Effect of increasing the proportion of dietary concentrate on gastrointestinal tract measurements and brush border enzyme activity in Holstein steers

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ABSTRACT

The aim of this study was to determine the time course for adaptation of the reticulo-rumen, omasum, abomasum, and small intestine in response to an abrupt increase in the proportion of grain in the diet. Adaptive responses include tissue and digesta mass, small intestinal length, and brush border enzyme activity in the duodenum, proximal jejunum, and ileum. Twenty-five Holstein steers (213 ± 23 kg; 5 to 7 mo of age) were blocked by body weight, and within block were randomly assigned to 1 of 5 treatments: the control diet (CTRL; 92% chopped grass hay and 8% mineral and vitamin supplement on a dry matter basis) or a moderate grain diet (MGD; 50% chopped grass hay, 42% rolled barley grain, and 8% mineral and vitamin supplement) that was fed for 3 (MGD3), 7 (MGD7), 14 (MGD14), or 21 d (MGD21). Dry matter intake was limited to 2.25% of body weight to ensure that changes in dry matter intake did not confound the results. On the last day of the dietary exposure, steers were slaughtered 2 h after feeding. Reticulo-rumen tissue mass and ruminal epithelium mass in the ventral sac of the rumen were not affected by the MGD. Wet reticulo-ruminal digesta mass decreased from CTRL to MGD7 and then increased, but reticulo-ruminal digesta dry matter mass did not differ between treatments. Omasal mass, omasal tissue mass, and omasum digesta mass decreased linearly with the number of days fed MGD, but abomasal tissue mass tended to increase linearly. Duodenal tissue mass tended to increase linearly, and ileal length increased linearly with the number of days fed MGD. Lactase activity in the proximal jejunum increased linearly and maltase activity in duodenum tended to increase linearly with days fed MGD. Amino-

peptidase N activity in the proximal jejunum increased cubically with days fed MGD, and dipeptidylpeptidase IV activity in ileum tended to decrease from CTRL to MGD14 and then tended to increase. Adaptation to a diet with a greater proportion of concentrate involves changes in the mass and length of regions of the gastrointestinal tract and brush border enzyme activity. These changes take place gradually over at least 3 wk.

Key words: starch, adaptation, forestomach, intestine

INTRODUCTION

To increase dietary energy density, diets for high-yielding dairy cows and growing and finishing beef cattle commonly include substantial quantities of grain (Steele et al., 2011; Schwaiger et al., 2013). Although this feeding strategy is necessary to achieve high levels of production, feeding highly fermentable diets poses a challenge for the gastrointestinal tract (GIT) epithelium in ruminants (Steele et al., 2011; Liu et al., 2013; Metzler-Zebeli et al., 2013). Consumption of highly fermentable carbohydrates increases the rate of short-chain fatty acid (SCFA) production in the rumen, which may lead to SCFA accumulation in the rumen and a reduction in ruminal pH (Aschenbach et al., 2011). Additionally, diets with moderate to high grain content may increase lactic acid production in the rumen and reduce saliva secretion, further reducing ruminal pH (Liu et al., 2013; Chibisa et al., 2016). This situation, commonly referred as subacute rumen acidosis, may lead to less efficient fiber digestion (Calsamiglia et al., 2002; Zebeli et al., 2008), reduced GIT barrier function (Wilson et al., 2012; Liu et al., 2013), translocation of pathogens and endotoxins into the systemic circulation (Plaizier et al., 2008; Li et al., 2012), and liver abscesses (Nagaraja and Titgemeyer, 2007; Plaizier et al., 2008). Furthermore, as the proportion of grain in the diet increases, the amount of starch entering and fermented in the hindgut also increases (Harmon, 2009; Brake et al., 2014). Increased hindgut fermentation can lead to a reduction in large intestinal pH, an increase in endotoxin

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concentration in the digesta, and a corresponding reduction in the expression of tight-cell junction proteins (Li et al., 2012; Metzler-Zebeli et al., 2013).

Cattle should be gradually adapted to diets that are more fermentable (Steele et al., 2011; Schwaiger et al., 2013). Reasons for this include the fact that microbial adaptation and morphological adaptation of the ruminal epithelium require 3 to 9 wk (Dirksen et al., 1985; Tajima et al., 2001; Bannink et al., 2008), and that functional adaptation, although much faster, proceeds over at least 3 wk after a dietary change (Etschmann et al., 2009; Schurmann et al., 2014; Walpole et al., 2015). Although it has been rarely investigated, small and large intestinal tissue mass increases in the first week of lactation, a period associated with an increase in concentrate intake (Reynolds et al., 2004). Therefore, adaptation of the lower regions of the GIT may also require time. However, under practical conditions, cattle often receive substantial increases in rapidly fermentable carbohydrates over a relatively short period. Examples of scenarios in which such rapid dietary change occurs include transition from the dry period to lactation, weaning, arrival at a feedlot, and adaptation to a finishing diet.

The GIT of cattle adapts to an increased proportion of grain in the diet at both morphological and cellular (functional) levels. Increased consumption and the subsequent fermentation of rapidly fermentable carbohydrate results in greater proliferation of ruminal epithelial cells, greater ruminal papillae dimensions, and, in consequence, greater surface area for SCFA absorption (Goodlad, 1981; Bannink et al., 2008; Malhi et al., 2013). The increased surface area of the ruminal epithelium also translates to greater reticulo-ruminal tissue mass (Stobo et al., 1966; Reynolds et al., 2004; Malhi et al., 2013). Furthermore, concentrate intake increases the permeability of ruminal epithelium to SCFA (Amat et al., 2014) by increasing passive and anion exchange pathways for SCFA absorption (Schurmann et al., 2014). Moderate to high grain intake has also been associated with greater abomasal and small intestinal tissue mass (Stobo et al., 1966; Reynolds et al., 2004), indicating a greater importance of those regions of the GIT when the plane of nutrition increases. Additionally, supplementation with concentrate has been reported to increase mRNA expression of sugar transporters in the small intestinal epithelium (Li et al., 2016), and increased provision of concentrate (and its associated greater energy intake) increases brush border enzyme activity (Kreikemeier et al., 1990). On the other hand, lower omasal tissue mass has been observed when the proportion of forage in the diet decreased (Hamada et al., 1976; Bailey, 1986; McLeod and Baldwin, 2000). As a result, adaptation of not only the reticulo-rumen but

also the omasum, abomasum, and intestine may be important in determining the efficiency of dietary nutrient use, especially when grain intake is rapidly increased.

