Animal Nutrition 20 (2025) 430–443

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Effects of peroxidized lipids on intestinal morphology, antioxidant capacity and gut microbiome in piglets

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ARTICLE INFO

Article history:
Received 23 October 2023
Received in revised form
5 November 2024
Accepted 20 November 2024
Available online 27 December 2024

Keywords:
Peroxidized lipid
Gut microbiota
Diarrhea
Oxidative stress
Piglet

ABSTRACT

This study investigated the effect of peroxidized lipids on piglets' growth performance, intestinal morphology, inflammatory reactions, oxidative stress in the liver, duodenum, jejunum, ileum, and colon, and ileal microbiota. Twenty piglets (Duroc × [Landrace × Yorkshire]; age = 21 d old, BW = 6.5 ± 1 kg) were randomly assigned to two groups with 10 replicates per group and one piglet per replicate. The control group was fed 6% fresh soybean oil and the peroxidized soybean oil (PSO) group fed 6% PSO. The experimental feeding period lasted 24 d. The study found no impact on ADFI, ADG and gain to feed ratio ($P > 0.05$). However, the PSO group increased the diarrhea index and the serum levels of lactate dehydrogenase triglycerides, cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol ($P < 0.05$), along with decreased concentrations of alanine aminotransferase and blood urea nitrogen ($P < 0.05$). For oxidative enzymes, PSO increased the concentration of F2-isoprostane in urine ($P = 0.032$), malondialdehyde (MDA) in the duodenum ($P = 0.001$) and jejunum ($P = 0.004$), decreased thiobarbituric acid reactive substances (TBARS) in the liver ($P = 0.001$) but increased TBARS in duodenum ($P = 0.001$), and carbonylated proteins in the duodenum ($P = 0.003$). For antioxidant enzymes, PSO decreased superoxide dismutase (SOD) in the liver ($P = 0.001$), colon ($P = 0.002$), and jejunum ($P = 0.015$), along with glutathione peroxidase (GSH-Px) in the liver ($P = 0.008$) and NAD(P)H:quinone oxidoreductase 1 (NQO1) in ileum ($P = 0.001$). For inflammatory reactions, PSO increased interleukin (IL)-1β concentrations in the duodenum and colon, and IL-10 in the jejunum, while decreasing IL-4 concentration in the duodenum ($P < 0.05$). For intestinal morphology and ileal microbiota, PSO increased ileal crypt depth, while decreasing the crypt-to-villus ratio ($P < 0.05$). Peroxidized soybean oil increased the relative abundance of *Prevotella, Clostridium_sensu_stricto_1, Clostridium_sensu_stricto_6, Pasteurella* and *Klebsiella* ($P < 0.05$). In conclusion, this study revealed that PSO worsened diarrhea, increasing the ileal crypt depth and the relative abundance of harmful microbiota, and induced oxidative stress and inflammation in the intestines and liver, primarily in the jejunum and ileum.

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Peer review under the responsibility of Chinese Association of Animal Science and Veterinary Medicine

Production and Hosting by Elsevier on behalf of KeAi

1. Introduction

Soybean oil, a supplemental energy source in pig nutrition used to improve feed efficiency and provide additional energy, contains high level of unsaturated fatty acids and low levels of long-chain fatty acids (Holman and lipids, 1954; Lindblom et al., 2018). Given

https://doi.org/10.1016/j.aninu.2024.11.015

its rich unsaturated fatty acid content, soybean oil is susceptible to lipid peroxidation when exposed to oxygen or subjected to thermal processing (Adam et al., 2008; Lin et al., 1989; Rosero et al., 2015).

Peroxidized lipids generate various secondary and tertiary peroxidative compounds, such as aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids and epoxy compounds (Lindblom et al., 2018; Overholt et al., 2018; Shurson et al., 2015; Wang et al., 2016). These aldehydes originating from peroxidation can interact with nucleotides, lipoproteins, proteins, glutathione (GSH), and DNA (Refsgaard et al., 2000), resulting in vivo DNA and protein damage, ultimately causing cell injury and apoptosis (Abraham et al., 2011; Chang et al., 2005; Esterbauer et al., 1991). In addition, peroxidized lipids reduce levels of glutathione peroxidase (GSH-GPX) and total antioxidant activity (T-AOC) in the serum and plasma (Silva-Guillen et al., 2020; Yuan et al., 2007), increased the thiobarbituric acid reactive substances (TBARS) in urine, elevated 8-hydroxy-2′-deoxyguanosine (8-OH-2dG) in the liver, and reduced in catalase activity (CAT) levels in the liver (Lindblom et al., 2018). Limited research has explored the impact of peroxidized lipids on pig intestines; however, an increase in malondialdehyde (MDA) and a reduction in T-AOC in the jejunum, causing an elevation in villus height and crypt depth in the jejunum has been demonstrated (Rosero et al., 2015). For the gut microbiota, feeding rats with heated perilla oil significantly enhances the relative abundance of *Akkermansia muciniphila*, while thermally oxidized sunflower oil and corn oil stimulates the proliferation of Bifidobacterium (Kwek et al., 2022; Ruan et al., 2023) and reduces the abundance of B*acteroides, Barnesiella, Clostridium_XIVa, Lactococcus, Parasutterella, Paraprevotella, Allobaculum, Acetatifactor, Clostridium_IV, Coprococcus, and Oscillibacter* (Kwek et al., 2022). Heating canola oil reduces *Prevotella* in rat microbiota while increase Bacteroides and *Allobaculum* (Zhou et al., 2016). Heating palm oil elevates *Lactobacillus* and *Roseburia* in the rat gut (Ruan et al., 2021). Peroxidized lipids can cause a reduction in species diversity and abundance within the gut microbiota (Kwek et al., 2022). These effects can lead to changes in the body's oxidative metabolism, causing oxidative stress (Breitzig et al., 2016; Chang et al., 2005; Ji et al., 2016) that detrimentally affects growth performance (Boler et al., 2012; DeRouchey et al., 2004; Rosero et al., 2015), digestion rate and the absorption of nutrients in the intestine (Engberg et al., 1996; Lindblom et al., 2018; Overholt et al., 2018).

However, investigation into the impact of peroxidized lipids on the pig's intestine remains incomplete. There is a lack of comprehensive understanding regarding the induction of oxidative stress by peroxidized lipids, the potential for triggering intestinal inflammation, the extent of damage to intestinal morphology, and alterations in the composition of gut microbiota in piglets. Additionally, it remains to be determined whether the liver, as a primary metabolic organ, will be similarly affected. In the current study, we assessed piglets' growth performance, intestinal morphology, inflammatory reactions, oxidative stress in the liver, duodenum, jejunum, ileum, and colon, and changes in the ileal gut microbiota. We measured antioxidant capacity indicators, including superoxide dismutase (SOD), GSH-GPX, NAD(P)H:quinone oxidoreductase 1 (NQO1), and T-AOC, as well as oxidative capability indicators, such as MDA, cortisol, TBARS, and carbonylated proteins, and F2-isoprostane (F2) levels in urine. This comprehensive approach addresses the need for a thorough assessment of peroxidized lipid-induced damage to piglets' bodies.

2. Materials and methods

2.1. Animal ethics statement

All animal experiments were conducted in compliance with the “Animal Research: Reporting of In Vivo Experiments” (ARRIVE) guidelines (https://arriveguidelines.org) and were approved by the protocol management and review committee of the institute of subtropical agriculture, Chinese academy of sciences (ISA2022006).

2.2. Dietary treatments

The peroxidized soybean oil (PSO) was produced by heating fresh soybean oil in a water bath at 90 °C. Each container was equipped with a submerged polyvinyl chloride pipe with 1-mm holes, allowing a continuous airflow through the oil at a constant rate of 12 L/min for 120 h. The peroxide value of the oil was determined in accordance with China National Standard (GB 5009.227-2016). The composition of PSO is presented in Table 1.

2.3. Dietary formulation

The diet, characterized as a corn-soybean meal type, was formulated in accordance with (NRC, 2012) guidelines. Details of the specific ingredients and nutrient composition are provided in Table 2.

