Long-term reticuloruminal pH dynamics and markers of liver health in early-lactating cows of various parities fed diets differing in grain processing

E. Humer,^{*1} A. Khol-Parisini,^{*1} L. Gruber,[†] J. Gasteiner,[†] Sh. M. Abdel-Raheem,^{*2} and Q. Zebeli^{*3}

^{*}Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, Vetmeduni Vienna, Veterinärplatz 1, 1210 Vienna, Austria

[†]Institute of Livestock Research, Agricultural Research and Education Centre Raumberg-Gumpenstein, Raumberg 38, 8952 Irdning, Austria

ABSTRACT

The present study aimed to investigate the long-term effect of feeding barley grain steeped in lactic acid (La) with or without thermal treatment on reticuloruminal pH dynamics and metabolic activity of the liver in 12 primiparous and 18 multiparous early-lactating dairy cows. All cows were included on d 21 postpartum and sampled until d 90 postpartum. Cows were fed a diet based on differently processed ground barley grain: untreated grain (control diet, CON), or grain treated with 1% La alone for 24 h before feeding (La), or with an additional oven-heating at 55°C for 12 h (LaH). The reticuloruminal pH and temperature were measured via indwelling sensors that allowed for continuous (every 10 min) and long-term measurement from d 21 to 80 postpartum. Blood samples were taken on d 21, 40, and 90 of lactation and analyzed for liver enzymes aspartate aminotransferase (AST), gamma-glutamyltransferase, and glutamate dehydrogenase, as well as bilirubin, bile acids, and serum amyloid A. Dry matter intake was higher in multiparous cows (20.7 ± 0.27 kg/d) compared with primiparous cows (18.2 ± 0.33 kg/d), but was not affected by dietary treatment. Overall, the relatively short duration (51 ± 5 min/d) of reticuloruminal pH <5.8 suggests low risk of subacute ruminal acidosis throughout the experiment. Results indicated that La treatment of barley, with or without heat, lowered the time duration of pH <5.8 compared with CON, but only in primiparous cows (from 118 ± 13 to 46 ± 11 and 25 ± 11 min/d for CON, La, and LaH, respectively). In multiparous cows, the opposite effect of feeding the La-treated barley on time duration of pH <5.8 (11 ± 8 vs. 46 ± 9 vs. 57 ± 9 min/d for CON, La, and LaH,

respectively) was observed. Multiparous cows generally showed higher pH readings and shorter periods in which the ruminal pH dropped below the threshold of pH 5.8. The reticuloruminal temperature was not affected by dietary treatment, whereas parity affected the time duration of reticuloruminal temperature $>39.5^\circ\text{C}$, being 60 ± 19 min/d shorter in primiparous cows. The measured activities of the liver enzymes AST, gamma-glutamyltransferase, and glutamate dehydrogenase, as well as bilirubin, bile acids, and the acute phase protein serum amyloid A, were not affected by grain feeding. Additionally, only one small effect of parity on investigated serum variables was noticed, showing slightly but significantly higher values of AST in multiparous (80.5 ± 1.4 U/L) compared with primiparous cows (76.0 ± 1.7 U/L). In conclusion, our results indicate greater risk for primiparous cows to develop subacute ruminal acidosis-like conditions during early lactation than multiparous cows. The study also suggests limited benefits of feeding processed barley grain with La with or without thermal treatment to modulate ruminal tolerance of grain feeding, whereby differing effects in primiparous cows were observed compared with multiparous cows.

Key words: rumen pH, parity, liver health, grain processing, lactic acid

INTRODUCTION

Inclusion of large amounts of grain in the diet is a common practice in dairy cows during early lactation. This feeding practice is useful to alleviate the energy deficit but also keeps the cows at high risk of developing rumen fermentation disorders such as SARA. Subacute ruminal acidosis is characterized by intermittent and moderate drops of ruminal pH in response to highly fermentable diets (Plaizier et al., 2008; Zebeli and Metzler-Zebeli, 2012). Because this disorder lacks overt clinical signs, its diagnosis relies mostly on ruminal pH measurements; however, single-point measurements of rumen pH are not reliable for predicting the

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¹These authors contributed equally to this paper.

²Present address: Department of Animal Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

³Corresponding author: Qendrim.Zebeli@vetmeduni.ac.at

risk of SARA compared with measuring the duration of pH below certain SARA thresholds (Penner et al., 2007), which can be obtained by continuous monitoring of rumen pH (Penner et al., 2007; Klevenhusen et al., 2014). Subacute ruminal acidosis is a prevalent metabolic disorder of cattle, and metabolic stress associated with SARA primarily afflicts the energy and lipid metabolism in different body tissues, most importantly in the liver and the mammary gland (Bionaz et al., 2007; Zebeli and Ametaj, 2009; Chang et al., 2015). A long duration of a low ruminal pH may result in the activation of the acute phase response (**APR**), commonly perceived as increased concentrations of acute phase proteins such as serum amyloid A (**SAA**) in the blood of affected cows (Plaizier et al., 2008).

