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High-fat intake induces gut microbiota disorders, inflammatory responses and oxidative stress in *Nyctereutes procyonoides*

Zhaoming Li[1†], Chengwei Wei[1,2†], Jie Yang[1], Yuan Geng[1], Mengran Zhu[1], Tianchao Xu[1] and Mengyao Guo[1*]

Abstract

The *Nyctereutes procyonoides* is highly regarded in the farming and leather industries because of the high value of its fur, which renders artificial feeding a crucial aspect. However, high-fat diets have always been associated with a variety of digestive disorders. This study aimed to investigate the impact of high-fat diets on the gut microbiota and the mechanisms of gut damage in *Nyctereutes procyonoides*. 16S rRNA sequencing demonstrated that high-fat diets caused diarrhea and intestinal damage through alterations in the gut microbiota: a decrease in the abundance of Firmicutes, an increase in the abundance of *Proteobacteria* and *Actinobacteria*, and an increase in the abundance of *Enterococcaceae*, *Escherichia coli-Shigella*, *Clostridium* and *Lactobacillus*. Subsequently, changes in metabolic pathways, such as amino and fatty acid pathways, were identified by KEGG and COG enrichment analysis, and the TLR4/NF-κB/NLRP3 inflammatory signaling pathway was shown to be activated by high-fat diets. In addition, high-fat diets lead to the accumulation of ROS and MDA and reduce the activity of the antioxidant enzymes GSH-PX and SOD. Correspondingly, the levels of proinflammatory cytokines (IL-6, IL-1β and TNF-α) were significantly increased, and the apoptosis and necrosis signaling pathways of colonic cells were detected, causing a dramatic decrease in the expression of intestinal tight junction proteins (Occludin, E-cadherin, ZO-1 and ZO-2). In conclusion, high-fat diets altered the structure of the *Nyctereutes procyonoides* gut microbiota community and led to colon damage. This study provides new insights into the intestinal health of *Nyctereutes procyonoides*.

Highlights

1. High-fat diets result in disruption of Nyctereutes procyonoides intestinal flora.
2. High-fat diets cause functional and metabolic disturbances in the intestinal flora.
3. Disorders of gut microbiota induce inflammatory response, oxidative stress, and intestinal barrier disruption in the *Nyctereutes procyonoides* colon.

Keywords *Nyctereutes procyonoides*, High-fat diets, Gut microbiota, Inflammatory responses, Oxidative stress, Intestinal barrier

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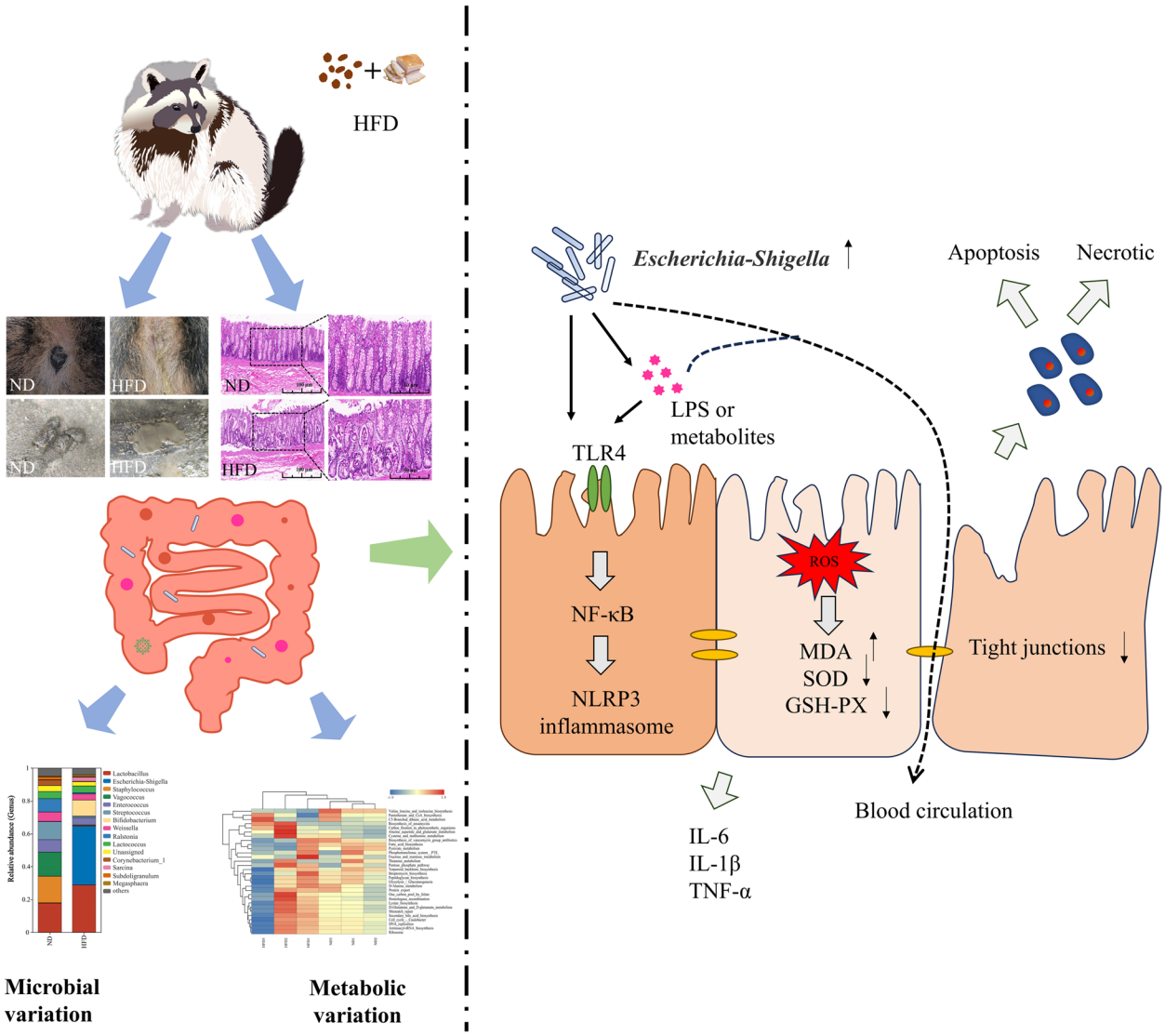
Graphical Abstract



Introduction

Nyctereutes procyonoides is an omnivorous canine with high economic value as a fur animal (Niiranen et al. 2021). Dietary fat can provide *Nyctereutes procyonoides* with essential fatty acids, which provide energy and promote the absorption of fat-soluble vitamins. If the proportion of fat in the ration is imbalanced, the growth and development of young *Nyctereutes procyonoides* will be hindered, affecting the quality of the fur and thus increasing the breeding cost and affecting the economic benefits (Zhao et al. 2022; Zhong et al. 2022).

Nyctereutes procyonoides is widely distributed in many provinces in China, including Heilongjiang, Shandong and Henan, where related research is progressively covering various fields (Qin et al. 2020). The P. procyonoides gut microbiota is mainly composed of *Bacteroidetes* and *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, etc. (Yuan et al. 2021; Seel et al. 2023; Liu et al. 2020). The composition of gut microbiota differs between wild and domestic *Nyctereutes procyonoides*. Domesticated *Nyctereutes procyonoides* had approximately 50% *Firmicutes* and approximately 35% *Bacteroidetes*, whereas wild

Nyctereutes procyonoides had approximately 70% *Firmicutes* and approximately 10% *Bacteroidetes* (Li et al. 2024; Yuan et al. 2023). Differences in diet and habitat may be responsible for the differences in gut microbiota composition between them. *Fusobacterium* were significantly more abundant in *Nyctereutes procyonoides* fed meat-based diets than in those fed corn-based diets, but the number of *Lactobacillus* was significantly lower (Li et al. 2024). The impact of the gut microbiota on health is primarily attributed to the regulation of the intestinal barrier, immune response, and metabolic homeostasis (Shi et al. 2020). Disturbances in the gut microbiota can lead to the development of various diseases, such as obesity, diabetes, fatty liver, and neurodegenerative diseases (Wang et al. 2023a, b). Different dietary patterns, specific dietary components, and functional dietary factors have a profound impact on the structure, composition, and function of the gut microbiota, thereby affecting overall health (Zhang et al. 2021; Berding et al. 2023).

