Effects of feeding unlimited amounts of milk replacer for the first 5 weeks of age on rumen and small intestinal growth and development in dairy calves

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ABSTRACT

The development of the gastrointestinal tract in newborn calves is essential for sufficient nutrient uptake. An intensive milk feeding during the neonatal period may impair the rumen development in calves. The aim of this study was to investigate effects of milk replacer (MR) feeding in unlimited amounts for the first 5 wk of age on the gastrointestinal growth and development in preruminant calves at wk 9 of age. Twenty-eight newborn Holstein and Holstein × Charolais crossbred calves (19 male and 9 female) were fed MR ad libitum (ADLIB) or in restricted amounts (6 L per day; RES) until wk 5 of age. Thereafter, the MR intake of ADLIB was gradually reduced at wk 6 and 7, and all calves received 6 L of MR per day until wk 9 of age. In wk 9, calves were slaughtered and carcass and organ weight as well as rumen papilla size in the atrium, ventral sac, and ventral blind sac, and villus size of the mucosa in the small intestine (duodenum; proximal, mid, and distal jejunum; and ileum) were determined. The expression of mRNA associated with the local insulin-like growth factor (IGF) system was measured in the rumen epithelium. Ad libitum MR feeding increased MR intake and growth in ADLIB without influencing concentrate intake compared with RES. Carcass weight in wk 9 was greater in ADLIB than in RES. The density of the rumen papillae in the atrium and ventral blind sac was greater in RES than in ADLIB calves, but surface area of the epithelium was not different between groups in the investigated regions of the rumen. The mRNA abundance of *IGF1* in the atrium tended to be greater and the *IGFR1* mRNA abundance in the ventral sac tended to be lower in the ADLIB than in the RES feeding group. The rumen pH and volatile fatty acid concentrations were not affected by MR feeding intensity. In mid-jejunum, villus circumference was

greater in ADLIB than in RES calves. In the distal jejunum, villus surface area and the villus height/crypt depth ratio were greater and the villus circumference and height tended to be greater, whereas crypt depth was smaller in ADLIB than in RES calves. The findings from this study indicate that ad libitum MR feeding for 5 wk of age followed by its gradual reduction promotes growth performance without any negative influence on gastrointestinal growth and development in dairy calves at 9 wk of age.

Key words: calf, milk feeding intensity, gastrointestinal development, insulin-like growth factor system

INTRODUCTION

Ad libitum milk or milk replacer (MR) feeding programs during the early preweaning period result in a greater nutrient intake and allow calves to achieve a higher ADG and BW than restricted milk feeding (Hammon et al., 2002; Jasper and Weary, 2002; Macari et al., 2015). New automated feeding systems allow calves to drink large amounts of milk or MR per day by several small portions and facilitate the weaning process without detrimental effects on the digestive system (Hammon et al., 2002; Khan et al., 2011; de Passillé and Rushen, 2016). The enhanced postnatal growth performance has long-lasting developmental effects and may promote health and individual milk production (Khan et al., 2011; Bach, 2012; Van Amburgh and Soberon, 2013). However, intensive milk feeding regimens are still under discussion due to the possible negative effect on solid feed intake and rumen development (Drackley, 2008; Gelsinger et al., 2016; Khan et al., 2016), but when calves receive up to 6 L of milk/d they do not reach their growth potential during the pre-weaning period (Hammon et al., 2002; Schäff et al., 2016; Frieten et al., 2017). When comparing growth performance in dairy calves with the more natural situations in beef calves, an elevated milk and solid feed intake at the same time should be feasible when milk and solid feed intake are distributed over the day by automated feed-

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ing systems (Egli and Blum, 1998; Maccari et al., 2015; de Passillé and Rushen, 2016). An adequate nutrient supply from liquid and solid feeds is probably most important for optimal dairy performance in later life (Gelsinger et al., 2016) and sufficient concentrate intake in the pre-weaning period is necessary to maintain constant growth and weight gain after weaning (Drackley, 2008; Bach, 2012). Therefore, there is great interest in developing pre-weaning feeding protocols that enable an accelerated growth performance and adequate maturation of the rumen function at once.

Body and organ growth is regulated by the systemic and local expression of the somatotrophic axis (Breier et al., 2000; Le Roith et al., 2001; Hammon et al., 2012). Enhanced MR feeding stimulates the systemic somatotrophic axis in the preruminant calf (Bartlett et al., 2006; Maccari et al., 2015; Schäff et al., 2016). On the other hand, the local IGF and insulin system affects gastrointestinal growth and development in the preruminant calf (Blum, 2006; Penner et al., 2011; Hammon et al., 2012) and the local IGF and insulin receptor gene expression in ruminal epithelial cells is under nutritional control in ruminants (Shen et al., 2004; Naeem et al., 2012; Connor et al., 2014). However, it is not known whether an ad libitum MR feeding program influences the local gene expression of the IGF and insulin system in the ruminal epithelium of the preruminant calf. We recently published our data on the IGF-I and IGF binding proteins (**IGFBP**) as well as insulin plasma concentrations in calves, resulting in elevated IGF-I and insulin plasma concentrations during and after the intensive MR feeding period (Schäff et al., 2016). In the present study we hypothesized that the intensive MR feeding for 5 wk of age followed by gradual reduction of its intake has no detrimental effects on the gastrointestinal growth and development, especially in the rumen, and that the intensive MR feeding stimulates the local IGF system in the ruminal epithelium of the preruminant calf. Therefore, we investigated feed intake, gastrointestinal growth and epithelial size, ruminal VFA concentrations, and the mRNA abundance of IGF-I, IGFBP, and the receptors for IGF-I and insulin in the ruminal epithelium at wk 9 of age before weaning to characterize differences of the gastrointestinal growth and development with respect to intensive MR feeding in the first 5 wk of age.