An abrupt increase in fermentable carbohydrate intake may have a detrimental effect on ruminal function, because it leads to a reduction in pH, damage of the ruminal epithelium, and less efficient SCFA absorption (Krehbiel et al., 1995; Steele et al., 2011; Schwaiger et al., 2013). At least several days are needed for the ruminal epithelium to recover after bout of ruminal acidosis (Steele et al., 2011; Schwaiger et al., 2013), but less efficient SCFA absorption may be observed for up to the next 3 mo (Krehbiel et al., 1995). This may delay adaptation of the reticulo-rumen (Schwaiger et al., 2013) and other regions of the GIT to dietary change. However, so far, only a limited number of studies have evaluated the time course of changes in the structure and function of the GIT in cattle in response to dietary change (Reynolds et al., 2004; Etschmann et al., 2009; Steele et al., 2011). Knowledge of how the GIT adapts to increased dietary fermentability may increase our knowledge of factors that limit the efficiency of nutrient use in intensively fed cattle.

We hypothesized that the tissue mass of the reticulo-rumen, abomasum, and small intestine, small intestinal length, and brush border enzyme activity would increase and omasal tissue mass would decrease in response to an abrupt exposure to a diet containing a higher proportion of grain. We further hypothesized that those changes would progress gradually over at least 21 d. The aim of this study was to determine the time course for adaptation of the reticulo-ruminal, omasal, and abomasal tissue mass, small intestinal mass and length, and intestinal brush border enzyme activity in response to an abrupt increase in the proportion of grain in the diet. We also investigated the time course of adaptation for the reticulo-ruminal, omasal, and abomasal digesta mass.

MATERIALS AND METHODS

The steers used for this study were cared for in accordance with the Canadian Council for Animal Care guidelines, and all experimental procedures were pre-approved by the University of Saskatchewan Animal Research Ethics Board (protocol 20100021).

Steers and Experimental Diets

Steers used for this study and the experimental procedures have been described elsewhere (Plaizier et al., 2014; Schurmann et al., 2014; Walpole et al., 2015). Briefly, 25 Holstein steers (213 ± 23 kg; 5 to 7 mo of age) fed a common high-forage diet (92% grass hay,

8% barley-grain-based vitamin and mineral supplement, on a DM basis) for at least 5 wk were blocked by BW and randomly assigned to 1 of 5 treatments: the control diet (**CTRL**; 92% chopped grass hay and 8% mineral and vitamin supplement with barley grain used as a carrier; DM basis) or a moderate grain diet (**MGD**; 50% chopped grass hay, 42% rolled barley grain, and 8% mineral and vitamin supplement) that was fed for 3 (**MGD3**), 7 (**MGD7**), 14 (**MGD14**), or 21 d (**MGD21**). Diets were fed at 2.25% of BW once per day (0800 h) and were formulated to meet nutrient requirements to achieve a growth rate of at least 0.5 kg/d (NRC, 2000). Because diets differed in forage-to-concentrate ratio but DMI was held constant, diets were not isoenergetic. Steers were kept in individual pens (12.2 m²) with floors covered with rubber mats for at least 14 d before slaughter. Once in the individual pens, steers' feed intake was limited. Feed intake was recorded daily (with the exception of the day of slaughter), and water was available ad libitum throughout the study period.

Measurements and Sample Collection

On the last day of dietary exposure, steers were slaughtered 2 h after feeding by captive bolt stunning followed by pithing and exsanguination. The entire forestomach, abomasum, and small intestine were dissected. The reticulo-ruminal contents were removed, reticulo-ruminal tissue and digesta were weighed, and 2 representative samples of the reticulo-ruminal digesta (1 kg each) were collected and kept frozen (−20°C) for analysis. The omasal and abomasal regions were weighed full and after removal of the digesta. To facilitate the empty weight measurement, each stomach compartment was rinsed repeatedly with water, water was pressed by hand and shaken down 2 times, and then each compartment was weighed individually. Then, 2 pieces of whole tissue (5 × 5 cm) from the ventral sac of the rumen were collected and kept frozen (−20°C) for analysis. The small intestine was divided into the duodenum (with the end determined by the ligament of Treitz), jejunum (with the end determined based on the ileocecal fold), and ileum (with the end at the ileocecal junction). Small intestinal tissues were carefully separated from the intestinal mesentery, emptied, measured, and weighed individually. Epithelial samples from the midpoint of the duodenum, the proximal jejunum (2 m from the end of duodenum), and the midpoint of the ileum were scraped using a microscope slide. The samples were then frozen in liquid nitrogen and stored at −80°C. Ruminant tissue was completely thawed, and the epithelium was manually stripped from the muscle layers. Both epithelium and

muscle layers were dried in a forced-air oven at 105°C until they achieved a constant weight to determine epithelium and muscle DM. Reticulo-ruminal digesta samples were dried in a forced-air oven (at 55°C until they achieved a constant weight) to determine digesta DM concentration and reticulo-ruminal DM content. Reticulo-ruminal DM and moisture content were calculated as follows: reticulo-ruminal DM content (kg) = reticulo-ruminal digesta content (kg) × reticulo-ruminal digesta DM concentration (%) / 100 (%) and reticulo-ruminal moisture content (kg) = reticulo-rumen digesta (kg) − reticulo-ruminal digesta DM content (kg).

