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

A. Stauder,¹ E. Humer,¹ V. Neubauer,^{1,2,3} N. Reisinger,⁴ A. Kaltenecker,¹ and Q. Zebeli^{1*}

¹Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

²Institute for Food Safety, Food Technology and Veterinary Public Health, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

³FFoQSI GmbH – Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, Technopark 1C, 3430 Tulln, Austria

⁴Biomim Research Center, Biomim Holding GmbH, Technopark 1, 3430 Tulln, Austria

ABSTRACT

During early lactation, both primiparous (PP) and multiparous (MP) cows are commonly fed diets rich in starch and low in forages to support their high energy requirements. Yet, the PP cows experience this dietary challenge for the first time, which might result in higher odds for them to develop rumen and systemic health disorders. The primary objective of this study was to evaluate the effect of decreasing the amount of forages in the diet on chewing and sorting behaviors and rumen and systemic health variables in PP and MP dairy cows. Twenty-four lactating Simmental cows [8 PP, average dry matter intake (DMI) of 19.1 ± 1.1 kg/d; 16 MP, average DMI of 22.5 ± 1.1 kg/d] with a body weight of 737 ± 90 kg and 50 ± 22 days in milk were used in this study. Cows were first fed a total mixed ration with 60% forage and 40% concentrate [on a dry matter (DM) basis] considered marginal in forages for 2 wk. Then, cows were switched to a diet low in forages with 40% forage and 60% concentrate (on a DM basis) for 4 wk. Reticular pH was measured continuously with wireless pH-sensors inserted into the reticulum to calculate the subacute ruminal acidosis (SARA) index. Chewing activity was measured with noseband-sensor halters, and feed sorting was measured weekly. Blood samples were collected weekly and analyzed for metabolic and inflammation markers. Switching PP and MP cows from a marginal to low-forage diet decreased the time spent eating and ruminating per kilogram of DM. Primiparous cows chewed longer per kilogram of DMI than MP cows. Also, the PP cows sorted more

pronounced for longer particles and against fine particles than MP cows did. Despite higher rumination activity per kilogram of DMI and the adaptive sorting behavior, the PP cows spent on average 4.6 h/d longer below a pH of 5.8 and had a higher SARA index (i.e., area pH <5.8/DMI) than MP cows, especially during the first week of the low-forage diet (9.5 vs. 4.8). The concentration of liver enzymes increased with the low-forage diet, which was especially pronounced in the PP cows. In conclusion, this study demonstrated greater susceptibility of PP cows to SARA and liver damage than MP cows fed the same diets. Although PP cows demonstrated greater chewing and ruminating activity per kilogram of DMI, as well as adapted sorting behavior in favor of large particles during the low-forage high-starch feeding, they developed more severe signs of SARA. This suggests higher forage fiber requirements for PP cows and the need for improved feeding strategies to mitigate rumen fermentation disorders during early lactation in these cows.

Key words: subacute ruminal acidosis, physically effective fiber, sorting, parity, feeding behavior

INTRODUCTION

In early lactation, dairy cows have increased demands for dietary energy and nutrients to support milk production and counteract body tissue losses. This increased demand for energy is commonly achieved by increasing the level of starchy concentrates in diets fed at that time. Indeed, starchy concentrates are energy-dense, palatable, and their inclusion in the diet maximizes the energy supply of early-lactation cows. However, feeding large amounts of concentrates occurs at the expense of forages, which are the main dietary source of physically effective fiber (peNDF). Cows require peNDF in the diet for rumination, salivary secretion, and rumen

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*Corresponding author: gendrim.zebeli@vetmeduni.ac.at

health (Beauchemin and Yang, 2005; Zebeli et al., 2012; Humer et al., 2018). Furthermore, the starch-rich lactation diet, being rapidly fermentable, generates large amounts of VFA in the rumen. Increased accumulation of VFA, decreased salivation, and lack of adaptation of the rumen epithelia and the microbiome increase the risk of an intermittent drop of the ruminal pH, termed as SARA in dairy cows (Aschenbach et al., 2011). Dietary factors, including the contents of peNDF and starch in the diet and DMI level, are known as risk factors for SARA (Zebeli et al., 2012; Humer et al., 2018). In addition, most cows sort for fine particles, which are richer in starch and other nutrients and taste better, but are easily fermentable, thus further enhancing the risk of SARA (Miller-Cushon and DeVries, 2017). Sub-acute ruminal acidosis is associated with decreased feed intake and milk production, diarrhea, rumen epithelial damage, inflammation, liver abscesses, and increased odds for dairy cows to develop other metabolic diseases (Plaizier et al., 2008).

Studies show that primiparous cows (**PP**), especially those in early lactation, are at a greater risk of SARA (Krause and Oetzel, 2005). Potential reasons for this may be the increased sorting for fine particles (Leonardi et al., 2005; DeVries et al., 2011) and an unprepared rumen for concentrate-rich feed (Humer et al., 2018). Nevertheless, many dairy farms feed early-lactation heifers the same diet as early-lactation multiparous (**MP**) cows (Contreras-Govea et al., 2015). When PP cows are kept separated from older cows they have increased eating times, more meals per day, increased DMI, and longer lying times (Grant and Albright, 2001). In mixed groups, however, the PP cows have to compete with MP cows, which are usually higher in hierarchy at the feeding bunk, and thus PP have difficulty getting access to small, more frequent meals throughout the day (Grant and Albright, 2001; Krause and Oetzel, 2006).

With replacement rates for heifers exceeding 40% in several dairy herds (Overton and Dhuyvetter, 2020), a better understanding of their eating and sorting behaviors, and predictions of their responses to dietary change (especially during early lactation), is of primordial importance to better shape healthier feeding strategies. There are only a few studies evaluating feeding, sorting, and ruminating behavior, as well as rumen and systemic health disorders in PP cows, that focus on diets differing in forage and starch levels. We hypothesized that decreasing the dietary forage level and increasing starch content during the time around the peak of lactation affects eating and sorting behaviors of PP cows more negatively compared with MP cows, and would reflect in impaired ruminal pH, metabolic

health, and liver health variables. The main objective of this study was therefore to evaluate sorting and chewing behaviors, changes in ruminal acidosis index, and systemic health variables in PP and MP cows fed different concentrations of forages and starch.

