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Research article

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ABSTRACT

High-energy diets increase the risk of subacute ruminal acidosis (**SARA**) in ruminants. Ruminants with SARA show behavioural modifications. However, behavioural changes due to high-energy diet are often confounded with the behavioural changes due to SARA per se. Here, we aimed to disentangle diet-induced effects from SARA-induced effects on cow behaviour. We fed Holstein cows with either a low-starch diet (10.5% starch) or a high-starch diet (31.5% starch) while monitoring their SARA status. Control cows (n = 14) received the low-starch diet for 60 days. Challenge cows (n = 14) received the same low-starch diet except for 10 days when they were gradually switched from the low- to the high-starch diet and the next 14 days when they were fed the high-starch diet only. The eCow rumen bolus and the CowView activity-collar sensors were used to track the rumen pH and cows' activities. DM intake (**DMI**) and milk yield of each cow were assessed on a daily basis. SARA status was defined based on a relative decrease in ruminal pH and pH variability. The high-starch diet induced SARA more often than the low-starch diet (SARA on 81% of days when receiving high-starch diet vs 8% of days when receiving low-starch diet). Cows on the high-starch diet also showed decreased milk yield and spent less time eating but ate more quickly (Challenge vs Control cows during the challenge period: milk yield 20.0 vs 18.2 L/d; % time spent eating, 22.5 vs 27.6; eating rate, 77.1 vs 69.6 g DMI/min; P (diet × period) <0.001 in all cases). Cows experiencing SARA during transition or challenge periods also tended to show lower milk yield, less time spent eating, and an increase in eating rate regardless of diet (Challenge vs Control cows: milk yield, −0.5 and −0.3 L/d, P (SARA) = 0.03; % time spent eating, −1.4 and −0.84, P(SARA) = 0.02; eating rate, +4.9 and +3.2 g DMI/min, P(SARA) = 0.06; P (diet × SARA) > 0.50). Based on these findings, an increase in eating rate, especially when combined with a decrease in milk yield, should alert farmers to the risk of ruminal acidosis.

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Implications

Subacute ruminal acidosis is associated with behavioural changes in cows regardless of cow diet. Sensor systems can detect such changes. A decrease in time spent eating without a decrease in feed intake (i.e. an increased eating rate) combined with a decrease in milk yield should alert farmers to the risk of ruminal acidosis. Precision livestock farming systems should integrate such alerts to help farmers prevent nutritional disease.

Specification table

Subject	Behaviour and Health management
Specific subject area	Identifying behavioural indicators of rumen dysfunction in dairy cows
Type of data	Table, figure

(*continued on next page*)

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[1] Both authors are to be considered as first authors since they provided an equal amount of work on both the experimental work and manuscript writing.

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How data were acquired	Activity Collar, GEA *Farm Technologies* (D) Rumen bolus, eCow (UK) Ruminal pump, Aameerlaan (Fr) Gas chromatograph, Agilent (USA)SAS Analytics pro 9.0 (USA)
Data format	Aggregated data at hourly scale and expressed as activities.
Parameters for data collection	Data were obtained in an experimental farm
Description of data collection	The present dataverse contains four datasets. Only dataset 2 was used for the present paper.
Data source location	Herbipôle Experimental Unit,INRAE, Auvergne-Rhône Alpes (Fr) https://doi.org/10.15454/1.5572318050509348E12 GPS location: 45.303560°N, 2.837178°E
Data accessibility	Repository name: Data INRAE Direct URL to data: https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=https://doi.org/10.57745/MH3CIN An associated Dataset containing raw data on activities is also available on another dataverse: https://entrepot.recherche.data.gouv.fr/file.xhtml?persistentId=https://doi.org/10.15454/52J8YS/HDKEGY&version=1.0
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Introduction

Dairy farms often have to feed high-energy diets in order to achieve high production levels. However, in many high-yielding dairy cows, the transition to a high-energy diet creates a greater risk of developing subacute ruminal acidosis (**SARA**) (Martin et al., 2006). A study on 315 cows in 26 herds in Germany found a 20% prevalence of SARA (Kleen and Cannizzo, 2012). SARA is characterised by a lower and more variable ruminal pH and abnormal rumen fermentation that induce changes in the synthesis of volatile fatty acids, i.e. an increase in propionate-to-acetate ratio (Sauvant and Peyraud, 2010). SARA can lead to diarrhoea, liver abscesses, and lameness (Martin et al., 2006), which all put the welfare of animals at risk and result in depressed milk production and an inverted milk fat-to-protein ratio (Zschiesche et al., 2023).

