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Lard promotes intestinal stem/progenitor cell proliferation and differentiation with improved gut microbiota

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ABSTRACT: The effects of dietary fats on the intestinal barrier have been widely studied, yet their role in intestinal repair remains unclear. Previous studies have primarily focused on fat quantity (e.g., high-fat vs. low-fat diets) and quality (e.g., saturated vs. unsaturated fats), while neglecting the importance of structural properties. This study evaluated the impact of dietary fats with varying *sn*-2 palmitate levels on intestinal repair using a DSS-induced murine injury model under moderate intake levels. The results indicated that lard, rich in *sn*-2 palmitate, significantly lowered intestinal permeability, reduced crypt damage, and decreased apoptosis in mice compared to fats low in *sn*-2 palmitate (corn oil and beef tallow). Immunohistochemical analysis demonstrated that lard significantly increased the number of SOX9, Ki67, MUC2, and PAS-positive cells per crypt, indicating enhanced proliferation and differentiation of intestinal stem/progenitor cells, thereby facilitating intestinal repair. Untargeted metabolomics analysis revealed distinct metabolic profiles of intestinal digestive products derived from dietary fats, with lard leading to enrichment of metabolites such as diacylglycerols, palmitoylcarnitine, and adhumulinic acid. The microbial results showed that lard improved α-diversity and increased the abundance of beneficial bacteria such as *unclassified_Lachnospiraceae, unclassified_Muribaculaceae*, and *Ligilactobacillus*. KEGG pathway analysis further demonstrated that lard enriched microbial pathways associated with replication and repair, translation, and amino acid metabolism. Our findings reveal that moderate intake of lard promotes intestinal repair, which is associated with beneficial shifts in metabolite profiles and gut microbiota composition. These results highlight the significance of the *sn*-2 palmitate structure in dietary fat interventions for promoting intestinal repair.

Keywords: Dietary fats, lard, intestinal repair, intestinal stem/progenitor cells, intestinal metabolites, gut microbiota

1. Introduction

The intestinal epithelial barrier is constituted by a monolayer of columnar epithelial cells and is crucial for organisms to maintain homeostasis. When the integrity of this barrier is compromised, toxins, pathogenic bacteria, and other harmful substances from the intestinal lumen can enter the circulatory system, contributing to the development of various diseases such as inflammatory bowel disease, autoimmune disorders, and even central nervous system pathologies[1-2]. The intestinal epithelium undergoes rapid renewal every 3-5 days and exhibits a remarkable ability for repair following injury, a process mainly driven by the proliferation of intestinal stem/progenitor cells[3]. Intestinal stem cells, residing at the base of the crypts, undergo transition

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into highly proliferating progenitor cells, which subsequently differentiate into fully functional epithelial cells. When intestinal injury occurs, the proliferation and differentiation of intestinal stem/progenitor cells are crucial for intestinal regeneration and repair, which is essential for maintaining the normal structure and function of intestine[4].

Dietary fats provide essential macronutrients for the human body, occupying an important position in the human dietary structure. In general, dietary fats provide a wide range of health benefits for various organs and cells within the body, including cell regeneration, protein signaling, energy balance, and the maintenance of homeostasis[5]. The fatty acids released during fat digestion are not only absorbed by intestinal epithelial cells but also serve as signaling molecules that help maintain gut homeostasis[6-7]. Both the amount and type of dietary fat influence the intestinal response to injury[6,8]. For example, in a sodium dextran sulfate (DSS)-induced colitis model, a high-fat diet was associated with exacerbated intestinal damage, while low-dose n-3 polyunsaturated fatty acids more effectively alleviated intestinal injury compared to higher doses in mouse models[9]. Intestinal regeneration and repair are crucial for maintaining intestinal homeostasis and overall health. However, few studies have investigated how different dietary fats influence on these processes. Existing research has primarily focused on the quantity of fat (e.g., high-fat vs. low-fat) and conventional quality aspects (e.g., saturated vs. unsaturated), while largely overlooking structural variations in triglycerides[7]. Notably, the structural configuration of triacylglycerol—such as the *sn*-2 positioning of palmitic acid—can significantly affect gut homeostasis and intestinal health[7]. This specific structure enhances nutrient absorption and promotes beneficial gut microbiota, thereby contributing to intestinal barrier integrity[4,10]. The specific effects of structurally distinct dietary fats, particularly those rich in *sn*-2 palmitate, on intestinal repair mechanisms remain unexplored.

In this study, we selected three dietary fats—lard, beef tallow (beef), and corn oil (corn)—as typical fats to investigate how moderate intake of fats with varying levels of *sn*-2 palmitate differentially impacts intestinal repair following injury. The fatty acid profiles of these fats and the *sn*-2 positional distribution of palmitic acid in their triglycerides are presented in Table S1 and Table S2, respectively[11,12]. We hypothesize that lard, which contains a high level of *sn*-2 palmitate[11], may be more beneficial for intestinal repair, and we aim to elucidate the underlying mechanisms.

2. Materials and methods

2.1 Animal experimental design

Sixty male C57BL/6J mice weighing 14-18 g (six-week-old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The mice were bred and maintained under a 12 h light/dark cycle in a SPF barrier facility, with a temperature maintained at (23 ± 1)°C and a humidity level ranging from 50% to 60%. Food and water were offered without restriction. After one week of acclimation, the mice were assigned to three separate groups at random: lard, beef and corn groups. Lard was purchased from Beijing Ershang Dahongmen Meat Products Co., Ltd, beef from Chongqing Shuaike Food Co., Ltd, and corn from COFCO Fulingmen Food Marketing Co., Ltd. The experimental diets were formulated to provide

20% of energy from fat, with detailed compositions outlined in Table 1. This level aligns with WHO guidelines, which recommend that total fat intake should not exceed 30% of total energy intake for adults[13], and can therefore be considered a moderate and nutritionally appropriate level. After 8 weeks of feeding, all mice were administered 2% dextran sulfate sodium (36,000-50,000 Da, MP Biomedicals) in their drinking water for 5 days, followed by a 3-day recovery period with normal water. At the end of the experiment, the mice were euthanized by cervical dislocation and subsequently subjected to dissection. The diagram illustrating the experimental design was presented in Fig. 1A. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of Shouke Life Biosciences (Beijing) Co., Ltd (approval No.: SKLF-DWLL-20230317-01).

Table 1 The composition of chow diets (%)

Ingredients (g/kg)	Lard group	Beef group	Corn group
Casein	20	20	20
Maize starch	37.6	37.6	37.6
Maltodextrin	13.2	13.2	13.2
Sugar	10	10	10
Cellulose	5	5	5
Vitamin mix	1	1	1
Mineral element mix	3.5	3.5	3.5
Soybean oil	2.5	2.5	2.5
Lard	6.65	0	0
Beef tallow	0	6.65	0
Corn oil	0	0	6.65
L-cysteine	0.3	0.3	0.3
Choline Bitartrate	0.25	0.25	0.25

2.2 Intestinal permeability assay

The intestinal permeability was determined by *in vivo* fluorescein isothiocyanate-dextran (FITC-dextran) (Sigma-Aldrich, St. Louis, MO) assay, following the previous method[14]. The mice were fasted for 4 h and then administered 100 μL of 40 mg/mL FITC-dextran solution. After 4 h, a 1 mL blood sample was collected from the orbital vein and centrifugated at 3000 × *g* for 10 min to obtain serum. The fluorescence intensity of the serum was measured using a spectrophotofluorometer with excitation and emission wavelengths set at 485 nm and 530 nm, respectively.

