Niacin mitigates rumen epithelial damage in vivo by inhibiting rumen epithelial cell apoptosis on a high concentrate diet

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Abstract

To investigate the effects of niacin on rumen fermentation, rumen epithelial antioxidant activity, and rumen epithelial cell apoptosis on high concentrate (HC) diets, nine male Hu sheep were randomly divided into: low concentrate diet (LC; concentrate : forage (C:F) = 20:80, high concentrate diet (HC; C:F = 80:20), and HCN diet (HC diet + niacin at 800 mg/kg diet air-dry matter). Compared with the LC group, the HC group had a lower rumen pH, increased volatile fatty acids and lactic acid in the rumen, reduced activity of antioxidant enzymes and total antioxidant capacity, and increased malondialdehyde content in the rumen epithelium ($P < 0.05$). Rumen epithelial papilla morphology was decreased, and apoptosis-related indicators and serum inflammatory cytokines were increased in the HC group over the LC group ($P < 0.05$). Compared with the HC diet, the HCN diet increased rumen pH, rumen epithelium antioxidant capacity, and rumen epithelial papilla morphology, decreased rumen lactate content, serum inflammatory cytokines, and apoptosis-related indicators ($P < 0.05$). Therefore, adding 800 mg/kg niacin helped protect against rumen epithelial damage by avoiding drastic changes in the rumen environment and improved rumen epithelial antioxidant capacity to inhibit rumen epithelial cell apoptosis in sheep on a HC diet.

Introduction

For ruminants, the integrity of the rumen epithelial structure is necessary for the executive function of rumen digestion, absorption, and epithelial barriers (John et al. 2011). Thus, maintenance of the integrity of rumen epithelium is crucial for the ruminant growth, immunity, and health. However, in current ruminant production, excess intake of high concentrate (HC) diets for ruminant frequently causes volatile fatty acids (VFA) accumulation in the rumen, which exceed VFA absorption of rumen epithelium (Li et al. 2019), leads to lower rumen pH and finally results in subacute ruminal acidosis (SARA) (Sun et al. 2018). The prolonged low pH condition promotes some rumen microorganism death, and some toxic metabolite release (e. g. lipopolysaccharides: LPS), which then results in the disruption of the ruminal epithelial structural integrity and further damages the barrier function of the ruminal mucosa (Mao et al. 2015; Plaizier et al. 2012; Sun et al. 2018). Subsequently, these abnormal metabolites and pathogenic microorganisms migrate into the blood through the damaged rumen mucosa, leading to immunosuppression and an inflammatory response in the animal, which ultimately affect the health and production performance of ruminants (Nagaraja and Titgemeyer 2007; Plaizier et al. 2012). Therefore, inhibiting SARA-induced rumen mucosal damage while maintaining the integrity of the ruminal epithelial cells is a formidable challenge that may improve ruminant health and production performance.

The balance between cellular apoptosis and proliferation is essential for maintaining the integrity and its normal function of mucous membrane cells. Nevertheless, excessive epithelial apoptosis disrupts the barrier functions of the intestinal epithelium and subsequently contributes to intestinal hyperpermeability (Günther et al. 2013). Moreover, recent research has shown that apoptosis and damage of rumen epithelium are simultaneous during long-term high-concentrate diet feeding (Dai et al. 2020). Herein, we speculated that inhibition of rumen epithelial cell apoptosis might improve the rumen mucosal barrier structure and function.

Niacin as a precursor for the coenzymes NAD and NADP *in vivo*, can participate in mitochondrial respiration and have redox functions. Niacin has been shown to raise intracellular poly ADP-ribose polymerase (PARP) and cell viability (Khafipour et al. 2009). Furthermore, niacin may increase the cellular content of glutathione, suppresses the efflux of cytochrome-C (Cyt-C), and repairs the shear of PARP-related proteins, resulting in inhibition of cellular apoptosis (Tang et al. 2008). It was reported that niacin had anti-oxidative and anti-inflammatory functions that would help against the oxidative stress and inflammation occur in periparturient cow or beef cattle fed HC diet (Bühler et al. 2018; Luo et al. 2019a). One previous study has reported that adding 40 mM niacin can inhibit rumen epithelial cells apoptosis induced by butyrate *in vitro* by reducing intracellular oxidative stress, inhibiting the activation of caspase-3 and p53, and repairing DNA damage (Luo et al. 2019b). However, the effect of niacin on rumen epithelial cell apoptosis in ruminants on a HC diet *in vivo* has not been reported.

