Direct Effect of Lipopolysaccharide and Histamine on Permeability Barrier of Rumen Epithelium

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Research

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Abstract

Background: Disruption of the ruminal epithelium barrier occurs during subacute ruminal acidosis due to low pH, hyper-osmolality, and increased concentrations of lipopolysaccharide and histamine in ruminal fluid. However, the individual roles of lipopolysaccharide and histamine in the process of ruminal epithelium barriers disruption are not clear. The objective of the present investigation was to evaluate the direct effect of lipopolysaccharide and histamine on barrier function of the ruminal epithelium.

Results: Compared with control (CON), lipopolysaccharide (LPS) increased the short-circuit current (I_{sc}) (88.2%, $P = 0.0022$), transepithelial conductance (Gt) (29.7%, $P = 0.0564$) and the permeability of fluorescein 5(6)-isothiocyanate (FITC) (1.04-fold, $P = 0.0047$) of ruminal epithelium. The apparent permeability of LPS was 1.81-fold higher than CON ($P = 0.0005$). The mRNA abundance of *OCLN* in ruminal epithelium was decreased by LPS (1.1-fold, $P = 0.0473$).

Conclusions: The results of the present study suggested that histamine plays a direct role in the disruption of ruminal epithelium barrier function while lipopolysaccharide without acidic pH has no significant effect on the permeability of rumen tissues.

Background

Factors contributing to the RE barrier function include the epimural microbiome, a continuously shedding mechanical barrier of highly keratinized cells, and tight-cell junctions, desmosomes, and gap junctions in the more basal cell strata (Graham and Simmons, 2005). It is well known that ruminal conditions that occur during SARA disrupt the RE barrier function, but a complete understanding of factors influencing the response are still limiting. Past research has reported that exposure to acidic and hyperosmotic conditions independently (Penner et al., 2010) and additively (Penner et al., 2010; Wilson et al., 2012) increase permeability to paracellular permeability markers such as mannitol. In vivo, however, increased concentrations of LPS, short chain fatty acids (SCFA), and biogenic amines (e.g. histamine) occur concurrently with decreased pH and hyperosmotic conditions with SARA (Liu et al., 2013; Mao et al., 2016; Stefanska et al., 2018). Experiments by Meissner et al. (2017) and Greco et al. (2018) indicated that the increases in permeability of the RE are moderate when pH is reduced without co-presence of SCFA, indicating that the direct effect of pH on RE barrier function is as not strong as originally thought and that factors affecting cell function may be critical to the process. As such, research is needed to understand the contribution of multifaceted effects occurring in vivo (Aschenbach et al., 2019).

Endotoxins such as LPS and biogenic amines such as histamine (HIS) are two important microbial metabolites found in the rumen during SARA. Zhao et al. (2018) reported that SARA increased the concentration of LPS in ruminal fluid and blood, and reported that LPS exposure upregulated the expression of inflammatory pathways and production of proinflammatory cytokines in RE. The ability of RE cells to initiate a pro-inflammatory response when exposed to LPS was further confirmed by Kent-

Dennis et al. (2020) and warrants further investigation as inflammation may compromise epithelial barrier function.

In addition to LPS, Pilachai et al. (2012) confirmed a negative relationship between ruminal HIS concentration and ruminal pH under high concentrate diets. Aschenbach et al. (1998) first showed that application of HIS in relevant dosages (10 and 100 μM) impairs differentiation of RE cells in culture and that when exposed to acidic pH (pH 4.5), histamine translocation across the ruminal epithelium was markedly increased (Aschenbach and Gäbel, 2000). Sun et al. (2017) suggested that HIS could activate an inflammatory pathway in cultured RE cells via NF- κ B. Moreover, it has been suggested that low ruminal pH and the presence of Gram Negative Bacilli (GNB) products (e.g. cell-free LPS, HIS) negatively affects the expression of RE cadherins leading to increased permeability of the RE (Zebeli and Metzler-Zebeli, 2012). However, the direct effect of the HIS and LPS on barrier function of RE in vivo or ex vivo are still not clear. Therefore, the objective of the present study was to evaluate the direct effect of LPS and HIS on the RE barrier function measured ex vivo.