Brush Border Enzyme Activity

The total protein content and activity of selected brush border enzymes in the intestinal epithelial scrapings were determined as described previously (Górka et al., 2011). Briefly, 1 g of epithelial scrapings was homogenized with 5 mL of distilled water and centrifuged for 5 min at 1,000 × *g* at 4°C to obtain a supernatant. The supernatant was used to determine the total protein and brush border enzyme activity. The total protein content was determined according to Hartree (1972) using bovine serum albumin as a standard. Lactase and maltase activities were determined according to Dahlqvist (1984) with minor modifications (Górka et al., 2011). Amino-peptidase A, amino-peptidase N, and dipeptidyl-peptidase IV activity were determined using L-glutamyl-*p*-nitroanilide, L-leucyl-*p*-nitroanilide, and L-glycyl-*p*-nitroanilide (Bachem, Budendorf, Sweden) as substrates, respectively (Maroux et al., 1973; Nagatsu et al., 1976). Each sample was run in duplicate. Analysis of each enzyme was conducted in a temperature-controlled laboratory using 1 set of reagents and was completed on consecutive days until all samples were analyzed. The intraassay CV were 1.77 ± 1.08, 1.83 ± 1.43, 2.17 ± 1.75, 6.86 ± 3.38, 6.31 ± 3.01, and 6.85 ± 3.12 for total protein, maltase, lactase, amino-peptidase N, amino-peptidase A, and dipeptidyl-peptidase IV analysis, respectively. Enzymatic activity was presented as enzymatic units normalized for total protein content in the supernatant ($U \times 10^{-3}/\text{mg}$ of protein), with 1 enzymatic unit expressing 1 μmol of glucose or *p*-nitroanilide released/min of reaction at 37°C.

Statistical Analysis

Data were analyzed as a randomized block design using the MIXED procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). The statistical model included the fixed effect of treatment and the random effect of block and can be described as follows:

$$Y_{ij} = \mu + \rho_i + \alpha_j + e_{ij}$$

where Y_{ij} = an observation value for GIT measurements and brush border enzyme activities taken from block i and treatment j ; μ = overall mean for the population; ρ_i = random effect of block i , where i = block 1, 2, 3, 4, or 5; α_j = fixed effect of treatment j , where j = CTRL, MGD3, MGD7, MGD14, or MGD21; and e_{ij} = error term specific for the steer assigned to j th treatment in the i th block.

The hypotheses were tested using preplanned orthogonal (CTRL vs. MGD) and polynomial contrasts (linear, quadratic, and cubic effects) accounting for unequal spacing in the duration of time steers were fed the MGD. Polynomial contrasts included the CTRL treatment. Significance was declared when $P \leq 0.05$, and tendencies were discussed when $0.10 \leq P < 0.05$.

RESULTS

Data on BW, DMI, and reticulo-ruminal fermentation have been presented in a companion paper (Schurmann et al., 2014). Briefly, BW and DMI were not different between treatments ($P \geq 0.47$). We observed almost no refusals, but feed intake on the last day of study (slaughter day) was not recorded. Minimum, mean, and maximum reticular pH decreased from 6.57, 6.90, and 7.22 for CTRL to 6.08, 6.59, and 7.05 for MGD7 and increased thereafter to 6.31, 6.79, and 7.19 for MGD21 (quadratic, $P \leq 0.01$). Total SCFA 2 h after feeding (slaughter point) was not different between treatments ($P \geq 0.25$). However, the molar proportion of acetate decreased cubically from 67.8% for CTRL to 63.6% for MGD7, followed by an increase to 64.0% for MGD14 and MGD21 (quadratic, $P < 0.01$). The molar proportion of butyrate increased from 9.6% for CTRL to 13.0% for MGD14 and then decreased to 11.9% for MGD21 (quadratic, $P < 0.01$). The molar proportion of propionate was not affected by days fed MGD.

Reticulo-ruminal tissue mass was not affected by MGD ($P \geq 0.33$; Table 1). However, reticulo-ruminal digesta wet mass decreased from CTRL to MGD7 and then increased (quadratic, $P = 0.03$), and reticulo-ruminal digesta DM content increased linearly with number of days fed MGD ($P < 0.01$). Reticulo-ruminal digesta DM mass did not differ between treatments, but digesta moisture mass decreased from CTRL to MGD7 and then increased (quadratic, $P = 0.02$).

Omasal mass, omasal tissue mass, and omasal digesta mass decreased ($P \leq 0.02$), but abomasal tissue mass tended to ($P = 0.07$) increase linearly with number of days fed MGD. Duodenal tissue mass tended to ($P = 0.06$) increase linearly, and ileal length increased ($P = 0.02$) linearly with number of days fed MGD. We

detected no effect on other measurements of the abomasum and small intestine.

The dry mass of the epithelium from the ventral sac of rumen was not affected by MGD, but the dry mass of the muscle increased from CTRL to MGD14 and then decreased (quadratic, $P = 0.02$; Table 2). As a result, the ratio of epithelial dry mass to muscle dry mass decreased from CTRL to MGD14 and then increased (quadratic, $P = 0.01$). Epithelial DM content increased linearly ($P = 0.03$) with number of days fed MGD, but muscle DM content increased from CTRL to MGD7 and then decreased (quadratic, $P = 0.03$).