Hu sheep is a predominant breed for meat-producing in China because of its high meat production performance and good meat quality, which has been reared on a large scale in intensive production systems (Wang et al. 2017). While intensive Hu sheep farming are prone to develop SARA, therefore, to study the effects of niacin on the prevention and treatment of SARA in Hu sheep are thus of great importance. This study aimed to investigate the effects of the supplementation of niacin on rumen fermentation, rumen epithelial antioxidant activity, blood inflammatory cytokines, apoptotic index of rumen epithelium cells, and apoptosis-related gene expressions in sheep fed a HC diet.

Materials And Methods

Animal treatments and experimental diets

Nine, 7 month old, healthy, male Hu sheep of a similar body weight (34 ± 3 kg) with permanent rumen fistulas were randomly allocated to three diets: a low concentrate diet (LC; the ratio of concentrate and forage (C:F) was 20:80, a high concentrate diet (HC; C:F was 80:20) and the HC and niacin diet (HCN, HC diet + niacin at 800 mg/kg diet air-dry matter). The niacin (purity $\geq 99\%$) was provided by the Tianjin Zhongrui Pharmaceutical Co., Ltd. China, and was non-bypassed niacin. Each group consisted of three replicates with one sheep per replicate (pen). The test period lasted 11 days, including 8 days of the pre-trial period and 3 days of the experimental period. The sheep of the LC group were fed with the LC diet during the 11 days of test period. The sheep of the HC and HCN groups were fed with the LC diet during the first 7 days of the pre-trial period. Feed was then withdrawn for 1 days, and then sheep received the HC diet and HCN diet for 3 days of the experimental period to induce subacute acidosis (Goad et al. 1998). The diet was designed based on the Chinese Feeding Standards for Meat Sheep (NY/T 816–2004). The LC diet was formulated to meet the nutrient requirements of 0.05 kg daily gain in 30–35 kg male sheep. The HC diet was formulated to meet the nutrient requirements of 0.2 kg daily gain in 30–35 kg male sheep. The composition and nutrient levels of the LC and HC diet are presented in Table 1. In the HCN diet, niacin was mixed into the concentrate. After that, the concentrate was combined with the milled forage to make a granular total mixed ration (TMR). The amount of niacin added to the HC diet referred to our previous study (Luo et al. 2017). The TMR was provided twice daily.

Table 1
Composition and nutrient levels of experimental diet (air-dry basis, %)

Items	Diet ¹	
	LC	HC and HCN
<i>Ingredients</i>		
Peanut straw	80.0	20.0
Corn meal	12.0	52.3
Wheat bran	3.3	13.6
Soybean meal	1.0	4.0
Cottonseed	2.0	8.0
Limestone	0.2	0.6
Salt	0.5	0.5
Premix ²	1.0	1.0
Total	100	100
<i>Nutrient levels</i>		
Dry matter	89.32	86.92
Crude protein	11.78	14.57
NDF	51.38	24.26
ADF	35.79	13.74
Calcium	0.59	0.64
Phosphorus	0.25	0.46
DE ³ (MJ/kg)	10.78	14.49
¹ Diet: LC = low concentrate diet; HC = high concentrate diet.		
² The premix provided per kilogram of diet: 6500 IU of VD3, 32000 IU of VE, 236000 IU of VA, 475 mg of copper as copper sulphate, 1575 mg of zinc as zinc oxide, 11.5 mg of selenium as sodium selenite, 29.5 mg of iodate as calcium iodate, 3125 mg of iron as iron sulphate, 2435 mg of manganese as manganous oxide, 6.25 mg of cobalt as cobalt chloride, 60 mg of Mg as magnesium oxide.		
³ DE was calculated value, while others were measured values.		

Feed intake

In the test period, the feed weight and 2 h postprandial residual feed weight of each sheep were recorded accurately to calculate the daily feed intake. The differences in feed intake of the three groups each day were analysed.

Samples collection

The blood, rumen fluid, and rumen abdominal bursa samples were collected. At the end of the feeding experiment, after a 12 hr fast (the next morning), before morning feeding, blood sample was taken from the sheep jugular vein, centrifuged (3000 *g*, 10, 4°C) to obtain serum samples, and then stored at – 20°C.