Early detection of SARA would bring a huge improvement to animal health management at the farm level, but it remains a challenge. Cattle struggling to cope with a high-energy diet may display irregular patterns of production or intake, but such modifications are not specific to SARA and may reflect other health disorders. SARA is characterised by changes in ruminal pH. Such changes can be detected by invasive methods (sampling of rumen content) or by placing an expensive pH bolus in the rumen. Villot et al. (2020) recently showed that combining behavioural indicators (ruminating time, number of drinking bouts) with a few peripheral indicators (typically pH, urea, and glucose) measured in various matrices (such as faeces, milk, blood, saliva, urine) offers a viable alternative to these invasive or expensive methods. However, this approach still demands time, investment, and lab analyses.

Ruminants quickly show behaviour changes in response to SARA challenge. First, feeding behaviour is modified during SARA episodes (Mialon et al., 2008, Commun et al., 2009, Villot et al., 2020), with animals spreading their meals more evenly over the day and decreasing their concentrate intake. Second, SARA episodes can also trigger changes in general activity and social behaviour. For instance, sheep fed SARA-inducing high-energy diets are more apathetic and more aggressive against each other (Silberberg et al., 2013). However, in most studies, it is difficult to distinguish SARA-induced behavioural modifications from diet-induced behavioural modifications. The ability to identify behavioural modifications that are specific to SARA onset would make it possible to take remedial action early on, and thus help to preserve animal welfare.

Animal behaviour can now be monitored continuously on-farm using sensor systems developed for precision livestock farming. These systems can record patterns of feeding behaviour and other activities, such as standing, walking, resting, and so on, over extended periods of time (for a review, see Buller et al., 2020). Here, we set out to determine whether early sensor-captured modifications in dairy cow behaviour can indicate risk for SARA, independently of the diet. We analysed modifications in ruminal pH, rumen concentrations of fatty acids, milk production and milk fat-to-protein ratio to identify SARA status, and modifications in cow activity captured by a real-time locating system (**RTLS**) to identify changes in behaviour related to SARA in cows fed a moderately high-energy or a low-energy diet.

Material and methods

The study was conducted at the INRAE's 'Herbipôle' experimental facility (https://doi.org/10.15454/1.5572318050509348E12, Marcenat, France) from February to May 2015, on cows housed indoors.

Animals and experimental design

We used a total of 28 Holstein dairy cows in a blocked factorial design. The cows were divided into 'Challenge' cows (n = 14) and 'Control' cows (n = 14) of similar BW (654 ± 56 kg), body condition score (1.45 ± 0.25 on a 0–5 scale), lactation stage (93 ± 2.9 days-in-milk) and parity distribution (1–4 lactations). The experiment lasted 9 weeks, divided into four periods: initial (25 days), transition (10 days), high-starch challenge (15 days), and recovery (10 days) (Fig. 1) for the challenge cows. However, the 'Control' cows remained on the same basal low starch diet throughout the experimentation.

Housing and diets

All the cows were housed together in a free-stall pen equipped with 28 cubicles and 28 individual troughs. Each cow was fitted with an electronic transponder (Dairy gate®, EFEI, Villeroy, France) in the ear tag that paired each cow to a given electronic-access feeding gate. The feeds used were natural pasture hay, wrapped hay, milk production concentrate, and a cereal mix (Table 1). The low-starch diet contained 75% forage and 25% concentrate, thus providing 10.5% starch. The high-starch diet contained 54% forage and 46% concentrate, thus providing 31.5% starch. All cows were fed the low-starch diet, except Challenge-group cows that were fed high-starch diet during the transition period followed by the high-starch challenge. The forage-to-concentrate ratio of the diet

