Effects of induced subacute ruminal acidosis on milk fat content and milk fatty acid profile

F. Enjalbert, Y. Videau, M. C. Nicot and A. Troegeler-Meynadier

INRA, Université de Toulouse, Tissus Animaux, Nutrition, Digestion, Ecosystème et Métabolisme, Chemin de Borde-Rouge, Auzeville, Castanet-Tolosan Cedex, France; INPT-ENSAT, Castanet-Tolosan Cedex, France; ENVT, Toulouse Cedex, France

Summary

Two lactating dairy cows fitted with a rumen cannula received successively diets containing 0%, 20%, 34% and again 0% of wheat on a dry matter basis. After 5, 10 and 11 days, ruminal pH was measured between 8:00 and 16:00 hours, and milk was analysed for fat content and fatty acid profile. Diets with 20% and 34% wheat induced a marginal and a severe subacute ruminal acidosis respectively. After 11 days, diets with wheat strongly reduced the milk yield and milk fat content, increased the proportions of C8:0 to C13:0 even- or odd-chain fatty acids, C18:2 n-6 and C18:3 n-3 fatty acids but decreased the proportions of C18:0 and *cis*-9 C18:1 fatty acids. Wheat also increased the proportions of *trans*-5 to *trans*-10 C18:1, the latter exhibiting a 10-fold increase with 34% of wheat compared with value during the initial 0% wheat period. There was also an increase of *trans*-10, *cis*-12 C18:2 fatty acid and a decrease of *trans*-11 to *trans*-16 C18:1 fatty acids. The evolution during adaptation or after return to a 0% wheat diet was rapid for pH but much slower for the fatty acid profile. The mean ruminal pH was closely related to milk fat content, the proportion of odd-chain fatty acids (linear relationship) and the ratio of *trans*-10 C18:1/*trans*-11 C18:1 (nonlinear relationship). Such changes in fatty acid profile suggested a possible use for non-invasive diagnosis of subacute ruminal acidosis.

Introduction

Subacute ruminal acidosis (SARA) is a common problem in dairy herds, because of diets with a high content of fermentable carbohydrates used to meet the high demand of animals for energy. Subacute ruminal acidosis can result in several disorders, especially depressed fibre digestion, depressed appetite, laminitis and low milk fat content (see review by Kleen et al., 2003).

At a herd level, SARA can be diagnosed by measurements of ruminal pH on samples obtained from some cows via ruminocentesis (Garrett et al., 1999). In addition, other diagnosis criteria have been evaluated. Enemark et al. (2004) demonstrated that, among a set of parameters involving rumen, blood, urine and milk analysis, only the ruminal proportion of propionate was a good predictor of rumen pH. The milk fat content, whose low values are often interpreted within herds as a consequence of SARA, only partly correlates with rumen pH (Allen, 1997), and is difficult to interpret in early lactation cows because of interactions with body fat mobilization, so that milk fat depression cannot be simply considered as a sequel of SARA (Kleen et al., 2003).

High-concentrate diets, which can lead to SARA, are known to result in changes of the fermentation pattern in the rumen, with possible changes in the

mammary production of fatty acids. First the production of propionate is strongly increased (Bauman and Griinari, 2001), propionate being recognized as a precursor of odd-chain fatty acids in milk (Massart-Leen et al., 1983). Second, the biohydrogenation pathway of C18:2n-6 shifts from *trans*-11 to *trans*-10 intermediates (Griinari et al., 1998). The objective of this study was to describe the modifications of milk fat content and milk fatty acid profile, focusing on odd-chain and *trans*-10 fatty acids, triggered by an induced SARA followed by a recovery period in dairy cows.

