



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Mechanisms of crude *Idesia polycarpa* oil in regulating metabolic syndrome via the gut-liver axis: a multidimensional functional analysis based on dietary fat source variations

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ABSTRACT: *Idesia polycarpa* crude oil (*I. polycarpa* crude oil), rich in linoleic acid, has long been consumed in southwestern China, but its health effects remain unclear. At the same time, the impact of low-fat/high-carbohydrate diets (LFD) on metabolic disorders is still debated. This study used GC-MS, 16S rRNA sequencing, metabolomics, and biochemical/histological analyses to compare *I. polycarpa* crude oil with olive, lard, rapeseed, and soybean oils in mice fed LFD or high-fat/low-carbohydrate diets (HFD). Mice consuming *I. polycarpa* crude oil showed more severe liver and intestinal injury than those fed olive oil, lard, or soybean oil, but milder than rapeseed oil, with effects dependent on diet. Under LFD, the *I. polycarpa* crude oil group (LID) exhibited higher *Lactobacillus* abundance (0.3126 vs. 0.0046, 0.0328, 0.0137 %), and Rumphellolide B (2.38 vs. 0.04, 0.07, 0.04) than olive, lard, and rapeseed oils, but not soybean oil. Under HFD, the *I. polycarpa* crude oil group (HID) showed markedly increased *Gastranaerophilales* abundance (5.01×10^{-5} vs. 0.0033, 0.0016, 0.0012, 0.0005 %) and vanillin (58.21 vs. 4.21, 4.13, 4.13, 2.90) compared with all other oils. The five dietary oils did not result in significant differences in antioxidant enzyme activity (SOD, GSH-Px), cytokine levels (IL-4, IL-6, TNF- α), or intestinal permeability between the LFD and HFD groups. Among the oils tested, beechwood crude oil induced milder symptoms compared with rapeseed oil, but more pronounced symptoms than olive oil, lard, and soybean oil. Although certain indicators did not differ significantly between the LFD and HFD groups, the overall health benefits of LFD were less favorable than commonly presumed.

Keywords: Gut microbiota; *Idesia polycarpa* crude oil; Metabolomics; High-fat diet; Low-fat diet.

1. Introduction

According to the latest WHO statistical report, the global population affected by obesity has surpassed the one billion marks. Demographic breakdown reveals 650 million adults constitute the largest proportion, followed by 340 million adolescents (aged 10-19) and 39 million children (aged 5-19). Epidemiological projection models indicate that if current growth patterns persist, distinct weight distribution trends will emerge among global adults by 2030: Clinically defined obesity is projected to affect one in five adults (20 %), while nearly four out of ten (38 %) will fall into the overweight category^[1, 2]. As a metabolic

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Received 10 April 2025

Received in revised form 31 May 2025

Accepted 11 October 2025

initiator of multisystem diseases, obesity is closely linked to diabetes, cardiovascular diseases, non-alcoholic fatty liver disease (NAFLD), chronic kidney disease, and male reproductive dysfunction^[3-7]. The traditional "positive energy balance" theory posits that chronic caloric surplus triggers pathological changes through adipose tissue hyperplasia and ectopic lipid deposition^[8, 9], a process modulated by multidimensional factors including genetic susceptibility, gut microbiota, environmental pollutants^[10-12], and dietary patterns^[13, 14]. Notably, even under isocaloric conditions, high-fat diets with distinct fatty acid profiles exhibit significant differential effects on obesity progression^[13, 14].

According to United Nations data, global agricultural output must increase by 75 % by 2050 to meet the demands of population growth^[15]. Although China sustains nearly 20 % of the world's population with less than 9 % of the planet's arable land, mounting pressure on food security has emerged alongside domestic dietary restructuring^[16]. Currently, constrained by limited arable land resources, China still relies heavily on soybean imports to support domestic soybean meal and oil production^[17]. Against this backdrop, Grain self-sufficiency (GSS) has become a critical concern globally, particularly in China^[18]. Thus, developing oil crops that are non-arable-land-dependent is of paramount importance.

Idesia polycarpa Maxim (*I. polycarpa*, Flacourtiaceae) demonstrates significant potential as a high-yield, high-efficiency oilseed crop. Its whole fruit oil content ranges from 26.26% to 43.6%^[19], with a yield per unit area of 2.25 to 3.75 tons per hectare, comparable to high-yield oil palm (2.84 tons per hectare), and significantly surpasses traditional oilseed crops such as soybeans (0.47 tons per hectare) and olives (0.32 tons per hectare)^[20, 21]. Compared to animal fat production, which requires three times the land resources of plant-based oilseed crops, *I. polycarpa* has a clear advantage in land efficiency. Additionally, *I. polycarpa* exhibits rapid growth (3–5 meters per year) and strong ecological adaptability (altitude range of 500–3,000 meters)^[22]. These high-yield, efficient, adaptable characteristics endow *I. polycarpa* with the potential to become a premium oilseed resource.

Epidemiological studies confirm that reducing saturated fatty acid (SFA) intake and optimizing diets toward polyunsaturated fatty acid (PUFA) dominance effectively lowers coronary heart disease risk^[23, 24], driving a global shift toward plant-derived PUFA in oil consumption^[25, 26]. The lipid composition of *I. polycarpa* crude oil is highly nutritious and functional. Its core advantage lies in its extremely high content of PUFAs, accounting for 64.01% to 71.72%^[27]. Among these, the content of linoleic acid, a key metabolic regulator, is particularly notable, reaching 63.07% to 70.69%, significantly exceeding that of soybean oil (55.33%) and olive oil (12.48%)^[19, 27]. Further research indicates that *I. polycarpa* crude oil not only contains conjugated linoleic acid (CLA), which regulates lipid metabolism and has anti-inflammatory and anti-cancer properties, but is also rich in natural antioxidants such as tocopherols and phytosterols^[27-31]. These functional components, combined with the high PUFA content, create a unique "nutritional-functional" synergistic effect, significantly enhancing its health benefits. Additionally, as a traditional edible oil in China, its long-term edibility safety has been confirmed through toxicological assessments^[32, 33].

Currently, research on the differences between *I. polycarpa* crude oil and other edible oils has primarily focused on comparative analyses of fatty acid composition and bioactive components^[27]. However, little is known about the metabolic processes of *I. polycarpa* crude oil in the body, its direct effects on gut microbiota structure, and its unique role in lipid metabolism. For decades, high-fat diets were widely regarded as the most critical factor contributing to metabolic diseases. However, a growing body of research now demonstrates that low-fat/high-carbohydrate diet is also a major contributing factor to metabolic disorders^[34]. However, this debate has persisted because low-fat diet, which is traditional weight-loss diet, typically require that fat or saturated fat intake account for less than 30% or 10% of total energy intake^[35].

To comprehensively address two issues, this study innovatively established a dietary intervention model based on five types of oils (*I. polycarpa* crude oil, olive oil, rapeseed oil, soybean oil, and lard) and two fat levels (low-fat and high-fat), and conducted a systematic study using C57BL/6J mice. By integrating multi-dimensional analyses of hepatic lipid deposition, serum lipoprotein homeostasis, small intestinal mucosal barrier integrity, gut microbiota diversity, and metabolomics, this study aims to address two core scientific questions: (1) whether *I. polycarpa* crude oil has health advantages compared to olive oil, lard, rapeseed oil, and soybean oil under low-fat or high-fat dietary patterns; and (2) under experimental conditions with the five types of dietary oils held constant, this study systematically compared the effects of low-fat, high-carbohydrate and high-fat, low-carbohydrate dietary patterns on multiple health metrics, including liver health, body weight, visceral fat, four blood lipid parameters, oxidative stress, inflammatory factors, gut microbiota, and metabolite composition. Without employing complex modeling, we directly assessed the significance of differences in these metrics between the two dietary regimes, aiming to objectively evaluate similarities and differences in their metabolic effects. Based on these findings, we will make further improvements with the aim of developing *I. polycarpa* crude oil into a safe and high-quality new source of edible oil, providing a feasible solution to future food security challenges.

In this study, neither the high-fat low-carbohydrate diet nor the low-fat high-carbohydrate diet was assigned a specific control diet. The primary objective was to compare the effects of *I. polycarpa* crude oil with those of other edible oils, rather than benchmarking against a single traditional “control oil.” Although both dietary patterns represent conventional high-fat and low-fat models, respectively, the experiment was not designed to simulate disease models but instead consistently focused on evaluating the comparative effects of *I. polycarpa* crude oil relative to other edible oils.

2. Materials and Methods

2.1 Materials

I. polycarpa Maxim fruits were purchased from Jianshi County, Enshi City, Hubei Province, China. Dietary oils including olive oil, rapeseed oil, lard, and soybean oil were provided by Trophic Animal Feed High-tech Co., Ltd. (Nantong, Jiangsu, China). Methanol, formic acid, and ammonium acetate were purchased from Thermo Fisher Scientific (Thermo Fisher, USA). Xylene was purchased from Tianjin Fuyu

Fine Chemical Co., Ltd. (Tianjin, China). Other analytical pure reagents were purchased from Aladdin (Shanghai, China).

2.2 *I. polycarpa* crude oil Extraction

Fresh *I. polycarpa* fruits were selected and defective specimens were removed. The fruits were placed in a heat pump drying oven (Boxun Medical Biological Instrument Corp, Shanghai, China) at 55 °C until reaching constant weight. Subsequently, the dried fruits were destoned, and crude oil was extracted using a mechanical oil press.

The extracted oil was centrifuged at 5000 rpm for 20 minutes (centrifuge: Heal Force Bio-Meditech Holdings Ltd, Shanghai, China) to obtain purified oil.

