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Ameliorative Effect of Dietary Selenium Supplementation on Fermentation Pattern, Epithelial Histomorphometry and Apoptotic Genes Expression in Rumen of Goats Fed High Concentrate Diet

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Key words
Apoptosis, Fermentation, Goats, Lipopolysaccharides, Rumen, Selenium

ABSTRACT

Selenium (Se) plays a vital role in antioxidant systems and preventing cellular damage. High concentrate (HC) diets cause abnormal rumen fermentation, increased short chain fatty acids (SCFA) production and lipopolysaccharides (LPS), leading to epithelial damage in rumen of goats. This study evaluated the effect of dietary Se on rumen fermentation and rumen epithelial integrity in goats. Thirty cross-bred goats (12-16 weeks, 11-13 kg) were randomly distributed to three groups: LC (low concentrate diet), HC (high concentrate diet), and HC-SY (HC + Se-yeast @ 0.5 mg/kg diet) for 10 weeks. Rumen fluid and epithelial tissue samples were analyzed for SCFA, pH, lipopolysaccharides (LPS). HC and HC-SY groups showed increased ($P < 0.05$) total SCFA and reduced ($P < 0.05$) pH. LPS levels were significantly decreased ($P < 0.001$) in HC-SY than other groups. Histomorphometry of rumen epithelium showed improved papillae growth and epithelial strata in group HC-SY than HC. Apoptotic genes *Bad, Caspase-8, were* downregulated ($P < 0.001$) while *Bcl-xl* were upregulated ($P < 0.01$) in HC-SY. In conclusion, HC diet supplemented with dietary Se showed ameliorative effect on fermentation pattern, epithelial histomorphometry by alleviating LPS level and apoptotic genes expression in rumen of goats compared to HC diet.

INTRODUCTION

Selenium (Se) is an essential micronutrient which is necessary for animals due to its vital role in various metabolic processes and biochemical activities (Ahsan *et al*., 2014). Supranutritional Se supplementation may also improve the productivity of animals (Memon *et al*., 2024). Animals fed concentrates diets exhibit greater Se absorption into body tissues due to passage of Se through the GIT compared to those fed roughage-based diets (Samo *et al*., 2018). A supranutritional level of Se improved the growth performance and musculoskeletal health in sheep subjected to heat stress (Chauhan *et al*., 2016), alleviated level of oxidative stress (OS) in several disease conditions and toxicity models (Ahsan *et al*., 2014; Zakeri *et al*., 2021).

Fattening ruminants receive high-concentrate (HC) diets containing starch which is readily fermented by ruminal microbes to produce volatile fatty acids (VFAs) that fulfill the energy requirement of the animal for maximizing its growth and productivity (Boerman *et al*., 2015; Chen *et al*., 2022). Feeding ruminants with HC diet for an extended

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period may result in digestive disruptions and intricate systemic issues due to the elevated levels of non-structural carbohydrates in diet with such highly fermentable feeds (Chen *et al*., 2023; Tao *et al*., 2015). Rumen is predisposed to abnormal changes as well as systemic diseases induced by grain rich diets (Shahid *et al*., 2020; Zhang *et al*., 2020) due to an unusual rise in rumen fermentation that decreases the rumen pH attributable to the retention of excessively produced short-chain fatty acids (SCFAs) beyond absorption capacity of rumen epithelium (Li *et al*., 2019). During ordinary circumstances, the penetration of endotoxins and antigens through the ruminal epithelium is limited (Balda and Matter, 2009) owing to the physical, microbial, and immune barriers that inhibit the transmission of these substances across the rumen (Shen *et al*., 2019). SCFA has pronounced effect on physical barriers in rumen, compromising the rumen epithelial tissue integrity for the regeneration of rumen epithelial cells and the expression distribution of tight junction proteins (Greco *et al*., 2018; Ma *et al*., 2021). Decreased pH due to excessive SCFA production against HC diet induces a significant release of lipopolysaccharides (LPS) that alter the gut permeability (Emmanuel *et al*., 2007; Gozho *et al*., 2005) followed by epithelial damage concomitant with escalated concentration of reactive oxygen species (ROS) (Tao *et al*., 2014). Feeding HC diet for an extended period further induces the proinflammatory response resulting in severe epithelial insults in rumen that trigger apoptotic cell death (Dai *et al*., 2022). Given the positive effects associated with supplementation of Se, particularly under challenging conditions, we hypothesized that feeding goats with supplementation of Se-yeast could improve fermentation pattern, rumen epithelial histomorphometry by alleviating LPS level and apoptosis induced by the HC diet. To our knowledge, no studies have assessed the impact of supplemental dietary Se on rumen fermentation patterns, histomorphometry, LPS, and apoptosis in goats fed HC diet. The present study was conducted to assess the effect of dietary Se supplementation against HC diet-induced changes on rumen fermentation, LPS, histomorphological changes and apoptotic genes expression in rumen epithelium of goats.