Materials and methods

Animals and diets

Two lactating (mid-lactation) Holstein cows (third lactation, 600 kg body weight), equipped with a rumen cannula, were used in this experiment. Three diets (Table 1), based on maize silage and supplemented with 0% (W0), 20% (W20) and 34% (W34) of wheat on a dry matter basis were used. There were four successive periods: an initial baseline period with W0 diet (BW0), a second period with W20 diet, a third period with W34 diet and a final recovery period with W0 diet (RW0). Each diet was offered during 11 days. The diet was distributed in two 10 kg dry matter meals, at 8:00 and 17:00 hours. All constituents except wheat were mixed and wheat was top dressed on the mixture of other ingredients.

Table 1 Ingredients and chemical composition of the diets (% of dry matter except for net energy for lactation, Mcal/kg of dry matter)

	Diets		
	W0	W20	W34
Ingredients			
Whole plant maize silage	65.5	69.3	54.8
Dehydrated alfalfa	9.5	0.0	0.0
Maize	1.9	0.0	0.0
Wheat	0.0	20.3	34.2
Canola meal	7.0	0.0	0.0
Sunflower meal	6.3	0.0	0.0
Soybean meal	8.5	9.0	95
Mineral–vitamin mix	1.3	1.4	1.5
Nutrient value and chemical composition			
Net energy for lactation	1.65	1.65	1.72
Crude fat	3.34	2.6	2.4
Crude protein	14.8	12.1	13.0
Starch	26.0	39.2	43.6
Neutral detergent fibre	36.1	32.4	28.5
Acid detergent fibre	20.2	15.8	13.4
Ash	6.0	5.2	5.0

W0, diet with 0% wheat; W20, diet with 20% wheat; W34, diet with 34% wheat.

Samples and laboratory analysis

Milk samples were taken at the evening milking and samples of ruminal contents were taken on days 10 and 11 in the first period, and on days 5, 10 and 11 in the three subsequent periods, before the morning meal, and 1, 2, 3, 4, 6 and 8 h after the morning meal. Milk fat content was determined using the Roese–Gottlieb method (AOAC, 1996). Milk samples for fatty acid analysis were freeze dried (Virtis Freezemobile 25; Virtis, Gardiner, NY, USA). Fatty acids were methylated according to the method of Park and Goins (1994) except that the solution of 14% of borontrifluoride was replaced by a solution of methanol–acetylchloride (10:1). Fatty acid profiles were analysed by gas–liquid chromatography (Agilent 6890N, equipped with a model 7683 auto injector; Network GC System, Palo Alto, CA, USA). The column was a fused silica capillary (CPSil88, 100 m × 0.25 mm ID, 0.2 μm film thickness; Chrompack-Varian, Middleburg, the Netherlands). Flame ionization detector temperature was maintained at 260 °C and the injector was at 255 °C. Hydrogen was the carrier gas with a constant pressure (23.2 psi). The samples were injected with an automatic injector, in 0.5 μl of hexane and a split ratio of 1:50. Initial temperature of the oven was 60 °C, held for 1 min, increased by 20 °C/min to 150 °C, held at 150 °C for 10 min, increased by 2 °C/min to 175 °C, held at 175 °C for 20 min, increased by 10 °C/min to a final temperature of 225 °C and maintained at 225 °C for 10 min.

Calculations and statistical analysis

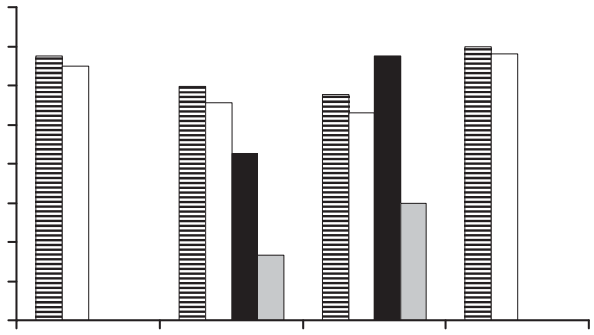
Mean ruminal pH between 8:00 and 17:00 hours was calculated as (pH0 + pH1 + pH2 + pH3 + pH4 + 2 × pH6 + 2 × pH8)/9, in which pHi was the pH measured i hours after the meal. Time of pH below 6.0 was calculated considering that each pH value below 6.0 from 0 to 4 h after the meal corresponded to 1 h of low pH, and values below 6.0 at 6 and 8 h after meal corresponded to 2 h of low pH. Summated pH depression below 6.0, which strongly correlates with depression in fibre digestion (Istasse et al., 1986), was calculated on a similar basis.