2.4. Experiment design and animal management

The necessary sample size was calculated using Power Analysis and Sample Size Software (PASS; NCSS 2021 Statistical Software 2021), aiming for a power of 0.8 and an alpha of 0.05, suggesting 20 per group. Due to costs, facility constraints, and typical animal nutrition research sizes (6-8), a sample size of 10 per group was chosen. This experiment employs a single-factor randomized block design (RBD), with one pig as the experimental unit (one data point per pig). After 7 d pre-fed period, 20 healthy 28 d piglets (Duroc × [Landrace × Yorkshire]; 6.5 ± 1.0 kg) were randomly assigned to two groups based on initial BW and sex ($n = 10$; 5 male and 5 female in each group). In the same group, the initial weights did not differ significantly between males and females. Since the weight measurements for piglets are taken after castration, gender is not considered an influencing factor. The PSO group received a diet containing 6% PSO, while the control group received a diet containing 6% fresh soybean oil. Each group, comprising 10 replicates with one pig per replicate, was observed over an experimental period of 24 d. All animals were housed in a fully enclosed pig farm, where a stable environmental temperature of 28 °C was maintained using artificial heating lamps. The piglets were raised in farrowing pens with plastic slatted floors. The pens had a height of 0.6 m, a length of 1.8 m, a width of 1.7 m, and were positioned above the ground at a distance of 50 to 60 cm. The pigs were individually housed in separate pens with free access to feed and water, each equipped with a circular tray feeder with a height of 10 cm. Individual BW was measured on d 0, 10, 20, and 24. The ADFI and ADG were recorded, and from these measurements, the gain to feed ratio

Table 1
Production conditions and composition of PSO.

Item	Value
Heating temperature, °C	90
Time heated, h	120
Acrolein, mg/kg	16.01
2,4-Decadienal, mg/kg	2824.58
4-Hydroxy-2-nonenal, mg/kg	654.07
Vitamin E, mg/100 g	<0.120
Peroxide value of PSO, mEq/kg	240
Peroxide value of fresh soybean oil, mEq/kg	2.82

PSO = peroxidized soybean oil.

Table 2
The ingredients and nutrient composition of the diet (%).[1]

Item	Phase 1		Phase 2	
	Control	PSO	Control	PSO
Ingredients				
Corn 261	58.26	58.26	57.82	57.82
Soybean meal 340	15.6	15.6	18.00	18.00
Soy protein concentrate	9.61	9.61	7.40	7.40
Whey powder	3.00	3.00	4.00	4.00
Peroxidized soybean oil	0.00	6.00	0.00	6.00
Soybean oil	6.00	0.00	6.00	0.00
Limestone	0.25	0.25	0.25	0.25
Calcium formate	0.60	0.60	0.60	0.60
Dicalcium phosphate	1.36	1.36	1.10	1.10
Choline chloride	0.18	0.18	0.18	0.18
Citric acid	2.00	2.00	2.00	2.00
Zinc oxide	0.02	0.02	0.02	0.02
Salt	0.83	0.83	0.60	0.60
Lysine 78%	0.65	0.65	0.52	0.52
DL-Methionine	0.32	0.32	0.26	0.26
Threonine	0.23	0.23	0.18	0.18
Tryptophan 98%	0.04	0.04	0.02	0.02
Calcium propionate	0.05	0.05	0.05	0.05
Multi-vitamins[2]	0.10	0.10	0.10	0.10
Copper sulfate pentahydrate	0.05	0.05	0.05	0.05
Manganese sulfate monohydrate	0.03	0.03	0.03	0.03
Zinc sulfate monohydrate	0.03	0.03	0.03	0.03
Ferrous sulfate monohydrate	0.06	0.06	0.06	0.06
Zeolite powder	0.50	0.50	0.50	0.50
Choline chloride	0.18	0.18	0.18	0.18
Preservative	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00
Analyzed nutrient compositions				
Dry matter	88.56	89.73	86.02	94.96
Crude protein	21.17	20.32	20.36	20.37
Ether extract	3.24	2.96	3.35	3.64
Crude ash	7.99	7.76	7.26	7.08
Crude fiber	1.53	2.54	2.91	3.41
Calcium	0.69	0.65	0.69	0.66
Phosphorus	0.50	0.45	0.45	0.38
Iron	0.01	0.01	0.02	0.01
Zinc	0.12	0.11	0.01	0.02
Gross energy, MJ/kg	18.60	18.72	18.89	19.05
Lysine	0.73	0.69	0.58	0.59
Methionine	0.36	0.35	0.28	0.28
Threonine	0.47	0.39	0.42	0.39
Tryptophan	0.34	0.30	0.38	0.36
Calculated nutrient compositions				
Metabolizable energy, kcal/kg	3354.20	3376	3259.70	3629
Net energy, kcal/kg	2431.80	2447.60	2363.30	2631

PSO = peroxidized soybean oil.
[1] The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO. Phase 1 diets were fed to piglets from 0 to 14 d and phase 2 diets were fed to piglets from 14 to 24 d. The ingredients were presented on as-fed basis, and the analyzed nutrient composition was presented on dry matter basis. The ingredients were presented on as-fed basis, and the analyzed nutrient composition was presented on dry matter basis.
[2] One-kilogram vitamin premix contained the following: 32,500,00 IU of vitamin A, 10,000,00 IU of vitamin D_3, 80,000 mg of vitamin E, 10,000 mg of vitamin K_3, 10,000 mg of vitamin B_1, 25,000 mg of vitamin B_2, 8000 mg of vitamin B_6, 75 mg of vitamin B_{12}, 600 mg of D-biotin, 5000 mg of folic acid, 100,000 mg of nicotinamide, 50,000 mg of D-pantothenic acid.

(G:F) was calculated as ADG/ADFI. The diarrhea index was monitored daily based on the criteria outlined in Table S1 (Yuan, 2007).

2.5. Sample collection

On the 24th day of the experiment, all piglets were fasted at 20:00 and euthanized by exsanguination at 08:00 the following morning. Venous blood samples were drawn into non-heparinized vacuum tubes, allowed to clot at room temperature for 30 min, and then centrifuged at 3000 × *g* at 4 °C for 10 min to isolate the serum, which was subsequently stored at −80 °C for further analysis. The intestinal tract was segmented to obtain duodenum, jejunum, ileum and colon, which were then flushed with 0.9% saline to remove the digesta content. Mucosa samples were gently scraped with a microscope slide, immediately frozen in liquid nitrogen, and stored at −80 °C. Sections of approximately 2 cm from the duodenum, jejunum, ileum, and colon were harvested and fixed in 4% formaldehyde for morphological analysis. Ileal contents were placed in sterile tubes, quickly frozen in liquid nitrogen, and stored at −80 °C for future 16S rRNA sequencing. Urine samples were collected from the urethra and stored at 4 °C for subsequent analyses. A liver sample was cut, rinsed with 0.9% saline, rapidly frozen in liquid nitrogen, and then stored at −80 °C.

2.6. Feed nutrition analysis

The feed were sampled and analyzed for crude protein (CP; N × 6.25) following China National Standard (GB/T 24318-2009), crude fiber (CF) following China National Standard (GB/T 6434-2006), ether extract (EE) following China National Standard (GB 6433-2006), and crude ash content using China National Standard (GB/T 6438-2007). Dry matter (DM) was determined according to method 930.15 (AOAC, 2005). Gross energy (GE) was measured using an automatic calorimeter (5E-AC8018; Kaiyuan, Changsha, China) according to the method of 9831 (ISO, 1998). Trace minerals were quantified according to China National Standard, including calcium (GB/T 6436-2018), phosphorus (GB/T 6437-2018), iron and zinc (GB/T 13885-2017). Amino acids, including lysine, methionine, and threonine, were quantified according to China National Standard (GB/T 18246-2019). Tryptophan was measured according to Inner Mongolia Autonomous Regional Standard (DB15/T 2037-2020).

2.7. Serum biochemical analysis

Serum parameters including cholesterol (CHOL), total protein (TP), alanine aminotransferase (ALT), high-density lipoprotein cholesterol (HDL-C4), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), triglycerides (TG), blood urea nitrogen (BUN) and immunoglobulin M (IgM), and low-density lipoprotein cholesterol (LDL-C3) were measured using Cobas c311 analyzer (Roche Diagnostics Operations, Inc., Switzerland).

2.8. Antioxidant capacity analysis

Samples from the liver, duodenum, jejunum, ileum, and colon mucosa were mixed with 1 mL of phosphate-buffered saline (PBS) containing 0.01 mol/L phosphate, 0.0027 mol/L KCl, and 0.137 mol/L NaCl. These were then ground using a Servicebio KZ-III-F lapping instrument (Wuhan Servicebio Technology, Co., Ltd., Hubei, China) and centrifuged at 5000 × *g* for 10 min. The resulting supernatant was used for ELISA and total protein concentration assays, while thawed serum samples were directly used for assays without further centrifugation. The biochemical assay kits were used to measure superoxide dismutase (SOD) and GSH-GPX from Beijing Boxbio Science & Technology Co., Ltd., as well as NQO1 and total antioxidant activity (T-AOC) from Shanghai Zcibio Technology Co., Ltd. The OD values were determined using the infinite 200 PRO multimode reader (Tecan Trading AG, Switzerland), and concentrations were calculated based on the instruction manual and absorbance from standard curves. The BCA protein assay kit (Lot No. 092721220221, enhanced BCA protein assay kit, Beyotime Biotechnology, Shanghai, China) was used to measure total protein concentration.