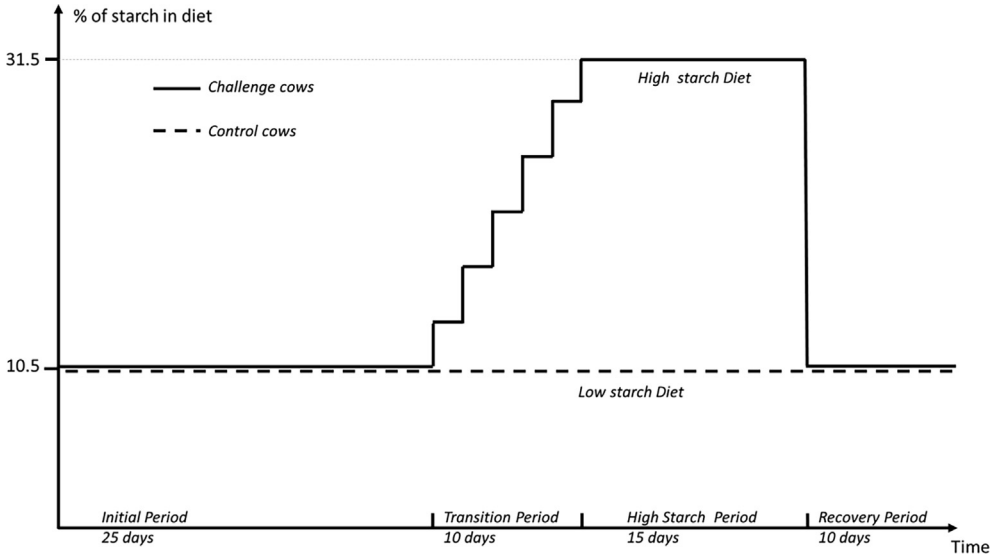

Fig. 1. Flowchart of the experimental design. Control-group of dairy cows (n = 14) received a low-starch diet throughout all experimental periods. Challenge-group of dairy cows (n = 14) received a basal low-starch diet except during a high-starch-diet transition period and the following high-starch challenge period. The low-starch diet was based on a 75% forage/25% concentrate diet containing 10.5% starch, whereas the high-starch diet was based on a 54% forage/46% concentrate diet containing 31.5% starch. During the transition period, the forage-to-concentrate ratio was modified every two days so that starch content was regularly increased in regular 2.1% increments.

given to Challenge cows was reduced by 4.2% every two days during the 10-day transition period and remained at 54% during the high-starch period (Fig. 1). Feed was distributed twice a day: 60% in the morning after milking at 0800 h, and 40% in the afternoon at 1600 h. The amount of feed provided to each cow was adjusted based on daily refusals to ensure 5% of refusals based on their previously established maximum spontaneous intake, so the cows were never feed-restricted. All cows had *ad libitum* access to fresh water and salt licks (pure salt) through the experiment.

Measurements

Ruminal fermentation parameters

Rumen content was sampled via a gastric pump for cattle (Ammerlaan, Loué, France) one time at the end of the high-starch period. Cows were restrained at the individual troughs at 0800 h, between milking and feeding. To avoid saliva pollution, the first 1L of rumen juice was pumped and discarded before collecting a 200 mL sample of rumen juice.

Table 1
Experimental diets – ingredients and chemical composition.

Item	High-starch diet	Low-starch diet
Forage-to-concentrate ratio (%)	54/46	75/25
Ingredients (% DM)		
Hay	14.0	19.0
Wrapped hay	40.0	56.0
Concentrate for production	0	25.0
Barley/corn/wheat cereal mix	46.0	0
Chemical composition of diet (g/100 g DM)		
Organic matter	94.7	93.3
Cellulose	18.2	25.5
NDF	39.4	52.1
ADF	21.6	30.4
Starch	31.5	10.5
CP	14.2	17.4

Control-group of dairy cows (n = 14) received a low-starch diet throughout all experimental periods. Challenge-group of dairy cows (n = 14) received a basal low-starch diet except during a high-starch-diet transition period and the following high-starch challenge period.

The samples were processed as described in Silberberg et al. (2013). Briefly, the rumen juice was filtered through a 400-µm nylon cloth, and two 800-µL subsamples of clear rumen fluid were collected and added to 500 µL of 0.5 N HCl containing 2% (w/v) metaphosphoric acid and 0.4% (w/v) crotonic acid, then snap-frozen and stored at 20 °C until analysis. Volatile fatty acid (VFA) content was analysed by gas-chromatography, and the ammonia content was determined by spectrophotometry (Morgavi et al., 2003).

Determination of ruminal pH and subacute rumen acidosis status

Ruminal pH was monitored using weighted rumen boluses (Farm bolus, eCow, Exeter, UK). We calibrated each bolus in buffer solutions at pH4 and pH7 maintained at 39 °C prior to insertion. A bolus was inserted into each cow's rumen using a purpose-designed balling gun as per the manufacturer's instructions and then left in the reticulum. Each bolus was set to record a mean pH over 15 minutes (giving 96 datapoints per day). Data were downloaded every 15 days using the eCow handset (smartphone + antenna) and its android application. The data on rumen pH kinetics were then processed as described in Villot et al. (2018). Briefly, the rumen pH of each cow was computed to remove drift over time, and then normalised around 0 to remove overall differences between cows, thus affording 'NpH kinetics' per day over the whole experiment. A cow was considered as SARA-positive on a given day if NpH decreased by more than 0.3 for more than 50 min and the NpH varied by more than 0.8 or its SD was above 0.2, as described in Villot et al. (2018). Using these thresholds, we labelled each cow × day as 'SARA' when the conditions for SARA were met, or 'No-SARA' otherwise.