Only data obtained after 10 and 11 days of adaptation were used for statistical analysis and will be referred as data after 11 days of adaptation. Effects of diets on ruminal pH and milk composition were

analysed by the general linear procedure of SYSTAT (version 9, 1998; SPPS Inc., Chicago, IL, USA). The statistical model statement contained the effects of cow and diet. Correlation coefficients between mean ruminal pH and milk components were calculated. Differences were considered significant at a value of $p < 0.05$.

Results

The evolutions of mean ruminal pH, pH nadir, duration of ruminal pH below 6.0 and summation of pH depression below 6 throughout the experiment are represented in Fig. 1. Table 2 summarizes the pH values observed at the end of each period. Ruminal pH from 1 to 8 h after feeding was significantly lowered with the W20 and W34 diets compared with W0 diets during BW0 and RW0 periods. Ruminal pH was lower with W34 diet compared with W20 diet 2 and 6 h after meal. Moreover, ruminal pH did not fall below 6.0 between the morning and the evening meals during the two control periods. By contrast, it was below 6.0 during 4.5 and 7.0 h with W20 and W34 diets respectively. Summated pH depression between 8:00 and 17:00 hours switched from 0 with W0 diets to 1.7 and 3.0 with W20 and W34 diets respectively. Mean ruminal pH during the 9 h post-feeding was very close to 6.0 with W20 diet and was below 6.0 with W34 diet. The pH nadir was strongly correlated with mean pH ($r = 0.99$). It was above 6.5 during BW0 and RW0 periods, and was 5.6 and 5.3 with diets W20 and W34 respectively. After 5 days of adaptation (results not shown), pH values were similar to values observed after 11 days of adaptation, except during period RW0.



Milk yield was 26.5, 20.3, 15.1 and 12.8 kg/day during BW0, W20, W34 and RW0 periods respectively. Milk fat content (Table 3) was divided by 2 with W34 diet compared with BW0 period, and then returned to baseline values after 11 days during RW0 period.

The profile of milk fatty acids is given in Table 3. Compared with W0 diet during BW0 period, both W20 and W34 diets increased the proportions of C10:0 and C12:0, and strongly increased the proportions of C13:0 and C15:0 odd-chain fatty acids, particularly C13:0 whose proportions were more than three times higher with W20 and W34 diets than during BW0 period. W34 diet resulted in higher values than W20 diet for C15:0 and total odd-chain fatty acids, but only W20 diet significantly differed from BW0 period for C8:0 and C11:0. During RW0 period, values for both even- and odd-chain saturated fatty acids were no more different from values observed during BW0 period, except C6:0 and C14:0 which exhibited lower proportions. W34 diet increased the proportions of C14:1 and C16:1 and the latter remained high during RW0 period.

Among C18 fatty acids, both W20 and W34 diets significantly decreased the proportion of C18:0 and *cis*-9 C18:1, and W34 increased the proportion of C18:2n-6, C18:3n-3, *cis*-11 C18:1 and total *trans* C18:1 fatty acids. Among *trans* C18:1, W34 diet increased the proportions of *trans*-5 to *trans*-10, this latter exhibiting a 10-fold increase compared with values obtained during BW0 period. On the contrary, W34 diet decreased the proportions of *trans*-11, *trans*-12 and *trans*-16 positional isomers. The *trans*-10 C18:1/*trans*-11 C18:1 ratio was 36-fold higher with W34 diet than during BW0 period, but