2.3 Histologic analysis

Fresh colonic tissues were isolated and fixed in 4% paraformaldehyde for 24 h. The tissues were dehydrated, embedded, sliced into a thickness of 5 μm and then subjected to hematoxylin & eosin (H&E) staining. The degree of crypt injury was assessed using a previously established scoring method[15].

2.4 TUNEL Staining

The TUNEL assay was performed with a TUNEL Apoptosis Detection Kit (Beyotime, Shanghai, China). Sections were deparaffinized, rehydrated, and then subjected to a 30 min treatment with 20 μg/mL DNase-free proteinase K at 37°C. This was followed by incubation with the TUNEL reaction mixture for 1 h at 37°C in

the dark. Subsequently, the samples were stained with DAPI in the dark for 5 min and then examined under a fluorescence microscope (Carl Zeiss, Jena, Germany).

2.5 Immunohistochemistry

After deparaffination and rehydration, tissue sections were subjected to antigen retrieval using citrate buffer and inactivation of endogenous peroxidases. They were then blocked at room temperature. The tissue sections were then incubated with primary antibody at 4°C overnight, and immunostaining was performed using the streptavidin-peroxidase method. Following DAB staining, hematoxylin counterstaining, dehydration, and transparentization, the tissue sections were examined under a microscope. The primary antibodies used in this study included Ki67, SOX9, and MUC2 (Abcam, Cambridge, MA, USA).

2.6 Periodic Acid-Schiff (PAS) staining

PAS staining was performed using a Glycogen PAS staining reagent (Leagene, Beijing, China). Following deparaffination and rehydration, tissue sections were treated with periodic acid for 8 min, and then stained with Schiff's reagent in the dark for 15 min. The sections were subsequently differentiated in acidic ethanol and examined under a light microscope.

2.7 Non-targeted metabolomics

50 mg of intestinal content was weighed and homogenized with 500 μL of methanol. The resulting mixture was vortexed for 30 minutes and then centrifuged at 12,000 r/min at 4 ℃ for 10 min. The supernatant was filtered through a 0.45 μm membrane and then analyzed by ultra high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS). The UHPLC analysis was performed utilizing a Vanquish UHPLC system (Thermo Fisher Scientific, USA), and MS analysis was conducted on a Quadrupole-Orbitrap™ Mass Spectrometer (Thermo Fisher Scientific, USA). Differential metabolites were screened by combining variable importance in projection (VIP > 1.0) derived from the orthogonal partial least squares discriminant analysis (OPLS-DA) model with statistical significance ($P < 0.05$). Metabolite was annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/) and the Human Metabolome database (HMDB, http://www.hmdb.ca/).

2.8 16S rRNA sequencing analysis

Total colon content DNA was extracted, and the concentration and purity of the DNA sample were assessed using an ultramicro-spectrophotometer. Additionally, the integrity of the extracted DNA was examined by electrophoresis. Amplification of the V3-V4 hypervariable regions via PCR was carried out as detailed in previous study[4]. Subsequently, the amplification products were purified, quantified, and standardized to construct a sequencing library. Finally, qualified libraries were sequenced on the NovaSeq 6000 system (Illumina, USA).

2.9 Statistical analysis

Data analysis was conducted with SPSS version 23.0 and GraphPad Prism 8. Data were expressed as mean ± standard deviation (SD). Statistical significance was assessed using one-way analysis of variance

(ANOVA) followed by Tukey's *post hoc* test. A *P*-value < 0.05 was deemed statistically significant. When analyzing gut microbiota sequencing data, Kruskal-Wallis test and two-tailed Wilcoxon rank-sum test were performed using R Project. Metabolites with VIP > 1 and *P*-value <0.05, and fold change (FC) > 1.5 or FC < 2/3 were considered differential metabolites. To minimize false positives, the Benjamini-Hochberg method was applied to control the false discovery rate (FDR), which served as the key criterion for identifying differential metabolites.

3. Results

3.1 Impact of different dietary fats on DSS-induced intestinal injury phenotypes

During the eight-week feeding period, no significant differences were found in the body weight, food and water consumption among the different diet groups (Fig. 1B, C and D). Subsequently, an intestinal injury model was established (ethical approval number: SKLF-DWLL-20230317-01) to assess the impact of different dietary fats on DSS-induced intestinal epithelium injury. The body weights of the mice at the ninth week were significantly decreased ($P < 0.05$) (Fig. 1B), confirming that the intestinal injury model was successfully established. Notably, mice fed a lard diet exhibited significantly higher final body weights compared to those fed corn and beef diet ($P < 0.05$) (Fig. 1B). A hallmark of intestinal epithelial barrier dysfunction is the loss of selective permeability. The FITC-dextran method was employed to assess the integrity of the intestinal epithelial barrier in mice. As illustrated in Fig. 1E, mice fed lard exhibited lower intestinal permeability compared to those fed corn and beef following DSS-induced injury, indicating reduced damage to the intestinal epithelial barrier. However, no statistically significant differences were observed between the lard and beef groups, or between the corn and beef groups. H&E staining indicated that the colonic tissue in the lard-fed mice remained intact, exhibiting normal crypt morphology, whereas the corn group displayed the most severe structural damage, characterized by substantial crypt loss and destruction (Fig. 1F). The crypt damage score in beef-fed mice was slightly lower than that in the corn oil group, though the difference was not statistically significant. Notably, the lard-fed mice exhibited the lowest crypt damage score among all groups (Fig. 1G). TUNEL staining was performed to assess the level of apoptosis in the colonic epithelium. A large number of TUNEL-positive cells (red fluorescence) were observed in the colonic epithelium of both beef-fed and corn-fed mice (Fig. 1H). In contrast, the lard group exhibited a marked reduction in TUNEL-positive cells (Fig. 1I), indicating significantly lower levels of apoptosis compared to the beef and corn groups.