Due to technical failures, we only obtained a total of 1 390 cow × days of pH measurements out of the 1 680 cow × day datapoints, i.e. 795 Control-group cow × days and 595 Challenge-group cow × days.

Cow activity records

Cow activity was determined using the CowView RTLS (GEA Farm Technologies, Bönen, Germany). All the cows were fitted with tags fixed on a wearable collar that was kept held on top of the cow's neck by a counterweight. The tag emitted ultra-wideband

radio waves that were detected by an array of 15 antennas fixed to the ceiling of the barn. CowView determines the position of each cow by triangulation every second and infers cow activity based on the position captured: the cow is classified as 'resting' if detected in a cubicle and 'eating' if detected near the troughs, and otherwise, it is classified as 'in alleys'. When a cow was 'in alleys', the time spent licking a salt block was distinguished according to the proximity of the cow to the salt block.

Feed intake

Individual per-cow forage and concentrate intake was recorded daily from Monday to Friday throughout the experiment.

Milk production and composition

Cows were milked in a milking parlour at 0730 h and 1530 h. Individual milk yield was recorded daily. Milk composition (fat, protein, lactose, urea, somatic cell count) was determined once a week by mid-infrared spectroscopy (LIAL, Aurillac, France) on a milk sample pooled from the morning and evening milk.

Statistical analyses

Statistical data analysis was performed using SAS Analytics pro 9.0. Data on fermentative parameters were analysed using the GLM procedure with group as fixed effect. We performed repeated measures on feed intake, milk production and composition, and ruminal pH and activity, and then performed data analysis using the MIXED procedure, with day as the repeated factor and cow as the subject. We used the autoregressive covariance structure with group (Challenge vs Control), period (initial, transition, high-starch, recovery) and group × period interaction included as fixed factors. The values from the first two weeks of the initial period were averaged and used as covariate. To differentiate the effects of SARA from the effects of diet, SARA status (SARA vs No-SARA) and group × SARA status interaction were added to the model to analyse data on feed intake, activity, and milk production and composition during the transition-to-high-starch and high-starch-challenge periods.

When significant differences were detected, we tested for differences between means using the Tukey–Kramer multiple comparison test. Differences were declared as significant when $P < 0.05$.

Results

Rumen fermentations

During the high-starch period, Challenge cows had lower rumen acetate concentrations than Control cows but higher propionate and butyrate concentrations, resulting in a lower acetate-to-propionate ratio. Ruminal ammonia concentrations were 10 times lower in Challenge cows than in Control cows ($P < 0.001$; Table 2).

Rumen pH and subacute rumen acidosis episodes

In Control cows, pH indicators showed little variation between periods other than a smaller SD of NpH during the initial period than during later periods and more time spent with NpH lower by 0.3 or more during the high-starch period than during the recovery period during the high-starch period than during the recovery period (Table 3). There was no difference between Challenge cows and Control cows during the initial period, other than a slightly lower NpH range. Challenge cows had a more variable NpH (range and standard variation) and spent more time with NpH decreased by 0.3 or more (SARA-positive) during the transition-to-high-starch period and high-starch period compared to the initial period and to Control cows. During the recovery period, their NpH variations and the time they spent with NpH decreased by 0.3 or more again decreased, and the values of NpH variations were similar to those of the initial period or to those of Control cows whatever the period. At that time, the values for the time spent with NpH decreased by 0.3 or more were even lower than their initial values or those of Control cows.

During the initial period and the recovery period, 6–11% of cows were tagged as SARA-positive (initial period: 19 out of 325 cow × days in Control cows and 32 out of 293 cow × days in Challenge cows; recovery period: 8 out of 132 cow × days in Control cows and 8 out of 103 cow × days in Challenge cows). These proportions did not change significantly between periods in Control cows (max 16% cow × days under SARA, with 7 out of 113 cow × days during the transition period and 27 out of 141 cow × days during the high-starch period) whereas they increased very significantly in Challenge cows during the transition period (49%, 51 out of 104 cow × days) and the high-starch period (81%, 117 out of 145 cow × days). The observed proportion of cow × days under SARA was higher than expected by chance only in Challenge cows and during the transition-to-high-starch and high-starch periods ($\chi^2 = 498$, df = 7, $P < 0.001$).

