Effects of subacute ruminal acidosis on colon epithelial morphological structure, permeability and expression of key tight junction proteins in a dairy goat model

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Research

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Abstract

Background: The hindgut epithelial barrier plays an important role in maintaining absorption and immune homeosrasis in ruminants. However, there is little information available on changes of colon epithelial barrier structure and function following grain-induced subacute ruminal acidosis (SARA). The objective of this study was to investigate the effects of SARA on colon epithelial morphological structure, permeability and gene expression involved in epithelial barrier function using dairy goats as a ruminant model.

Methods: Twelve mid-lactating Sannan dairy goats (62.13 ± 4.76 kg) were randomly divided into either control (n = 6) or SARA treatment (n = 6). Control goats were fed a mixed diet (non-fiber carbohydrates/neutral detergent fiber (NFC/NDF) =1.15) for 60 days, SARA induction goats were given 4 mixed diets with NFC to NDF ratios of 1.15, 1.49, 2.12 and 2.66 to induce development of SARA. The duration of each diet was 15 d including 12 d for adaption and 3 d for sampling. Continuous ruminal pH recordings were utilized to diagnose the severity of SARA. Additionally, Plasma and colonic tissue were collected to evaluate the epithelial tissue morphological structure, permeability and expression of tight junction (TJ) protein using histological techniques, Ussing chamber, real-time PCR and western blotting.

Results: Profound disruption in the colonic epithelium was mainly manifested as the electron density of TJ decreased, intercellular space widened and mitochondria swelled in SARA dairy goats. Epithelial Isc, Gt and the mucosal-to-serosal flux of FD4 ($P < 0.05$) were increased, PD was decreased in SARA dairy goats compared with the control. SARA increased the plasma LPS ($P < 0.001$), D-lactic acid contents ($P = 0.025$) and DAO activity ($P = 0.003$). Increased gene and protein expression of claudin-1 and occludin ($P < 0.05$) were observed in colonic epithelium of SARA goats.

Conclusion: Overall, the data of present study demonstrate that SARA can impair the barrier function of the colonic epithelium in dairy goats, which is associated with severe epithelial structural damage and increased permeability and changes in the expression of TJ proteins.

Background

In modern intensive production system, a large proportion of easily fermentable carbohydrates in ruminant diet is usually fed to meet the energy requirements for supporting high milk yields and rapid weight gain [1–3]. However, rapid transition to these diets and inadequate buffering can cause decreased ruminal pH, increased the accumulation of organic acids, and altered the microbial population in gastrointestinal tract (GIT) and subsequently result in digestive disorders such as subacute ruminal acidosis (SARA) [4–6]. Exposure to SARA may also increase the risk of low dry matter intake and milk quality, as well as diarrhea, laminitis, hepatic abscesses and other diseases, which ultimately lead to significant financial losses in the dairy industry [7–9]. Thus, maximizing milk production without incurring SARA is a challenging for most dairy producers.

It is known that GIT is the main place for digestion and absorption of nutrients, and the GIT epithelium serves as an important barrier that can prevent the paracellular transportation of toxic compounds into

the portal circulation [10, 11]. In case of SARA, increased LPS, high acidity and hyperosmolality of the digesta can damage the barrier function and increase the rumen epithelial permeability, leading to the increased uptake of LPS, which initiates a systemic acute phase response [12–14]. Several studies conducted in cow and goat demonstrated that grain-rich diets and grain-induced SARA increased ruminal epithelial permeability and compromised rumen epithelial barrier function [11, 15, 16].

In comparison to the rumen histological structure with multilayered squamous epithelium [17, 18], the large intestine, which is much more “leaky”, being covered by a single layer of epithelial cells, is another region where microbial fermentation occurs [10, 19]. Fermentable carbohydrates in the hindgut typically provides 5 ~ 10% of dietary energy, but certain conditions such as SARA can result in hindgut excessive fermentation [20]. It has been shown that the increase of hindgut luminal acidity, coupled with elevated lipopolysaccharide, might cause damage to the intestinal lining and contribute to SARA symptoms [21, 22]. This indicates that the effects of SARA extend beyond the rumen, While little information is currently available about the consequences of SARA on the hindgut epithelial barrier function.

Therefore, the objective of this study was to investigate the effects of SARA on the colon epithelial morphological structure, permeability and key tight junction protein expression related to barrier function in dairy goats. We established a SARA model that is closer to real-life production conditions by increasing gradually the dietary of NFC to NDF ratios to reduce rumen pH to a conventional level.

Materials And Methods

The experimental design and procedures were approved by the Animal Care and Use Committee for Livestock issued by the Institute of Animal Science, Chinese Academy of Agricultural Sciences.

Animals and Experimental Design

Twelve ruminally fistulated mid-lactating Sannan dairy goats with approximately 62.13 ± 4.76 kg body weight were used in this study. All animals were fed an all-hay diet ad libitum for 2 wk prior to the start of this experiment to ensure adaptation to the diet. After dietary adaptation period, dairy goats were randomly allocated to either control (n = 6) or SARA treatment (n = 6), and placed in individual pens with free access to water. The control goats were fed a basal diet [non-fiber carbohydrates/neutral detergent fiber (NFC/NDF) = 1.15] for 60 days, and SARA induction goats gradually switched to the experimental diets with different NFC/NDFs (1.15, 1.49, 2.12 or 2.66, each for 15 days) to induce development of SARA for a period of 60 days. The ingredients and nutrient compositions of diets with NFC to NDF ratios of 1.15, 1.49, 2.12 and 2.66 are shown **in** Table 1. The dairy goats were fed these diets at 6:00 a.m. and 6:00 p.m.