MATERIALS AND METHODS

Experimental design, animal feeding and management

Thirty cross-bred goats aging between 3-4 months, having bodyweight of 11-13 kg were kept in separate pens having an area of 3 × 3 sq. ft. Three dietary groups (n=10/group) were made where each group received the feedings as low concentrate (LC; roughage:concentrate 65:35), high concentrate (HC; roughage:concentrate 35:65), and HC diet supplemented with Se-yeast (HC-SY; roughage:concentrate 35:65 + Se @ 0.5 mg/kg diet). Dietary Se was supplemented at 0.5 mg/kg in diet as Se-yeast (Sel-Plex®, Alltech®, USA). The experiment lasted for 10 weeks including 4 weeks of adaptation. Animals were fed at 0800 h and 1700 h with *ad libitum* water access throughout the experiment. Se level in the diet of LC group was adjusted by including the exogenous Se to match the treatment amount of Se in the diet of HC group. Se at 0.5 mg/kg of diet was supplemented in the diets of HC-SY group. Se concentrations in the experimental diets were determined by wet microwave digestion, measuring the absorption against the standards using the inductively coupled plasma optical emission spectroscopy (ICP-OES Optima 2100-DV, Perkin Elmer, USA) according to the procedures previously described by Taylor (2005). The composition of diets and level of Se in each diet is presented in Table I.

Table I. Composition of diets fed to animals in treatment groups.

Items	Treatments		
	LC diet	HC diet	HC-SY diet
Ingredients (% of DM)			
Corn	25.6	25	25
Wheat bran	-	30.7	30.7
Soybean meal	7.4	2.2	2.2
Rapeseed meal	-	4	4
Limestone	0.5	1.5	1.5
DCP	0.8	0.7	0.7
Salt	0.4	0.4	0.4
Premix[a]	0.4	0.4	0.4
Se (mg/kg diet)			
Background Se in diet	0.035	0.15	0.15
Added	0.115	-	0.50
Total level	0.15	0.15	0.65

Goats were fed diets i.e. low concentrate (LC), high concentrate (HC), and HC with selenium (HC-SY) diets for 10 weeks. DM, Dry Matter; DCP, digestible crude protein. [a]Per kg of premix = Vitamin A 6000 U; Vitamin D2 500U; Vitamin E 80 mg; Cu 6.25 mg; Fe 62.5 mg; Zn 62.5 mg; Mn 50 mg; I 0.125 mg; Co 0.125 mg; Mo 0.125 mg. Selenium (Se) was supplemented as Se-yeast (SY) feed grade added to HC diets.

Slaughtering

At the end of experimental trial, each goat was slain in an isolated slaughter room within the Ruminant Research Unit. Goats were appropriately restrained in lateral recumbency and slaughtered by decapitation. Immediately after the exsanguination, complex stomach

was exteriorized through abdominal incision, isolated from other visceral organs, and taken into a clean tub.

