Applied Animal Behaviour Science 292 (2025) 106800

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Behavioral and systemic differences in Holstein cows with known severity of rumen acidosis during low and high-grain feeding

Rana Muhammad Atif [a,b], Patrick Biber [a,b], Thomas Hartinger [a,b], Ezequias Castillo-Lopez [a,b], Nicole Reisinger [c], Qendrim Zebeli [a,b,*]

[a] Centre for Animal Nutrition and Welfare, University of Veterinary Medicine, Vienna 1210, Austria
[b] Christian Doppler Laboratory for Innovative Gut Health Concept of Livestock, Vienna 1210, Austria
[c] dsm-firmenich, Animal Nutrition & Health R&D Center Tulln, Tulln 3430, Austria

ARTICLE INFO

Keywords:
Lying behavior
Rumination
Feed sorting
Lactation
Eating behavior
Blood variables

ABSTRACT

This study investigated behavioral and systemic differences in second-lactation Holstein cows with known susceptibility to subacute ruminal acidosis (SARA) when transitioned from a low to high-grain diet. Eighteen cows (9 SARA-susceptible, 9 SARA-resistant; 646 ± 59 kg body weight) were used in a longitudinal experimental design, with two experimental runs. In each run, the cows were first fed a 40 % concentrate diet for 2 weeks followed by a 65 % concentrate diet for 4 weeks. Behavioral parameters (chewing, lying, eating, ruminating, feed sorting), salivary characteristics, milk production, and blood metabolites were evaluated. SARA-resistant cows exhibited greater rumination activity (rumination time, chews per bolus, chews per minute; $P < 0.05$), higher body weight ($P = 0.04$), and elevated serum total protein ($P = 0.01$), despite no differences in ruminal pH indices. The dietary shift to high-grain significantly affected most behavioral, production, and physiological parameters ($P < 0.01$), including increased sorting for physically effective fiber (peNDF), eating rate, meal frequency, milk yield, milk protein, blood glucose, and salivary osmolality, alongside reductions in rumination time, eating duration, milk fat, and blood lipid metabolites. Significant SARA type × diet interactions were observed in rumination indices, eating behavior (visit size, number of meals), blood glucose, and non-esterified fatty acids concentrations, with SARA-resistant cows showing improved metabolic adaptation during the high-grain phase. In conclusion, cows previously identified as SARA-resistant demonstrated enhanced behavioral and metabolic resilience to high-grain diets, suggesting that prior SARA status may influence adaptation strategies during dietary transitions. Additional research is suggested to evaluate prolonged impact of high-grain diets on behavioral and production parameters across different SARA phenotypes.

1. Introduction

Chewing behavior is physiologically essential for ruminants, as it helps feed degradation and stimulates saliva production, which buffers rumen acidity. Dairy cows typically spend up to 16 h per day chewing, mostly ruminating (Beauchemin, 2018). However, this behavior strongly depends on diet composition. Accordingly, lowering the forage-to-grain ratio reduces chewing time due to decreased physically effective fiber (peNDF) intake, which diminishes saliva secretion and rumen buffering capacity (Nørgaard et al., 2010; Zebeli et al., 2012). Indeed, the feeding of such diets increases the risk of subacute rumen acidosis (SARA; Plaizier et al., 2008). The SARA is a metabolic disorder of dairy cattle, and cows suffering from SARA consistently have reduced milk fat content due to altered rumen fermentation profiles (lower acetate and higher propionate) that disrupts milk fat synthesis (Kleen et al., 2003; Plaizier et al., 2008). Beyond production losses, SARA elevates the risk for lameness, hoof lesions, displaced abomasum, systemic inflammation, and liver abscesses, thus impairing cow's health and welfare (Garrett et al., 1999; Krause and Oetzel, 2006; Enemark, 2008; Kofler et al., 2023). Hence, chewing behavior serves as a direct indicator of dietary fiber adequacy and overall cattle welfare (Zebeli et al., 2012).

While there is substantial data linking chewing behavior with SARA, the relationships between SARA and lying behavior remains unexplored, especially alongside chewing activity. It is known that cows prefer to ruminate while lying down, often on their left side to stabilize the rumen, making lying behavior an important welfare criterion (Albright,

* Corresponding author at: Centre for Animal Nutrition and Welfare, University of Veterinary Medicine, Vienna 1210, Austria.
E-mail address: Qendrim.zebeli@vetmeduni.ac.at (Q. Zebeli).

https://doi.org/10.1016/j.applanim.2025.106800
Received 24 March 2025; Received in revised form 22 August 2025; Accepted 26 August 2025
Available online 28 August 2025

1993; Acatincai et al., 2009; Tucker et al., 2019). Discomfort from SARA may alter these behaviors. Exploring the connection between chewing and lying behavior can enhance understanding of cow welfare and behavioral adaptations under dietary stress such as high-grain feeding and SARA. In addition, SARA is also known to affect feed sorting, potentially as a cow's strategy to mitigate rumen disturbances caused by grain-rich diets, leading to altered nutrient intake profiles (Coon et al., 2019; Castillo-Lopez et al., 2021a; Rivera-Chacon et al., 2022b).

Recently, research has revealed that despite uniform feeding and management cows differ in their susceptibility to SARA (Gao and Oba, 2014; Nasrollahi et al., 2017; Hartinger et al., 2024). Variability in chewing, eating, rumination, feed sorting may explain part of these differences. For example, although ruminal pH drop primarily depends on the peNDF and starch content of the diet (Zebeli et al., 2012), eating behavior (i.e., frequency and size of meals) can directly affect the magnitude of pH fluctuations during the day (Macmillan et al., 2017), and hence can modulate SARA severity. Besides, it is known that primiparous cows face a higher SARA risk, especially in early lactation, compared to multiparous cows, which are better adapted to high-grain diets (Krause and Oetzel, 2005; 2006; Stauder et al., 2020). Thus, behavioral and physiological responses are relevant to SARA susceptibility, likely differing between first and second lactations. However, behavioral and physiological aspects of the persistence of SARA susceptibility across lactations have not yet been evaluated.

The main objective of this study was to evaluate whether cows differing in susceptibility to SARA show distinct responses in chewing, lying, feed sorting and eating behavior, and if this is also reflected in changes in rumen, systemic and milk parameters, when transitioned from low to high-grain diets in their second lactation. We hypothesized that SARA susceptibility will persist through the second lactation, with marked differences in chewing, lying, feed sorting, eating behavior, which will be reflected in rumen and systemic parameters related to cow's health and production performance. We further hypothesized that this effect would be greater during the high-grain feeding period.

2. Material & methodology

All procedures including animal handling and treatments were approved by the Institutional Ethics and Animal Welfare Committee of the University of Veterinary Medicine, Vienna, and national authority according to § 26 of the Law for Animal Experiments, Tierversuchsgesetz 2012-TVG (Protocol # 2022–0.276.659).

2.1. Housing, animals, and experimental design

The experiment was carried out at the research dairy farm Kremesberg of Vetmeduni, Vienna (Pottenstein, Lower Austria). In total, 18 Holstein cows in their second lactation (646 ± 59 kg, of average body weight, 87 ± 57 DIM), were used in a longitudinal experimental design. These 18 cows were selected based on the severity of SARA observed during their first lactation (Hartinger et al., 2024). In the latter report, the cows showed different severity to SARA when monitored via wireless sensors for 13 weeks, despite consuming a diet with the same ingredient and chemical composition. Among those cows, 18 were selected to be used in the present trial. All the cows were housed together all the time. Their health was monitored regularly by professional veterinarian and their milk data was recorded daily during morning and evening milking, by an electronic recorder (deLaval Corp., Eugendorf, Austria). Selected cows were identified and classified into 1 of 2 categories; 1) 9 cows experiencing high SARA severity (45 % of the ruminal pH < SARA threshold (pH < 5.8 for more than 330 min/day (Zebeli et al., 2008)), on average; which we nominated SARA-susceptible cows and 2) 9 cows that experienced low SARA severity (8 % of the ruminal pH < SARA threshold (pH < 5.8 for more than 330 min/day (Zebeli et al., 2008)), on average; which we nominated SARA-resistant cows. Within each SARA category, there were 6 cows with rumen cannula, to facilitate evaluation of rumen physiology. The cows were used in a longitudinal experimental design, in 2 experimental runs, each lasting 42 days and separated by 21-days washout period. Before the start of experiment, cows were housed in the experimental area for 7 days, for adaptation to feed bins. The first week (week 0) was used as a baseline period, and the sampling started from week 1 (supplementary figure 1). The cows were first fed a 40 % concentrate diet (low-grain diet, dry matter (DM) basis) for first 3 weeks; then with 65 % concentrate diet (high-grain diet, DM basis) for 4 weeks. This concentrate mixture contained highly degradable grains such as wheat/triticale, corn grain, and formulated to meet cow's requirements for energy and all nutrients. Diets were formulated according to instructions of (GfE, 2001) (Supplementary Table 1). This concentrate was prepared by a feed company (Königshofer, Lower Austria, Austria).

