A high-grain diet alters the omasal epithelial structure and expression of tight junction proteins in a goat model

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ABSTRACT

The omasal epithelial barrier plays important roles in maintaining nutrient absorption and immune homeostasis in ruminants. However, little information is currently available about the changes in omasal epithelial barrier function at the structural and molecular levels during feeding of a high-grain (HG) diet. Ten male goats were randomly assigned to two groups, fed either a hay diet (0% grain; n = 5) or HG diet (65% grain; n = 5). Changes in omasal epithelial structure and expression of tight junction (TJ) proteins were determined via electron microscopy and Western blot analysis.

After 7 weeks on each diet, omasal contents in the HG group showed significantly lower pH (P < 0.001) and significantly higher concentrations of free lipopolysaccharides (LPS; P = 0.001) than the hay group. The goats fed a HG diet showed profound alterations in omasal epithelial structure and TJ proteins, corresponding to depression of thickness of total epithelia, stratum granulosum, and the sum of the stratum spinosum and stratum basale, marked epithelial cellular damage, erosion of intercellular junctions and down-regulation in expression of the TJ proteins, claudin-4 and occludin. The study demonstrates that feeding a HG diet is associated with omasal epithelial cellular damage and changes in expression of TJ proteins. These research findings provide an insight into the possible significance of diet on the omasal epithelial barrier in ruminants.

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Introduction

Intensive production systems in ruminants encourage inclusion of a relatively large proportion of grains or easily fermentable carbohydrates in the diet, to support high milk yields or rapid weight gain. Although these feeding practices might enhance cost efficiency in the short term, they are not necessarily optimal for ruminant health and welfare. The long-term consumption of high-grain (HG) diets has been associated with diseases such as liver abscesses, septic emboli and chronic inflammation, possibly due to the translocation of microbes or microbial components from the digestive tract into the circulation (Gozho et al., 2007; Khafipour et al., 2009; Li et al., 2012). This might be a consequence of relatively high concentrations of lipopolysaccharide (LPS) developing in the gastrointestinal tract (Plaizier et al., 2008, 2012; Khafipour et al., 2009; Li et al., 2012), which can compromise gastrointestinal epithelial barrier function (Emmanuel et al., 2007; Steele et al., 2011; Plaizier et al., 2012; Klevenhusen et al., 2013; Liu et al., 2013).

Changes to the rumen epithelium, associated with a HG diet, have been reported previously (Steele et al., 2011; Klevenhusen et al., 2013; Liu et al., 2013), although little information is currently available in terms of the effect of HG diets on omasal epithelial barrier function. The omasum has a stratified squamous epithelium consisting of a multilayered structure (stratum corneum, granulosum, spinosum and basale) and the junctional complex, that forms a barrier between the luminal contents and the internal milieu (Stumpff et al., 2011). Its role as a permeable barrier is to facilitate absorption of nutrients, water and electrolytes, while at the same time preventing uptake of toxins and invasion by microbes (Gareau et al., 2008; Turner, 2009; Stumpff et al., 2011).

Under normal physiological conditions, only a limited amount of LPS and other microbial components penetrate the gastrointestinal epithelial barrier (Gareau et al., 2008). However, under certain circumstances, the barrier properties of gastrointestinal epithelia can be adversely affected, resulting in increased epithelial permeability and translocation of LPS into the circulation (Gareau et al., 2008). A previous study has demonstrated that omasal epithelium from concentrate-fed sheep exhibited a tendency to increased paracellular permeability compared with hay-fed sheep (Martens et al., 2004). However, little information is currently available with respect to changes in the omasal epithelial barrier at the structural and molecular levels, during feeding of a HG diet.

Tight junctions (TJ), located in the stratum granulosum and spinosum, play a key role in maintaining the normal epithelial barrier
and preventing translocation of LPS and other microbial components (Turner, 2009; Stumpff et al., 2011). The TJ consists of transmembrane proteins (claudins and occludin) that mediate adhesive functions and are linked to underlying plaque proteins, such as zonula occludens-1 (ZO-1), which in turn anchor to the cytoskeleton (Fanning and Anderson, 1998). Western blot analysis and immunofluorescence has shown that the native epithelium of both the rumen and omasum express the same profile of TJ proteins (claudin-1, claudin-4, claudin-7 and occludin) (Stumpff et al., 2011). Our recent study demonstrated that feeding a HG diet induced rumen epithelial barrier dysfunction, involving both cellular damage and changes in expression and distribution of TJ proteins (Liu et al., 2013). The present study was designed to investigate changes to the omasal epithelial structure, and expression of TJ proteins, when goats were fed a HG diet.