Rumen fermentation characteristics and lipopolysaccharide determination

Rumen digesta contents were collected into individual containers under aseptic conditions and strained using four layered cheesecloth to collect rumen fluid. The pH of rumen fluid was instantly detected using a pH meter (Hanna pH 211, Hanna Instruments, Inc., RI, US). An aliquot of 50 mL rumen fluid was preserved with 5% $HgCl_2$ (v/v, 1/20) to avoid any further gas formation. Rumen fluid and the physiological saline solution (0.9% w/v of NaCl) with ratio of 1:1 was rigorously mixed, centrifuged for 15 minutes at 3000 × g. Two portions of the supernatants were separated. The first part was utilized to analyze short-chain fatty acids (SCFAs) using capillary column gas chromatography (Model: GC-14B, Shimadzu, Tokyo, Japan; Capillary column size: 30 mm × 0.32 mm × 0.25 mm film thickness; Temperature of column: 110°C; Temperature of injector: 180°C; Temperature of detector: 180°C) according to the procedure demonstrated by Malhi *et al*. (2013). Second portion of the supernatant was used to detect LPS using Chromogenic Endpoint Tachypleus Amebocyte Lysate Assay Kit (Chinese Horseshoe Crab Reagent Manufactory Co. Ltd., Xiamen, China). Pre-treated supernatants were diluted unless the concentration of LPS reached between 0.1 and 1.0 endotoxin unit per mL (EU/mL) comparable to the reference endotoxin in accordance with the procedure adopted from Gozho *et al*. (2005).

Histomorphometry of rumen epithelium

A 1 cm^2 segment of rumen tissue was excised from the atrium ruminis of each animal, washed with physiological saline solution, and fixed in 4% paraformaldehyde solution for 24 to 48 h to carry out for the determination of histomorphometry. Tissue samples were kept in running tap water overnight to remove excessive fixation followed by dehydration in ascending concentrations of ethanol, clearance in pure xylene and finally embedding tissue blocks in paraffin wax. Sections were made having 5-7 µm thickness, using a microtome and stained with hematoxylin and eosin. Histomorphometrical characteristics were determined using DigiPro 4.0 (Labomed, USA) as described by Shahid *et al*. (2020).

Measurement of expression of apoptosis-related genes using PCR

Tissue samples of rumen epithelium were processed for total RNA extraction using acid guanidinium thiocyanate-phenol-chloroform method, according to Chomczynski and Sacchi (2006). Total RNA concentration was analyzed at 260 and 280 nm with a nanodrop spectrophotometer. The absorbance ratio of each sample ranging between 1.91 and 2.29 showed maximum RNA purity. The relative mRNA expression was determined by performing real-time PCR using the MyiQ2 2-color real-time PCR detection system (Bio-Rad Laboratories Inc., Hercules, CA). Real-time PCR was performed in a final volume of 20 µl containing 1x iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA), cDNA template, using the combination of forward and reverse primers for the target genes that are being targeted (Table II), with sterile water for volume adjustment. To denature the cDNA, a first cycle of 30s at 95°C was applied. This procedure followed further with 40 PCR cycles to denature cDNA for 10s at 95°C, and the annealing and extension of primers for 30s at 55°C. Prior to execution of the PCR on ready samples, the amplification efficiencies of each primer were precisely calculated using a standard dilution series. At the end of each PCR, a melt curve analysis was performed. Gene expression was normalized to GAPDH ($\Delta Ct = Ct_{target} - Ct_{GAPDH}$). After normalization with GAPDH, the expressions values of relative mRNA for the targeted genes were calculated by using formula $2^{-\triangle\triangle Ct}$ as illustrated by Livak and Schmittgen (2001).

Table II. Primer sequences and GenBank accession number specific to genes of interest.

Gene	Primer sequence 5' to 3'	Accession No.	Size (bp)
GAPDH	F GGGTCATCATCTCTGCACCT R GGTCATAAGTCCCTCCACGA	HM043737.1	180
Bcl-xl	F TCCATCTCCGATTCAGTCCCT R TGAAGCGCATTGGAGATG	XM_006498612	142
Bad	F TTTCGGAAGACTGAGGTCTGAT R CGGCGAAGTTAGGGTTAATCTC	XM_004019650.3	185
Caspase-8	F GGCTCCTCTGAGATGCTG R TGCTCCCGTGCTATGCTA	NM-001045970	149

GAPDH mRNA, Glyceraldehyde 3-phosphate dehydrogenase ribosomal RNA; *Bcl-xl*, B-cell lymphoma-extra large; *Bad,* Bcl2 associated agonist of cell death; *Caspase*, cysteine-aspartic proteases. The first primer listed for each gene is the forward primer and the second primer is the reverse primer.