Dairy cow diets should contain sufficient forages rich in physically effective NDF (**peNDF**) to prevent SARA (Yang and Beauchemin, 2007; Zebeli et al., 2012). However, during early lactation, diets typically contain lower proportion of forage compared with late-lactation diets due to the energy deficit and the need to alleviate it by highly fermentable, energy-rich diets (Zebeli et al., 2010). One promising approach to prevent SARA without the need to change the diet's forage proportion is to feed cereal grains or cultivars with low ruminal degradability (Silveira et al., 2007a,b; Nasrollahi et al., 2012). Studies conducted by Iqbal et al. (2009, 2012) found, for the first time in cattle, that treating barley grain with lactic acid (**La**) alleviated the risk of SARA when fed to late-lactation cows. Processing of barley grain with La has also been shown to enhance the content of slowly digestible starch and fibers and to trigger the hydrolysis of phytate P in more recent studies (Deckardt et al., 2014, 2015; Metzler-Zebeli et al., 2014). An additional thermal processing of barley with 50 to 55°C and La showed synergistic effects in terms of starch composition, its microscopic structure, and fiber fractions as well as in the hydrolysis of phytate P (Metzler-Zebeli et al., 2014; Harder et al., 2015a,b). However, less is known about feeding La-treated grains in early-lactating dairy cows. Several events such as the sudden reduction of dietary peNDF and the lack of an adequate adaptation of the rumen to a highly fermentable diet make early-lactating dairy cows more susceptible to SARA (Penner et al., 2007; Zebeli et al., 2008; Bannink et al., 2012). Apart from the stage of lactation, SARA susceptibility may also be influenced by parity, with first production heifers being differently sensible to dietary changes than multiparous cows that have experienced more high-grain diets.

Because of the aforementioned beneficial effects of La and thermal treatments on the carbohydrate fractions of barley such as greater content of slowly digestible starch and fibers, we hypothesized that feeding of

treated barley modulates reticuloruminal pH dynamics in early-lactating dairy cows. Another hypothesis of the study was that the effects of barley processing on reticuloruminal pH and metabolic activity of the liver are different in primiparous vs. multiparous dairy cows due to their presumably different sensibility against fermentable diets. Therefore, the aim of this study was to evaluate long-term dynamics of reticuloruminal pH and temperature, and the metabolic activity of the liver in early-lactating primiparous and multiparous dairy cows fed differently processed barley grain.

MATERIALS AND METHODS

Animals and Experimental Design

The longitudinal trial was conducted at the Dairy Research Facilities of the Institute of Livestock Research, Agricultural Research and Education Centre Raumberg-Gumpenstein, Austria, from June 2012 to March 2013. A total of 30 (12 primiparous and 18 multiparous) early-lactating cows, including 25 Holstein and 5 Simmental cows, were used. At the beginning of the trial, at d 20 postpartum, Holstein cows weighed 599 ± 56.4 kg (mean $\pm$ SD) and produced 28.4 ± 5.7 kg milk/d, whereas Simmental cows weighed 708 ± 73.4 kg and produced on average 25.3 ± 4.2 kg milk/d. Cows were blocked by expected calving date, breed and parity and were randomly allocated to 1 of 3 different feeding groups (10 cows in each group) using a longitudinal block-randomized design. The feeding experiment started at d 21 postpartum, and cows were sampled for this study until d 90 postpartum. The 3 experimental diets were alike, except for the processing of the barley, which was ground with a hammer mill (Ultra 110/150, Gruber Machineries, Gaspoltshofen, Austria) at 3-mm screen and fed as such for the untreated control group (**CON**), ground and steeped 1:1 (wt/vol) in 1% L-lactic acid (Brenntag, Vienna, Austria) for 24 h at room temperature (i.e., La), or ground and steeped in L-lactic acid plus heating at 55°C for 12 h (**LaH**). Preparation of La solutions and chemical treatment of barley grain has been described previously (Deckardt et al., 2014). The chemical composition of the untreated and treated barley grains is shown in Table 1.

All diets were formulated to meet the energy and nutrient requirements of dairy cows of 650 kg of BW, producing 35 kg milk/d with 4% fat, and 3.3% protein (GfE, 2001). Diets were designed to provide (DM basis) 50% forage (15% meadow hay, 15% grass silage, 20% corn silage), and 50% concentrate [38.5% barley grain (CON, La, or LaH), and 11.5% of a protein-mineral-vitamin mix (**PMV**)]. The PMV contained extracted soybean- and rapeseed meal in equal proportions,

Table 1. Analyzed chemical composition of barley grains untreated (CON), or treated with 1% lactic acid (La), or with 1% lactic acid and heat (LaH)

Item ¹	Barley grain		
	CON	La	LaH
% of DM unless stated			
DM, % fresh matter	87.1	48.1	48.2
OM	96.7	97.3	97.4
CP	13.2	11.6	11.7
Ether extract	2.6	2.1	2.2
aNDFom	19.6	20.3	20.7
Starch	61.1	59.3	60.7
ME, MJ/kg of DM	12.1	12.7	12.6
NE _L , MJ/kg of DM	7.52	8.01	7.89

¹The contents of ME and net NE_L of the barley grains were calculated according to Gruber et al. (2014). aNDFom = amylase-treated NDF determined on an organic matter basis.

limestone, salt, and a commercial mineral-vitamin premix for lactating dairy cows. The analyzed chemical composition of the forages and the PMV is shown in Table 2, and the chemical composition and particle size (PS) distribution of the experimental diets (accounting for different intakes by each group) are shown in Table 3. All cows had free access to water. Cows were fed ad libitum in individual Calan gates, being offered 110% of the average amount of feed consumed on the 3 preceding days (realized average daily feed refusals of 5 to 10%). Fresh feed was offered twice a day (0430 and 1600 h), whereby the cows received their roughage (hay and grass silage) portion first, followed by their barley allowance and corn silage, subsequently being offered each component again for at least 30 min or until finished. A computer-regulated feeding station was centrally located in the stable to individually and continuously offer the proportion of PMV.