2.3 Animal Models and Experimental Design

This study utilized 100 male C57BL/6J mice (6-week-old, 17 ± 1 g body weight) were purchased from Henan Sukeibisi Biological Technology Co., Ltd. (Certification Nos. SCXK(YU)2020-0005 and SCXK(YU)2024-0001). The mice were housed in an SPF-grade animal facility at Enshi Central Hospital Animal Experiment Center (Facility License SYXK(E)2023-0138) under controlled conditions (25 ± 2 °C, 55 ± 5 % humidity, 12-hour light-dark cycle). The protocol was approved by the Institutional Animal Care and Use Committee (IACUC-202406002), after a 1-week acclimation period, the mice were randomly divided into 10 groups (n=10 per group): five low-fat, high-carbohydrate diet subgroups (LFD, 10 % fat, 3.6 kcal/g, TP23402) and five high-fat, low-carbohydrate diet subgroups (HFD, 60 % fat, 5.1 kcal/g, TP23400), with each subgroup receiving exclusive dietary lipids from *I. polycarpa* crude oil, olive oil, lard, rapeseed oil, or soybean oil (TROPIC Animal Feed High-Tech Co., Ltd., Nantong, China). Over 8 weeks, daily food intake and weekly body weight were monitored, with oral glucose tolerance test (OGTT) conducted in week 7. Terminal procedures included D-xylose gavage followed by euthanasia via intraperitoneal Tribromoethanol injection 1 hour later. Serum samples were stored at -80 °C, while visceral fat and liver tissues were collected for cryopreservation and HE staining. Body composition analysis encompassed body weight, length, and organ measurements, with detailed dietary components provided in Table 1.

Table 1. Feed composition

Compound Name	High-fat diet (TP 23400)	Low-fat feed (TP 23402)
Protein (casein, L-cysteine)	201	
Protein (casein, L-cysteine)		141
Carbohydrates (dextrin, sucrose)	320	
Carbohydrates (dextrin, sucrose, corn starch)		722
Fat (<i>I. polycarpa</i> crude oil, Olive Oil, Lard, Rapeseed Oil, Soybean Oil)	341	
Fat (<i>I. polycarpa</i> crude oil, Olive Oil, Lard, Rapeseed Oil, Soybean Oil)		40
Fiber (Cellulose)	71	50
Minerals (Mineral Mix, M1021) and Vitamins (Vitamin Mix, V1010)	67	47
Antioxidant (TBHQ)	0.066	0.008
Total	1000	1000
Energy, kcal/g	5.1	3.6

Protein energy source (%kcal)	14 %	14 %
Carbohydrate energy source (%kcal)	26 %	76 %
Fat energy source (%kcal)	60 %	10 %
Total	100 %	100 %

Note: This data is provided by TROPIC Feed Technology Co., Ltd.

2.4 Composition of fatty acids in *I. polycarpa* crude oil, olive oil, lard, rapeseed oil, and soybean oil

The fatty acid composition of *I. polycarpa* crude oil, olive oil, lard, rapeseed oil, and soybean oil were analyzed using gas chromatography-mass spectrometry (GC-MS). Fatty acid percentages were quantified via chromatographic peak integration using a normalization method. Briefly, 100 μ L of each oil was dissolved in 2 mL n-hexane, mixed with 1 mL 1 mol/L KOH-methanol solution, and reacted at 60 °C for 40 min. After cooling, 3 mL saturated NaCl solution was added, followed by centrifugation (3500 r/min, 10 min). The n-hexane layer was filtered through a 0.45 μ m organic membrane for GC analysis. Chromatographic conditions included an Agilent 123-5533 DB-5MS column (30 m $\times$ 320 μ m $\times$ 1 μ m; Agilent, USA) with an injector temperature of 250 °C and flow rate of 1.0 mL/min. The temperature program was: initial 80 °C (3 min), ramp at 8 °C/min to 150 °C (7 min), then 10 °C/min to 240 °C (10 min final hold), as described previously^[36].

2.5 Serum Parameter Analysis

Serum biochemical parameters (TC, TG, HDL-C, LDL-C) were analyzed using a Mindray BS-240VET fully automated biochemical analyzer (Mindray, Shenzhen, China). Oral glucose tolerance test (OGTT) performed according to Zhang et al^[37], where mice were fasted for 12 h and gavaged with 40 % glucose solution (2 g/kg, LEAGENE, Beijing, China) at the end of week 7, with blood glucose levels measured via tail vein sampling (Roche glucometer, Roche, Switzerland) at 0, 15, 30-, 45-, 60-, and 120-minutes post-administration; Serum insulin levels quantified using ELISA kits (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China). Intestinal absorption capacity was evaluated using a modified protocol^[38]: After 12-hour fasting, mice received 8 % D-xylose solution (800 mg/kg, Aladdin) by gavage, followed by serum collection 1 hour later for D-xylose concentration determination using Jiancheng Bioengineering detection kits.

2.6 Biochemical and Inflammatory Marker Analyses

Oxidative Stress Markers: Approximately 0.05 g of liver tissue was homogenized in ice-cold extraction buffer (kit-provided) using a pre-chilled mortar. Following homogenization, the lysate was centrifuged at 10,000 rpm for 10 min (4 °C), and the supernatant was maintained on ice for subsequent assays. Malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) levels were quantified using commercial ELISA kits (Michy Biomedical Technology Co., Ltd., Suzhou, China) according to manufacturer protocols, with absorbance measured on an Infinite M200 Pro microplate reader (TECAN, Switzerland).

Inflammatory Cytokines: Precisely 0.05 g ($\pm$ 0.001 g) of liver tissue was transferred to a 2 mL grinding tube containing grinding beads. After adding 450 μ L saline, samples were homogenized (60 Hz, 60

sec/cycle, 4 cycles) at -20°C , followed by centrifugation at 5,000 rpm for 10 min. The supernatant was collected under ice-cooled conditions. Interleukin-4 (IL-4), IL-6, IL-10, IL-1 β , and tumor necrosis factor- α (TNF- α) levels were determined using ELISA kits (DUMABIO Co., Ltd., Shanghai) with measurements performed on the Infinite M200 Pro system (TECAN).

2.7 Histological Analysis

Liver and small intestinal tissues were fixed in 4 % paraformaldehyde (PFA) for histological analysis. For hematoxylin and eosin (H&E) staining, tissues were dehydrated in a graded ethanol series, paraffin-embedded, and sectioned at 4 μm thickness. Sections were dewaxed by heating (60°C , 1 h), rehydrated, stained with hematoxylin (Solarbio Co., Ltd., Beijing, China) and eosin, dehydrated via ethanol-xylene gradients, and mounted with neutral balsam. For lipid visualization, Oil Red O staining was performed by fixing tissues in 4 % PFA, incubating in 60 % isopropanol (3 min), staining with Oil Red O (Sigma-Aldrich, USA; 15 min), differentiating in 60 % isopropanol, and counterstaining nuclei with hematoxylin (20 sec). Stained sections were imaged at 200 $\times$ magnification using an Olympus BX53 microscope, and lipid deposition/histopathology were quantified using Aperio ImageScope software (v12.4.6). NASH^[39] was assessed using the NASH Clinical Research Network histological scoring system. The NAS is an unweighted composite score based on the degree of steatosis (0-3 points), lobular inflammation (0-3 points), and hepatocellular ballooning (0-2 points). In this system, a NAS score of ≥ 5 is defined as the diagnostic threshold for NASH. Detailed scoring criteria are provided in Table S2.

2.8 Fecal DNA Extraction, PCR Amplification, Sequencing, and Data Analysis

The collected stool samples were sequenced and analyzed by Wekemo Tech Group Co., Ltd (Shenzhen, China). The data were analyzed on the online platform of Wekemo Bioincloud

(<https://www.bioincloud.tech>). Fecal DNA was extracted using the CTAB method (Noblerlyder Co., Beijing), with purity and concentration verified via 1 % agarose gel electrophoresis. The V3–V4 regions of the 16S rRNA gene were amplified using primers 341F/806R, purified (Tiangen Biotech Universal DNA Recovery Kit), and sequenced on the Illumina NovaSeq 6000 platform. Raw reads were processed in QIIME2 (v1.22.0) using the DADA2 plugin for quality filtering, denoising, merging, and chimera removal to generate amplicon sequence variant (ASV) tables^[40]. Taxonomic assignment was performed against the SILVA database (v138, 97 % similarity) via the QIIME2 feature-classifier plugin^[41, 42], with contaminants removed using the feature-table plugin. Through the Wekemo Bioincloud online analysis platform (<https://www.bioincloud.tech>)^[43], we conducted Alpha diversity (observed ASVs., Chao1, Shannon, Simpson) and beta diversity (Bray-Curtis) metrics were calculated, followed by visualization via PCoA and NMDS. Supervised partial least squares discriminant analysis (PLS-DA) was conducted using the Wekemo Bioincloud online analysis platform to model microbiota-group relationships. Spearman correlations between microbial taxa and metabolic parameters were computed and visualized as heatmaps using Pheatmap (v1.0.12).

2.9 Untargeted Metabolomic Profiling of Gut Microbiota

Fecal metabolites were profiled using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) on a Q Exactive™ HF-X system (Thermo Fisher Scientific) equipped with a Hypersil Gold C18 column (100 × 2.1 mm, 1.9 μm). Chromatographic separation was achieved at 40 °C with a mobile phase gradient transitioning from 98 % aqueous (0.1 % formic acid) to 100 % methanol over 12 minutes at 0.2 mL/min, under the following gradient: 0–3 min (98 % A, 2 % B), 3–10 min (15 % A, 85 % B), 10–10.1 min (0 % A, 100 % B), and 10.1–12 min (98 % A, 2 % B). Mass spectra were acquired in positive/negative ionization modes (m/z 100–1500) using electrospray ionization (ESI) with optimized parameters (spray voltage: 3.5 kV; ion transfer tube: 320 °C; MS/MS data-dependent scanning). Raw data were processed via Wekemo Bioincloud online analysis platform (<https://www.bioincloud.tech>)^[43] for orthogonal partial least squares-discriminant analysis (OPLS-DA) to identify group-specific metabolic differences. Significantly altered metabolites ($VIP \geq 1$, $P < 0.05$) were annotated using the KEGG Compound Database and mapped to enriched pathways, elucidating diet-induced metabolic perturbations. The values presented in the text are the metabolite content of the peak area after "in-sample correction".