The cows were kept in a free-stall barn equipped with 15 deep litter cubicles (2.6 × 1.25 m, straw litter) and a deep-bedded pack area (15.7 × 8 m). The cows had ad libitum access to water and a salt block during the complete experiment (except sampling time). The feed bins were randomly allocated to the cows, before the start of the experiment, so that each cow had access to only one feed bin throughout the experiment. Each cow was trained to access the allocated feed bin, by using an ear tag transponder. Each feed bin was equipped with electronic scale (Insentec, B.V. Marknesse, Netherlands), validated by Chapinal et al. (2007); thus individual feed intake was recorded throughout the experiment. This feeding technique allowed data collection related to eating behavior and feed sorting behavior of each cow. There was no competition for feed bunk, as each cow had her own feed bin with ad libitum access. Each cow was used as an experimental unit. The feed was prepared daily in the morning, using an automated feeding system (Trioliet Triomatic T15, Oldenzaal, the Netherlands) and was offered to the cows in their individuals feeding bins, and refilled again in the afternoon. Because of low moisture content in the feed ingredients used, calculated amount of water was added to the diet during mixing, targeting DM content of total mixed ration (TMR) approximately 45 %. DM content of silages and TMR were determined weekly by drying samples at 103°C for 24 h, to ensure appropriate inclusion of water in the diet, to fulfill the desired DM content.

2.2. Feed sampling for analysis of chemical composition

Fresh feed samples were collected in week 1 and week 4 of each run. Forages and TMR were immediately stored at −20°C, while concentrate ingredients were stored at room temperature for later analysis. All nutrient analysis of feed samples were evaluated in duplicate according to the German Handbook of Agricultural Experimental and Analytical Methods (VDLUFA, 2012). The DM of wet feed samples was measured by forced-air drying at 65 °C for 48 h. Then the samples were ground through 0.5 mm sieve for proximate nutrient analysis (Ultra Centrifugal Mill ZM 200, Retsch, Haan, Germany). The residual water was subsequently analyzed by oven drying at 103 °C for 4 h (method 3.1). Ash was measured by combustion in a muffle furnace overnight at 580 °C (method 8.1). Crude protein (CP) was estimated with Kjeldhal method and ether extract (EE) using the Soxhlet extraction system (Extraction System B-811, Büchi, Flawil, Switzerland). Similarly, for neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations (methods 6.5.1 and 6.5.2, respectively) were estimated with sodium sulfite and reported exclusive residual ash following the official analytical methods using the Fiber Therm FT 12 (Gerhardt GmbH Co. KG, Königswinter, Germany) with heat stable α-amylase for NDF analysis. Non-Fiber carbohydrates content was calculated as 100 – (% CP + % NDF + % EE +% ash).

2.3. Evaluation of chewing behavior

Chewing behavior (rumination and eating pattern) and drinking time was monitored in all cows in week 0 (baseline week), week 1, week

4 and week 6, using noseband sensor halters (RumiWatch System, ITIN + HOCH GmbH, Fütterungstechnik, Liestel, Switzerland) validated by Kröger et al. (2016). Briefly, halters were placed on the cows for adaptation, approximately 12 h before the start of data collection and remained for 3 full consecutive days in each week. At the end of each measurement period, the recorded raw data was transferred through the interface software Rumiwatch Manager (version 2.2.0.0; ITIN + HOCH GmbH, Switzerland) and was processed using the evaluation software Rumiwatch converter (version 0.7.3.2). Chewing activity included rumination time (min/day), eating time (min/day), total chewing time (min/day), rumination bolus (n/day), rumination chews (n/day), rumination chews per minute, and rumination chews per bolus. Moreover, using the dry matter intake (DMI) data, chewing index (min/kg DMI) rumination time per kg DMI and eating time per kg DMI was also calculated.

2.4. Evaluation of lying behavior

Lying behavior was monitored in all cows in week 0 (baseline week), week 1, week 4 and week 6, using data loggers (HOBO Pendant G Acceleration Data Logger, Onset Computer Corp., Bourne, MA). These measurements were taken in the same 3 days used for measurements of chewing activity. Loggers were attached to medial side of right hindleg of the cow using a self-adherent bandage (UKAL cohesive flexible bandage, France). Prior to the attachment, each logger was fixed to a silicon mat to avoid chafing. The recording interval was set at 30 s. After 3 days of data collection, the loggers were removed, and raw data were downloaded using the software HOBOware PRO. Lying data was processed using the Ledgerwood's algorithms for lying/standing bouts and laterality (Ledgerwood et al., 2010). Lying behavior variables were calculated on daily basis and included: standing time (h/day), total lying time (h/day), lying time on right side (h/day), lying bouts on right side (n/day), lying time on left side (h/day), lying bouts on left side (n/day), total lying bouts (n/day), average bout time lying on right side (h/day), average bout time lying on left side (h/day).

Moreover, data of chewing and lying behavior were combined. To perform these calculations, both parameters were matched in 10-minute intervals. This enabled the calculation of rumination time while standing or lying according to Rivera-Chacon et al. (2022b). For these calculations, we considered a minimum time of 9.5 min to assume that the cows were lying for complete 10-minute interval. Rumination time data included rumination standing time (min/day), rumination lying total time (min/day), rumination lying on left (min/day), rumination lying on right (min/day).

2.5. Evaluation of eating behavior

Eating behavior was recorded for each cow on weekly basis. Eating behavior included valid visits (n/day), visit duration (min/visit), visit size (kg DMI/visit), meals (n/day), meal duration (min/meal), meal size (kg DMI/meal), and eating rate (g DM/minute). A valid visit was defined when a cow stayed at a feed bin for minimum 4.5 min and consumed at least 200 g of dry matter. In addition, if the time interval between two consecutive visits was less than 29.5 min, these visits were considered a part of a single meal. This time interval was measured based on the methods described by Tolkamp et al. (1998) and DeVries et al. (2003). Eating rate was calculated as per protocol of Beauchemin et al. (2008).

2.6. Evaluation of feed sorting behavior and particle size distribution

Feed sorting was assessed in week 2 and week 4, using an approach similar to Haselmann et al. (2019). Feed sorting of each cow was expressed through change in particle size distribution (as-is basis) of the provided TMR in relation to the refusals. Feed selection of each particle size was calculated as the percentage of the actual intake (as-fed) from the particle intake (as-fed) expressed by the selection index, according to Leonardi and Armentano (2003). The predicted intake of a specific particle fraction was calculated as the product of as-fed intake and as-fed proportion of this specific fraction in this TMR offered. A subsample of the diet and refusal was collected and frozen at −20°C for chemical composition. Sorting of peNDF was also evaluated. To do so, the NDF content of the diets and feed refusals were taken into account to estimate the sorting index.

Particle size distribution of the TMR was determined by using the method described by Kononoff et al. (2003), using a modified Penn State Particle Separator equipped with 3 screens (19.0, 8.0 and 1.18 mm) and a pan. Physically effective fiber (peNDF) and physically effectiveness factor (pef) were calculated as described by Beauchemin and Yang (2005). Shortly, the peNDF content of the diet was calculated by multiplying the NDF content of the diet by its pef. The pef (ranging from 0 to 1) was calculated as sum of the proportions of particles retained on the corresponding sieves (19.0 and 8.0 mm sieves for pef > 8 mm; and 19.0, 8.0 and 1.8 mm sieves for pef > 1.18 mm).

2.7. Measurement of body weight and body condition scoring

Body weight (BW) and body condition scoring (BCS) was measured in all cows in week 0 (baseline week), first day of week 3 and last day of week 6.

Body weight in all cows was measured by using an electronic digital scale, when cows were coming back from milking parlor, after morning milking and before the morning feeding. Measurements taken on first day of week 3 were considered as low-grain period measurements as it was end of low-grain diet period and start of high-grain diet period and the measurements taken at last day of week 6 as high-grain period measurements.

The BCS (using scale from 1 to 5) was always measured by the single individual, and a scoring method similar to Wildman et al. (1982) was used but reported to the quarter point.

2.8. Evaluation of milk composition and production

Lactation performance was evaluated in all cows throughout the experiment. Cows were milked twice a day at 0500 h and 1600 h in a 4 × 4 tandem milking parlor (DeLaval GmbH, Eugendorf, Austria). Milk yield was recorded by an electronic recorder (DeLaval Corp., Tumba, Sweden) daily, during the entire period of the trial. Milk samples were collected from morning and evening milking by in-line samplers in every week, to measure milk composition and then stored in a 50 ml tubes. Approximately 10–15 ml of the morning milk samples were stored with a conservation liquid (Eco Bronysolv GK 145, ANA.LI.TIK. Vienna, Austria) and refrigerated at 4 °C until evening milking. The milk samples collected in the evening were added in approximately equal amounts to those of the morning, uniformly mixed, and stored at 4 °C until analyzed for fat, protein, lactose, milk urea nitrogen (MUN), somatic cell count (SCC) and pH with infrared spectrophotometry (CombiFoss TM7, Foss, Hillerod, Denmark). The energy corrected milk (ECM) was calculated as: ECM = [(12.86 x kg of fat) + (7.04 x kg of protein) + (0.3246 x kg of milk)] (Maltz et al., 2013) and 4 % fat corrected milk (4 % FCM) was calculated as: 4 % FCM = 0.4 x kg of milk + 15 x kg fat (Hall, 2023).