MATERIALS AND METHODS

All procedures involving animal handling and treatment were approved by the institutional ethics committee of the University of Veterinary Medicine (Vetmeduni) Vienna and the national authority according to §26 of the Law for Animal Experiments, Tierversuchsgesetz 2012-TVG (GZ: 68.205/0023-V/3b/2018).

Housing, Animals, and Experimental Design

The experiment was carried out at the research dairy farm of Vetmeduni Vienna (Pottenstein, Austria). Twenty-four lactating Simmental cows, 8 PP and 16 MP (average lactation number = 4.1 ± 1.9 ; mean $\pm$ SD), were enrolled in this study. All cows were housed in a freestall barn equipped with 12 deep litter cubicles (2.6×1.25 m, straw litter) and a deep-bedded pack-area (10×8 m). Cows had ad libitum access to water and a salt block. At the start of the trial, the cows weighed 737 ± 90 kg and were 50 ± 22 DIM. The PP and MP cows were assigned into 3 experimental runs, with each run lasting 42 d. Before starting the trial, the cows were adapted to the experimental barn area and trained to access the individual feeding troughs for approximately 1 wk. Troughs controlled and recorded individual feed intake with computer-regulated access gates and electronic weighing scales (Insentec B.V., Marknesse, the Netherlands; validated by Chapinal et al., 2007). At the start of the experiment, cows were first fed a diet with 60% forage and 40% concentrate (on a DM basis) considered marginal in forage and lower in starch (**M** diet, Table 1) for 2 wk (**Mwk1** and **Mwk2**, d 1–14). Then cows were switched to a diet considered low in forages and higher in starch (**L** diet), containing 40% forage and 60% concentrate (on a DM basis) for 4 wk (**Lwk1–Lwk4**, d 15–42). The TMR fed during the experiment contained forage (mix of 50% grass silage and 50% corn silage, on a DM basis) and pelleted concentrate mixture (Table 1). The TMR was prepared once daily with an automatic feeding system (Trioliet Triomatic T15, Oldenzaal, the Netherlands) and offered ad libitum to ensure more than 10% feed refusals (actual orts averaged $28.1 \pm 1.36\%$ of the offered feed DM). Fresh TMR was offered daily at 0730 and 1430 h in equal proportions. Before morning feeding, troughs were emptied and cleaned thoroughly.

Table 1. Ingredient composition of the offered TMR and analyzed nutrient composition and particle size distribution of the 60% forage diet (M diet) or 40% forage diet (L diet)

Item (% of DM unless otherwise stated)	M diet	L diet
Ingredient		
Grass silage	30.0	20.0
Corn silage	30.0	20.0
Barley grain	25.2	37.8
Soybean meal	6.0	9.0
Corn	3.6	5.4
Rapeseed meal	3.2	4.8
Mineral-vitamin premix ¹	0.8	1.2
Limestone	0.48	0.72
Beet pulp	0.4	0.6
Monocalcium phosphate	0.2	0.3
Sodium chloride	0.12	0.18
Nutrient composition ² (±SD)		
DM, % of fresh matter	47.6 ± 0.6	50.2 ± 1.1
OM	93.0 ± 0.2	93.0 ± 0.3
CP	16.5 ± 0.3	18.2 ± 0.7
Ether extract	1.94 ± 0.3	1.94 ± 0.3
aNDFom	33.2 ± 0.3	29.4 ± 0.4
ADFom	20.8 ± 0.6	17.2 ± 0.2
Starch	26.3 ± 1.0	32.0 ± 0.8
Particle size distribution ³ (±SD)		
>19 mm	7.71 ± 4.12	6.83 ± 1.83
8.0–19.0 mm	44.30 ± 6.29	42.10 ± 6.05
1.18–8.0 mm	42.30 ± 5.13	45.40 ± 5.83
Pan	5.63 ± 1.20	5.70 ± 1.37
PEF ⁴ >8	0.52 ± 0.05	0.49 ± 0.06
peNDF ⁵ >8	17.3 ± 1.67	14.3 ± 1.78

¹The mineral-vitamin premix contained 13.5% Ca; 9% Mg; 5% P; 1.5% Na; 1,800,000 IU of vitamin A/kg; 300,000 IU of vitamin D/kg; 7,500 mg of vitamin E/kg; 70 mg of vitamin B₁/kg; 180 mg of vitamin B₂/kg; 145 mg of vitamin B₆/kg; 1,800 µg of vitamin B₁₂/kg; 1,800 mg of niacin/kg; 305 mg of pantothenic acid/kg; 36 mg of folate/kg; 11,800 mg of choline/kg; 13,500 mg of manganese (II) oxide/kg; 19,800 mg of zinc oxide/kg; 4,500 mg of copper (II) sulfate/kg; 450 mg of iodine (calcium iodine)/kg; 120 mg of selenic (sodium selenide)/kg; and 195 mg of cobalt (II) carbonate/kg.

²aNDFom = ash-free amylase-treated neutral detergent fiber; ADFom = ash-free acid detergent fiber.

³For particle size analysis a 3-screen Penn State Particle Separator (PSPS; model C24682N, Nasco, Fort Atkinson, WI) was used.

⁴PEF = physically effective factor

⁵peNDF = physically effective NDF.

Feed Sampling and Analysis

Dry matter analysis was done once per week by drying 100 g of TMR, collected from all feeding troughs, for 4 h in an oven at 104°C; diet composition was adjusted for changes in DM content of silages. Fresh feed samples from each animal's feeding trough were collected in Mwk1 (d 5), Mwk2 (d 12), Lwk1 (d 19), Lwk2 (d 26), Lwk3 (d 33), and Lwk4 (d 40). We performed a particle size analysis and determination of the DM performed for these samples from each individual feeding trough. A pooled sample from all feeding troughs was used for nutrient analysis. Twenty-four hours after the sampling of fresh feed, samples of the remaining feed

from each animal's trough were collected to perform particle size analysis and determine the DM and NDF.