Table 2 Effect of diets on ruminal pH

Hours after meal	Diets				
	BW0	W20	W34	RW0	SEM
0	6.96	6.74	6.84	7.13	0.12
1	6.73^{a}	6.10^{b}	6.00^{b}	7.06^{a}	0.09
2	6.80^{a}	6.23^{b}	5.75^{c}	6.88^{a}	0.09
3	6.77^{a}	5.78^{b}	5.36^{b}	6.86^{a}	0.11
4	6.74^{a}	5.68^{b}	5.36^{b}	6.97^{a}	0.12
6	6.73^{b}	5.80^{c}	5.49^{d}	7.11^{a}	0.07
8	6.68^{a}	5.88^{b}	5.85^{b}	6.87^{a}	0.13

Values in the same row with different superscripts differ ($p < 0.05$).
BW0, diet with 0% wheat, baseline period; W20, diet with 20% wheat; W34, diet with 34% wheat; RW0, diet with 0% wheat, recovery period.

Table 3 Effect of diets on milk fat content and milk fatty acid profile

	Diets				
	BW0	W20	W34	RW0	SEM
Fat content (g/100 g of milk)	4.41[a]	3.31[b]	2.24[c]	4.11[ab]	0.22
Fatty acids (% of total fatty acids)					
C6:0	1.89[a]	2.00[a]	1.43[b]	1.47[b]	0.08
C8:0	0.86[bc]	1.16[a]	1.00[ab]	0.72[c]	0.06
C10:0	1.82[b]	3.07[a]	2.70[a]	1.64[b]	0.15
C11:0	0.05[b]	0.24[a]	0.14[b]	0.16[ab]	0.03
C12:0	2.11[b]	3.86[a]	3.85[a]	1.57[b]	0.19
C13:0	0.05[b]	0.15[a]	0.17[a]	0.04[b]	0.01
C14:0	11.26[a]	14.82[a]	11.31[a]	7.58[b]	0.84
C14:1	0.87[b]	1.88[ab]	3.14[a]	1.36[b]	0.34
C15:0	0.82[c]	1.37[b]	1.99[a]	0.96[c]	0.08
C16:0	27.07	26.19	26.79	25.48	0.82
C16:1	1.76[b]	2.38[ab]	5.15[a]	4.22[a]	0.20
C17:0	0.55	0.62	0.73	0.70	0.06
C18:0	9.40[a]	5.37[b]	3.61[b]	7.65[a]	0.50
cis-9 C18:1	24.83[b]	19.23[c]	16.96[c]	33.58[a]	1.30
cis-11 C18:1	0.55[c]	0.92[b]	1.48[a]	0.87[bc]	0.08
total *trans* C18:1	3.54[b]	2.88[b]	6.14[a]	2.82[b]	0.27
trans-5 C18:1	0.017[b]	0.015[b]	0.028[a]	0.015[b]	0.002
trans-6 to *trans*-8 C18:1	0.50[b]	0.43[b]	0.69[a]	0.36[b]	0.04
trans-9 C18:1	0.25[bc]	0.21[c]	0.55[a]	0.27[b]	0.01
trans-10 C18:1	0.42[b]	0.64[b]	4.19[a]	1.00[b]	0.23
trans-11 C18:1	1.25[a]	0.79[b]	0.36[c]	0.52[c]	0.05
trans-12 C18:1	0.58[a]	0.50[ab]	0.21[c]	0.37[b]	0.03
trans-15 C18:1	0.20	0.18	0.06	0.07	0.04
trans-16 C18:1	0.30[a]	0.22[b]	0.08[c]	0.21[b]	0.02
C18:2n-6	1.97[b]	2.31[b]	5.02[a]	2.48[b]	0.41
trans-10, *cis*-12 C18:2	0.012[b]	0.008[b]	0.157[a]	0.041[b]	0.011
cis-9, *trans*-11 C18:2	0.81[a]	0.83[a]	0.61[b]	0.55[b]	0.03
C18:3n-3	0.40[b]	0.35[b]	0.58[a]	0.37[b]	0.04
total odd-chain fatty acids	1.48[d]	2.38[b]	3.03[a]	1.80[c]	0.07
trans-10 C18:1/*trans*-11 C18:1	0.34[b]	0.81[b]	12.22[a]	2.14[b]	1.05

Values in the same row with different superscripts differ ($p < 0.05$).
BW0, diet with 0% wheat, baseline period; W20, diet with 20% wheat; W34, diet with 34% wheat; RW0, diet with 0% wheat, recovery period.

was not affected by W20 diet. Similarly, W34 diet strongly increased the proportion of *trans*-10, *cis*-12 C18:2 and slightly but significantly decreased the proportion of *cis*-9, *trans*-11 C18:2.