Table 1

Ingredient and composition of experimental diets with NFC to NDF ratios of 1.15, 1.49, 2.12 and 2.66

Item	Diet (NFC to NDF ratios)			
	1.15	1.49	2.12	2.66
Ingredients, % of DM				
Alfalfa	17.98	9.88	4.22	1.61
Hay	34.59	32.99	27.37	21.72
Corn	27.71	37.86	52.13	59.95
Soybean meal	3.57	7.52	8.80	9.73
Wheat bran	13.68	8.75	3.94	3.01
NaCl	0.15	0.15	0.15	0.15
Limestone	0.90	1.14	1.35	1.53
Premix[1]	1.42	1.71	2.05	2.30
Total	100.00	100.00	100.00	100.00
Nutrient levels[2]				
NE_L (MJ/kg)	11.95	12.49	13.11	13.32
CP	12.10	12.48	12.20	12.44
NFC	41.51	46.19	52.60	55.94
NDF	36.03	30.96	24.82	20.99
ADF	22.25	18.27	13.87	11.06
Ca	0.69	0.69	0.68	0.71
P	0.35	0.35	0.34	0.36
Ca:P	1.97	1.97	2.00	1.97
Concentrate: forage	47:53	57:43	68:32	77:23

[1] The premix consisted of the following ingredients per kg of diet: Vitamin A 500 000 IU, Vitamin D_3 100 000 IU, VE 3 500 mg, Fe 10 670 mg, Cu 1 700 mg, Zn 9 900 mg, Mn 5 000 mg, I 150 mg, Se 60 mg, Co 80 mg.

[2] Nutrient levels were estimated from the current goat foods.

Ruminal pH Measurement

Ruminal pH technology and methodology described by Sun et al [16] was used to measure ruminal pH every minute during the last three days of each induction period. Briefly, Ruminal pH was measured continuously throughout SARA induction period by ruminal pH measuring and recording system (S651CD, America Sensorex company, America Jenco). The probes were presented before and after incubation in the rumen using pH buffers 7.0 and 4.0 (Sensorex, Garden Grove, CA) at 39 °C. The daily ruminal pH was summarized as maximum, minimum, and mean values and averaged over a 3-d period. In addition, daily duration below pH 5.8 and 5.5 were calculated for each goat and averaged over a 3-d period, and pH 5.5 was used to as a threshold of determination of dairy goats incidence of SARA.

Samples Collection

After dairy goats induced SARA all animals were slaughtered by exsanguination after overnight fasting. a segment of the colon wall was removed immediately and washed three times with PBS buffer. The tissue samples were harvested and prepared for Ussing chamber studies within 15 min after slaughter, subsequently, the other part of colonic epithelium was separated from the muscular layers by blunt dissection rapidly transferred into liquid nitrogen and then placed in freezer at -80 °C until further for extracting RNA and proteins. Blood samples were collected from jugular vein into 5 mL vacuum tubes containing sodium heparin and immediately mixed to avoid coagulation. Plasma was obtained after centrifugation at 3000 × g for 15 min and then stored at -20 °C until analysis.

Histology (or Morphology) and Ultrastructure

Specimens of colonic wall were prepared for histological examination by fixing in 4% paraformaldehyde solution, dehydrating via ethanol, embedding in paraffin, and sectioning. Epithelial injury was assessed after hematoxylin and eosin (H&E) staining which has been described previously [17, 18].

Colonic tissues were separated and rapidly fixed with 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide, and embedded in resin. Five nanometer sections were cut by ultrathin microtome and stained by uranyl acetate and lead citrate. The ultrastructure of epithelial intercellulars were observed by transmission electron microscope (JEM-1010, Japan).

Ussing Chamber Measurements

The electrophysiological properties and permeability of the colonic epithelium were investigated with the isolated, intact colonic epithelium using the Ussing chamber technique. In the present experiment, approximately a 50-cm^2 piece of colonic wall tissue from the ventral sac were excised carefully and rinsed by immersion in buffer solution (37 °C). The removed epithelium was then stripped off from the muscle layer, cut into square (about 1 cm^2) and later mounted between the two halves of the incubation chambers (EM-CSYS-6, USA) with an exposed area of 1 cm^2 each. Both halves of the chambers were rapidly filled with buffer solution (Table 2) and were gassed with carbogen gas (95% O_2 and 5% CO_2) at 37 °C. The time from killing the dairy goats to mounting tissues in the Ussing chambers should be completed within 40 min.