2.10 Statistical Analysis

All data were analyzed and visualized using OriginPro 2024 (OriginLab Corporation, Massachusetts, USA). Continuous variables are expressed as mean ± standard deviation (SD). Two-way ANOVA was employed to examine the main effects of oil type (Factor A), diet (Factor B), and their interaction. When a significant interaction was detected ($P < 0.05$), simple effects analyses were performed: differences in oil type were compared within each diet using one-way ANOVA followed by post hoc multiple comparisons, at the same time differences in diet were compared within each oil type using one-way ANOVA^[44]. If the interaction was not significant but a main effect was, Tukey's HSD test was applied for post hoc comparisons of that main effect. No post hoc analyses were conducted when neither main effects nor the interaction reached significance. Prior to ANOVA, data were tested for normality and homogeneity of variance; if these assumptions were violated, the Kruskal–Wallis test with Dunn's post hoc correction was used instead. Statistical significance was defined at a 95% confidence level ($P < 0.05$). Within-group differences are indicated by lowercase letters (a, b, c) for the low-fat, high-carbohydrate diet and uppercase letters (A, B, C) for the high-fat, low-carbohydrate diet. A “#” symbol denotes significant differences between LFD and HFD under the same oil treatment.

3. Result

3.1 Fatty Acid Composition of Dietary Oils

After analyzing the fatty acid composition of *I. polycarpa* crude oil, olive oil, lard, rapeseed oil, and soybean oil, it was observed that the fatty acid profile of *I. polycarpa* crude oil is more similar to that of olive oil than to the other three edible oils. However, some differences were noted: Elaidic acid was more abundant in olive oil (28.86 ± 6.57 %) ($P = 0.0148$), whereas *cis*-linoleic acid was higher in *I. polycarpa* crude oil (48.45 ± 2.87) ($P < 0.0001$). Notably, Elaidic acid was not detected in *I. polycarpa* crude oil, and

trans-linoleic acid was absent in olive oil. Erucic acid was not detected in either *I. polycarpa* crude oil or olive oil. In comparison with lard, *I. polycarpa* crude oil contained a higher content of *cis*-linoleic acid ($48.45\% \pm 2.87\%$) ($P < 0.0001$), while lard exhibited higher levels of Palmitic acid ($26.01\% \pm 0.67\%$) ($P = 0.0051$) and Oleic acid ($20.00 \pm 10.00\%$) ($P = 0.0047$). When compared to rapeseed oil, *I. polycarpa* crude oil also showed greater amounts of *cis*-linoleic acid ($48.45\% \pm 2.87\%$) ($P < 0.0001$) and Palmitic acid ($26.01 \pm 0.67\%$) ($P < 0.0001$), whereas rapeseed oil had higher concentrations of Oleic acid ($23.21\% \pm 0.40\%$) ($P = 0.0014$) and Erucic acid ($0.45 \pm 0.01\%$) ($P = 0.0293$). Relative to soybean oil, both oils were rich in *cis*-linoleic acid, but *I. polycarpa* crude oil contained more Palmitic acid and Palmitoleic acid. Notably, linoleic acid—a key polyunsaturated fatty acid—accounted for nearly 50% of the total fatty acids in both *I. polycarpa* crude oil and soybean oil, a proportion significantly higher than that in the other three oils (Table 2).

Table 2 Main composed of fatty acids from *Idesia polycarpa* crude oil, Olive oil, Lard, Rapeseed oil and Soybean oil

Serial number	Fatty acids	Abbreviation	Different edible oils				
			IPCO	OO	Lard	RO	SO
1	Pentadecanoic acid	C _{15:0}	0.11 ± 0.03^a	0.08 ± 0.07^a	0.07 ± 0.12^a	0.08 ± 0.00^a	0.11 ± 0.09^a
2	Palmitic acid	C _{16:0}	22.13 ± 1.93^b	19.8 ± 0.75^{bc}	26.01 ± 0.67^a	12.33 ± 0.21^d	18.83 ± 0.32^c
3	Palmitoleic acid	C _{16:1}	4.70 ± 1.78^a	2.36 ± 0.42^{ab}	2.03 ± 1.03^{ab}	1.24 ± 0.02^{ab}	0.64 ± 0.22^b
4	Stearic acid	C _{18:0}	3.28 ± 0.07^{ab}	3.69 ± 0.34^{ab}	12.15 ± 0.61^a	2.94 ± 0.05^b	4.39 ± 0.16^{ab}
5	Elaidic acid	C _{18:1n9t}	/	28.86 ± 6.57^a	12.64 ± 1.39^{ab}	2.61 ± 0.05^{ab}	2.35 ± 2.23^{ab}
6	Oleic acid	C _{18:1n9c}	2.01 ± 0.59^c	10.11 ± 0.98^{bc}	20.00 ± 10.00^{ab}	23.21 ± 0.40^a	3.80 ± 1.39^c
7	<i>trans</i> -linoleic acid	C _{18:2n6t}	0.99 ± 0.40^a	/	/	1.08 ± 0.37^a	0.58 ± 0.01^{ab}
8	<i>cis</i> -linoleic acid	C _{18:2n6c}	48.45 ± 2.87^b	/	0.97 ± 0.85^c	1.92 ± 0.03^c	54.1 ± 2.32^a
9	Heneicosanoic acid	C _{21:0}	0.37 ± 0.07^a	0.16 ± 0.14^{bc}	0.05 ± 0.04^c	0.20 ± 0.00^{abc}	0.29 ± 0.01^{ab}
10	Docosanoic acid	C _{22:0}	0.42 ± 0.02^{ab}	0.92 ± 0.03^{ab}	0.13 ± 0.01^b	1.72 ± 0.03^{ab}	2.43 ± 0.05^a
11	Erucic acid	C _{22:1(n-9)}	/	/	0.15 ± 0.02^{ab}	0.45 ± 0.01^a	0.08 ± 0.01^{ab}

Note: Different letters in the same industry indicate significant differences ($P < 0.05$). Data in this table were analyzed using Kruskal-Wallis test with Dunn's post hoc correction ($n = 3$). *I. polycarpa* crude oil (IPCO); Olive oil (OO); Rapeseed oil (RO); Soybean oil (SO).

3.2 The effect of different edible oils in high-fat and low-fat diets on mouse body weight and visceral fat

After the adaptation period, no statistically significant differences were observed in the initial body weights of the mice in each group. Long-term observation showed that the body weights of both the HFD and LFD groups increased over time, with the HFD group exhibited significantly higher body weights than the LFD group starting from week 6 (Figure 1A). At the end of the experiment, while maintaining the same basal dietary structure (low-fat or high-fat), compared with other edible oils, *I. polycarpa* crude oil showed no significant differences in body weight, body length, Lee's index, weight gain, food intake, energy intake, food efficiency ratio, visceral fat weight (including epididymal fat and perirenal fat), and body fat percentage ($P > 0.05$) (Figure 1, Table 3).

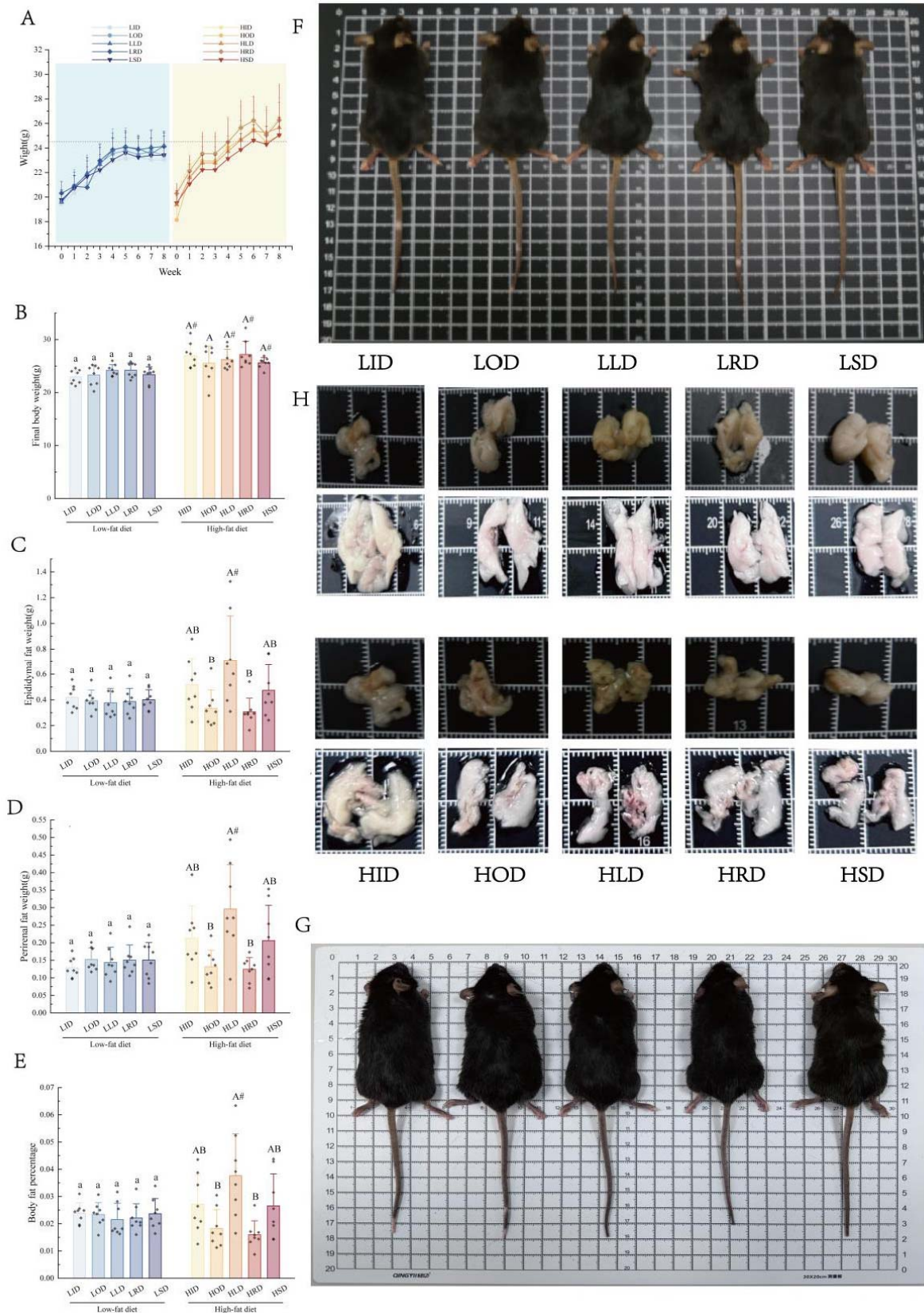


Figure 1. The effect of different edible oils in HFD and LFD on mouse body weight and visceral fat. Body weight growth curves (A); Terminal body weight (B); Epididymal fat weight (C); Perirenal fat weight (D); Body fat percentage (E); Morphological features of low-fat diet groups (F); Morphological features of high-fat diet groups (G); Gross morphology of epididymal and perirenal fat pads (H). The two-way ANOVA test showed that the interactions of the above indicators were significant. Within-group significant differences are denoted by lowercase letters (a, b, c) for the low-fat, high-carbohydrate diet (LFD) and uppercase letters (A, B, C) for the high-fat, low-carbohydrate diet (HFD). A # symbol indicates significant differences between the LFD and HFD groups within the same oil treatment.