Lactase activity in the proximal jejunum increased ($P = 0.02$; Table 3) linearly, and maltase activity in the duodenum tended to increase ($P = 0.08$) linearly with days fed MGD. Furthermore, maltase activity in the proximal jejunum tended to be greater for MGD than for CTRL ($P = 0.09$). Aminopeptidase N activity in the proximal jejunum increased ($P = 0.01$) cubically with days fed MGD and tended to ($P = 0.09$) be greater for MGD than for CTRL. Dipeptidylpeptidase IV activity in ileum tended to decrease from CTRL to MGD14 and then tended to increase (quadratic, $P = 0.07$).

DISCUSSION

Previous studies have suggested that several weeks are needed for the ruminant GIT to adapt to diets with greater fermentability, mainly because several weeks are needed for ruminal microflora adaptation (Tajima et al., 2001; Fernando et al., 2010) and enlargement of the surface area of the ruminal epithelium to promote nutrient absorption (Dirksen et al., 1985; Bannink et al., 2008; Dieho et al., 2016). Furthermore, the tissue mass of the omasum, abomasum, and small intestine (Stobo et al., 1966; Reynolds et al., 2004), secretion of digestive enzymes (Kreikemeier et al., 1990), and expression of genes encoding nutrient transporters (Liu et al., 2013; Metzler-Zebeli et al., 2013) are also affected by dietary factors and may also require time to adopt to dietary changes. However, in commercial settings, cattle often experience abrupt increases in fermentable carbohydrate intake. Therefore, although the rate of adaptation is important, understanding the transient responses associated with adaptation of the GIT is also necessary, because it relates to the efficiency of nutrient use and the health of cattle on these diets. In this study, we investigated changes in GIT tissue mass and length, digesta mass, and brush border enzyme activity over a 21-d adaptation period following an abrupt increase in the proportion of grain in the diet. Results describing the effects of treatments used in this study on SCFA absorption (Schurmann et al., 2014), urea flux across the ruminal epithelium (Walpole et al., 2015), and en-

Table 1. Effect of number of days fed a moderate grain diet (MGD) on gastrointestinal tract measurements

Item	Treatment ¹						Contrast <i>P</i> -value			
	CTRL	MGD3	MGD7	MGD14	MGD21	SEM	CTRL vs. MGD	Linear	Quadratic	Cubic
Reticulo-rumen full, kg	45.04	39.96	38.54	38.73	40.82	2.03	0.02	0.26	0.04	0.39
Reticulo-rumen tissue, kg	4.08	4.35	4.19	4.34	4.38	0.26	0.33	0.41	0.82	0.76
Reticulo-rumen digesta, kg	40.96	35.62	34.34	34.39	36.45	1.86	0.01	0.20	0.03	0.34
Reticulo-rumen digesta DM, %	15.25	16.70	17.56	17.71	17.86	0.65	<0.01	0.01	0.09	0.35
Reticulo-rumen digesta DM, kg	6.21	5.92	6.04	6.06	6.47	0.29	0.77	0.39	0.32	0.90
Reticulo-rumen digesta moisture, kg	34.74	29.69	28.30	28.33	29.98	1.67	<0.01	0.12	0.02	0.30
Omasum full, g	4,958	4,879	4,132	4,228	3,550	292	0.03	<0.01	0.82	0.43
Omasum tissue, g	2,030	2,260	2,055	2,084	1,762	102	0.93	0.02	0.10	0.93
Omasum digesta, g	2,928	2,619	2,077	2,144	1,788	220	<0.01	<0.01	0.28	0.28
Abomasum full, g	3,058	2,468	2,528	3,000	2,813	283	0.27	0.78	0.52	0.10
Abomasum tissue, g	881	851	910	982	964	54	0.41	0.07	0.66	0.35
Abomasum digesta, g	2,177	1,617	1,618	2,018	1,849	253	0.17	0.95	0.42	0.10
Duodenum tissue, g	129	126	163	158	154	13	0.12	0.06	0.13	0.99
Jejunum tissue, g	3,148	3,174	3,358	3,384	3,244	205	0.47	0.56	0.33	0.85
Ileum tissue, g	422	350	451	440	418	48	0.89	0.62	0.64	0.53
Whole intestine tissue, ² g	3,699	3,650	3,972	3,982	3,816	234	0.51	0.47	0.32	0.76
Duodenum, m	0.50	0.53	0.52	0.55	0.57	0.04	0.42	0.22	0.99	0.99
Jejunum, m	21.57	18.34	19.96	20.45	20.05	1.70	0.15	0.95	0.54	0.15
Ileum, m	1.07	0.98	1.24	1.49	1.56	0.18	0.24	0.02	0.81	0.52
Whole intestine, ² m	23.14	19.85	21.73	22.50	22.19	1.73	0.22	0.69	0.57	0.13

¹CTRL = 0 d on moderate grain diet; MGD3 = 3 d on moderate grain diet; MGD7 = 7 d on moderate grain diet; MGD14 = 14 d on moderate grain diet; MGD21 = 21 d on moderate grain diet; n = 5 per treatment.

²Small intestine.

Table 2. Effect of number of days fed a moderate grain diet (MGD) on epithelial and muscle mass in the ventral sac of the rumen

Item	Treatment ¹					Contrast <i>P</i> -value				
	CTRL	MGD3	MGD7	MGD14	MGD21	SEM	CTRL vs. MGD	Linear	Quadratic	Cubic
Dry mass of whole tissue, g	3.68	3.75	4.00	3.97	3.28	0.35	0.84	0.39	0.11	0.70
Dry mass of epithelium, g	2.06	1.92	1.95	1.94	1.84	0.19	0.35	0.38	0.94	0.57
Dry mass of muscle, g	1.61	1.83	2.05	2.03	1.45	0.21	0.31	0.53	0.02	0.85
Epithelium/muscle ² , g/g	1.27	1.06	1.01	0.97	1.03	0.10	0.09	0.73	0.01	0.93
Epithelium DM, %	17.83	17.84	18.81	18.17	18.97	0.36	0.14	0.03	0.83	0.23
Muscle DM, %	18.90	19.53	21.12	20.17	19.78	0.62	0.06	0.38	0.03	0.32

¹CTRL = 0 d on moderate grain diet; MGD3 = 3 d on moderate grain diet; MGD7 = 7 d on moderate grain diet; MGD14 = 14 d on moderate grain diet; MGD21 = 21 d on moderate grain diet; n = 5 per treatment.