During the experiment, cows were located in a free stall. All procedures involving animal handling and treatment were in accordance with national regulations

for animal use in research and approved by the authority according to §9ff, Law for Animal Experiments (GZ FA10A-78Gu-19/2012-2).

Feed Sampling and Chemical Analyses

Amounts of feed offered and refused were recorded daily to determine the feed intake of cows by difference. Feed and orts samples were collected weekly. They were mixed and aliquots were used for analyses of nutrient composition and PS distribution. Dry matter was determined after oven drying for 72 h at 55°C. The ground samples (1-mm screen) were analyzed for N using the Kjeldahl method, and ether extract and ash were measured according to the official methods (Naumann and Bassler, 2012). The NDF and ADF were determined using the methods described by Van Soest et al. (1991), with heat-stable α -amylase used in the NDF procedure. The starch contents of the barley grains were determined enzymatically according to the analysis procedure [AOAC (2002) Official Method 2002.02; AACC (2002) 101 Method 32–40] provided by the Megazyme Kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland). Calculation of energy concentration of feedstuffs was based on their crude nutrient composition and in vivo digestibility coefficients of individual ingredients determined with the method described by Gruber et al. (2014). The PS distribution was determined using a Penn State Particle Separator with 3 sieves (Kononoff et al., 2003). Particles retained on each fraction were oven dried at 55°C. The physical effectiveness factors (**pef**, i.e., the cumulative proportion of feed DM retained on sieves of the Penn State Particle Separator) were nominated as **pef**_{>8} and **pef**_{>1.18}, respectively. The physically effective NDF of 2 (**peNDF**_{>8}) and 3 sieves (**peNDF**_{>1.18}) were calculated by multiplying the fraction on **pef**_{>8} and **pef**_{>1.18} with the NDF content (Kononoff et al., 2003), respectively.

Table 2. Analyzed chemical composition of forages and protein-mineral-vitamin mix (PMV)

Item, ¹ % of DM unless otherwise stated	Hay	Corn silage	Grass silage	PMV ²
OM	88.6	95.1	86.9	84.1
CP	13.5	6.9	13.9	39.5
Ether extract	2.4	3.3	3.8	2.0
aNDFom	45.0	44.9	47.1	16.7
ADFom	28.6	26.7	33.1	13.4
NFC	27.7	40.0	22.1	25.9
NE _L , MJ/kg of DM	5.40	6.26	5.65	6.77

¹NFC [= 100 – (% NDF + % CP + % ether extract + % ash)] and NE_L according to (Gruber et al., 2014). aNDFom = amylase-treated NDF determined on an organic matter basis; ADFom = ADF determined on an organic matter basis.

²The PMV contained 45.48% soybean meal, 45.48% rapeseed meal, 5.04% limestone, 2.00% salt and 2.00% mineral-vitamin supplement for lactating dairy cows (contained per kg: Ca 6%, P 12%, Mg 10%, Na 8%, Mn 1,500 mg, Zn 5,700 mg, Cu 800 mg, vitamin A 750,000 IU, vitamin D₃ 75,000 IU, vitamin E 3,000 mg).

Table 3. Analyzed nutrient composition and particle size distribution of the diets fed to primiparous (Prim) and multiparous (Mult) early-lactating dairy cows from d 21 to 80 postpartum¹

Item, ² % of DM unless stated	CON		La		LaH	
	Prim	Mult	Prim	Mult	Prim	Mult
DM, % of fresh matter	67.9	66.9	52.6	52.9	52.8	53.5
OM	91.9	92.4	92.2	92.6	91.4	92.3
CP	15.3	14.6	14.5	15.2	14.5	14.6
Ether extract	2.8	2.8	2.6	2.6	2.6	2.7
aNDFom	32.0	33.2	32.5	33.2	32.7	33.3
ADFom	18.3	19.2	18.9	19.4	18.6	19.0
NFC	41.8	41.8	42.6	41.6	41.6	41.7
NE _L , MJ/kg of DM	6.55	6.51	6.75	6.70	6.70	6.64
Particle size distribution, g/kg of DM retained on sieves						
19 mm	352	324	312	286	311	276
8 mm	114	132	120	141	105	131
1.18 mm	449	453	529	530	527	539
Pan	85	92	39	43	58	55
pef _{>8}	0.47	0.46	0.43	0.43	0.42	0.41
pef _{>1.18}	0.92	0.91	0.96	0.96	0.94	0.95
peNDF _{>8} , % of DM	15.0	15.3	14.0	14.3	13.7	13.7
peNDF _{>1.18} , % of DM	29.4	30.2	31.2	31.9	30.7	31.6

¹Experimental diets differed in barley grain processing, being either unprocessed (CON), or processed with 1% lactic acid (La), or with 1% lactic acid and heat (LaH); Diet components are shown in Table 4.

²NFC [= 100 – (% NDF + % CP + % ether extract + % ash)] and NE_L according to (Gruber et al., 2014); aNDFom = amylase-treated NDF determined on an organic matter basis; ADFom = ADF determined on an organic matter basis; pef = physically effective factor; peNDF = physically effective NDF (Kononoff et al., 2003).