For nutrient analysis, samples of the TMR were first dried at 65°C in a forced-air oven for 48 h. Afterward, samples were grinded through a 0.5-mm screen (Ultra Centrifugal Mill ZM 200, Retsch, Haan, Germany) before analysis. Dry matter was determined by oven-drying at 103°C for 4 h and ash was analyzed by combustion in a muffle furnace overnight at 580°C. Crude protein was analyzed using the Kjeldahl method (method 4.1.2; Neumann and Bassler, 2012) and ether extract was analyzed using the Soxhlet extraction (method 5.1.1; Neumann and Bassler, 2012) system. The NDF and ADF were determined with sodium sulfite and reported exclusive of residual ash following the official analytic methods of VDLUFA (Neumann and Bassler, 2012) using the Fiber Therm FT 12 (Gerhardt GmbH & Co. KG, Königswinter, Germany) with heat-stable α-amylase in case of NDF. Starch content was determined with a commercially available kit (Megazyme, Wicklow, Ireland).

Blood Sampling and Analyses

Blood samples were collected shortly before the morning feeding from the jugular vein in Mwk1 (d 7), Mwk2 (d 14), Lwk1 (d 21), Lwk2 (d 28), Lwk3 (d 35), and Lwk4 (d 42). To collect blood samples, we used serum evacuated tubes for serum collection (9 mL, Vacuette, Greiner Bio-One, Kremsmünster, Austria) and K3EDTA vacutainer tubes (9 mL, Vacuette, Greiner Bio-One) and sodium fluoride vacutainer tubes (6 mL, Vacuette, Greiner Bio-One) for plasma collection. Immediately after collection, plasma tubes were stored in the fridge at a temperature of 4°C for 2 h and serum tubes were allowed to clot for 2 h at room temperature. All tubes were then centrifuged at 2,000 × *g* at 4°C for 15 min (Centrifuge 5804 R, Eppendorf, Hamburg, Germany), serum and plasma pipetted into 2-mL tubes (Eppendorf), and stored at −20°C and −80°C (depending on target parameter) for further analysis.

The concentrations of aspartate aminotransferase (AST), alkaline phosphatase, gamma-glutamyl-transferase (GGT), glutamate dehydrogenase (GLDH), BHB, nonesterified fatty acids (NEFA), bilirubin, triglycerides, albumin, cholesterol, calcium (Ca), inorganic phosphorus (iP), and magnesium (Mg) were measured in serum samples and glucose in plasma samples (sodium fluoride) using standard enzymatic colorimetric assays. The analyses were performed on a fully automated analyzer for clinical chemistry (Cobas 6000/c501; Roche Diagnostics GmbH, Vienna, Austria). Cortisol concentration (serum sample) was measured with a DEH3388 Cortisol ELISA, 96 well kit

(Demeditec Cortisol ELISA kit, Demeditec Diagnostics GmbH, Kiel, Germany). The intraassay variation for all blood chemistry assays was $\leq 5\%$. Haptoglobin and serum amyloid A concentration in serum samples were analyzed using a commercially available cow ELISA kit (Life Diagnostics Inc., West Chester, PA) and a multispecies ELISA kit (Tridelta, Maynooth, County Kildare, Ireland), respectively. The ELISA was performed according to the manufacturer's instructions. Samples were analyzed in duplicate.

Milk Sampling and Analyses

Cows were milked twice a day, between 0700 and 0740 h and between 1740 and 1820 h in a tandem milking parlor and milk yield was recorded automatically (Alpro, DeLaval Inc., Kansas City, MO). For milk component analysis, milk samples were collected in Mwk1 (d 7), Mwk2 (d 14), Lwk1 (d 21), Lwk2 (d 28), Lwk3 (d 35), and Lwk4 (d 42). On each day, morning and afternoon samples were pooled before analysis. Milk components, including fat, protein, lactose, SCC, milk urea nitrogen, pH, fat:protein, and solids not fat, were determined using a CombiFoss FT (Foss Electric, Hillerød, Denmark). Energy-corrected milk was calculated as: $(0.38 \times \text{milk fat } \% + 0.21 \times \text{milk protein } \% + 0.95) \times \text{kg of milk}/3.2$ (GfE, 2001).

Reticulum pH

To monitor reticular pH continuously over several days, all cows received an individual wireless pH-transmitting unit (smaXtec, Graz, Austria). These sensors were previously validated by Klevenhusen et al. (2014). These wireless pH-transmitting units (3.5 cm i.d., 12 cm long, 210 g) were inserted into the reticulum via the esophagus using a balling gun on d 6. Before insertion, the units were calibrated by a 1-point calibration using a pH 7.0 buffer, following the company's instruction protocol. The pH was measured continuously every 10 min. Mean and minimum pH values, as well as the duration (min) and area below a threshold of 5.8, were calculated per cow and day. Moreover, the SARA index was calculated as the area under pH 5.8 $[(5.8 - \text{pH}) \times \text{min}]$, normalized by DMI.

Measurement of Chewing Activity

Chewing activity was measured with rumination halters (RumiWatch System, ITIN + Hoch GmbH, Liestal, Switzerland). Measurements were conducted on each cow from 0000 to 2359 h during 3 consecutive days of Mwk1 (d 4–6), Mwk2 (d 11–13), Lwk2 (d 25–27), and Lwk4 (d 39–41) to enable repeated observations

for each animal. The functioning, data recording, and validation of the nose-band sensors halters have been reported in detail by Kröger et al. (2016). Chewing data included diurnal ruminating and eating patterns, the duration of eating and ruminating, as well as the number of ruminating boli (n/d) and chews per bolus (n/bolus). Chewing data and feed intake data of the same day were used for calculation of the chewing indices (min per kg of DMI, NDF, and peNDF intake) for each chewing category.