Statistical analysis

The data obtained thus statistically evaluated using one-way analysis of variance. Differences among the means were assumed significant at 95% probability ($P < 0.05$). Results were presented in terms of mean ± standard error of the mean. All the data was statistically analyzed using statistical software IBM® SPSS® Statistics (version 27.0.1; IBM Corp®, USA).

RESULTS

Rumen fermentation pattern

Molar concentrations of acetate (Ac), propionate (Pr), butyrate (Bu), and total short chain fatty acids (TSCFA) were increased significantly ($P < 0.05$) in rumen fluid, whereas the pH reduced ($P < 0.05$) both in HC and HC-SY than in LC (Table III). Goats in HC-SY group had lower molar concentration of Ac compared to HC ($P < 0.05$). However, Ac:Pr ratio was significantly reduced ($P < 0.05$) in HC-SY than HC, however there was no statistically a significant difference ($P > 0.05$) was found among HC and LC. TSCFA and Ac concentrations, and Ac:Pr ratio were significantly decreased ($P < 0.05$) by 6.96%, 16.25%, and 23.14% respectively, whereas the pH was significantly raised ($P < 0.05$) by 0.496 units in HC-SY to that of group HC (Table III).

Table III. Effect of LC, HC and HC-SY diets on SCFA (Molar concentration) and pH in rumen fluid of goat.

Items	Treatments		
	LC	HC	HC-SY
Acetate (mmol)	52.07 ± 1.62^{c}	67.71 ± 0.94^{a}	58.24 ± 0.99^{b}
Propionate (mmol)	18.29 ± 0.80^{b}	23.09 ± 0.70^{a}	24.41 ± 0.54^{a}
Butyrate (mmol)	9.10 ± 0.35^{b}	12.90 ± 0.38^{a}	14.29 ± 0.26^{a}
TSCFA (mmol)	79.47 ± 1.45^{b}	103.70 ± 1.03^{a}	96.95 ± 1.16^{a}
Ac:Pr ratio	2.87 ± 0.18^{a}	2.95 ± 0.11^{a}	2.39 ± 0.08^{b}
pH	6.50 ± 0.09^{a}	5.82 ± 0.07^{b}	6.31 ± 0.09^{c}

Ac:Pr = acetate to propionate ratio, TSCFA = total short chain fatty acid. Goats were fed low LC, HC and HC-SY diets for a period of 10 weeks. Total Se concentration in LC, HC and HC-SY diets were 0.15, 0.15 and 0.65 mg/kg diet, respectively. Values are means ± S.E and $^{a, b, c}$ different letters on the bars exhibit the difference among groups with $P < 0.05$.

LPS concentration in rumen digesta

LPS concentration in rumen digesta was significantly escalated ($P < 0.001$) by 214.62% in goats in groups HC than LC, whereas it was significantly raised ($P < 0.001$) by 104.19% in group HC-SY than in LC. However, the HC diet supplemented with Se (HC-SY) significantly decreased ($P < 0.001$) the LPS concentration by 54.08% compared with group HC (Fig. 1).

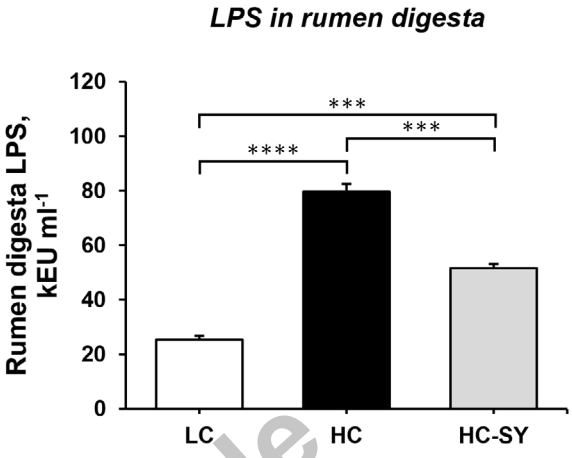

Fig. 1. Effects of LC, HC, and HC-SY diets on LPS contents in rumen digesta. The level of LPS contents calculated as endotoxin unit per ml (EU/ml). Goats were fed low concentrate (LC), high concentrate (HC), and HC with Se (HC-SY) diets for a period of 10 weeks. Total Se concentration in LC, HC, and HC-SY diets were 0.15, 0.15, and 0.65 mg/kg diet, respectively. Values are means ± S.E whereas, asterisk indicates statistical difference between among groups (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$).