Epithelial permeability was measured by the fluxes of fluorescein isothiocyanate dextran 4 kDa (FD4-100MG, Sigma-Aldrich, USA) across the epithelium as described previously by Overman et al. [25] and Hering et al. [26]. After a 20 min equilibration of the epithelium with the buffer solution open-circuit conditions, tissues were short-circuited and 8 µL FD4 were added to the mucosal side of each chamber at the final concentration of 0.75 mmol/L. After equilibration for 20 min, samples of 200 uL were taken from the serosal side at 20-min intervals over a 1-h period and stored at -20 °C and later analyzed for concentrations of FD4 via fluorescence at excitation 492 nm and emission 520 nm, respectively. Electrophysiological parameters were continuously recorded with the aid of a computer-controlled analysis software for automatic data.

Table 2
Composition of the buffer solution used in the Ussing chamber

Composition	Concentration(mmol/L)
NaCl	80.0
KCl	5.0
$NaHCO_3$	25.0
$NaH_2PO_4 \times H_2O$	0.4
$Na_2HPO_4 \times 2H_2O$	2.4
$C_3H_5NaO_2$	10.0
$C_4H_7NaO_2$	5.0
$C_2H_3NaO_2 \times 3H_2O$	25.0
$CaCl_2 \times 2H_2O$	1.2
$MgCl_2 \times 6H_2O$	1.2

Lipopolysaccharide, D-lactic Acid and Diamine Oxidase Analysis

The levels of Lipopolysaccharide (LPS), D-lactic acid and Diamine oxidase (DAO) used as indices of colonic mucosal injury in dairy goats. ELISA Kits were used to determine LPS and D-lactic acid contents in plasma samples. The activity of DAO was analysed by using the method of Hu et al. [27].

Quantitative Real-Time PCR

Total RNA was extracted from colon samples of each dairy goat using TRIzol® reagent (Takara, Japan). A Nanodrop 2000 Spectrophotometer (Thermo, USA) was used to assess concentration and quality the

RNA. Then, RNA were treated with RNase-Free DNase and reverse-transcribed to the first-strand cDNA according to PrimeScript™ RT reagent Kit (Takara, Japan). All the primers selected to study the expression of genes related to TJs as listed **in** Table 3, which were designed and synthesized in Shanghai Sangon Biotech Co., Ltd. Quantitative real-time PCR was performed using a TB Green™ Premix Ex Taq™ II Kit (Takara, Japan) via StepOne Plus Real-Time PCR system (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is not affected by the experimental factors, was used as the reference gene. The relative expression values of genes were calculated by $2^{-\Delta\Delta Ct}$ method.

Table 3
primer sequences for qRT-PCR

Gene	GenBank Accession No.	Primer sequence (5'-3')	PCR Products (bp)
β-actin	NM_001314342.1	F: GGCTACAGCTTCACCACCAC R: GGAAGGAAGGCTGGAAGAGAG	211
Claudin-1	XM_005675123.3	F: ACAGCACTCTGCAAGCAACC R: TTCTGTGCCTCGTCGTCTTC	124
Claudin-4	XM_005697785.2	F: CCGCCACGAAACAACAAG R: GGGAGAAACAAAGACGAAAGGA	129
Claudin-7	XM_018064556.1	F: CGAGCCCTAATGGTGGTTTC R: GCCTTCTTCACTTTGTCGTCTC	107
Occludin	XM_018065681.1	F: AGCAGCAGCGGTAACTTGG R: CGTCGTGTAGTCTGTTTCATAGTGG	108
ZO-1	XM_018066118.1	F: CCGAATGAAACCACACACAAA R: TCCACGCCACTGTCAAACTC	104

Western Blotting Analysis

The total protein of colonic tissue was extracted as directed by the instructions of a total protein extraction kit (Keygen Biotech, Nanjing, China). Briefly, approximately100 mg of frozen colonic tissue was minced and homogenized in 1 mL ice-cold RIPA protein isolation buffer. Then, the protein concentration was determined via the BCA protein assay kit (Thermo, USA). 50 μg of total protein extract from each sample was later loaded onto 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto the nitrocellulose membranes (Bio Trace, Pall Co., USA) in Tris-Glycine buffer. After transfer, The membranes were saturated with 5% (wt/vol) nonfat milk powder (Yili, China) prepared in Tris-Buffered-Saline with Tween (TBST) for 1 h at room temperature and were then incubated with primary antibody overnight at 4 °C. The primary antibodies employed were rb-anti-claudin-1, m-anti-claudin-4, m-anti-claudin-7, m-anti-occludin, rb-anti-ZO-1 and β-actin (Abcam, UK, dilutions of 1:1000 for claudin-1, claudin-4, occludin and ZO-1, 1:200 for claudin-7, 1:4000 for β-actin).

After several washes with TBST, membranes were incubated with in a 1:5000 dilution of an anti-rabbit or anti-mouse horseradish peroxidase (HRP)-conjugated secondary antibodies (Thermo, USA). Finally, the blot was washed and detected by enhanced chemiluminescence (ECL) using the SuperSignal West Dura Extended Duration Substrate (Thermo, USA). The signals were recorded with an imaging System (Bio-Rad, USA), and the results were later analyzed by Quantity One software (Bio-Rad, USA). The β-actin was used as an internal control, which exhibited no difference between the groups. The protein expression value was the ratio of the densitometry units of TJ protein and β-actin.