2.9. Sampling of blood for metabolic profile

Blood was collected from jugular vein of all cows once a week, before the morning feeding. Blood samples were collected using serum and sodium fluoride (NaF) vacutainers, (approximately 8 ml of blood sample per tube), and analysis was performed similar to Stauder et al. (2020). Serum tubes and NaF coated tubes were kept for 1.5 h at room temperature and ice respectively, for clotting. After 1.5 h, tubes were centrifuged at 2000 x g at 4 °C for 15 min (Centrifuge 5804 R, Eppendorf) to obtain the serum, which were pipetted out into 2 ml eppendorf tubes, and immediately stored at −80°C, until further analysis. Blood parameters, including total protein, albumin, bilirubin, cholesterol, non-esterified fatty acids (NEFA), triglycerides, beta hydroxybutyrate (BHBA), urea and glucose were measured using a conventional large-scale analyzer for clinical chemistry at the laboratory of the Central Clinical Pathology Unit, University of Veterinary Medicine, Vienna, using the standard enzymatic colorimetric analyses for clinical chemistry (Cobas 6000/c504; Roche Diagnostics GmbH, Vienna, Austria). Briefly, kits from Roche Diagnostics (Basel, Switzerland) were used to measure blood components; namely glucose (kit glucose HK, 20767131322), total proteins (kit total protein Gen.2, 03183734190), albumin (kit albumin Gen.2, 03183688122), triglycerides (kit triglycerides, 20767107322), Non esterified fatty acids (kit NEFA, WA434–91795 + WA436–91995), Betahydroxybutyrate (kit D-3-Hydroxybutyrate, RB 1007) and cholesterol (kit cholesterol Gen.2, 03039773190). The intra-assay co-efficient of variation was < 5 % for all blood variables.

2.10. Recording of ruminal pH

During the first run of the experiment, ruminal pH of cannulated cows was recorded using the eCow ruminal pH indwelling sensors (eCow Devon Ltd, United Kingdom) according to manufacturer's instructions (Neubauer et al., 2018), and following the procedures of Castillo-Lopez et al. (2021b) with minor changes for improvement and accuracy of measurements. Briefly, pH systems were first activated by putting in water at 35 °C for 15 min. Data collection, communication with the stationary module and download function were verified, prior to insertion of sensors into ventral sac of rumen. The pH systems were calibrated weekly, in buffers with 4.0 and 7.0, for almost 60 min, also data was downloaded on weekly basis. This procedure was performed while placing the sensors in water at 35 °C.

However, due to high failure rate of the eCow pH systems, we changed to the SmaXtec pH sensor system (SmaXtec, GmbH, Graz, Austria) in the second experimental period. The pH measurements were conducted according to Klevenhusen et al. (2014). Briefly, the sensors were calibrated by a calibration buffer of 7.0, following the company's instruction protocols and then, sensors were manually inserted into the ventral sac of the rumen via the ruminal cannula, in cannulated cows. The sensors were inserted into rumen using nasogastric tube, in non-cannulated cows. The data collected from pH sensors was summarized by calculating maximum, mean, minimum pH, the difference between maximum and minimum pH, the period of time pH below 6.0 and 5.8, as well as area under the pH curve pH < 6.0 and 5.8. In addition, acidosis index during the sampling period was calculated by two methods; firstly by calculating the time that ruminal pH was below 5.8 per kg DMI, and then by calculating the area under the curve where pH < 5.8 per kg of DMI (Gao and Oba, 2014).

2.11. Collection of saliva samples and analysis of salivary composition

Saliva samples were collected in all cows in week 2, week 3 and week 6, to evaluate salivary physico-chemicals characteristics as described in Castillo-Lopez et al. (2021a).

2.12. Statistical analysis

The number of replicates per treatment was set after a priori power analysis performed using Proc POWER (Two-Sample t Test for Mean Difference with Unequal Variances) of SAS. Data of a similar study by Castillo-Lopez et al. (2023b) were used to establish minimal detectable difference and the variation for this power analysis, using chewing behaviour as a response variable. By doing so, a minimal detectable difference of 3.0 between the groups was assumed, whereas the intergroup standard deviations were set between 1.0 and 1.8. The power analysis with a 0.05 two-sided significant level suggested that a group size of 9 animals would have < 85 % ($1 - \beta = 86.8$ %) power to detect an intergroup difference (exact alpha = 0.0453). Based on these recommendations of the power analysis, we selected 9 animals in each SARA group for this experiment.

Data were checked for influential outliers using the Cook's distance in SAS. The data for every variable were tested for normal distribution using the Shapiro-Wilk test with Proc Univariate of SAS. When normality was not met, data was transformed (i.e. log or square root transformation) following evaluation with the boxcox statement in the Transreg procedure of SAS, which determines the transformation mode. Then, data was subjected to analysis of variance using Proc Mixed of SAS considering the following fixed and random effects. The following statistical mixed model was used:

$Y_{jklm} = \mu + X_j + d_k + s_l + a_m(s) + I_{kl} + e_{jklm}$,

Y_{jklm} = response variable,

μ = overall mean,

X_j = covariate assumed to be measured without error,

d_k = fixed effect of the diet k

s_l = fixed effect of the SARA-type susceptibility

$a_m(s)$= random effect of the cow m within run,

I_{kl} = interaction between diet and SARA-type susceptibility.

e_{jklm}= residual error.

Thus, the measurements taken before the initiation of each period (adaptation week) were used as covariates. Measurements taken on different days and runs on same animal were considered as repeated measures using respective repeated statements (AR(1), CS) in the ANOVA. The variance-covariance structure was included to take into account that covariance among adjacent time points decays with time. Data are reported as LSMEANS, and the transformed data were transformed back after the ANOVA. The PDIFF option in SAS was used to perform multiple comparisons of the LSMEANS and p-values were corrected using TUKEY. The largest standard error of the means (SEM) of the data before transformation was reported. Statistical significance was declared at $P \leq 0.05$ and had a tendency at $0.05 < P < 0.10$.

3. Results

3.1. Chewing behavior

The analysis indicated that SARA-type affected rumination time ($P < 0.01$), rumination chews ($P < 0.01$), and rumination chews per minute ($P < 0.01$), and tended to decrease rumination chews per bolus ($P = 0.06$) being higher in SARA-resistant cows compared to SARA-susceptible cows (Table 1).

As far as effect of diet is concerned, rumination time ($P < 0.01$), eating time ($P < 0.01$), total chewing time ($P < 0.01$), rumination bolus ($P < 0.01$), rumination chews ($P < 0.01$), rumination chews per minute ($P < 0.01$), ruminate chews per bolus ($P < 0.01$), chewing index ($P < 0.01$), ruminate time per kg of DMI ($P < 0.01$), were lower in the cows during the high-grain period.

The interaction between SARA-type and diet was observed in the number of rumination chews per bolus ($P < 0.01$; Fig. 1A) and rumination time per kg of DMI ($P < 0.01$; Fig. 1B). Accordingly, the decrease of the number of rumination chews per bolus and rumination time per kg DMI during the high-grain diet were less in SARA-resistant than in SARA-susceptible cows.

3.2. Eating behavior

SARA-type did not show any effect ($P > 0.05$) on eating behavior throughout the experiment, but there was an effect of diet (Table 1). Total number of valid visits per day ($P < 0.01$), visit duration ($P < 0.01$), meal duration ($P < 0.01$) and meal size ($P < 0.01$) were lower in all the cows during high-grain diet period. While visit size ($P < 0.01$), number of meals per day ($P < 0.01$) and eating rate ($P < 0.01$) were higher in all the cows, during high-grain diet period. The interaction between SARA-type and diet was found in visit size

Table 1
Chewing behavior and eating behavior in mid-lactating Holstein cows differing in subacute rumen acidosis susceptibility in their first lactation fed with low-grain and high-grain diets during the second lactation.

	SARA-Susceptible		SARA-Resistant		SEM	P-values		
Parameter	Low-grain	High-grain	Low-grain	High-grain		SARA-type	Diet	SARA*Diet interaction
Rumination time, min/day	595	506	611	553	14.4	0.04	< 0.01	0.11
Eating time, min/day	204	164	184	146	19.2	0.27	< 0.01	0.91
Total chewing time, min/day	825	665	825	709	22.3	0.38	< 0.01	0.11
Rumination bolus, #/day	616	521	616	560	15.5	0.19	< 0.01	0.07
Rumination chews, #/day	38087	30767	40014	34884	1088	0.01	< 0.01	0.13
Rumination chews per minute	70.8	68.2	72.2	69.5	0.58	0.03	< 0.01	0.92
Rumination chews per bolus	61.6^{Aa}	57.6^{Bb}	62.30^{Aa}	60.8^{Aa}	1.28	0.06	< 0.01	0.01
Chewing index, min/kg DMI	37.9	29.0	36.8	30.7	1.45	0.66	< 0.01	0.10
Rumination time, min/kg DMI	28.1^{Aa}	22.0^{Bb}	28.2^{Aa}	24.3^{Ab}	0.70	0.13	< 0.01	0.03
Eating time, min/kg DMI	8.33	8.62	8.01	7.84	1.18	0.44	0.93	0.72
Drinking time, min/day	5.06	8.39	6.32	7.44	0.14	0.72	< 0.01	0.11
Valid visit, #/day	15.8	13.1	14.9	13.1	0.44	0.33	< 0.01	0.15
Visit duration, min	14.3	12.4	15.5	13.4	0.04	0.13	< 0.01	0.97
Visit size, kg DMI	1.33^{b}	1.68^{a}	1.44^{b}	1.67^{a}	0.07	0.59	< 0.01	0.04
Meals, n/day	7.11^{a}	7.15^{a}	6.87^{b}	7.37^{a}	0.23	0.99	< 0.01	0.02
Meal duration, min	31.1	22.6	33.5	23.7	1.59	0.14	< 0.01	0.56
Meal size, kg DMI	3.09	3.05	2.98	2.97	0.04	0.53	< 0.01	0.81
Eating rate, g DM/mint	88	129	89	126	3.22	0.72	< 0.01	0.34