Histomorphometry of rumen epithelium

Compared to group HC the papillae height increased ($P < 0.0001$) in group HC-SY and LC, whereas papillae thickness and epithelial thickness showed no statistical difference ($P > 0.05$) among all groups, though it slightly improved in HC-SY than in HC. Number of cell layers in stratum corneum (SC) increased ($P < 0.01$) in group HC-SY and ($P < 0.001$) in LC than in HC. Likewise, number of cell layers in stratum germinativum (SGv) increased ($P < 0.001$) in group HC-SY and ($P < 0.0001$) in LC than that of HC (Table IV).

mRNA expression of apoptotic genes in rumen epithelial tissue

mRNA expression of apoptotic regulator genes of *Bcl* family and *Caspase-8* in rumen epithelial tissue of goats are shown in Figure 2. Compared with HC group, the goats fed HC-SY diet upregulated ($P < 0.01$) the expression level of *Bcl-xl*. Whereas the mRNA expression level of *Bad* were downregulated ($P < 0.001$) in group HC-SY than in HC. Compared with LC, the HC diet upregulated ($P < 0.05$) the mRNA expression of *Caspase-8*, whereas the dietary Se supplementation in group HC-SY downregulated ($P < 0.001$) the HC diet-induced mRNA expression of *Caspase-8*.

Table IV. Effect of LC, HC and HC-SY diets on histomorphometric analysis of rumen epithelium in goats.

Items	Treatments		
	LC	HC	HC-SY
Papillae height (µm)	1451.83 ± 19.70[a]	1106.46 ± 17.14[b]	1334.86 ± 14.45[a]
Papillae thickness (µm)	355.04 ± 8.25	344.85 ± 6.81	351.48 ± 6.76
Epithelial thickness (µm)	191.04 ± 5.03	179.18 ± 7.25	188.12 ± 5.46
Thickness of epithelial strata (No. of cell layers)			
Stratum corneum	3.43 ± 0.18[a]	2.63 ± 0.11[b]	3.25 ± 0.10[a]
Stratum germinativum	5.81 ± 0.17[a]	4.42 ± 0.12[c]	5.32 ± 0.11[b]

Goats received LC, HC and HC-SY diets for a period of 10 weeks. Total Se concentration in LC, HC and HC-SY diets were 0.15, 0.15 and 0.65 mg/kg diet, respectively. Values are means ± S.E. The significance was considered at $P < 0.05$

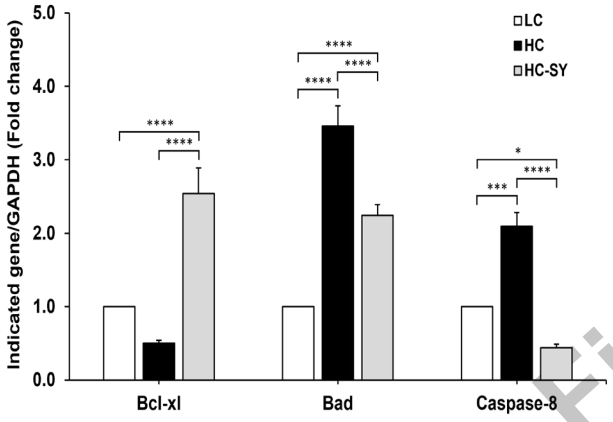

Fig. 2. Effects of LC, HC, and HC-SY diets on the expression of apoptotic genes i.e. *Bcl-xl, Bad* genes in rumen epithelium of goats. *Bcl-xl,* B-cell lymphoma-extra-large; *Bad*; *Caspase-8,* cysteine-aspartic protease-8; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. Goats were fed low concentrate (LC), high concentrate (HC), and HC with Se (HC-SY) diets for a period of 10 weeks. Total Se concentration in LC, HC, and HC-SY diets were 0.15, 0.15, and 0.65 mg/kg diet, respectively. Values are means ± S.E whereas, asterisk indicates statistical difference between among groups (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$).