Statistical Analysis

Ruminal pH was analyzed by performing general linear model repeated measures using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Means were compared via one-way ANOVA to analyze the colon epithelial permeability, blood biochemical index, and TJ mRNA and protein expression between groups in SAS 9.2 statistics for Windows. If a significant treatment effect was observed, the significance of the differences between groups was determined using Duncan's multiple comparisons test. All values are presented as the means and SEM. Differences were considered significant at $P < 0.05$, and a tendency was considered at $0.05 \leq P < 0.10$.

Results

Responses of Ruminal pH During Induction Protocol

As shown **in** Table 4, the SARA goats had a lower mean ruminal pH compared with control goats ($P < 0.05$). The maximum decreased from 6.91 to 6.24 ($P < 0.05$) and the minimum decreased from 5.74 to 5.29 ($P < 0.05$). The duration ruminal pH below 5.8 and 5.5 per day lasted for 7.35 h and 4.71 h in SARA goats, respectively, and were significantly higher than control goats ($P < 0.01$), which was consistent with the experimental definition of subacute ruminal acidosis proposed by Kleen et al [28] and Penner et al [29]. This means that the SARA induction protocol was successful.

Table 4
Effects of SARA on ruminal pH values in dairy goats

Item	Control	SARA	SEM[1]	*P* value
Ruminal pH				
Maximum	6.91[a]	6.24[b]	0.281	0.035
Minimum	5.74[a]	5.29[b]	0.233	0.020
Mean	6.14[a]	5.37[b]	0.254	0.037
Time of duration (h/d)				
< 5.8	1.56[b]	7.35[a]	0.450	< 0.01
< 5.5	0[b]	4.71[a]	0.673	0.018
< 5.2	0	0	-	-
[a,b] Means in a row with different superscript letters differ significantly ($p < 0.05$).				
[1] Pooled standard error of mean, n = 6.				

Morphology and Ultrastructure of the Colonic Epithelium

It can be seen from the HE staining results that SARA could cause a certain degree of damage to the colon epithelium of dairy goat. Compared with the control goats, severe indentations and cellular damage were observed on the surface of colon epithelium in SARA goats (Fig. 1A **and B**).

The ultrastucture of TJ in colon epithelium was detected by Transmission Electron Microscopy (TEM) to determine the colon TJ status. As shown in Fig. 2A **and B**, TJ structure of colon epithelium in control goats was integrity and displayed narrow ridge line, while TJ electronic density were decreased and intercellular space between epithelial cells widen as well as swelled mitochondria and broke or disappeared some mitochondria ridge were observed in SARA goats.

Colon Epithelial Permeability

Table 5 shows the permeability of colonic epithelium in dairy goats, which is reflected by electric-physiology parameters and the mucosal-to-serosal fluxes of FD4 measured in Ussing chambers. SARA increased colon epithelial Isc, Gt ($P < 0.05$) and decreased PD ($P < 0.05$). Moreover, the data of marker molecule FD4 flux from the mucosal to serosal indicated that a higher permeability of dairy goats in SARA goats compared with the control goats ($P < 0.05$).

Table 5
Electric-physiology parameters and FD4 flow of colonic epithelium of dairy goats suffered from SARA

Item	Control	SARA	SEM[1]	*P* value
Isc, mA/(cm^2 × h)[2]	0.73[b]	1.68[a]	0.077	0.019
Gt, mS/(cm^2 × h)[3]	4.76[b]	5.91[a]	0.136	0.048
PD, mV/(cm^2 × h)[4]	2.02[a]	0.51[b]	0.041	0.026
FD4, mM/(cm^2 × h)[5]	0.19[b]	0.41[a]	0.039	0.031
[a,b] Means in the same row with different superscripts differ significantly ($p < 0.05$).				
[1] Pooled standard error of mean, n = 6.				
[2] Isc = short-circuit current.				
[3] Gt = tissue conductance.				
[4] PD = potential difference.				
[5] FD4 = Fluorescein isothiocyanate dextran 4 kDa.				

Lipopolysaccharide, D-lactic Acid and Diamine Oxidase

Plasma LPS, D-lactic acid contents and DAO activity in dairy goats are presented **in** Table 6. During SARA, markedly increase were observed for LPS ($P < 0.001$), D-lactic acid contents ($P = 0.025$) and the activity of DAO ($P = 0.003$) in comparison with control goats.

Table 6
Lipopolysaccharide (LPS), D-lactic acid contents and Diamine oxidase (DAO) activity in plasma of dairy goats suffered from SARA

Item	Control	SARA	SEM[1]	*P* value
LPS, EU/mL	28.01[b]	64.31[a]	0.570	< 0.001
D-lactic acid, mmol/L	2.62[b]	3.76[a]	0.135	0.025
DAO, U/L	2.65[b]	3.93[a]	0.035	0.003
[a,b] Means in the same row with different superscripts differ significantly ($p < 0.05$).				
[1] Pooled standard error of mean, n = 6.				