Reticuloruminal pH and Temperature

To monitor rumen pH continuously, all cows received an indwelling wireless pH-transmitting unit (smaXtec Animal Care Sales GmbH, Graz, Austria). These units (3.5 cm i.d., 12 cm long, and weighing 210 g) were manually inserted into the reticulorumen via the esophagus (Gasteiner et al., 2012) in all cows on d 17 postpartum. Prior to this, the units were calibrated by a 2-point calibration using buffers of pH 4.0 and 7.0 following the company's instruction protocol. The units measured pH and temperature every 10 min and transmitted the data in real time to a basis station using the ISM band (433 MHz). Antennas installed in the barn and milking parlor picked up the sensor signals. Both data of pH and temperature were collected using an analog to digital converter and stored in an external memory chip. As the guaranteed working time of the sensors was 60 d, the measurements were taken continuously until d 80 postpartum from each cow. One concern of the indwelling sensors is that they provide data of pH only in the site they are located, namely in the reticulum as they are located in the reticulum within 24 h after administration (Gasteiner et al., 2009). The sensors used in this study were recently evaluated and have shown to satisfactorily reflect the pH of the free rumen liquid (Klevenhusen et al., 2014). Thus, the term reticuloruminal pH is used here. Daily mean, minimal, and maximal pH values, standard deviation, and dura-

tion during which the pH was below thresholds of 5.8 and 6.0 were calculated. Furthermore, diurnal changes (measurements throughout 1 d taken at 10-min intervals) were analyzed separately. Data of temperature were analyzed for diurnal changes. In accordance to the pH data, daily mean temperature and duration during which temperature was above a threshold of 39.5°C were computed and analyzed separately.

Blood Sampling and Analyses

Blood samples were collected shortly before the morning feeding from the coccygeal vein on experimental d 21 (baseline), 40, and 90. Serum 10-mL evacuated tubes (Vacuette, Greiner Bio-One, Kremsmünster, Austria) were used to collect blood samples. Immediately after collection, blood samples were allowed to clot for 30 min at 25°C; subsequently serum was separated by centrifuging at $3,000 \times g$ at 4°C for 20 min. Serum samples were stored at –20°C until analyses. Concentrations of SAA were determined by a commercially available ELISA bovine kit according to the method described previously (Klevenhusen et al., 2013). In brief, serum samples for SAA were initially diluted 1:200, and samples with optical density values above the range of the standard curve were diluted further (1:400 or 1:250) and reanalyzed. All samples were tested in duplicate and the optical density values were read by an iMark microplate absorbance reader (Bio-Rad Laboratories

GmbH, Vienna, Austria) at 450 nm. The concentrations of bile acids, bilirubin, and liver enzymes such as aspartate aminotransferase (**AST**), glutamate dehydrogenase (**GLDH**), and gamma-glutamyltransferase (**GGT**) in the serum were measured by a conventional large-scale analyzer for clinical chemistry at the laboratory of the Central Clinical Pathology Unit, University of Veterinary Medicine, Vienna. The standard enzymatic colorimetric analyses with a fully automated autoanalyzer for clinical chemistry (Cobas 6000/c501; Roche Diagnostics GmbH, Vienna, Austria) was used. The intraassay variation was controlled by limiting the coefficient of variation to $\leq 10\%$ for SAA and $< 5\%$ for other blood variables.

Statistical Analyses

Statistical analyses were performed using PROC MIXED of SAS (version 9.2, SAS Institute Inc., Cary, NC). To evaluate the effect of cow's parity, cows were classified in 2 different lactation groups: primiparous ($n = 12$) or multiparous ($n = 18$). The model included the fixed effects of block, diet, parity, sampling time (i.e., day or time of the day at 10-min intervals), as well as the diet $\times$ time and diet $\times$ parity interaction. The baseline measurements were considered as covariate in the model. The data gained from the same cow but at different times were considered as repeated measures in the model with a first-order autoregressive variance-covariance matrix. Comparisons among treatments were evaluated by Tukey's test. Degrees of freedom were estimated with the method of Kenward-Roger. Furthermore, the significance of the overall effect of the La treatment was tested for all variables (orthogonal contrast involving the average of the 2 La-treated groups vs. control). Associations among pH and temperature measurements were studied by performing a Pearson correlation (PROC CORR of SAS). The significance level was set at $P \leq 0.05$, and a trend was considered up to $0.05 < P < 0.10$ level.

RESULTS

Nutrient Composition of Barleys and Diets, and Feed Intake

The processing of barley with La and LaH resulted in minor changes in the composition of crude nutrients and energy content (Table 1). Treatments with La and LaH slightly reduced ash, starch, and CP contents, but increased amylase-treated NDF determined on an organic matter basis (**aNDFom**) and NE_L contents of the treated barleys; however, these differences in nutrient

and energy contents were less visible in the consumed diets (Table 3).

Effects of barley feeding on DMI from d 21 to 80 of lactation are shown in Table 4. Total DMI was not affected by barley treatment ($P = 0.44$), whereas primiparous cows consumed less ($P < 0.01$) feed (18.2 kg of DM/d), including barley grain ($P < 0.01$), compared with their multiparous counterparts (20.7 kg of DM/d). Relative intake of the different dietary ingredients did not differ among feeding groups either (Table 4). However, primiparous cows consumed a higher ($P = 0.03$) proportion of barley (38.8%) compared with multiparous cows (36.5%), and this happened at the expense of hay (14.7% in primiparous cows compared with 16.0% in multiparous cows, Table 4).

