period, respectively). Acidosis index was calculated as the area under reticulo-ruminal pH 6.0 (pH × min/d) divided by DMI. Full red bars represent the HT10s cows and open green bars represent LT10s cows. One cow died at the beginning of the increasing rapidly fermentable carbohydrate period through an accident (not due to the treatment) leading to 5 HT10s observations for the low rapidly fermentable carbohydrate period and 4 HT10s for the increasing and high rapidly fermentable carbohydrate period. HT10: 15 cows with the highest milk fat C18:1 *trans*-10 concentration in the third week of lactation (>0.31 g/100 g of FA) of the cohort of 125 animals. LT10: 15 cows with milk fat C18:1 *trans*-10 concentration in the third week of lactation below 0.24 g/100 g of FA and stratified with the HT10 group for parity. Dots during the low rapidly fermentable carbohydrate period were the outliers. The error bar indicates the maximum and minimum value of the acidosis index. x indicates the mean value of the acidosis index. Color version available online.

and C18:1 *trans*-10 to C18:1 *trans*-11 ratio) over the 4 WIM suggests that inter-animal variation enlarged for these indicators when concentrate intake increased. Accordingly, these indicators might be of particular interest to differentiate between animals that are more or less susceptible to SARA development as differences enlarged upon a concentrate challenge.

In a first attempt to assess whether milk FA could be indicative to distinguish animals that were more or less susceptible to SARA development, we categorized cows based on proportions of milk fat C18:1 *trans*-10 in the third WIM when this milk FA, on average, reached its peak proportion. This was an expert-driven selection, based on the direct link between rumen pH and the proportion in the milk of this FA isomer (Enjalbert et al., 2008; Colman et al., 2013). C18:1 *trans*-10, together with C15:0, are of particular interest as these FA are linked to both the level of rumen pH as well as the rumen pH variation (Colman et al., 2012). Compared with other SARA indicative milk FA, C18:1 *trans*-10 is assumed to be least affected by lactation stage because its accumulation in and release from the adipose tissue is limited (Mosley et al., 2002). Indeed, C18:1 *trans*-10 particularly accumulates in the rumen of dairy cows when high levels of concentrate are fed and these periods are most often characterized by high milk yield, but only limited lipogenesis in the adipose tissue. Hence, storage of this FA in adipose tissue is limited (Hostens et al., 2012). For other milk FA that also have been identified as SARA indicators and are expected to increase upon concentrate build-up [e.g., milk fat C17:0 (Enjalbert et al., 2008)], the increase might have been masked as C17:0 in early lactation partly originates from the mobilization of body reserves (Craninx et al., 2008).

Fifteen HT10 cows were selected that showed the highest proportions of milk fat C18:1 *trans*-10 in the third WIM within the herd. To minimize potential confounding effects, parity was taken into account when selecting the corresponding cows for the LT10 group as, for example, eating and ruminating behavior, which could affect SARA susceptibility, might differ between primiparous and multiparous cows (Maekawa et al., 2002; Penner et al., 2007). Thus, some cows that had lower milk fat C18:1 *trans*-10 proportions were not selected in the LT10 group due to lack of a corresponding HT10 cow in terms of parity.

Together with the higher proportion in milk fat of C18:1 *trans*-10 in HT10 cows, proportions of other SARA indicative milk FA, for example, C15:0 and C18:2 *cis*-9,*trans*-11 as well as the C18:1 *trans*-10 to C18:1 *trans*-11 ratio also were increased in those animals as compared with LT10 cows. These correlations between SARA indicative milk FA were also reported in previously published research (Colman et al., 2010). Moreover, based on a multivariate PCA analysis, the HT10 and LT10 cows largely differentiated based on the PC1 score, which was positive for all HT10 cows

Table 9. Comparison of milk fatty acids (g/100 g of fatty acids; FA) and milk fat content (g/100 g of milk) between the HT10s and LT10s group during the SARA challenge trial (experiment 2)

	HT10s[1]			LT10s[1]				*P*-value		
Item	LFC[3]	IFC[3]	HFC[3]	LFC[3]	IFC[3]	HFC[3]	SEM[2]	Group	Period	Group × period
Milk fat content	3.68	3.56	3.29	4.06	4.00	3.67	0.307	0.126	0.462	0.978
iso C14:0	0.072	0.072	0.060	0.088	0.085	0.079	0.006	0.002	0.177	0.918
C15:0	1.34	1.44	1.80	1.12	1.16	1.38	0.198	0.054	0.136	0.858
iso C15:0	0.236	0.239	0.168	0.238	0.246	0.222	0.013	0.247	0.531	0.602
*anteiso C*15:0	0.496	0.517	0.341	0.445	0.467	0.340	0.029	0.065	0.561	0.891
iso C16:0	0.178	0.184	0.513	0.203	0.223	0.483	0.015	0.002	0.647	0.614
C17:0	0.523	0.532	0.517	0.504	0.479	0.461	0.045	0.252	0.373	0.910
iso C17:0	0.358	0.363	1.80	0.340	0.338	1.38	0.019	0.340	0.839	0.802
anteiso C17:0	0.484	0.521	0.591	0.432	0.443	0.537	0.032	0.022	0.568	0.902
C18:1 *trans*-10	0.376	0.431	0.478	0.290	0.305	0.305	0.063	0.017	0.655	0.810
C18:1 *trans*-11	0.932	0.909	0.764	0.985	0.945	0.738	0.042	0.541	<0.001	0.566
C18:2 *cis*-9,*trans*-11	0.441	0.426	0.405	0.420	0.400	0.341	0.042	0.265	0.313	0.869
trans-10 to *trans*-11[4]	0.406	0.483	0.633	0.295	0.325	0.420	0.074	0.012	0.067	0.808

[1]HT10s and LT10s cows: 5 cows selected from the HT10 group and 5 cows selected from LT10 group, respectively, based on milk FA data obtained during experiment 1. HT10: 15 cows with the highest milk fat C18:1 *trans*-10 concentration in the third week of lactation (>0.31 g/100 g of FA) of the cohort of 125 animals. LT10: 15 cows with milk fat C18:1 *trans*-10 concentration in the third week of lactation below 0.24 g/100 g of FA and stratified with the HT10 group for parity.