Materials and methods

Analysis of omasum contents

The effects of diet on omasal contents in goats fed either hay or a high grain (HG) diet group during the feeding trial.

Table 1 shows the analysis of omasum contents at the end of the dietary trial. The group fed a HG diet showed increased concentrations of propionate (P = 0.026) and butyrate (P = 0.001) in the HG diet group, pH (P < 0.001), acetate concentration (P = 0.001) and free LPS (EU/g of wet matter) (P = 0.001).

Results

Analysis of omasal contents

In the HG diet group, pH (P < 0.001), acetate concentration (P = 0.001) and free LPS (EU/g of wet matter) (P = 0.001).

Table 1

<table>
<thead>
<tr>
<th>Component</th>
<th>Hay (n = 5)</th>
<th>HG (n = 5)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.26 ± 0.10</td>
<td>5.43 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total SCFA (mM)</td>
<td>26.04 ± 0.95</td>
<td>25.75 ± 1.96</td>
<td>0.895</td>
</tr>
<tr>
<td>Acetate (mM)</td>
<td>20.87 ± 0.66</td>
<td>14.84 ± 0.98</td>
<td>0.001</td>
</tr>
<tr>
<td>Propionate (mM)</td>
<td>3.96 ± 0.11</td>
<td>7.50 ± 1.30</td>
<td>0.026</td>
</tr>
<tr>
<td>Butyrate (mM)</td>
<td>0.78 ± 0.16</td>
<td>2.74 ± 0.34</td>
<td>0.001</td>
</tr>
<tr>
<td>Others⁎ (mM)</td>
<td>0.42 ± 0.10</td>
<td>0.67 ± 0.09</td>
<td>0.093</td>
</tr>
<tr>
<td>Acetate: Propionate</td>
<td>5.27 ± 0.08</td>
<td>2.14 ± 0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free LPS (EU/g of wet matter)</td>
<td>4217 ± 422</td>
<td>6924 ± 254</td>
<td>0.001</td>
</tr>
</tbody>
</table>

EU, endotoxin unit.

⁎ Others = isobutyrate + valerate + isovalerate.
acetate:propionate ratio \((P < 0.001)\) were significantly lower than in the hay-fed group. The goats fed a HG diet had a higher free LPS concentration in the omasal digesta \((P = 0.001)\) compared with the hay-fed goats.

**Histology, morphology, and ultrastructural analysis of omasal epithelium**

The goats fed a HG diet had more omasal papillae, compared with the hay-fed goats \((105 \pm 7 \text{ versus } 60 \pm 3 \text{ per cm}^2; P < 0.001)\). Representative histological sections of omasal epithelium from goats fed hay or HG diet are shown in Figs. 1A, B. In the HG diet-fed goat (Fig. 1B), sloughing of the stratum corneum was evident and adhesion between cells of the stratum corneum and stratum granulosum appeared to be compromised, as shown by large gaps between cells. Evaluation of cross sections of the omasal papillae, viewed under light microscopy, revealed that reduced thickness of total omasal epithelium \((P = 0.005)\), stratum granulosum \((P < 0.001)\) and the sum of the stratum spinosum and stratum basale \((P = 0.036)\) were evident in HG diet-fed animals (Table 2). No significant difference was observed in the thickness of the stratum corneum \((P = 0.629)\) comparing hay and HG groups (Table 2).

Scanning electron micrographs of omasal epithelium from all goats in the hay group revealed relatively large conical papillae and longitudinal grooves on the inter-papillary surface (Fig. 1C). In comparison, the omasal papillae were claw-like in shape and the longitudinal groove-like structures were not clearly visible in the group fed the HG diet (Fig. 1D). Desquamation was apparent, throughout the surface of the omasal leaves in both groups but was more pronounced in the HG diet-fed goats (Fig. 1D).