Materials And Methods

Treatments and Design

Three treatments [CON (control), LPS (lipopolysaccharide), HIS (histamine)] were compared using a complete randomized design. The 3 treatments were allocated to 6 chambers with 2 technical replicates for each treatment. The experiment was repeated 8 times ($n = 8$), each with tissue sourced from a different steer.

Ruminal Epithelia Sample Collection and Preparation

Ruminal tissue samples for this experiment were collected from a commercial abattoir (Beijing, China). A 15 cm² area of ruminal tissue from the ventral sac were excised from healthy feedlot Simmental steers within 10 min after slaughter ($n = 8$). Tissues were then washed in a pre-heated buffer solution at 37 to 39°C adjusted to pH 7.4 using HCl (6 mol/L) or NaOH (6 mol/L) as needed. The buffer solution consisted of CaCl₂×2H₂O (1.2 mM), MgCl₂×6H₂O (1.2 mM), NaCl (80.0 mM), NaHCO₃ (25.0 mM), NaH₂PO₄×H₂O (0.40 mM), Na₂HPO₄×2H₂O (2.4 mM), KCl (5.0 mM), Na-acetate (25.0 mM), Na-propionate (10.0 mM), and Na-butyrate (5.0 mM). The osmolarity of the buffer solution was 315.2 mOsmol/L. To maintain the activity of the tissues and enable respiration of the tissues during the transportation, the rumen tissue was stored in an insulated container with 37 to 39°C buffer solutions continuously gassed with carbogen (95% O₂/5% CO₂) until processing and mounting the tissues in the Ussing chamber (KINGTECH, China). Once at the laboratory, the serosal and muscular layers were separated gently by hand. The maximum possible fibrous tissue was removed from the mucosa without injuring the tissue. Rumen tissue samples collected from a single animal were run on an individual day. At the time of slaughter, rumen whole digesta was collected and squeezed through 4 layers of muslin cloth to separate rumen fluid from the solid digesta. Following straining, pH was measured and samples were collected to determine the

concentration of NH_3N and VFA as previously described (Gao et al., 2017). The results were shown in Table 1. The time from slaughter to mounting of tissues in the chambers was approximately 50 min.

Table 1
Ruminal pH, ammonia-N, and VFA in rumen fluid from the cows used in this study

Items	Mean	Maximum	Minimum	Standard Deviation
pH	6.53	6.86	6.18	0.23
NH_3N (mg/dL)	8.66	16.71	2.93	4.77
Total VFA (mmol/L)	51.13	56.37	45.97	3.31
Acetate (mmol/L)	31.80	35.98	27.09	3.37
Propionate (mmol/L)	11.46	13.23	10.35	1.07
Butyrate (mmol/L)	6.14	7.14	5.07	0.74
Isobutyrate (mmol/L)	0.51	0.76	0.15	0.21
Valerate (mmol/L)	0.59	0.89	0.28	0.19
Isovalerate (mmol/L)	0.63	1.12	0.26	0.26

Operation Procedures of Ussing chamber

One hour before the rumen tissue collection, the lucite Ussing chambers were heated and assembled. Subsequently, 10 mL of buffer solution was added to both reservoirs of the Ussing chamber, and the chambers were connected to a 95% O_2 /5% CO_2 airlift system to facilitate buffer mixing. Once the buffer solution in the system attained a temperature of 39°C, fluid resistance was measured and adjusted, using an automatic computer-controlled voltage-clamp device (voltage/current clamp, VCC MC6 Plus, KINGTECH, China), to correct for the inherent fluid resistance. The instrument was then switched to open-circuit conditions. The current and voltage electrodes (KINGTECH, China) contained 4% noble agar and filled with 3 M KCl.