After blood sampling, before morning feeding, approximately 100 mL of rumen fluid samples were collected from the upper, middle, and lower sites in the rumen. The rumen fluid samples were mixed homogeneously and then filtered through four layers of cheesecloth and immediately measured for pH using a Leici PHS-3C pH meter (Leici, Shanghai, China). Three replicates of each sample were measured to calculate the average value of rumen fluid pH. Then, a subsample 8 mL of rumen fluid was used for VFA analysis (mixed with 2 mL of 25% (wt/vol) metaphosphoric acid). Another subsample of rumen fluid was used for lactic acid analysis. These samples were frozen at – 20°C.

All sheep were sacrificed following electrical stunning and exsanguination after blood sampling and rumen fluid collection. The abdominal cavity of the sheep was opened and partial samples of rumen epithelium were excised from the caudoventral blind sac and washed three times in precooled phosphate buffered saline. The rumen epithelium samples were divided into two portions, the first sample was placed in 10% neutral buffered formalin at room temperature for 24 hr to examine tissue morphology. The other subsample of rumen epithelium was minced with scissors and frozen in liquid nitrogen in 2 mL tubes and stored at – 80°C for antioxidant activity analysis and determination of apoptosis-related gene expression.

Volatile fatty acids and lactic acid determination

The VFA content in the rumen fluid was evaluated by high-performance liquid chromatography (HPLC). Briefly, after thawing, the rumen fluid was mixed in a 1:1 ratio with chromatographic grade methanol and then centrifuged (12,500 *g*, 4°C, 10 min). The supernatant was filtered through a 0.45 µm filter, injected into a Waters-2489 Alliance HPLC system (Waters, Milford, MA, USA) with a Kromasil 5 µm C18 column (250 × 4.6 mm) (Feinano, Tianjin, China). Mobile phase A, HPLC grade methanol, mobile phase B, phosphate buffer (KH₂PO₄/H₃PO₄; 20 mmol/L, pH 2.37), mobile A/mobile B, 15%/85%. Column temperature: 30°C; injection volume: 10 µL; UV detection: 214 nm; characteristic running time: 15 min; flow-rate: 1.0 mL/min. The sample measurement was set to auto-sequence injection. Peaks were identified and quantified using standard curves established according to the method described by Zhang et al. (2010). Each sample was measured three replicates to calculate the average values of VFA.

The rumen fluid lactic acid concentrate was measured using a commercial lactic acid kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Each sample was measured three replicates to calculate the average value.

Rumen epithelium antioxidant activity determination

For antioxidant activity assays, frozen rumen epithelium samples were homogenized in ice-cold physiological saline (1:9) for 1 min and then centrifuged (2,700 *g*, 4°C, 10 min). The supernatants were used to measure the total antioxidant capacity (T-AOC), the activity of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), and the content of malondialdehyde (MDA). The content of total protein in the rumen epithelium was determined using a total protein kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instructions of the manufacturer. Antioxidant indices in the rumen epithelium were determined by commercial CAT, GSH-Px, SOD, T-AOC, and MDA kits (Nanjing Jiancheng Bioengineering Institute), and were normalized by total protein concentration in the rumen epithelium according to the instructions of the manufacturer. Each sample was measured three replicates to calculate the average values of those antioxidant indices.

Morphological structure of rumen epithelium

To determine the structure of the rumen epithelium, the rumen epithelial samples in 10% neutral buffered formalin were taken out and then dehydrated, paraffin-embedded, sliced, haematoxylin-eosin stained and sealed. Photographs were taken of the sections using a microscope (Motic BA210, Xiamen, China) at a magnification of 100×. The pictures, including villus height, width, and cuticular thickness, were analysed using the Image-ProPlus6.0 analysis system. The papilla width was measured at the middle of the villus. The papilla length was measured from the base of the villus to the highest point of the villus. Five fields were randomly captured in each section.

Serum inflammatory cytokines determination

The contents of serum interleukin-1 β (IL-1 β), IL-4, IL-6, IL-10, and tumour necrosis factor- α (TNF- α) were measured using commercial sheep IL-1 β , IL-4, IL-6, IL-10, and TNF- α Elisa kits (Nanjing Jiancheng Bioengineering Institute). Each sample was measured three replicates to calculate the average values of the serum inflammatory cytokine levels.

Rumen epithelium cell apoptotic index analysis

To analyse the apoptotic index of rumen epithelial cells, the rumen epithelial samples in 10% neutral buffered formalin were taken out and then dehydrated, paraffin-embedded, and sliced. The TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling) staining of rumen epithelial cells was performed using the TUNEL Apoptosis Detection kit (Wuhan Boster Biological Technology, Ltd., Wuhan, China). Photographs were taken of the sections with a microscope (Motic BA210, Xiamen, China) at a magnification of 400 ×. The TUNEL-positive brown cells were considered apoptotic cells. The apoptotic index was calculated and five views were captured in each section.