During the recovery period, except a high increase of *cis*-9 C18:1, proportions of C18 fatty acids were between values observed with W34 and BW0 diets. In most cases, they did not significantly differ from BW0 values, but *trans*-11 C18:1 and *cis*-9, *trans*-11 C18:2 still exhibited low proportions, which did not differ from values observed with the W34 diet.

For most milk fatty acids, values observed after 5 days of adaptation (not shown) were between values observed at the end of the previous period and values observed at the end of the period.

Milk fat content, C18:0 and *cis*-9 C18:1 were positively related to ruminal pH (Table 4), while even-chain saturated fatty acids with 8–14 carbons, C13:0, C15:0, total odd-chain fatty acids, *trans*-6 to *trans*-10 C18:1, *trans*-10, *cis*-12 C18:2 and the *trans*-10 C18:1/*trans*-11 C18:1 ratio were negatively correlated with mean ruminal pH.

Discussion

Ruminal pH

The pH nadir was slightly above 5.5 with W20 diet, and around 5.3 with W34 diet, which corresponded to marginal and severe SARA respectively (Duffield et al., 2004). The decrease in ruminal pH when the proportion of concentrate increased was rapid, because of similar values after 5 or 11 days of adaptation (data not shown). On the contrary, the recov-

Table 4 Correlation coefficients between mean ruminal pH and milk fat content or individual milk fatty acid percentages

Milk fat content	0.86**
Fatty acid profile	
C6:0	−0.05
C8:0	−0.72**
C10:0	−0.84**
C11:0	−0.48
C12:0	−0.91**
C13:0	−0.98**
C14:0	−0.64**
C14:1	−0.61**
C15:0	−0.88**
C16:0	0.23
C16:1	−0.22
C17:0	−0.06
C18:0	0.77**
cis-9 C18:1	0.81**
cis-11 C18:1	−0.61**
total *trans* C18:1	−0.62**
trans-5 C18:1	−0.22
trans-6 to *trans*-8 C18:1	−0.69**
trans-9 C18:1	−0.56*
trans-10 C18:1	−0.62**
trans-11 C18:1	0.43
trans-12 C18:1	0.22
trans-15 C18:1	0.17
trans-16 C18:1	0.43
C18:2n-6	−0.50
trans-10, *cis*-12 C18:2	−0.58*
cis-9, *trans*-11 C18:2	−0.17
C18:3n-3	−0.44
total odd-chain fatty acids	−0.89**
trans-10 C18:1/*trans*-11 C18:1	−0.60*

*$p < 0.05$, **$p < 0.01$.

ery of ruminal pH after return to the W0 diet in the final period was slower, suggesting that the disturbances of ruminal ecosystem because of SARA were not immediately and easily reversible, as reported by Krause and Oetzel (2005).

Milk production

The effects of W20 and W34 diets on milk yield were much more important than observed in other attempts to induce SARA (Krause and Oetzel, 2005; Rustomo et al., 2006). Returning to W0 diet did not result in an improvement of milk yield, which explains the low correlation coefficient ($r = 0.25$, $p > 0.05$) between mean ruminal pH and milk yield. This could be due to the slow recovery to a normal digestion, as discussed above.