Superscripts a,b show differences in between diets of same SARA types
Superscripts A,B show differences in between SARA types of same diets

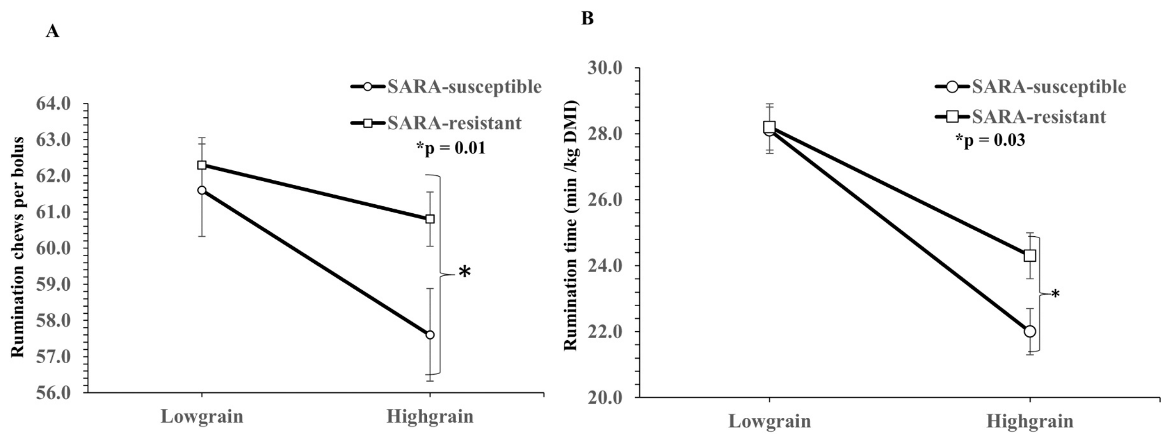

Fig. 1. Interaction effect of Diet* SARA severity in mid-lactating Holstein cows, differing in Subacute rumen acidosis severity in their first lactation fed with low-grain and high-grain diets during their second lactation, on (A) Rumination chews per bolus and (B) Rumination time per KGDMI.

(P = 0.04) and meals per day (P = 0.02), so that visit size was higher in SARA-resistant cows during low-grain only. On the other hand, the meal number per day was lower in SARA-resistant cows during low-grain but increased during high-grain.

3.3. Lying behavior

All variables of lying behavior (Table 2) were not affected by SARA-type (P > 0.05). Diet had effect on some of variables. For example, total

Table 2
Lying behavior and rumination time in mid-lactating Holstein cows differing in subacute rumen acidosis susceptibility in their first lactation fed with low-grain and high-grain diets during the second lactation.

	SARA-Susceptible		SARA-Resistant		SEM	P-values		
Parameters	Low-grain	High-grain	Low-grain	High-grain		SARA-type	Diet	SARA*Diet Interaction
Standing time, h/d	12.5	12.6	12.5	12.7	0.28	0.81	0.54	0.89
Lying time on right, h/d	1.09	0.97	0.70	0.94	0.24	0.37	0.64	0.21
Lying bouts, right side, #/d	5.56	4.66	3.47	4.77	0.97	0.41	0.64	0.06
Lying time on left side, h/d	10.4	10.2	10.7	10.2	0.36	0.62	0.19	0.55
Lying bouts, left side, #/d	13.8	15.1	13.3	16.3	0.83	0.63	< 0.01	0.15
Total lying bouts, #/d	19.6	20.9	17.3	21.5	1.62	0.64	< 0.01	0.13
Average bout time lying on right, h/d	0.17	0.18	0.16	0.17	0.03	0.75	0.62	0.96
Average bout time lying on left, h/d	0.73	0.69	0.81	0.64	0.05	0.79	< 0.01	0.08
Total lying time, h/d	11.5	11.4	11.5	11.3	0.25	0.92	0.26	1.00
Ruminate standing time, min/day	167	172	169	172	14.2	0.93	0.62	0.89
Ruminate lying time, min/day	405	323	408	328	13.6	0.73	< 0.01	0.91
Ruminate lying right, min/day	19.5	22.5	19.4	24.8	14.7	0.83	0.19	0.75
Ruminate lying left, min/day	372	300	368	299	15.0	0.86	< 0.01	0.91

number of lying bouts were higher in the cows during high-grain period. Similarly, the number of lying bouts on left side were also higher during high-grain period. However, the average bout time lying on left side (hours/d) was lower during this feeding period. As far as interaction between SARA-type and diet is concerned, all the variables of lying behavior were not affected.

There was an effect of diet on rumination lying total time ($P < 0.01$) and rumination time lying left ($P < 0.01$). The rumination time total time while lying was 19.9 % lower in high-grain period. Similarly, ruminating time while lying on left side was 19.2 % lower during high-grain period.

3.4. Feed sorting behavior

The SARA-type and SARA x diet interaction did not affect the feed particle sorting behavior of the cows ($P > 0.05$), and selection index of peNDF ($P > 0.05$) (Table 3). However, diet affected selection index for long particles ($P < 0.01$) and for peNDF ($P < 0.01$), being higher in high-grain period as compared to low-grain period.

3.5. Dry matter intake, BW, BCS, and milk variables

Data of DMI, BW, BCS and milk production are shown in Table 4. Data showed no effect of SARA-type ($P = 0.66$) and SARA × diet interaction ($P = 0.91$) on DMI. However, diet affected DMI ($P < 0.01$) as cows ate on average 1.4 kg more DM during high-grain period, as compared to low-grain period (Table 4). On the other hand, the SARA-type affected BW ($P = 0.04$), whereby the SARA-resistant cows had higher BW as compared to SARA-susceptible cows. Similarly, there was a SARA x diet interaction ($P < 0.05$) on BW, so that the SARA-resistant cows gained more BW only during the high-grain period. No effect of SARA-type and SARA x diet interaction was observed on BCS ($P > 0.05$). Diet showed effect on BW ($P < 0.05$) and BCS ($P = 0.05$). The cows had higher BW and BCS during high-grain period as compared to low-grain period.

We did not observe any effect ($P > 0.05$) of SARA-type on all variables of milk production (Table 4). Diet affected most of the variables such as milk yield ($P < 0.01$), ECM ($P = 0.01$), milk protein concentration ($P < 0.01$), milk urea nitrogen ($P = 0.01$), somatic cell count ($P < 0.01$) and fat free dry matter ($P < 0.01$), which were all higher during the high-grain period, while milk fat concentration ($P < 0.01$), milk fat to protein ratio ($P < 0.01$), and milk pH ($P = 0.01$) decreased during the high-grain period.

3.6. Blood parameters

SARA-type did not affect blood parameters ($P > 0.05$) except of total protein ($P = 0.01$) and a tendency for albumin ($P = 0.06$), being higher in SARA-resistant cows compared to SARA-susceptible cows (Table 5). There was no interaction between SARA-type and diet on the blood variables ($P > 0.1$), except of glucose ($P < 0.01$) and NEFA ($P = 0.01$). Glucose concentration increased more and NEFA decreased more in SARA-resistant cows during high-grain, as compared to in low-grain feeding.

A diet effect was found in blood variables such as BHBA ($P < 0.01$), cholesterol ($P < 0.01$), NEFA ($P < 0.01$) and triglycerides ($P < 0.01$), all being lower in high-grain periods. On the other hand, blood glucose ($P < 0.01$) and urea nitrogen ($P < 0.01$) were higher in high-grain periods.

3.7. Rumen pH and saliva variables

The SARA-type showed no effect ($P > 0.1$) on all rumen pH variables of the second lactation (Table 6). Similarly, there was no interaction between SARA-type and diet on all rumen pH variables ($P > 0.1$). However, feeding the high-grain diet showed an effect on rumen pH variables. Cows had higher maximum pH ($P < 0.01$), but lower minimum pH ($P < 0.01$) and longer duration of pH below 6.0 ($P < 0.01$) during the high-grain period.

The physico-chemical characteristics of saliva were unaffected by SARA-type, but high-grain diet showed some effects (Table 6). During the high-grain period, salivary mean pH was higher ($P < 0.01$), salivary mucin concentration was lower ($P = 0.03$), and salivary osmolality was increased ($P = 0.01$), compared to low-grain period. An interaction between SARA-type and diet was found in saliva phosphate concentration ($P = 0.03$), where saliva phosphate was higher in SARA-resistant cows during high-grain period as compared to in low-grain period.