High-fat diets are a contributing factor to the development of inflammatory bowel disease (IBD) (Lewis and Abreu 2017). Short-term high-fat diets increase the number of proinflammatory macrophages in the colon lamina propria, leading to an imbalance in M1/M2 macrophage polarization and the onset of IBD (Wang et al. 2020). It is important to note that this is a causal relationship, not just a correlation. Research conducted on a rat model with high-fat diets has demonstrated that disorders of the gastrointestinal microbiota, specifically the production of LPS, can compromise the integrity of the intestinal barrier (Zhang et al. 2020). The LPS-induced activation of the TLR4/NF-κB signaling pathway is a key factor in causing inflammation in the colon (Tian et al. 2021; Chen et al. 2022; Xu et al. 2024). Additionally, oxidative stress can also induce intestinal damage (Bhattacharyya et al. 2014; Zhu et al. 2023). Our research verified that high-fat diets activate the NADPH oxidase subunit gp91 and promote reactive oxygen species (ROS) induction in colonic epithelial and lamina propria cells (Li et al. 2019), activating the pro-apoptotic pathway and potentially resulting in cell death and damage to the intestinal barrier (Li et al. 2019; Zeng et al. 2023).

Higher economic benefits hinge on supplementing large amounts of fat during the winter season, which promotes fur growth and development in *Nyctereutes procyonoides* (Zhao et al. 2023). However, excessive fat intake can be detrimental to the body. For example, high-fat diets significantly influence the microbial composition of the gastrointestinal tract, disrupting metabolic homeostasis (Wang et al. 2023a, b) and leading to intestinal disorders such as gastritis, colorectal inflammation, or cancer (Tong et al. 2021). To date, no studies have investigated the effects or hazards of high-fat diets on *Nyctereutes procyonoides.* Therefore, the objective of our study was to examine the effects of excessive fat intake on the gut microbiota, inflammatory damage, the gut barrier, and the antioxidant capacity of *Nyctereutes procyonoides*. These findings may contribute to the rational, scientific, and standardized rearing of fur-bearing animals, ultimately improving economic efficiency.

Results

Experimental procedure and general results

The *Nyctereutes procyonoids* were acclimatized to the feeding conditions described above for 7 d, and all animals were fed a normal diet. After becoming accustomed to the environment, the ND group was fed a normal ration, and the HFD group was fed an excess of fat on top of the normal ration. The conditions of the *Nyctereutes procyonoidess* were observed and recorded, and samples were collected after 10 d of feeding (Fig. 1A). During the test period, the HFD group showed symptoms of diarrhea (Fig. 1B). Histopathological examination (Fig. 1C) indicated that the ingestion of excess fat can lead to colonic tissue damage and inflammatory cell infiltration. These results suggest that a HFD causes significant colonic inflammation.

Effects of high-fat diets on the structure of the *Nyctereutes procyonoides* gut microbiota

To investigate the effect of high-fat diets on the structure of the *Nyctereutes procyonoides* gut microbiota, 16S high-throughput sequencing was used to analyze the intestinal contents of *Nyctereutes procyonoides* in this work. The species abundance bar graphs (Fig. 2A and B) show that the bacterial abundance in the high-fat diet group exhibited notable variations at different taxonomic levels at the phylum and genus levels compared with that in the normal diet group. Additionally, the gut microbiota of the HFD group displayed a decrease in the abundance of the phylum *Firmicutes* and an increase in the abundance of the phyla *Proteobacteria* and *Actinobacteria*. At the genus level, the abundances of *Escherichia coli-Shigella* spp., *Clostridium* spp., *Bifidobacterium* spp., and *Lactobacillus* spp. tended to increase, while the abundances of *Streptococcaceae* and *Staphylococcaceae* decreased. Venn diagrams of shared or endemic species revealed 395 OTUs in the ND and HFD groups, among which 290 OTUs were endemic to the ND group and 240 OTUs were endemic to the HFD group (Fig. 2C). By comparing the HFD group and the ND group, linear discriminant analysis effect size (LEfSe) was used to identify species that exhibited significant differences in abundance. The abundances of *Enterobacteriaceae* and *Escherichia*

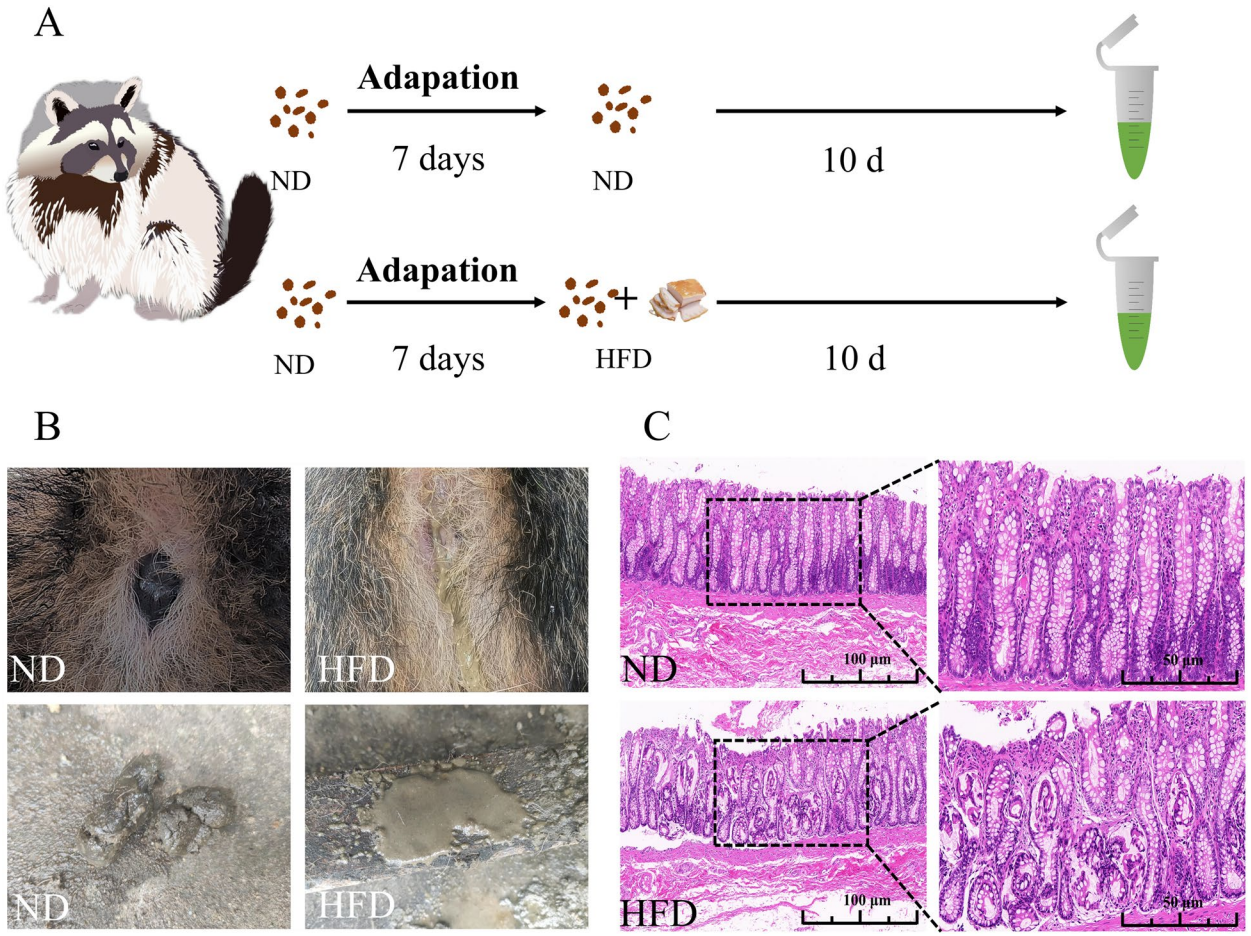


Fig. 1 Experimental procedure and general results. **A** *Nyctereutes procyonoides* were acclimatized under experimental feeding conditions for 7 d, the ND group was fed a normal ration, the HFD group was fed a normal ration with an excessive amount of fat, and the samples were collected after 10 d of feeding. **B** Diarrhea status of *Nyctereutes procyonoides*. **C** Histopathological examination of the colon; the scale indicates 50 μm in **B** and 100 μm in **C**

coli-Shigella spp. tended to increase in the HFD group compared with those in the ND group.