Reticuloruminal pH and Temperature Changes

Effects of barley feeding on the reticuloruminal pH and temperature changes from d 21 to d 80 of lactation are shown in Table 5. In cows receiving LaH barley, the mean, maximum, and minimum reticuloruminal pH values were lowered by 0.05 units compared with CON and La ($P < 0.01$). Figure 1a shows daily mean pH values of the 3 dietary treatment groups. Slightly lower pH-values were measured in LaH cows ($P < 0.01$), whereas no differences between lactation days were observed ($P = 0.71$). As shown in Figure 1b, mean reticuloruminal temperature was 0.07°C higher in La cows compared with CON and LaH ($P < 0.01$), whereas no effect of lactation day was recorded ($P = 0.65$). Diurnal variations of mean reticuloruminal pH as well as temperature on 2 exemplary days (d 40 and 60) are shown in Figure 2. Values were averaged over all treatment groups as no effect of the barley processing method was observed. However, reticuloruminal pH as well as temperature were affected by time ($P < 0.01$). The pH profile showed that the pH maximum occurred just before the offering of fresh feed and declined until 6 to 7 h thereafter. Additionally, abrupt drops in temperature were noticed when fresh feed was offered. The same was true for water intake, when observing temperature curves of single cows (data not shown). The duration of pH below the threshold of 6.0 was not influenced by barley treatment and averaged 231 min/d (Table 5). In contrast, the daily period of pH values < 5.8 was 21 min shorter ($P = 0.02$, Figure 3a) in the La groups. The time of pH below both thresholds was not different between lactation days ($P > 0.10$), whereas time below 5.8 was interactively affected by lactation day and treatment ($P = 0.03$). Interestingly, an interaction between parity and treatment group was observed for the minimum pH, showing the lowest

Table 4. Diet components and DMI in primiparous (Prim) and multiparous (Mult) cows fed diets based on untreated barley grain (CON), or barley grain steeped in 1% lactic acid solution (La), or steeped in 1% lactic acid solution with heat (LaH) for 12 h from d 21 to 80 postpartum

Item	CON			La			LaH			P-value ¹		
	Prim	Mult		Prim	Mult		Prim	Mult		Diet	Par	Diet × Par
Diet component, % of DM												
Barley grain	39.3	37.3		39.1	36.2		38.1	36.0		0.59	0.03	0.92
Corn silage	18.4	19.7		18.9	20.2		19.9	19.4		0.83	0.32	0.50
Grass silage	16.4	17.1		16.3	16.6		15.9	16.5		0.76	0.28	0.95
Hay	14.5	16.4		14.7	15.3		15.0	16.4		0.68	<0.01	0.32
Protein-mineral mix	11.4	9.5		11.0	11.7		11.1	11.7		0.27	0.83	0.42
DMI, kg/d										0.49		
Barley grain	7.30	7.81		6.77	7.85		6.73	7.31		0.10	<0.01	0.43
Corn silage	3.45	4.02		3.28	4.29		3.53	3.93		0.97	<0.01	0.48
Grass silage	3.05	3.49		2.82	3.55		3.55	3.33		0.14	<0.01	0.22
Hay	2.72	3.34		2.56	3.26		2.67	3.30		0.66	<0.01	0.94
Protein-mineral mix	2.06	1.90		1.88	2.46		2.32	2.32		0.29	<0.01	0.01
Total	18.5	20.6		17.3	21.4		18.8	20.1		0.44	<0.01	0.13

¹Effect of dietary treatment (Diet), effect of parity (Par), interactive effect of dietary treatment and parity (diet × Par), and overall effect of lactic acid (Con).

values in primiparous cows of the CON group ($P < 0.01$), resulting in the longest duration of pH <6.0 and 5.8 in these cows ($P < 0.01$). Finally, in primiparous CON cows the duration of the reticuloruminal pH <5.8 was on average 83 min/d longer when compared with the 2 other treatment groups (Table 5, $P < 0.01$). On the contrary, in multiparous cows the opposite effect was observed, showing on average a 41 min/d shorter duration of reticuloruminal pH <5.8 in CON cows compared with the cows of the 2 other treatments ($P < 0.01$). Time duration of temperature >39.5°C was neither affected by barley treatment ($P = 0.31$) nor by lactation day ($P = 0.27$, Figure 3b). However, an interaction was observed between lactation day and treatment ($P = 0.04$).

Parity affected certain pH and temperature variables. Although the mean pH was not affected (Figure 4a), the maximum pH was higher in multiparous cows compared with first-lactation heifers ($P < 0.01$). The duration of pH <6.0 was on average 66 min/d shorter in multiparous cows ($P = 0.02$). Additionally, the time during which pH was below the threshold of 5.8 was reduced by 25 min/d in cows in second or higher lactation ($P < 0.01$, Figure 5a). Mean reticuloruminal temperature was 0.06°C higher in multiparous cows compared with primiparous cows ($P < 0.01$, Figure 4b). Furthermore, the time during which temperature was >39.5°C was on average 60 min/d longer in multiparous cows compared with first-lactation heifers ($P < 0.01$, Figure 5b).

Serum Variables

Barley treatment and parity showed minor effects on liver-associated variables (Table 6). No effect of the barley processing technique was observed. Moreover, only small effects of parity on investigated serum variables were noticed. Aspartate aminotransferase showed 6% higher values in cows in the second or higher lactation compared with primiparous cows ($P = 0.05$). Concentrations of bilirubin decreased with sampling day ($P < 0.01$), whereas none of the investigated serum variables was interactively affected by sampling day and diet.