The isolated epithelium was cut into 6 pieces approximating 2 cm² and mounted between two-halves of the Ussing chamber with an exposed area of 1.27 cm² each, and then the clamps were assembled between the lucite chambers. A silicon washer, placed on each side of the Ussing chamber was used to prevent edge damage of the tissue. The buffer solution (pH 7.4) was re-added (10 mL to each side) and the airlift were reconnected after the clamps were assembled. Tissues were allowed to equilibrate for 15 min under open-circuit conditions, then the instrument was switched to short-circuit conditions. During the study, transepithelial short-circuit current (I_{sc}), as a measure of net ion transport, and transepithelial conductance (G_t) were continuously recorded with the aid of an automatic computer-controlled voltage-

clamp device (voltage/current clamp, VCC MC6 Plus, KINGTECH, China). Tissues were randomly assigned to 1 of 3 treatments with 2 technical replicates within from each steer. For all chambers, 8 μL FITC (final concentration 0.2 mM, Sigma-Aldrich China Ltd.) was added to the mucosal side as a permeability marker. Treatments included a negative control (no further addition), a treatment where HIS was added to the mucosal side to achieve a final concentration of 20 μM (8 μL histamine, Sigma-Aldrich China Ltd.), or a treatment where LPS was added to the mucosal side to achieve a final concentration of 1 $\mu\text{g}/\text{mL}$ (10 μL from E. coli B:055, Sigma-Aldrich China Ltd.). Samples (100 μL) were taken from the mucosal side of the chambers immediately after FITC, HIS, and LPS were added to detect the initial concentrations. Additional samples were collected at 20, 40, 60, and 80 min from the serosal compartment of the chambers for detection of permeability of FITC, LPS, and HIS (2 samples per time per chamber, one for FITC detection, and the other one for LPS and histamine detection). All the samples were collected using pyrogen free tubes. The fluorescence intensity of the FITC samples was measured after dilution in water 1:5 using a fluorescence spectrometer (Tecan Infinite 200 Pro, Tecan, Austria). The samples for LPS and HIS detection were stored at -20°C until analysis. At 100 min, the tissues mounted in the chambers were collected and stored in liquid nitrogen until RNA extraction.

Determination of LPS

Cell-free Lipopolysaccharide concentration in the buffer samples were determined by a commercially available Limulus amebocyte lysate assay (Xiamenhoushiji, Xiamen, China) as previously described by Liu et al. (2013). A control standard endotoxin containing 10 ng endotoxin/vial (Xiamenhoushiji) was used to prepare standard solutions. All of the samples were tested in duplicate, and the optical density values were read on a microplate spectrophotometer (Tecan Infinite 200 Pro, Tecan, Austria) at a wavelength of 405 nm.

Determination of Histamine

The concentration of HIS in the serosal and mucosal side of the chambers were determined using commercial Elisa kit (MLBio, Shanghai, China). The detection was performed following the manufacturer's instructions. In principle, the wells of the microtiter plates were pre-coated with the antibody (anti-histamine). Samples, standard, and horseradish peroxidase (HRP) conjugated with anti-histamine were successively added, incubated, removed, and plates were washed thoroughly. The substrate tetramethylbenzidine (TMB) was used for color development, which was converted to blue by HRP catalysis and finally to yellow by acid. There was a positive correlation between the color depth and the histamine concentration in the samples. The optical density values were read on a microplate spectrophotometer (Tecan Infinite 200 Pro, Tecan, Austria) at a wavelength of 450 nm.

Real-Time Quantitative PCR

About 300 mg RE tissues were homogenized in TRIzol reagent (QIAGEN, Germany) and total RNA was isolated by phase separation. To eliminate potential DNA contamination, isolated RNA was treated with RNase-Free DNase Set (QIAGEN, Germany) and further purified using a RNeasy Mini Kit (QIAGEN, Germany). RNA concentration was determined using Nanodrop 1000 spectrophotometer (NanoDrop