2.9. Oxidative capacity analysis

ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were employed to measure the concentration of malondialdehyde (MDA), cortisol, TBARS and carbonylated proteins in the supernatant of liver, duodenum, jejunum, ileum, and colon mucosa. F2-isoprostane (F2) was measured in urine. The methods were performed according to the manufacturer's protocols.

2.10. Immune responses analysis

The levels of interleukin -1β (IL-1β), tumor necrosis factor-alpha (TNF-α), interleukin-4 (IL-4), and interleukin-10 (IL-10) in the supernatants from the liver, duodenum, jejunum, ileum, and colon mucosa were measured using ELISA kits (Jiangsu Meimian Industrial Co., Ltd). The methods were performed according to the manufacturer's protocols (Tang et al., 2020).

2.11. Morphological analysis

After 72 h of fixation in 4% paraformaldehyde, the duodenum, ileum, jejunum, and colon tissues were dehydrated and processed according to Servicebio's protocols (Wuhan Servicebio Technology, Co., Ltd., Wuhan, China), which included embedding in paraffin and sectioning. The 4 μm-thick paraffin sections were stained with hematoxylin and eosin (H&E). Villus height, crypt depth, and the villus-to-crypt (V:C) ratio were measured following Wang et al. (2021), using an image processing and analysis system (FLUOCA FCSnap, FluoCa Scientific Pte. Ltd, Singapore) at 10× magnification across four fields of view per slide (top, bottom, left, and right). Within each of these fields, 15 individual measurements are taken for both the villi and the crypts, totaling 30 measurements per field. The average of these 15 measurements for villi and 15 for crypts is calculated separately, resulting in two averages per field. Thus, for each slide, eight data points are generated (four villi and four crypts). Then, the mean value of these data points is used for further statistical analysis as the experimental unit is each pig.

2.12. Extraction of DNA and 16S microbiome sequencing

According to the manufacturer's protocol, fecal DNA was isolated using the TGuide S96 magnetic soil/stool DNA kit (Tiangen Biotech Co., Ltd., Beijing, China), incorporating a bead-beating step to facilitate DNA extraction. The quantification of DNA concentration was achieved through the application of the Qubit dsDNA HS assay kit and the Qubit 4.0 fluorometer (Invitrogen, Thermo Fisher Scientific, Oregon, USA). Amplification of the V4-V5 region of the 16S rRNA gene was conducted using a universally applied primer set, comprising the forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and the reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Both forward and reverse primers for the 16S rRNA gene were appended with sample-specific PacBio barcode sequences, facilitating multiplexed sequencing. The choice of barcoded primers was predicated on their ability to diminish chimera formation, a notable improvement over alternative protocols that involve the addition of primers in a secondary PCR reaction. PCR amplification was carried out for 25 cycles using the KOD ONE PCR master mix (TOYOBO Life Science). The protocol included an initial denaturation at 95 °C for 2 min, followed by 25 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min and 30 s, with a final extension at 72 °C for 2 min. The amplified PCR products were purified with agencourt AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using the Qubit dsDNA HS assaykit and Qubit 4.0 fluorometer (Invitrogen, Thermo Fisher Scientific, Oregon, USA). Amplicons were combined in equal molar ratios, and SMRTbell libraries were created using the SMRTbell Express Template Prep Kit 2.0, following the guidelines provided by Pacific Biosciences. These libraries, generated from pooled and barcoded samples, were sequenced on a PacBio Sequel II 8M cell with the Sequel II Sequencing Kit 2.0. Notably, the library preparation and sequencing phases included negative controls but did not incorporate mock communities.

2.13. Statistical analysis

Statistical analyses were conducted using SPSS version 27. An independent samples *t*-test was performed to evaluate the differences in means between the groups. Prior to conducting the *t*-tests, data normality was assessed using the Shapiro-wilk test, which is suitable for small sample sizes. Histograms and Q–Q plots were also generated to visually inspect the distribution of the data. These preliminary tests ensure the appropriateness of parametric testing. In cases where data did not meet the assumption of normality, non-parametric alternatives such as the Mann Whitney U test were employed. The level of significance was set at $P < 0.05$ for all tests. Bioinformatics analysis for this study was performed using BMK cloud provided by Biomarker Technologies Co., Ltd., Beijing, China. Initially, raw sequencing reads were processed through SMRT Link software (version 8.0), applying filters for minimum passes (≥ 5) and minimum predicted accuracy (≥ 0.9) to generate circular consensus sequencing (CCS) reads. The CCS sequences were then matched to their corresponding samples using barcode identification with lima (version 1.7.0), based on barcode identification. Circular consensus sequencing reads lacking primers or falling outside the length range of 1200 to 1650 bp were excluded using Cutadapt (version 2.7) for forward and reverse primer recognition and quality control. Clean reads were then analyzed to produce amplicon sequence variants (ASVs) using dada2, discarding ASVs with a relative abundance below 0.005%. Taxonomic classification of the operational taxonomic units (OTUs) was conducted using the Naive Bayes classifier within QIIME2 (Bolyen et al., 2019), referencing the SILVA database (release 132) and setting a confidence threshold at 70% (Quast et al., 2013). Alpha diversity metrics were computed and visualized using QIIME2 and R software. The similarity of microbial communities across different samples, representing beta diversity, was analyzed using QIIME, employing techniques such as Principal Coordinate Analysis (PCoA), heat maps, UPGMA, and nonmetric multidimensional scaling (NMDS) for this purpose. Significant taxonomic differences among groups were identified using the linear discriminant analysis (LDA) effect size (LEfSe), with a logarithmic LDA score of 4.0 as the significance threshold. Microbiome dissimilarities across various factors were examined using redundancy analysis (RDA) in R with the 'vegan' package. Total variance is decomposed using distance matrices, like Euclidean, to conduct PERMANOVA (Adonis) analysis. This approach quantifies the degree of sample variability attributable to different group factors, and permutation tests are employed to evaluate the statistical significance of these distinctions. Bug base is a computational tool designed to infer functional attributes and phenotypic characteristics from complex microbiome datasets (Ward et al., 2017). This algorithm enhances our understanding of microbial communities by normalizing OTUs against predicted 16S ribosomal RNA gene copy numbers and by utilizing an extensive database of precomputed phenotypic information. For each microbiome sample, Bug base quantitatively estimates the relative abundance of phenotypic traits, employing a comprehensive threshold range from 0 to 1 in increments of 0.01. It determines the optimal coverage threshold to accurately represent each phenotypic feature within the dataset. Subsequently, the algorithm

outputs a detailed prediction table at the organism level. The RNA-seq that supports this study's findings is available from the NCBI SRA database with accession numbers PRJNA1061470.

3. Results

3.1. The composition of PSO

Table 1 delineates the compositional attributes of PSO utilized in the study. The oil was subjected to a heating temperature of 90 °C over a period of 120 h. Analytical measurements revealed the presence of acrolein at 16.01 mg/kg and 2,4-decadienal at 2824.58 mg/kg. Additionally, the quantity of 4-HNE was at 654.07 mg/kg. The vitamin E content was found to be less than 0.120 mg per 100 g of PSO. The peroxide value of PSO was high at 240 mEq/kg. Conversely, fresh soybean oil had a much lower peroxide value of 2.82 mEq/kg.

3.2. Effects of PSO on growth performance and diarrhea index

The growth performance and diarrhea index are shown in Table 3. Compared with the control group, there was no difference of ADG, ADFI and G:F ($P > 0.05$; Table 3). The diarrhea index was higher in the PSO group compared to the control group ($P = 0.001$; Table 3).

3.3. Effects of PSO on blood biochemistry

As described in Table 4, the concentrations of LDH, TG, CHOL, LDL-C3, and HDL-C4 increased in the PSO group, while ALT and BUN were decreased compared to the control group ($P < 0.05$; Table 4). There were no significant differences in the concentrations of TP, AST, ALP, and LGM between the control group and the PSO group ($P > 0.05$; Table 4).

3.4. Effects of PSO on serum antioxidant and oxidative capacity

Serum antioxidant and oxidative capacity was presented in Table 5. No differences were observed in the serum activities of MDA, cortisol, and SOD between the PSO group and the control group ($P > 0.05$; Table 5).

3.5. Effects of PSO on liver and intestinal antioxidant capability

Figure 1 presents the comparative analysis of antioxidant activities in the liver, duodenum, jejunum, ileum, and colon. SOD activity was significantly decreased in the PSO group compared to the control group; specifically in the liver, colon, and jejunum ($P < 0.05$; Fig. 1A). Glutathione peroxidase activity in the PSO group showed a significant decrease in the liver and a significant increase in the duodenum ($P < 0.05$; Fig. 1B). NAD(P)H:quinone oxidoreductase 1 activity also decreased significantly in the liver and ileum of the PSO group, whereas the duodenum displayed a significant increase in this activity ($P < 0.05$; Fig. 1C). Additionally, a reduction in T-AOC activity was observed in the duodenum of the PSO group in comparison to the control group ($P = 0.001$; Fig. 1D).