Analysis of differences in α and β diversity indices

Figure 3A shows the dilution curve for α diversity, and when the curve flattened out, the amount of sequencing data was adequate. The Chao1 (Fig. 3B) and ACE (Fig. 3F) indices revealed that the total number of microbial OTUs in the HFD group was less than that in the ND group. The PD_whole_tree (Fig. 3C), Shannon_2 (Fig. 3D), and Simpson's (Fig. 3E) indices indicated that the diversity of the gut microbial community in the HFD group was lower than that in the ND group. These α-diversity-related indices indicated that the microbial community of the gut contents of the ND group had a greater abundance and greater evenness than that of the HFD group. The flatness of the rank-abundance curve (Fig. 3G) indicated the degree of homogeneity of the species composition. As shown in the figure, the HFD group had a lower degree of homogeneity of the species composition than the ND group. PCA (Fig. 3H) based on the Euclidean algorithm showed that there was greater variability between the two groups at the OTU level. PCoA (Fig. 3I) was performed with the unweighted UniFrac algorithm to evaluate the degree of similarity between the samples, and the results indicated that the similarity between the samples of the two groups was greater for the normal and high-fat diet groups. A heatmap of the beta diversity of the samples (Fig. 3J) generated using the Bray–Curtis algorithm was used to visualize the branching distance between the ND group and the HFD group. Cluster analysis of beta diversity (Fig. 3K) was performed using the canberra algorithm, which revealed low similarity between the ND and HFD groups.

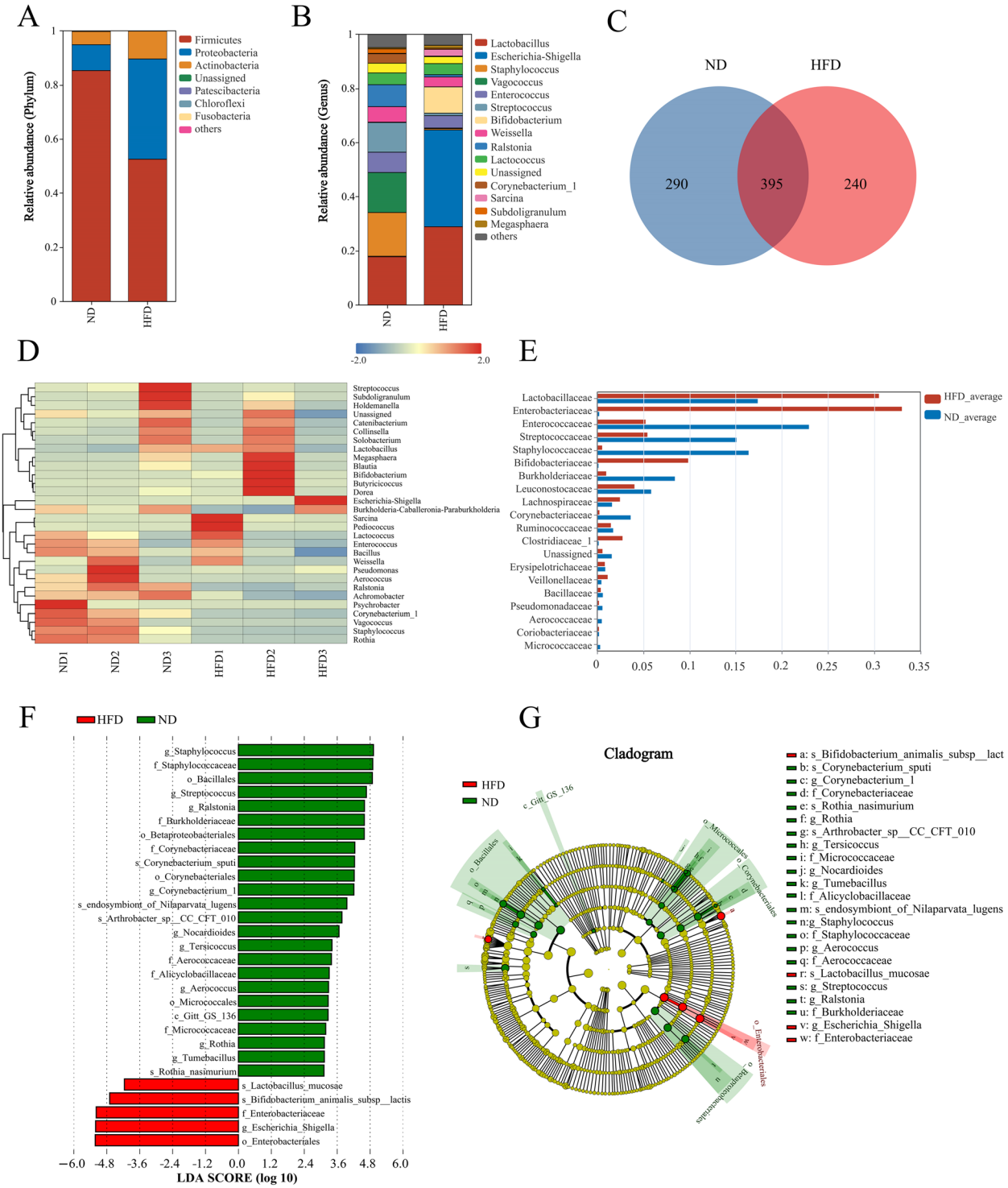


Fig. 2 Species community analysis of the colon contents of *Nyctereutes procyonoides*. **A** Classification of intestinal flora at the phylum level. **B** Classification of the gut microbiota at the genus level. **C** Shared and endemic OTU counts. **D** Species abundance clustering heatmap. **E** Comparison of the top 20 differentiating bacteria between the two groups. **F** LEfSe analysis (LDA = 4), $p < 0.01$. **G** Species branching evolution map