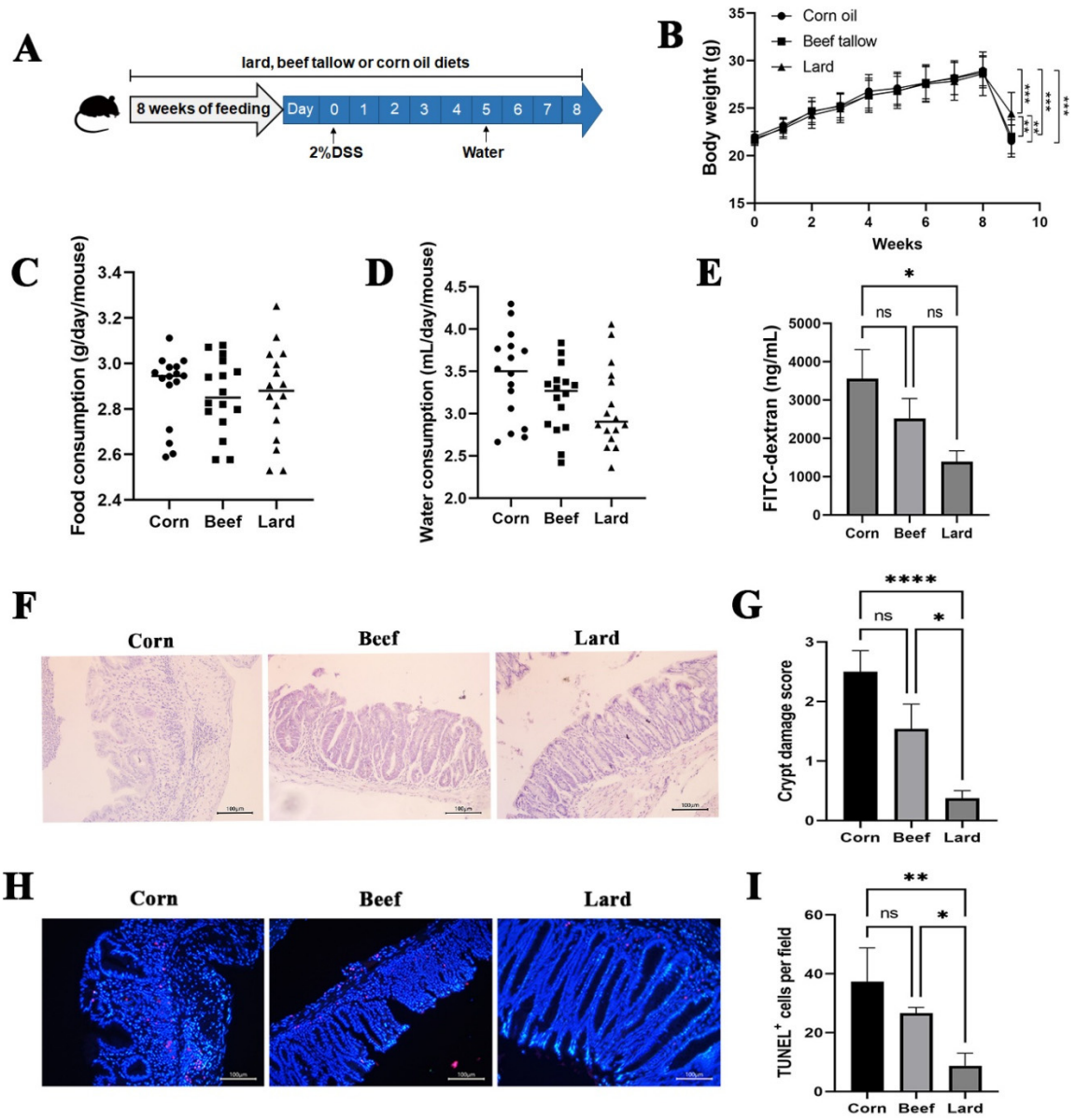

Fig. 1. Effect of different dietary fats on dextran sodium sulfate (DSS)-induced intestinal injury phenotypes. (A) Schematic of the experimental design. (B) Body weight of mice. (C) Average daily food intake per mice. (D) Average daily water intake per mice. (E) Serum FITC-dextran level. (F) Representative hematoxylin & eosin (H&E) images of colonic tissue and (G) Quantification of crypt damage score. (H) Representative images of TUNEL staining and (I) quantification of TUNEL$^+$ cells per field. Data are expressed as mean ± SD (n=10). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.2 Lard enhanced intestinal repair by promoting intestinal stem/progenitor cell proliferation and differentiation

The proliferation and differentiation of intestinal stem/progenitor cells is crucial for facilitating the repair of the intestinal epithelium following injury[16]. Ki67, a biomarker for cell proliferation, is highly expressed during the proliferative phase of the cell cycle[17]. As illustrated in Fig. 2A and 2C, Ki67 immunohistochemical staining revealed that, in comparison to the lard group, the number of Ki67-positive cells within the crypts of the beef and corn-fed mice was significantly decreased ($P < 0.05$). Additionally, SOX9 is a biomarker for intestinal stem/progenitor cells[18]. SOX9-positive cells were distributed at the base

of each crypt (Fig. 2B and 2D). Consistent with the Ki67 findings, both the beef and corn groups exhibited a marked decrease in SOX9-positive cells relative to the lard group ($P < 0.05$).

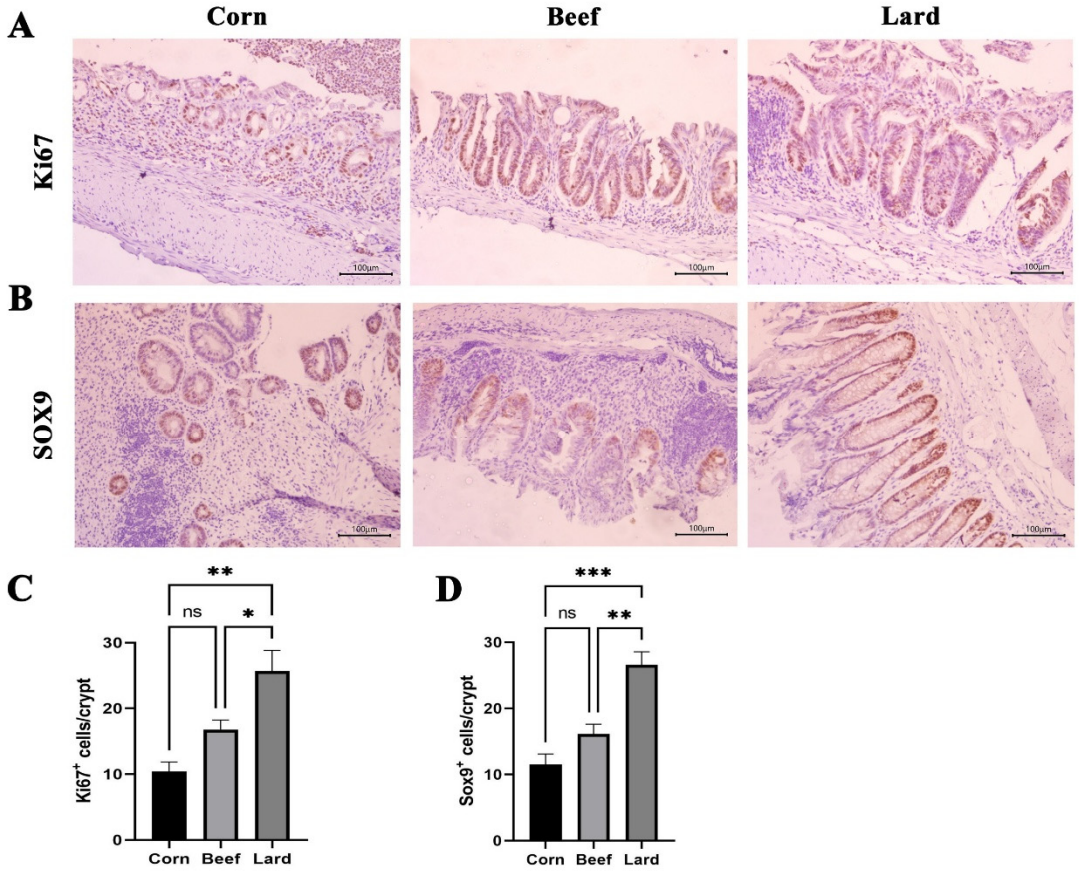

Fig. 2. Effect of different dietary fats on proliferation of intestinal stem/progenitor cells. (A) Representative immunohistochemistry images of Ki67 staining in colonic tissues. (B) Representative immunohistochemistry images of SOX9 staining in colonic tissues. (C) Quantification of $Ki67^+$ cells per crypt. (D) Quantification of $SOX9^+$ cells per crypt. Data are expressed as mean ± SD (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Intestinal stem/progenitor cells differentiate into various functional cell types when simultaneously undergoing proliferation. Goblet cells, predominantly located in the colon, were detected using glycogen PAS staining. Fig. 3A and 3C depicted that the lard-fed group exhibited a significantly greater number of PAS-positive cells within the intestinal crypts compared to the beef and corn-fed groups. Subsequently, we assessed the expression of MUC2, a mucin produced and secreted by goblet cells. The findings indicated that, in comparison to the beef and corn groups, the lard-fed mice demonstrated a marked rise in MUC2-positive cells per crypt (Fig. 3B and 3D). These data suggested that lard promoted the proliferation and differentiation of intestinal stem/progenitor cells compared with beef and corn.