Table 3
Effects of PSO on growth performance and diarrhea index.

Item	Treatments[1]		*P*-value
	Control diet	PSO diet	
Initial BW, g	7.35 ± 0.228	7.35 ± 0.198	0.997
ADFI, g	597.02 ± 32.881	561.44 ± 28.358	0.428
ADG, g	344.46 ± 34.244	308.52 ± 20.355	0.349
G:F	1.68 ± 0.108	1.76 ± 0.081	0.543
Diarrhea index	1.33 ± 0.125^{b}	2.27 ± 0.134^{a}	0.001

PSO = peroxidized soybean oil; G:F = gain to feed ratio.
Values are represented by mean ± SEM. Different superscript letters within a row denote significant differences ($P < 0.05$).
[1] The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

Table 4
Effects of PSO on the blood biochemistry.

Item	Treatments[1]		*P*-value
	Control diet	PSO diet	
TP, g/L	55.3 ± 0.327	55.01 ± 0.692	0.405
ALT, U/L	63.24 ± 3.692^{a}	44.68 ± 2.471^{b}	0.001
AST, U/L	58.25 ± 4.366	65.63 ± 13.388	0.614
ALP, U/L	398.88 ± 41.451	356.88 ± 25.685	0.404
LDH, U/L	544.57 ± 13.446^{b}	657.33 ± 48.719^{a}	0.036
BUN, mmol/L	2.41 ± 0.213^{a}	0.86 ± 0.155^{b}	0.001
TG, U/L	0.22 ± 0.022^{b}	0.49 ± 0.056^{a}	0.001
CHOL, mmol/L	0.58 ± 0.053^{b}	1.98 ± 0.112^{a}	0.001
LDL-C3, mmol/L	0.27 ± 0.052^{b}	1.13 ± 0.072^{a}	0.001
HDL-C4, mmol/L	0.29 ± 0.022^{b}	1.06 ± 0.080^{a}	0.001
IgM, g/L	0.36 ± 0.013	0.32 ± 0.011	0.063

PSO = peroxidized soybean oil; TP = total protein; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; BUN = blood urea nitrogen; TG = triglyceride; CHOL = cholesterol; LDL-C3 = low-density lipoprotein cholesterol ; HDL-C4 = high-density lipoprotein cholesterol ; IgM = immunoglobulin M.
Values are represented by mean ± SEM. Different superscript letters within a row denote significant differences (*P*-value <0.05).
[1] The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

3.6. Effects of PSO on liver and intestinal oxidative capability

Figure 2 illustrates the variations in oxidative activities in liver, duodenum, jejunum, ileum, and colon. For MDA activity, there was an increase observed in both the duodenum and jejunum in the PSO group ($P < 0.05$; Fig. 2A). For the activity of carbonylated proteins, an increase was noted in the duodenum of the PSO group ($P = 0.003$; Fig. 2B). TBARS activity in the PSO group increased in the duodenum and decreased in the liver ($P < 0.05$; Fig. 2C). F2-isoprostane activity was higher in the PSO group compared to the control group ($P = 0.032$; Fig. 2D).

3.7. Effect of PSO on liver and intestinal immune responses

Figure 3 presents the impact of the PSO diet on immune responses in the liver, duodenum, jejunum, ileum, and colon. IL-1β concentration in the PSO group saw an increase in both the duodenum and colon compared to the control group ($P < 0.05$; Fig. 3A). However, TNF-α concentrations did not show a difference between the control and PSO groups ($P > 0.05$; Fig. 3B). In the jejunum, the concentration of IL-10 was higher in the PSO group than in the control group ($P = 0.010$; Fig. 3C). A notable decrease in IL-4 concentration was observed in the duodenum of the PSO group ($P = 0.002$; Fig. 3D).

Table 5
Effects of PSO on serum antioxidant and oxidative capability.

Item	Treatments[1]		*P*-value
	Control diet	PSO diet	
MDA, nmol/mL	3.26 ± 0.702	3.18 ± 0.301	0.935
Cortisol, ng/mL	25.25 ± 7.174	31.49 ± 3.520	0.450
SOD, U/mL	52.91 ± 12.910	60.53 ± 8.814	0.635

PSO = peroxidized soybean oil; MDA = malondialdehyde; SOD = superoxide dismutase.
Values are represented by mean ± SEM.
[1] The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

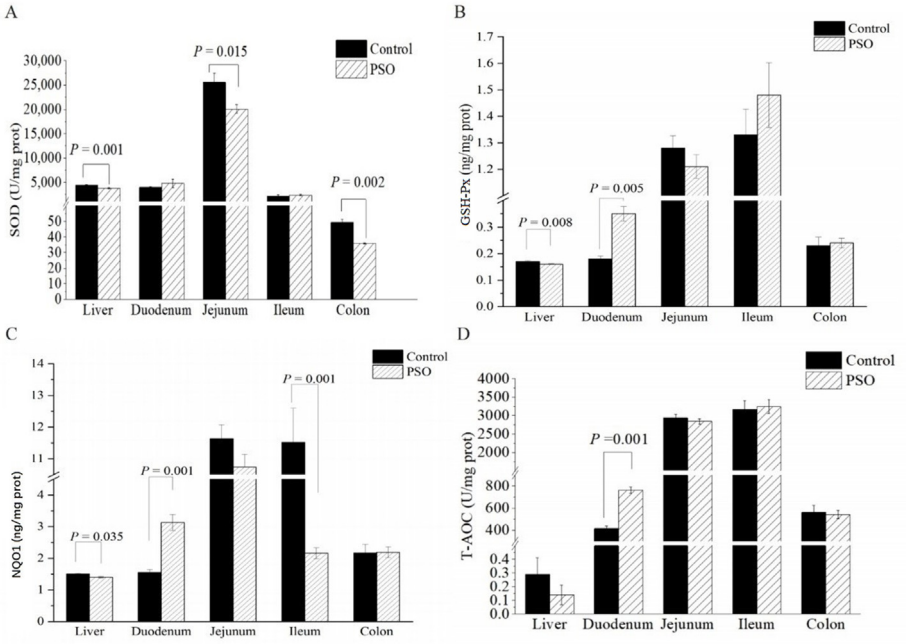

Fig. 1. Effects of PSO on liver and intestinal antioxidant capability. (A) Superoxide dismutase (SOD), (B) glutathione peroxidase (GSH-Gx), (C) NAD(P)H:quinone oxidoreductase 1(NQO1) and (D) total antioxidant activity (T-AOC) values in the liver, duodenum, ileum, colon, and jejunum. PSO = peroxidized soybean oil. Values are represented by mean and SEM. The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

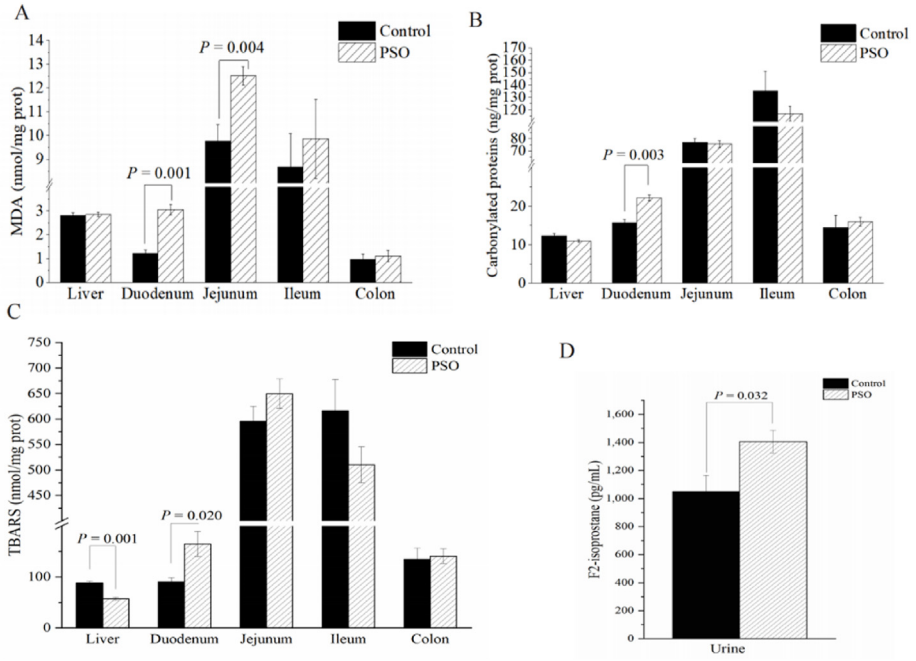

Fig. 2. Effects of PSO on liver and intestinal oxidative capability. (A) Malondialdehyde (MDA), (B) carbonylated proteins, (C) thiobarbituric acid reactive substances (TBARS) values in the liver, duodenum, ileum, colon, and jejunum, and (D) F2-isoprostane activity in urine. PSO = peroxidized soybean oil. Values are represented by mean and SEM. The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

3.8. Effect of PSO on intestinal histomorphology

Figure 4 illustrates the histomorphology of the small intestine through light micrographs. In the PSO group, there was a significant increase in ileal crypt depth compared to the control group ($P = 0.001$; Fig. 4B). The ileal V:C in the PSO group decreased ($P = 0.004$; Fig. 4B). There were no differences in villus height between the control group and the PSO group ($P > 0.05$; Fig. 4B).