MATERIALS AND METHODS

Animals, Feeding, and Diets

The experimental procedures were carried out according to the German Animal Welfare law and the animal care guidelines of the State Government in

Mecklenburg-Western Pomerania, Germany (LALLF M-V/TSD/7221.3-2.1-011/12).

The experimental setup was recently described in a companion paper (Schäff et al., 2016). Briefly, 28 German Holstein ($n = 5$) or German Holstein $\times$ Charolais crossbred calves ($n = 23$) were fed either ad libitum (**ADLIB**; 3 Holstein and 11 Holstein $\times$ Charolais; 8 male and 6 female) or restricted (**RES**; 2 Holstein and 12 Holstein $\times$ Charolais; 11 male and 3 female) amounts of milk reconstituted from MR (SalvaLac MiraPro 45, Salvana Tiernahrung, Klein-Offenseth Sparrieshoop, Germany; 125 g of powder/L; Supplemental Table S1; <https://doi.org/10.3168/jds.2017-13247>) for the first 5 wk of life by an automated feeder (Förster Technik GmbH, Engen, Germany). In wk 6 and 7 the available amount of MR for ADLIB calves was reduced step by step to 6 L per day and kept constant afterward. The RES group received up to 6 L per day for the whole experimental time by automated feeder (Förster Technik GmbH). Calves were housed on straw bedding in one pen with 2 compartments and 2 feeding stations for young calves (up to 3 wk of age) and older calves (from wk 4 of age on), respectively. Calves of both groups were mixed in the same compartments and used the same feeding stations for young and older calves, respectively. All calves had free access to water and concentrate (Kälber Start 18/3, Vollkraft Mischfuttermwerke, Karstädt, Germany; Supplemental Table S1; <https://doi.org/10.3168/jds.2017-13247>). Concentrate was provided by automated feeders (Förster Technik GmbH).

Measurement of daily feed intake and weekly weight gain as well as prophylactic treatments were described recently (Schäff et al., 2016). The nutrient compositions of the MR and concentrate were analyzed according to the Weender standard procedure (Naumann and Bassler, 2004). Total intake of ME was calculated according to Drackley (2008).

Tissue Sampling and Analyses

Calves were slaughtered before weaning on d 60 of age [exact age was (LSM $\pm$ SE) 59.8 ± 0.6 d for ADLIB and 60.1 ± 0.7 d for RES calves]. Empty rumen, omasum, and abomasum weight as well as weight and length of the small intestine were determined. Samples of the rumen [atrium ruminis (atrium), saccus ventralis (ventral rumen sac), and saccus cecus caudoventralis (ventral blind sac)] and small intestine (duodenum; proximal, mid, and distal jejunum; and ileum) were collected and cut in 1-cm² pieces and fixed in 4% formaldehyde solution for morphometrical measurements. Additional samples from the 3 rumen areas were stored at -80°C for gene expression measurements.

Ruminal fluid (50 mL) was sieved through a tea strainer to remove feed particles larger than mesh size (0.3 mm), and the pH was measured immediately after collecting (Elektrode SenTix81, Wissenschaftlich Technische Werkstätten, Weilheim, Germany) in the slaughterhouse. The ruminal fluid was centrifuged ($4,347 \times g$, 4°C , 10 min; Heraeus Multifuge 3 S-R, Kendro Laboratory Products, Osterode, Germany) and was stored at -20°C until measurement of VFA. After thawing, the rumen fluid was again centrifuged ($4,347 \times g$, 4°C , 10 min). The supernatant (5 mL) was transferred in another vial, 2 mL of internal standard solution (1 L consisting of 5 g of 4-methylpentanoic acid, Merck-Schuchardt, Hohenbrunn, Germany) and 200 g of phosphoric acid (≥ 85 wt. % in H_2O ; Sigma-Aldrich) were added and mixed immediately, then again centrifuged ($4,347 \times g$, 4°C , 10 min). The resulting supernatant was transferred into 2 tubes (1.5 mL) and centrifuged at room temperature ($15,700 \times g$, 20 min; Centrifuge 5415R, Eppendorf, Hamburg, Germany). Supernatant was transferred to autosampler vials and used for determination of VFA (acetate, propionate, and butyrate) by GC on a GC-17A (Shimadzu Corporation, Kyoto, Japan; Ryan, 1980).