Particle Size Analysis, Sorting Index, and peNDF

For particle size analysis, a 3-screen Penn State Particle Separator was used (model C24682N, Nasco, Fort Atkinson, WI), which separated the samples into 4 fractions including long (>19 mm), medium (8–19 mm), short (1.18–8 mm), and fine (<1.18 mm) particles.

The sorting index of each Penn State Particle Separator fraction was calculated according to Leonardi and Armentano (2003) by dividing the actual amount of feed consumed of each fraction by the predicted amount of feed consumed of that fraction, expressed as a percentage. The actual amount of feed for every fraction was calculated by subtracting the DM refused from the DM offered. The predicted amount of feed consumed was calculated by multiplying the DMI of the total diet by every fraction in the feed TMR. If the sorting index equaled 100, no sorting of the particle fraction occurred; if the sorting index was <100 , the animal sorted against this fraction; a sorting index >100 indicated that the animal sorted in favor for this fraction. The physical effectiveness factors (PEF; i.e., the cumulative proportion of feed DM retained on sieves of the Penn State Particle Separator) was nominated as PEF >8 mm. The peNDF of the 2 sieves with particles >8 mm (peNDF >8 mm) was calculated by multiplying the fraction of PEF >8 with the NDF content (GfE, 2001).

Statistical Analyses

Statistical analyses were performed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). For each variable tested, the model included the fixed effects of parity (PP, MP) and phase (Mwk1–2, Lwk1–4) as well the interaction between parity and phase, whereas DIM was used as a covariate. For diurnal mean pH, eating, and chewing data, the hour and interactions between hour and feeding phase, as well as interactions between hour and parity, and 3-way interactions between hour, feeding phase, and parity were considered. The individual cows and the experimental run were considered as random effects. The data obtained on the same cow but at different times (i.e., day

and hour) were considered as double-repeated repeated measurements. The covariance structure of the repeated measurements for each variable was modeled separately according to the lowest values of fit statistics based on the Bayesian information criteria. The Kronecker product of a completely unrestricted variance-covariance matrix and a first-order autoregressive variance-covariance matrix was used to account for double repeated measures taken on individual cows across times. The degrees of freedom were estimated with the method of Kenward-Roger. A $\log_{10}$ transformation was used for milk SCC for better homogeneity of the distribution of residuals. Means shown in the table for SCC were then back-transformed for better understanding. The significant level was set at $P \leq 0.05$ and a trend was set as $0.05 < P \leq 0.10$.

RESULTS

Feed Intake and Reticular pH

The DMI increased during Lw2 ($P < 0.01$), whereby the PP cows consumed an average of 3.4 kg of DM/d less compared with MP cows ($P < 0.01$) during the whole trial.

The diurnal pH profile was affected by the time of the day and the parity ($P < 0.01$), whereas no 3-way interaction between time of the day, feeding phase, and parity was observed. Figure 1a illustrates the diurnal mean pH among feeding phases, showing the highest pH before the morning feeding and a concomitant drop thereafter, reaching a nadir at about 1000 h. Figure 1b shows the diurnal pH profile difference between PP and MP cows. Until 0800 h, reticular pH increased in both groups, but MP showed overall higher pH values. After the morning feeding, reticular pH decreased until 1100 in both groups, and then increased until the afternoon feeding, but was more pronounced in MP cows.

The time duration below pH 5.8 was longest in Lwk1 ($P < 0.01$). Figure 1c shows the duration of pH below 5.8 for PP and MP cows. There was an overall trend for differences according to parity, with PP cows spending more time under pH 5.8 than MP (708 min in PP and 431 min in MP cows; $P < 0.10$). Further, PP cows had a higher SARA index, especially during Lwk1 (9.5 vs. 4.8 pH $\times$ min/kg; data not shown). The largest area of pH below 5.8 for all cows was found in Lwk1 ($P = 0.02$), which correlates with the highest SARA index in Lwk1 ($P = 0.01$; Table 2).

Sorting

An overall effect of parity on the sorting behavior was detected ($P < 0.01$; Table 3). More specifically, PP

cows consumed more long- (115 vs. 109%; $P = 0.05$) and medium-size (112 vs. 109%; $P = 0.02$) particles than MP cows. On the other hand, these cows consumed fewer fine (33 vs. 59%; $P < 0.01$) and short particles (94 vs. 96%; $P = 0.06$) than MP cows. During L-feeding, all cows showed higher amounts of leftovers in the individual feeders, but PP cows had less leftovers (26.5 vs. 29.8%) than MP cows (Table 3).

Chewing Behavior

The total time spent eating was affected neither by parity nor by parity $\times$ feeding (Table 4). A more detailed analysis of the diurnal eating behavior revealed an effect of the time of the day ($P < 0.01$), the interaction between time and day ($P < 0.01$; Figure 2a), and time and parity ($P < 0.01$; Figure 2b). Peak of eating occurred at 0800 h, which was more pronounced in the M-feeding phases, but did not differ among parities. Further peaks were observed at 1800 (after the evening milking) and 0500 h. Only PP cows had a fourth peak of eating in the afternoon at 1400 h (when fresh feed was provided in the afternoon).

All eating indices revealed higher values for PP cows compared with MP cows ($P \leq 0.02$), as well as interactive effects between phase and parity ($P \leq 0.02$). Though PP cows decreased the eating time per kilogram of DM and NDF during L-feeding phases, no such effect was seen in MP cows (Table 4).

Similar to the daily eating pattern, the daily ruminating pattern was affected by the time of the day ($P < 0.01$; Figure 2c), interaction between time and day ($P < 0.01$; Figure 2a), as well as time and parity ($P < 0.01$; Figure 2b). Primiparous cows ruminated on average 21 min/h, whereas the MP cows ruminated 19 min/h during the whole day, only interrupted by eating activity (Figure 2d).