Gene Expression of Tight Junction Proteins in the Colonic Epithelium

The results obtained from the preliminary analysis of the Claudins, Occludin and ZO-1 mRNA relative expression of dairy goats are summarised **in** Table 7. The mRNA expression of Claudin-1 and Occludin in the colonic epithelium markedly increased in SARA dairy goats ($P < 0.05$). However, expression of Claudin-4, Claudin-7 and ZO-1 had no significant difference between SARA and control dairy goats ($P > 0.05$).

Table 7
mRNA expression of tight junction proteins of colonic epithelium of dairy goats suffered from SARA

Item	Control	SARA	SEM[1]	*P* value
Claudin-1	0.98[b]	1.65[a]	0.128	0.030
Claudin-4	1.01[a]	1.21[a]	0.073	0.826
Claudin-7	1.03[a]	0.88[a]	0.046	0.583
Occludin	0.93[b]	1.54[a]	0.055	0.016
ZO-1	0.91[a]	0.83[a]	0.047	0.590
[a,b] Means in the same row with different superscripts differ significantly ($p < 0.05$).				
[1] Pooled standard error of mean, n = 6.				

Tight Junction Proteins Expression in the Colonic Epithelium

Figure 3 shows the proteins expression of Claudin-1 and Occludin in the colonic epithelium was significantly increased in SARA goats than those in control goats ($P < 0.05$), whereas Claudin-4, Claudin-7 and ZO-1 proteins expression in the colonic epithelium were not altered by SARA ($P > 0.05$).

Discussion

SARA is one of the most important metabolic disorders in ruminant production, which has a certain negative impact on rumen fermentation, animal welfare, productivity and farm profitability [30–32]. At present, time for pH below optimal level is used as a critical indicator to determine the severity of SARA. However, there are no uniform criteria for the rumen pH threshold and duration below a threshold. It has been reported that the continuous decline in pH threshold ranging from 5.8 to 5.5 exceeds 3 h/d is defined as SARA [33–35]. Deleterious effects of SARA, such as the increase of LPS concentrations in rumen and acute phase proteins in blood [14], are often observed with pH critical value depression below 5.6 for at least 3 h [5, 7, 36]. Kleen et al. [28] recommended pH 5.5 as the threshold for SARA. Based on

the above references, we decided pH 5.5 as the threshold of SARA incidence. Among all goats, maximun pH, minimum pH, mean rumen pH, duration pH below 5.8 and 5.5 range from 6.91 to 6.24, 5.74 to 5.29, 6.14 to 5.37 and from 1.56 to 7.35 and 0 to 4.71 h/d, respectively, without a drop in ruminal pH below 5.2. when the dietary NFC/NDF was 2.66, indicating that SARA model was induced successfully of dairy goats in the current study.

Due to the structural differences between the epithelium of rumen and large intestine epithelium, and the hindgut lacks natural defense against SARA, it would be easier compromised by microbes and their products. Argenzio and Meuten [37] showed that acetic acid (0.1 M) had a time- and pH dependent ability to damage the colonic epithelium in pigs. In production practice, intestinal epithelial barrier dysfunction is frequently found in high-yield ruminants due to long-term feeding of high-grain diet. In this study, our data demonstrated that a profound structural disruptions of colonic epithelium was observed in SARA goats depicted by electron density of TJ decreased, intercellular space widened and mitochondria swelled. In contrast, histological structure and the intercellular ultrastructure were normal in the colonic epithelium of control goats. The results of the present study are in accordance with previous studies conducted in high grain-fed goat omasum [38], goat colon and cecum [39, 40], indicating the disruption and expansion of tight junction in the hindgut tissues. As previous reported on the rumen epithelial barrier [21, 41–43], grain-induced SARA can disrupt rumen epithelial structure and integrity in dairy cows and goats, resulting in breakdown of rumen epithelial barrier function.

The integrity of the intestinal epithelial barrier plays a central role in maintaining the digestive system and even the entire animal health, which is damaged or dysfunctional can increase epithelial permeability [44]. The ussing chamber was first proposed when studying the ion transfer of epithelial tissues. Its main function is to detect changes in the electric current signal of ion channel of the whole cell membrane through microelectrodes to reflect the intestinal epithelial permeability or drug absorption [45, 46]. This technology provides a short-term culture method for isolated tissues, which can truly reflect the real-time conditions of the animal's intestinal environment and is simple to operate. To date, it has become the main method to study the permeability of gastrointestinal tract by detecting the electrophysiological parameters to reflect the activity of epithelial tissue and the ratio of isotopic or fluorescein labeled flow-through epithelium[25, 47] .

The increase of Isc, as a measure for active epithelial ion transfer, as well as Gt, as a measure of passive ion “leakage” means an increase of both transport capacity and permeability cross the epithelium. PD reflects the activity of intestinal epithelium. Previous studies have suggested that high grain diets can increase the Isc and Gt of ruminal epithelium, and flow rate of marker molecules of different sizes such as HRP and FITC, leading to weakened barrier function [11]. Penner et al. [48] reported that a mild episode of SARA did not negatively affect epithelial barrier function in the short term. However, with the increase of acidification in vitro, a decrease of barrier function was found. In the present study, we monitored intestinal permeability in terms of Isc, Gt of the colonic epithelium and flux of FD4. The flux of intact FD4 across the intestinal epithelium occurs mainly through paracellular pathways [49, 50]. Our results indicated that a concurrent increase of the Isc, Gt and flux of intact FD4 across the colonic epithelium in

SARA goats was observed, which showed that SARA induced higher epithelial permeability and barrier dysfunction. These results are in good agreement with the results of Sun et al [16] from this same experiment, who reported that SARA increased paracellular permeability of ruminal epithelium and compromised ruminal barrier. Therefore, our results combined with previous publications suggested that SARA not only impaired rumen epithelial barrier function of dairy goats, but also disrupted the integrity of the intestinal epithelium, resulting in an increase in permeability of colonic epithelium.