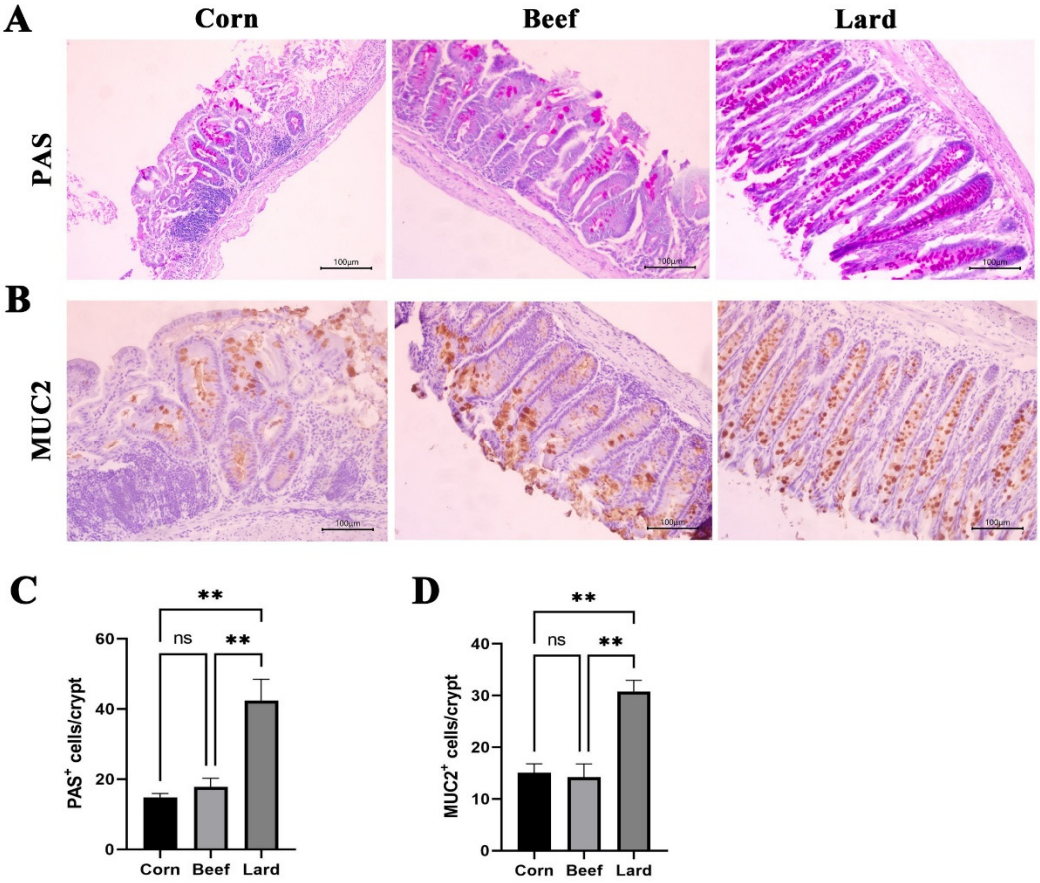

Fig. 3. Effect of different dietary fats on differentiation of intestinal stem/progenitor cells. (A) Representative images of PAS staining in colonic tissues. (B) Representative immunohistochemistry images of MUC2 staining in colonic tissues. (C) Quantification of PAS^+ cells per crypt. (D) Quantification of $MUC2^+$ cells per crypt. Data are expressed as mean ± SD (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.3 The metabolic profiles of intestinal digestive products of dietary fats

Untargeted metabolomics was employed to analyze the metabolic profiles of intestinal digestive products derived from dietary fats. The OPLS-DA results demonstrated clear separations between the metabolic profiles of the corn and lard groups, as well as between the beef and lard groups (Fig. 4A and B). Differential metabolites were screened based on VIP values (VIP > 1.0) derived from the OPLS-DA model, combined with *P* values ($P < 0.05$) obtained from statistical tests. The lard exhibited significant differences in the abundance of 69 metabolites compared to corn group, with 44 metabolites up-regulated and 25 metabolites down-regulated (Fig. 4C). Additionally, 46 metabolites showed significant abundance differences between the lard and beef groups, with 31 up-regulated and 15 down-regulated metabolites (Fig. 4D). A heatmap was subsequently generated to visualize all metabolites with significant abundance variations. As illustrated in Fig. 4E, the lard group exhibited a significant upregulation of several metabolites compared to the corn group, including DG(14:0/20:4(8Z,11Z,14Z,17Z)/0:0), DG(8:0/18:3(10,12,15)-OH(9)/0:0), DG(16:0/22:6(4Z,8Z,10Z,13Z,16Z,19Z)-OH(7)/0:0), palmitoylcarnitine, adhumulinic acid, (E)−11−Hexadecenoic acid, and cis−12−octadecenoic acid. In contrast, N−acetyltaurine and nirogacestat were significantly down-regulated. As shown in Fig. 4F, compared to the beef group, metabolites in the lard

group, such as DG(8:0/18:3(9,11,15)-OH(13)/0:0), DG(16:0/20:4(5Z,8Z,11Z,14Z)/0:0), DG(13:0/PGF1alpha/0:0), palmitoylcarnitine, adhumulinic acid, indoleacetyl glutamine, phenylalanyl−prolyl−arginine, and 5−hydroxy−coniferaldehyde, were significantly up-regulated, while geneticin, γ−glutamylglutamic acid, and 8−hydroxyguanosine were significantly down-regulated.