Samples of the rumen epithelium of the atrium, ventral sac, and ventral blind sac were taken for determination of papilla length, width, density, and surface (Zitnan et al., 1998). In the intestine, villus circumference, cut surface area, height, and crypt depth were measured and the villus height-to-crypt depth ratio was calculated in the mucosa of the duodenum, proximal, mid and distal jejunum, and ileum as described previously (Zitnan et al., 2008). Briefly, samples were fixed in a 4% neutral formaldehyde solution, rinsed with water, dehydrated in a graded series of ethanol (30, 50, 70, 90%, and absolute ethanol), cleared in benzene, and saturated with and embedded in paraffin. Of each sample, sections of $5 \mu\text{m}$ in thickness (10 slices of each sample) were stained with hematoxylin and eosin. The size of the papilla (length, width, density, and surface) and villi (circumference, cut surface area, height) as well as crypt depth were determined in 30 papillae (rumen) and 30 villi with corresponding crypts (intestine), respectively, by the computer-operated Image C picture analysis system (Imtronic, Berlin, Germany) and the IMES analysis program using a color video camera (Sony 3 CCD, Sony Electronics, Tokyo, Japan) and a light microscope (Axiolab, Carl Zeiss, Jena, Germany). Rumen papilla length was measured from the apex to the base of the papillae and papillae width was measured at mid-papillae length. Number of papillae per cm^2 was estimated with a video camera by an image analysis system. The total surface of papillae per cm^2 was determined as length $\times$ width $\times$ 2,

multiplied by the number of papillae per cm^2 (Hofmann and Schnorr, 1982). In the intestine, villus height was measured from the villus tip to the villus-crypt interface and the crypt depth was measured from the villus-crypt opening to the lamina propria. Villus circumference was measured from the beginning of the villus base all around the perimeter to the end of the villus. Cut surface area of the villus was determined using a computer-operated Image C picture analysis system and the IMES analysis program (Zitnan et al., 2005). Accuracy of either 30 papillae or 30 villi was tested in previous studies, and the coefficient of variation for measurements in the intestine could be reduced lower than 20%, if at least 30 villi and crypts were evaluated (Blättler et al., 2001).

Tissue preparation for mRNA quantification was recently described by Schäff et al. (2016). Briefly, total RNA was extracted from nitrogen-frozen, homogenized powdered tissue of the atrium, ventral rumen sac, and ventral blind sac with Trizol Reagent (Life Technologies, Darmstadt, Germany). Integrity and quality of total RNA was confirmed by gel electrophoresis and by measuring the RNA integrity number with a bioanalyzer using the Agilent RNA 6000 Nano Kit (Agilent Bioanalyzer 2100, Hamburg, Germany). Mean RNA integrity number for the atrium, ventral rumen sac, and ventral blind sac were (mean $\pm$ SE) 7.9 ± 0.1 , 7.4 ± 0.1 , and 6.8 ± 0.1 , respectively. The quantity and quality of the total RNA were also measured by the optical density via spectrophotometer (NanoPhotometer, Implen GmbH, Munich, Germany) at 260:280, which was 1.8 except for one sample of ventral rumen sac, which was therefore excluded from further analyses. For cDNA synthesis, 750 ng of RNA was reverse-transcribed with 200 U of Reverse Transcriptase MMLV-RT RNase (H-) Point Mutant (Promega, Madison, WI) and 250 pmol of random hexamer primer (Metabion International AG, Planegg-Steinkirchen, Germany). The cDNA was diluted 1:4 with DEPC water and aliquots were stored at -80°C . Specific primers were used to measure the mRNA expression of insulin-like growth factor 1 (*IGF1*), insulin-like growth factor binding protein 1 (*IGFBP1*), insulin-like growth factor binding protein 2 (*IGFBP2*), insulin-like growth factor binding protein 3 (*IGFBP3*), insulin-like growth factor binding protein 4 (*IGFBP4*), insulin-like growth factor 1 receptor (*IGF1R*), insulin receptor (*INSR*), and sodium/glucose cotransporter 1 (*SLC5A1*). Primers were designed with Primer3 v.0.4.0 (Untergasser et al., 2012) or taken from literature as shown (Supplemental Table S2; <https://doi.org/10.3168/jds.2017-13247>).

Real-time quantitative reverse-transcription PCR was performed using FastStart DNA Master Plus SYBR Green I MasterMix (Roche Diagnostics, Mannheim,

Germany) with 2 μ L of cDNA. Each cDNA sample was analyzed in duplicate. To verify specific PCR products, a melting curve analysis was performed after the last amplification cycle. Furthermore, the product purity and size were confirmed using agarose gel electrophoresis, showing a single band at the expected size, followed by sequencing (ABI 3130 Genetic Analyzer, Life Technologies). The efficiency was calculated using LinRegPCR 2013 software (Ruijter et al., 2009). Samples with an efficiency below 1.8 were discarded, and the preparation was repeated. Data of one calf of the ADLIB group for ventral rumen sac were excluded from analysis because of poor quality. For each sample, a crossing point (quantification cycle) value, which represents the intersection of a fixed threshold above background fluorescence and the amplification curve, was determined by LightCycler analysis software 4.05 (F. Hoffmann-La Roche AG, Basel, Switzerland). The quantification cycle values were imported to qBase+ software and the selection of appropriate reference genes and the quantification of the data were performed using this software (Biogazelle NV, Zwijnaarde, Belgium; Saremi et al., 2012). Low-density lipoprotein receptor-related protein 10 (*LRP10*), emerin (*EMD*), and hippocalcin-like protein 1 (*HPCAL1*) served as reference genes. The geometric mean of the reference gene abundances was used for normalization. Data are presented as the ratio of the copy numbers of genes of interest and the geometric mean of the reference genes' abundances.