Cow activity

Control cows slightly increased their time spent eating and slightly decreased their eating rate across periods, from 26.5% time spent eating per day and 75 g DM intake (**DMI**)/min in the initial period and transition period to 30% and 63 g DMI/min during the recovery period (Table 3). During the initial period, there were no differences between Challenge and Control cows in time spent eating and eating rate. During transition-to-high-starch and high-starch periods, Challenge cows reduced their time spent eating from 26.0 to 22.9% and increased their eating rate to 80 g DMI/min compared to their initial level. During the recovery period, Challenge cows increased their time spent eating to just above their initial level and reduced their eating rate to below their initial level. However, in the recovery period, Challenge cows continued to spend less time eating than control Cows, and whatever the group, SARA-positive cows spent less time eating than no-SARA cows (23.8 vs 24.9% of the time, $P = 0.027$; Table 4).

Time spent near the salt block tended to vary with period and group. All cows spent a roughly similar time near the salt block (0.31–0.37% of the time; Table 3) in the initial period and the recovery period and spent more time (0.49% of the time) near the salt block during the transition period, whatever their group. In the high-starch period, Challenge cows continued to spend more time near the salt lick (0.78% of the time) whereas Control cows resumed initial levels of time. There was no SARA-status effect on time spent near salt licks (Table 4).

Over the whole experiment, there were no between-group differences in time spent resting or in alleys (Table 3). Control cows spent more time in alleys when they were under SARA, whereas Challenge cows did not (Table 4).

Feed intake

Control cows did not change their feed intake during the experiment, other than eating more concentrates during the transition period (7.9 kg DMI/d) than during other periods (6.7–7.1 kg DMI/d) (Table 3). During the initial period, Challenge cows ate similar amounts of forage and concentrates to Control cows. During the transition and the high-starch periods, Challenge cows decreased their forage intake and increased their concentrate intake compared to their initial levels and to Control cows (over 10 kg/d con-

Table 2
Rumen fermentation parameters of Control and Challenge cows during the high-starch period.

Item	Treatments		SE	*P*-value	Treatments		SE	*P*
	Challenge	*Control*			*Challenge*	*Control*		
	In mM				In % of total			
Total volatile fatty acids	99.1	106	3.72	0.2				
Acetate-to-propionate ratio	3.13	4.24	0.152	<0.001				
Acetate	61.0	73.5	2.97	0.006	61.3	69.2	1.27	<0.001
Propionate	20.1	17.4	0.898	0.044	20.2	16.4	0.613	<0.001
Butyrate	13.9	12.4	1.03	0.326	14.2	11.6	0.902	0.057
Isobutyrate	0.68	0.59	0.048	0.221	0.69	0.57	0.044	0.061
Isovalerate	1.32	0.83	0.121	0.009	1.34	0.80	0.121	0.004
Valerate	1.47	1.06	0.165	0.094	1.49	1.00	0.171	0.055
Caproate	0.77	0.39	0.072	<0.001	0.80	0.37	0.76	<0.001
Ammonia	0.11	1.08	0.102	<0.001				

Control-group of dairy cows (n = 14) received a low-starch diet throughout all experimental periods. Challenge-group of dairy cows (n = 14) received a basal low-starch diet except during a high-starch-diet transition period and the following high-starch challenge period.
Presented values are presented as means ± SE. Statistical differences were assessed using the MIXED procedure in SAS with Tukey adjustment. Statistical differences were declared significant at $P < 0.05$.

Table 3
Rumen pH, activity, intake and milk production in cows in a basal low-starch diet fed a high-starch diet during a high-starch challenge (14 challenge cows) vs control cows fed a constant low-starch diet (14 control cows).