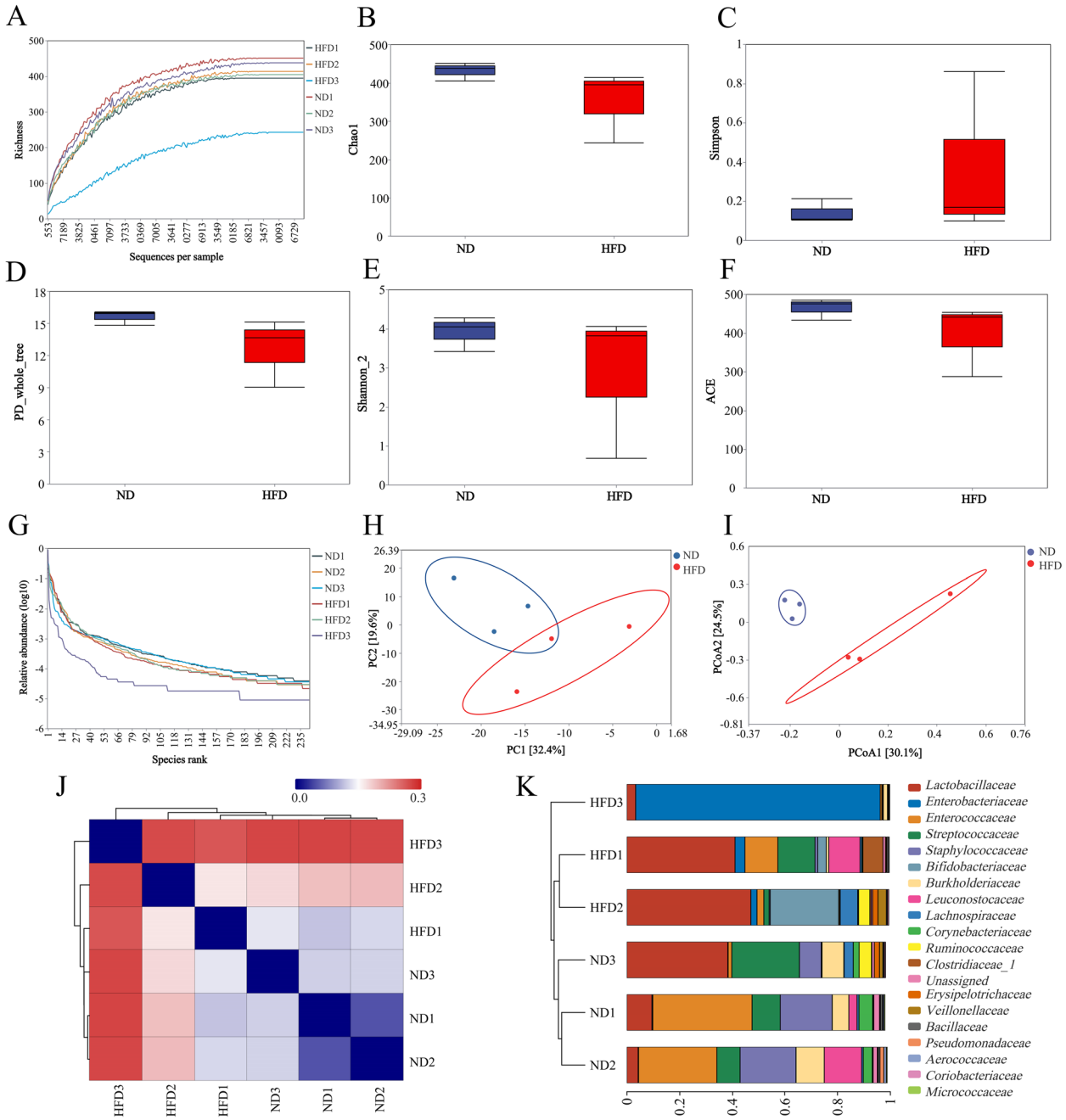


Fig. 3 Analysis of alpha and beta diversity. **A** dilution curve. **B** Chao1 index. **C** Shannon index. **D** PD_whole_tree index. **E** Simpson index. **F** ACE index. **G** Rank-abundance curve. **H** PCA of β diversity. **I** PCoA of β diversity. **J** Sample distance heatmap. **K** Cluster analysis of β diversity

Prediction of the functions of the *Nyctereutes procyonoides* gut microbiota

Based on 16S rRNA sequencing data, relative abundance and differences in microbial functional categories were predicted for the ND and HFD groups. We used COG and KEGG to analyze the metabolic and biosynthetic pathways of the gut microbiota after high-fat diets and to identify functions that may change. PCA of the COG and KEGG results showed differences in microbiota function between the ND and HFD groups (Fig. 4A and B). Venn diagrams (Fig. 4C) revealed 348 functions specific to the ND group and 129 functions specific to the HFD group, with 6774 functions overlapping between the two groups. Significant alterations were observed in the functional

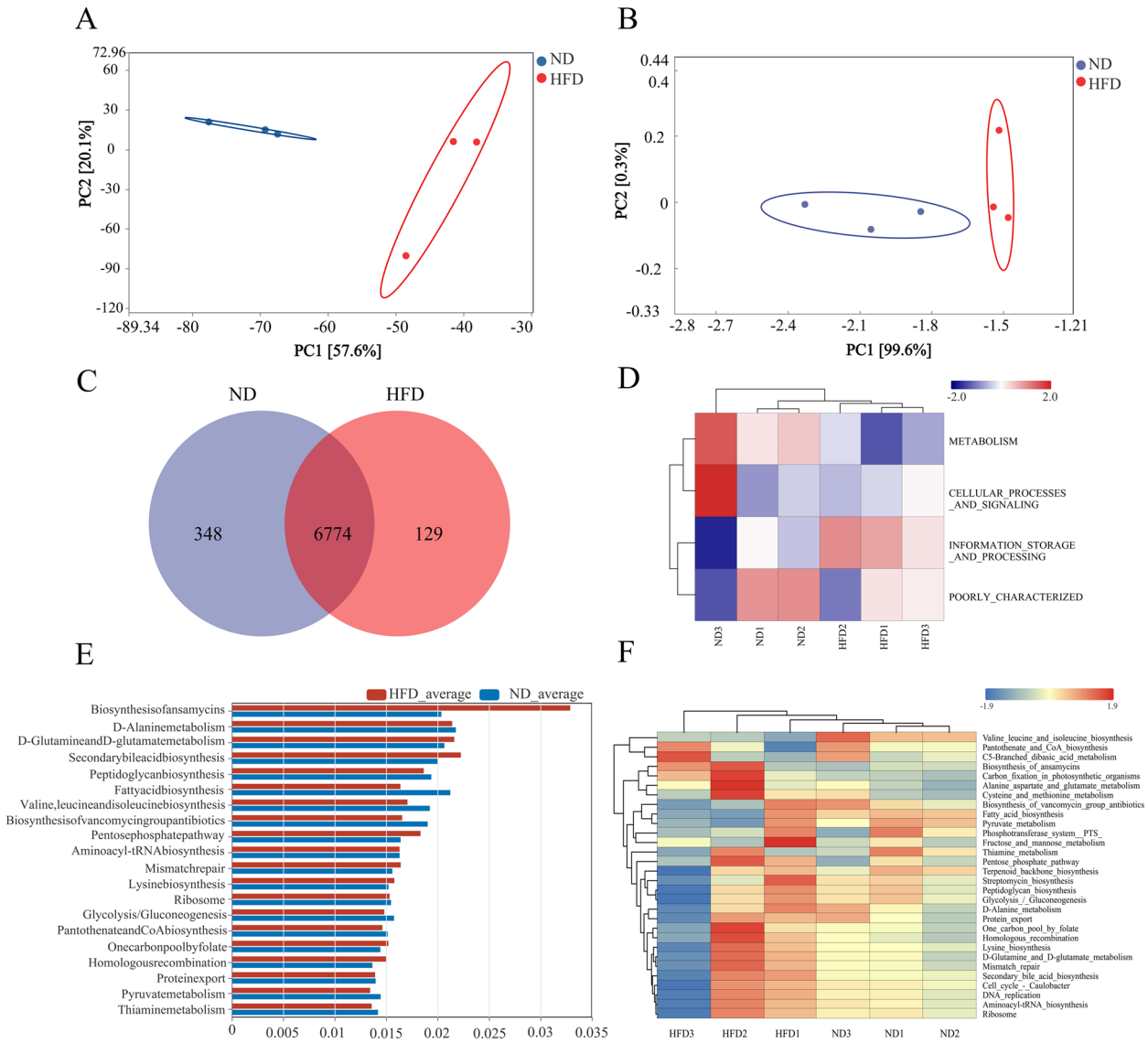


Fig. 4 16S functional prediction analysis of pathways and metabolic differences in the intestinal microbiota. **A** COG functional analysis-PCA. **B** KEGG functional analysis-PCA. **C** COG shared and unique pathway analysis. **D** COG functional abundance clustering heatmap. **E** KEGG functional multigroup interpathway difference analysis. **F** KEGG functional pathway clustering analysis

abundance of alanine, aspartate, glutamate, lysine, cysteine, and methionine metabolism and peptidoglycan biosynthesis (Fig. 4D - F). Additionally, the functions of bile acid and secondary bile acid metabolism, fatty acid synthesis, and pyruvate metabolism were associated with inflammation and oxidative stress, which were also altered in the HFD group.