²Dry weight ratio.

dotoxin concentration in regions of the GIT (Plaizier et al., 2014) have been reported in companion papers. Briefly, the results of those studies showed that sodium flux, mucosal-to-serosal acetate and butyrate flux, and serosal-to-mucosal urea flux across rumen epithelium increased with the number of days fed MGD (Schurmann et al., 2014; Walpole et al., 2015). Those functional changes preceded structural adaptation of the ruminal epithelium (e.g., enlargement of rumen papillae), indicating that functional adaptation occurs before tissue growth and associated enlargement of surface area for nutrient absorption. Furthermore, MGD increased LPS concentration in the digesta of reticulo-rumen, ileum, cecum, and colon (Plaizier et al., 2014). Taking into account the fact that LPS may directly compromise epithelial barrier function (Chin et al., 2006; Liu et al., 2013), regions more distal than the reticulo-rumen can be challenged with an abrupt increase in the proportion of grain in the diet (Plaizier et al., 2014).

In commercial conditions, rapid increases in grain intake take place during transition from the dry period to lactation and at weaning or on entering a feedlot. In most cases, the increase in concentrate intake is a result of increased proportion of grain in the diet and feed intake. To partially separate the effect of DMI and the proportion of grain in the diet, we restricted DMI in this study. Although a constant DMI was not fully representative of commercial settings, it did allow us to determine the adaptive response independent of changes induced by DMI.

Adaptation of the Reticulo-Rumen

Because moderate increases in the fermentable carbohydrate content of diets stimulate ruminal papillae growth (Dirksen et al., 1985; Bannink et al., 2008; Dieho et al., 2016) and result in greater rumen epithelial mass (Reynolds et al., 2004; Malhi et al., 2013), we expected that the reticulo-ruminal tissue mass would increase as adaptation of the ruminal epithelium to the MGD progressed. However, we detected no effects of the MGD for papillae dimensions and density (Schurmann et al., 2014) or for the weight of the ruminal epithelium from the ventral sac. This lack of response was most likely due to the timeline required for ruminal epithelial proliferation, and because epithelial growth and muscle mass may occur divergently. For example, past studies have suggested that approximately 3 to 9 wk are required for ruminal papillae to adapt to diets with a moderate level of fermentable carbohydrates (Dirksen et al., 1985; Bannink et al., 2008; Dieho et al., 2016). That said, these studies evaluated adaptation in response to dietary change coupled with increased DMI in the postpartum phase of the transition period for

Table 3. Effect of number of days fed a moderate grain diet (MGD) on brush border enzymes activity ($U \times 10^{-3}/\text{mg}$ of protein)

Item	Treatment ¹					Contrast <i>P</i> -value				
	CTRL	MGD3	MGD7	MGD14	MGD21	SEM	CTRL vs. MGD	Linear	Quadratic	Cubic
Lactase										
Duodenum	17.94	14.53	11.96	18.13	18.71	4.12	0.64	0.53	0.41	0.34
Proximal jejunum	24.65	27.02	28.16	39.36	38.21	6.02	0.11	0.02	0.70	0.40
Ileum	1.20	0.81	0.35	0.99	0.76	0.33	0.21	0.68	0.35	0.16
Maltase										
Duodenum	10.94	10.85	10.38	11.57	13.48	1.28	0.62	0.08	0.28	0.94
Proximal jejunum	18.67	24.87	29.49	24.07	31.34	4.41	0.09	0.12	0.67	0.15
Ileum	16.36	14.55	14.23	13.69	17.33	1.79	0.47	0.65	0.11	0.86
Aminopeptidase N										
Duodenum	12.71	14.51	12.25	12.76	13.98	1.15	0.61	0.78	0.51	0.48
Proximal jejunum	13.78	16.45	22.70	13.35	25.82	1.23	0.09	0.06	0.62	0.01
Ileum	52.24	57.25	51.96	54.09	61.37	7.54	0.64	0.50	0.64	0.70
Aminopeptidase A										
Duodenum	3.41	3.62	4.15	4.12	3.91	0.50	0.35	0.44	0.36	0.92
Proximal jejunum	5.25	5.33	7.36	4.47	9.37	1.29	0.45	0.17	0.37	0.11
Ileum	38.62	41.93	32.81	33.17	44.41	7.03	0.95	0.78	0.25	0.59
Dipeptidylpeptidase IV										
Duodenum ²	ND ²	ND	ND	ND	ND	—	—	—	—	—
Proximal jejunum	0.90	0.00	4.48	0.00	1.96	1.62	0.64	0.78	0.58	0.16
Ileum	20.38	20.62	15.94	11.76	22.34	3.80	0.53	0.85	0.07	0.27

¹CTRL = 0 d on moderate grain diet; MGD3 = 3 d on moderate grain diet; MGD7 = 7 d on moderate grain diet; MGD14 = 14 d on moderate grain diet; MGD21 = 21 d on moderate grain diet; n = 5/treatment.

²ND = not detected.