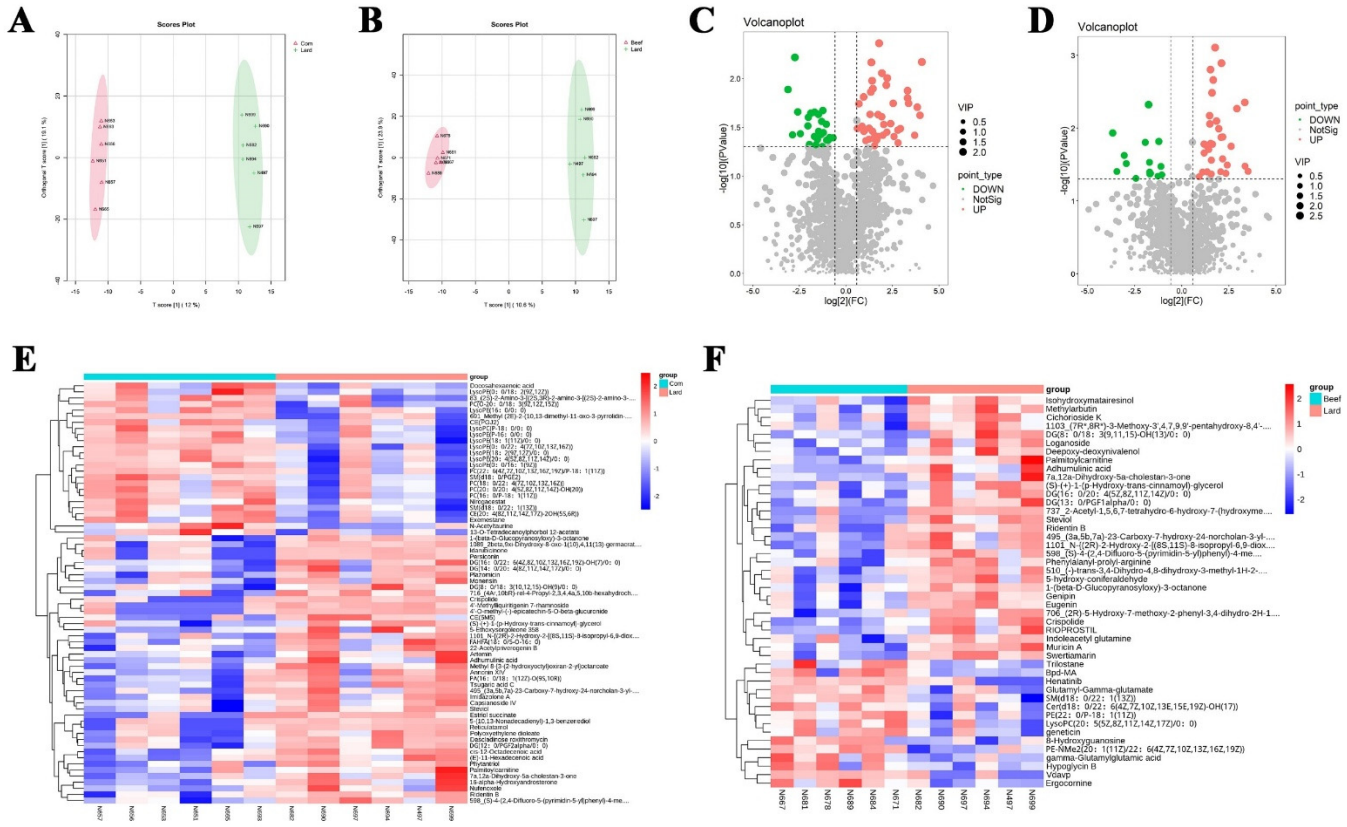

Fig. 4. The metabolic profiles of intestinal digestive products of dietary fats. (A-B) Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) of intestinal metabolites between the lard and corn groups, or between the lard and beef groups. (C-D) Volcanic plot of differential metabolites between the lard and corn groups, or between the lard and beef groups. (E-F) The abundance heatmap cluster analysis of differential metabolites between the lard and corn groups, or between the lard and beef groups. Metabolites with the Variable importance in the projection (VIP) value of OPLS-DA model >1 and the *P* values < 0.05 were considered to be significantly different. Data are expressed as mean ± SD (n = 6).

The metabolites identified in this study were classified according to the HMDB, indicating that 63% of the metabolites were categorized as lipid and lipid-like molecules, 13% as organic acids and derivatives, and 7% as organoheterocyclic compounds and organooxygen compounds (Fig. 5A). In addition, KEGG pathway enrichment analysis showed that the most significantly enriched pathways were related to lipid metabolism, amino acid metabolism, and the biosynthesis of other secondary metabolites (Fig. 5B). Furthermore, enrichment analysis focusing on KEGG pathways was performed for the metabolites with differential abundance. As illustrated in Fig. 5C, the differential metabolites were mainly associated with pathways in sphingolipid metabolism, sphingolipid signaling pathway, and protein digestion and absorption.

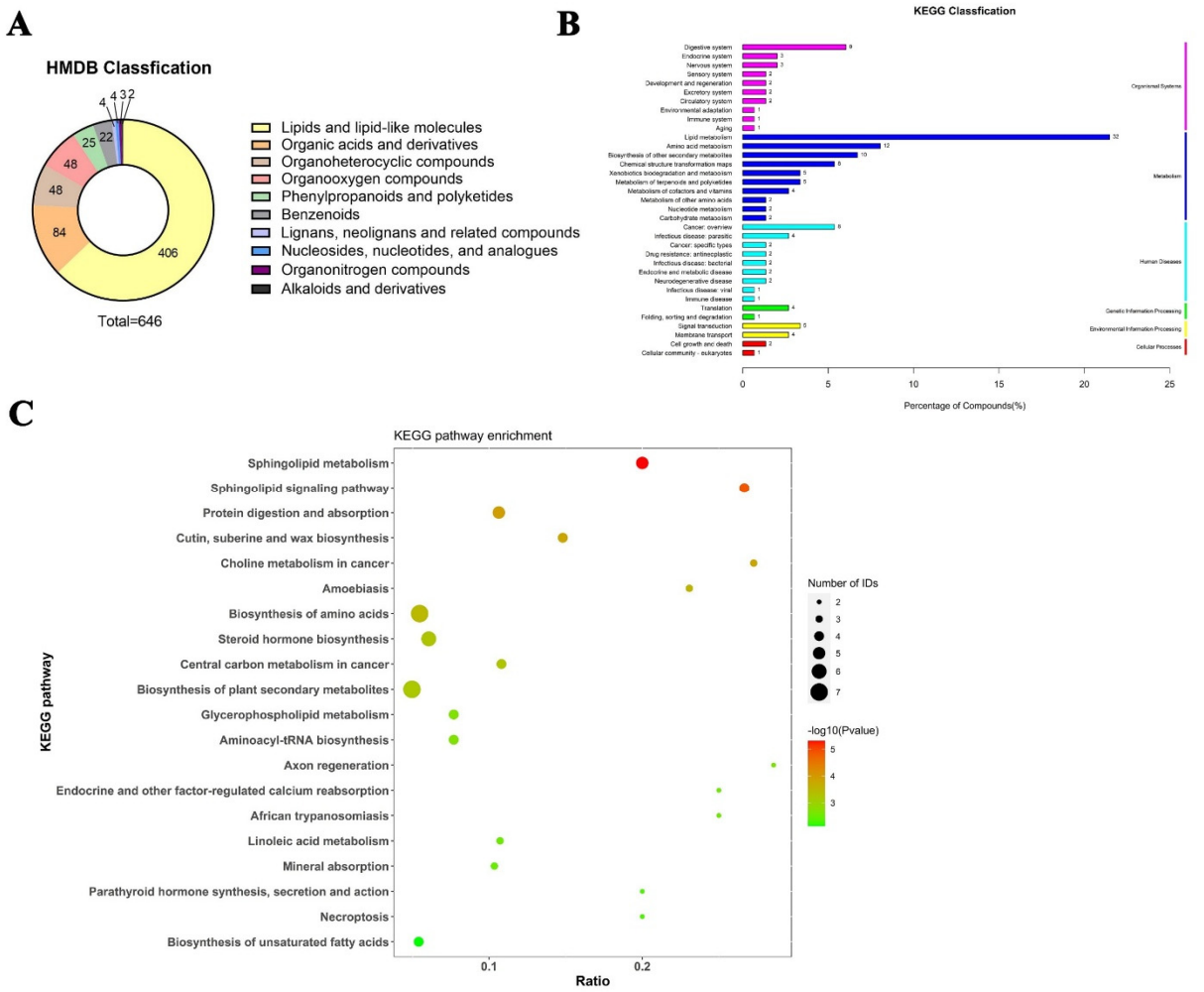

Fig. 5. Intestinal metabolite classification and metabolic pathways. (A) HMDB compound classification of differential metabolites. (B) KEGG compound classification of differential metabolites. (C) Diagram of KEGG pathway enrichment.