4. Discussion

The study aimed to test the hypothesis that susceptibility to SARA persists into the second lactation, manifesting significant differences in chewing, lying, feed sorting, eating behavior, and a range of systemic variables, including salivary, blood and milk parameters, in cows fed both low and high-grain diets. Notably, SARA-resistant cows exhibited longer daily rumination times and higher rumination indices, while all chewing parameters were reduced during high grain feeding in both SARA groups, likely due to low $peNDF_{>8\ mm}$ content (13.8 %) and high starch concentration (33.2 %) of the diet, which are known risk factors for SARA development (Khorrami et al., 2021). Although these differences in rumination are biologically meaningful, as increased rumination time is associated with improved ruminal pH, greater fiber digestibility, leading to higher milk fat yield (Zebeli et al., 2008), these relationships explain only a portion of the variation in production outcomes (Souza et al., 2022). Fiber digestibility is not reported in current study, but research has shown that diets higher in peNDF increase chewing, ruminal pH and fiber digesting enzyme activity, reducing SARA risk (Zebeli et al., 2008; Cao et al., 2021). It has also to be mentioned that although increased chewing and rumination promote salivary secretion, their net effect on rumen buffering might still be

Table 3
Feed/Particle sorting behavior in mid-lactating Holstein cows differing in subacute rumen acidosis susceptibility in their first lactation fed with low-grain and high-grain diets during the second lactation.

Parameters	SARA-Susceptible		SARA-Resistant		SEM	P-values		
	Low-grain	High-grain	Low-grain	High-grain		SARA-type	Diet	SARA*Diet Interaction
Long	83	96	83	96	4.0	0.95	< 0.01	0.98
Medium	100	101	102	100	2.0	0.94	0.68	0.39
Short	103	102	106	101	2.0	0.66	0.08	0.30
Fine	97	96	100	94	2.0	1.00	0.10	0.23
[a]peNDF	96	99	95	101	1.0	0.69	< 0.01	0.45

Values = 100 no sorting
Values < 100: sorting against
Values > 100: sorting for
[a] Physically effective NDF

Table 4
Dry matter intake, body weight and milk composition of mid-lactating Holstein cows differing in subacute rumen acidosis susceptibility in their first lactation fed with low-grain and high-grain diets during the second lactation.

Parameter	SARA-Susceptible		SARA-Resistant		SEM	P-values		
	Low-grain	High-grain	Low-grain	High-grain		SARA-type	Diet	SARA*Diet Interaction
Dry matter intake, kg	21.7	23.1	21.5	22.9	0.38	0.66	< 0.01	0.91
Body weight, kg	640.5^{b}	658.9^{aB}	644.1^{b}	681.2^{aA}	5.25	0.04	< 0.05	< 0.05
Body condition score	3.11	3.22	3.16	3.23	0.06	0.63	0.05	0.64
Milk yield, kg/day	35.8	38.9	35.1	37.9	1.35	0.55	< 0.01	0.77
4 % Fat corrected milk, kg/day	33.4	34.8	33.6	33.0	1.67	0.64	0.56	0.12
Energy-corrected milk, kg/day	36.0	38.2	35.8	36.6	1.60	0.58	0.01	0.26
Fat%	3.60	3.20	3.70	3.00	0.19	0.57	< 0.01	0.09
Protein, %	3.00	3.30	3.10	3.30	0.08	0.66	< 0.01	0.85
Fat to protein ratio	1.20	1.00	1.20	0.90	0.06	0.45	< 0.01	0.16
Lactose, %	4.90	4.90	5.00	4.90	0.05	0.50	0.82	0.11
Milk Urea N, mg/dL	14.3	16.1	8.4	14.9	1.64	0.17	0.01	0.08
pH	6.57	6.55	6.59	6.57	0.01	0.26	0.01	0.43
Somatic cell count ($*10^3$/ml)	17.6	23.6	16.1	27.4	14.8	0.88	< 0.01	0.13
Fat free dry matter, %	8.7	9.0	8.8	9.0	0.08	0.61	< 0.01	0.28

Superscripts a,b show differences in between diets of same SARA types
Superscripts A,B show differences in between SARA types of same diets

Table 5
Blood parameters related to production in mid-lactating Holstein cows differing in subacute rumen acidosis susceptibility in their first lactation fed with low-grain and high-grain diets during the second lactation.

	SARA-Susceptible		SARA-Resistant		SEM	P-values		
Parameters	Low-grain	High-grain	Low-grain	High-grain		SARA-type	Diet	SARA*Diet Interaction
Total protein, g/dl	7.66	7.69	7.99	7.95	0.09	0.01	0.88	0.43
Albumin, g/dl	3.85	3.89	4.00	4.01	0.06	0.06	0.26	0.56
Glucose, mg/dl	63.8^{Ab}	66.7^{Ba}	62.6^{Ab}	69.6^{Aa}	0.76	0.49	< 0.01	< 0.01
Bilirubin, mg/dl	0.06	0.07	0.06	0.06	0.01	0.59	0.52	0.44
Beta-Hydroxybutyrate, mmol/L	0.40	0.31	0.36	0.31	0.02	0.50	< 0.01	0.13
Non-esterified fatty acids, mmol/L	0.14^{Ba}	0.13^{Aa}	0.18^{Aa}	0.13^{Ab}	0.01	0.25	< 0.01	0.01
Cholesterol, mg/dl	184	174	184	173	8.03	0.99	< 0.01	0.92
Triglycerides, mg/dl	11.3	10.1	11.2	9.4	0.43	0.40	< 0.01	0.15
Blood Urea N, mg/dl	21.8	30.4	21.5	30.1	1.1	0.82	< 0.01	0.92

Superscripts a,b show differences in between diets of same SARA types
Superscripts A,B show differences in between SARA types of same diets

Table 6
Ruminal pH and salivary characteristics of mid-lactating Holstein cows differing in subacute rumen acidosis susceptibility in their first lactation fed with low-grain and high-grain diets during the second lactation.

Parameters	SARA-Susceptible		SARA-Resistant		SEM	p-value		
	Low-grain	High-grain	Low-grain	High-grain		SARA-Type	Diet	SARA*Diet Interaction
Ruminal pH variables Mean ruminal pH	6.39	6.38	6.39	6.37	0.05	0.69	0.18	0.69
maximum ruminal pH	6.79	6.90	6.81	6.91	0.04	0.36	< 0.01	0.36
minimum ruminal pH	6.02	5.97	5.94	5.87	0.07	0.56	< 0.01	0.56
Duration < pH 6.0, minutes	23.1	61.0	42.9	66.9	38.8	0.14	< 0.01	0.14
Duration < 5.8, minutes	7.20	7.80	13.6	11.8	24.8	0.55	0.83	0.55
Area < pH 6.0, minute x pH	5.84	8.77	9.91	11.37	16.7	0.46	0.08	0.46
Area < pH 5.8, minute x pH	1.89	1.26	2.37	1.70	6.46	0.95	0.10	0.95
Salivary characteristics Saliva mean pH	8.60	8.74	8.59	8.76	0.05	0.99	< 0.01	0.56
Buffer capacity, mol of HCL per Δ pH	0.02	0.02	0.02	0.02	0.00043	0.88	0.76	0.37
Saliva mucin, ug/ml	639	538	1147	568	761	0.22	0.03	0.18
Saliva lysozyme activity, U/ml/min	6.43	6.39	6.56	7.58	2.47	0.63	0.73	0.71
Saliva osmolality, mOsmol/kg	241	255	242	260	34.7	0.59	0.01	0.79
Saliva total protein, ug/ml	192	190	201	182	43.5	0.98	0.66	0.73
Saliva phosphate, mM/L	12.0	11.1	10.9	12.4	0.78	0.88	0.49	0.03
Saliva bicarbonate, mM/L	90.8	88.3	92.7	92.7	3.05	0.61	0.25	0.65

modest, especially in diets rich in starch (Dijkstra et al., 2012; Beauchemin, 2018). Indeed, the benefits in chewing behaviour were not reflected in most of the salivary and ruminal pH variables measured in this study. In fact, with exception of the maximum pH, the other ruminal pH metrics such as minimal pH decreased and duration of pH below 6 increased during high-grain diet, independent of the SARA-type. However, our data indicate that the improved rumination indices in SARA-resistant cows, although statistically significant, were not enough to modulate ruminal pH of these cows. It seems that at this high dietary starch level (33.2 %), the slightly improved rumination-induced salivation and rumen buffering may not be enough to modulate ruminal pH variables, likely because of the release of large amounts of VFA in the rumen, which cannot be neutralized by saliva buffers alone. It is known that the ruminal absorption of ionic VFA plays an increasing role in buffering the rumen than saliva, when cattle are fed high-grain diets (Dijkstra et al., 2012). On the other hand, the data of eating behavior showed that the cows increased the number of meals but lowered the meal size during high-grain feeding and independent of SARA-type. This might also have resulted in lower VFA production rate with a timely neutralization, as suggested by Gregorini et al. (2012), showing that cows change their feeding behavior towards smaller, more frequent meals to mitigate the harmful effects of highly fermentable starch-rich diets.