In the present study, We also used blood biochemical indicators to measure the barrier function of intestinal epithelium. Similar to the study of Andersen et al. [51] and Khafipour et al. [4], grain-induced SARA increased the concentration of LPS in peripheral blood. When the intestinal permeability increased abnormally due to some sort of disruption, allowing a large amount of LPS, D-lactic acid and DAO in the lumen to enter the blood circulation system. SARA could provide a stress on the hindgut epithelium via increasing the acidity and the LPS concentration of digesta. However, previous studies indicated that SARA did not increase LPS in peripheral blood [5, 7], which were inconsistent with the results of our study. Lactation can pose a stress on the ruminants and it may be one of the reasons for the difference [5]. In addition, our data also suggested that SARA significantly increased plasma D-lactic acid content and DAO activity. LPS, D-lactic acid and DAO in plasma have been considered as circulating markers for reflecting the degree of intestinal barrier injury [52, 53]. Thus, the changes of these indicators further suggested that SARA compromised the barrier function of the intestinal epithelium in dairy goats.

TJ is the most important connection between epithelial cells and also the key to form the intestinal mucosal barrier, which is mainly composed of TJ proteins [54]. Numerous studies have demonstrated the enrichment or defect of TJ proteins are causally related to a variety of human diseases, indicating that TJ proteins serve crucial role in human physiology [55–57]. In this study, with the disruption and expansion of intercellular TJs morphology, expression of Claudin-1 and Occludin were markedly up-regulated in the colonic epithelium of dairy goats during SARA, while no significant difference was observed in the expression of Claudin-4, Claudin-7 and ZO-1.

Upregulation of key TJ proteins would be expected to result in an increase of barrier function, but this was not the case in our study. The claudins comprise a multigene family, and the different claudins have diverse functions depending on cell type and the host organism [58]. Poritz et al [59] revealed that Claudin-1 doesn't localize to the TJ and may not contribute to the barrier function. The presence of Claudin-1 in the nucleus and cytoplasm may demonstrate that it has other functions. As previous reports, Claudin-1 expression has been confirmed to be upregulated in most types of malignancies, such as colorectal cancer [60–62]. Therefore, we speculate that the upregulation of Claudin-1 did not lead to an increase in intestinal epithelial barrier function. Occludin has a transmembrane region and may play a role in determining the function and structure of the paracellular barrier. In the present study, the increase of Occludin in colonic epithelium was basically consistent with the results of studies by Pederozoli et al. [63] and Tao et al. [41]. Reasons for the difference may be related to severity of SARA challenge, experimental animals, different dietary structures and the degree of damage to the intestinal epithelium. However, a recent study demonstrated that occludin depletion in intestinal epithelial cells in vitro and in

vivo leads to an increase in paracellular flux of larger-sized molecules, suggesting that occludin plays a crucial role in the maintenance of intestinal epithelial TJ barrier function, through the large-channel TJ pathway [64], which is inconsistent with the results of the present study. Therefore, the true causal relationship between TJ structural disruption and Occludin increase in the colonic epithelium of dairy goats suffered from SARA is unclear and needs further study.

Conclusions

This study demonstrate that SARA is involved in disruption of colonic epithelial barrier function as indicated by damage of morpthological integrity and changes in the expression of TJ proteins, which may increase colonic epithelial paracellular permeability, thereby increasing the risk of translocation of microbes and toxic compounds. A better understanding of the injury mechanism of SARA to the intestinal epithelium and its regulatory measures should be practical significance for improving the high yield of ruminants.

Abbreviations

SARA: Subacute ruminal acidosis; NFC: Non-fiber carbohydrates; NDF: Neutral detergent fiber; TJ: Tight junction; GIT: Gastrointestinal tract; Isc: Short-circuit current; Gt: Tissue conductance; PD: Potential difference: FD4: Fluorescein isothiocyanate dextran 4: LPS: Lipopolysaccharide; DAO: Diamine oxidase; ZO-1: Zonula occludens-1; HRP: Horseradish peroxidase.