[2]Due to the death of one animal, the experimental design was unbalanced and SEM differs between groups; the largest SEM is presented.

[3]LFC = low rapidly fermentable carbohydrate period; IFC = increasing rapidly fermentable carbohydrate period; HFC = high rapidly fermentable carbohydrate period.

[4]C18:1 *trans*-10 to C18:1 *trans*-11 ratio.

and negative for most LT10 cows. However, these differences between groups were not due to concentrate intake ($P = 0.776$, Table 7).

The HT10s cows were characterized by higher proportions of milk fat C15:0, *anteiso* C15:0, C18:1 *trans*-10, and the C18:1 *trans*-10 to C18:1 *trans*-11 ratio, both in experiment 1 as well as experiment 2. Inversely, milk fat proportion of iso C14:0 and iso C16:0 generally were lower in HT10s cows compared with LT10s cows, both in experiment 1 and 2. This indicates that the milk FA differences between groups were stable, even though these experiments were separated in time (>1 yr; Supplemental Table S2; https://doi.org/10.3168/jds.2018-14903).

Based on the mean reticulo-ruminal pH parameters at group level, SARA was only induced in HT10s group in experiment 2. Although LT10s cows received relatively higher amounts of RFCH and DMI was similar for both groups, at group level, the LT10s cows did not suffer from SARA during any of the 3 subperiods in experiment 2. Accordingly, HT10s cows seemed more prone to SARA development when facing a RFCH challenge as evidenced by the lower pH values. Such interanimal differences were also documented by Gao and Oba (2014) with 16 late-lactating dairy cows offered the same diet consisting of 35% forage and 65% concentrate. Tolerant cows had a mean rumen pH of 6.47 in that study, whereas the mean rumen pH of susceptible cows was 6.02. Strikingly, for most of the rumen pH variation, no group × period interaction was observed (range of reticulo-ruminal pH and duration of reticulo-ruminal pH <6.0), indicating HT10s and LT10s cows differed in all 3 periods.

Despite the higher amount of wheat-rich concentrate during the HFC period in experiment 2, the mean reticulo-ruminal pH parameters increased and the acidosis index decreased for both groups. A possible reason could be the structural adaptation of the animals to RFCH [e.g., through adaptation of the rumen epithelium and absorption rate during a carbohydrate challenge (Odongo et al., 2006; Steele et al., 2011), through adaptation of the rumen microbiota, or both (Mao et al., 2013; Huo et al., 2014)].

Hence, the SARA challenge trial validated the selection based on the proportions of milk FA. Milk fat C18:1 *trans*-10, C15:0, and the change in C18:1 *trans*-11 during the concentrate build-up in early lactation presumably allowed identification of cows that are more or less susceptible to SARA development as 8 out of 10 cases were correctly classified when we look into individual cow data. Indeed, most of the cows (8 out of 10 for the LFC period and 8 out of 9 for the IFC and HFC periods) differed in acidosis index as we presumed. Moreover, experiment 2 was performed almost one and a half years after experiment 1 and with cows being in a different lactation stage (156 ± 70 DIM). Nevertheless, differences in milk FA between HT10s and LT10s cows remained the same in both experiments, indicating that these milk FA could be reliable indicators during long-term monitoring. During 3 subperiods of experiment 2, animals received increasing amounts of RFCH. However, differences between HT10s and LT10s cows in SARA-indicative milk FA were maintained throughout experiment 2, indicating these milk FA allowed to distinguish SARA susceptible and unsusceptible cows when animals are on a comparable diet. The one exceptional LT10s cow showed a low rumen pH during experiment 2, whereas the milk FA proportion in experiment 1 resembled the ones of the other LT10 cows. However, this milk FA pattern was preserved in the second experiment (detailed data not shown), indicating a mismatch between the milk FA pattern and reticulo-ruminal pH. In this case, further research is required to elucidate reasons for such a mismatch in some cows.

Overall, the current findings show the possibility to select susceptible and unsusceptible cows for SARA development primarily based on the proportion of milk fat C18:1 *trans*-10, as well as milk fat C15:0 and changes in C18:1 *trans*-11. Given this proof of concept, a multivariate and robust model should be developed in the future. Moreover, the current selection of cows was based on the more extreme (highest vs. lowest) milk C18:1 *trans*-10 proportions. Future investigations should reveal whether this approach also allows identification of SARA susceptibility on a more continuous scale. Additionally, to be of practical relevance, these diagnostic milk FA should be determined routinely. Earlier research by our group indicated Raman spectroscopy showed potential for the determination of individual and grouped *trans*-(mono) UFA in milk fat (Stefanov et al., 2011). Given the results of the current trial, it might be of major interest to further invest in such a routine technology.

CONCLUSIONS

Based on the results of the current 2 experiments, it is possible to distinguish cows with relatively higher susceptibility for SARA from a cohort of cows receiving a similar diet. These cows are characterized by a higher proportion in milk fat of C18:1 *trans*-10, C15:0, and C18:1 *trans*-10 to C18:1 *trans*-11 ratio.

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