Apoptotic index = (number of apoptotic cells / number of total cells) × 100% Eq. 1

Rumen epithelium apoptosis-related genes expressions

Total RNA was extracted from frozen rumen epithelium using Trizol reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. The purity and quantity of total RNA were measured using a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd. China) at 260 and 280 nm, and the 260/280 ratios at 1.9–2.0 and the 260/230 ratios at 2.0–2.2 were used in the subsequent polymerase chain reaction (PCR). Total RNA was transcribed to cDNA using RevertAID reverse transcriptase (Thermo Scientific), according to the manufacturer's instructions. The mRNA expressions of apoptosis-related genes were analyzed as described by Li et al. (2021). Primers used for mRNA expression were presented in Table 2.

Table 2
PCR amplification primer design

Gene ¹	Primer sequence (5' to 3')	Product size (bp)	GenBank accession no.
GAPDH	Forward: 5'-AGGTTGTCTCCTGCGACTTCA-3' Reverse: 5'-CCCTGTTGCTGTAGCCGAAT-3'	132	NM_001190390.1
Fas	Forward: 5'-CTCTGAGGGGCTGAGATTGA-3' Reverse: 5'-GTTTGCCAGGAGGACAAGG-3'	107	NM_001123003.1
Bcl-2	Forward: 5'-TGTTTGATTTCTCCTGGCTGT-3' Reverse: 5'-ACTGCTTTCACGAACCTTTTG-3'	145	XM_012103831.2
Bax	Forward: 5'-TTCCGACGGCAACTTCAAC-3' Reverse: 5'-GAGCACTCCAGCCACAAAGA-3'	244	XM_015100639.1
Caspase8	Forward: 5'-AAAATGCCCTTCCCTTGTTG-3' Reverse: 5'-CTTCCCTCTGTTCTGAGTCGGT-3'	110	XM_012142500.2
Caspase3	Forward: 5'-GCAGCAAACCTCAGGGAAA-3' Reverse: 5'-CATGGCTTAGAAGCACGCA-3'	154	XM_015104559.1
Caspase9	Forward: 5'-TGTTGCCGTTTCCTTCTCC-3' Reverse: 5'-CTAGCACTTCGCTTCTGGTG-3'	111	XM_015099300.1
p53	Forward: 5'-CAGGAGACATTTTCCGACTTG-3' Reverse: 5'-TCATCCAGCCAGGTGACAA-3'	122	NM_001009403.1
¹ GAPDH = glyceraldehyde-3-phosphate dehydrogenase; Fas = actor associated suicide; Bcl-2 = B-cell lymphoma-2; Bax = BCL2-Associated X.			

Statistical analysis

All data analyses were performed by One-Way ANOVA using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). The significance of differences among treatments was evaluated using Tukey's tests. All results were presented as the mean $\pm$ standard deviation (SD). *P*-values < 0.05 were considered statistically significant.

Results

Feed intake

The feed intake of the LC group did not change, while that of the HC and HCN group fell gradually during the 3 d of the experimental period (Fig. 1). On the first day, the HC and HCN group had significantly higher (16.7% and 26%, respectively) feed intake than the LC group ($P < 0.05$). Then the HC and HCN groups had numerically lower (24% and 6.5%, respectively) feed intake than the LC group on the second day, and a significantly lower (37.7% and 26.7%, respectively) feed intake than the LC group on the third day ($P < 0.05$).

Rumen fermentation

The rumen pH value was lower in the HC and HCN groups than in the LC group ($P < 0.05$), while the rumen pH was higher in the HCN group than the HC group ($P < 0.05$) (Table 3). The HC and HCN groups had a higher content of acetate, propionate, butyrate, total VFA, and lactate in the rumen fluid than the LC group ($P < 0.05$), while the content of acetate, propionate, butyrate, total VFA, and lactate in the rumen fluid were lower in the HCN group than the HC group ($P < 0.05$). The HC group had a lower acetate/propionate ratio than the LC group ($P < 0.05$), and there was no difference between the HC group and the HCN group or the LC group and HCN group.