Milk fat content and milk fatty acid profile

Low milk fat content is one of the most usual effects of SARA in dairy cows (Nocek, 1997), although this effect is not always observed when SARA is experimentally induced (Keunen et al., 2003; Cottee et al., 2004). In our experiment, milk fat content was strongly affected by induced SARA. Several theories have been proposed to explain diet-induced milk fat depression (Bauman and Griinari, 2001). The most recent theory involved the specific role of *trans*-10, *cis*-12 C18:2 fatty acid produced during biohydrogenation of C18:2n-6, which decreased the expression of genes that encode for most enzymes related to milk fat content, including enzymes involved in mammary uptake of arterial fatty acids, *de novo* synthesis of fatty acids in the mammary gland and desaturation of fatty acids by Δ-9 desaturase (Baumgard et al., 2002). Abomasal infusions of *trans*-10, *cis*-12 C18:2 strongly decreased milk fat content, with a nonlinear negative relationship between the proportion of this fatty acid in milk fat and the change in milk fat yield (de Veth et al., 2004). In our experiment, the W34 diet, but not the W20 diet, significantly increased the proportion of *trans*-10, *cis*-12 C18:2 in milk fat, but both W20 and W34 diets decreased milk fat content. Moreover, the return to W0 diet during RW0 period resulted in a complete recovery of milk fat content, but with a numerically higher proportion of *trans*-10, *cis*-12 C18:2 than during BW0 period. This suggests that the *trans*-10, *cis*-12 C18:2 fatty acid cannot account for all modifications of milk fat content in cows with SARA, as outlined by Loor et al. (2005).

Previous observations showed that high-concentrate diets induce a reduction of short- and medium-chain fatty acids as a result of reduced *de novo* synthesis (Bauman and Griinari, 2001). On the contrary, in our experiment, proportions of C8:0–C12:0 even-chain fatty acids were increased by W20 and W34 diets. Induced SARA strongly inhibited both milk yield and milk fat content, so that compared with BW0 period, milk fat production was reduced by 43% and 71% with W20 and W34 diets respectively. For this reason, even if the proportions of short- and medium-chain fatty acids in milk fat were increased, their productions were decreased, which is consistent with a reduced *de novo* mammary synthesis.

Odd-chain saturated fatty acids are synthesized by the mammary gland by elongation of propionate (Emmanuel and Kennelly, 1985) so that propionate infusion in the rumen increased their proportions in milk fat (Rigout et al., 2003). Similarly, the proportion of odd-chain fatty acids, particularly C15:0, has already been shown to be positively related with the molar proportion of propionate among ruminal volatile fatty acids (Vlaeminck et al., 2006). The ruminal production of propionate is known to be enhanced by high-concentrate diets, so that the proportions of C5:0–C17:0 odd-chain fatty acids (Apper-Bossard et al., 2006) or C13:0 and C15:0 fatty acids (Gaynor et al., 1995) have been shown to be dependent on the percentage of concentrates in the diet. In our experiment, the strong increase of C11:0, C13:0 and C15:0 proportions with W20 and W34 diets could be due to an increased production of rumen propionate because of wheat starch fermentation.

The biohydrogenation of polyunsaturated fatty acids first requires an isomerization to conjugated linoleic or linolenic acids, followed by one (C18:2n-6) or two (C18:3n-3) reductions into *trans* C18:1 fatty acids, and finally a reduction to C18:0. In our experiment, proportions of C18:2n-6 and C18:3n-3 in milk increased with W20 and W34 diets, proportion of total *trans* C18:1 increased with W34 diet and proportion of C18:0 strongly decreased with W20 and W34 diets. On the whole, these results suggested that SARA resulted in modifications of the extent of the different ruminal biohydrogenation steps. Low ruminal pH is known to inhibit the isomerization step (Troegeler-Meynadier et al., 2006), which is consistent with the higher proportion of C18:2n-6 and C18:3n-3 in milk fat in our experiment. Increased proportion of total *trans* C18:1 has already been reported with high-concentrate diets (Gaynor et al., 1995; Bauman and

Griinari, 2001), and can be explained by the inhibition of the reduction of *trans* C18:1–C18:0 when ruminal pH was low (Troegeler-Meynadier et al., 2006), resulting also in the decreased proportion of C18:0 in milk fat observed with W20 and W34 diets.