DISCUSSION

Rumen fermentation pattern

The present study showed a significant elevation in SCFA molar concentrations (mmol) of Ac, Pr, Bu, and TSCFA in rumen fluid, whereas ruminal pH simultaneously decreased in goats fed HC diet with or without supplemental Se. These results are consistent with the previous investigations that there is an inverse relationship between SCFA and pH, both in rumen and hind gut of ruminants fed grain-rich diet (Pang *et al*., 2022; Tao *et al*., 2017; Wang *et al*., 2018; Ye *et al*., 2016). Fermentation process in gut is negatively impacted by long-term HC diet feeding, as evidenced by the linear association between the length of HC diet intake and the elevated fermentation rate (Wang *et al*., 2018). Such relationship between SCFA and pH triggers the mechanism of cell lysis and increase in toxin release resulting in subacute ruminal acidosis (SARA) (Li *et al*., 2019). However, we observed that the ruminal fermentation behavior in goats intaking the Se supplemented HC diet did not show a significant effect compared to those fed HC diet. Our current findings are aligned with previous studies, since depending on the dose and type of feed offered, dietary Se supplementation alters the patterns of fermentation in ruminants. Ruminal SCFA rises in cows fed dietary Se at up to 0.3 mg/kg diet; however, no increase in SCFA levels occurs with increasing dietary Se supplementation to 0.45 mg/kg diet (Wang *et al*., 2009). Lamb fed either 50% or 70% corn based HC diets showed the unaffected rumen fermentation patterns in response to gradual increase of sodium selenite (Na_2SeO_3) ranging 0.3 to 0.9 mg/kg diet (Razo-Rodríguez *et al*., 2013). These outcomes can be explained by a higher rate of fermentation due to the HC diet reaching a saturation level that potentially masked the effects of supplemental Se.

LPS level in rumen epithelium

In the present study we found that the HC diet boosted the LPS level in rumen fluid, correlating with an increased rate of rumen fermentation. It has been documented that feeding HC diet for an extended period supports the translocation of rumen-derived LPS to the bloodstream (Guo *et al*., 2017; Shah *et al*., 2022). Moreover, 60-90% HC diet significantly increased the LPS level both in colon and blood of goats (Tao *et al*., 2017; Wang *et al*., 2021). Upregulation of LPS with the decreased pH in rumen fluid was observed in cows fed grain-rich diet for up to 18 weeks (Dai *et al*., 2022). Likewise, elevated LPS

content was observed in colonic digesta fluid taken from the young goats fed up to 65% grain-rich diets (Samo *et al*., 2020; Ye *et al*., 2016). Increase in LPS levels due to HC diets is associated with the lowering luminal pH that induces bacteriolysis that not only releases endotoxins but also liberates LPS in the gut lumen (Mao *et al*., 2016; Plaizier *et al*., 2017). This study showed that the HC diet supplemented with dietary Se initiated an inhibitory effect on increasing level of LPS. The specific mechanism of LPS inhibition by dietary Se supplementation is unknown. It appears that Se may have contributed to reduce the release of LPS by preventing the bacteriolysis. Previous investigations have shown that dietary treatment with Se reduces LPS production and protects the colonic epithelial barriers in goats exposed to the stress of a diet rich in concentrate (Samo *et al*., 2020), and protective effect on jejunal damage in pigs experiencing heat stress (He *et al*., 2022). Either reduction or inhibition of the LPS production in ruminants may be due to the regulation of rumen pH by supplementing diet with Se (Aschenbach and Gäbel, 2000).