Another known mechanism that cows use to counteract the impact of highly fermentable starch-rich diets on the rumen milieu is feed sorting (Coon et al., 2019; Castillo-Lopez et al., 2021a; Rivera-Chacon et al., 2022b). Feed sorting, especially against long particles, is linked to increased SARA risk and reduced rumination (Gao and Oba, 2014; Coon et al., 2019). Cows at higher risk for SARA tend to sort more, especially when fed diets with longer straw particles, which can lead to less balanced intake of peNDF and lower milk fat yields (Coon et al., 2019). We initially expected different sorting behavior of the cows based on the SARA-type in this lactation, but this was not the case. However, during the high-grain feeding period, the cows sorted for long particles and this somehow increased both the proportion of large particles and the $peNDF_{>8\ mm}$ intake, which may have contributed to regulate the ruminal pH and to mitigate potential negative effects on animal health and behavior. Other data of our study showed that SARA-type had no effect on all parameters of lying behavior, providing evidence that previous susceptibility to SARA has no influence on lying behavior of the cows in the second lactation, and so disapproving our hypothesis. Lying behavior, is often correlated with rumination and overall cow comfort. Interestingly, the total number of lying bouts increased during the high-grain feeding. Preferring lying on the left side is known in cows as an adaptive position for them for increase rumination efficiency, as this position might support the rumination process, due to better alignment of the esophagus with the rumen contents (Albright, 1993). However, shorter times spent ruminating while lying on the left side might be interpreted as an expression of impaired welfare of cows during the high-grain feeding.

Other findings of this research showed that SARA-type did not affect the DMI, BCS and milk variables, but the SARA-resistant cows had higher BW, and these cows gained around 40 kg BW during the 4 weeks in high-grain feeding. Previously, the SARA-resistant cows showed lower DMI and milk yield but no differences in the BW during first lactation (Castillo-Lopez et al., 2025). No effect on the performance of the SARA-type suggests no persistence of this effect across lactations. The reason for the BW gain of the SARA-resistant cows during the high-grain feeding is not clear, as the DMI and the energy intake of both SARA groups were similar. At the same time, the SARA-resistant cows showed a tendency for lowered milk fat concentration with the diet changed from low to high-grain feeding. The blood analysis also showed that glucose increased, whereas the NEFA decreased stronger during the dietary change from low to high-grain feeding in SARA-resistant cows. This might suggest a tendency for higher body energy deposition, decreased mobilisation and likely also decreased milk fat excretion of the SARA-resistant cows when they were switched from low to high-grain diet, as compared to their SARA-susceptible counterparts. The phenomenon of increasing body fat/energy accretion is common in mid-lactation cows aiming to support body reserves as they reach positive energy balance with DMI being at its peak and milk production beginning to decline (van Beukelen et al., 1985). However, since the groups were in similar mid-lactation phase, this body mass accretion change in SARA- resistant cows might be rather linked to their metabolic type.

As expected, feeding the high-grain diet increased the milk yield including milk protein concentration and MUN contents, and BW and BCS, while decreasing fat concentration, fat to protein ratio and the pH of the milk. During the high-grain feeding, the cows experienced a strong depression of milk fat and the fat to protein ratio dropped below 1. Milk fat depression (MFD) is a known sequela of feeding high-grain diets and SARA, caused by changes in ruminal biohydrogenation, particularly the trans-11 to trans-10 shift (Alves and Bessa, 2014; Dewanckele et al., 2019) and overall drop of the lipogenic precursors for the *de novo* mammary milk fat synthesis, mainly acetate (Lock et al., 2008; Toral et al., 2015). Mammary *de novo* synthesis, milk fatty acid alterations stem also from changes in the blood uptake of long chain fatty acids originating from adipose tissue mobilization (Bionaz and Loor, 2008).

Our data of blood variables showed that SARA-resistant cows had higher total protein and a tendency for higher albumin in their blood serum. Serum albumin is one of the major proteins in blood, synthesized in the liver and having a critical role in maintaining osmotic pressure, transport of hormones, fatty acids, and drugs, and has antioxidant properties. So, higher serum total protein and albumin levels can be interpreted of better liver function and metabolic health (Allison, 2012; Soeters et al., 2019), probably linked to a superior metabolic type of SARA-resistant cows. The SARA is known to induce systemic inflammation in ruminants, primarily through ruminal translocation of lipopolysachharides (LPS) originating from Gram negative bacteria, leading to systemic immune activation (Plaizier et al., 2012, 2022; Zhao et al., 2018) and liver damage (Rivera-Chacon et al., 2022a).

As expected, the feeding of high-grain diets increased serum glucose concentration due to higher starch intake that results in increased ruminal propionate, being converted to glucose, by the process of hepatic gluconeogenesis (Reynolds, 2006). This enhanced glucogenic state during high-grain feeding lowered both the lipolysis and ketogenesis, while increasing lipogenesis, as reflected by lower NEFA and BHBA, and the increased BW of the cows. The decreased concentrations of triglycerides and cholesterol during the high-grain feeding period may also be due to lowered lipolysis and increased lipogenesis in the adipose tissues. Another possibility is also either decreased cholesterogenesis due to lowered rumen acetate (Liepa et al., 1978) or increased hepatic clearance of cholesterol via biliary route (Ametaj et al., 2010) in response to high-grain feeding. However, these latter processes were similar in both SARA types.

Regarding, salivary characteristics, we found no effect of SARA-type on all parameters, except a tendency for increased salivary phosphate in SARA-resistant cows during high-grain feeding. Salivary phosphate along with bicarbonate has a role in proton neutralisation, ultimately balancing the ruminal pH (Aschenbach et al., 2011). Similarly, a slight increase in salivary pH found during high-grain period might be due to increased saliva osmolality, which could be a host adaptation for ruminal pH regulation (Allan et al., 2000; Castillo-Lopez et al., 2021a; Rivera-Chacon et al., 2022b). Salivary mucin concentration dropped during the high-grain period, which could be due to low rumination activity, as reported in a recent study that high-grain feeding tended to reduce salivary content of mucins, due to reduction in chewing activity (Castillo-Lopez et al., 2023a). These changes suggest alteration in the cow's body, adjusting for digestion of highly fermentable high-grain diets. Such adaptive response may help cows to protect from ruminal acidosis ensuring efficient digestion of high-grain diets.

5. Conclusion

Collectively, the data indicate that SARA-resistant cows coped better with high-grain diets than SARA-susceptible cows by enhancing their ruminating behavior and modulating the metabolic state in the second lactation. The latter consisted in maintaining a higher body mass, obviously by increasing systemic glucose availability and body energy accretion vs. mobilisation, and by maintaining higher serum protein/albumin levels, as well as a tendency to decrease milk fat excretion.

However, SARA-resistant type did not affect several behavior parameters of the cows including lying or feed sorting behavior and did not alleviate most of the changes in saliva, rumen, blood, and milk variables related to high-grain feeding conditions. The study also reinforced the negative impact of high-grain feeding on cow behavior and welfare, most importantly by impairing rumination while lying, and lowering ruminal pH, and milk fat content. Although the cows attempted to counteract the high-grain feeding by sorting it in favor of long particles and peNDF, this was not enough considering the high-grain diets, being very low in peNDF and high in starch, in this research. Further research should focus on evaluating the observed changes in the metabolic state of SARA-resistant cows during early lactation, particularly in terms of increased rumination and the energy accretion, and its associated implications for cow health.

Funding

This research was funded by the Austrian Federal Ministry for Digital and Economic Affairs, and the National Foundation for Research, Technology and Development through the Christian Doppler Research Society. BIOMIN Holding GmbH, which is part of dsm-firmenich, financially supports the Christian Doppler Laboratory for Innovative Gut Health Concepts of Livestock as business partner.

CRediT authorship contribution statement

Qendrim Zebeli: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Ezequias Castillo-Lopez:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Formal analysis. **Nicole Reisinger:** Writing – review & editing, Software, Resources, Conceptualization. **Patrick Biber:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Thomas Hartinger:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Data curation. **Rana Muhammad Atif:** Writing – original draft, Visualization, Validation, Project administration, Investigation.

Declaration of Competing Interest

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

Acknowledgments

The authors acknowledge the help and support of Raul Rivera-Chacon, Claudia Lang, Marlene Schmidt in sampling throughout the experiment. The authors also thank the excellent technical support and cooperation of Anita Dockner, Manfred Hollman, Sabine Leiner, Suchitra Sharma, Simone Koger, Thomas Schwarcz-Enzinger (Centre for Animal Nutrition and Welfare) and Elmar Draxler (Vetfarm Vetmeduni, Vienna). The first author thanks the Higher Education Commission (HEC), Pakistan for the financial support for the doctoral programme in collaboration with Austrian Agency for International Cooperation (OeAD) in Education and Research, Austria.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2025.106800.

References

Acatincai, S., Gavojdian, D., Czister, L.T., Tripon, I., Alungel, A., Popian, C., 2009. Study regarding rumination behavior in multiparous Romanian black and White cows during summer season. Lucr Stiint. Zooteh. Biotehnol 42, 191–193.

Albright, J.L., 1993. Feeding behavior of dairy cattle. J. Dairy Sci. 76, 485–498. https://doi.org/10.3168/jds.S0022-0302(93)77369-5.

Allan, B., Dennis, M., Nyvad, B., Nauntofte, B., 2000. The buffer capacity and buffer systems of human whole saliva measured without loss of CO2. Arch. Oral. Biol. 45, 1–12. https://doi.org/10.1016/S0003-9969(99)00119-3.

Allison, R.W., 2012. Laboratory evaluation of the liver. Vet. Hematol. Clin. Chem. Ed. 2, 401–424.