High-fat diets induce inflammatory injury through the TLR4/NF-κB/NLRP3 pathway

The results demonstrated that excessive fat intake resulted in significantly greater mRNA and protein levels of TLR4 (Fig. 5A and J), Myd88 (Fig. 5B and K), NLRP3 (Fig. 5D and N), and ASC (Fig. 5E and O) in colon tissues and significantly increased protein expression levels of phosphorylated IKBα (Fig. 5L) and phosphorylated p65 (Fig. 5M) compared to those in controls. In addition, the mRNA levels of the proinflammatory cytokines IL-6 (Fig. 5F), IL-1β (Fig. 5G), and TNF-α (Fig. 5H) and their contents in tissues (Fig. 5P-R) were significantly elevated compared with those in the control group. This observation revealed that high-fat diets triggered the activation of the TLR4/NF-κB/NLRP3 inflammatory pathway, thereby elevating the

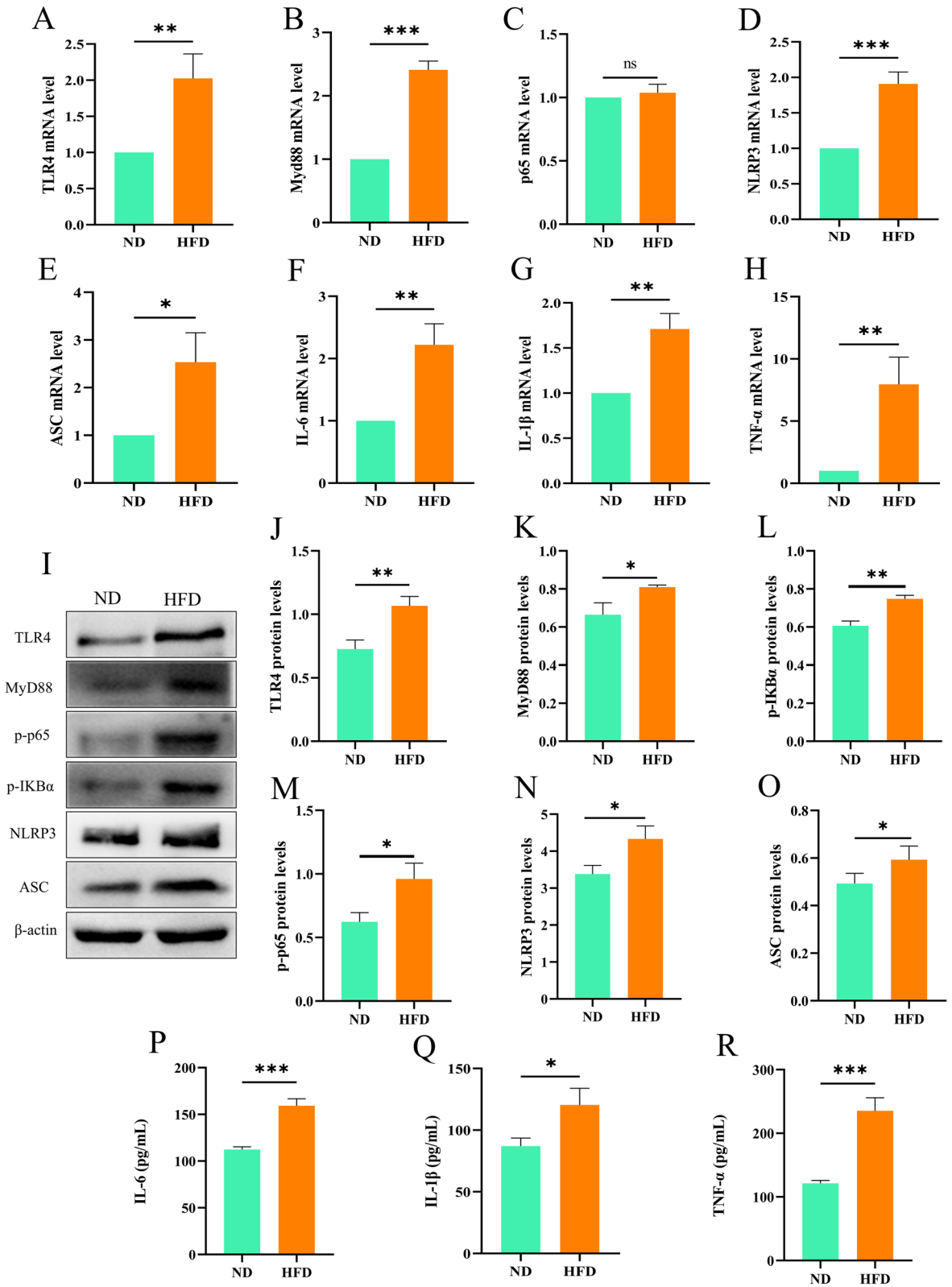


Fig. 5 A high-fat diet caused inflammatory injury in the colon of *Nyctereutes procyonoides*. **A-H** Fluorescence quantitative PCR was used to measure the mRNA expression levels of TLR4, Myd88, p65, NLRP3, ASC, IL-6, IL-β and TNF-α, and GAPDH was used as a housekeeping gene for uniform quantification. **I-N** The protein expression levels of TLR, MyD88, p-p65, p-IKBα, NLRP3, and ASC. β-Actin was used as a housekeeping protein for uniform quantification. **N-P** ELISA detection of IL-6, IL-1β and TNF-α levels in colon tissues. ns $P > 0.05$, * $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$