DISCUSSION

This study was primarily undertaken to monitor long-term reticuloruminal pH dynamics and metabolic activity of the liver in early-lactating primiparous and multiparous dairy cows fed a diet based on barley grain processed with La, with or without thermal treatment. All cows were included on d 21 postpartum and sampled until d 90 postpartum, whereby pH and temperature data were analyzed from d 21 to d 80 postpartum as

Table 5. Effect of dietary treatment and parity (Prim = primiparous; Mult = multiparous) on reticuloruminal pH and temperature in cows from d 21 to d 80 of lactation fed untreated barley (CON), barley steeped in 1% lactic acid solution (La), or steeped in 1% lactic acid solution with heat (LaH)

Item	CON		La		LaH		P-value ¹				
	Prim	Mult	Prim	Mult	Prim	Mult	SEM	Diet	Par	Diet × Par	Con
pH											
Mean	6.20	6.26	6.23	6.23	6.18	6.17	0.004	<0.01	0.28	0.13	0.15
Maximum	6.51	6.57	6.53	6.59	6.48	6.52	0.004	<0.01	<0.01	0.81	0.38
Minimum	5.79 ^c	5.91 ^a	5.89 ^a	5.86 ^{ab}	5.83 ^{bc}	5.81 ^{bc}	0.005	<0.01	0.21	<0.01	0.97
SD ²	0.155 ^b	0.142 ^c	0.135 ^d	0.161 ^a	0.138 ^d	0.156 ^a	0.14	0.95	<0.01	<0.01	0.81
Time <6.0, min/d	370 ^a	97 ^d	246 ^{bc}	221 ^{bc}	177 ^c	277 ^b	0.37	0.98	0.02	<0.01	0.92
Time <5.8, min/d	118 ^a	11 ^c	46 ^b	46 ^b	25 ^{bc}	57 ^b	0.97	0.07	<0.01	<0.01	0.02
Temperature											
Mean	38.78	38.84	38.87	38.89	38.75	38.86	0.004	<0.01	<0.01	0.11	0.07
Time >39.5°C, min/d	207	275	218	266	180	243	4.65	0.31	<0.01	0.91	0.99

^{a-d}Means within a row with different superscripts differ ($P \leq 0.05$).¹Effect of dietary treatment (diet), effect of parity (Par), interactive effect of dietary treatment and parity (diet × Par), overall effect of lactic acid (Con).²Standard deviation of measurements within a day.

the indwelling sensors allowed for measurement for as many as 60 d.

Depression of ruminal pH occurs early postpartum due mainly to dramatic dietary changes, whereby SARA is considered a prevalent metabolic disorder during this period (Plaizier et al., 2008). The durations of pH below thresholds of 5.6 or 5.8 for longer than 3 or 5.4 h/d, respectively, are considered as safer indicators of SARA than single measurements of pH (Penner et al., 2007; Plaizier et al., 2008; Zebeli and Metzler-Zebeli, 2012). Overall, the short duration (average time: 51 min/d) of reticuloruminal pH <5.8 suggests a low risk of SARA in the cows of this study. Despite the fact that cows were in early lactation and fed almost 50% concentrate in the diet, this could be explained by low DMI and a relatively high concentration of peNDF (>1.18) in all diets, averaging 30.8%, which is assumed to be sufficient in maintaining a mean ruminal pH of above 6 (Zebeli et al., 2008).

Overall results showed limited effects of feeding barley processed with La or LaH on reticuloruminal pH. Accordingly, the differences of 0.05 pH units between LaH compared with CON and La is in fact below the pH accuracy level (± 0.2 units). However, data revealed important differences when analyzed separately by parity. Indeed, an interesting finding of the study was that La and LaH treatments of barley shortened the time duration of pH <5.8 by 83 min/d in primiparous cows compared with their CON counterparts. This result confirms, at least in part, findings reported by Iqbal et al. (2009), who also observed a slightly shorter duration in which rumen pH was <5.8 (2.4 vs. 3.9 h/d) in primiparous late-lactating cows fed barley steeped in 0.5% La compared with cows fed control barley grain. The reason(s) why this grain treatment improved the ruminal tolerance of barley in primiparous cows by shortening their time duration of rumen pH <5.8 compared with control cows, but not in multiparous cows, is not clear. Nevertheless, these results suggest that primiparous cows may benefit from La treatments. It is also possible that primiparous cows had a greater SARA challenge than multiparous cows, as evidenced by a longer duration of pH <5.8 in those cows. The processing of barley with La and LaH resulted in few changes in the composition of crude nutrients such as reduced ash, total starch, and CP contents, and also increased the aNDFom content of the treated barleys, although these chemical changes of barleys were leveled up in the whole diets, which provided almost similar crude nutrient composition to the cows. Most importantly, treatments of barley grain with La and LaH have been shown to reduce the rate of enzymatic starch digestion, alter its microscopic structure (Deckardt et al., 2014; Harder et al., 2015a,b), and also modulate

Figure 1. Effect of dietary treatment on mean reticularuminal pH (a) and temperature (b) in lactating cows fed barley (CON), barley steeped in 1% lactic acid solution (La), or steeped in 1% lactic acid solution with heat (LaH). Error bars represent SEM.