Table 3
Effects of diet treatments on the rumen fermentation of sheep

Items ²	Diet ¹		
	LC	HC	HCN
Ruminal pH	6.85 ± 0.05 ^a	5.95 ± 0.08 ^c	6.28 ± 0.05 ^b
Acetate (mmol/L)	35.39 ± 1.18 ^c	70.24 ± 5.87 ^a	50.34 ± 2.92 ^b
Propionate (mmol/L)	8.75 ± 1.35 ^c	28.76 ± 9.03 ^a	15.11 ± 2.87 ^b
Acetate/Propionate	4.11 ± 0.05 ^a	2.59 ± 0.61 ^b	3.43 ± 0.66 ^{ab}
Butyrate (mmol/L)	4.27 ± 0.44 ^c	18.85 ± 4.69 ^a	7.84 ± 1.13 ^b
TVFA (mmol/L)	48.42 ± 2.47 ^c	117.86 ± 19.50 ^a	73.29 ± 3.60 ^b
Lactate acid (mmol/L)	0.21 ± 0.22 ^c	0.43 ± 0.05 ^a	0.30 ± 0.02 ^b
Mean values with different letters were significantly different ($P < 0.05$).			
¹ Diet: LC = low concentrate diet; HC = high concentrate diet; HCN = HC + 800 mg/kg niacin in the feed concentrate.			
² TVFA = total volatile fatty acids.			

Rumen epithelium antioxidant activity

The activity of CAT, GSH-Px, SOD, and T-AOC of rumen epithelium was lower in the HC and HCN groups than in the LC group ($P < 0.05$), while the activity of CAT, GSH-Px, and T-AOC was higher in the HCN group compared to the HC group ($P < 0.05$) (Table 4). However, there was no difference in SOD activity between the HC and the HCN group. Compared to the LC group, the HC and HCN group had a higher content of MDA ($P < 0.05$), while the content of MDA was lower in the HCN group than the HC group ($P < 0.05$).

Table 4
Effects of diet treatments on the rumen epithelium antioxidant activity of sheep

Items ²	Diet ¹		
	LC	HC	HCN
CAT (U/mg of protein)	3.62 ± 0.18 ^a	1.65 ± 0.12 ^c	2.55 ± 0.15 ^b
GSH-Px (U/mg of protein)	538.28 ± 33.86 ^a	144.02 ± 24.46 ^c	235.37 ± 25.18 ^b
SOD (U/mg of protein)	233.99 ± 16.38 ^a	162.65 ± 10.17 ^b	171.52 ± 24.56 ^b
T-AOC (U/mg of protein)	2.34 ± 0.15 ^a	0.59 ± 0.13 ^c	1.43 ± 0.28 ^b
MDA (nmol/mg of protein)	1.66 ± 0.11 ^c	3.59 ± 0.22 ^a	2.19 ± 0.15 ^b
Mean values with different letters were significantly different ($P < 0.05$).			
¹ Diet: LC = low concentrate diet; HC = high concentrate diet; HCN = HC + 800 mg/kg niacin in the feed concentrate.			
² CAT = catalase; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; T-AOC = total antioxidant capacity; MDA = malondialdehyde.			

Rumen epithelium papilla morphology

In the LC group, normal ruminal mucosa was observed. There was mucosal shedding and superficial submucosal congestion in the sheep fed with the HC diet. In the HCN group, only mild mucosal shedding was observed. The papilla length, width (Fig. 2b), and cuticular layer thickness (Fig. 2c) of rumen epithelium were lower in the HC and HCN groups than in the LC group ($P < 0.05$), while the HCN group had a higher papilla length, width, and cuticular layer thickness than the HC group ($P < 0.05$) (Fig. 2).

Serum inflammatory cytokines

Compared with the LC group, the HC and HCN groups had higher levels of IL-1 β , IL-4, IL-6, IL-10, and TNF- α in the serum ($P < 0.05$), while the levels of IL-1 β , IL-4, IL-6, IL-10, and TNF- α in the serum were lower in the HCN group than in the HC group ($P < 0.05$) (Table 5).

Table 5
Effects of diet treatments on the serum inflammatory cytokines of sheep

Items ²	Diet ¹		
	LC	HC	HCN
IL-1 β (ng/L)	14.09 $\pm$ 2.66 ^c	55.45 $\pm$ 2.33 ^a	31.54 $\pm$ 3.77 ^b
IL-6 (ng/L)	70.86 $\pm$ 2.90 ^c	532.28 $\pm$ 28.64 ^a	297.17 $\pm$ 58.93 ^b
TNF- α (ng/L)	123.65 $\pm$ 15.13 ^c	484.75 $\pm$ 33.78 ^a	250.73 $\pm$ 30.85 ^b
IL-4 (ng/L)	167.08 $\pm$ 62.22 ^c	982.30 $\pm$ 13.74 ^a	388.31 $\pm$ 73.67 ^b
IL-10 (pg/mL)	161.84 $\pm$ 48.09 ^c	562.36 $\pm$ 26.45 ^a	276.93 $\pm$ 52.17 ^b
Mean values with different letters were significantly different ($P < 0.05$).			
¹ Diet: LC = low concentrate diet; HC = high concentrate diet; HCN = HC + 800 mg/kg niacin in the feed concentrate.			
² IL-1 β = interleukin-1 β ; TNF- α = tumor necrosis factor- α .			