Rumen histomorphometry and apoptosis

Concurrently with decreasing pH and elevated LPS level in rumen, the histometric analysis of papillae in rumen epithelium of goats in all groups showed increased papillae height, and slight rumen papillae thickness in ruminal epithelium was observed in group HC-SY. Previous research has found that larger papillae are related with an improve in epithelial tissue mass (Malhi *et al*., 2013). Rumen papillae height and thickness of rumen epithelium increased in goats fed a diet supplemented with organic Se (Shahid *et al*., 2020). Increased germinal epithelial size and number of sertoli cell were found with some hypertrophic and hyperplastic effects due to proliferative changes in testis of young goats when fed grain rich diet supplemented with organic Se (Soomro *et al*., 2025). To determine whether the epithelial thickness was caused by cellular hypertrophic or hyperplastic effects, we measured the number of cell layers forming the epithelial strata as well as cell density within these layers. Se treated goats had a larger number of cell layers developing and stratum germinativum (SGv), which was linked to enhanced epithelial cell density. The greater thickness of these layers in groups HC-SY showed that the rumen epithelium was better protected and had a higher absorptive capacity than goats in control (Kauffold *et al*., 1977).

HC diet initiates the gut epithelium to undergo cell death via apoptotic pathways, endangering the integrity of mucosa that, consequently, damage the epithelium (Droin and Green, 2004; Tao *et al*., 2015). The highly specialized mechanism of apoptosis is controlled by various distinct proteins, most notably the *Bcl-2* family and *Caspases* (Trachootham *et al*., 2006). The *Bcl-2* family including both *Bad* and *Bcl-xl* proteins naturally occur in a stable state but any significant alteration in the *Bcl2* and *Bax* ratio (*Bcl2/Bax*) can modulate the apoptotic rate (Droin and Green, 2004; Tian *et al*., 2016). HC diet containing 35% concentrate lowered the expression of *Bcl2/Bax* ratio in comparison to a 10% concentrate containing LC diet by increasing the mRNA expression of *Caspase-8,* (Gui and Shen, 2016). In this experiment we observed that HC diet feeding resulted to cell death of rumen epithelium through apoptotic changes with a higher mRNA expression ratio of *Bad* than that of group LC, concomitantly with improved *Bcl-xl* expression in Se treated HC diet. Previous studies demonstrate that *Bad* with *Bcl-xl* protein form heterodimers, any conformational alteration prevents *Bak* from heteromerizing with *Bcl-xl* to exhibit an inhibitory effect on apoptosis (Hinds *et al*., 2007). Moreover, *Bcl-xl* as an anti-apoptotic member of *Bcl2* family showed an inhibitory effect against apoptosis that reduced colonic apoptotic changes (López-Oliva *et al*., 2013).

Apoptosis is induced through two distinct caspase-dependent pathways: The extrinsic pathway, which interacts with death receptors, and the intrinsic pathway, which involves the mitochondria (Droin and Green, 2004). Binding of a ligand to death receptors that trigger programmed demise of the cell via *Caspase-8* via extrinsic pathway. In the present study, rumen epithelial apoptosis was observed with upregulation of *Caspase-8* concurrently with *Bad*, via both the intrinsic and extrinsic apoptotic pathways induced against feeding HC diet. Gui and Shen (2016) illustrated that HC diet tends to increase mRNA expression of *Caspase-8* and showed lessen *Bcl2/Bax* expression ratio in contrast to LC diet. Similarly, the goats fed grain-rich diet escalated the mRNA expression of *Caspase-8* and *Bax* in colonic epithelium (Hua *et al*., 2017; Samo *et al*., 2020; Tao *et al*., 2015).

CONCLUSIONS

HC diet caused an improved SCFA and LPS concentration, rumen epithelial histomorphometry, concurrently by upregulating the mRNA expression of apoptotic genes *Bad* and *Caspase-8*. Finally, inducing rumen epithelial injuries in goats. However, the HC diet containing dietary Se mitigated the LPS level, downregulated the mRNA expression of apoptotic genes of *Bad* and *Caspase-8* concomitantly by upregulating the anti-apoptotic genes *Bcl-xl*. Hence reduced the HC diet-induced epithelial injuries in rumen of goats.

DECLARATIONS

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IRB approval

The proposal of current study received approval from the Board of Advanced Studies and Research (BASR) as Ref # DAS/1869) during year 2023.

Ethical approval

This research was carried out at Ruminant Research Unit situated in Livestock Experimental Station, Sindh Agriculture University, Tandojam, Pakistan in accordance with the guidelines of animal care and slaughtering; prior to approval by the Institutional Ethical Committee (Approval # DAS/1869 of 2023).

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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