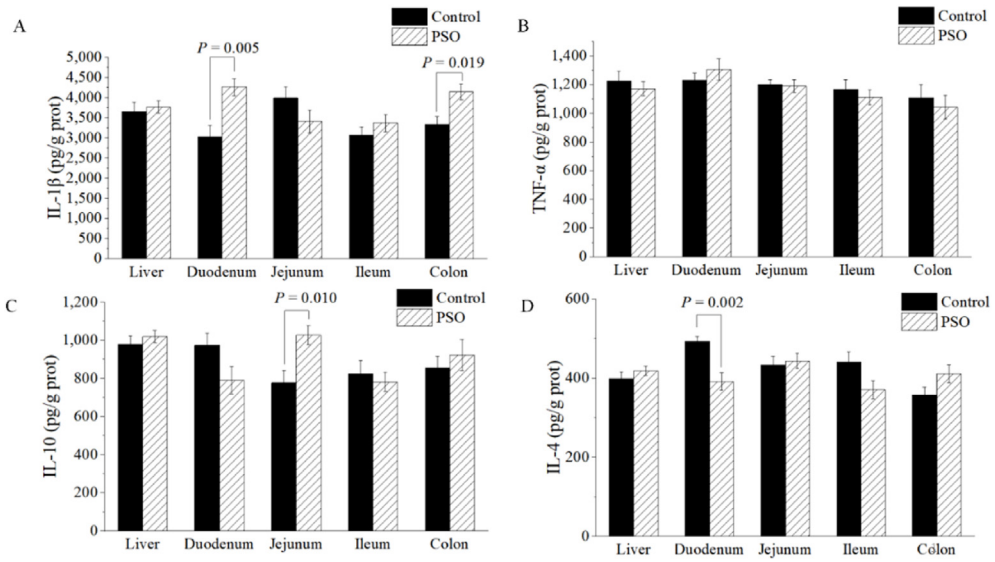

Fig. 3. Effects of PSO on serum, liver and intestinal immune responses. (A, B) The indices of pro-inflammatory factors and (C, D) anti-inflammatory factors. IL-1β = interleukin-1β; TNF-α = tumor necrosis factor-alpha; IL-4 = interleukin-4; IL-10 = interleukin-10; PSO = peroxidized soybean oil. Values are represented by mean and SEM. The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

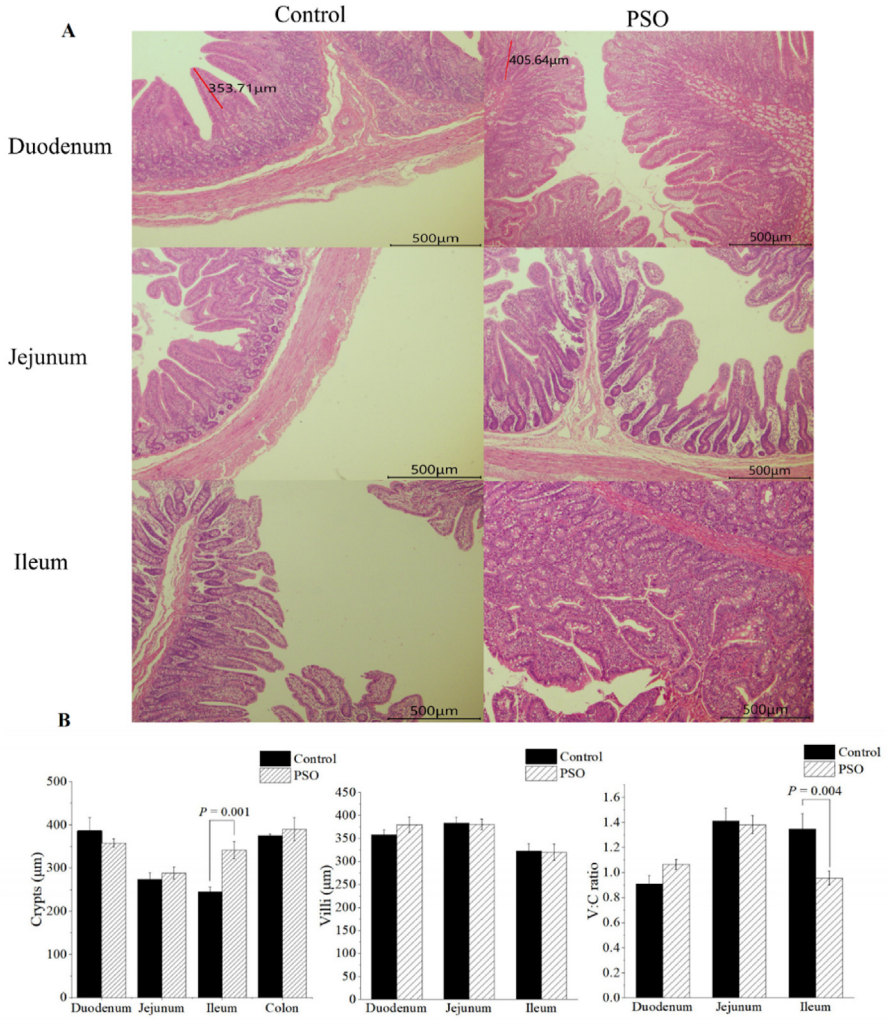

Fig. 4. Effects of PSO on the intestinal histomorphology. Values are represented by mean and SEM. (A) Hematoxylin and eosin (H&E) staining of crypt and villus. (B) Data of crypt depth, villus height and V:C ratio across different intestinal section. PSO = peroxidized soybean oil; V:C ratio = crypt-to-villus ratio. The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

3.9. Diversity changes in gut microbiota

Sequencing data quality assessment and rarefaction curves indicated that the sequencing results could reflect the microbial diversity and bacterial community information in the samples (Fig. S1 and Table S2). No significant differences were observed in the alpha-diversity indices (ACE, Chao1, PD-whole-tree, Shannon, and Simpson) between the control group and the PSO group ($P > 0.05$; Fig. 5A to E).

3.10. Effects of PSO on gut microbiota beta-diversity composition

Microbial community analysis depicted in Fig. 6 demonstrated distinct separations in microbiota structure between the control and PSO groups, as evidenced by beta-diversity analyses using both PCoA and NMDS (Fig. 6A and B). These graphical representations indicate a clear divergence in microbial populations attributable to the dietary interventions. The PERMANOVA (Adonis) statistical test reinforced this observation, confirming differences between the two groups ($P = 0.019$; Fig. 6C). Firmicutes and Proteobacteria were found to be the most prevalent phyla, collectively forming a substantial portion of the microbial composition, with Firmicutes being the more dominant in both groups (Fig. 6D). This dominance is reflective of the gut microbiota's typical structure in swine, where these phyla often predominate. The genus-level analysis revealed a shift in microbial diversity between the control and PSO groups. The PSO group was characterized by a higher relative abundance of genera such as *Prevotella* and *Limosilactobacillus*, among others, which are indicative of a response to the PSO diet. In contrast, the control group's microbiota was marked by a diverse array of genera including *Actinobacillus* and *Campylobacter*, pointing towards a more varied microbial ecosystem when fed a standard diet (Fig. 6E).

3.11. Effects of PSO on gut microbial t-test and LEfSe analysis

The PSO diet induced alterations in the microbial composition at the phylum level. Specifically, there was a decrease in the relative abundance of Deferribacterota and Actinobacteriota in the PSO group as compared to the control group ($P < 0.05$; Fig. 7A). At the genus level, there was an increase in the relative abundance of *Clostridium_sensu_stricto_6*, *Terrisporobacter*, and *Intestinibacter* in the PSO group ($P < 0.05$; Fig. 7B). Conversely, the control group maintained higher abundances of several genera, including *Blautia*, *Enterobacter*, and members of the *Erysipelotrichaceae* family ($P < 0.05$; Fig. 7B). The LEfSe analysis (Fig. 7C and D) identified two microflorae, specifically *g_Clostridium_sensu_stricto_6* and *s_Clostridium_bornimense*, that were more abundant in the PSO group. The LDA scores indicate these genera are statistically significant and potentially relevant biomarkers of the PSO group's microbial community. The cladogram visually represents the taxonomic levels from phylum down to genus or species, highlighting where significant differences are detected. The branches and nodes are marked to show the taxa that are significantly enriched in the PSO group, with *g_Clostridium_sensu_stricto_6* and *s_Clostridium_bornimense* standing out as key differentiators from the control group.