Table. 3 Growth indexes of different grease mice on high-fat and low-fat diets

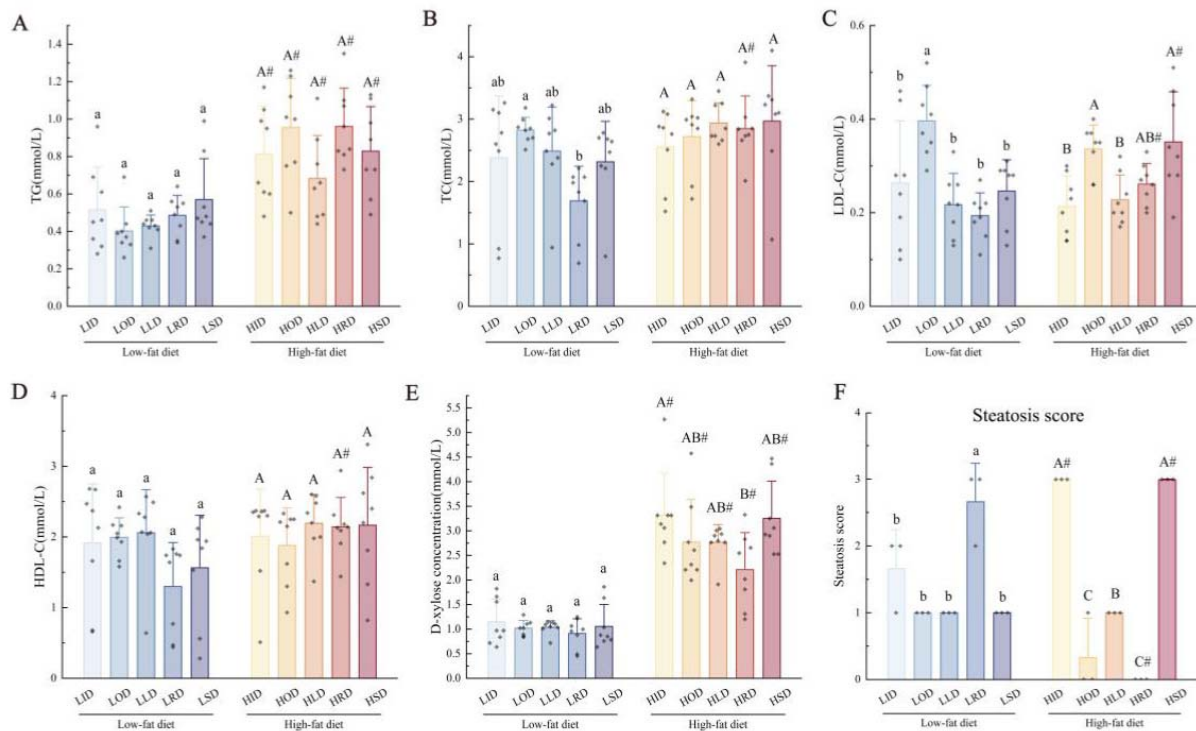
Indexes	Low-fat diet					High-fat diet				
	IPCO	OO	Lard	RO	SO	IPCO	OO	Lard	RO	SO
Body length (cm)	8.94 ± 0.33 ^a	9.01 ± 0.27 ^a	9.00 ± 0.32 ^a	9.23 ± 0.30 ^a	8.89 ± 0.21 ^a	9.23 ± 0.11 ^{A#}	9.27 ± 0.18 ^{A#}	9.34 ± 0.22 ^{A#}	9.35 ± 0.13 ^A	9.34 ± 0.14 ^{A#}
Lee's efficiency	318.23 ± 12.93 ^a	316.77 ± 6.19 ^a	321.49 ± 9.47 ^a	313.72 ± 9.42 ^a	321.69 ± 4.73 ^a	324.7 ± 10.30 ^A	319.35 ± 6.60 ^A	317.96 ± 7.23 ^A	321.26 ± 6.43 ^A	315.67 ± 6.86 ^A
Weight gain (g)	3.98 ± 1.32 ^a	3.81 ± 1.97 ^a	4.71 ± 0.84 ^a	3.98 ± 2.14 ^a	3.74 ± 1.69 ^a	6.48 ± 1.77 ^{A#}	6.88 ± 2.39 ^{A#}	6.76 ± 1.83 ^{A#}	6.78 ± 2.19 ^{A#}	5.84 ± 1.09 ^{A#}
Feed intake (g/week)	18.44 ± 0.99 ^a	18.70 ± 0.89 ^a	18.72 ± 1.14 ^a	19.05 ± 1.32 ^a	18.48 ± 0.79 ^a	16.56 ± 1.15 ^{A#}	16.72 ± 1.49 ^{A#}	16.29 ± 1.54 ^{A#}	16.96 ± 1.23 ^{A#}	16.85 ± 1.67 ^{A#}
Energy intake (kcal/week)	66.40 ± 3.57 ^a	67.32 ± 3.19 ^a	67.39 ± 4.10 ^a	68.57 ± 4.74 ^a	66.54 ± 2.84 ^a	92.74 ± 6.45 ^{A#}	93.60 ± 8.37 ^{A#}	91.25 ± 8.60 ^{A#}	94.98 ± 6.91 ^{A#}	94.37 ± 9.38 ^{A#}
Feed efficiency (%)	0.027 ± 0.009 ^a	0.026 ± 0.013 ^a	0.031 ± 0.006 ^a	0.027 ± 0.014 ^a	0.026 ± 0.012 ^a	0.050 ± 0.014 ^{A#}	0.052 ± 0.018 ^{A#}	0.053 ± 0.014 ^{A#}	0.051 ± 0.016 ^{A#}	0.044 ± 0.008 ^{A#}

Note: The two-way ANOVA test showed that the interactions of the above indicators were significant. Within-group significant differences are denoted by lowercase letters (a, b, c) for the low-fat, high-carbohydrate diet (LFD) and uppercase letters (A, B, C) for the high-fat, low-carbohydrate diet (HFD). A # symbol indicates significant differences between the LFD and HFD groups within the same oil treatment. For data that did not conform to normal distribution, the Kruskal-Wallis test with a Dunn post hoc correction was used (n = 6). *I. polycarpa* crude oil (IPCO); Olive oil (OO); Rapeseed oil (RO); Soybean oil (SO).

This study compared the effects of different dietary oils as the sole fat source on metabolic parameters in mice fed either LFD or HFD. Compared with HFD-fed mice, those fed *I. polycarpa* crude oil diet showed significant reductions in body weight (22.95 vs. 26.41 g, $P = 0.0182$), body length (8.94 vs. 9.23 cm, $P = 0.0216$), weight gain (3.98 vs. 6.48 g, $P = 0.0063$), weekly food intake (66.40 vs. 92.74 g/week, $P = 0.0182$), weekly energy intake (66.40 vs. 92.74 kcal, $P = 0.0182$), and feed efficiency (0.027 % vs. 0.050 %, $P = 0.0315$). No significant differences were observed in visceral fat mass or body fat percentage ($P > 0.05$). Among the olive, rapeseed, and soybean oil groups under LFD conditions, no differences in visceral fat or body fat percentage were found. However, these groups exhibited significant decreases in body weight (rapeseed: 24.21 vs. 27.18 g, $P = 0.0106$; soybean: 23.39 vs. 25.61 g, $P = 0.0031$), weight gain (olive: 3.81 vs. 6.88 g, $P = 0.0142$; rapeseed: 3.98 vs. 6.78 g, $P = 0.0214$; soybean: 3.74 vs. 5.84 g, $P = 0.0105$), weekly food intake (olive: 18.70 vs. 16.71 g/week, $P = 0.0060$; rapeseed: 19.05 vs. 16.96 g/week, $P = 0.0056$; soybean: 16.85 vs. 18.48 g/week, $P = 0.0258$), weekly energy intake (olive: 67.32 vs. 93.60 kcal, $P < 0.0001$; rapeseed: 68.57 vs. 94.98 kcal, $P < 0.0001$; soybean: 66.54 vs. 94.37 kcal, $P < 0.0001$), and feed efficiency (olive: 0.026 % vs. 0.052 %, $P = 0.0046$; rapeseed: 0.027 % vs. 0.051 %, $P = 0.0137$; soybean: 0.026 % vs. 0.044 %, $P = 0.0029$). Additionally, LFD mice in the soybean group had shorter body length (8.89 vs. 9.34 cm, $P < 0.0001$). Notably, no significant reduction in visceral fat was observed in HFD mice fed olive or rapeseed oil. In the lard group, compared with LFD mice, significant reductions were found in body weight (24.18 vs. 26.23 g, $P = 0.0192$), perirenal fat weight (0.144 vs. 0.297 g, $P = 0.0011$), epididymal fat weight (0.378 vs. 0.708 g, $P = 0.0233$), body fat percentage (0.022 vs. 0.038, $P = 0.0093$), feed efficiency (0.031 % vs. 0.053 %, $P = 0.0416$), and weekly energy intake (67.39 vs. 91.25 kcal, $P < 0.0001$), while weekly feed intake increased significantly (18.72 vs. 16.29 g, $P = 0.0091$).