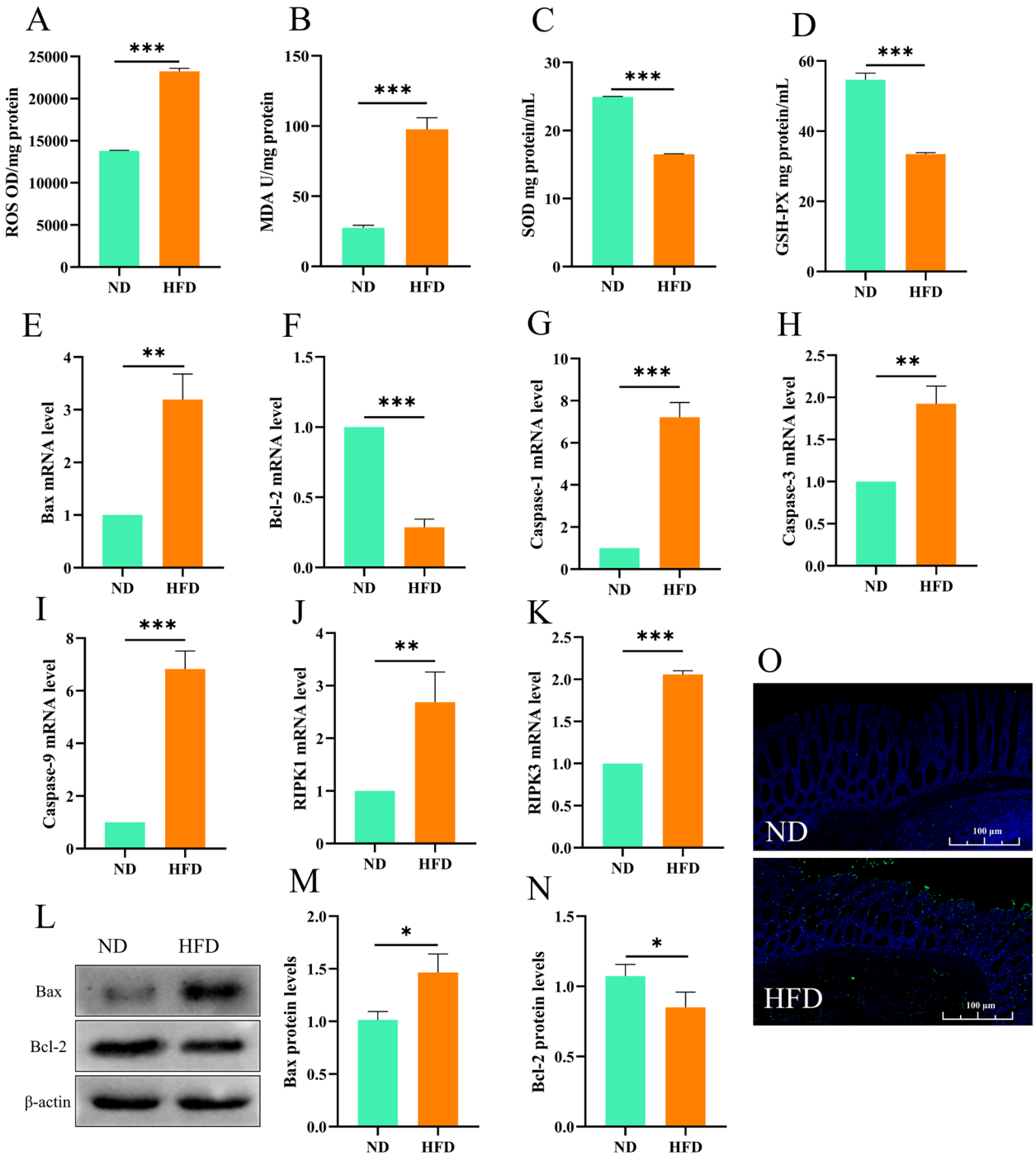


Fig. 6 A high-fat diet led to colonic oxidative stress and colonic cell death. **A** ROS, fluorescence value/mg protein. **B** MDA level. **C** SOD. **D** GSH-PX. **E-I** The mRNA levels of Bax, Bcl-2, caspase-1, caspase-3, and caspase-9, which are associated with apoptosis. **J-K** The mRNA levels of RIPK1 and RIPK3, which are associated with cellular necrosis. GAPDH was quantified uniformly as a housekeeping gene. **L-N** The protein levels of Bax and Bcl-2 were detected by Western blotting, and the level of β-actin, a housekeeping protein, was quantified. **O** Fluorescence image of TUNEL-stained colon tissue; the scale bar indicates 100 μm. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

concentrations of proinflammatory cytokines and leading to inflammatory injury.

Oxidative stress levels and antioxidant capacity of colon *cancer* cells

Compared to those in the normal diet group, the high-fat diet group exhibited significant increases in ROS (Fig. 6A) and malondialdehyde (MDA) (Fig. 6B) levels and significant decreases in the antioxidant indices glutathione peroxidase (GSH-PX) (Fig. 6C) and superoxide dismutase (SOD) (Fig. 6D), which suggested that oxidative stress occurs in the colon, reducing its antioxidant capacity. Additionally, increased mRNA levels of Bax (Fig. 6E), caspase-1 (Fig. 6G), caspase-3 (Fig. 6H), and caspase-9 and decreased mRNA levels of BCL-2 (Fig. 6F) were detected in the HFD group. Western blot (Fig. 6L) analysis revealed an increase in the protein level of the proapoptotic factor Bax (Fig. 6L-M) and a significant reduction in the protein level of the antiapoptotic factor BCL-2 (Fig. 6L-N). These results implied that high-fat diets induced the activation of apoptosis pathways in colonocytes. Furthermore, the mRNA levels of RIPK1 (Fig. 6J) and RIPK3 (Fig. 6K) were significantly elevated, indicating that the necrosis pathway was also activated in colonic cells.

Effect of high-fat diets on the intestinal barrier

High-fat diets can disrupt the intestinal barrier of *Nyctereutes procyonoides*. Excessive fat intake resulted in marked decreases in the mRNA expression levels of the tight junction-associated proteins Occludin, E-cadherin, ZO-1, and ZO-2 (Fig. 7A-D) in the colonic tissues compared with those in the control group. The Western blot results indicated that the protein expression levels of E-cadherin and Occludin were markedly reduced compared to those in the control group (Fig. 7E-G).

Discussion

The *Nyctereutes procyonoides* has long played an important role in the fur industry. To promote *the* growth and development of Nyctereutes procyonoides, high-fat ingredients are often added to the diet (Rebersek 2021).

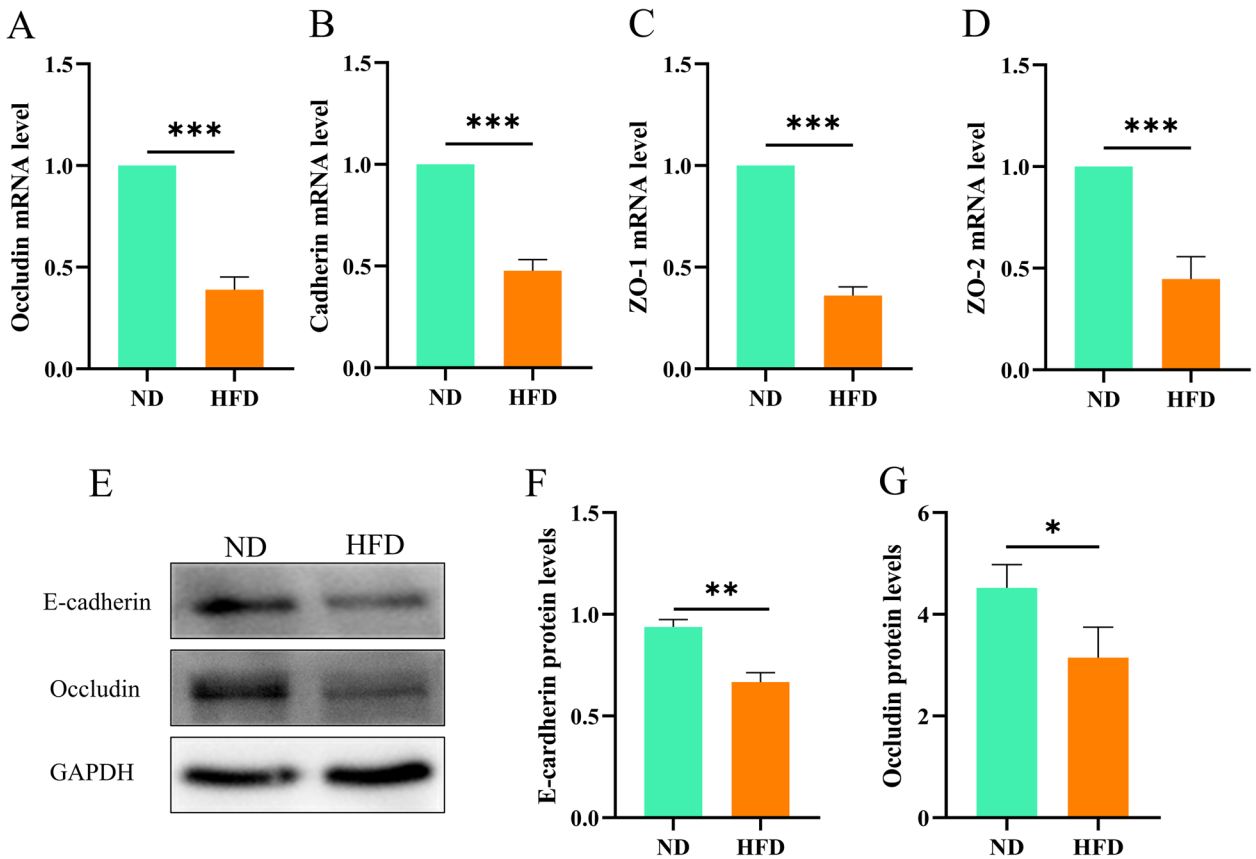


Fig. 7 Effects of a high-fat diet on the intestinal barrier of the colon of *Nyctereutes procyonoides*. **A-D** The mRNA expression levels of the tight junction-associated proteins Occludin, E-cadherin, ZO-1 and ZO-2 were detected by RT–qPCR, and GAPDH was used as a housekeeping gene for uniform quantification. **E–G** Protein expression levels of E-cadherin and Occludin. GAPDH was quantified uniformly as a housekeeping protein. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

However, increasing numbers of studies have shown that high-fat diets increase the risk of diseases such as inflammatory bowel disease (IBD) (Zhou et al. 2022; Qiao et al. 2023). However, detailed research on the effects of high-fat diets on *Nyctereutes procyonoides* is limited. In this study, we found that high-fat diets caused diarrhea, disturbances in the gut microbiota, damage to the gut barrier, inflammatory damage, and a reduction in the antioxidant capacity of the gut in *Nyctereutes procyonoides*.