Item	Group	Initial	Transition	High-starch	Recovery	SE	Period	Group	Period × group
Time spent with NpH < −0.3 (min/d)	Challenge	46.2^{cd}	193^{ab}	244.0^{a}	41.6^{e}	21.6	<0.001	0.002	<0.001
	Control	61.9^{cd}	47.4^{cd}	108.0^{bc}	43.4^{d}				
NpH range	Challenge	0.41^{e}	0.56^{b}	0.74^{a}	0.41^{de}	0.032	<0.001	0.752	<0.001
	Control	0.45^{bcd}	0.43^{cde}	0.50^{bc}	0.44^{cde}				
NpH SD	Challenge	0.130^{cd}	0.188^{b}	0.267^{a}	0.139^{cd}	0.005	<0.001	<0.001	<0.001
	Control	0.124^{d}	0.144^{c}	0.153^{c}	0.129^{cd}				
% time spent eating	Challenge	26.0^{b}	22.9^{a}	22.5^{a}	27.5^{c}	5.91	<0.001	<0.001	<0.001
	Control	26.9^{bc}	26.1^{b}	27.6^{c}	30.2^{d}				
% time spent in alleys	Challenge	20.1^{ab}	22.0^{ab}	23.0^{b}	20.7^{ab}	9.97	0.017	0.342	0.220
	Control	19.9^{a}	21.0^{ab}	21.3^{ab}	21.7^{ab}				
% time spent resting	Challenge	53.9^{bc}	55.1^{c}	54.5^{c}	51.8^{b}	11.03	<0.001	<0.001	0.314
	Control	53.1^{bc}	52.9^{bc}	51.1^{b}	48.1^{a}				
% time spentnear salt lick	Challenge	0.35^{a}	0.52^{b}	0.78^{b}	0.37^{a}	0.08	<0.001	<0.01	0.110
	Control	0.32^{a}	0.47^{b}	0.30^{a}	0.31^{a}				
Forage DMI (kg/d)	Challenge	19.0^{c}	15.2^{b}	13.2^{a}	15.3^{b}	0.475	<0.001	0.196	0.748
	Control	19.0^{c}	18.6^{c}	18.9^{c}	18.9^{c}				
Concentrate DMI (kg/d)	Challenge	6.9^{a}	10.6^{c}	10.9^{c}	6.8^{a}	0.264	<0.001	<0.001	<0.001
	Control	7.0^{a}	7.9^{b}	6.7^{a}	7.1^{a}				
Total DMI (kg/d)	Challenge	25.9^{c}	25.6^{bc}	24.3^{b}	22.1^{a}	0.311	<0.001	<0.001	<0.001
	Control	25.9^{c}	26.5^{c}	25.6^{bc}	26.0^{c}				
Eating rate (g DMI/min)	Challenge	71.0^{bc}	82.8^{d}	77.1^{cd}	58.8^{a}	1.83	<0.001	0.357	<0.001
	Control	74.9^{c}	75.4^{cd}	69.6^{bc}	63.1^{ab}				
Milk yield (L/d)	Challenge	20.3^{a}	19.3^{b}	18.2^{c}	17.9^{c}	0.296	<0.001	<0.001	<0.001
	Control	20.2^{a}	20.5^{a}	20.0^{ab}	20.2^{a}				
Milk fat-to-protein ratio	Challenge	1.20^{ab}	1.23^{a}	1.17^{b}	1.11^{c}	0.021	0.002	0.941	0.051
	Control	1.20^{ab}	1.18^{ab}	1.16^{bc}	1.16^{b}				

Control-group of dairy cows (n = 14) received a low-starch diet throughout all experimental periods. Challenge-group of dairy cows (n = 14) received a basal low-starch diet except during a high-starch-diet transition period and the following high-starch challenge period.
Presented values are presented as means ± SE. Statistical differences were assessed using the MIXED procedure in SAS with Tukey adjustment. Statistical differences were declared significant at $P < 0.05$.
$^{a-e}$Superscript letters indicate significant differences when the periods × group interaction was significant.
Abbreviation: DMI = DM Inake.

centrate). During the recovery period, Challenge cows resumed their initial intake of concentrates but still ate less forage than initially, thus resulting in a lower total intake. Control and Challenge cows always ate at a similar rate (between 59 and 83 g DMI/min) and both groups ate more slowly during the recovery period than before.

In the transition period and high-starch period, Challenge cows ate more concentrates when SARA-positive than when SARA-negative, whereas no such effect was observed in control cows (Table 4). There were no SARA status-related variations in forage intake. Eating rate tended to increase when cows were under SARA whatever the group.

Milk production

During the initial period, the milk production did not differ in quantity or quality between Control and Challenge cows (Tables 3 and 5). In Control cows, milk yield remained between 20 and 20.5 L/d and did not change across periods. In Challenge cows, milk yield decreased significantly from the initial period to the transi-

Table 4
Effects of SARA status during transition to high-starch and high-starch periods in cows receiving a high-starch diet (challenge, n = 14) vs a low-starch diet (control, n = 14). A cow was considered as under SARA on a specific day if its normalised pH (NpH) was decreased by more than 0.3 for more than 50 min and NpH range was over 0.8 or NpH SD was above 0.2.