3.3 Effects of Different Edible Oils on Hepatic Lipid Levels and Steatosis in Mice

Serological analysis revealed that under both low-fat (LFD) and high-fat (HFD) dietary conditions, *I. polycarpa* crude oil (LID/HID groups) had no significant effects on serum triglyceride (TG), total cholesterol (TC), or high-density lipoprotein cholesterol (HDL-C) levels compared to olive oil, lard, rapeseed oil, or soybean oil (Figure 2A, B, D). However, under both LFD and HFD conditions, it significantly reduced low-density lipoprotein cholesterol (LDL-C) levels compared to olive oil (0.264 vs. 0.396 mmol/L; $P = 0.0294$). Conversely, under HFD conditions, the LDL-C levels in the *I. polycarpa* crude oil group were significantly lower than those in the soybean oil group (LFD: 0.264 vs. 0.396 mmol/L; $P = 0.0242$; HFD: 0.214 vs. 0.336 mmol/L; $P = 0.0078$) (Figure 2C). Additionally, under HFD conditions, it significantly increased intestinal permeability compared to rapeseed oil (2.212 vs. 3.314 mmol/L; $P = 0.0401$; Figure 2E). Histopathological examination showed that under both dietary patterns, rapeseed oil caused the most severe damage to liver and intestinal tissues; *I. polycarpa* crude oil induced relatively mild intestinal damage, characterized by mucosal sloughing and widened intercellular spaces. In the liver, this oil caused moderate steatosis under LFD conditions (second in severity only to rapeseed oil), while under HFD conditions, it led to severe steatosis with prominent macrovesicles—suggesting lipid solubility or metabolic abnormalities—which were not observed with other oils (Figure 2F–H). NAS scores further indicated that under LFD conditions, the liver damage caused by *I. polycarpa* crude oil was comparable to that of olive oil, lard, and soybean oil but less severe than rapeseed oil; under HFD conditions, its NAS scores were higher than those of olive oil, lard, and rapeseed oil, most closely resembling soybean oil (Figure 2I). Although the type of oil generally did not alter serum lipid profiles or intestinal absorption, it significantly influenced histopathological outcomes. *I. polycarpa* crude oil uniquely reduced LDL-C levels compared to certain oils and induced characteristic histological lesions, though its severity was lower than that of rapeseed oil.



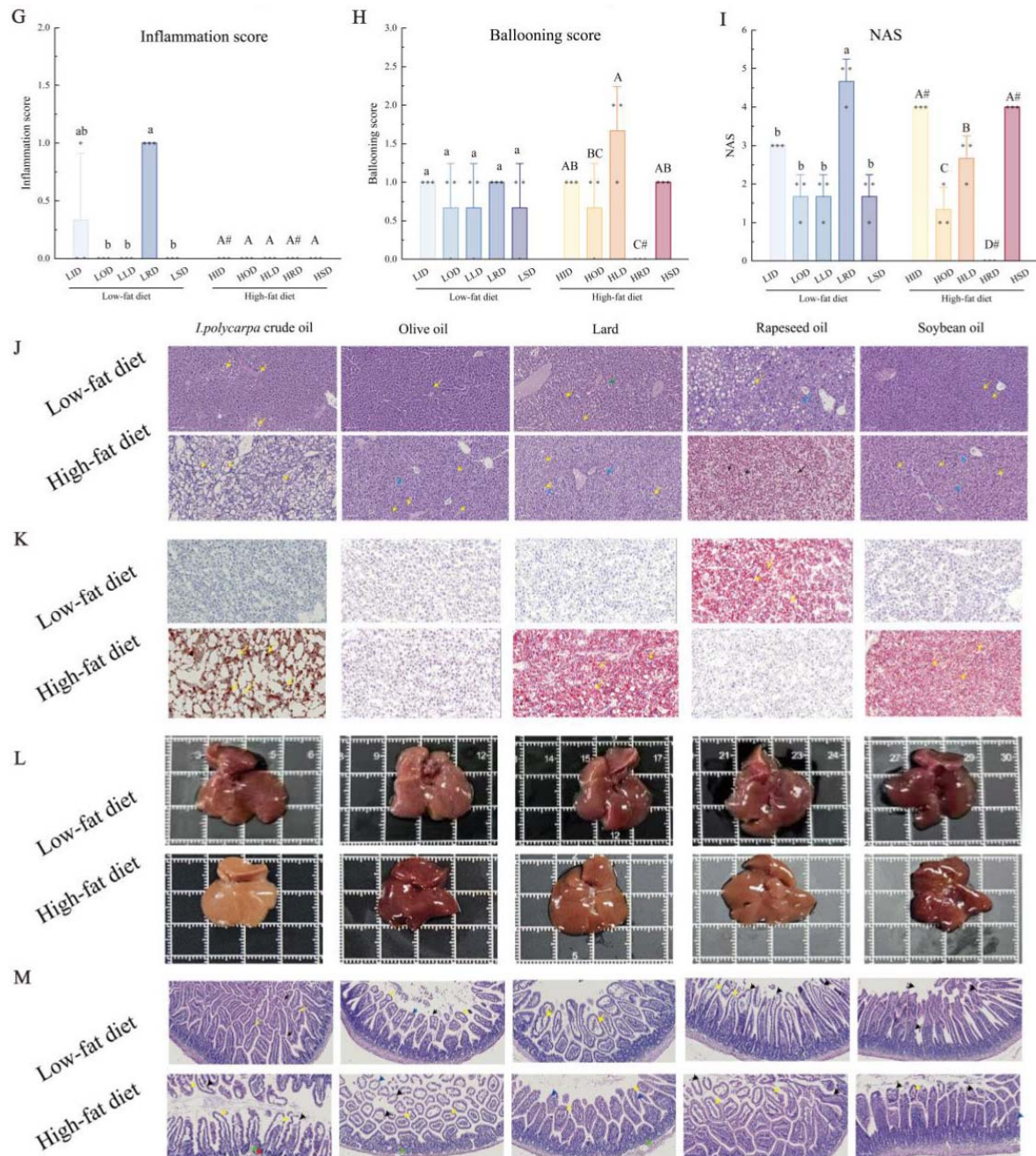


Figure 2. The effects of different edible oils in different dietary structures on lipid levels and liver and small intestine health in mice. Serum TC level (A); Serum TG level (B); Serum LDL-C level (C); Serum HDL-C level (D); Serum D-xylose level (E); Steatosis score (F); Inflammation score (G); Ballooning score (H); NAS (I); Hepatic HE staining (J) (200 × magnification); Hepatic Oil Red O staining (K) (200 × magnification); Gross liver morphology (L). Central vein and portal vein congestion (red arrows); Hepatocyte edema (blue arrows); Hepatocyte steatosis (yellow arrows); Focal hepatocyte necrosis (black arrows); Connective tissue hyperplasia (white arrows); Inflammatory cell infiltration (gray arrows); Sinusoidal dilatation (green arrows). Jejunal HE staining (200×) (M). Shedding of villous mucosal epithelial cells (black arrows); Separation of villous mucosal epithelium from the lamina propria (yellow arrows); Edema of mucosal epithelial cells (blue arrows); Vascular dilatation (green arrows); Inflammatory cell infiltration (red arrows). The two-way ANOVA test showed that the interactions of the above indicators were significant. Within-group significant differences are denoted by lowercase letters (a, b, c) for the low-fat, high-carbohydrate diet (LFD) and uppercase letters (A, B, C) for the high-fat, low-carbohydrate diet (HFD). A # symbol indicates significant differences between the LFD and HFD groups within the same oil treatment.

This study investigated the effects of different dietary oils, used as the sole fat source, on blood lipid parameters and D-xylose concentrations in rats fed either LFD or HFD. When *I. polycarpa* crude oil was used, no significant differences were observed in TC, HDL-C, and LDL-C between the LFD and HFD groups

($P > 0.05$), but the LFD group showed significantly lower TG (0.516 vs. 0.814 mmol/L, $P = 0.0273$) and D-xylose concentrations (1.147 vs. 3.314 mmol/L, $P < 0.0001$). Similarly, olive oil showed no differences in TC, HDL-C, or LDL-C ($P > 0.05$), yet TG (0.403 vs. 0.955 mmol/L, $P < 0.0001$) and D-xylose (1.019 vs. 2.770 mmol/L, $P < 0.0001$) were significantly reduced in the LFD group. For lard, TC, HDL-C, LDL-C, and D-xylose showed no significant differences ($P > 0.05$), but TG (0.486 vs. 0.961 mmol/L, $P = 0.0005$) and D-xylose (1.036 vs. 2.765 mmol/L, $P < 0.0001$) were significantly lower with the LFD. When rapeseed oil was used, LFD significantly reduced TG (0.486 vs. 0.961 mmol/L, $P < 0.0001$), TC (1.690 vs. 2.848 mmol/L, $P = 0.0005$), HDL-C (1.299 vs. 2.143 mmol/L, $P = 0.0066$), LDL-C (0.194 vs. 0.261 mmol/L, $P = 0.0113$), and D-xylose (0.913 vs. 2.212 mmol/L, $P = 0.0005$). With soybean oil, there were no significant differences in TC and HDL-C ($P > 0.05$), but TG (0.570 vs. 0.829 mmol/L, $P = 0.0401$), LDL-C (0.246 vs. 0.351 mmol/L, $P = 0.0340$), and D-xylose (1.055 vs. 3.253 mmol/L, $P < 0.0001$) were significantly lower in the LFD group. Across all oil types (*I. polycarpa* crude oil, olive oil, lard, rapeseed oil, and soybean oil), increasing the dietary oil proportion induced consistent liver and intestinal histopathological changes, while serum lipid and D-xylose responses varied by oil type. Notably, no oil maintained full tissue stability: *I. polycarpa* crude oil aggravated hepatic steatosis and intestinal inflammation; olive oil alleviated hepatic steatosis but elevated serum TG; lard altered liver morphology yet improved intestinal lesions; rapeseed oil caused severe hepatic necrosis; and soybean oil improved liver pathology but worsened intestinal integrity. Based on NAS scores, olive oil and lard showed no significant difference between LFD and HFD, *I. polycarpa* crude oil and soybean oil exacerbated liver injury, while rapeseed oil paradoxically reduced the score due to extensive necrosis under HFD. Collectively, these findings demonstrate that higher dietary oil intake inevitably disrupts tissue homeostasis, and specific risks must be considered: rapeseed oil poses hepatotoxicity, soybean oil compromises intestinal integrity, *I. polycarpa* crude oil promotes chronic inflammation, whereas olive oil exhibits the most favorable safety profile.

3.4 Blood Glucose and Insulin Levels

As shown in Figure 3A, during the initial 0–15 min period, blood glucose levels in mice fed a high-fat diet (HFD) increased more rapidly than in those fed a low-fat diet (LFD). When comparing subgroups, no statistically significant differences were observed in glucose tolerance area under the curve (AUC) or insulin levels between the LID group and the LOD, LLD, or LSD groups ($P > 0.05$). However, the LID group exhibited a significantly lower glucose tolerance AUC compared to the LRD group (977.3 vs. 1197.75, $P = 0.0457$). Similarly, no significant differences in glucose tolerance AUC or insulin levels were found among the HID, HOD, HLD, HRD, and HSD groups ($P > 0.05$). These findings suggest that overall insulin sensitivity was not affected by dietary fat type under either dietary pattern.