Statistical Analyses

All results are presented as least squares means (LSM) with their standard error. Performance and tissue data were evaluated with the Mixed Model procedure of SAS software, version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC) with MR feeding intensity, time (i.e., week of life for performance data) or region (for measurements in tissues), and respective interaction as well as sex and breed as fixed effects. Because calves were not balanced for sex and breed, the outcome concerning genetic and sex effects with respect to intensive MR feeding is of minor value in this study. All pair-wise differences of LSM were tested with the Tukey-Kramer procedure. The slice statement was used for partitioned analysis of interactions of the LSM. Data from rumen fluid were generated using the General Linear Model procedure of the SAS software with MR feeding intensity as the main effect. Differences of data with P -values < 0.05 were defined as significant, and P -values < 0.1 were considered as trends.

The CORR procedure of SAS software was used to calculate and test Pearson correlations by tissue region

between data on epithelial gene expression and data generated from morphometric and VFA measurements in the rumen.

RESULTS

Feeding Intake and Growth Performance

Data for milk and concentrate intake for wk 1 to 8 of age were first presented as fresh matter intake by Schäff et al. (2016). Milk intake increased ($P < 0.001$) up to wk 5 of age in ADLIB calves, but did not change in RES calves, as planned, and was much greater ($P < 0.001$) in ADLIB than in RES calves for the first 6 wk of life (Figure 1A). Concentrate intake increased ($P < 0.001$) from wk 5 onward in both groups to the end of the study, but did not differ between groups (Figure 1B). Total DMI (MR and concentrate intake) as well as ME intake (first published by Schäff et al., 2016) increased ($P < 0.001$) in ADLIB calves until wk 5, decreased ($P < 0.001$) thereafter between wk 5 and 7, and increased again ($P < 0.001$) to the end of the study, whereas in RES calves total DMI and ME intake continuously increased up to the end of the study (Figure 1C, 1D). Total ME intake was greater ($P < 0.05$) in wk 1 and total DMI and ME intake were greater from wk 2 to 6 ($P < 0.001$) and tended to be greater in wk 8 ($P = 0.1$; $P = 0.09$) in ADLIB than in RES calves. Data for growth performance were recently published in a companion paper (Schäff et al., 2016). The BW at birth was similar between the groups (LSM for birth weight was 44.0 ± 2.2 kg), increased in both groups during the experimental period, and was greater in ADLIB than in RES from wk 4 on (LSM of the BW at the end of the study was 80.2 ± 2.2 kg for ADLIB and 71.6 ± 2.5 kg for RES; Schäff et al., 2016). At slaughter, BW tended to be greater ($P < 0.1$) and carcass weight was greater ($P < 0.05$) in ADLIB than in RES calves, but weights (absolute and relative to BW) of the rumen, omasum, abomasum, and small intestine and the length of the small intestine were not affected by MR feeding intensity (Table 1). There were no significant effects on DMI and body and organ weight with respect to sex and breed.

Epithelial Growth, mRNA Abundance of the IGF System, and Short-Chain Fatty Acid Concentration in the Rumen

The papilla length, width, and surface were greatest ($P < 0.001$) in the atrium of the rumen, whereas the density of the rumen papillae was greatest ($P < 0.001$) in the ventral blind sac and was greater ($P < 0.05$) in the ventral sac than in the atrium in both groups

Figure 1. Dry matter intake of milk (A) and concentrates (B), total DMI (C), and total ME (D) of calves fed milk ad libitum (■) or restricted (○) for the first 5 wk of age. Data for ME were first published by Schäff et al. (2016). Values are shown as LSM $\pm$ SE; n = 14 per group; * indicates group effect $P < 0.05$; ** indicates group effect $P < 0.01$; *** indicates group effect $P < 0.001$.

Table 1. Body weight, weight of carcass, and absolute and relative (to BW) weight of rumen, omasum, abomasum, and small intestine and length of the small intestine on d 60 of age in calves fed milk ad libitum (ADLIB) or restrictedly (RES) for the first 5 wk of life¹

Item	Feeding intensity		SE	P-value
	ADLIB	RES		
BW	83.9	77.7	2.9	0.09
Hot carcass, left side, ² kg	22.4	20.2	0.60	0.02
Rumen, kg	1.10	1.09	0.07	0.9
Rumen, g/kg of BW	13.2	14.0	0.7	0.3
Omasum, kg	0.41	0.37	0.04	0.4
Omasum, g/kg of BW	4.87	4.70	0.45	0.8
Abomasum, kg	0.43	0.46	0.04	0.6
Abomasum, g/kg of BW	5.29	5.83	0.56	0.4
Small intestine, kg	2.67	2.46	0.13	0.13
Small intestine, g/kg of BW	31.8	33.4	1.15	0.2
Small intestine, m	19.5	18.2	0.91	0.3

¹Values are presented as LSM $\pm$ SE; n = 14 per group.