Rumen epithelial cell apoptotic index

Compared with the LC group, the HC and HCN group had a higher apoptotic index of rumen epithelial cells ($P < 0.05$), while the apoptotic index was lower in the HCN group than in the HC group ($P < 0.05$) (Fig. 3).

Rumen epithelial apoptosis-related genes expressions

The mRNA expression of *Fas*, *Bcl-2*, *Bax*, *Caspase-3*, *Caspase-8*, *Caspase-9*, and *p53*, and the ratio of *Bcl-2/Bax* expression in the rumen epithelium was higher in the HC and HCN group than the LC group ($P < 0.05$), while mRNA expression of the genes and the ratio of *Bcl-2/Bax* expression were lower in the HCN group than the HC group ($P < 0.05$) (Table 6).

Table 6

Effects of diet treatments on the apoptosis-related genes expressions in rumen epithelium of sheep

Items ²	Diet ¹		
	LC	HC	HCN
Fas	0.61 ± 0.17 ^c	2.16 ± 0.26 ^a	1.08 ± 0.23 ^b
Bcl-2	0.63 ± 0.12 ^c	3.11 ± 0.24 ^a	1.35 ± 0.12 ^b
Bax	0.45 ± 0.03 ^c	1.13 ± 0.11 ^a	0.78 ± 0.09 ^b
Caspase-3	0.62 ± 0.14 ^c	2.80 ± 0.17 ^a	1.37 ± 0.28 ^b
Caspase-8	0.65 ± 0.19 ^c	2.31 ± 0.15 ^a	1.15 ± 0.19 ^b
Caspase-9	0.32 ± 0.04 ^c	1.59 ± 0.26 ^a	0.63 ± 0.04 ^b
p53	0.73 ± 0.15 ^c	2.52 ± 0.18 ^a	1.27 ± 0.15 ^b
Bcl-2/Bax	1.38 ± 0.21 ^c	2.76 ± 0.11 ^a	1.76 ± 0.19 ^b
Mean values with different letters were significantly different ($P < 0.05$).			
¹ Diet: LC = low concentrate diet; HC = high concentrate diet; HCN = HC + 800 mg/kg niacin in the feed concentrate.			
² Fas = actor associated suicide; Bcl-2 = B-cell lymphoma-2; Bax = BCL2-Associated X.			

Discussion

In modern ruminant production, an increased proportion content in the diet is widely used to improve production performance and economic benefits. Nevertheless, HC diets tend to induce adverse effects on feed intake (Nagaraja and Titgemeyer 2007) and lead to an accumulation of VFA, reducing buffering capacity in the rumen (Li et al. 2019). This promotes lower rumen pH, reduces ruminal microbial diversity and ultimately results in SARA (McCann et al. 2016; Oba and Allen 2000). In this study, the results showed that the HC diet reduced the rumen pH in sheep. Although the rumen pH value did not reach a rumen acidosis standard of below 5.8 (Gozho et al. 2005), digestive disturbances still occurred, as evidenced by the reduced feed intake and high content of lactic acid in the rumen of the sheep. The HC diet supplemented with niacin increased the rumen pH and decreased the lactic acid compared to the HC diet. As previously reported, niacin supplementation in HC diets of beef cattle can improve ruminal pH and propionate concentration, decrease the content of acetate, and promote the growth of rumen microorganisms (Doreau and Ottou 1996; Luo et al. 2019a; Niehoff et al. 2008). The beneficial effect of niacin may be partially ascribed to the regulation of niacin of the growth and community diversity of rumen microorganisms by inhibiting starch utilization and stimulating fibre degradation (Luo et al. 2017).

It is unclear if a higher concentration of niacin would fully reverse the effects of a HC diet. However, the previous *in vitro* study in our research group showed that the addition of 20–80 mM niacin attenuated cellular damage induced by butyrate and promoted cell proliferation. Higher concentrations (100 mM) of niacin lead to low cell viability (Luo et al. 2019b).