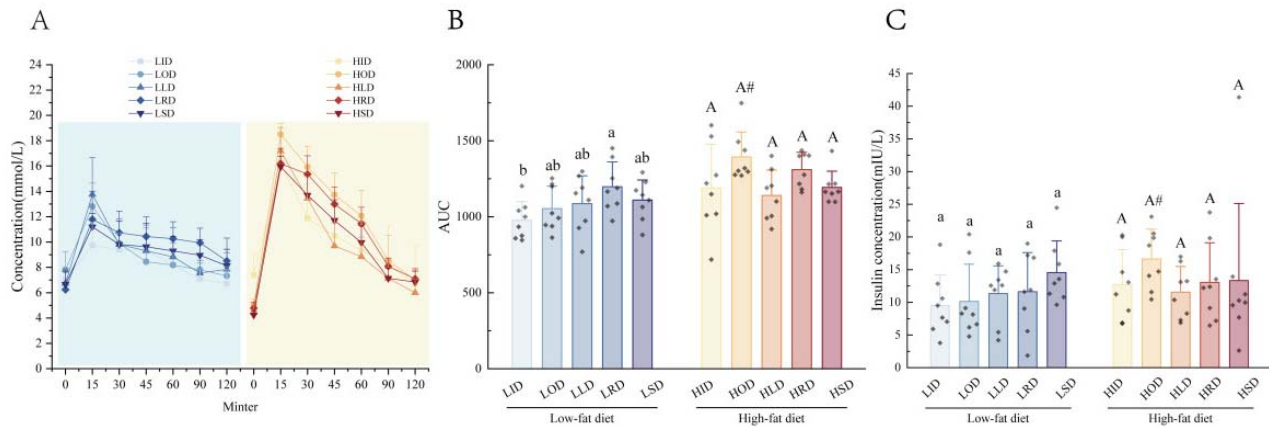


Figure 3. Effects of different edible oils on blood glucose and insulin concentrations in mice with different dietary structures ($n = 8$). Blood glucose kinetics (A); Glucose tolerance AUC (Area Under the Curve) (B); Serum insulin concentration (C). The two-way ANOVA test showed that the interactions of the above indicators were significant. Within-group significant differences are denoted by lowercase letters (a, b, c) for the low-fat, high-carbohydrate diet (LFD) and uppercase letters (A, B, C) for the high-fat, low-carbohydrate diet (HFD). A # symbol indicates significant differences between the LFD and HFD groups within the same oil treatment, If it is not distinctive, we will not mark it.

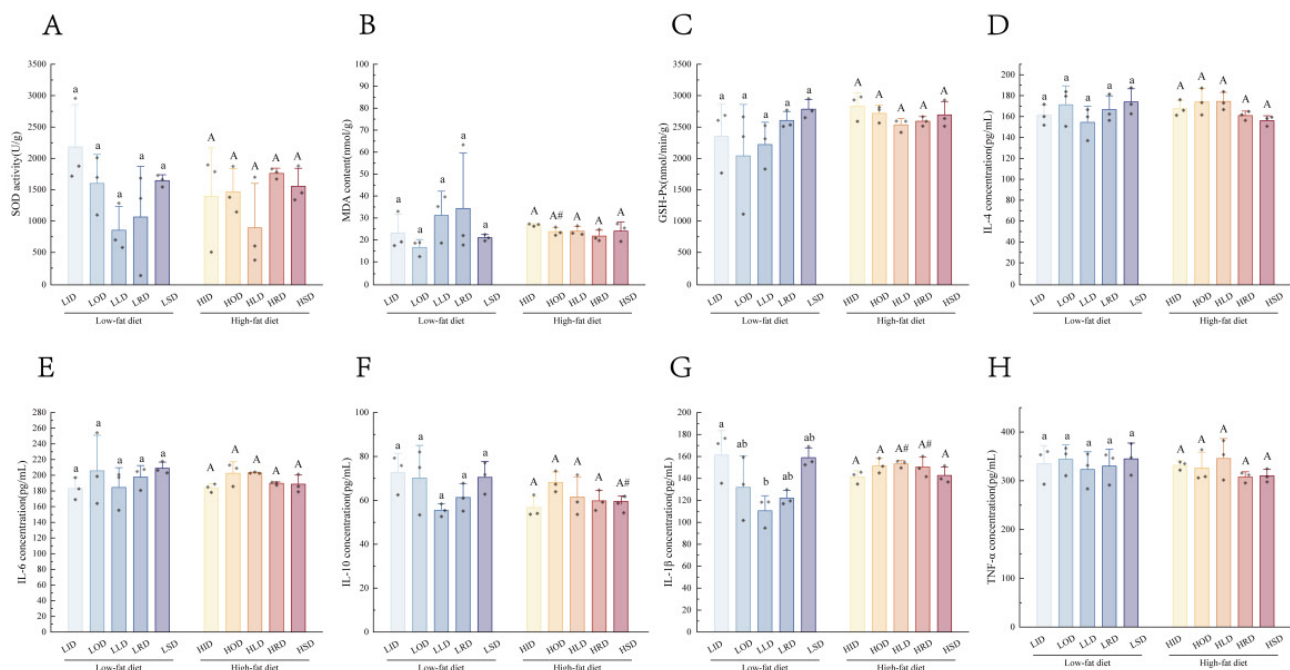


Figure 4. The effects of different edible oils in different dietary structures on oxidative stress and inflammatory factors in mouse livers. Hepatic SOD activity (A); Hepatic MDA content (B); Hepatic GSH-Px activity (C); Hepatic cytokine concentrations: IL-4 (A); IL-6 (B); IL-10 (C); IL-1 β (D); TNF- α (E). The two-way ANOVA test showed that the interactions of the above indicators were significant. Within-group significant differences are denoted by lowercase letters (a, b, c) for the low-fat, high-carbohydrate diet (LFD) and uppercase letters (A, B, C) for the high-fat, low-carbohydrate diet (HFD). A # symbol indicates significant differences between the LFD and HFD groups within the same oil treatment.

Further analysis (Figures 3B and 3C) showed that increasing the proportion of *I. polycarpa* crude oil, lard, rapeseed oil, or soybean oil in the diet did not significantly alter glucose tolerance AUC or insulin levels ($P > 0.05$). Notably, when rapeseed oil was the sole dietary fat source, the LFD group displayed a significantly lower glucose tolerance AUC (1053.71 vs. 1393.25, $P = 0.0048$) and insulin level (10.142 vs. 16.621 mIU/L, $P = 0.0249$) compared with the HFD group.

3.5 Analysis of Hepatic Oxidative Stress and Inflammation

This study evaluated the effects of different dietary fats on the hepatic status of mice by measuring oxidative stress markers (SOD, MDA, GSH-Px) and inflammatory cytokines (IL-4, IL-6, IL-10, IL-1 β , TNF- α). Under LFD conditions, no significant differences were observed among the groups in SOD, MDA, GSH-Px activity, or IL-4, IL-6, IL-10, TNF- α levels ($P > 0.05$), but IL-1 β levels in the LID group (161.26 pg/mL) were significantly higher than in the LLD group (110.53 pg/mL, $P = 0.0407$). Under HFD conditions, HID and other groups also showed no significant differences in these parameters ($P > 0.05$) (Figure A-H).

Trend analysis indicated that increasing the proportions of *I. polycarpa* crude oil, olive oil, lard, rapeseed oil, or soybean oil had no significant effect on SOD, GSH-Px activity, or IL-4, IL-6, TNF- α levels ($P > 0.05$). However, under olive oil diet, MDA concentration in the LOD group (16.56 pg/mL) was significantly lower than in the HFD diet (23.74 pg/mL, $P = 0.0385$); under soybean oil diet, IL-10 concentration in the LSD group (70.52 pg/mL) was significantly higher than in the HFD diet (56.05 pg/mL, $P = 0.0357$). Moreover, significant differences in IL-1 β levels were observed between LFD and HFD patterns under lard and rapeseed oil conditions: LLD group was significantly lower than HLD group (110.53 vs. 153.27 pg/mL, $P = 0.0012$), and LRD group was significantly lower than HRD group (122.08 vs. 150.33 pg/mL, $P = 0.0133$). Apart from these findings, no other significant differences were detected ($P > 0.05$).

3.6 Differences in Gut Microbiota Among Group

3.6.1 Alpha, Beta, Phylum-Level and Genus-Level Diversity Analysis of Gut Microbiota Across Groups

To investigate the effects of dietary oils on gut microbiota, we performed 16S rDNA sequencing (V3–V4 region) on three samples per group, generating 2,926,751 high-quality tags across 30 samples. Bioinformatics analysis clustered sequences into operational taxonomic units (OTUs) at a $> 97\%$ similarity threshold, identifying 3,505 OTUs in total with 120 OTUs shared universally; results were visualized in a petal diagram. Rarefaction curves confirmed sufficient sequencing depth at 20,000 reads (Figure 5A). Alpha diversity analysis (Chao1, Shannon, and Simpson indices) revealed no significant differences between the LID group and other low-fat subgroups (LOD, LLD, LRD, LSD), or between the HID group and high-fat subgroups (HOD, HLD, HRD, HSD) ($P > 0.05$). At the phylum level, the top 10 bacterial phyla exhibited distinct distributions across groups (Figure 5G). Analysis of six dominant phyla (*Firmicutes*, *Bacteroidetes*, *Actinobacteriota*, *Verrucomicrobiota*, *Desulfobacterota*, *Deferribacterota*) and the *Firmicutes*/*Bacteroidetes* (F/B) ratio (a metabolic homeostasis marker; Figure S1A-G) showed no significant abundance differences between LID and other low-fat subgroups, or between HID and high-fat counterparts ($P > 0.05$). Similarly, the stacked bar plot analysis of the top 10 bacterial genera—with a focus on six dominant groups: *Muribaculaceae*, *Bacteroides*, the *Eubacterium-fissicatena-group*, *Akkermansia*, *Bifidobacterium*, and *Dubosiella* (Figure 5H, S1H–M)—revealed no significant differences among the low-fat groups ($P > 0.05$). In the high-fat group, no significant differences were observed between HID and HOD, HLD, HRD, or HSD in the relative abundances of *Muribaculaceae*, *Bacteroides*, the *Eubacterium-fissicatena-group*, or *Akkermansia*. However, the relative abundance of *Dubosiella* was significantly lower in HID compared to

HLD, HRD, and HSD. Moreover, the relative abundance of *Bifidobacterium* in HID was also significantly lower than that in HOD.