²First published by Schäff et al. (2016).

(Table 2). The density of the rumen papillae in atrium and ventral sac was greater ($P < 0.05$) in RES than in ADLIB calves. The papilla size was not affected by breed or by sex.

A tendency ($P < 0.1$) to interaction for abundance of IGF1 mRNA between treatment and rumen section was shown, most likely due to greater IGF1 abundance ($P < 0.1$) in the atrium of ADLIB than of RES calves, and in ADLIB calves mRNA abundance was greater ($P < 0.05$) in the atrium than in the ventral blind sac (Table 3). The rumen segment tended to affect ($P < 0.1$) mRNA abundance of *IGFBP3* and *IGFBP4* and affected ($P < 0.05$) mRNA abundance of *IGFR1*. The *IGFR1* mRNA abundance was greater ($P < 0.05$) in the ventral blind sac than in the atrium (all calves), and in the ventral sac tended to be greater ($P < 0.1$) in RES than in ADLIB calves. The mRNA abundance of *IGFBP1*, *IGFR1*, and *INSR* were affected by breed,

Table 2. Papilla length, width, density, and surface in rumen of calves fed milk either ad libitum (ADLIB) or restrictedly (RES) for the first 5 wk of life¹

Item	Feeding intensity			<i>P</i> -value		
	ADLIB	RES	SE	Rumen segment	Feeding intensity	Segment × intensity
Papilla length, mm						
Atrium ruminis	3.23 ^A	3.13 ^A	0.10	0.001	0.6	0.09
Ventral sac	2.26 ^B	2.14 ^B	0.10			
Ventral blind sac	2.10 ^B	2.15 ^B	0.10			
Papilla width, mm						
Atrium ruminis	1.04 ^A	1.02 ^A	0.03	0.001	0.4	0.8
Ventral sac	0.75 ^B	0.70 ^B	0.03			
Ventral blind sac	0.73 ^B	0.70 ^B	0.03			
Papilla surface, mm ² /cm ²						
Atrium ruminis	551 ^A	566 ^A	21.4	0.001	0.9	0.5
Ventral sac	332 ^B	317 ^B	21.4			
Ventral blind sac	317 ^B	329 ^B	21.4			
Papilla density, no./cm ²						
Atrium ruminis	82.9 ^{C,b}	88.9 ^{C,a}	2.4	0.001	0.04	0.6
Ventral sac	98.8 ^{B,b}	104.8 ^{B,a}	2.4			
Ventral blind sac	104.6 ^A	108.9 ^A	2.4			

^{A-C}For each parameter, LSM within a column with different uppercase letters differ ($P < 0.05$) among rumen segments.

^{a,b}LSM within a row with different lowercase letters differ ($P < 0.05$) between groups.

¹Values are presented as LSM ± SE; n = 14 per group.

showing a greater mRNA abundance ($P < 0.05$ or lower) in Holstein × Charolais than in purebred Holstein calves, respectively (data not shown). The mRNA abundance of *SLC5A1* did not provide any significant differences.

The mRNA abundance of *IGF1* was negatively correlated with papilla width ($r = -0.41$; $P < 0.05$) in the ventral blind sac and to papilla length ($r = -0.47$; $P < 0.01$) and surface ($r = -0.40$; $P < 0.05$) in the ventral sac of the rumen, but was positively related to papilla density in the ventral blind sac and ventral sac ($r = 0.38$; $P < 0.05$, respectively).

The rumen pH (LSM ± SE was 5.94 ± 0.13) and concentrations of VFA were not affected by group, breed, or sex. Concentrations of acetate, propionate, butyrate, and total VFA were 38.0 ± 2.5 , 21.8 ± 2.4 , 17.3 ± 3.0 , and 83.3 ± 6.5 mmol/L (LSM ± SE), respectively (Supplemental Table S3; <https://doi.org/10.3168/jds.2017-13247>). The mRNA abundance of *IGF1* in the atrium was positively related to acetate ($r = 0.46$; $P < 0.05$), butyrate ($r = 0.34$; $P < 0.1$), and total VFA concentration ($r = 0.41$; $P < 0.05$), and *IGF1* mRNA abundance in the ventral sac was positively related to rumen butyrate concentration ($r = 0.39$; $P < 0.05$). The concentration of butyrate in the rumen was positively related to plasma IGF-I concentration ($r = 0.42$; $P < 0.05$). The plasma IGF-I concentration was recently published in a companion paper (Schäff et al., 2016) and in Supplemental Table S3 (<https://doi.org/10.3168/jds.2017-13247>).