Differences among parities revealed higher ruminating indices in PP cows ($P < 0.01$). Although the number of ruminating chews per bolus did not differ among feeding phases, PP cows chewed more frequently per bolus compared with MP cows ($P = 0.02$). A trend toward a generally lower number of ruminated boli in L ($P = 0.07$) was observed, but this variable was not affected by parity. Similar to the aforementioned parity effects, all total chewing indices were higher in PP cows compared with MP cows ($P < 0.01$).

Milk Components

Both PP and MP cows yielded more milk during the L diet ($P < 0.01$; Table 5). The PP cows yielded less milk and ECM compared with MP cows ($P < 0.01$) over the whole experiment. Protein yield, lactose yield, and

Figure 1. Diurnal pH dynamics during the experimental weeks for all cows (a); diurnal pH averaged over all days of the experiment for primiparous (PP) and multiparous (MP) cows (b); duration of reticular pH <5.8 per experimental day in PP and MP cows (c). Days in (c) with * indicate significant differences among parities ($P \leq 0.05$); days with † indicate a difference by trend among parities ($0.05 < P \leq 0.10$). Mwk1 and Mwk2 = marginal forage diets wk 1 and 2 (d 1–14); Lwk1–Lwk4 = low-forage diets wk 1–4 (d 15–42). Error bars = SEM.

Table 2. Dry matter intake and reticular pH in primiparous (PP) and multiparous (MP) cows fed 60% forage diet for 2 wk (Mwk 1–2) or 40% forage diet for 4 wk (Lwk 1–4)

Item	Parity			Feeding phase					P-value		
	PP	MP		Mwk1	Mwk2	Lwk1	Lwk2	Lwk3	Lwk4	SEM	Phase × parity
DMI, kg/d	19.1	22.5		19.6 ^b	19.7 ^b	19.9 ^b	21.9 ^a	21.6 ^a	22.1 ^a	1.06	<0.01
Time pH < 5.8, min/d	708	431		512 ^b	594 ^{ab}	673 ^a	536 ^b	546 ^b	554 ^b	137.6	<0.01
Mean pH	5.84	5.97		5.90 ^{bc}	5.88 ^{bc}	5.85 ^c	5.94 ^a	5.91 ^{ab}	5.92 ^{ab}	0.073	<0.01
Minimum pH	5.41	5.51		5.20 ^b	5.52 ^a	5.49 ^a	5.56 ^a	5.51 ^a	5.49 ^a	0.074	<0.01
Area under pH 5.8 ¹	141	81.3		108 ^{ab}	105 ^b	137 ^a	96.8 ^b	99.2 ^b	121 ^{ab}	36.78	0.02
SARA index	7.35	3.72		5.61 ^{ab}	5.50 ^b	7.12 ^a	4.59 ^b	4.76 ^b	5.63 ^b	1.785	0.01
Diurnal variation of pH ²	0.193	0.230		0.275 ^a	0.194 ^{cd}	0.183 ^d	0.204 ^{bc}	0.199 ^c	0.216 ^b	0.0124	<0.01

^{a–d}Values with different superscripts indicate differences between feeding phases ($P \leq 0.05$).

¹pH × min/d.

²Standard deviation of measurements within a day.

Table 3. Feed particle sorting behavior in primiparous (PP) and multiparous (MP) cows fed 60% forage diet for 2 wk (Mwk 1–2) or 40% forage diet for 4 wk (Lwk 1–4)

Sorting index ¹	Parity			Feeding phase					P-value		
	PP	MP		Mwk1	Mwk2	Lwk1	Lwk2	Lwk3	Lwk4	SEM	Phase × parity
>19 mm	115.4	109.0		106.8 ^b	107.5 ^b	113.4 ^a	114.5 ^a	117.0 ^a	113.9 ^a	3.01	<0.01
8.0–19 mm	112.4	109.2		109.3	109.9	113.0	109.2	112.3	111.3	2.48	0.02
1.18–8.0 mm	93.8	96.1		96.7 ^a	93.0 ^b	93.5 ^b	96.3 ^a	94.6 ^{ab}	95.5 ^{ab}	2.39	0.10
Pan	33.1	58.8		57.7 ^a	54.2 ^a	23.7 ^b	48.0 ^a	39.5 ^b	52.6 ^a	17.3	<0.01
Feed refusals, %	26.5	29.8		25.5 ^a	26.6 ^{ab}	27.6 ^{bc}	28.7 ^c	29.8 ^c	30.9 ^c	1.36	<0.01

^{a–c}Values with different superscripts indicate differences between feeding phases ($P \leq 0.05$).

¹The sorting index was calculated as the ratio of actual intake to predicted intake for particles retained on each sieve of the separator. A sorting index above 100 indicates sorting for particles, and a sorting index below 100 indicates sorting against particles (Leonardi et al., 2005).

²The proportion of feed refusals relative to the offered feed on DM basis.

Table 4. Eating and ruminating behaviors in primiparous (PP) and multiparous (MP) cows fed 60% forage diet for 2 wk (Mwk 1–2) or 40% forage for 4 wk (Lwk 1–4)