3.4 Lard improved gut microbiota composition and function

The gut microbiota significantly impacts the process of intestinal regeneration and repair through modulating the function of intestinal stem/progenitor cells[19]. To assess the differential impacts of different dietary fats on intestinal microbiota, we conducted an analysis utilizing 16S rRNA gene sequencing. As illustrated in Fig. 6A, the lard-fed mice demonstrated a higher Chao1 index compared to those fed corn or beef diet, indicating that lard increased microbial community richness in mice with intestinal injury. Non-metric Multi-dimensional Scaling (NMDS) suggested a distinct clustering among the three groups; however, β diversity was not significantly affected by dietary fats (Fig.6B). The ternary plot presented in Fig. 6C illustrated the distribution of various bacterial phyla. It was indicated that *Muribaculaceae, Lactobacillaceae,* and *Lachnospiraceae* were predominant in the lard group, while *Enterobacteriaceae* and *Peptostreptococcaceae* were more abundant in the corn group. Additionally, *Beijerinckiaceae* and *Erysipelotrichaceae* were notably enriched in the beef group. The proportional histogram displayed the relative abundance at the genus level among three groups (Fig. 6D). Following intestinal injury, *Escherichia_Shigella* was the predominant bacterial genus, followed by *Bacteroides*. The corn group exhibited significantly greater abundance of *Escherichia_Shigella* in comparison to the lard-fed mice (Fig.

6E). The abundance of *Ligilactobacillus*, *unclassified_Lachnospiraceae,* and *unclassified_Muribaculaceae* in the lard group was greater than that in the corn or beef groups (Fig. 6F, G, and H). A Venn diagram illustrating the distribution of all operational taxonomic units (OTUs) among the three groups, revealing that 257 OTUs were common to all groups. The corn, beef, and lard groups had 476, 870, and 1,716 unique OTUs, respectively (Fig. 6I).

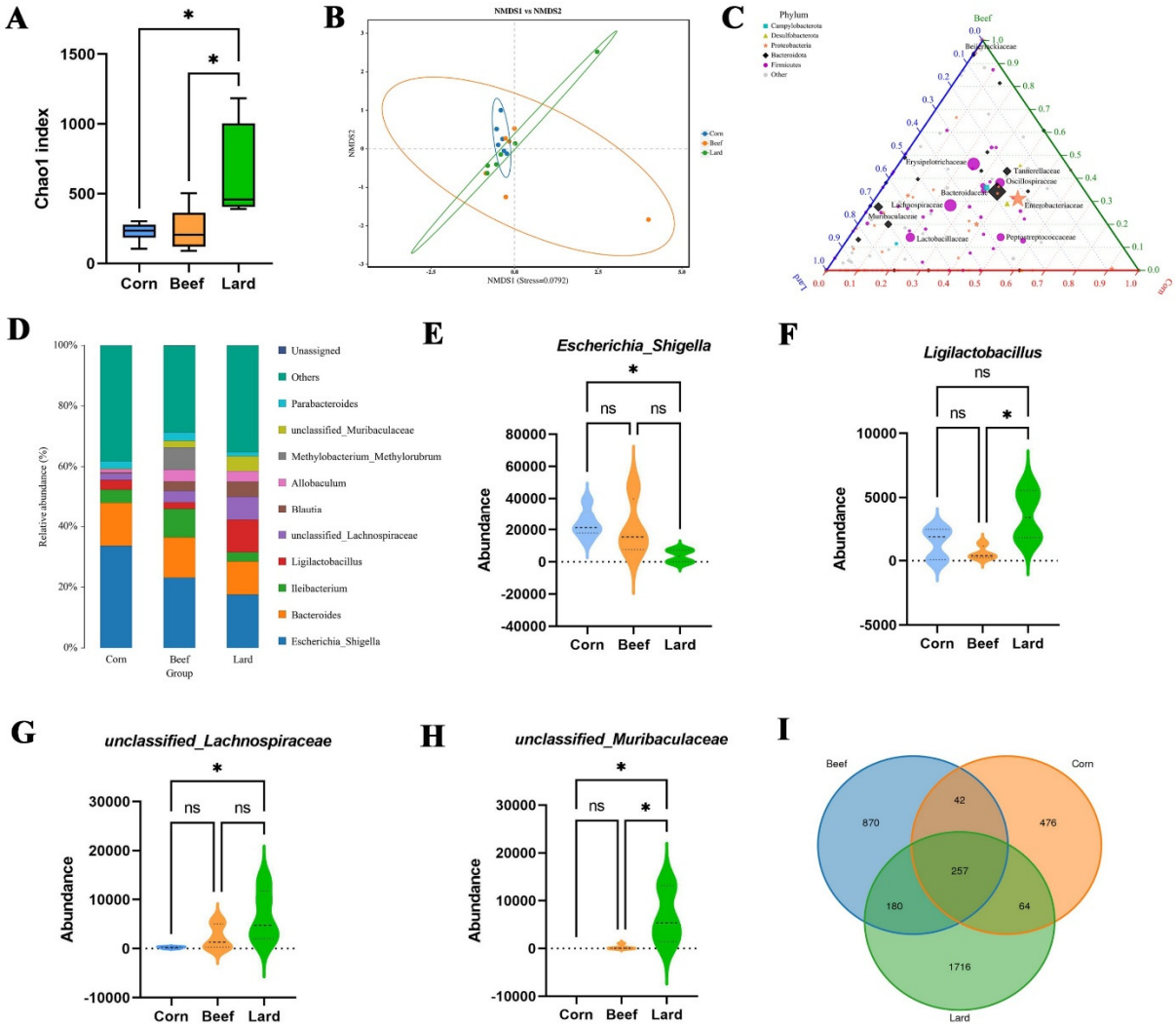

Fig. 6. Effect of different dietary fats on gut microbiota composition. (A) Alpha diversity analysis of gut bacterial richness (Chao1 index). (B) Non-metric Multi-dimensional Scaling (NMDS) analysis. (C) A ternary plot illustrates the different bacterial phyla. The spatial distance between each circle and the corresponding vertices indicates the relative abundance of microorganism within three groups. (D) Distribution of the top 10 bacterial taxa averaged at the genus level. Abundances of (E) *Escherichia_Shigella*, (F) *Ligilactobacillus*, (G) *unclassified_Lachnospiraceae*, (H) *unclassified_Muribaculaceae* at the genus level. (I) The Venn diagram represents the distribution of shared OTUs among three groups. Data are expressed as mean ± SD (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Using PICRUSt2 to predict the functions of gut microbiota (Fig. 7), we conducted a differential analysis of KEGG metabolic pathways between the groups. When contrasted with the corn group, the lard group showed significant enrichment in the following pathways: global and overview maps, translation, amino acid metabolism, replication and repair, metabolism of cofactors and vitamins, nucleotide metabolism, and glycan biosynthesis and metabolism (Fig. 7A). Conversely, the corn group showed enrichment in carbohydrate metabolism and membrane transport. In comparison to the beef group, the lard group was enriched in global

and overview maps, translation, amino acid metabolism, replication and repair, metabolism of cofactors and vitamins, biosynthesis of other secondary metabolites, and nucleotide metabolism (Fig. 7B). In contrast, the beef group exhibited significant enrichment in pathways associated with membrane transport, carbohydrate metabolism, and signal transduction.