	Group							
	Control		Challenge			*P*-value		
	SARA	No-SARA	SARA	No SARA	SE	Diet	SARA	Group × SARA
% time								
Eating	25.49	26.89	22.07	22.91	0.47	<0.001	0.027	0.561
In alleys	24.09[b]	21.44[a]	22.07[ab]	22.21[ab]	0.78	0.502	0.096	0.056
Resting	50.07	51.61	55.87	55.38	0.90	<0.001	0.540	0.214
Spent near salt licks	4.41	4.75	6.59	6.32	0.103	0.016	0.895	0.606
DMI								
Forage DMI (kg/d)	18.3	18.5	14.1	14.9	0.457	<0.001	0.196	0.478
Concentrate DMI (kg/d)	7.05[a]	7.05[a]	11.0[c]	9.77[b]	0.233	<0.001	0.006	0.006
Total DMI (kg/d)	25.3	25.6	25.9	24.3	0.528	0.234	0.609	0.278
Eating rate (g DMI/min)	73.3	68.4	82.1	78.9	2.618	0.006	0.064	0.705
Milk yield (L/d)	19.9	20.2	18.6	19.1	0.312	0.008	0.030	0.663

Control-group of dairy cows (n = 14) received a low-starch diet throughout all experimental periods. Challenge-group of dairy cows (n = 14) received a basal low-starch diet except during a high-starch-diet transition period and the following high-starch challenge period.
Presented values are presented as means ± SE. Statistical differences were assessed using the MIXED procedure in SAS with Tukey adjustment. Statistical differences were declared significant at $P < 0.05$.
[a–c]Superscript letters indicate significant differences when the periods × group interaction was significant.
Abbreviation: DMI = DM Inake, SARA = SubAcute Ruminal Acidosis.

Table 5
Milk production and milk quality.

Item	Group	Initial	Transition	High-starch	Recovery	SE	*P*-value		
							Period	Group	Period × Group
Milk composition									
Lactose (g/L)	Challenge	48.5	Nd	48.9	47.9	0.560	0.473	0.582	0.613
	Control	48.5	Nd	48.1	47.9	0.560			
Urea (mg/L)	Challenge	188[a]	Nd	116[c]	148[b]	5.552	<0.001	<0.001	<0.001
	Control	196[a]	Nd	184[a]	190[a]	5.552			
Cells (*1 000/mL)	Challenge	54.2[abc]	49.0[bc]	66.2[ab]	80.8[a]	0.02	0.929	0.390	0.003
	Control	66.2[ab]	54.2[abc]	49.0[abc]	40.1[c]	0.02			

Control-group of dairy cows (n = 14) received a low-starch diet throughout all experimental periods. Challenge-group of dairy cows (n = 14) received a basal low-starch diet except during a high-starch-diet transition period and the following high-starch challenge period.
Presented values are presented as means ± SE. Statistical differences were assessed using the MIXED procedure in SAS with Tukey adjustment. Statistical differences were declared significant at $P < 0.05$.
[a–e]Superscript letters indicate significant differences when the periods × group interaction was significant.
Nd: Not determined.

tion period (at −1 L/d) and continued to decrease from the transition-to-high-starch period to the high-starch period (−1 L/d), resulting in lower milk yield values in Challenge cows than Control cows from the transition period to the recovery period. Whatever the group, cows produced less milk when SARA-positive (Control cows, −0.3 L/d; Challenge cows, −0.5 L/d; $P = 0.030$).

Milk fat-to-protein ratio and milk urea decreased in Challenge cows over time and in comparison with Control cows but only toward the end of the experiment (i.e. during the recovery period for milk fat-to-protein ratio (Table 3) and during the high-starch and recovery periods for urea (Table 5). During the recovery period, somatic cell counts were higher in milk from Challenge cows than in milk from Control cows (80 800 vs 40 100 cells/mL, $P = 0.003$).

Authors' point of view

A high-starch diet affects ruminal pH and modifies cow behaviour, making cows spend less time eating but eat more quickly. A decrease in the time spent eating and an increase in eating rate are also observed, although to a lesser extent, in cows experiencing SARA, regardless of diet. In cows not submitted to a high-starch diet, SARA episodes led to less time spent eating and more time spent standing.

Increasing the proportion of concentrate to 46% affects rumen function and milk production. The high-starch diet made ruminal pH vary more during the day, with longer periods of 0.3-point decrease in NpH compared to baseline. The risk of SARA in cows receiving the high-starch diet was confirmed by a lower acetate-to-propionate ratio in rumen contents, depressed milk production, and a lower fat-to-protein ratio in milk, all of which are known markers of acidosis (Sauvant and Peyraud, 2010).