3.12. Effect of PSO on gut microbiota metabolic phenotypes at the genus level

A metabolic phenotype analysis of the gut microbiota was conducted using Bug base (Fig. 8). In the PSO group, the relative abundance of *anaerobic*, *facultatively_anaerobic*, *forms_biofilms* and *gram_positive* were higher compared to the control group. Conversely, the relative abundance of *aerobic*,

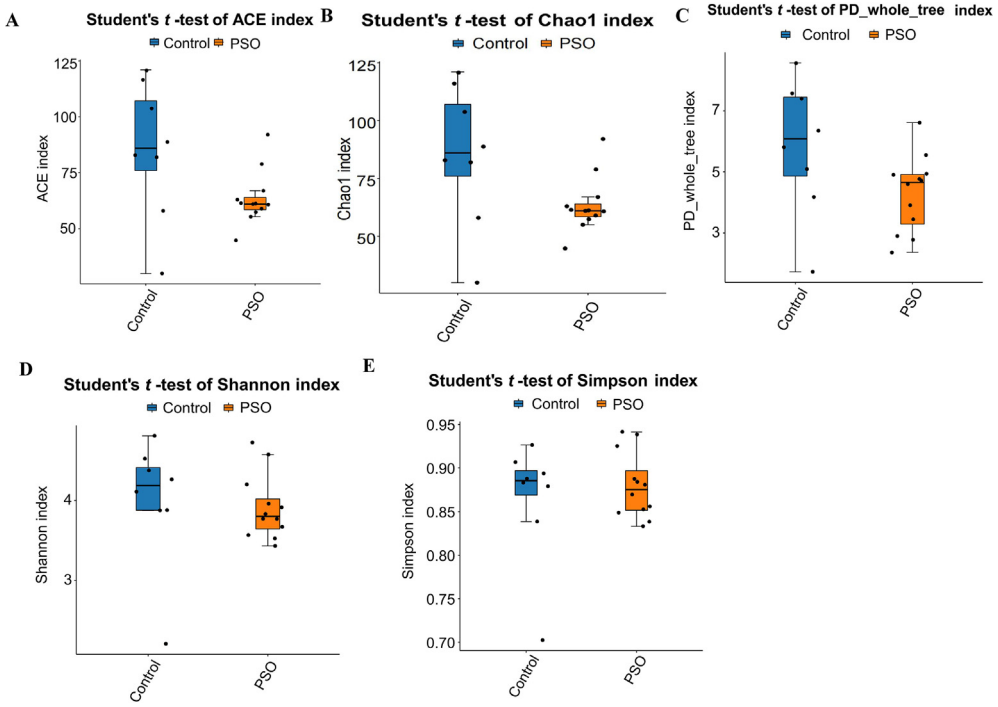

Fig. 5. Effects of PSO on alpha diversity in gut microbiota. (A–E) Indices for alpha diversity and richness were evaluated. After assessing normality with the Shapiro–Wilk test, either paired *t*-tests or independent t-tests were applied. Control = fresh soybean oil; PSO = peroxidized soybean oil. The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

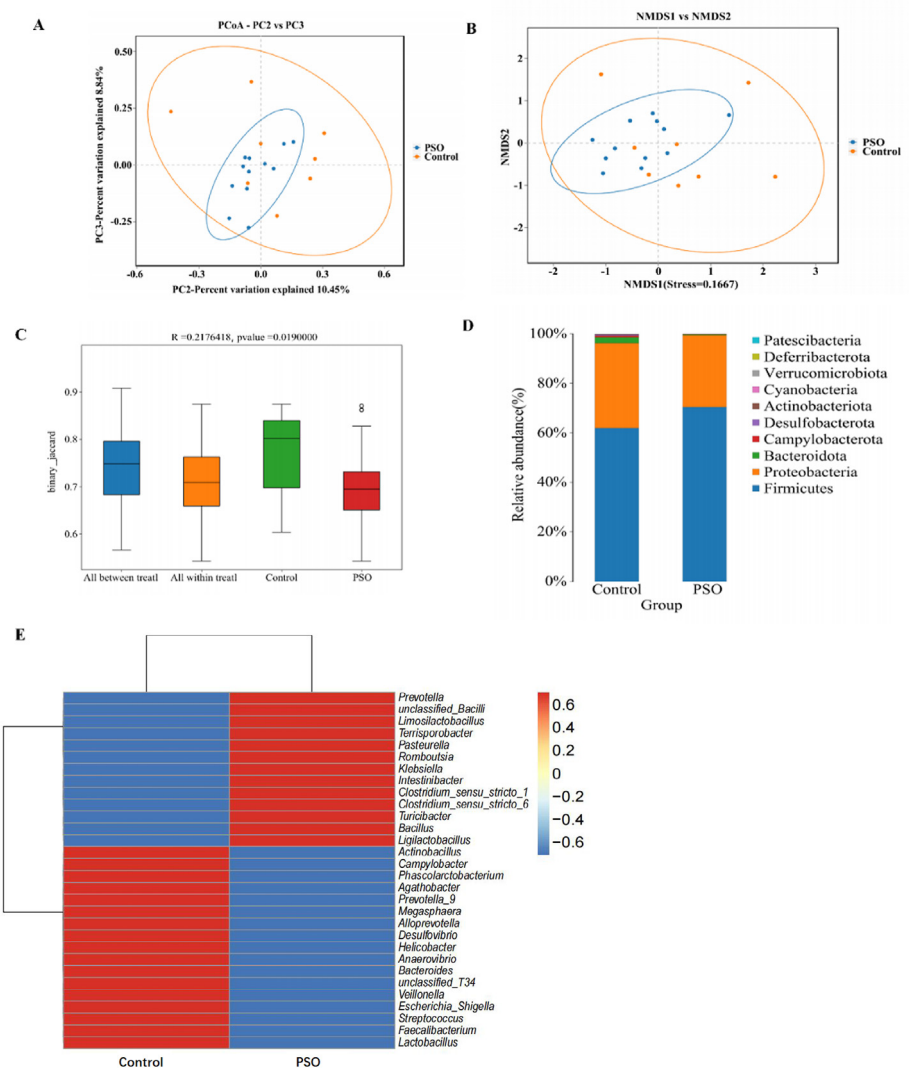

Fig. 6. Effects of PSO on gut microbiota beta-diversity composition. (A, B) Principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS) showing the microbial community structure in the ileum. (C) PERMANOVA indicates significant differences in microbiota at beta diversity. R = analysis of similarities. (D) Taxa distribution shows the relative abundance at the phylum level. (E) The heatmap depicts the relative abundance at the genus level. PSO = peroxidized soybean oil. The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

contains_mobile_elements, *gram_negative*, *potentially_pathogenic* and *stress tolerant* was lower.

4. Discussion

The formation of lipid peroxidation products is contingent upon the duration and intensity of heat treatment, as well as the presence of oxygen (St. Angelo et al., 1996). In the context of this investigation, fresh soybean oil subjected to a heating process at 90 °C for 120 h exhibited a peroxide value of 240, markedly higher than the peroxide value of 2.82 in unprocessed fresh soybean oil. Consistent with previous studies (Kerr et al., 2020; Lindblom et al., 2018; Overholt et al., 2018; Shurson et al., 2015), 2,4-decadienal was the most abundant aldehyde, followed by 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde in the aldehyde compounds we analyzed. The oxidized oil likely led to the depletion of beneficial nutrients such as vitamin E (Hanson et al., 2016). This study also observed a decrease in vitamin E levels in the oxidized oil to below 0.120 mg/100 g.

The detrimental impact of peroxidized lipids on animal growth performance is well-established. Pigs fed peroxidized lipids decreased ADFI (approximately 5%) and ADG approximately 6%) compared to those on un-peroxidized lipid diets (Hung et al., 2017). However, no significant effects on G:F were observed in nursery pigs fed peroxidized white grease, finisher pigs provided a peroxidized corn oil diet (Boler et al., 2012) and nursery pigs subjected to PSO diets (Rosero et al., 2015). Hence, the responses to peroxidized lipid diets on animal growth performance remain a topic of debate. In this study, feeding peroxidized lipids slightly decreased ADFI and ADG, consistent with observations from previous studies (Kerr et al., 2020). However, Overholt et al. (2018) reported a significant decrease in ADG and G:F. Limited research exists on the impact of peroxidized lipids on diarrhea, with Kerr et al. (2020) reporting no detrimental effect on the fecal scores of pigs due to lipid source or peroxidation status (Kerr et al., 2020). However, this study found that the PSO group had significantly more severe diarrhea compared to the control group.