Holstein cows. Combined with the results of previous studies, our findings suggest that without a corresponding increase in DMI, increasing the fermentability of the diet has little effect on stimulating papillae proliferation (Schurmann et al., 2014) and ruminal epithelial tissue mass. We cannot exclude the possibility that the duration of our study was too short to detect changes in papillae morphology and ruminal tissue mass. However, when feed intake was limited, as in the present study, ruminal papillae length and width did not change, even after 6 wk of exposure to a moderate- or high-grain diet (30 to 60% in DM; Metzler-Zebeli et al., 2013). That said, increased absorption of sodium (Etschmann et al., 2009; Schurmann et al., 2014), acetate and butyrate uptake (Schurmann et al., 2014), and urea secretion (Walpole et al., 2015) occurred within 1 wk following an abrupt dietary change. Others have also reported increased expression of genes encoding for proteins involved in SCFA absorption and intracellular pH regulation as an acute response to greater dietary fermentability (Metzler-Zebeli et al., 2013). These studies and the results from the present study suggest that changes in cellular activity occur acutely and may be prioritized over tissue growth, at least for short-term adaptation (Etschmann et al., 2009). Tissue growth and the associated enlargement of the surface area for nutrient absorption can be justified predominantly when functional adaptation is insufficient to ensure efficient nutrient absorption and when increased dietary fermentability and possibly DMI are sustained.

Another explanation for the lack of differences in reticulo-ruminal tissue mass is that it can be affected by the tissue mass of both the rumen and the reticulum. The tissue mass of the reticulum decreases with high-grain diets (McLeod and Baldwin, 2000), and a similar decrease after the abrupt change from CTRL to MGD could have contributed to a lack of difference in reticulo-ruminal tissue mass between the CTRL and MGD steers in this study. Unfortunately, we did not measure the tissue mass of the reticulum and rumen separately.

Because reticulo-ruminal tissue mass may be affected not only by epithelial mass but also by muscle mass (Harrison et al., 1960; Hamada et al., 1976), we measured epithelial and muscle mass in the ventral sac of the rumen. However, we observed the lowest muscle DM mass for CTRL and MGD21 and also MGD3, and these treatments corresponded with the heaviest digesta wet mass. Thus, muscle DM mass was most likely affected by tissue (muscle) stretching. This is plausible considering that we collected tissue samples 2 h after feeding. Observed differences in muscle DM mass between treatments in the present study should be interpreted with caution.

Previous studies have reported higher reticulo-ruminal DM content and lower reticulo-ruminal moisture content, longer DM retention time in the rumen, and lower fluid flow in ruminants fed moderate- to high-grain diets compared with those fed mostly forage (Colucci et al., 1990; Jaakkola and Huhtanen, 1993; Chibisa et al., 2016). Longer digesta retention time in the rumen, particularly fiber retention time, allows for partial compensation of reduced structural carbohydrate digestion when diets contain moderate to high grain inclusion, at least when feed intake is restricted (Colucci et al., 1990; Jaakkola and Huhtanen, 1993). Although we did not record DMI before slaughter in the present study, DMI among days was constant, so results on the day of slaughter should represent a reasonable estimate of the adaptive response over time. Nevertheless, our sampling approach allowed for only a single sampling time point. Therefore, precise interpretation of changes for reticulo-ruminal digesta mass with advancing days fed MGD could not be provided. That said, reticulo-ruminal DM mass was not affected, but reticulo-ruminal moisture mass decreased after the shift to MGD. Furthermore, after an initial decrease, reticulo-ruminal moisture mass increased from d 14 to 21 after the shift to MGD (quadratic pattern of change). This resembled the pattern of change for reticulo-ruminal pH observed in the present study (Schurmann et al., 2014). Diets with a moderate to high grain inclusion rate decrease liquid outflow from the rumen (Colucci et al., 1990; Jaakkola and Huhtanen, 1993; Chibisa et al., 2016). As a result, the lower reticulo-ruminal moisture mass in MGD steers may indicate lower fluid inflow, because of reduced saliva secretion or water intake. Saliva secretion decreases when the proportion of grain in the diet increases (Maekawa et al., 2002; Chibisa et al., 2016). Additionally, cattle transitioned to high-grain diet gradually alter their diet selection and sort for longer dietary particles (DeVries et al., 2014), which should increase chewing activity (Zebeli et al., 2008) and thus saliva secretion. It is thought that this altered sorting behavior is a compensatory mechanism to help regulate ruminal pH with high-grain diets. Although not measured in this study, the observed changes in reticulo-ruminal digesta mass may be attributed to changes in saliva secretion during the adaptation period and may help to explain observed changes in reticulo-ruminal pH (Schurmann et al., 2014), due to the known importance of saliva secretion for ruminal pH stabilization (Aschenbach et al., 2011; Chibisa et al., 2016). However, changes in water intake after the abrupt change to MGD and its effect on reticulo-ruminal digesta moisture mass cannot be excluded.

Combined, the results of this study confirm that adaptation of the rumen of cattle in response to an abrupt

dietary change (without a change in DMI) is limited, as indicated by a transient decrease in reticulo-ruminal pH (Schurmann et al., 2014) and reticulo-ruminal digesta mass shortly after the dietary change, followed by a gradual increase with advancing days. Nevertheless, adaptation of the reticulo-ruminal tissue mass may be of little importance when feed intake is limited or the duration of exposure is short. Furthermore, the results of this and accompanying studies (Schurmann et al., 2014; Walpole et al., 2015) indicate that ruminal adaptation proceeded throughout the study period. Considering that the primary roles of the reticulo-rumen are nutrient digestion and absorption of SCFA, the transient changes during adaptation may also affect the time course of adaptation for more distal regions of the GIT.