Declarations

Acknowledgments

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Author' contributions

MYW, HLH, MG and MX conceived and designed the study; YL, YYS, LWS, MZ and XLZ provide assistance with animal feeding and specimen sampling; MYW and XLZ wrote the manuscript; MYW and HLH performed the statistical analysis and participated in the revision of the manuscript. All authors critically read the manuscript and gave final approval for submission.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The experimental design and procedures were approved by the Animal Care and Use Committee for Livestock issued by the Institute of Animal Science, Chinese Academy of Agricultural Sciences.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

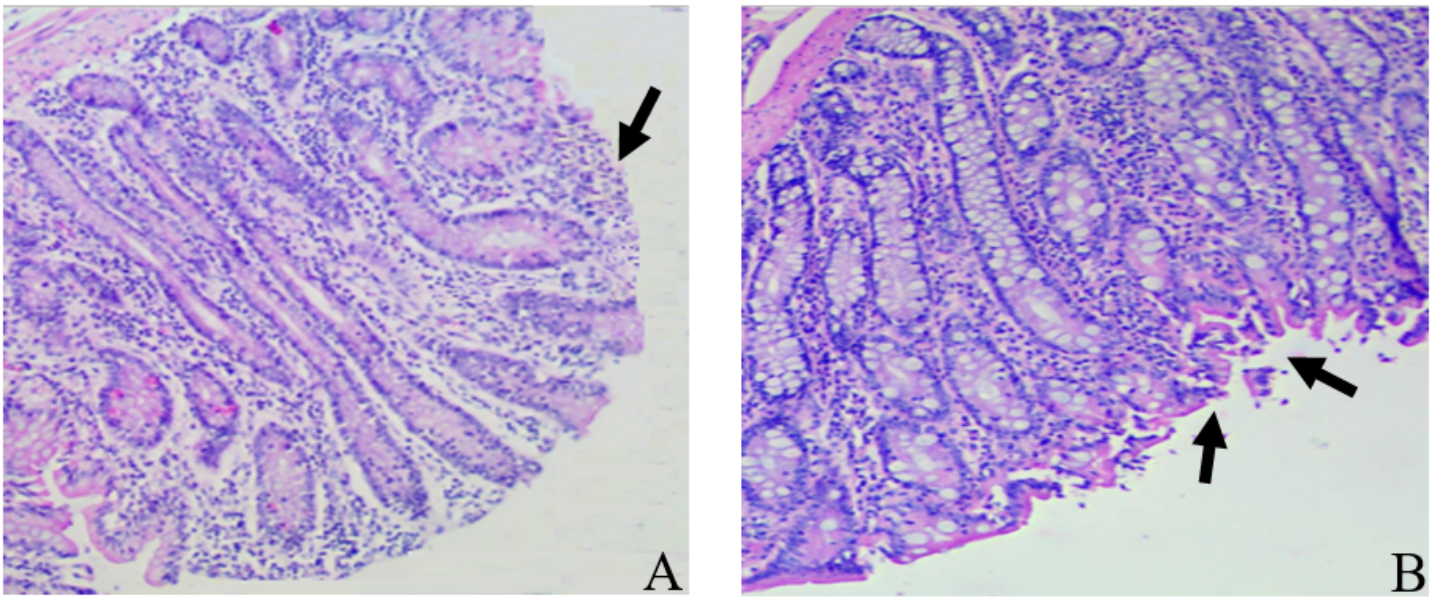

Figure 1

Comparisons of the morphological structure of the colonic epithelium between control and SARA dairy goats. Colonic epithelium from each group were processed for morphological observation: colon section of the (A: scale bar = 100 μm) control goats; (B: scale bar = 100 μm) SARA goats. Arrow indicates the damage of the colonic epithelium.