Fig. 7. Effect of different dietary fats on gut microbiota function. (A) KEGG metabolic pathway differential analysis between the lard group and corn group. (B) KEGG metabolic pathway differential analysis between the lard group and beef group.

3.5 Correlation analysis of intestinal metabolites, gut microbiota, and intestinal repair markers

As shown in Fig. 8, Spearman's correlation analysis showed that proliferation markers (Ki67 and SOX9) were positively correlated with *unclassified_Lachnospiraceae*, *unclassified_Muribaculaceae*, DG(16:0/22:6(4Z,8Z,10Z,13Z,16Z,19Z)-OH(7)/0:0), 5−hydroxy−coniferaldehyde, palmitoylcarnitine, (E)−11−hexadecenoic acid, phenylalanyl−prolyl−arginine, and DG(14:0/20:4(8Z,11Z,14Z,17Z)/0:0). In addition, the differentiation markers (PAS and MUC2) were positively correlated with *Ligilactobacillus*, adhumulinic acid, DG(8:0/18:3(10,12,15)-OH(9)/0:0). In contrast, Ki67, SOX9, PAS, and MUC2 were negatively correlated with *Escherichia_Shigella.* Further analysis demonstrated strong positive correlations between *unclassified_Muribaculaceae*, *unclassified_Lachnospiraceae* and DG(14:0/20:4(8Z,11Z,14Z,17Z)/0:0), DG(16:0/22:6(4Z,8Z,10Z,13Z,16Z,19Z)-OH(7)/0:0), 5−hydroxy−coniferaldehyde, palmitoylcarnitine, (E)−11−Hexadecenoic acid as well as phenylalanyl−prolyl−arginine. Conversely, *Escherichia_Shigella* showed significant negative correlations with these metabolites.

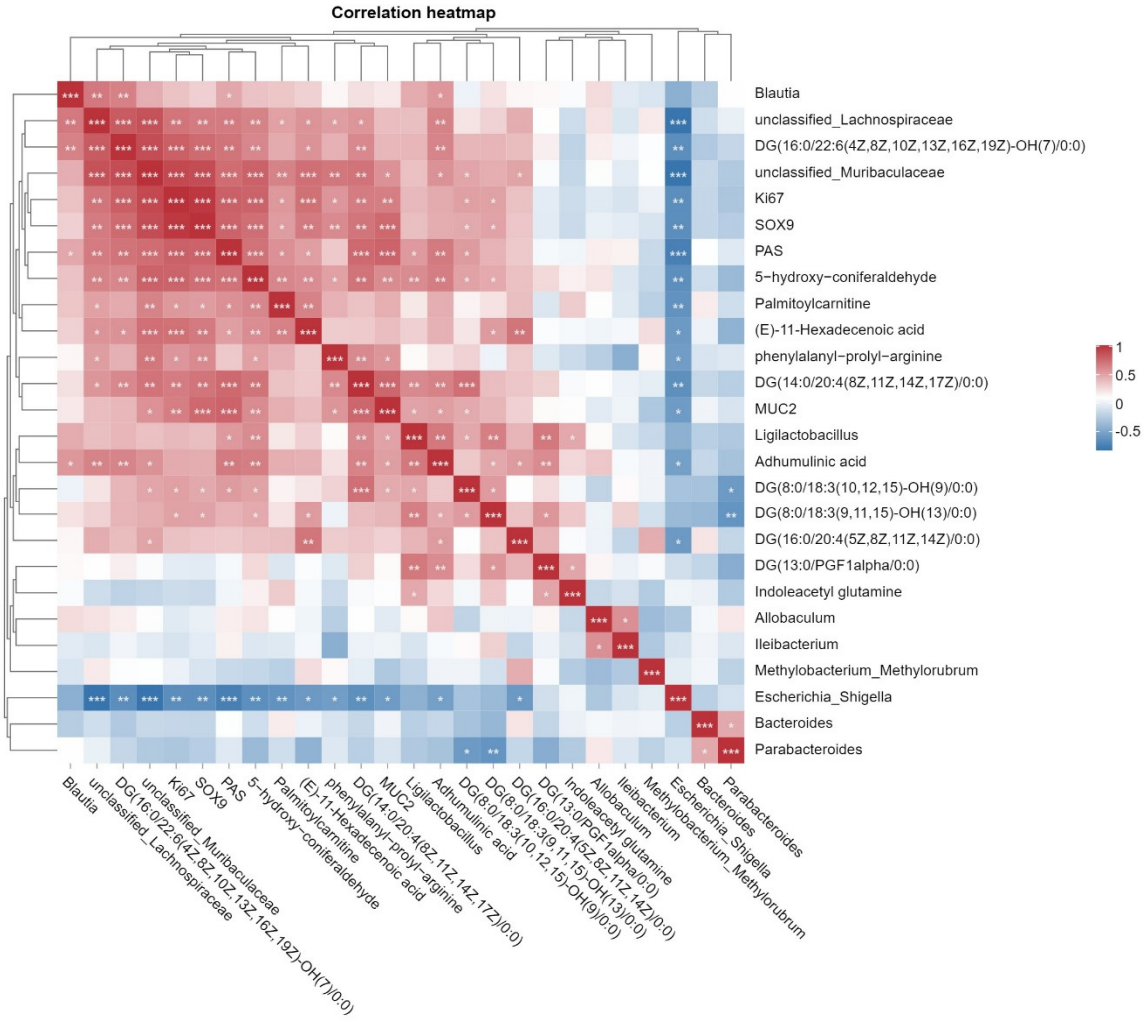

Fig. 8. Spearman's correlation analysis between intestinal metabolites, gut microbiota, and intestinal repair markers. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

4. Discussion

Intestinal epithelial renewal is a rapid and coordinated process driven by the proliferation and differentiation of intestinal stem/progenitor cells. Impaired intestinal stem/progenitor cell function disrupts epithelial repair, compromises the intestinal barrier and perturbs homeostasis, thereby increasing susceptibility to inflammatory bowel disease and other disorders[20]. It is well established that the intestinal cells are responsive to dietary signals[21]. Notably, dietary fat signaling has been shown to influence the function of intestinal stem/progenitor cells[6]. However, few studies focused on the moderate intake of dietary fats with different structural characteristics on intestinal repair. Herein, we interrogated the effects of different dietary fats with different levels of *sn*-2 palmitate on intestinal repair following intestinal injury. Our study revealed that lard (58% *sn*-2 palmitate) enhanced the proliferation and differentiation of intestinal stem/progenitor cells, thereby promoting intestinal regeneration and repair. The proliferation of intestinal stem/progenitor cells is crucial to facilitating the repair of the intestinal epithelium following injury. An increase in the proliferation of stem/progenitor cells is likely to enhance the intestinal resistance to damage[17]. Conversely, impaired proliferation of stem/progenitor cells may exacerbate injury susceptibility[22].

Consequently, *sn*-2 palmitate-rich lard displayed less severe damage to the intestinal epithelial barrier compared to beef (17% *sn*-2 palmitate) and corn (2.3% *sn*-2 palmitate).