Item	Parity		Feeding phase				P-value		
	PP	MP	Mwk1	Mwk2	Lwk2	Lwk4	SEM	Phase	Parity
Eating									
min/d	285	246	260	270	264	269	43.9	0.66	0.16
min/kg of DMI	13.9	10.7	13.1 ^a	12.9 ^a	11.5 ^{ab}	11.7 ^b	1.93	0.03	0.01
min/kg of NDF intake	44.4	34.0	39.8	39.2	38.5	39.2	6.14	0.88	0.01
min/kg of peNDF ¹ intake	85.9	67.0	80.7 ^c	73.3 ^c	78.8 ^{yz}	73.0 ^{yz}	10.30	0.19	0.02
Ruminating									
min/d	494	460	488 ^a	488 ^a	469 ^{ab}	462 ^b	19.5	0.10	0.16
min/kg of DMI	25.1	20.3	25.4 ^a	24.1 ^b	20.8 ^c	20.2 ^c	0.81	<0.01	<0.01
min/kg of NDF intake	79.9	64.6	76.7 ^a	72.8 ^b	70.7 ^b	69.0 ^b	3.67	<0.01	<0.01
min/kg of peNDF intake	158	131	158 ^a	135 ^b	151 ^a	134 ^b	8.22	0.01	<0.01
Ruminating boli, no./d	508	472	505 ^a	502 ^a	480 ^{ab}	474 ^b	22.8	0.07	0.11
Ruminating chews/bolus	63.1	57.2	59.8	60.4	60.1	60.3	2.28	0.84	0.02
Total chewing									
min/d	753	676	726	734	702	697	41.1	0.22	0.03
min/kg of DMI	37.2	28.9	37.0 ^a	35.4 ^b	30.2 ^c	29.5 ^c	1.57	<0.01	<0.01
min/kg of NDF intake	119	92.1	112 ^a	107 ^b	103 ^{bc}	100 ^c	4.99	<0.01	<0.01
min/kg of peNDF intake	235	188	232 ^a	200 ^{bc}	220 ^{ab}	195 ^c	10.2	<0.01	<0.01

^{a-c}Values with different superscripts indicate differences between feeding phases ($P \leq 0.05$).^{yz}Values with different superscripts indicate differences by trend between feeding phases ($0.05 < P \leq 0.10$).¹peNDF = physically effective NDF

milk urea nitrogen increased when cows were switched to the L diet, whereas the opposite was observed for the fat to protein ratio and milk pH ($P < 0.01$). The MP cows had higher milk protein yield (kg/d) and lactose yield (kg/d) compared with PP cows ($P < 0.01$), but a lower lactose concentration and milk pH ($P \leq 0.02$; Table 5).

Blood Variables

Serum concentration of liver enzymes increased by feeding the L diet, especially in PP cows (Figure 3). Though AST did not differ among parities (Figure 3a), interactions between parity and feeding phase for GLDH (Figure 3b), GGT (Figure 3c), and alkaline phosphatase (Figure 3d), revealed a stronger increase of these variables with the L diet in PP cows compared with MP cows. The concentration of cholesterol, cortisol, NEFA, bilirubin, triglycerides, and Ca was higher when cows received the M diet, whereas glucose, iP, and Mg concentration increased when cows were switched to L diets (Table 6). Primiparous cows had higher concentrations of glucose, cortisol, and iP ($P \leq 0.03$), and serum amyloid A tended to be lower in PP cows ($P = 0.08$) compared with MP.

DISCUSSION

The aim of the study was to determine whether the effects of decreasing amounts of forages and increasing starch content in the diet on feed intake, chewing activity, sorting behavior, risk of ruminal acidosis, and markers of liver health were different in PP vs. MP cows. As expected, the PP cows consumed less DM compared with MP cows, likely due to a smaller rumen linked to a smaller body size and weight, which consequently limits meal size and rumen fill in PP cows (Dado and Allen, 1994). In our study, total eating and ruminating time per day was not affected by parity; therefore, PP cows spent more time eating and ruminating per kilogram of DM, NDF, and peNDF. The total chewing time per day and chewing time related to intake of DM, NDF, and peNDF was higher for PP cows. Previously, a threshold of 31 min total chewing time/kg of DMI was suggested to maintain physiological pH conditions (Sudweeks et al., 1981; Tafaj et al., 2006), which was exceeded by PP cows, but not by MP cows. Similarly, Beauchemin and Rode (1994, 1997) found PP cows eat and ruminate longer per kilogram of DM and NDF intake, however Maekawa et al. (2002) reported MP cows eating (min/d and min/kg of DMI) and ruminate (min/d) longer. According to Maekawa et al. (2002), the relationship between parity and time spent eating and ruminating depends on the diet.

Figure 2. Diurnal eating activity in the feeding phase (a); average diurnal eating activity of primiparous (PP) and multiparous (MP) cows during all phases (b); diurnal ruminating activity during the feeding phases (c); average diurnal ruminating activity of PP and MP cows during all phases (d). Days with * indicate significant differences among phases and parities ($P \leq 0.05$); days with † indicate a difference by trend among phases and parities ($0.05 < P \leq 0.10$). Error bars = SEM.

Figure 2 (Continued). Diurnal eating activity in the feeding phase (a); average diurnal eating activity of primiparous (PP) and multiparous (MP) cows during all phases (b); diurnal ruminating activity during the feeding phases (c); average diurnal ruminating activity of PP and MP cows during all phases (d). Days with * indicate significant differences among phases and parities ($P \leq 0.05$); days with † indicate a difference by trend among phases and parities ($0.05 < P \leq 0.10$). Error bars = SEM.

Table 5. Lactation performance and milk variables in primiparous (PP) and multiparous (MP) cows fed 60% forage diet for 2 wk (Mwk 1–2) or 40% forage for 4 wk (Lwk 1–4)

^{a-c}Values with different superscripts indicate differences between feeding phases ($P \leq 0.05$).¹ECM calculated as $(0.38 \times \text{milk fat \%} + 0.21 \times \text{milk protein \%} + 0.95) \times \text{kg of milk}/3.2$ (GfE, 2001).