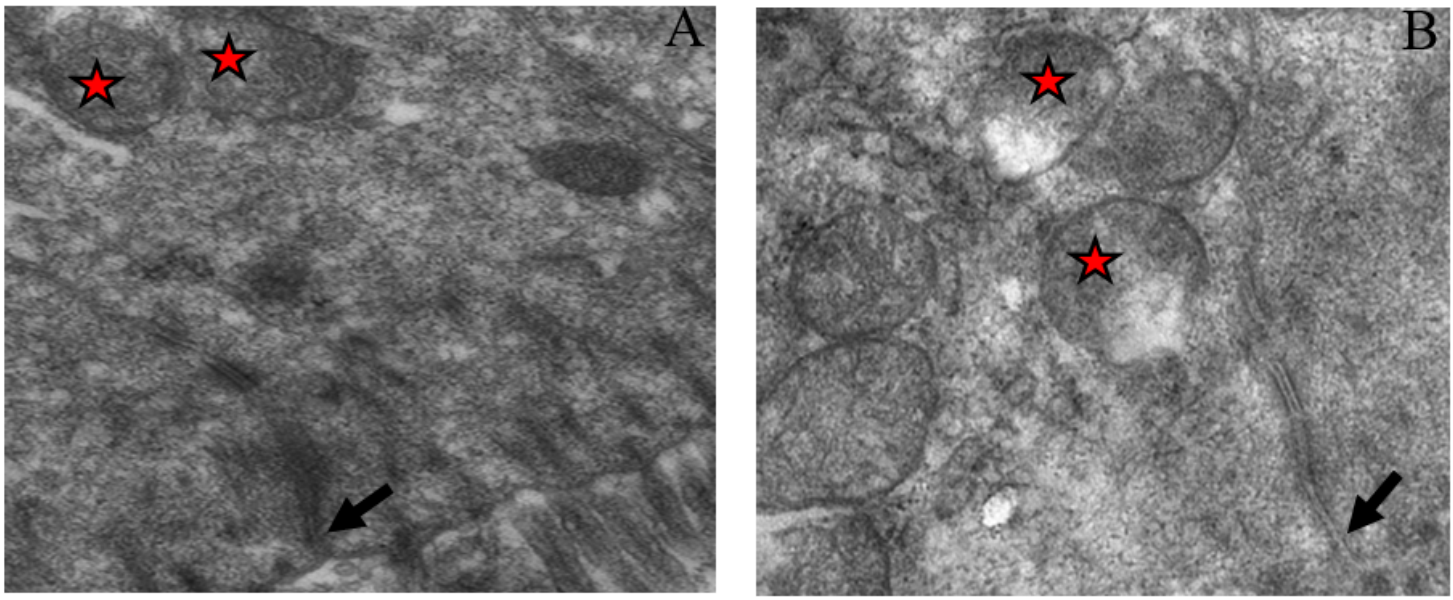

Figure 4

Comparisons of ultrastructural structure of the colonic epithelium between control and SARA dairy goats. Colonic epithelium from each group were processed for ultrastructure observation: colon section of the

(A: scale bar = 600 nm) control goats; (B: scale bar = 400 nm) SARA goats. The black arrow indicates tight junction, the red star represents mitochondria.

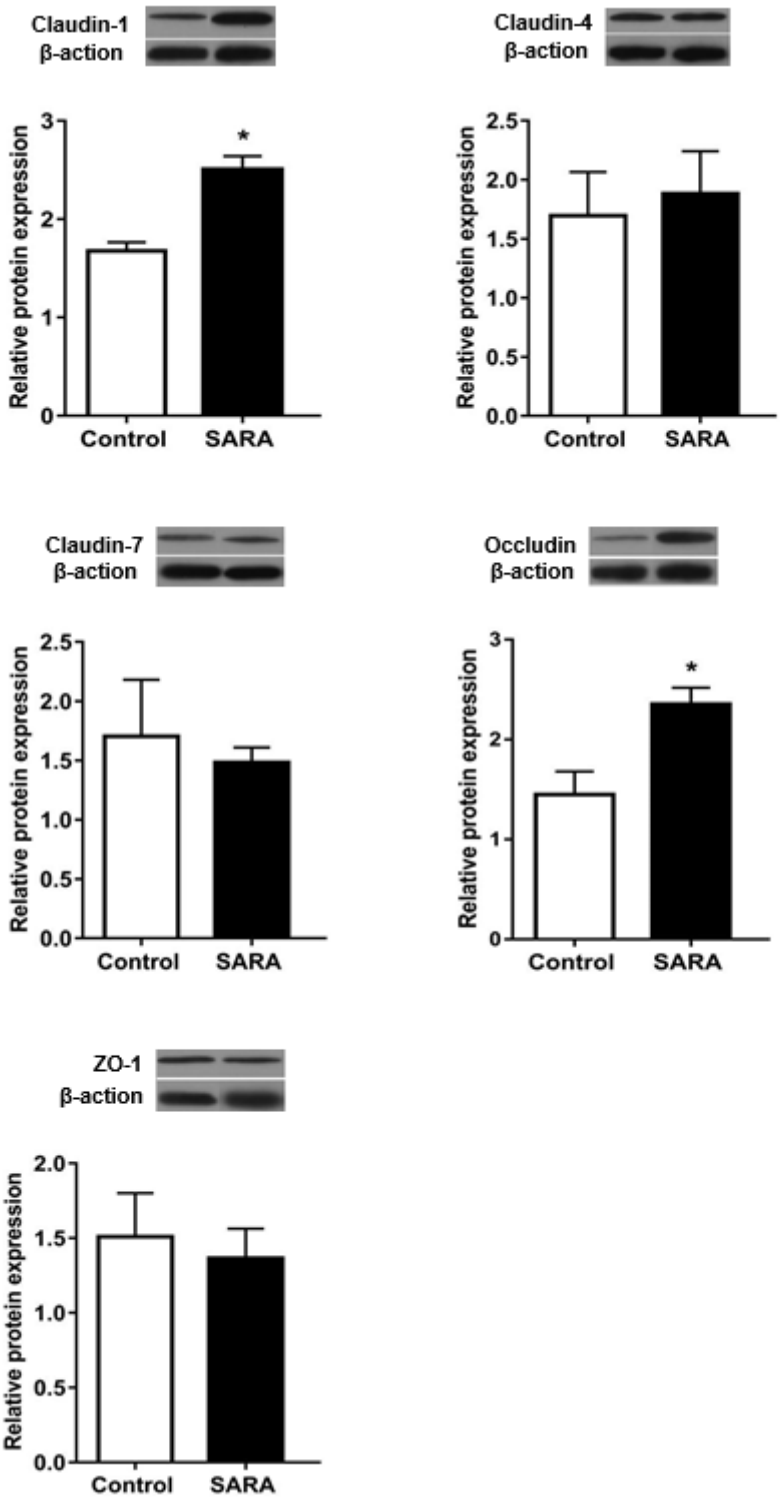

Figure 7

Relative tight junction proteins expression in the colonic epithelium between control and SARA goats .Values are mean ± SEM (n = 6). *indicates $p < 0.05$.