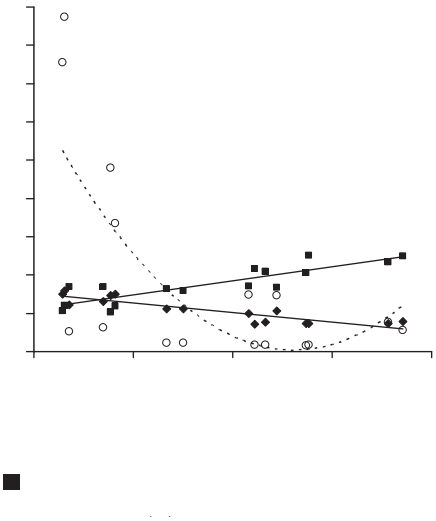
These quantitative biohydrogenation modifications were associated with qualitative effects: the profile of positional isomers was modified by W20 and W34 diets towards increased proportions of *trans*-5 to *trans*-10 C18:1 and decreased proportions of *trans*-11 to *trans*-16 C18:1. Previous studies switching from 35% to 65% of concentrate in the diet of lactating dairy cows did not result in such modifications (Loor et al., 2005; Shingfield et al., 2005). There was only a strong increase in the proportions of *trans*-10 C18:1 and *trans*-10, *cis*-12 C18:2 fatty acids, consistent with our observations and with the shift of biohydrogenation pathway because of low-fibre diets (Griinari and Bauman, 1999) or low ruminal pH (Troegeler-Meynadier et al., 2003).

The highest values of the *trans*-10 C18:1/*trans*-11 C18:1 ratio in cows with a mean ruminal pH over 6.0 were around 3.0 and were observed during RW0 period in one of the two cows: it could be explained by an incomplete return to a normal state of ruminal digestion, even after 11 days of W0 diet. The lowest values in cows with a mean ruminal pH below 6.0 were around 1.1 and were observed with W20 diet in one of the two cows. In this case, a low *trans*-10 C18:1 production in spite of a low ruminal pH would be consistent with the fact that *trans*-10 C18:1 production was not strictly dependent on ruminal pH but can also relate to the starch content of the diet (Loor et al., 2004).

Prediction of ruminal pH

Milk fat content, proportions of C12:0, C13:0, C15:0 and total odd-chain saturated fatty acids, which exhibited a linear relationship with mean ruminal pH, remained in a two- to threefold range, making difficult the determination of a cut-off point for SARA diagnosis. Moreover, previous studies have shown that when considering several herds or experiments, milk fat content and ruminal pH were not closely related, a 6.0 pH being observed to be associated with milk fat contents ranging from 27 to 58 g/kg (Allen, 1997; Enemark et al., 2004).

On the contrary, the *trans*-10 C18:1/*trans*-11 C18:1 ratio (Fig. 2) had very different average values between cows with mean ruminal pH over 6.0 (1.08) or below 6.0 (8.53). Such a pattern could more probably allow the establishment of a cut-off point between normal and SARA cows. This should take into account the interaction between ruminal pH or concentrate level and dietary supply of unsaturated fatty acids (Griinari et al., 1998), and the fact that maize silage-based diets resulted in higher *trans*-10 C18:1 proportions than grass silage-based diets (Shingfield et al., 2005; Nielsen et al., 2006).



Conclusions

Induced SARA resulted in strong modifications of milk fatty acid profile, so that fatty acid profile could be used as a tool for diagnosis of SARA. Among the possible criteria, changes in odd-chain saturated fatty acids and the *trans*-10 C18:1/*trans*-11 C18:1 ratio could be good candidates for this diagnosis. Moreover, since *trans*-10 C18:1 fatty acid originates from rumen fermentation, its detection in plasma lipids could be used in non-lactating cattle. Further studies will be necessary to assess, at cow or at herd level, thresholds, sensitivity and specificity of the method.

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