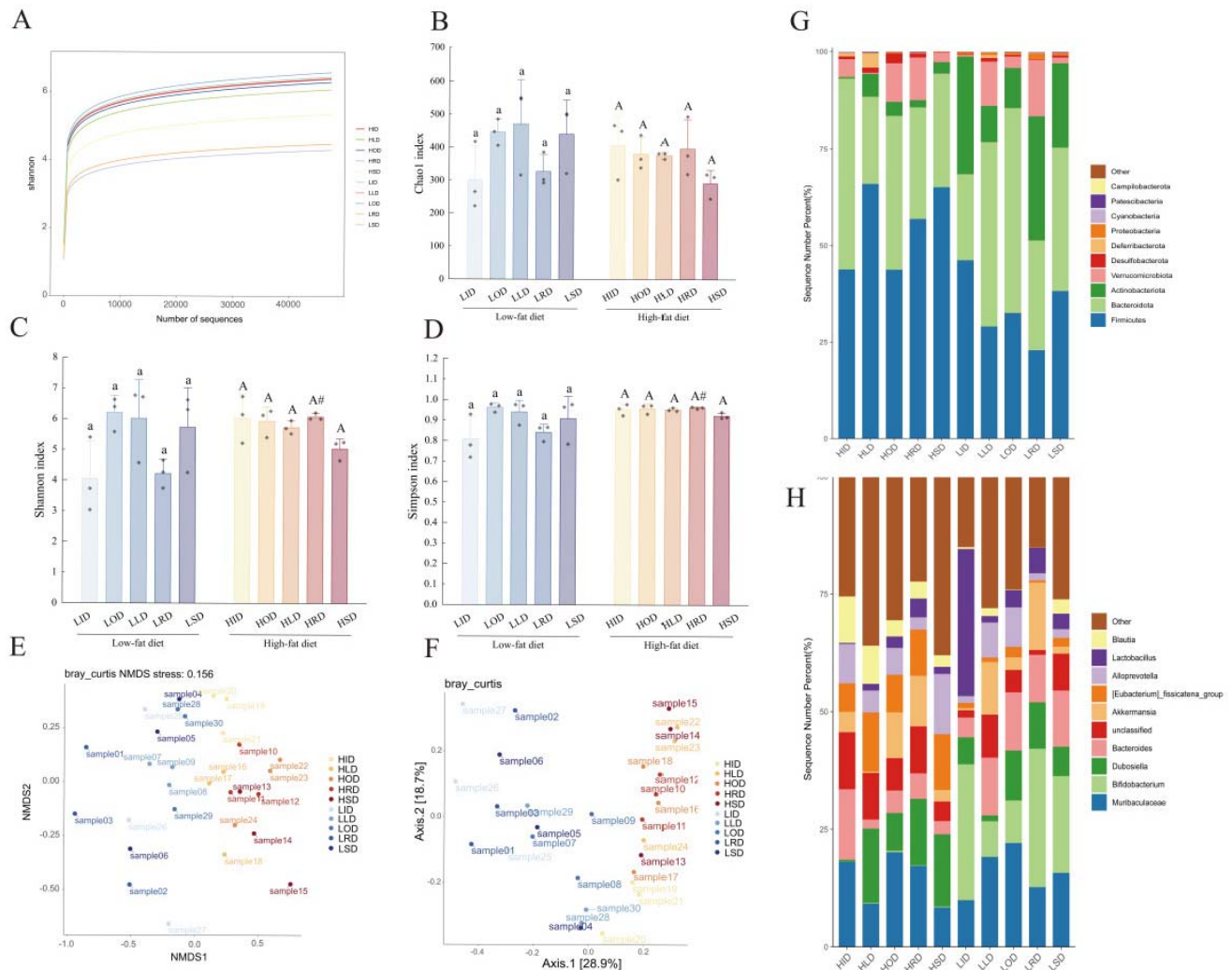


Figure 5. Effects of different edible oils in different dietary structures on the diversity, specificity, phylum and genus levels of intestinal microbiota in mice. Shannon rarefaction curve (A); Chao1 index (B); Shannon index (C); Simpson index (D); NMDS plot based on Bray-Curtis's dissimilarity (E); PCoA plot based on Bray-Curtis's dissimilarity (F); The top 10 most abundant bacterial phyla in the gut microbiota of each group (G); Top 10 most abundant bacterial genera in the gut microbiota of each group (H). The two-way ANOVA test showed that the interactions of the above indicators were significant. Within-group significant differences are denoted by lowercase letters (a, b, c) for the low-fat, high-carbohydrate diet (LFD) and uppercase letters (A, B, C) for the high-fat, low-carbohydrate diet (HFD). A # symbol indicates significant differences between the LFD and HFD groups within the same oil treatment.

Beta diversity analysis (NMDS stress value = 0.156; PCoA) revealed significant clustering of microbial communities between the LFD and HFD groups (Figure 5E, F), indicating that basal dietary fat content is a major driver of gut microbiome structure. Further analysis demonstrated that increasing the proportions of *I. polycarpa* crude oil, olive oil, lard, rapeseed oil, and soybean oil distinctly altered the structure and diversity of the gut microbiome. Among these, *I. polycarpa* crude oil showed the most unique effects: higher supplementation was correlated with increased α -diversity and reduced abundances of *Actinobacteriota*, *Dubosiella*, and *Bifidobacterium*. In contrast, elevated levels of olive oil, lard, or soybean oil generally decreased α -diversity, raised the F/B ratio, and promoted the growth of the *Eubacterium-fissicatena-group*. Rapeseed oil had more complex impacts; although it enhanced α -diversity, it also reduced the abundance of

Figure 6. LEfSe analysis revealed differences in gut microbiota biomarkers between the *I. polycarpa* crude oil group and other oil groups under LFD regimen. Phylogenetic distribution of functional biomarkers across groups. PLS-DA ROC curves of OTUs across groups (A); PLS-DA ordination plot of OTUs across groups (B); Biomarker differences between LID and LRD (C); Biomarker differences between LID and LSD (D); Biomarker differences between LID and LOD (E); Biomarker differences between LID and LLD (F).

Conversely, *Bifidobacteriaceae* levels declined significantly ($P < 0.05$) in four oil types (*I. polycarpa* (HID vs. LID, 0.0004 vs. 0.2888 %), rapeseed (HRD vs. LRD, 0.0013 vs. 0.2942 %), olive (HOD vs. LOD, 0.0032 vs. 0.0907 %), soybean (HSD vs. LSD, 0.0019 vs. 0.2057 %)) but remained stable in lard-fed mice. Enrichment of SCFA-producing taxa occurred consistently, yet exhibited oil-specific patterns: *Lachnospiraceae* increased under *I. polycarpa*, rapeseed, lard, and olive oils, while *Clostridia* expanded with *I. polycarpa*, rapeseed, and olive oils. Distinct oil-unique alterations were also identified: lard exclusively reduced *Bacteroidaceae* and *Verrucomicrobiales* while enriching *Erysipelotrichaceae* and *Dubosiella*; soybean oil specifically enriched *Prevotellaceae* and *Alloprevotella*; olive oil depleted *Muribaculum* (an effect unobserved with other oils); and rapeseed oil increased *Muribaculaceae* and *Blautia* (Figure S2E-I).

3.7 Untargeted Metabolomic Profiling of Gut Microbiota Under Different Dietary Structures

3.7.1 Analysis of Metabolic Composition Differences Between Groups

Rigorous quality assurance (QA) and quality control (QC) analyses ensured data reproducibility and precision. A total of 3,125 metabolites were detected, comprising 1,945 features in positive ion mode and 1,180 in negative ion mode, which were subsequently analyzed using multivariate statistics. In both ionization modes, Principal Component 1 (PC1) values for all samples fell within $3 \times$ standard deviation limits, confirming stringent control over sample preparation and instrument performance. Heatmaps of the top 30 metabolites by abundance revealed distinct profiles across the ten experimental groups in both positive (Figure 7C) and negative ion modes (Figure 7D). PCA analysis showed partial overlap between the HLD group and some LSD/LLD samples in positive ion mode (PC1: 18.6 %, PC2: 13.3 %, PC3: 9.3 %), while other groups clustered distinctly (Figure 8A). In negative ion mode, PCA similarly highlighted inter-group metabolic divergence (PC1: 18.2 %, PC2: 12.9 %, PC3: 9.3 %) (Figure 8B). Notably, *I. polycarpa* crude oil significantly reduced levels of Phenacetin, 12-ketolithocholic acid, Stercobilin, 9-Hydroxyoctadecanoic acid, and L-isoleucyl-L-proline in positive ion mode, as well as 12-hydroxystearic acid in negative ion mode, compared to other oils (Figure 7C, D).