Research indicates that rats consuming diets enriched with oxidized lipids and their byproducts demonstrate hepatic impairment, manifested through elevated levels of serum or plasma total LDH, ALT, and AST (Abarikwu et al., 2018; Dhibi et al., 2011; Hamsi et al., 2015; Narayanankutty et al., 2022). Additionally, the intake of

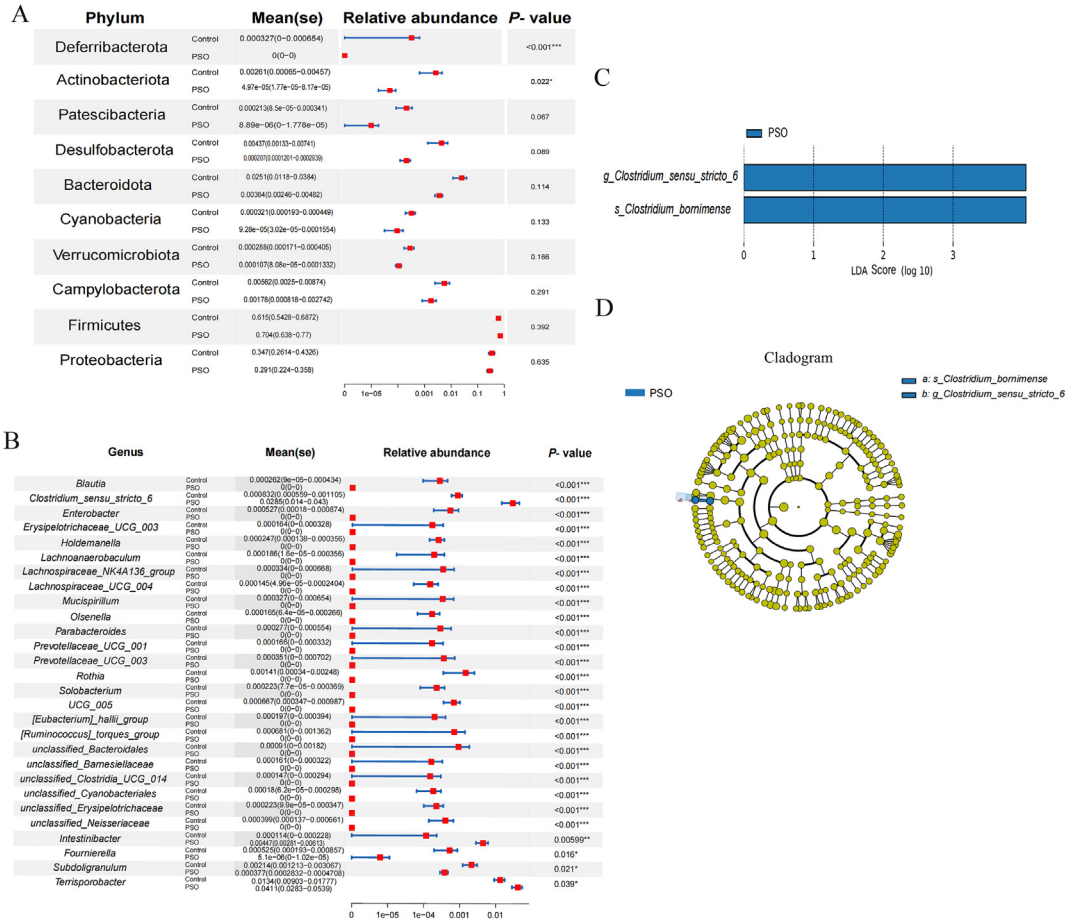

Fig. 7. Effects of PSO on gut microbial *t*-test and LEfSe analysis. (A) The *t*-test bar plot shows significant differences in microbiota at the phylum level. (B) The *t*-test bar plot shows significant differences in microbiota at the genus level. (C) Histogram of the linear discriminant analysis (LDA) scores, highlighting biomarker taxa with LDA scores >3. (D) Cladogram obtained from LEfSe analysis, indicating the phylogenetic distribution of microbiota. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. PSO = peroxidized soybean oil. The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

peroxidized fats has heightened levels of total cholesterol, triglycerides, and LDL-cholesterol in the blood (Chacko and Rajamohan, 2011; Zeb and Ullah, 2015). This study corroborates these findings, as peroxidized lipids increased the serum concentrations of LDH, TG, CHOL, LDL-C3 and LDL-C4, and decreased ALT and BUN. This phenomenon is presumably attributable to the presence of volatile and noxious compounds in oxidized oils that can compromise biological membranes (Aruna et al., 2005; Ashida et al., 1991; Hayam et al., 1995, 1997). A particularly noteworthy result is the significant absorption and reactivity of α, β-unsaturated hydroxyaldehydes, such as 4-HNE, posing a potential threat to liver function (Grootveld et al., 1998; Kanazawa and Natake, 1986). The rise in ALT is non-specific, thus the observed decrease in ALT in this study may be related to different animals and oil sources, with a few studies also reporting a decrease in ALT following the consumption of peroxidized fats (Dhibi et al., 2011). The introduction of peroxidized lipids in diets has been implicated in the stimulation of oxidative stress (Kode et al., 2004; Penumathsa et al., 2006). This phenomenon is characterized by elevated hepatic levels of MDA and TBARS (Acikgoz et al., 2011; Lindblom et al., 2018; Lu et al., 2014; Narayanankutty et al., 2022), coupled with diminished activities of antioxidant enzymes such as SOD, CAT, and GPx (Dhibi et al., 2011). In alignment with these findings, our study observed a substantial decrease in the activities of SOD and GSH-GPX in the liver, alongside a significant increase in the concentrations of MDA and SOD.

Peroxidized lipids damage the intestinal epithelial mucosa (Rosero et al., 2015) and induce oxidative stress in the gut. This phenomenon is characterized by a reduction in the activities of SOD and T-AOC (Chang et al., 2019; Kerr et al., 2020; Ringseis et al., 2007; Silva-Guillen et al., 2020) and an increase in MDA, carbonylated proteins, and TBARS in the intestine (Acikgoz et al., 2011; Lindblom et al., 2018; Ringseis et al., 2007; Yuan et al., 2007). The current research similarly found that peroxidized lipids markedly elevated the concentrations of SOD and MDA, and decreased NQO1 in the intestine. Additionally, this study also revealed that GSH-GPX, NQO1 and T-AOC activities in the PSO group were significantly increased in the duodenum (Konieczka et al., 2018). This variation may be attributed to the different segments of the intestine. For instance, the jejunum is more likely to experience DNA damage in epithelial cells than the duodenum (Konieczka et al., 2018). This may be attributed to the considerably shorter transit time of digesta through the duodenum compared to the jejunum, leading to a reduced contact duration between peroxidized lipids and the duodenal environment. Unlike the jejunum and ileum, the duodenum may have a high capability of detoxifying hydroperoxides, as the consumption of peroxidized lipids did not disrupt the duodenal GSH/GSSG balance. This suggests that the duodenum may have strong redox capabilities. However, in the duodenum, MDA, carbonylated proteins and TBARS activities also increased in the PSO group. This may be due to the fact that the redox capacity and oxidative stress in the duodenum are regulated by distinct