Technologies, US). The OD260/OD280 values were $2.1 \geq 1.9$. RNA integrity was assessed using 2100 Bioanalyzer (Agilent Technologies, US) and the RNA 6000 Nano Kit (Agilent Technologies, US). The RNA integrity number values were ≥ 8.0 . After isolation, 2 μg of total RNA was used for reverse transcription (RT) using PrimeScript RT Master Mix (TaKaRa, Japan). The reaction system included 10 μL 5 $\times$ PrimeScript RT Master, 10 μL RNA template, and 30 μL RNase-free dH_2O . The cDNA concentration was measured and all samples were diluted to 50 ng/ μL using diethylpyrocarbonate (DEPC) water to a final volume of 1000 μL . The real-time quantitative PCR reaction was performed on QuantStudio 7 Flex (Life Technologies, US) using TB Green Premix Ex Taq (TaKaRa, Japan) with a 20 μL system including 10 μL TB Green Premix, 1.6 μL of primer (forward and reverse primer were premixed), 0.4 μL of ROX Reference Dye II (50X), 2 μL of cDNA template, and 6 μL of DNase Free dH_2O . Six reference genes were tested *ACTB*, *UXT*, *DBNDD2*, *RPS9*, *DDX54*, and *HMBS* as suggested by Die et al. (2017) using geNorm (V3.5, Ghent University Hospital Center for Medical Genetics, Ghent, Belgium). A ranking of candidate genes was shown according to their stability (expressed in geNorm M values) from most unstable genes at the left (high M value, *HMBS*) to the best reference genes at the right (low M value, *RPS9*). The best normalization factor was obtained by using all 6 candidate references, V-value = 0.278). The relative expression of 4 target genes (*OCLN*, *CLDN1*, *CLDN4*, and *TJP1*) related to barrier function of the RE were analyzed. Primer-pair sequences are reported in Table 2. Final RTqPCR data were obtained by $2^{-\Delta\Delta\text{Ct}}$ method.

Table 2
Gene names and primer sequences for real-time quantitative PCR analysis

Gene	Sequence	Accession number
<i>ACTB (actin beta)</i>	Forward: ACTGTTAGCTGCGTTACACC <hr/> Reverse: GTCACCTTCACCGTTCCA	NM_173979.3
<i>UXT (ubiquitously expressed prefoldin like chaperone)</i>	Forward: CACATGTTGCTAGAGGGGCT <hr/> Reverse: TCAGTGCTGAGTCTCTGGGA	NM_001037471.2
<i>DBNDD2 (dysbindin domain containing)</i>	Forward: GTGGAGCTTATCGACCTGGG <hr/> Reverse: GGAGTTGGTGGAGGGTCTTC	NM_001130748.1
<i>RPS9 (ribosomal protein S9)</i>	Forward: TGCTGGATGAGGGCAAGATG <hr/> Reverse: GCAGGCGTCTCTCCAAGAAA	NM_001101152.2
<i>DDX54 (DEAD-box helicase 54)</i>	Forward: AAGAAGCGGTTTGTGGGACA <hr/> Reverse: CTGATGTAGCGGCCACTCTC	XM_002694516.6
<i>HMBS (hydroxymethylbilane synthase)</i>	Forward: ACCGCGCTCTCTAAGATTGG <hr/> Reverse: CCTCTCAAAGCATGCTCCA	XM_024975251.1
<i>OCN (occludin)</i>	Forward: TAAGTTGGAGACGCTTTC <hr/> Reverse: TAGGTGGATATTCCCTGA	NM_001082433.2
<i>CLDN1 (claudin 1)</i>	Forward: CGTGCCTTGATGGTGATT <hr/> Reverse: TTCTGTGCCTCGTCGTCT	NM_001001854.2
<i>TJP1 (tight junction protein 1, zonula occludens 1)</i>	Forward: TAGTTGAGCGAAATGAGAAA <hr/>	XM_024982009.1

Gene	Sequence	Accession number
	Reverse: AGTTGAGTTGGGCAGGAC	
<i>CLDN4 (claudin 4)</i>	Forward: CCTTCATCGGCAGCAACA	NM_001014391.2
	Reverse: AACAGCACGCCAAACACG	

Calculations and Statistical Analysis

The apparent permeability coefficient (Papp) of FITC, LPS, and HIS from the mucosal-to-serosal side were calculated for each treatment as described by Mani et al. (2013):

$$P_{app} = \frac{dQ}{(dt \times A \times C_0)}$$

Where: dQ/dt = transport rate (µg/min); C₀ = initial concentration in the donor chamber (µg/ml); A = exposed surface area of the membrane (cm²).