Intestinal Mucosa Size

Villus circumference, cut surface area, and height as well as the villus height/crypt depth ratio increased ($P < 0.001$) along the small intestine, increased from duodenum to mid-jejunum, showed the greatest size in mid-jejunum, and decreased thereafter to the distal jejunum or ileum (villus height/crypt depth ratio), whereas crypt depth was lowest ($P < 0.001$) in the mid-jejunum and greatest in duodenum and ileum (Table 4). In mid-jejunum, villus circumference was greater ($P < 0.05$) in ADLIB than in RES calves. In distal jejunum, cut surface area of the villi and the villus height/crypt depth ratio were greater ($P < 0.05$) and the villus circumference and height tended to be greater ($P < 0.1$), whereas crypt depth was smaller ($P < 0.05$) in ADLIB than in RES calves. The measured villus parameters (circumference, cut surface area, and height) were greater ($P < 0.05$) in Holstein × Charolais than in purebred Holstein calves, respectively (data not shown).

DISCUSSION

Feeding of unlimited amounts MR for the first 5 wk of age enhanced feed intake and body growth in dairy calves when compared with restricted MR feeding (750 g of MR powder/d; Schäff et al., 2016), as also seen in previous studies (Hammon et al., 2002; Jasper and Weary, 2002; Maccari et al., 2015). The intensive milk

Table 3. Relative mRNA expression in rumen papillae of calves fed milk either ad libitum (ADLIB) or restrictedly (RES) for the first 5 wk of life¹

Item ²	Feeding intensity			<i>P</i> -value		
	ADLIB	RES	SE	Rumen segment	Feeding intensity	Segment × intensity
<i>IGF1</i>						
Atrium ruminis	4.24	3.48	0.34	0.3	0.6	0.1
Ventral sac	3.58	3.67	0.34			
Ventral blind sac	3.43	3.59	0.34			
<i>IGFBP1</i>						
Atrium ruminis	1.54	1.53	0.11	0.9	0.9	0.8
Ventral sac	1.53	1.50	0.11			
Ventral blind sac	1.52	1.61	0.11			
<i>IGFBP2</i>						
Atrium ruminis	1.66	1.77	0.15	0.8	0.5	0.7
Ventral sac	1.69	1.66	0.15			
Ventral blind sac	1.65	1.84	0.15			
<i>IGFBP3</i>						
Atrium ruminis	2.25	2.14	0.24	0.09	0.5	0.5
Ventral sac	2.23	2.24	0.24			
Ventral blind sac	2.00	1.87	0.24			
<i>IGFBP4</i>						
Atrium ruminis	2.26	2.12	0.24	0.09	0.5	0.4
Ventral sac	2.23	2.57	0.24			
Ventral blind sac	2.48	2.74	0.24			
<i>IGF1R</i>						
Atrium ruminis	1.95	1.97	0.16	0.03	0.4	0.3
Ventral sac	1.91	2.27	0.16			
Ventral blind sac	2.31	2.29	0.16			
<i>INSR</i>						
Atrium ruminis	2.02	2.10	0.13	0.5	0.4	0.9
Ventral sac	2.06	2.20	0.13			
Ventral blind sac	2.17	2.21	0.13			
<i>SLC5A1</i>						
Atrium ruminis	3.36	3.17	0.60	0.4	0.9	0.9
Ventral sac	3.53	3.71	0.60			
Ventral blind sac	4.00	3.90	0.60			

¹Values shown are LSM ± SE; n = 14 per group.

²*IGFBP1* = insulin-like growth factor binding protein 1; *IGFBP2* = insulin-like growth factor binding protein 2; *IGFBP3* = insulin-like growth factor binding protein 3; *IGFBP4* = insulin-like growth factor binding protein 4; *IGF1R* = insulin-like growth factor 1 receptor; *INSR* = insulin receptor; *SLC5A1* = sodium-dependent glucose cotransporter 1.

feeding programs often compromised solid feed intake (Kristensen et al., 2007; Gelsinger et al., 2016; Khan et al., 2016). However, solid feed intake during the first 4 wk of age is of minor importance in dairy calves and several studies indicated an improved solid feed intake (concentrate or forage) after the intensive milk feeding period (Jasper and Weary, 2002; Khan et al., 2007; Maccari et al., 2015). In addition, automated feeding systems allow individual weaning of calves, resulting in an improved solid feed intake (de Passillé and Rushen, 2016). In the present study, the concentrate intake was not affected by the intensive MR feeding and was similar between RES and ADLIB calves when MR intake was reduced in ADLIB calves after wk 5 of age. Some previous studies also indicated no impaired concentrate intake during the intensive milk feeding period using automated feeding systems (Maccari et al., 2015; Korst et al., 2017). One reason for this finding might be the allocation of MR and concentrate in small portions by

automated feeding systems. Finally, differences in feed intake and their effects on body growth and energy metabolism in the present study were driven by the differences in MR intake during the first 5 wk of age as discussed in the companion paper (Schäff et al., 2016).