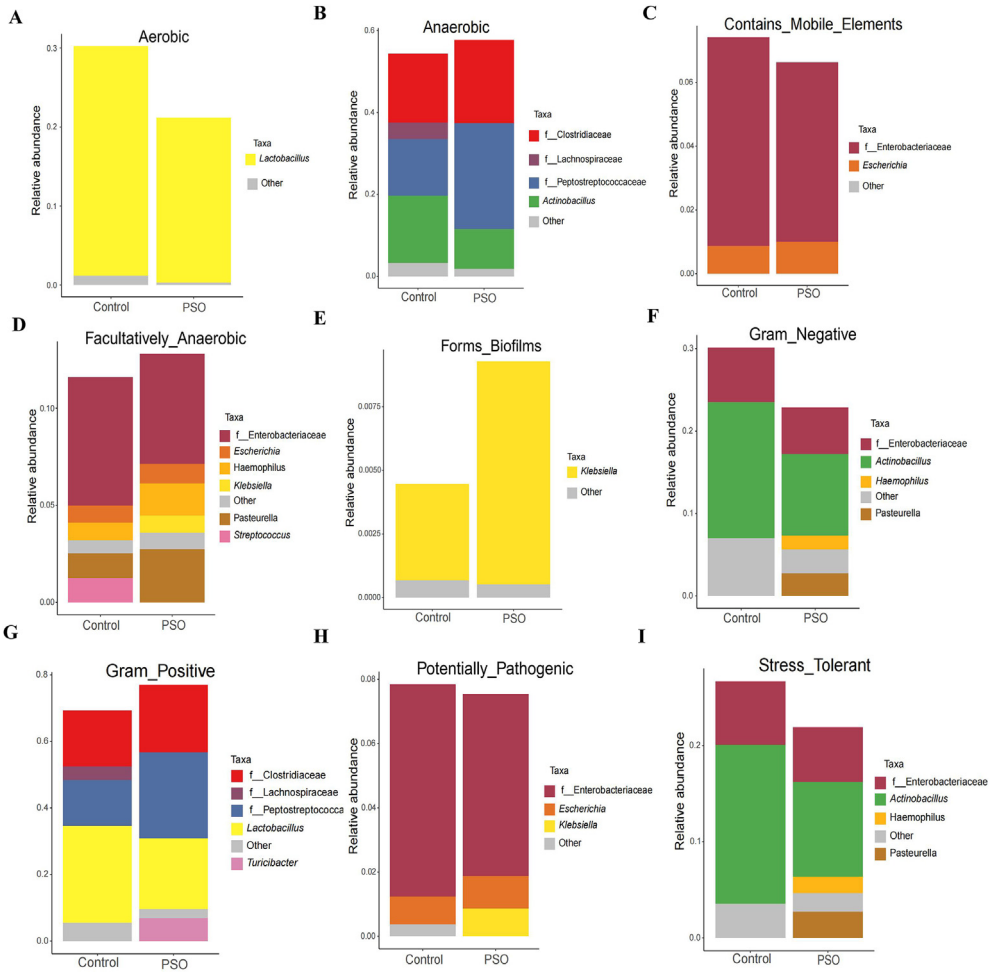

Fig. 8. Effects of PSO on gut microbiota metabolic phenotypes at the genus level. (A–I) Bug base analysis revealed various indices related to microbial phenotypes. The Wilcoxon signed-rank test or Mann–Whitney U-test was used for statistical comparisons. PSO = peroxidized soybean oil. The control group was fed 6% fresh soybean oil and the PSO group fed 6% PSO.

pathways. NAD(P)H:quinone oxidoreductase 1 and other antioxidant enzymes are induced through the activation of the Nrf2 Keap1 pathway (Piotrowska et al., 2021). F2-isoprostane, specific products resulting from lipid peroxidation, known for their stability and reliability in urine (Montuschi et al., 2004), significantly increased in the PSO group in this study. These findings support that peroxidized lipids dietarily induce oxidative stress, as reflected in parameters assessing oxidative status. In the evaluation of oxidative stress biomarkers, the SEM for GSH, QNO1, MDA and carbonylated proteins in certain tissues was observed to be less than 5%. This consistency aligns with findings from a wide range of studies across different regions (Ahasan et al., 2019; Cervantes et al., 2024; Chedea et al., 2019; Chen et al., 2020; Deng et al., 2020; Liu et al., 2021; Marin et al., 2018; Martin et al., 2011; Schwarz et al., 2017; Shi et al., 2017; Sierzant et al., 2019; Su et al., 2018; Yin et al., 2019; Zheng et al., 2013). This stability may be attributed the fact that the experiment primarily focused on tissue biomarkers rather than those in blood. Blood biomarkers are subject to rapid degradation due to their short half-lives once cytokines are secreted. Conversely, biomarker measurements in tissues tend to be more stable (Tarrant, 2010).

Two proinflammatory factors were assessed, revealing an increase in IL-1β concentration in both the duodenum and colon of the PSO group. In contrast, no effects on TNF-α concentration were observed in the examined intestinal segments. A previous study also reported that peroxidized lipids did not increase TNF-α concentration in the jejunum (Rosero et al., 2015). In the jejunum, there was a significant increase in the anti-inflammatory factor IL-10, while in the control group, a significant increase in IL-4 was observed in the duodenum. Previous studies reported that peroxidized lipids induced oxidative stress in pigs without causing inflammation (Ringseis et al., 2007). However, this study revealed that peroxidized lipids induced inflammation and oxidative stress simultaneously. Polar compounds, such as 4-HNE and acrolein, have been identified as potential factors contributing to increased crypt depth and intestinal damage (Rosero et al., 2015; Zhang et al., 2019). Concentrations of 4-HNE ranging from 2 to 5 μM could induce cell proliferation and participate in regulating various cellular processes (Dalleau et al., 2013; Ji et al., 2016; Milkovic et al., 2015). Moreover, acrolein has been found to increase both crypt depth and villus height, disrupting the balance of intestinal epithelial homeostasis. Oxidized intestinal redox status has been linked to promote cell growth and proliferation (Tian et al., 2007), and peroxidized lipids have been reported to increase crypt depth and villus height (Rosero et al., 2015). In this study, crypt depth increased in the PSO group, while the V:C ratio in the ileum decreased. This damage may be attributed to the presence of secondary products, such as aldehydes, ketones, and alcohols, generated during the peroxidation process.

Evidence indicates a significant role of gut microbiota in the damage to gut histological structures due to oxidized oil. In the present study, feeding oxidized oil was found to decrease alpha diversity, as evidenced by decreased Chao1 and ACE values. This finding aligns with previous studies that showed deep-fried edible oil intake leads to a reduction in the Chao1 and ACE indices (Yan et al., 2020; Zhou et al., 2016), indicating a lower bacterial community richness in piglets from the oxidized oil group. In the current study, the dominant bacteria in both groups at phylum level were Firmicutes and Proteobacteria, which is in agreement with previous studies (Holman et al., 2017; Wang et al., 2019). Feeding peroxidized lipids has been observed to increase the relative abundance of Firmicutes and Proteobacteria, while decreasing that of Bacteroidetes, with no significant differences at the phylum level (Zhou et al., 2016; Zhu et al., 2021). Similar results were found in this study, as PSO led to an increase in Firmicutes and a decrease in Bacteroidetes. However, at the phylum level, significant changes were observed, with the PSO diet leading to a notable decrease in Deferribacterota and Actinobacteriota. Deferribacterota has been associated with the inhibition of the inflammatory response and shown to decrease in mice on a high-fat diet (Yu et al., 2023). Actinobacteriota, known for its potentially positive impacts on health (Gryaznova et al., 2022), tends to decrease in cases of ulcerative colitis. Previous studies on peroxidized lipids did not report findings related to Deferribacterota and Actinobacteriota, but they were observed in our study. This could be due to the specific animal breeds and oil sources we used. At the genus level, 13 dominant bacteria were observed in PSO group. Five species (*Clostridium_sensu_stricto_1, Clostridium_sensu_stricto_6, Prevotella, Pasteurella* and *Klebsiella)* with high abundance and prevalence are discussed further. Both *Clostridium_sensu_stricto_1* and *Clostridium_sensu_stricto_6* belong to the *Clostridium* genus and have the potential to cause diarrhea and ulcerative colitis (Leffler and Lamont, 2015; Sun et al., 2018). *Clostridium_sensu_stricto_1* is significantly associated with necrotizing enterocolitis (Yang et al., 2019). The abundance of *Clostridium_sensu_stricto_6* significantly increased in the piglet's intestines exposed to oxidative stress induced by PSO in previous study. *Pasteurella, Prevotella,* and *Klebsiella* are all associated with inflammation. *Pasteurella* is typically linked to chronic and acute infections in animals (Wilson and Ho, 2013). *Prevotella*, associated with autoimmune diseases and gut inflammation, has been found to reduce IL-18 production, potentially exacerbating intestinal inflammation (Iljazovic et al., 2021; Tett et al., 2021). *Klebsiella*, a pathogenic bacterium, increased IL-1β expression in the intestine, inducing colitis in mice and disrupting intestinal barriers (Hering et al., 2019). *Klebsiella* also increased the activities of MDA and 4-HNE in the colon, while reducing the concentration of glutathione and SOD and catalase activity. These dominant bacteria may have a potential connection to the exacerbation of diarrhea in the PSO group of this study.

5. Conclusion

Feeding peroxidized lipids affects piglets' production performance, intestinal microbiota, and histological characteristics in different ways. This study found that PSO did not significantly impact piglet production performance, but it could exacerbate diarrhea. Moreover, compared to the control group, PSO potentially damaged the liver, reducing its antioxidant capacity. The intake of peroxidized lipids potentially harms intestinal tissue structure, with PSO increasing ileal crypt depth and inducing intestinal oxidative stress and immune responses, primarily in the jejunum and ileum. More importantly, feeding peroxidized lipids reduced microbial richness and increased the relative abundance of harmful bacteria such as *Clostridium_sensu_stricto_1, Clostridium_sensu_stricto_6, Prevotella, Pasteurella and Klebsiella.*

CRediT authorship contribution statement

Mengxuan Tang: Data curation, Formal analysis, Software, Writing – original draft, Conceptualization, Methodology. **Yuliang Wu:** Data curation. **Chen Guang Olnood:** Data curation. **Yundi Gao:** Data curation. **Fei Wang:** Data curation. **Zicheng Zhang:** Data curation. **Can Peng:** Data curation. **Xihong Zhou:** Conceptualization, Supervision. **Chunxia Huang:** Data curation. **Xia Xiong:** Supervision, Writing – review & editing. **Yulong Yin:** Funding acquisition, Supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

This work was funded by National Natural Science Foundation of China (32130099).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.11.015.

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