Transmission electron micrographs of omasal epithelia, demonstrated that hay-fed goats displayed integrity and normal nuclei, mitochondria and intercellular junctions (Figs. 2A–C). For the HG diet-fed goats, fragments of plasma membranes with remnants of junctional components (desmosomes and gap junctions) were interposed as an almost continuous strip between the cells of the stratum granulosum (Fig. 2E). Further abnormalities included nuclear breakdown, mitochondria with indistinct cristae and swelling, numerous vacuoles present and vague intercellular boundaries (Figs. 2D–F).

**Expression of TJ proteins in omasal epithelial tissue**

Expression of claudin-1, claudin-4, occludin and ZO-1 was assessed by Western blotting (Figs. 3A, B). Results demonstrated that there was lower expression of claudin-4 \((P = 0.017)\) and occludin \((P = 0.007)\), comparing HG diet-fed and hay-fed groups. No significant differences were observed for expression of claudin-1 \((P = 0.923)\) or ZO-1 \((P = 0.069)\) comparing hay and HG diet-fed groups.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Hay ((n = 5))</th>
<th>HG ((n = 5))</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of total epithelium ((\mu m))</td>
<td>101.03 ± 4.24</td>
<td>86.13 ± 2.75</td>
<td>0.005</td>
</tr>
<tr>
<td>Thickness of stratum granulosum ((\mu m))</td>
<td>11.35 ± 0.48</td>
<td>11.26 ± 0.44</td>
<td>0.629</td>
</tr>
<tr>
<td>Thickness of stratum spinosum/basale ((\mu m))</td>
<td>22.31 ± 0.64</td>
<td>18.01 ± 0.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thickness of stratum spinosum/basale ((\mu m))</td>
<td>67.37 ± 3.96</td>
<td>56.86 ± 2.31</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Fig. 1. Histology of omasum epithelium, comparing goats fed hay or a HG diet. Light microscopy cross section of omasal epithelium from a representative hay-fed goat (A) or HG-diet goat (B). Scale bar = 50 \(\mu m\). Scanning electron micrograph of omasal epithelium from a representative hay-fed goat (C) or HG-diet goat (D). Scale bar = 1 mm.
Discussion

The epithelial barrier of the gastrointestinal tract plays important roles in absorption of nutrients and host defence against infection. The results of our study have demonstrated that feeding a HG diet can cause omasal epithelial barrier dysfunction, involving both cellular damage and changes in expression of TJ proteins. These findings provide insight into the role of TJ proteins (particularly claudin-4 and occludin) in omasal epithelial homeostasis and might also inform new strategies to prevent omasum dysfunction as a consequence of feeding a HG diet.

In the present study, feeding of a HG diet increased the density of omasal papillae, which would suggest adaptation of the omasal epithelium to the enhanced substrate density of the diet, by growth of the papillae, as has been documented to occur in the rumen (Sakata and Tamate, 1979; Zitnan et al., 1998; Shen et al., 2004). The HG diet-fed goats showed significant alteration in their omasal epithelial structure, depicted by loss of epithelial thickness, intercellular junction erosion and cellular necrosis. These results are similar to those reported by Gaebel et al. (1989), who demonstrated that rapidly reducing the pH from 7.4 to 5.5 caused intercellular junction erosion and cellular necrosis in the deeper cell layers of the sheep rumen mucosal epithelium. The similarity of these findings suggests that high acidity may be one of the major insults responsible for causing the omasal epithelial abnormalities observed.

TJ proteins in the gastrointestinal epithelium are highly dynamic structures, playing important roles in physiological homeostasis (Turner, 2009). Our study demonstrated that the omasal epithelial TJ proteins, claudin-4 and occludin, were depleted by feeding a HG diet. These changes in TJ proteins might also be associated with high acidity in the rumen. The HG diet-fed goats showed significant alteration in their omasal epithelial structure, depicted by loss of epithelial thickness, intercellular junction erosion and cellular necrosis. These results are similar to those reported by Gaebel et al. (1989), who demonstrated that rapidly reducing the pH from 7.4 to 5.5 caused intercellular junction erosion and cellular necrosis in the deeper cell layers of the sheep rumen mucosal epithelium. The similarity of these findings suggests that high acidity may be one of the major insults responsible for causing the omasal epithelial abnormalities observed.