Cows fed a high-starch diet spent 18.5% less time eating during the day compared to Control cows fed the low-starch diet, but they nevertheless ingested similar amounts of food. These results are coherent with a previous study on male bulls fed a 92%-concentrate diet that spent less time eating but at a higher eating rate than bulls fed a 43%-concentrate diet (Mialon et al., 2008). The shorter time spent eating comes from a reduction in chewing (Gonzalez et al., 2012).

Challenge cows in the high-starch period increased their time spent near salt blocks, presumably licking it. Control cows also increased their time spent close to the salt block (although less than challenge cows) during the transition period, probably due to their slightly increased concentrate intake. Similar results have been reported in sheep fed high-starch diets (Phy and Provenza, 1998, Commun et al., 2012). This behaviour is interpreted as a way to limit the decrease in ruminal pH (Commun et al., 2012).

When cows are returned to a low-starch diet after being fed a high-starch diet, they do not return to the behaviour and status they exhibited before the diet t. Challenge cows that had received the high-starch diet still spent less time eating than the Control cows during the recovery period when they went back onto the previous same low-starch diet, and so they consequently ate less food, and especially less forage, than Control cows. These results may be explained by a contrast in diet appetence when Challenge cows are switched back from a high-starch diet to the initial diet. During the recovery period, the Challenge cows still produced quantitatively less milk with a qualitatively lower fat-to-protein ratio compared to Control cows. Acidotic challenges significantly modify ruminal microbial populations (e.g. in sheep submitted to 5-day acidosis challenges; Silberberg et al., 2013). We assume that the rumen of Challenge cows needed time to re-adapt to the initial diet.

In this experiment, some cows not submitted to the high-starch diet still experienced SARA for a few days during the periods when other cows received the high-starch diet. We suspect that although access to troughs was controlled electronically, the cows not provided with the high-starch diet managed to 'steal' some of the concentrates provided to their Challenge counterparts. Nevertheless, such cows did not express exactly the same pattern of behaviour modifications when they were under SARA. Indeed, they still spent more time feeding and less time near the salt blocks than cows receiving the high-starch diet, which suggests that they did not eat as much concentrate as the cows receiving the high-starch diet. The fact that SARA episodes were observed in both groups of cows (high-starch diet or low-starch diet) allows us to distinguish diet effects from SARA effects.

SARA episodes were associated with an increased eating rate. Whatever the diet offered, on the day they were under SARA, cows spent less time eating but did not decrease their feed intake, and Challenge-group cows even ate more concentrate. This resulted in a higher eating rate in both Control and Challenge cows. The timing of the activity changes needs to be considered as a factor: it is likely that a reduction in eating time with no reduction (or even an increase) in intake induces SARA (as found here) but that a prolonged SARA episode leads to a decrease in time spent eating and in intake until normal pH is resumed (Minami et al., 2021).

The decreased time spent eating when under SARA was compensated for time spent standing in Control cows only. This increase in standing activity seems consistent with Dorokhov et al. (2021) who reported that cows under the SARA challenge show a decrease in time spent active, although they did not specify what they counted as 'activity', which could therefore include both eating and standing but not eating.

SARA episodes were associated with a reduction in milk yield. Whatever the diet, cows produced less milk when they were under SARA. Although significant in statistical terms, the decrease was moderate in absolute terms (0.3–0.5 L/d) and represented only 1.5–2.5% of total milk yield. We were unable to run a finer-grained analysis of milk fat-to-protein ratio in relation to SARA status because the milk analyses were only performed once a week.

Conclusion

In conclusion, SARA is associated with detectable behavioural changes, in particular, an increase in eating rate resulting from a decrease in time spent eating without a decrease in feed intake. This behavioural change can be captured by sensors that measure eating time and intake. An increase in eating rate, especially if combined with a decrease in milk yield, should alert farmers to a risk of ruminal acidosis.

Further studies are needed to investigate the precise timing of sensor-detectable behavioural changes, especially any behaviour changes that occur before SARA and their time lag from behavioural change to SARA, and whether such changes result in a decrease in feed intake and how long the depressed intake lasts.

Ethics approval

The study was conducted at the INRAE's 'Herbipôle' experimental facility (https://doi.org/10.15454/1.5572318050509348E12, Marcenat, France) in accordance with the French Ministry of Agriculture guidelines on animal research and all applicable European guidelines and regulations. The protocol was approved by the French Ministry for Higher Education and Research animal care and use committee under agreement APAFIS#366.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Declaration of interest

The authors declare they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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