The gut microbiota is influenced by the host's genetics, gut wall structure, age, diet and environmental conditions (Fan et al. 2021). Previous studies have suggested that the gut microbiota plays an irreplaceable role in host nutrition, immunity, metabolism, and maintenance of the intestinal barrier (Ganesh and Versalovic 2015; Qiao et al. 2023). Our study showed that high-fat diets mediated alterations in the gut microbiota of *Nyctereutes procyonoides*. It consisted mainly of *Firmicutes*, *Proteobacteria* and *Actinobacteria*, with excess fat intake resulting in a decrease in the abundance of *Firmicutes* and an increase in the abundance of *Proteobacteria* and *Actinobacteria*. These observed variations were similar to those in the gut microbiota of mice overfed N-6 fatty acids (Selmin et al. 2021), indicating that there was an increase in the abundance of *Enterococcaceae*, *Escherichia coli-Shigella*, *Clostridium* and *Lactobacillus* at the genus level, which may be the main causes of diarrhea in *Nyctereutes procyonoides* (Zewdie et al. 2020). In addition, *Escherichia coli-Shigella* can overcome multiple obstacles to infect colonic epithelial cells, enter the bloodstream, and disseminate to other organs (Pasqua et al. 2017). *Lactobacillus* is considered a beneficial bacterium that inhibits the NF-κB pathway and mitigates intestinal damage (Wu et al. 2020). However, interestingly, our study revealed that the abundance of *Lactobacillus* in the gut of *Nyctereutes procyonoides* in the HFD group was elevated, but this variation needs to be further investigated.

Based on high-fat diet-induced gut microbiota disorders in *Nyctereutes procyonoides*, we analyzed the changes in the metabolic functions of the gut microbiota using kyoto encyclopedia of genes and genomes (KEGG) enrichment and found that gut microbiota disruption led to abnormalities in metabolic pathways, including alanine, aspartate, glutamate, lysine, cysteine and methionine metabolism; bile acid metabolism; and fatty acid synthesis. Previous studies have demonstrated that elevated levels of lysine and glutamate inhibit apoptosis and promote the proliferation of colonic mucosal cells (Xu et al. 2023a,b,c). Disturbed methionine metabolism may be associated with the accumulation of reactive oxygen species (Liu et al. 2023). Our data indicated that high-fat diet-induced intestinal barrier damage, colonic apoptosis, and oxidative stress may be associated with disturbances in amino acid metabolism. Bile acid metabolism and short-chain fatty acid synthesis are strongly associated with gut microbiota homeostasis, lipid metabolism, immunity, and inflammation (Jia et al. 2018; Ikeda et al. 2022). In conjunction with the aforementioned study, it could be postulated that bile acid and fatty acid metabolism may present a potential target for the treatment of *Nyctereutes procyonoides* gut damage.

It has been reported that increased numbers of *Clostridium perfringens* in the gut microbiota cause the production of large amounts of deoxycholic acid (DCA), which in turn activates macrophages to cause colonic inflammation (Dong et al. 2023a,b; Wang et al. 2020). Notably, *Shigella* can produce LPS, whose translocation is strongly associated with the TLR4/NF-κB pathway and NLRP3 inflammatory vesicle activation. (Paciello et al. 2013; Chen et al. 2021). To further validate the relationship between gut microbiota disorders and colonic inflammation, we examined the TLR4/NF-κB/NLRP3 pathway and proinflammatory cytokines and found that inflammation was induced in diseased animals with gut microbiota disorders; these findings were further confirmed by KEGG enrichment analysis.

Oxidative stress is a significant factor in the development of intestinal damage (Wu et al. 2024; Malesza 2021). For instance, high-fat diets stimulate the expression of ROS and gp91 in the mitochondria of colonic mucosal cells (Lee et al. 2021), whereas excess ROS reduce the protective properties of the intestinal mucus layer. ROS peroxidation and MDA production can disrupt the integrity of the intestinal mucosa (Li et al. 2019). In our study, we found that high-fat diets caused a decrease in the levels of SOD and GSH-PX, which are critical antioxidants in the colon tissues of Nyctereutes procyonoides. Conversely, the levels of ROS and MDA, which are indicators of oxidative stress (Lv et al. 2024; Liu et al. 2024), were elevated. These findings suggest that oxidative stress and a decrease in antioxidant capacity occur in the intestines of *Nyctereutes procyonoides* following high-fat diets.

The intestinal barrier consists of the mucus layer, intestinal epithelial cells, tight junctions, immune cells and the gut microbiota (Cao et al. 2022). Under normal dietary conditions, the intestinal barrier system maintains homeostasis, and the intestine utilizes a variety of strategies to prevent the migration of foreign bodies from the lumen to the lamina propria (Wan et al. 2023). Additionally, it has been shown that high-fat diets lead to enrichment of bile acid metabolism in the KEGG pathway analysis of the gut microbiota and affect the expression of the tight junction-associated proteins Occludin and E-Cadherin (Rohr et al. 2020). In this study, we found that high-fat diets contributed

to colonic tissue damage, intestinal mucosal disruption, and decreased mRNA expression of the tight junction-associated proteins ZO-1, ZO-2, Occludin and E-Cadherin in *Nyctereutes procyonoids*. Disturbance of the gut microbiota results in the activation of the TLR4/NF-κB pathway by deleterious factors such as LPS, leading to an increase in the proinflammatory cytokines IL-6, IL-1β, and TNF-α and pathological damage to the intestinal barrier (Wang et al. 2022). In addition, the activation of the necrosis and apoptosis pathways in colonic cells was detected in the HFD group, which is equally crucial for damage to the intestinal barrier.

Conclusion

In conclusion, high-fat diets lead to gut microbiota disturbance, functional impairment, and metabolic pathway changes in *Nyctereutes procyonoides*. Disruption of the gut microbiota triggered inflammatory injury through the TLR4/NF-κB/NLRP3 pathway, compromised the integrity of the intestinal barrier, induced intestinal cell death, caused oxidative stress in the intestine and reduced its antioxidant capacity. Our research demonstrated that maintaining gut microbial homeostasis is a vital factor in the gut health of Nyctereutes procyonoides.

Methods

Animals and diet

Thirty *Nyctereutes procyonoides* (65 ± 5 d, 3 ± 0.5 kg) were divided into normal diet (ND) and high-fat diet (HFD) groups of 15 each. The feeding conditions and housing conditions have recently been described in detail (Xu et al. 2023a, b, c). The experimental animal housing consisted of *Nyctereutes procyonoides maintained* at 25°C and 50% humidity with 12 h of light and 12 h of darkness, and the animals were provided food and water. The normal diet nutrients included crude protein (≥ 24.0%), crude fiber (≤ 6.0%), crude ash (≤ 8.0%), lysine (≥ 1.4%), calcium (0.5 ~ 2.0%), total phosphorus (≥ 0.5%), sodium chloride (0.5 ~ 1.5%), and moisture (≤ 14.0%). The daily intake of the normal ratio was 5% of the body weight. Lard was added to the normal diet to form a high-fat diet, and the ratio of lard to normal diet was 1:2.5. At the end of the experiment, the *Nyctereutes procyonoides* were euthanized, and colon tissues and colon contents were collected. Three samples were randomly drawn from each group for subsequent tests. All experiments in this study were approved by the Ethics Committee of Northeastern Agricultural University (NEAUEC20220340).