Adaptation of the Omasum

The importance of the omasum for digestion and absorption in ruminants is often neglected. However, up to 10% of NDF can be digested in the omasum (Smith, 1984; Ahvenjärvi et al., 2000), and up to 15% of SCFA produced in the rumen are absorbed before they reach the duodenum, suggesting significant SCFA absorption in the omasum (Rupp et al., 1994). We observed that the omasal digesta mass for MGD steers was less than for CTRL steers, and this likely reflected lower NDF intake. The reduced omasal digesta mass we observed corresponded with results of other studies that reported lower mass in ruminants as the proportion of grain in the diet increased (Bailey, 1986). Furthermore, lower omasal digesta mass explains a reduction in omasal tissue mass after the shift to the MGD, simply because less tissue (epithelium, muscles) is needed when the amount of digesta is reduced. In line with results of the present study, others have observed lower omasal tissue mass for ruminants fed high-grain diets compared to high-forage diets (Hamada et al., 1976; Bailey, 1986; McLeod and Baldwin, 2000). However, it is not clear whether the changes observed for the omasum were due to reduced muscle mass, epithelial mass, or both. Feeding high-concentrate diets (65% of DM) to goats decreased omasal digesta pH, increased endotoxin concentration in the omasum, and impaired barrier function of the omasal epithelium as indicated by a thinner epithelium, sloughing of the stratum corneum, and reduced expression of tight-cell junction proteins (Liu et al., 2014). It is likely that the reduction in omasal tissue mass we observed was at least partially due to reduced epithelial thickness and mass. Moreover, although a substantial reduction (13%) in omasal tissue weight occurred within 21 d, the omasal tissue mass decreased predominantly between d 14 and 21 after the

dietary change, and the linear response indicates that the retrogressive adaptive response was not complete.

Assuming that 5 to 10% of dietary fiber is digested in the omasum, it can be predicted that the omasal digesta retention time does not exceed several hours (Ahvenjärvi et al., 2000). Because the dietary change to MGD reduced NDF intake, we could expect that the corresponding reduction in omasal digesta mass should occur immediately in response to dietary change (e.g., d 3 to 7) and be less pronounced thereafter (d 7 to 21). However, this was not the case in the present study. Although omasal digesta mass decreased by almost 30% in the first week after the shift to the MGD, we also observed a substantial decrease (by 17%) between d 14 and 21. We can provide at least 2 explanations for this pattern of change. First, the increased proportion of grain in the diet may have reduced the omasal digesta pH, as shown in goats (Liu et al., 2014), and this may have negatively affected the digestion of fiber already entrapped between omasal leaves, resulting in longer digesta retention time in the omasum. Second, at least part of the starch consumed was fermented in the omasum. Although the fermentation rate of starch (%/h) is higher than that of fiber (Zicarelli et al., 2011), the omasal microflora would need time to adapt to the dietary change.

Taken together, our results showed gradual but sustained reduction in the omasal digesta mass that occurred for as long as 3 wk after an abrupt shift to the MGD. The reduction in digesta mass corresponded to a reduction in omasal tissue mass that occurred predominantly after 14 d of exposure to the MGD. Taking into account the fact that omasal digesta and tissue mass were still decreasing between d 14 and 21 after the shift to the MGD, 21 d was likely insufficient for omasal adaptation.

Adaptation of the Abomasum

Few studies have evaluated the adaptation of the abomasum in cattle in response to dietary change. One study reported a substantial increase in abomasal tissue mass during the transition from the dry period to lactation (Reynolds et al., 2004). This change corresponded with DMI that was higher after parturition compared with the dry period, and that continued to increase in early lactation. The increased DMI was suggested as a partial explanation for a demand for greater abomasal tissue mass (Reynolds et al., 2004). On the other hand, Bailey (1986) found no difference in abomasal tissue mass in steers fed ad libitum high- or low-concentrate diets, and McLeod and Baldwin (2000) found lower abomasal tissue mass in sheep fed a high-concentrate diet compared with sheep fed a high-forage diet. It is

important to note that in the study of McLeod and Baldwin (2000), energy intake was equal between the treatments (and thus DMI was higher for the sheep fed a high-concentrate diet). To partially separate the role of DMI and dietary fermentability, we kept DMI constant for all treatments in the present study. Nevertheless, increasing the proportion of grain in the diet could increase microbial protein synthesis in the rumen and the amount of microbial and total protein flowing to the abomasum, at least when feed intake is limited (Jaakkola and Huhtanen, 1993; Lascano et al., 2016). Taking into account the important role of the abomasum in protein digestion, this situation could explain the tendency we observed for a linear increase in abomasal tissue mass following the dietary shift to the MGD. Furthermore, abomasal infusions of both protein and non-structural carbohydrates affect the secretion of GIT regulatory peptides (Swanson et al., 2004; Relling and Reynolds, 2008). This, in turn, may affect abomasal tissue growth, based on the trophic effect of GIT peptides (Guilloteau et al., 2009). Future studies are needed to evaluate the regulating factors that promote adaptation of the abomasum and how nutrient supply to the abomasum alters its function.

Adaptation of the Small Intestine

Of the investigated sections of the small intestine, only duodenal tissue mass tended to increase after the dietary change to MGD. This increase occurred without an increase in duodenum length, suggesting that it was related to changes in the epithelium or muscle mass. However, assuming no change in intestinal length, 90% of the increase in intestinal mass is accounted for by epithelial growth (McLeod and Baldwin, 2000), such as an increased villi size (Zitnan et al., 2003; Wang et al., 2009). In line with the tendency for greater duodenal mass we observed in the present study, Zitnan et al. (2003) found longer villi and greater crypt depth in the duodenum of bulls fed high-concentrate diets compared to bulls fed low-concentrate diets. We interpreted the tendency for greater duodenal mass after the shift to a MGD to be the result of epithelial growth.