Recent reports have highlighted the importance of fatty acid positional distribution in triglycerides in modulating intestinal injury response[7,23]. Our previous study demonstrated that 1,3-dioleoyl-2-palmitoylglycerol (*sn*-2 palmitate) exhibited a protective role in DSS-induced colitis via modulating gut microbiota and maintaining intestinal epithelial barrier integrity in a mouse model[4]. Therefore, we speculated that the enhanced intestinal repair observed in lard-fed mice may be attributed to its high *sn*-2 palmitate content. Nevertheless, whether *sn*-2 palmitate is the decisive factor remains to be fully elucidated. Future studies will evaluate whether a similar reparative effect occurs when mice are fed with *sn*-2 palmitate triglyceride in beef or corn. Furthermore, previous studies have proposed that saturated fat may exert a more pronounced protective effect on the intestinal epithelial barrier compared to unsaturated fat following intestinal injury[9,24-26]. Given that beef and lard contain similar levels of saturated fatty acids—both substantially higher than those in corn—this compositional difference may also contribute to the reduced intestinal damage observed in beef-fed compared to corn-fed mice.

It has been shown that metabolites derived from dietary fats influence signaling pathways associated with intestinal repair[19]. Our study revealed that lard significantly enriched multiple intestinal metabolites, including diacylglycerols (DG), palmitoylcarnitine, adhumulinic acid, (E)−11−hexadecenoic acid, cis−12−octadecenoic acid, indoleacetyl glutamine, phenylalanyl−prolyl−arginine, and 5−hydroxy−coniferaldehyde. Among these, DG(16:0/22:6(4Z,8Z,10Z,13Z,16Z,19Z)-OH(7)/0:0), 5−hydroxy−coniferaldehyde, palmitoylcarnitine, (E)−11−hexadecenoic acid, phenylalanyl−prolyl−arginine, and DG(14:0/20:4(8Z,11Z,14Z,17Z)/0:0) were positively correlated with the proliferation of intestinal stem/progenitor cells. Adhumulinic acid and DG(8:0/18:3(10,12,15)-OH(9)/0:0) were positively correlated with the differentiation of intestinal stem/progenitor cells. The key metabolites, DG, are primarily generated during the hydrolysis of lard triglycerides by pancreatic lipase. Palmitoylcarnitine is a mitochondrial β-oxidation intermediate derived from palmitic acid, which is released during the digestion of lard. In addition, (E)−11−hexadecenoic acid likely arises from microbial isomerization of dietary fatty acids, suggesting a microbiota-dependent origin. It has been demonstrated that adhumulinic acid showed strong associations with tight junction proteins related to the intestinal epithelial barrier[27]. Dietary fats rich in DG have been shown to improve intestinal microbiota and reduce intestinal inflammation[28-29]. Endogenous DG are known to regulate cellular responses, including proliferation, differentiation and apoptosis, thereby exerting a protective effect against intestinal epithelial injury[30]. Palmitoylcarnitine, as a crucial intermediate in fatty acid β-oxidation, elevated levels directly reflect enhanced fatty acid oxidation activity, which supports intestinal stem cell repair[31]. In the future, we will explore which key metabolites play a crucial role in promoting intestinal repair in lard. Furthermore, the KEGG enrichment analysis indicated that the differential metabolites were primarily involved in sphingolipid metabolism. Sphingolipids have been reported to enhance MUC2 expression and promote the proliferation of intestinal crypt stem/progenitor cells,

thereby facilitating intestinal repair[32-34]. This suggests that the sphingolipid metabolism pathway is likely to play a crucial role in the mechanisms through which lard promotes intestinal regeneration and repair.

Dietary fats play a significant role in shaping gut microbial communities, thereby influencing host intestinal health[7]. The intestinal microbiota is vital for preserving the functionality of the intestinal epithelial barrier through direct and indirect interactions with intestinal epithelial cells[35-37]. Furthermore, evidence demonstrates that gut microbiota critically supports intestinal stem/progenitor cell function during injury and repair, partly through direct contact and modulation of gene expression in these cells[38-39]. Our results indicated that the lard diet enhanced microbial community richness compared to the corn and beef groups. However, different dietary fats did not alter the β diversity in mice with intestinal injury. These findings were aligned with our earlier investigations, which indicated that dietary fats showed no significant effect on the β diversity of intestinal microbiota in mice subjected to a high-fat diet[40]. Lard contains unique fatty acid profiles that may act as energy substrates or signaling molecules for specific bacteria[4,41-42]. Notably, we observed a significant positive correlation between *unclassified_Muribaculaceae* and two specific DGs: DG(14:0/20:4(8Z,11Z,14Z,17Z)/0:0) and DG(16:0/22:6(4Z,8Z,10Z,13Z,16Z,19Z)-OH(7)/0:0). This finding is consistent with a recent study demonstrating that dietary DG supplementation modulates gut microbiota composition, particularly by increasing the abundance of *unclassified_Muribaculaceae*[43]. It is speculated that DGs released during the digestion of lard may be metabolized by bacterial enzymes, thereby driving shifts in microbial community structure. Moreover, the abundance of *Lactobacillaceae*, *unclassified_Lachnospiraceae* and *unclassified_Muribaculaceae* showed a notable rise in the lard group compared to the corn and beef groups. It was reported that *sn*-2 palmitate could promote the proliferation of *Lactobacillaceae* and *unclassified_Muribaculaceae*[4], which may partially explain the enrichment of these beneficial bacteria in the lard group. In addition, our findings demonstrated that *unclassified_Lachnospiraceae* and *unclassified_Muribaculaceae* were positively correlated with the proliferation and differentiation of intestinal stem/progenitor cells. This aligns with prior research demonstrating that these butyrate-producing bacteria such as *Lachnospiraceae* and *Muribaculaceae*, generate butyric acid which serves as the primary energy source for colonic epithelial cells, promoting intestinal stem cell proliferation, and stimulating MUC2 secretion to facilitate mucosal repair[44,45]. Additionally, beneficial *Lactobacillaceae* have been shown to play a regulatory role in intestinal epithelial regeneration and repair[46]. Furthermore, KEGG pathway analysis revealed that lard enriched microbial metabolic pathways associated with replication and repair, translation, amino acid metabolism, and nucleotide metabolism, indicating that lard may enhance proliferation and repair capability[20]. The gut microbial profile aligned with improved intestinal regeneration and repair observed in the lard-fed group. Although our findings provide evidence for microbiota-mediated mechanisms in lard-induced intestinal repair, definitive establishment of causal relationships between specific microbial alterations and epithelial regeneration would require additional validation through microbiota-depletion and fecal microbiota transplantation studies. Future work will focus on elucidating the role of these microbial communities in mediating the

regenerative effects of *sn*-2 palmitate-enriched dietary fats, particularly their potential to modulate stem/progenitor cell proliferation and differentiation through microbial-metabolite signaling pathways.

5. Conclusions

In summary, our findings demonstrate that moderate intake of lard promotes intestinal repair following injury when compared to beef and corn. Mechanistically, lard digestion produces unique bioactive metabolites and favorably modulates gut microbiota composition. These synergistic effects may promote the proliferation and differentiation of intestinal stem/progenitor cell, ultimately accelerating epithelial regeneration and repair. This study offers novel insights into the beneficial effects of dietary fats rich in *sn*-2 palmitate, such as lard, on intestinal repair.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Furthermore, they affirm no association with any animal fat companies.

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