Feeding an HC diet to ruminants results in a massive release of bacterial endotoxins (such as LPS), which provoke rumen epithelial cells to generate a amount of reactive oxygen species (ROS) (Arroyo et al. 2017). The ROS causes oxidative damage in mitochondrial proteins, DNA, and lipids, and decrease the activities of GSH-Px and SOD (Martínez-Alfaro et al. 2011; Terpilowska and Siwicki 2019; Wang et al. 2015). In the present study, the HC diet decreased the activities of CAT, GSH-Px, SOD, and T-AOC and increased the MDA content in the rumen epithelium compared to the LC diet, which was consistent with previous work. Niacin is an important antioxidant. It can inhibit nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and reduce intracellular ROS production (Ganji et al. 2015). One previous review has summarized that niacin can effectively reduce the cellular ROS concentration, improve the activities of GSH-Px and CAT, and restore the activities of pyruvate dehydrogenase and succinate dehydrogenase (Ilkhani et al. 2016). Similarly, the HC diet supplemented with niacin increased the activities of CAT, GSH-Px, and T-AOC and decreased the MDA content.

The rumen mucosa, an important physiological barrier between rumen internal environment and blood circulation system, can effectively prevent various pathogens and harmful substances from entering blood (Plaizier et al. 2012). When rumen mucosal integrity is damaged, the functions of rumen digestion, absorption, and epithelial barriers are impaired (John et al. 2011). In this study, we observed that the rumen papillae length, width, and cuticular layer thickness in sheep fed with a HC diet were lower than in sheep fed a LC diet. This result was consistent with earlier reports suggesting that during SARA, exposure to an acidic environment coupled with high rumen LPS concentrations work together to cause rumen epithelial damage, resulting in a decrease in rumen papillae length, width, and cuticular layer (Hu 2008). In the present study, adding niacin to the HC diet increased the rumen papillae length, width, and cuticular layer thickness compared to the HC diet. The development of SARA is accompanied by inflammatory reaction, which due to that the destruction of ruminal epithelium, leading to abnormal harmful substances from the rumen entering the blood and resulting in an up-regulation of serum inflammatory cytokines by epithelial leakage (Dong et al. 2013; Plaizier et al. 2012). The results in the present study showed that the HC diet increased the serum inflammatory cytokines compared to the LC diet, and adding niacin to the HC diet decreased these factors. This effect may be partially due to niacin playing an anti-inflammatory role by reducing multiple inflammatory factors (including IL-6, TNF- α , and iNOS) via inhibition of the NF- κ B signaling pathway (Chen et al. 2009).

The molecular mechanism underlying niacin regulation of rumen epithelial papillae morphology is not clear. It is well known that the stability of rumen epithelial cells depends on a specific balance between cellular apoptosis and proliferation. In the current study, the HC diet increased the apoptotic index of rumen epithelial cells in sheep over the LC diet, which might suggest that the disruption of the rumen mucosal barrier is associated with rumen epithelial cell apoptosis induced by the HC diet. Dai et al.

(2020) demonstrated that a high grain diet could promote rumen epithelial cell apoptosis in dairy cows. During SARA, the rumen is in a state of abnormal stress due to the accumulation of organic acids and the decrease in pH, which may result in an increase in the apoptosis of rumen epithelial cells. Similarly, acetic acid-induced mucosal injury in a rat model is often used to study the therapeutic effects of anti-apoptotic drugs on gastric ulcers (Li et al. 2017; Nakamura et al. 2003). When the content of butyrate exceeds the rumen papillae absorbing ability, it will inhibit the proliferation of epithelial cells (Luo et al. 2019b). In this study, niacin supplementation in the HC diet decreased the apoptotic index of rumen epithelial cells. It is suggested that lower VFA (especially of butyrate) and higher ruminal pH in sheep in the HCN treatment may be responsible for stress relief in the rumen epithelium.