Alves, S.P., Bessa, R.J.B., 2014. The trans-10,cis-15 18:2: a missing intermediate of trans-10 shifted rumen biohydrogenation pathway? Lipids 49 (6), 527–541. https://doi.org/10.1007/s11745-014-3897-4.

Ametaj, B.N., Zebeli, Q., Iqbal, S., 2010. Nutrition, microbiota, and endotoxin-related diseases in dairy cows (suppl. especial). Rev. Bras. De. Zootec. 39, 433–444. https://doi.org/10.1590/S1516-35982010001300048.

Aschenbach, J.R., Penner, G.B., Stumpff, F., Gäbel, G., 2011. Ruminant nutrition symposium: role of fermentation acid absorption in the regulation of ruminal ph. J. Anim. Sci. 89 (4), 1092–1107. https://doi.org/10.2527/jas.2010-3301.

Beauchemin, K.A., 2018. Invited review: current perspectives on eating and rumination activity in dairy cows. J. Dairy Sci. 101 (6), 4762–4784. https://doi.org/10.3168/jds.2017-13706.

Beauchemin, K.A., Yang, W.Z., 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. J. Dairy Sci. 88, 2117–2129. https://doi.org/10.3168/jds.S0022-0302(05)72888-5.

Beauchemin, K.A., Eriksen, L., Nørgaard, P., Rode, L.M., 2008. Short communication: salivary secretion during meals in lactating dairy cattle. J. Dairy Sci. 91 (5), 2077–2081. https://doi.org/10.3168/jds.2007-0726.

van Beukelen, P., Wensing, T., Breukink, H.J., 1985. Some experiences with the feeding of chopped roughage to high producing dairy cows. Effects on chewing time, ruminal fermentation, milk fat production and on blood glucose and insulin levels. Z. F. üR. Tierphysiol. Tiere ährung und Futterm. 53 (1-5), 19–34. https://doi.org/10.1111/j.1439-0396.1985.tb00003.x.

Bionaz, M., Loor, J.J., 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genom. 9, 366. https://doi.org/10.1186/1471-2164-9-366.

Cao, Y., Wang, D., Wang, L., Wei, X., Li, X., Cai, C., Lei, X., Yao, J., 2021. Physically effective neutral detergent fiber improves chewing activity, rumen fermentation, plasma metabolites, and milk production in lactating dairy cows fed a high-concentrate diet. J. Dairy Sci. 104 (5), 5631–5642. https://doi.org/10.3168/jds.2020-19012.

Castillo-Lopez, E., Petri, R.M., Ricci, S., Rivera-Chacon, R., Sener-Aydemir, A., Sharma, S., Reisinger, N., Zebeli, Q., 2021a. Dynamic changes in salivation, salivary composition, and rumen fermentation associated with duration of high-grain feeding in cows. J. Dairy Sci. 104 (4), 4875–4892. https://doi.org/10.3168/jds.2020-19142.

Castillo-Lopez, E., Rivera-Chacon, R., Ricci, S., Petri, R.M., Reisinger, N., Zebeli, Q., 2021b. Short-term screening of multiple phytogenic compounds for their potential to modulate chewing behavior, ruminal fermentation profile, and ph in cattle fed grain-rich diets. J. Dairy Sci. 104 (4), 4271–4289. https://doi.org/10.3168/jds.2020-19521.

Castillo-Lopez, E., Pacífico, C., Sener-Aydemir, A., Hummel, K., Nöbauer, K., Ricci, S., Rivera-Chacon, R., Reisinger, N., Razzazi-Fazeli, E., Zebeli, Q., Kreuzer-Redmer, S., 2023a. Diet and phytogenic supplementation substantially modulate the salivary proteome in dairy cows. J. Proteom. 273, 104795. https://doi.org/10.1016/j.jprot.2022.104795.

Castillo-Lopez, E., Rivera-Chacon, R., Ricci, S., Khorrami, B., Haselmann, A., Reisinger, N., Zebeli, Q., 2023b. Dynamics of chewing and eating behavior, lying behavior, and salivary characteristics associated with duration of high grain feeding in cows with or with no phytogenic supplement. Appl. Anim. Behav. Sci. 261, 105877. https://doi.org/10.1016/j.applanim.2023.105877.

Castillo-Lopez, E., Hartinger, T., Farghaly, M.M., Reisinger, N., Lang, C., Klambauer, L., Huber, J., Zebeli, Q., 2025. Differences in severity of reticulo-rumen ph drop in primiparous Holstein cows fed the same diet during transition and early lactation: effects on performance, energy balance, blood metabolites, and reproduction. J. Anim. Sci. 103. https://doi.org/10.1093/jas/skae390.

Chapinal, N., Veira, D.M., Weary, D.M., von Keyserlingk, M.A.G., 2007. Technical note: validation of a system for monitoring individual feeding and drinking behavior and intake in group-housed cattle. J. Dairy Sci. 90 (12), 5732–5736. https://doi.org/10.3168/jds.2007-0331.

Coon, R.E., Duffield, T.F., DeVries, T.J., 2019. Short communication: risk of subacute ruminal acidosis affects the feed sorting behavior and milk production of early lactation cows. J. Dairy Sci. 102 (1), 652–659. https://doi.org/10.3168/jds.2018-15064.

DeVries, T.J., von Keyserlingk, M.A.G., Weary, D.M., Beauchemin, K.A., 2003. Measuring the feeding behavior of lactating dairy cows in early to peak lactation. J. Dairy Sci. 86, 3354–3361. https://doi.org/10.3168/jds.S0022-0302(03)73938-1.

Dewanckele, L., Jing, L., Stefańska, B., Vlaeminck, B., Jeyanathan, J., van Straalen, W. M., Koopmans, A., Fievez, V., 2019. Distinct blood and milk 18-carbon fatty acid proportions and buccal bacterial populations in dairy cows differing in reticulorumen ph response to dietary supplementation of rapidly fermentable carbohydrates. J. Dairy Sci. 102 (5), 4025–4040. https://doi.org/10.3168/jds.2018-15823.

Dijkstra, J., Ellis, J.L., Kebreab, E., Strathe, A.B., López, S., France, J., Bannink, A., 2012. Ruminal ph regulation and nutritional consequences of low ph. Anim. Feed Sci. Technol. 172 (1-2), 22–33. https://doi.org/10.1016/j.anifeedsci.2011.12.005.

Enemark, J.M.D., 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): a review. Vet. J. (Lond. Engl. 1997) 176 (1), 32–43. https://doi.org/10.1016/j.tvjl.2007.12.021.

Gao, X., Oba, M., 2014. Relationship of severity of subacute ruminal acidosis to rumen fermentation, chewing activities, sorting behavior, and milk production in lactating dairy cows fed a high-grain diet. J. Dairy Sci. 97 (5), 3006–3016. https://doi.org/10.3168/jds.2013-7472.

Garrett, E.F., Pereira, M.N., Nordlund, K.V., Armentano, L.E., Goodger, W.J., Oetzel, G.R., 1999. Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. J. Dairy Sci. 82, 1170–1178. https://doi.org/10.3168/jds.S0022-0302(99)75340-3.

GfE, 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere Nr., 8. DLG Verlag, Frankfurt (Main).

Gregorini, P., DelaRue, B., McLeod, K., Clark, C., Glassey, C.B., Jago, J., 2012. Rumination behavior of grazing dairy cows in response to restricted time at pasture. Livest. Sci. 146 (1), 95–98. https://doi.org/10.1016/j.livsci.2012.02.020.

Hall, M.B., 2023. Invited review: corrected milk: reconsideration of common equations and milk energy estimates. J. Dairy Sci. 106 (4), 2230–2246. https://doi.org/10.3168/jds.2022-22219.

Hartinger, T., Castillo-Lopez, E., Reisinger, N., Zebeli, Q., 2024. Elucidating the factors and consequences of the severity of rumen acidosis in first-lactation Holstein cows during transition and early lactation. J. Anim. Sci. 102. https://doi.org/10.1093/jas/skae041.

Haselmann, A., Zehetgruber, K., Fuerst-Waltl, B., Zollitsch, W., Knaus, W., Zebeli, Q., 2019. Feeding forages with reduced particle size in a total mixed ration improves feed intake, total-tract digestibility, and performance of organic dairy cows. J. Dairy Sci. 102 (10), 8839–8849. https://doi.org/10.3168/jds.2018-16191.

Khorrami, B., Khiaosa-Ard, R., Zebeli, Q., 2021. Models to predict the risk of subacute ruminal acidosis in dairy cows based on dietary and cow factors: a meta-analysis. J. Dairy Sci. 104 (7), 7761–7780. https://doi.org/10.3168/jds.2020-19890.

Kleen, J.L., Hooijer, G.A., Rehage, J., Noordhuizen, J.P.T.M., 2003. Subacute ruminal acidosis (SARA): a review. J. Vet. Med. A Physiol. Pathol. Clin. Med. 50, 406–414. https://doi.org/10.1046/j.1439-0442.2003.00569.x.

Klevenhusen, F., Pourazad, P., Wetzels, S.U., Qumar, M., Khol-Parisini, A., Zebeli, Q., 2014. Technican note: evaluation of a real time wireless ph measurement system relative to intraruminal differences of digesta in dairy cattle. J. Anim. Sci. 2014, 5635–5639. https://doi.org/10.2527/jas2014-8038.