The elevated body growth resulted in a greater carcass weight and an enhanced muscle and fat tissue growth in ADLIB than in RES calves at wk 9 of age (Schäff et al., 2016). In contrast to the carcass, the size of the gastrointestinal tract did not differ between groups in the present study and a reduced growth of the forestomach tissue could be expected with respect to previous studies (Baldwin et al., 2004). In contrast, intensive milk or MR feeding in preruminant calves stimulated the growth of the gastrointestinal tract in several studies (Khan et al., 2007; Naeem et al., 2012; Geiger et al., 2016). In the present study, the growth and development of the forestomach were not affected by ad libitum MR feeding for 5 wk followed by gradual

reduction of MR intake. The comparable VFA concentrations in the rumen of ADLIB and RES calves at the end of the experiment may support the finding of a similar rumen development in ADLIB and RES calves. There was probably a difference in forestomach development at the first month of age, when the BHB plasma concentration, which indicates ketogenesis from butyrate in the rumen wall (Baldwin et al., 2004; Khan et al., 2007; Penner et al., 2011), increased in RES but not in ADLIB calves (Schäff et al., 2016). However, the greater BHB plasma concentration in RES calves was transient and plasma BHB in ADLIB calves caught up from wk 6 of age onward (Schäff et al., 2016). This finding corresponded with the similar concentrate intake in both groups, because VFA production in the rumen of preruminant calves depends on concentrate intake (Khan et al., 2016). Therefore, the enhanced MR feeding in ADLIB calves during the first 5 wk of age did not compromise rumen development as measured on wk 9 of age. Obviously, there is an ontogenic response to rumen development, besides the well-known diet-

induced influences on rumen function (Baldwin et al., 2004; Khan et al., 2016). Factors other than the dietary input are presently under discussion that may affect rumen development, including ontogenic gene control of metabolic enzymes in the ruminal epithelium and microbial colonization in early life (Lane et al., 2002; Steele et al., 2016; Meale et al., 2017). However, the effect of intensive milk or MR feeding during the pre-weaning period on rumen growth and development after weaning depends on the weaning protocol, indicating the importance of step-down milk feeding schedules for postweaning rumen function (Khan et al., 2011; Steele et al., 2016, 2017).

A minor effect of intensive versus restricted MR feeding for 5 wk followed by gradual reduction of MR intake was also true for the development of the rumen papillae. The RES and ADLIB calves had the same papilla length, width, and surface in the atrium, ventral sac, and ventral blind sac of the rumen. This result was not surprising, because similar concentrate intake and VFA production in the rumen were most probably

Table 4. Villus circumference, cut surfaces area, and height and crypt depth in duodenum, proximal, mid, and distal jejunum, and ileum of calves fed milk either ad libitum (ADLIB) or restrictedly (RES) for the first 5 wk of life¹

Item	Feeding intensity		SE	P-value		
	ADLIB	RES		Gut segment	Feeding intensity	Segment × intensity
Villus circumference, μm						
Duodenum	1,340 ^C	1,316 ^C	20.0	0.001	0.15	0.001
Proximal jejunum	1,377 ^B	1,364 ^B	20.0			
Mid-jejunum	1,487 ^{A,a}	1,421 ^{A,b}	20.0			
Distal jejunum	1,401 ^B	1,358 ^B	20.0			
Ileum	1,391 ^B	1,373 ^B	20.0			
Villus cut surface area, μm^2						
Duodenum	64,758 ^C	63,133 ^C	1,492	0.001	0.2	0.04
Proximal jejunum	67,986 ^B	66,104 ^B	1,492			
Mid-jejunum	72,408 ^A	70,671 ^A	1,492			
Distal jejunum	69,334 ^{B,a}	65,551 ^{BC,b}	1,492			
Ileum	68,433 ^B	67,285 ^B	1,492			
Villus height, μm						
Duodenum	548 ^C	539 ^C	11.7	0.001	0.3	0.01
Proximal jejunum	575 ^B	570 ^B	11.7			
Mid-jejunum	611 ^A	592 ^A	11.7			
Distal jejunum	589 ^B	563 ^B	11.7			
Ileum	584 ^B	573 ^B	11.7			
Crypt depth, μm						
Duodenum	253 ^A	257 ^A	2.8	0.001	0.15	0.01
Proximal jejunum	232 ^B	234 ^{BC}	2.8			
Mid-jejunum	228 ^C	231 ^C	2.8			
Distal jejunum	230 ^{BC,b}	239 ^{B,a}	2.8			
Ileum	258 ^A	262 ^A	2.8			
Villus height/crypt depth						
Duodenum	2.18 ^C	2.10 ^C	0.07	0.001	0.2	0.001
Proximal jejunum	2.48 ^B	2.45 ^B	0.07			
Mid-jejunum	2.70 ^A	2.58 ^A	0.07			
Distal jejunum	2.57 ^{B,a}	2.37 ^{B,b}	0.07			
Ileum	2.27 ^C	2.19 ^C	0.07			

^{A-C}For each parameter, LSM within a column with different uppercase letters differ ($P < 0.05$) among gut segments.

^{a,b}LSM within a row with different lowercase letters differ ($P < 0.05$) between groups.