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Fig. 2. Transmission electron microscopy to determine effects of HG diet on omasal epithelial ultrastructure. Omasal epithelium of representative hay-fed goat (A, scale bar = 10 μm) and HG diet-fed goat (D, scale bar = 10 μm). Comparison of omasal epithelial ultrastructure of stratum spinosum and basale between hay-fed goat (B, scale bar = 5 μm) and HG diet-fed goat (E, scale bar = 5 μm). Omasal epithelial ultrastructure of junctional complexes in representative hay-fed goat (C, scale bar = 0.5 μm) and HG diet-fed goat (F, scale bar = 0.5 μm). SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum; N, nuclei; V, vacuoles; LP, lamina propria; M, mitochondria. Arrow = intercellular junction.

Fig. 3. Effects of HG diet on relative expression of TJ proteins in the omasal epithelium of goats. Western blot analysis of claudin-1, claudin-4, occludin and ZO-1 proteins in omasal tissue in response to hay versus HG diet. (A) Protein extracts of omasal epithelium samples were prepared and immunoblotted with specific antibodies. (B) Intensities of claudin-1, claudin-4, occludin, and ZO-1 were normalised to corresponding β-actin levels (means ± SEM, n = 4). *P < 0.05, **P < 0.01. MW, molecular weight; ns, not significant.
The present study revealed that feeding a HG diet resulted in mitochondrial swelling, which might be partly explained by the lower pH, believed to inhibit mitochondrial permeability transition (Gaebel et al., 1989; Teixeira et al., 1999). This structural damage to mitochondria could suppress ATP production (Gaebel et al., 1989), thereby affecting transcription and translation of claudin-4 and occludin, resulting in decreased expression of these proteins. The mechanisms behind the changes involving oesophageal TJ proteins needs to be further elucidated in vitro using an oesophageal cell culture model.

The intact oesophageal TJ barrier can maintain the large gradients for chloride and sodium (usually blood > oesophagus) and for potassium, hydrogen ions and SCFA (usually oesomus > blood) (Martens and Gabel, 1988; Schulteiss and Martens, 1999; Martens et al., 2004; Ali et al., 2006; Stumpfl et al., 2011). Mutation or deletion of individual TJ proteins can have profound effects on epithelial structure and function, with studies of monogastric animals demonstrating the ability of claudin-4 to influence sodium permeability (Van Itallie et al., 2001; Colegio et al., 2002). Thus, a deficiency in claudin-4 expression may cause sodium efflux, which would dissipate the gradient established by Na+/H+ exchanger-mediated transcellular transport, potentially resulting in cell-swelling and necrosis.

Occludin is widely accepted as a regulator of TJ assembly and function (Al-Sadi et al., 2011; Raleigh et al., 2011). A recent study demonstrated that occludin depletion in intestinal epithelial cells led to an increase in paracellular flux of larger-sized molecules, suggesting that occludin plays a crucial role in the maintenance of TJ barrier, through the large-channel TJ pathway (Al-Sadi et al., 2011). In our study, the loss of occludin, induced by feeding a HG diet, was consistent with gastrointestinal inflammation studies in the colon (Gassler et al., 2001; Zeissig et al., 2007; John et al., 2011) and rumen (Liu et al., 2013). The loss of oesophageal occludin could potentially increase transport of macromolecules, leading to translocation of LPS and potentially bacteria, which might impact on the cell layers of the oesophagus.

Evidence also exists that sheep, fed a high concentrate diet, exhibited a tendency to greater oesophageal epithelial permeability, compared to those fed hay (Martens et al., 2004). That finding is consistent with our proposal that permeability of oesophageal epithelium is increased during feeding of a HG diet, as suggested by the present study. Our previous study revealed that feeding goats a HG diet resulted in weakened barrier function of the rumen epithelium, and circulating LPS was detectable in the plasma (0.80 ± 0.20 EU/mL), while not detectable in hay-fed goats (Liu et al., 2013). Our results suggest that LPS translocation is not restricted to the rumen when animals are fed a HG diet and that disruption of the oesophageal TJ, associated with the broad range of oesophageal epithelial defects, may also lead to the translocation of large molecules such as LPS into the blood.

Conclusions

The results of the present study demonstrate that feeding goats a HG diet caused both oesophageal epithelial cellular damage and changes in the expression of TJ proteins, which may increase the oesophageal epithelial permeability, thereby increasing the risk of translocation of microbes and/or microbial products, such as LPS.