As a cascade reaction, apoptosis is regulated by intracellular genes and extracellular factors (Bergmann 2007). Among the many apoptosis-related genes, *Fas*, *Bcl-2*, and *Bax* have attracted more attention in the literature. *Fas* (also known as Apo-1/CD95) an one cell surface protein, belongs to the tumor necrosis factor (TNF)/nerve growth factor receptor family. *Fas* activation by *FasL* and its receptor (*Fas*-associated death domain, *FADD*) activate caspases-3, -8, and -9, leading to apoptosis (Freiberg et al. 1996; Hodge et al. 1998; Walker et al. 1998). *Bcl-2* is highly homologous to *Bax*. Expression of *Bcl-2* has been shown to suppress apoptosis, whereas *Bax* promotes apoptosis in response to different stimuli, and a high *Bcl-2*/*Bax* ratio indicates resistance to apoptosis (Zhang et al. 2011). A crucial tumor suppressor protein, *p53* plays a vital role in inducing cell cycle arrest, DNA repair, and apoptosis (Thakur et al. 2012). Previous studies have revealed that a medium concentrate diet (35% concentrate) increased the mRNA expression of apoptotic genes (*caspase-3*, *caspase-8*, *caspase-9*, *p53*, and *Bax*), and reduced the ratio of *Bcl-2* to *Bax* (*Bcl-2*/*Bax*) expression compared to a LC diet (10% concentrate) (Gui and Shen 2016). Similarly, in the present study, the HC diet increased the mRNA expression of rumen epithelium apoptosis-related genes (*Fas*, *Bcl-2*, *Bax*, *Caspase-3*, *Caspase-8*, *Caspase-9*, and *p53*) over the LC diet. However, *Bcl-2*/*Bax* expression in the HC group was elevated, probably because programmed cell death is not regulated by *Bcl-2* family proteins but rather depends on caspase activity and Cyt-C release. Niacin supplementation in the HC diet decreased the mRNA expression of apoptosis genes (*Fas*, *Bcl-2*, *Bax*, *Caspase-3*, *Caspase-8*, *Caspase-9*, and *p53*). The down-regulation of apoptotic genes by niacin may be partially because niacin promotes ATP generation, DNA synthesis, and repair, and thereby improves the levels of cell viability (Kennedy 2016). Furthermore, oxidative stress-mediated by ROS is a well-known inducer of cell apoptosis (Raj et al. 2011). In this study, niacin increased the antioxidant capacity of rumen epithelium cells. It reduced the ROS production, which may contribute to the inhibition of apoptosis induced by the HC diet. More studies establishing the SARA model *in vitro* will be conducted soon to observe the effects of niacin on DNA repair and ROS generation in rumen epithelial cells, and to investigate the associated molecular signalling pathways to uncover the molecular mechanism of niacin in regulating rumen epithelial cell apoptosis.

In conclusion, the HC diet reduced the rumen pH, decreased the rumen epithelial papillae morphology and size and antioxidant capacity, and increased the apoptotic index of rumen epithelial cells and apoptosis-related gene expression, promoting the inflammatory response in sheep. Adding 800 mg/kg niacin tended

to reverse the negative effects induced by the HC diet in terms of improving rumen epithelial papillae morphology and size, rumen epithelial antioxidant capacity, and inhibiting rumen epithelial cell apoptosis.

Declarations

Authors' contributions

Z.G., K.Ouyang. designed the overall study. Z.G., Y.L., C.X., D.L., Q.Q., K.P., X.X., M.Q. performed experiments. Z.G., Y.L. wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Data availability

The datasets generated and analyzed during the current study are not publicly available due to all the results in the form of means and statistics are presented in this paper, but are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Ethics approval

This study was approved by the Animal Care and Use Committee of the College of Animal Science and Technology of Jiangxi Agricultural University (JXAULL-2020-27).

Consent to participate

Authors have permission to participate.

Consent for publication

Authors have permission for publication.

Competing interests

The authors declare that they have no conflicts of interest.

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Figures

Figure 1

Effects of diet treatments on the dry matter intake of sheep LC = low concentrate diet; HC = high concentrate diet; HCN = HC + 800 mg/kg niacin in the feed concentrate. On the x-axis, -1 d mean the 7th day of the pre-trial period, 0 d mean the day of fast, and 1 d, 2 d, 3 d means the three days of the

experimental period. At the last stage of the pre-trial period, the feed intake of each groups was closed. Only the 7th day of the pre-trial period was showed on the table.

Figure 2

Effects of diet treatments on the rumen papilla morphology (a), papilla length and papilla width (b) and papilla cuticular layer thickness (c) in sheep (■): LC = low concentrate diet; (□): HC = high concentrate diet; (▨): HCN = HC + 800 mg/kg niacin in the feed concentrate. a,bMean values within different letters were significantly different ($P < 0.05$).

Figure 3

Effects of diet treatments on the rumen epithelium cell apoptosis (density plots) (a) and apoptosis index (b) in sheep. LC = low concentrate diet; HC = high concentrate diet; HCN = HC + 800 mg/kg niacin in the feed concentrate. a,b Mean values within different letters were significantly different ($P < 0.05$).