Intestinal epithelial growth is driven by nutrient availability (McLeod and Baldwin, 2000; Habold et al., 2004), and this suggests that nutrient flow to the duodenum likely increased in response to the MGD. The tendency for increased duodenal mass was also supported by a tendency for linearly increased abomasal tissue mass. With moderate- to high-grain diets, the amount of starch that bypasses the reticulo-rumen increases compared with high-forage diets (Reynolds, 2006; Li et al., 2012). Furthermore, the supply of rapidly fermented carbohydrates may increase microbial

protein synthesis and protein flow out of the rumen (Jaakkola and Huhtanen, 1993; Lozano et al., 2016). The fact that the duodenum is the first section of the intestine exposed to this increased nutrient flow may explain the adaptive response in this compartment of the GIT. However, the length of the duodenum accounts for only a small portion of the entire small intestine (less than 2.6% in this study) and its contribution to nutrient absorption is low. In this study, we did not observe changes in the mass or length of the jejunum, a region where substantial nutrient absorption is expected to occur.

Besides the adaptational changes in the duodenum, the results of this and other studies suggest that the ileum responds to increased dietary grain inclusion in ruminants (Wang et al., 2009; Liao et al., 2010). In contrast to the duodenum, we found that ileal mass was not affected but its length increased, indicating that ileal thickness was reduced after the shift to the MGD. When expressed as weight per unit of intestinal surface, epithelial weight was shown to be the greatest in the ileum of cattle relative to other regions of the small intestine (Kreikemeier et al., 1990). Furthermore, changes in small intestinal mass are predominantly a result of changes of epithelial mass and intestine length (McLeod and Baldwin, 2000). Therefore, a longer ileum without corresponding greater tissue mass indicates a thickening of epithelium in this section of the small intestine in response to the MGD. A linear increase for ileal length with days fed MGD was accompanied by increased LPS concentration in the ileal digesta (Plaizier et al., 2014). Greater intake of grain increases starch and simple sugar flow to the lower regions of the small intestine and colon (Li et al., 2012; Metzler-Zebeli et al., 2013; Brake et al., 2014), because of limited starch assimilation in ruminants (Harmon, 2009; Brake et al., 2014). This promotes gram-negative bacteria growth, which is associated with an increase in endotoxin concentration in the GIT (Li et al., 2012; Liu et al., 2013; Metzler-Zebeli et al., 2013), in turn negatively affecting the GIT epithelial structure (Chin et al., 2006; Liu et al., 2013; Metzler-Zebeli et al., 2013). It is unclear how ileal length and risk for LPS exposure are related, but increased ileum length may be also a part of adaptational changes to increase surface area for nutrient absorption. Liao et al. (2010) found that starch hydrolysate infusion into the abomasum led to an upregulation of sodium-dependent glucose transporter 1 and facilitated glucose transporter 2 in the ileum, indicating that besides adaptation of ileum length, functional adaptation of this region of GIT in response to diet occurred. The extent to which adaptation of the ileum determines the efficiency of nutrient use in cattle fed high-grain diets requires further study. However, a

linear increase in ileal length suggests that studies with a longer duration are needed to determine the time required for ileal adaptation.

Because functional adaptation of the small intestinal epithelium precedes structural adaptation and may be very rapid (Ferraris and Vinnakota, 1995), adaptation of brush border enzymes, if any, would be expected to occur within several days after the shift to the MGD. However, in the present study, activities of lactase in the jejunum, maltase in the duodenum, aminopeptidase N in the jejunum, and dipeptidylpeptidase IV in the ileum progressively increased with days on the MGD. This progressive increase indicates that adaptation of brush border enzymes in cattle is not a rapid process, also reported for pancreas secretions (Chittenden et al., 1984). The extended period required for brush border enzyme activity may be affected by the time required for preceding regions of the GIT to adapt to the dietary change, as already discussed. Duodenal infusion of nonessential amino acids in cattle decreased the flow of small α -glycosides to the ileum, indicating more efficient starch digestion as a result of greater maltase and isomaltase activity (Brake et al., 2014). Increased grain intake stimulates microbial protein synthesis and increases the amount of amino acids entering small intestine (Merchen et al., 1986; Jaakkola and Huhtanen, 1993; Lascano et al., 2016). However, microbial adaptation is not a rapid process (Tajima et al., 2001; Fernando et al., 2010). Gradual changes in the supply of amino acids (as well as other nutrients) entering small intestine after dietary change may lead to gradual changes in brush border enzymes activity. Furthermore, brush border enzyme activity increases would be initiated from the base of the villi, and the life span of enterocytes, depending on the section of the small intestine, varies from 2 to several days (Shirazi-Beechey et al., 1991; Fan et al., 2001). The division, maturation, and migration of enterocytes would present a lag for adaptation of brush border enzyme activity in response to dietary change.

Although the results of this study do not answer whether the total GIT activity of investigated brush border enzymes was affected by the MGD, the activity of lactase, maltase and aminopeptidase N did increase in the proximal section of the small intestine after the shift to the MGD. Kreikemeier et al. (1990) found that greater feed intake and the associated increased energy intake resulted in greater small intestinal brush border disaccharidase activity, but they detected no differences between animals fed diets high in forage or high in concentrate. In the current study, the MGD was associated with greater energy intake than the CTRL diet, providing additional evidence that brush border enzyme activity in cattle is affected by energy intake.

On the other hand, Zitnan et al. (2003) did not find differences in maltase or lactase activity in the duodenum, jejunum, and ileum in bulls fed a high-concentrate diet compared with bulls fed a low-concentrate diet.

CONCLUSIONS

Our results indicate that adaptation to diets with a moderate grain content involves a decrease in omasal digesta and tissue mass and an increase in abomasal and duodenal tissue mass, ileal length, and the activity of some brush border enzymes. Furthermore, most of the adaptive responses progressed throughout the study, suggesting that 3 weeks is not sufficient to complete the adaptive response.

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