The data were subjected to statistical analysis using MIXED PROC in SAS 9.4 (SAS Institute Inc., Cary, NC). Apparent permeability coefficient of FITC, HIS, and LPS from mucosal to serosal side, and electrophysiological parameters (Isc and Gt) were analyzed utilizing the follow model:

$$Y = \mu + b_k + \pi_l + \varphi_m + \pi\varphi_{ml} + \varepsilon_{kml}$$

Where μ = overall mean; b_k = the random effect of the k th tissue; π_l = the fixed effect of the l th sampling time (20 min intervals); φ_m = the fixed effect of the m th treatment; $\pi\varphi_{ml}$ = the interaction effect of the m th treatment by the l th time; ε_{ijk} = the random error and is $\sim N(0, \sigma_b^2)$. The model included tissue as repeated effect with compound symmetry as a covariance structure as it produced the least Akaike's and Bayesian Information Criterion values.

Gene expression data were analyzed with treatment as fixed effects and tissue as repeated effect with compound symmetry as a covariance structure. Differences between treatments were determined by least square means methods using the PDIFF option and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Results

Electrophysiological Parameters of LPS and Histamine Treated Rumen Epithelium

HIS increased the Isc of rumen tissue when compared with CON (Fig. 1A, **88.2%**, $P = 0.0022$). The Isc of tissue exposed to LPS was not different relative to the CON. Gt of HIS tended to higher than CON (Fig. 1B, **29.7%**, $P = 0.056$).

Permeation of FITC, LPS, and Histamine of Rumen Epithelium

Adding LPS to the mucosal side did not affect the Papp of the RE to FITC and FITC flux rate across RE, but HIS increased Papp ($P = 0.017$, 1.04-fold) and the flux rate ($P = 0.0223$, 71.45%) of rumen tissue to FITC compared with CON (Fig. 2A and Fig. 2B). The Papp of LPS from the mucosal-to-serosal side was 1.81-fold greater than that of HIS (Fig. 2D, $P = 0.0005$). As shown in Fig. 2C, the flux rate of HIS was 56.17 pmol/(cm²×h); the flux rate of LPS was 12.71 EU/(cm²×h).

Relative mRNA Abundance of Genes Associated with Tight Junction

As shown in Fig. 3A, *OCN* expression was less for HIS compared with CON (1.1-fold, $P = 0.0473$), while there were no differences in the expression of *CLDN1*, *CLDN4*, and *TJP1*.

Discussion

Subacute ruminal acidosis (SARA) is considered a major animal health and welfare issue in intensive ruminant production systems (Plaizier et al., 2008). While initially the focus was on low pH, it is now recognized that outcomes arising from SARA initiated by low digesta pH, increased SCFA concentration, and hyperosmolality (Owens et al., 1998; Hernandez et al., 2014; Humer et al., 2018). The previously mentioned changes in the rumen milieu coupled with rapid fermentation leads to the release of microbial metabolites and cell wall fragments such as HIS and LPS (Pilachai et al., 2012; Dong et al., 2013; Liu et al., 2013; Mao et al., 2016). Low pH, hyperosmolality, and accumulation of microbial associated molecular patterns (MAMPS) are considered as the potential triggers of ruminal epithelial barrier damage and the subsequent activation of systemic inflammation (Aschenbach et al., 2019). However, the study of Schurmann et al. (2014) reported that although ruminal acidosis was not induced, tissue conductance and mannitol flux, as a measure of permeability, linearly increased as calves were fed a diet containing 50% concentrate for 3, 7, 14, and 21 d suggesting that the RE barrier function could be modulated even in the absence of ruminal acidosis. Similarly, experiments by Meissner et al. (2017) and Greco et al. (2018) demonstrated that low pH alone does not increase the permeability of RE and further reported that the presence of VFA were responsible for the increase the permeability when coupled with low ruminal pH. Thus, there is a growing body of research suggesting that changes occurring concurrently with decreased ruminal pH during SARA may damage RE barrier function. However, the specific role of individual components is not clear.