¹Values are presented as LSM $\pm$ SE; n = 14 per group.

responsible for the similar rumen papilla size (Baldwin et al., 2004). Butyrate is the main driving force for papilla growth in the rumen (Guilloteau et al., 2009a; Górka et al., 2011; Penner et al., 2011), but butyrate concentration did not differ between groups in the present study. Only the density of the papillae in the atrium and ventral sac of the rumen was slightly greater in RES than in ADLIB calves. This finding may point at a somehow smaller papilla size, but not surface area, in RES than in ADLIB calves, although papilla length and width did not differ between groups. However, greater papilla density may point at a better rumen development in RES calves because of more papillae of the same size. Nevertheless, the intensive MR feeding during first 5 wk of age did not impair papilla growth in the rumen of ADLIB calves as indicated by measurements in wk 9 of age. The absence of differences in rumen papilla growth due to variable MR feeding was also published by Silper et al. (2014), but the difference in MR intake described in this study was much smaller than in the present study. The greater papilla size in the atrium compared with papilla size in the ventral sac and ventral blind sac of the rumen was in agreement with previous studies in calves and goats (Shen et al., 2004; Zitnan et al., 2005).

Increasing evidence from literature indicates that the rumen papilla growth is under the control of the local and systemic IGF system (Baldwin et al., 2004; Penner et al., 2011; Steele et al., 2016). The minor differences in gene expression with respect to the local somatotrophic axis due to intensive MR feeding corresponded to the lack of differences in papilla size between groups. However, Naeem et al. (2012) found a greater *INSR* gene expression in the rumen with enhanced MR and starter feeding. With respect to all 28 calves, some negative correlations were present between mRNA abundance of *IGF1* and papilla size in the ventral sac and ventral blind sac of the rumen, whereas the opposite effect (positive correlation) was the case for *IGFBP1* and *IGFBP2* mRNA abundance and papilla size in distinct rumen regions. On the other hand, plasma IGF-I concentration was greater in ADLIB than in RES calves at the end of the study (Schäff et al., 2016). There is probably an interaction between systemic and local action of the somatotrophic axis that both contribute to the regulation of rumen papilla growth (Le Roith et al., 2001; Shen et al., 2004; Steele et al., 2016). Because nutrient intake was the same in ADLIB and RES calves at the end of the study, no conspicuous differences in the local or systemic action of the somatotrophic axis could be expected. However, rumen VFA concentrations were positively related to the local *IGF1* mRNA abundance in the ventral sac, and rumen butyrate was positively related to plasma IGF-I concentration, indicating the

interaction of rumen development and local and systemic growth regulation in growing cattle (Penner et al., 2011).

Recent studies in preruminant calves indicated that intensive MR feeding affects intestinal growth (Geiger et al., 2016). The greater villus size and villus height/crypt depth ratio in the distal parts of the jejunum in ADLIB than in RES calves support these findings. It is well known that colostrum feeding affects small intestinal villus size when compared with iso-energetic formula or MR feeding (Blum, 2006; Blum and Baumrucker, 2008; Ontsouka et al., 2016). In addition, supplementation of butyrate, besides prolonged milk feeding, can affect villus growth in the preruminant calf (Guilloteau et al., 2009a,b). In a recent study, villus growth in the small intestine of calves was stimulated by both ad libitum MR feeding and butyrate treatment (Gerbert et al., 2017). Obviously, the response on villus size is prolonged in the small intestine when milk feeding is enhanced in dairy calves. Previous studies indicate a regulatory effect of the IGF system on intestinal mucosa growth (Blum, 2006; Blum and Baumrucker, 2008; Ontsouka et al., 2016), and possibly other para- and endocrine factors are also involved in intestinal mucosa growth regulation and development (Steele et al., 2016). Unfortunately, we did not study mucosal growth regulation by the local IGF system in the small intestine of these calves, but previous studies indicate an effect of the local somatotrophic axis on intestinal growth regulation in calves (Georgiev et al., 2003; Flaga et al., 2011). Villus size was affected by the genetic background of the calves in the present study, showing larger villi in Holstein $\times$ Charolais than in purebred Holstein calves. This finding is of interest because in a previous study, greater villi in the small intestine were reported in Charolais than in Holstein bulls (Zitnan et al., 2008). Further studies are necessary to validate a general trend of a genetic effect on villus size in cattle.

In conclusion, intensified MR feeding for a 5-wk period immediately after birth stimulated DMI and body growth and did not impair rumen development at wk 9 of age in dairy calves. Consistent with these findings, ruminal expression of genes related to the somatotrophic axis did not differ between feeding groups, which was in contrast to the stimulating effect on the systemic IGF-I and insulin system in these calves (Schäff et al., 2016). Therefore, ad libitum milk or MR feeding can be performed in dairy calves for several wk during the early postnatal period without the risk of an insufficient rumen function in further life. The intensified MR feeding for 5 wk affected the villus size in the distal part of the small intestine; this was potentially a result of the greater nutrient intake in ADLIB than

in the RES calves. Whether differences in distal villus size were regulated by the local somatotrophic axis needs further investigation.

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