Figure 2. Diurnal variation of reticuloruminal pH and temperature in lactating cows, averaged across all treatment groups on d 40 (a) and d 60 (b) of lactation. Error bars represent SEM.

rumen microbial activity, and lower the concentration of VFA in a semi-continuous culture fed 60% concentrate (Deckardt et al., 2015). Grain processing might be more effective in modulating ruminal pH when cows are fed grain-rich diets, challenging them to SARA conditions. Healthy cows are assumed to be less responsive

to dietary treatments compared with SARA-challenged cows, an argument that is further strengthened by the observed interaction between parity and treatment in this study. Evaluation of this hypothesis requires more research with cows fed diets richer in grain. However, it has to be emphasized that the opposite effect to that

Figure 3. Effect of dietary treatment on time reticuloruminal pH < 5.8 (a) and temperature > 39.5°C (b) in lactating cows fed barley (CON), barley steeped in 1% lactic acid solution (La), or steeped in 1% lactic acid solution with heat (LaH). Error bars represent SEM.

Figure 4. Effect of parity (Mult = multiparous, Prim = primiparous) on reticuloruminal pH (a) and temperature (b) in lactating cows. Error bars represent SEM.

Figure 5. Effect of parity (Mult = multiparous, Prim = primiparous) on time duration of reticuloruminal pH < 5.8 (a) and temperature > 39.5°C (b) in lactating cows. Error bars represent SEM.

Table 6. Effect of dietary treatment and parity (Prim = primiparous; Mult = multiparous) on serum variables in lactating cows fed barley (CON), barley steeped in 1% lactic acid solution (La), or steeped in 1% lactic acid solution with heat (LaH)

Item ¹	CON		La		LaH		P-value ²				
	Prim	Mult	Prim	Mult	Prim	Mult	SEM	Diet	Par	Diet × Par	Con
AST, U/L	74.92	79.53	75.10	83.66	77.99	78.40	1.43	0.74	0.05	0.31	0.52
GGT, U/L	22.10	21.92	25.17	26.26	26.26	22.20	0.81	0.19	0.63	0.66	0.14
GLDH, U/L	11.65	12.59	15.39	13.54	21.51	13.69	1.49	0.14	0.20	0.27	0.11
Bile acids, μmol/L	47.99	51.65	49.58	58.39	67.17	53.99	4.50	0.68	0.98	0.63	0.49
Bilirubin, mg/dL	0.04	0.07	0.07	0.08	0.10	0.08	0.01	0.18	0.65	0.35	0.11
SAA, mg/L	2.34	1.55	14.30	24.85	1.65	7.99	3.66	0.30	0.71	0.68	0.71

¹AST = aspartate aminotransferase; GGT = gamma-glutamyltransferase; GLDH = glutamate dehydrogenase; SAA = serum amyloid A.²Effect of dietary treatment (Diet), effect of parity (Par), interactive effect of dietary treatment and parity (diet × Par), overall effect of lactic acid (Con).

found for primiparous was observed in multiparous cows, whereby La and LaH cows showed an on average 41 min/d longer duration of pH <5.8 compared with the CON group. This contradictory observation of grain processing in primiparous versus multiparous cows is difficult to explain with the current data of pH dynamics. Clarification of the mechanisms behind the latter effects requires further investigations aiming to establish the effects of barley processing on reticulo-ruminal fermentation profile such as the dynamics of release, neutralization, and metabolism of VFA and lactate, and also chewing activity, events which are known as determinants of intra-reticuloruminal pH regulation (Allen, 1997; Zebeli et al., 2012).

Besides monitoring the effect of La-treated barley on pH dynamics, this study further aimed at investigating possible differences between first-production heifers and multiparous cows. The role of the parity in the risk of developing SARA has not been clarified yet. For example, Krause and Oetzel (2006) and Bramley et al. (2008) observed a decreased risk of developing SARA in multiparous cows compared with primiparous cows, but other studies have reported an opposite trend (Maekawa et al., 2002), or no relation between risk of ruminal acidosis and parity (Gröhn and Bruss, 1990). Generally, one could expect that primiparous cows should be at lower risk of SARA, due to their lower production potential and DMI (Krause and Oetzel, 2006). Indeed, primiparous cows consumed approximately 2.5 kg/d less feed DM than multiparous cows in this study. Although multiparous cows had on average 2% less barley grain at the expense of more hay and corn silage in their diets, these cows in general consumed significantly greater amounts of easily fermentable substrates (14.0 vs. 12.4 kg of DM/d), contained in concentrates and corn silage, with almost unchanged peNDF_{1.18} contents. Because of these feeding patterns, a greater risk of SARA for multiparous cows was expected. It is known that for each unit increase in the DMI, and hence in fermentable substrates and fiber, acid generation rather than acid neutralization processes are stimulated in the rumen of dairy cows (Allen, 1997). However, contrary observations were made in the present study. Multiparous cows showed shorter periods in which pH decreased below 6.0 and 5.8, respectively. The occurrence of SARA may be exacerbated in first-production heifers when compared with multiparous cows, because primiparous cows have not had previous long-term exposure to a highly fermentable lactation diet yet. One possible explanation might be that they may have fewer rumen papillae and less bacterial adaptation than mature cows (Penner et al., 2007; Bramley et al., 2008). This is probably the reason why feeding additional concentrate parturum is suggested to al-