In the present study, the Isc of the RE was increased by HIS compared with CON, which is similar to the changes of physiological parameters of RE during SARA where increased Isc and Gt were observed (Klevenhusen et al., 2013). However, these results differ from Aschenbach and Gäbel (2000) where no effect of HIS was detected on Isc. Aschenbach et al. (2000) illustrated a very efficient intraepithelial catabolism at a mucosal pH of 7.4. The catabolism of histamine seems to comprise a complex enzymatic pathway initiated by the diamine oxidase enzyme (DAO) (Sjaastad, 1967; Dickinson and Huber, 1972). Sun et al. (2017) demonstrated that histamine can activate of the NF- κ B inflammatory pathway and upregulate the expressions of the inflammatory cytokines (TNF- α , IL-6, and IL-1 β), a then induce the inflammatory response in bovine rumen epithelial cells. Thus, both efficient intraepithelial catabolism of histamine and induced inflammatory response in rumen epithelium seemingly indicated an induced metabolism in rumen epithelium which may be partly account for the increased Isc of RE under histamine.

The apparent permeability of HIS and LPS in RE were compared and the results showed that the Papp of HIS was less than that for LPS. Aschenbach et al. (2000) suggested that, at a mucosal pH of 7.4, permeability of the ruminal epithelium to histamine was very low. In addition, their study also demonstrated a very efficient intraepithelial catabolism of histamine (mucosal to serosal direction, 98.7%) at mucosal pH 7.4 and a significant secretory mechanisms from serosal to mucosal side (Aschenbach et al., 2000). Thus, their results in vitro approach established that the intact ruminal epithelium is a very effective barrier to luminal histamine (Aschenbach and Gäbel, 2000). LPS is thought to enter circulation by transport across the intestinal epithelium via not only paracellular pathways through the openings of intestinal tight junctions between two epithelial cells, but also by a transcellular pathway through lipid raft membrane domains involving receptor-mediated endocytosis (Berg, 1995; Drewe et al., 2001; Mani et al., 2012). Transcellular passive transportation is the predominate pathway of LPS absorption by intestinal mucosa (Drewe et al., 2001) and specific transport system for LPS was observed in colonic epithelial cells (Tomita et al., 2004). Furthermore, significantly increased translocation of LPS from the mucosal to the serosal side of rumen tissues under the presence of mucosal side LPS was observed by Emmanuel et al. (2007). Thus, in the present study, the higher Papp of LPS than HIS indicated that LPS seemingly can more easily pass through the gastrointestinal tract than HIS.

Supporting past research, we did not observe an effect of HIS on Gt under incubation conditions with a pH of 7.4 (Aschenbach and Gäbel, 2000). While Gt was not affected, HIS increased Papp and flux rate to FITC suggesting a direct role of HIS on altering RE permeability. Aschenbach et al. (2000) also reported that HIS receptors are broadly distributed and evidence of their localization within smooth muscle of the rumen (Ohga and Taneike, 1978) causing cessation of rumen contractions, increased vascular blood flow, and increased vascular permeability. In addition, HIS has been reported to increase permeability of the intestinal tract in rabbits (Kingham and Loehry, 1976; Miller et al., 1992) and permeability of HIS to cross the RE barrier increases with exposure to acidic pH (Aschenbach and Gäbel, 2000; Aschenbach et al., 2000); however, when measured in vivo under anesthesia, permeability of the rumen to HIS was low (Kay and Sjaastad, 1974). Overall, the results of the present study support previous research that HIS exposure may have a causative role in reducing the barrier function of the RE. In addition, the mRNA abundance of

OCN, one of the tight junctions, was downregulated in HIS compared with CON (Fig. 3). Epithelial barrier function is primarily dependent on tight junction (TJ) proteins that limit paracellular permeation (Marchiando et al., 2010). A variation in epithelial permeability can be related to a change in the general abundance of TJ proteins, including the localization and the interaction of the proteins (Markov et al., 2015, 2017). The study of Liu et al. (2013) suggested that downregulation of TJ protein (*CLDN-4* and *OCN*) as well as redistribution of *CLDN-1*, *CLDN-4*, and *OCN* out of the TJ caused the increased RE permeability. Thus, the downregulated mRNA abundance of *OCN* of the present study further implied a disrupted permeation barrier of RE by HIS.