Kofler, J., Hoefler, M., Hartinger, T., Castillo-Lopez, E., Huber, J., Tichy, A., Reisinger, N., Zebeli, Q., 2023. Effects of high Concentrate-Induced subacute ruminal acidosis severity on claw health in First-Lactation Holstein cows. Anim. Open Access J. MDPI 13 (8). https://doi.org/10.3390/ani13081418.

Kononoff, P.J., Heinrichs, A.J., Buckmaster, D.R., 2003. Modification of the penn state forage and total mixed ration particle separator and the effects of moisture content on its measurements. J. Dairy Sci. 86, 1858–1863. https://doi.org/10.3168/jds.S0022-0302(03)73773-4.

Krause, K.M., Oetzel, G.R., 2005. Inducing subacute ruminal acidosis in lactating dairy cows. J. Dairy Sci. 88, 3633–3639. https://doi.org/10.3168/jds.S0022-0302(03)73773-4.

Krause, K.M., Oetzel, G.R., 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: a review. Anim. Feed Sci. Technol. 126 (3-4), 215–236. https://doi.org/10.1016/j.anifeedsci.2005.08.004.

Kröger, I., Humer, E., Neubauer, V., Kraft, N., Ertl, P., Zebeli, Q., 2016. Validation of a noseband sensor system for monitoring ruminating activity in cows under different feeding regimens. Livest. Sci. 193, 118–122. https://doi.org/10.1016/j.livsci.2016.10.007.

Ledgerwood, D.N., Winckler, C., Tucker, C.B., 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. J. Dairy Sci. 93 (11), 5129–5139. https://doi.org/10.3168/jds.2009-2945.

Leonardi, C., Armentano, L.E., 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86, 557–564. https://doi.org/10.3168/jds.S0022-0302(03)73634-0.

Liepa, G.U., Beitz, D.C., Linder, J.R., 1978. Cholesterol synthesis in ruminating and nonruminating goats. J. Nutr. 108 (3), 535–543. https://doi.org/10.1093/jn/108.3.535.

Lock, A.L., Rovai, M., Gipson, T.A., de Veth, M.J., Bauman, D.E., 2008. A conjugated linoleic acid supplement containing trans-10, cis-12 conjugated linoleic acid reduces milk fat synthesis in lactating goats. J. Dairy Sci. 91 (9), 3291–3299. https://doi.org/10.3168/jds.2008-1071.

Macmillan, K., Gao, X., Oba, M., 2017. Increased feeding frequency increased milk fat yield and May reduce the severity of subacute ruminal acidosis in higher-risk cows. J. Dairy Sci. 100 (2), 1045–1054. https://doi.org/10.3168/jds.2016-11337.

Maltz, E., Barbosa, L.F., Bueno, P., Scagion, L., Kaniyamattam, K., Greco, L.F., de Vries, A., Santos, J.E.P., 2013. Effect of feeding according to energy balance on performance, nutrient excretion, and feeding behavior of early lactation dairy cows. J. Dairy Sci. 96 (8), 5249–5266. https://doi.org/10.3168/jds.2013-6549.

Nasrollahi, S.M., Zali, A., Ghorbani, G.R., Moradi Shahrbabak, M., Heydari Soltan Abadi, M., 2017. Variability in susceptibility to acidosis among high producing mid-lactation dairy cows is associated with rumen ph, fermentation, feed intake, sorting activity, and milk fat percentage. Anim. Feed Sci. Technol. 228, 72–82. https://doi.org/10.1016/j.anifeedsci.2017.03.007.

Neubauer, V., Humer, E., Kröger, I., Braid, T., Wagner, M., Zebeli, Q., 2018. Differences between ph of indwelling sensors and the ph of fluid and solid phase in the rumen of dairy cows fed varying concentrate levels. J. Anim. Physiol. Anim. Nutr. 102 (1), 343–349. https://doi.org/10.1111/jpn.12675.

Nørgaard, P., Nadeau, E., Randby, Å.T., 2010. A new nordic structure evaluation system for diets fed to dairy cows: a meta analysis. In Modelling nutrient digestion and utilisation in farm animals. Wageningen Academic, pp. 112–120.

Plaizier, J.C., Krause, D.O., Gozho, G.N., McBride, B.W., 2008. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. Vet. J. (Lond. Engl. 1997) 176 (1), 21–31. https://doi.org/10.1016/j.tvjl.2007.12.016.

Plaizier, J.C., Khafipour, E., Li, S., Gozho, G.N., Krause, D.O., 2012. Subacute ruminal acidosis (SARA), endotoxins and health consequences. Anim. Feed Sci. Technol. 172 (1-2), 9–21. https://doi.org/10.1016/j.anifeedsci.2011.12.004.

Plaizier, J.C., Mulligan, F.J., Neville, E.W., Guan, L.L., Steele, M.A., Penner, G.B., 2022. Invited review: effect of subacute ruminal acidosis on gut health of dairy cows. J. Dairy Sci. 105 (9), 7141–7160. https://doi.org/10.3168/jds.2022-21960.

Reynolds, C.K., 2006. Production and metabolic effects of site of starch digestion in dairy cattle. Anim. Feed Sci. Technol. 130 (1-2), 78–94. https://doi.org/10.1016/j.anifeedsci.2006.01.019.

Rivera-Chacon, R., Ricci, S., Petri, R.M., Haselmann, A., Reisinger, N., Zebeli, Q., Castillo-Lopez, E., 2022b. Effect of duration of High-Grain feeding on chewing, feeding behavior, and salivary composition in cows with or without a phytogenic feed supplement. Anim. Open Access J. MDPI 12 (15). https://doi.org/10.3390/ani12152001.

Rivera-Chacon, R., Castillo-Lopez, E., Ricci, S., Petri, R.M., Reisinger, N., Zebeli, Q., 2022a. Supplementing a phytogenic feed additive modulates the risk of subacute rumen acidosis, rumen fermentation and systemic inflammation in cattle fed acidogenic diets. Anim. Open Access J. MDPI 12 (9). https://doi.org/10.3390/ani12091201.

Soeters, P.B., Wolfe, R.R., Shenkin, A., 2019. Hypoalbuminemia: pathogenesis and clinical significance. Jpen. J. Parenter. Enter. Nutr. 43 (2), 181–193. https://doi.org/10.1002/jpen.1451.

Souza, J.G., Ribeiro, C.V.D.M., Harvatine, K.J., 2022. Meta-analysis of rumination behavior and its relationship with milk and milk fat production, rumen ph, and total-tract digestibility in lactating dairy cows. J. Dairy Sci. 105 (1), 188–200. https://doi.org/10.3168/jds.2021-20535.

Stauder, A., Humer, E., Neubauer, V., Reisinger, N., Kaltenegger, A., Zebeli, Q., 2020. Distinct responses in feed sorting, chewing behavior, and ruminal acidosis risk between primiparous and multiparous simmental cows fed diets differing in forage and starch levels. J. Dairy Sci. 103 (9), 8467–8481. https://doi.org/10.3168/jds.2019-17760.

Tolkamp, B.J., Allcroft, D.J., Austin, E.J., Nielsen, B.L., Kyriazakis, I., 1998. Satiety splits feeding behaviour into bouts. J. Theor. Biol. 194, 235–250. https://doi.org/10.1006/jtbi.1998.0759.

Toral, P.G., Chilliard, Y., Rouel, J., Leskinen, H., Shingfield, K.J., Bernard, L., 2015. Comparison of the nutritional regulation of milk fat secretion and composition in cows and goats. J. Dairy Sci. 98 (10), 7277–7297. https://doi.org/10.3168/jds.2015-9649.

Tucker, C.B., Jensen, M.B., de Passillé, A.M., Hänninen, L., Rushen, J., 2019. Invited review: lying time and the welfare of dairy cows. J. Dairy Sci. 104 (1), 20–46. https://doi.org/10.3168/jds.2019-18074.

VDLUFA, 2. 2012. VDLUFA-Methodenbuch Bd. III Die chemische Untersuchung von Futtermitteln. 3. Aufl. Handbuch der landwirtschaftlichen Versuchs- und Untersuchngsmethodik (VDLUFA-Methodenbuch)/Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalen. Hrsg. von Rolf Bassler Band III. VDLUFA-Verlag, Dermstadt.

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt Jr, H.F., Lesch, T.N., 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65, 495–501. https://doi.org/10.3168/jds.S0022-0302(82)82223-6.

Zebeli, Q., Dijkstra, J., Tafaj, M., Steingass, H., Ametaj, B.N., Drochner, W., 2008. Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal ph and milk fat production to composition of the diet. J. Dairy Sci. 91 (5), 2046–2066. https://doi.org/10.3168/jds.2007-0572.

Zebeli, Q., Aschenbach, J.R., Tafaj, M., Boguhn, J., Ametaj, B.N., Drochner, W., 2012. Invited review: role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. J. Dairy Sci. 95 (3), 1041–1056. https://doi.org/10.3168/jds.2011-4421.

Zhao, C., Liu, G., Li, X., Guan, Y., Wang, Y., Yuan, X., Sun, G., Wang, Z., 2018. Inflammatory mechanism of rumenitis in dairy cows with subacute ruminal acidosis. BMC Vet. Res. 14 (1), 135. https://doi.org/10.1186/s12917-018-1463-7.