leviate the risk of postpartum SARA in cows aiming to help them in increasing the absorptive capacity of rumen papilla and adapt better to high grain feeding postpartum (Stone, 2004). However, this was not supported by Penner et al. (2007), who were not able to detect ameliorative effects of feeding additional concentrate in primiparous cows on postpartum SARA development. Another reason for the differing SARA susceptibility might be related to higher chewing time in multiparous cows compared with primiparous cows. Thus, a lower daily saliva output in heifers compared with multiparous cows may result in reduced rumen buffering and consequently in lower pH compared with older cows (Maekawa et al., 2002; Bowman et al., 2003). Furthermore, Oetzel (2007) assumed that heifers need more time to self-regulate their own ruminal pH after they start consuming large amounts of cereal grains after parturition compared with multiparous cows. Additionally, first-lactation cows represent a largely uncultured population. Thus, it is also possible that the heifers that are unable to learn to self-regulate their own ruminal pH have not yet been removed from the herd, but will be removed in the following lactations due to complications of SARA (Oetzel, 2007).

In addition, parity affected reticuloruminal temperature. Although the differences of the mean reticuloruminal temperature of $+0.06^{\circ}\text{C}$ is below the measurement accuracy ($\pm 0.25^{\circ}\text{C}$), older cows spent 1 h more per day with a reticuloruminal temperature $>39.5^{\circ}\text{C}$, when compared with primiparous animals. Thus, it seems that the obtained longer duration of high reticuloruminal temperature in the present study was less directly associated with a higher risk of SARA, than rather to a higher heat production due to higher fermentation resulting from higher feed intake in multiparous cows. This is supported by the observation that no correlation was found between ruminal temperature and ruminal pH in this study ($r = -0.06$).

The present study also focused on liver-associated variables, as it is well known that metabolic stress associated with low ruminal pH during early lactation primarily affects liver function (Bionaz et al., 2007). For instance, in cattle affected by SARA, APR is typically expressed as an increase of the acute phase protein SAA (Plaizier et al., 2008; Iqbal et al., 2010). Furthermore, in early-lactation cows, the liver has to adequately adapt to cope with the increased flow of NEFA resulting from excessive lipolysis (Goff and Horst, 1997). In case of an increased lipid deposition in the liver, lesions of the liver tissue can be the consequence, and levels of enzymes that indicate liver injury (AST, GGT, and GLDH) are generally augmented (Bobe et al., 2004).

The measured activities of AST, GGT, and GLDH corresponded with previous reports (Wille et al., 2010; Lee et al., 2012). In general, liver-associated variables were not affected by barley feeding in this study. Because the values of presented enzyme activities were within reference intervals (Wille et al., 2010), no clear sign is found of impaired liver function of the present cows, which is also explained by low level of SARA and the cows' low production level. Thus, the present data suggest that the process of lipomobilization in these cows was not affected by diet, and was not strong enough to cause liver lesions in early-lactating cows. Although treatment with La and LaH enhanced energy content of barleys by almost 0.4 to 0.5 MJ/kg of DM, compared with CON barley, the total NE_L contents of the ingested diets were almost similar. The increase of NE_L content of La-treated barleys can be mainly explained by changes in the profile of degradation of its nutrients such as ash, CP, starch, phytate P, and soluble fiber (Deckardt et al., 2014; Metzler-Zebeli et al., 2014; Harder et al., 2015a). Also, the addition of 1% La in the soaking solution (resulting in 10 g of La/kg of barley) might have contributed to greater energy value of the treated barley. Assuming energy content of 15.1 kJ per g of La, the La treatment provided additional 0.15 MJ per each kg of barley (i.e., 1% La solution with ratio 1:1). Because the DMI of concentrate and forages remained unchanged, resulting in no difference in overall DMI among feeding groups, it is reasonable to assume that total NE_L intake also was similar among treatments. The similar energy intakes together with no SARA conditions likely explain no effect of diets on the process of lipomobilization, APR, and liver health in the cows of our study. Because the intake of barley grain was not significantly affected by treatment, it seems that treatment of grain with La and LaH does not negatively affect the palatability of the treated grain.

The concentrations of the acute phase protein SAA were not affected by dietary treatment and generally below recommended cutoff levels for identification of an APR in postparturient dairy cows (Humblet et al., 2006). Thus, the absence of SARA and its related inflammatory state in all treatment groups is further supported. Most liver-associated variables were not affected by parity, too. However, 6% higher activities of AST were found in multiparous cows compared with primiparous cows. This is supported by Wille et al. (2010) and Cozzi et al. (2011) who found only minor effects of the parity on enzyme activity. In general, elevated levels of certain liver enzymes in multiparous cows compared with primiparous cows could be the

result of an elevated productive stress compared with primiparous cows.

CONCLUSIONS

In conclusion, the present study showed contradictory effects of steeping barley grain in La with or without thermal treatment on ruminal tolerance of barley grain feeding, determined by long-term reticuloruminal pH dynamics; the treatment reduced the duration that reticuloruminal pH was <5.8 in primiparous cows but increased it in multiparous cows. As the risk of SARA seems to be greater in primiparous cows, our data indicate that more care should be taken in the practice in terms of feeding and management of this group of cows. The lack of pronounced effects of dietary treatment on liver-associated variables may be explained by the low production level of the cows and the fact that cows in the present study were not challenged by SARA conditions. Thus, it would be of interest to test the effects of feeding La-treated barley grain on dairy cows challenged with SARA conditions in future studies.

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