Histopathological examination

Small pieces of colon tissue were soaked in 4% formaldehyde, dehydrated and put into xylene solution to make them transparent, followed by paraffin embedding and

Table 1 Primers used in this study

Name	Sequence (5′ to 3′)	Product length (bp)
ASC	F: TTACCGGACAGCAGCCAAG	124
	R: CTCTGACAGGACTTTCCCATAC	
Bax	F: GAGTCCAGGCACCTCTTCCC	107
	R: CTGCTCGATCTTGGATGAAACCC	
BCL2	F: GAACTGTACGGCCCCACCATGC	89
	R: CAAGCTCCCACCAGGGCCAGA	
ZO-1	F: AGCAGAAGCCTCATCTCCAGT	113
	R: TAGGCCCCTCCAGTCTGACA	
ZO-2	F: CTCAACCTAAAGCAGCCCCAA	143
	R: TATCCCAACGTCATTGCCACCA	
Occludin	F: CCTTTTGTTTTATCGCTGCAT	105
	R: AGGCACTCAGTATTATTACAGT	
TLR4	F: TTCTCTAACATGCCAAACCTG	96
	R: TGGCATTTTATGTAGAACCTG	
Myd88	F: TACTGCCCCAGCGATATCCA	80
	R: ACACACAACTTCAGCCGAT	
p65	F: AGCTCCCCAGTCCTATCCCT	87
	R: ATCTGCCCCGAAGAAAAGACCA	
GAPDH	F: ATATTGTCGCCATCAATGACCC	116
	R: AAGTTTCCCGTTCTCAGCCTT	
TNF-α	F: CGACGTGCCAATGCCCTCC	115
	R: ATCCTTGGCCCTTGAAGAGGAC	
IL-6	F: GTGTGAAGACAGCAAGGAGG	93
	R: TGATTGAACCCAGATTGGAAGC	
IL-1β	F: GGATGGAAAGCCCACCCTAC	204
	R: TCCTGGCCACCTCTGGTATT	
NLRP3	F: AGTGCTGCCTTTCCTATCGG	103
	R: AAGTCTCCCAGGGCGTTG	
Caspase-1	F: TTAATGTCTCACGGCATCCTG	114
	R: GAGGCAGTGACGGTTGTTG	
Caspase-3	F: AAAATGATCTCACATGCGAAG	120
	R: AAATTATTCCTTCATCCCCAT	
Caspase-9	F: CTGGATGCCGTGTCTAGTTTG	97
	R: AGCCGCTCTTGGGATTTC	
RIPK1	F: ATGGACAGGCAGACGAAACC	92
	R: TGTGCAAAAGGGTCATGGGAG	
RIPK3	F: TTGCCTGATGTAAACCGAAAGG	111
	R: CGATGTCTGGGCCACTATCTC	
Claudin-1	F: AGTGTATGAAGTGCATGGAAGAC	98
	R: AACTAAAACAGCCAGACCTGC	
E-cadherin	F: ACCAGGTTTGGAACGGGAC	92
	R: GGCGTTTGGATCATCAGCATC	

cutting into thin slices. Then, they were deparaffinized and stained with hematoxylin & eosin (HE) (Dong et al. 2023a, b). The sections were microscoped, and the resulting images were captured using an imaging system.

Real-time quantitative PCR (RT–qPCR)

Colon tissues were ground to a powdered form in liquid nitrogen, total RNA was extracted according to the methods of Zhang. (Zhang et al. 2023), and cDNA synthesis and fluorescence quantitative PCR were performed based on the methods of Xu (Xu et al. 2023a, b, c). Primers were designed using oligo V. 7.6 (Table 1). The PCR amplification programme was as follows: 94°C for 30 s, 35 cycles of 94°C for 5 s, 60°C for 15 s, 72°C for 10 s, and 4°C for 15 min. Normalization was performed using the housekeeping gene GAPDH, and the results were calculated using the $2^{-\Delta\Delta ct}$ method: Δct (test) = CT (test) − CT (reference, mean), Δct (control) = CT (control) − CT (reference, mean), and ΔΔct = Δct (test) − Δct (control).

Western blot

This procedure was performed according to the method of Cui (Cui et al. 2023). Briefly, proteins from colon tissues were extracted using RIPA tissue lysis buffer (Beyotime, Shanghai, China), mixed with protein buffer, subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to nitrocellulose (NC) membranes (Biosharp, Hefei, China), which successively bind primary and secondary antibodies, and an enhanced chemiluminescence (ECL) system (Thermo Scientific, USA) was used for luminescence.

Enzyme-linked immunosorbent (ELISA) assay

Totally, 900 μL of precooled phosphate-buffered saline (PBS) solution was added to Nyctereutes procyonoides colon tissue, which weighed 0.1 g, and the tissue was ground using a tissue grinder (Sun et al. 2024). The supernatant was obtained by centrifugation at 8,000 × *g*. The expression levels of IL-6, IL-1β, and TNF-α were measured according to the instructions of the reagent vendors (Jiancheng Co., Nanjing, China).

TdT-mediated dUTP nick end labeling (TUNEL) assay

The One Step TUNEL Apoptosis Assay Kit (Beyotime, Shanghai, China) was used to detect apoptotic cells in colon tissue. Paraffin sections of colon tissue of 4 μm thickness were taken and reacted with 50 μL of proteinase K (50 μg/mL) for 10 min. Fifty microliters of TUNEL assay solution (TdT enzyme: dUTP fluorescent labeling solution, ratio 1:9) was added to each tissue section and then incubated at 37°C for 60 min. The tissue sections were washed with PBS and sealed. Fluorescence signals were observed using a fluorescence microscope (Xu et al. 2023; Zhang et al. 2023).

Reactive oxygen species and antioxidant enzyme measurements

A total of 0.1 g of *Nyctereutes procyonoides* colon tissue weighing was soaked in 900 μL of precooled saline to make a tissue homogenate, and the mixture was subsequently centrifuged at 2,500 rpm for 10 min. The supernatant was obtained to measure the protein concentration using the BCA method and assayed for ROS,GSH-PX), (SOD), and MDA according to the instructions of the reagent vendor (Jiancheng Co., Nanjing, China).

16S rRNA sequencing of the gut microbiota

Total DNA was extracted from the colonic contents of each group of *Nyctereutes procyonoides* by a Biospin DNA extraction kit (Biospin, Hangzhou, China). The DNA of the samples that passed the quality check was collected, and the V3-V4 variable region was amplified using the primers 338F (5′-ACTCCTACGGGAGG CAGCAG-3′) and 806R (5′-GGACTACHVGGGGTW TCTA AT-3′). The PCR amplification programme was 98°C for 3 min, followed by 30 cycles of 98 °C for 10 s, 50 °C for 30 s, 72 °C for 60 s, and 72°C for 10 min. The PCR products obtained were subsequently purified, and libraries were generated using the MagaBio Plus Bacterial Genomic DNA Purification Kit (Biospin, Hangzhou, China). High-throughput sequencing analysis of the gut microbiota was performed on the Illumina NovaSeq platform using double-ended amplification, followed by sequencing data processing using QIIME2 (V. 2019.7). Quality control, species annotation, and abundance analysis were performed using previous methods (Chengwei et al. 2024; Ran et al. 2024). Venn diagrams were generated to analyze the overlap of OTUs between samples. Alpha and beta diversity analysis based on OTU/ASV abundance tables was performed using usearch-alpha_div (V.10, http://www.drive5.com/usearch/) and the R package. Unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis was used to construct the cluster tree of the samples. A significant analysis of differences in species between groups was carried out using LEfse software with the default setting of a screening value of 4 for the LDA score ($p < 0.01$).

Statistical analysis

The sample data conform to normal distribution. The samples in this study were subjected to random sampling to maintain independence. The same set of samples was processed and tested three times independently. One-way analysis of variance (ANOVA) and t tests were used, and the data were analyzed using SPSS 26. The results from three independent experiments are expressed as the mean ± standard deviation (mean ± SD). A p value less than 0.05 was considered to indicate a significant difference.

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Authors' contributions
ZML and CWW: Conceptualization, data curation, formal analysis, investigation, methodology, software, visualization and writing of the original draft. JY: Formal analysis. YG: Visualization. MRZ: Investigation and methodology. TCX: Conceptualization. MYG: Conceptualization, funding acquisition, project administration and writing, reviewing and editing. All the authors have read and approved the manuscript.

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Availability of data and materials
The datasets generated during this study are available in the NCBI databases [https://www.ncbi.nlm.nih.gov/, PRJNA1063850].

Declarations

Ethics approval and consent to participate
All experiments in this study were approved by the Ethics Committee of Northeastern Agricultural University (NEAUEC20220340).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no known competing interests or personal relationships. This manuscript contains content that has never been published and has been read by all the authors.

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