Table 6. Blood metabolites in primiparous (PP) and multiparous (MP) cows fed 60% forage diet for 2 wk (Mwk 1–2) or 40% forage for 4 wk (Lwk 1–4)

Item ¹	Parity			Feeding phase						P-value			
	PP	MP		Mwk1	Mwk2	Lwk1	Lwk2	Lwk3	Lwk4	SEM	Phase	Parity	Phase × Parity
Glucose, mg/dL	69.3 ^A	65.0 ^B		63.5 ^c	66.0 ^{bc}	69.0 ^a	67.4 ^{ab}	68.9 ^a	68.4 ^{ab}	1.09	<0.01	<0.01	0.27
Cholesterol, mg/dL	118	123		133 ^a	122 ^{ab}	117 ^b	115 ^b	116 ^b	122 ^{ab}	15.9	0.06	0.59	0.23
Cortisol, µg/dL	2.68 ^A	1.81 ^B		2.89 ^a	NA ²	1.60 ^b	NA	NA	NA	0.738	<0.01	0.03	0.94
BHB, mmol/L	0.325	0.342		0.358	0.357	0.332	0.337	0.306	0.311	0.0267	0.27	0.60	0.51
NEFA, mmol/L	0.195	0.221		0.368 ^a	0.198 ^b	0.165 ^b	0.180 ^b	0.154 ^b	0.183 ^b	0.0676	<0.01	0.50	0.44
Bilirubin, mg/dL	0.0643	0.0897		0.1003 ^a	0.0684 ^b	0.0698 ^b	0.0734 ^b	0.0760 ^b	0.0741 ^b	0.01568	0.04	0.14	0.39
Triglycerides, mg/dL	10.8	10.3		13.6 ^a	11.4 ^b	10.3 ^{bc}	9.39 ^c	9.49 ^c	9.32 ^c	0.0767	<0.01	0.54	0.41
Albumin, g/dL	4.08	4.07		4.01 ^z	4.15 ^{yz}	4.02 ^z	4.00 ^z	4.19 ^z	4.08 ^{yz}	0.147	0.24	0.96	0.58
Ca, mmol/L	2.56	2.47		2.57 ^{ab}	2.54 ^{ab}	2.44 ^c	2.48 ^{bc}	2.50 ^{abc}	2.59 ^a	0.0625	0.03	0.13	0.05
Inorganic P, mmol/L	1.93 ^A	1.66 ^B		1.46 ^c	1.55 ^c	1.98 ^{ab}	2.05 ^a	1.86 ^b	1.86 ^b	0.117	<0.01	0.02	<0.01
Mg, mmol/L	1.26	1.28		1.15 ^b	1.15 ^b	1.29 ^a	1.22 ^a	1.39 ^a	1.30 ^a	0.046	<0.01	0.68	0.36
Hp, ng/mL	601	1,075		839 ^b	650 ^b	2,110 ^a	532 ^b	747 ^b	152 ^b	1,137.2	0.12	0.72	0.15
SAA, µg/mL	31.3 ^Z	65.6 ^Y		42.4	46.9	68.6	44.2	45.9	42.6	16.36	0.69	0.08	0.80

^{A,B}Values with different superscripts indicate differences between primiparous and multiparous cows ($P \leq 0.05$). ^{a-c}Values with different superscripts indicate differences between feeding phases ($P \leq 0.05$).

^{yz}Values with different superscripts indicate differences by trend between feeding phases ($0.05 < P \leq 0.10$).

^{xyz}Values with different superscripts indicate differences by trend between feeding phases ($0.05 < P \leq 0.10$).

¹NEFA = nonesterified fatty acids; Hp = haptoglobin; SAA = serum amyloid A.

²NA = not analyzed.

When presenting high-fiber diets, cows with greater intake capacity (i.e., MP cows) seem to chew cud more efficiently, requiring less chewing time per unit of DMI (De Boever et al., 1990). This might also explain the higher chewing indices during eating in PP cows when fed the M diet in comparison to MP cows. However, it should also be noted that heifers and calves have also higher eating and chewing activity. Suarez-Mena et al. (2013) presented eating activity for over 17 min/kg of DMI and chewing activity over 76 min/kg of DMI in Holstein heifers (12.5 ± 0.5 mo old; $344 \pm$ kg of BW). Muhammad et al. (2016) showed eating activity over 20 min/kg of DMI in young Holstein calves (4 and 5 mo old; 157 ± 33 kg of BW). Overall, there was no difference in number of ruminated boli per day when comparing PP and MP cows. However, the PP cows chewed more frequently per bolus than MP cows. As observed previously, SARA-susceptible cows chewed more per bolus than tolerant cows (Khiaosa-ard et al., 2018). Therefore, the higher number of chews per bolus might be an attempt of the PP cows to alleviate their stronger ruminal pH drop by the resulting enhanced saliva production.

In our study, the amount of leftovers was higher than predicted (reaching almost 30% of the daily DM feed offered), and more predominant during L-feeding and by MP cows. The increased leftovers during L-feeding and MP cows can be explained with an increasing DMI level of the cows during the L-feeding and the subsequent greater day-to-day feed supply to cows. Switching the cows from the M diet to the L diet affected their sorting behavior: cows displayed increased sorting for longer particles (the sorting index increased from approximately 107 to 117%). A few studies recorded that cows sort for long particles to meet peNDF requirements (Beauchemin and Yang, 2005; DeVries et al., 2009; Gao and Oba, 2016), as cows may change their sorting behavior to attenuate ruminal acidosis (Gao and Oba, 2016); this agrees with the results obtained in the work of Kröger et al. (2019). In the latter study, cows altered their sorting behavior after consuming an acidogenic diet with 60% concentrates mainly by sorting against very fine particles (<1.18 mm) in an attempt to avoid the drop of ruminal pH caused by this diet (Kröger et al., 2019). According to Beauchemin and Yang (2005), a finer diet yields a greater sorting effect for large particles. Interestingly, the PP cows sorted even stronger for long particles and against short particles compared with MP cows. Present results contrast with previous reports, which either showed that PP cows sort in favor of short and against long particles (Leonardi et al., 2005; DeVries et al., 2011) or did not find any difference (Leonardi and Armentano, 2003). Eventually, the different sorting responses among studies are mainly

due to different starch levels in the diet, which was higher in our study, and our cows were in greater risk of SARA than in previous studies. Out of these 3 studies, only Leonardi et al. (2005) measured ruminal pH, who indeed reported mean ruminal pH >6.0 for all dietary treatments containing 48% grain in the diet, which is much lower than the L diet of this study.