Little effects of LPS on permeability and electrophysiological character of rumen tissues was observed at pH 7.4 in the present study, which is similar with the results of Emmanuel et al. (2007) where the presence of LPS did not affect permeability of rumen tissue to ³H-mannitol at pH values of 5.5 and 6.5. However, at pH 4.5, the permeability of rumen tissues to ³H-mannitol was increased more than 6-fold by the presence of LPS (Emmanuel et al., 2007). Similar phenomenon also occurred in colon tissues, where no difference permeability of ³H-mannitol due to LPS at pH of 6.5 and 7.4, but permeability was increased at pH 5.5 (Emmanuel et al., 2007). Thus, consistent with previous results, this study suggested that separately increased mucosal side LPS without acidic pH has no significant effect on the permeability of rumen tissues.

Conclusions

Compared with CON, HIS increased the permeability of FITC and Isc of RE and decreased the mRNA abundance of *OCN* in RE. As such, the results of the present study suggested that HIS plays a direct role in the processing of the disruption of RE barrier function. While increased LPS without acidic pH has no significant effect on the permeability of rumen tissues.

Abbreviations

CON: control; DEPC: diethylpyrocarbonate; FITC: fluorescein 5(6)-isothiocyanate; GNB: Gram Negative Bacilli; Gt: transepithelial conductance; HIS: histamine; HRP: horseradish peroxidase; Isc: short-circuit current; LPS: lipopolysaccharide; Papp: apparent permeability coefficient; RE: ruminal epithelium; RT: reverse transcription; SARA: subacute ruminal acidosis; SCFA: short chain fatty acids; TMB: tetramethylbenzidine; VFA: volatile fatty acid.

Declarations

Ethics approval and consent to participate

All animal care and procedures were approved by the Animal Welfare and Ethical Committee of Institute of Animal Science, Chinese Academy of Agricultural Sciences (No. IAS20180115). The whole experiment

was conducted in strict accordance with the Directions for Caring of Experimental Animals from the Institute of Animal Science within the Chinese Academy of Agricultural Sciences (Beijing).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

STG and DPB contributed to the concept and design of the work. STG, ALTZL and WHL executed the experiments. STG and GBP contributed to the analysis and interpretation of the data. STG drafted the manuscript. LM and DPB contributed to the final approval of the version for publication. All the authors read and approved the final manuscript.

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Figures

Figure 1

Effects of LPS and HIS on electrophysiological parameters of rumen epithelial. A represent the short-circuit current of different treatments. B represent the tissue conductance of different treatments. Values with different letters (a, b and c) differ significantly ($P < 0.05$). Error bars represent the standard error of the mean (SEM). 8 tissues ($n = 8$) were used in this study.

Figure 2

Apparent permeability coefficient (Papp) and flux rate of fluorescein 5(6)-isothiocyanate (FITC), LPS, and histamine from mucosal to serosal side. A and B represents the flux rate and Papp of FITC. C and D represent the flux rate and Papp of LPS and HIS. The unit of LPS was shown in left Y-axis. The unit of HIS was shown in right Y-axis. Values with different letters (a, b and c) differ significantly ($P < 0.05$). Error bars represent the standard error of the mean (SEM). 8 tissues ($n = 8$) were used in this study.

Figure 3

Relative mRNA abundance genes relative to tight junction proteins: A) OCLN, B) CLDN4, C) CLDN1, D) TJP1. Values with different letters (a and b) differ significantly ($P < 0.05$). Black diamond and black line in each box represent the mean and the median of that treatment respectively. 8 tissues ($n = 8$) were used in this study.