According to the current definitions, the risk of SARA increases when ruminal pH drops below 5.8 for more than 5 to 6 h/d (Zebeli et al., 2008) or longer than 3 h when pH threshold is 5.6 (Plaizier et al., 2008). The cows in the current study experienced SARA throughout the trial, as they exceeded the threshold in each week. However, the SARA was less pronounced during the M-feeding and the pH drop was more severe during Lwk1. Accounting for the high-starch content of the present diets, a peNDF > 8 levels higher than 18% would have been required to avoid SARA (Humer et al., 2015), which was not reached, even in the M-phases. The severity of SARA was highest when cows started with the L diet as indicated by the mean pH, area under pH 5.8, and SARA index, due to the change to a diet lower in peNDF and higher in starch.

An interesting finding of the study was that PP cows were more susceptible to SARA, as demonstrated by the longer time duration of pH under 5.8 and higher SARA index. Maekawa et al. (2002) observed the opposite, with MP cows showing a higher risk of acidosis, explained by the higher DMI in MP cows. It is possible that this difference between studies is because their PP and MP had different DIM (146 ± 69 DIM in the study of Maekawa et al., 2002) and starch content in the diet, and therefore, in the SARA level. Indeed, more recent studies with SARA-inducing diets observed higher susceptibilities to SARA in PP (Krause and Oetzel, 2006; Bramley et al., 2008; Humer et al., 2015). Possible reasons might be because PP are naiver to high-concentrate diets as their rumen has not experienced such high starch amounts before (Humer et al., 2015). Along with that, their rumen papillae, therefore, absorption and neutralization capacity, and their rumen microbiome are less adapted to starch or might take more time to adapt to a nutritive change than in MP (Aschenbach et al., 2011).

All cows increased their DMI and produced more milk during the L-feeding phases. Reasons for the higher milk yield during the L diet could be either the higher DMI (and higher amounts of concentrate in the diet) or progressing lactation days of the cows. In a similar study, DeVries et al. (2011) observed increasing DMI but decreasing milk yield, and explained this effect from the cow's transition from peak lactation to peak of DMI. Milk yield of cows experiencing SARA is often negatively affected (Plaizier et al., 2008) or

not altered in cows (Gozho et al., 2007; Gao and Oba, 2016). When cows undergo SARA, a milk fat depression is often reported (Plaizier et al., 2008). The fat percentage was reduced with the L diet and the fat yield was affected only after 3 wk of the L diet. Gozho et al. (2007) found no changes in milk fat yield after a short-term SARA challenge, which might indicate that the length of feeding a starch-rich diet is also important to obtain responses in the milk fat yield depression. As reported previously, we observed an inversion of the milk fat to protein ratio, which is a common observation in cows experiencing SARA (Gozho et al., 2007; Plaizier et al., 2008).

Cows experiencing SARA, are at higher risk to develop several health problems such as rumenitis, liver abscesses, laminitis, and sole ulcers (Krause and Oetzel, 2006). Further problems are associated with ruminal LPS emerging into the systemic blood circulation. Lipopolysaccharides arise from the lysis of gram-negative bacteria, which increases during SARA (Nagaraja et al., 1978). Absorbed LPS enters the systemic circulation and is transported to the liver for detoxification (Andersen, 2003). In addition to detrimental effects due to SARA, it is known that the metabolic stress in early lactation affects liver health because the negative energy balance can lead to enhanced lipolysis, causing excessive lipid deposition and lesions in the liver, thereby increasing liver enzymes (AST, GGT, and GLDH; Humer et al., 2015). In the present study, all measured liver enzymes increased in the L-feeding phase, exceeding the reference ranges of healthy cows (Cozzi et al., 2011), with PP cows showing a stronger increase compared with MP cows. Due to the enhanced energy density and DMI in the respective phases, it can be assumed that the increase in liver enzymes was more related to metabolic stress associated with impaired rumen health than to excessive lipid mobilization. These results suggest that PP cows are even more susceptible to impaired liver function when diets high in starch are fed. Further, metabolites BHB and NEFA are indicators for liver health with relation to fat mobilization load. There was a reduction of BHB for all cows during the experiment by trend and a lower NEFA during the trial (Table 6). However, there were no differences comparing PP and MP cows. Elevated NEFA in the beginning of the trial suggested higher metabolization of body reserves (DeVries et al., 2011). DeVries et al. (2011) found similar results, where NEFA and BHB showed no differences among parities, yet Nasrollahi et al. (2017) found no differences in NEFA, but higher BHB in PP cows. The blood minerals Ca, P, and Mg were all affected by the feeding phases, but only iP was higher in PP cows. Higher iP concentration in PP cows was already described. As reviewed by Cozzi et al. (2011),

higher growth hormone activity, by promoting intestinal phosphate absorption, and renal re-absorption may cause higher iP concentrations.

CONCLUSIONS

This study showed that lowering the content of forages in the diet from 60 to 40% and increasing the starch content from roughly 26 to 32% impaired rumination activity and increased the risk of SARA in the Simmental cows, especially during the first week of the L-feeding. This rumen metabolic challenge was associated with milk fat depression and increased concentration of liver enzymes in the plasma. Interestingly, when the same low-forage high-starch diet was fed to PP cows, they had increased risk of developing SARA and a higher impairment of the liver health variables compared with MP cows. Although PP cows sorted stronger for long particles and chewed longer per kilogram of DMI than MP cows, this effort could not alleviate the deficiency of physically effective fiber in the diet due to forage shortage in the diet, and did not mitigate the risk of SARA and liver health damage. Taken together, our data suggest a greater susceptibility of PP cows to SARA and liver damage, indicating greater requirements for physically effective fiber of these cows than MP to maintain rumen health and mitigate SARA.

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ORCIDS

- A. Stauder <https://orcid.org/0000-0002-7948-1753>
E. Hummer <https://orcid.org/0000-0001-9776-0353>
V. Neubauer <https://orcid.org/0000-0002-4312-5781>
N. Reisinger <https://orcid.org/0000-0002-9207-452X>
A. Kaltenegger <https://orcid.org/0000-0002-5782-7178>
Q. Zebeli <https://orcid.org/0000-0001-5188-9004>