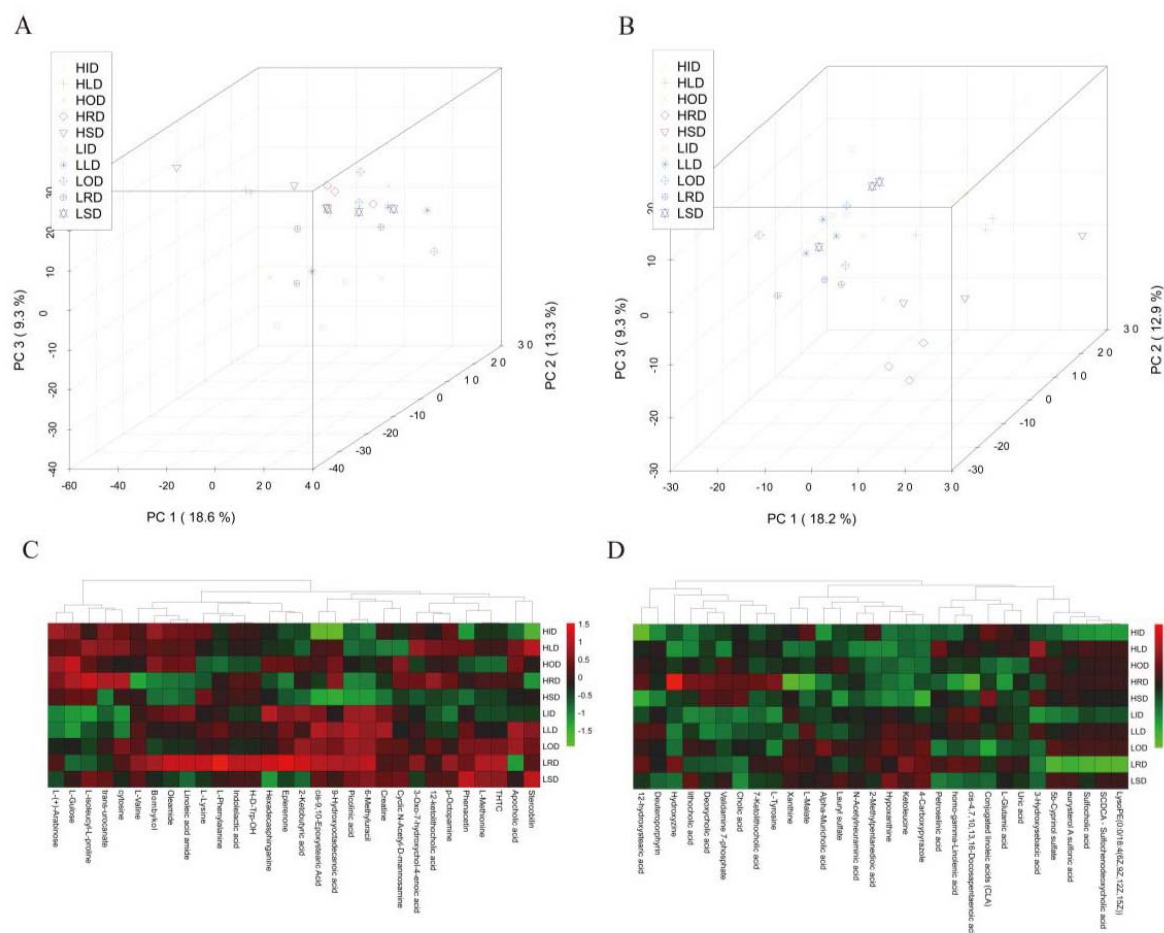


Figure 7. Differences in the composition of non-targeted metabolites between different groups under positive and negative ions. 3D PCA scatter plot of samples in positive ion mode across experimental groups (A); 3D PCA scatter plot of samples in negative ion mode across experimental groups (B); Heatmap of metabolite composition differences between groups in positive ion mode (C); Heatmap of metabolite composition differences between groups in negative ion mode (D).

As the intake of different oils increased, metabolite alterations exhibited both shared and distinct patterns. Commonly, picolinic acid decreased across multiple oils, including *I. polycarpa* crude oil, lard, and soybean oil. Similarly, cis-9,10-epoxystearic acid and 6-methyluracil were reduced in both *I. polycarpa* crude oil and soybean oil, while L-gulose consistently increased in *I. polycarpa* crude oil, lard, and rapeseed oil, and cytosine increased in *I. polycarpa* crude oil and lard. However, significant differences were observed: *I. polycarpa* crude oil also led to a decrease in 9-hydroxyoctadecanoic acid and an increase in L-(+)-arabinose. Lard uniquely reduced creatine. Rapeseed oil decreased oleamide, L-phenylalanine, linoleic acid amide, hexadecasphinganine, bombykol, and L-valine, while increasing L-isoleucyl-L-proline. Soybean oil additionally caused a decrease in phenacetin, with no metabolites showing an increase. In marked contrast, increasing olive oil intake did not induce any significant metabolite changes, differing significantly from all other oils.

3.7.2 Analysis of Metabolic Composition Differences Between Groups

Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) with thresholds ($P < 0.05$, $VIP > 1$) [45] was employed to identify group-specific metabolic signatures, effectively distinguishing LFD and HFD clusters in both positive (Figure 8A) and negative ion modes (Figure 9C). Model validation

confirmed robustness (positive mode: $R^2Y=0.98$, $Q^2=0.83$; negative mode: $R^2Y=0.99$, $Q^2=0.85$) (Figures 8B, D). Under LFD conditions, *I. polycarpa* crude oil uniquely upregulated Fellutanine D (40.13 vs. 0.77, 0.38, 2.35), Rumphellolide B (2.38 vs. 0.04, 0.04, 0.07), Tensyic acid A (0.22 vs. 0.07, 0.06, 0.03), and other metabolites while downregulating Dicranin (4.17 vs. 5.63, 28.51, 4.45) compared to olive oil, rapeseed oil, or lard (Figure 8E-H). In HFD groups, it elevated Methyl 3-hydroxy-4,5-dimethoxybenzoate (62.98 vs. 0.52, 0.31, 0.18, 0.19), Lys Ile Glu (128.40 vs. 1.58, 1.04, 1.41, 0.94), and Vanillin (58.21 vs. 4.21, 4.13, 4.13, 2.90) but suppressed Bortezomib (0.026 vs. 0.338, 0.314, 0.178, 0.032) relative to olive oil, lard, rapeseed oil and soybean oil (Figure 8J-M).

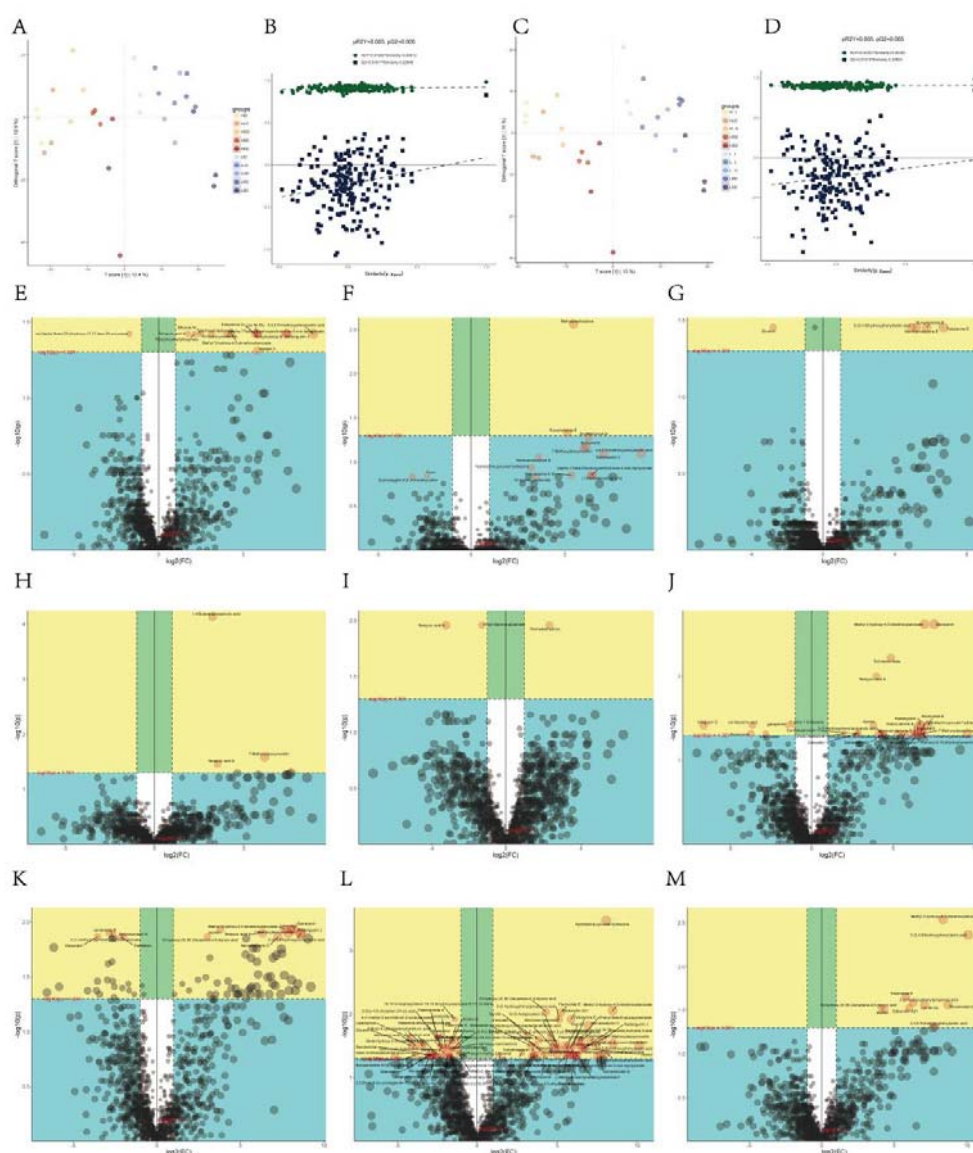


Figure 8. Effects of changes in intake from *I. polycarpa* crude oil on intestinal metabolites and comparative analysis with other edible oils. 3D OPLS-DA PCA scatter plot in positive ion mode (A); OPLS-DA permutation test plot in positive ion mode (B); 3D OPLS-DA PCA scatter plot in negative ion mode (C); OPLS-DA permutation test plot in negative ion mode (D). In the positive ion mode, metabolite differences identified by t-test ($P < 0.05$) for pairwise group comparisons: LID.VS.LOD (E), LID.VS.LLD (F), LID.VS.LRD (G), LID.VS.LSD (H), LID.VS.HID (I), HID.VS.HOD (J), HID.VS.HLD (K), HID.VS.HRD (L), HID.VS.HSD (M).

As the intake of *I. polycarpa* crude oil increased, the levels of Tensyuic acid A (HID vs. LID, 2.08 vs. 0.22) showed a dose-dependent increase, while Homostachydrine (HID vs. LID, 1.20 vs. 5.91) levels decreased (Figure 9I). In contrast, increased intake of olive oil, lard, and rapeseed oil did not result in significant changes in intestinal metabolites. However, increased intake of soybean oil led to elevated levels of Histidylprolineamide (HSD vs. LSD, 0.19 vs. 0.03), Fascicularone A (HSD vs. LSD, 4.83 vs. 0.97) (Figure S4).

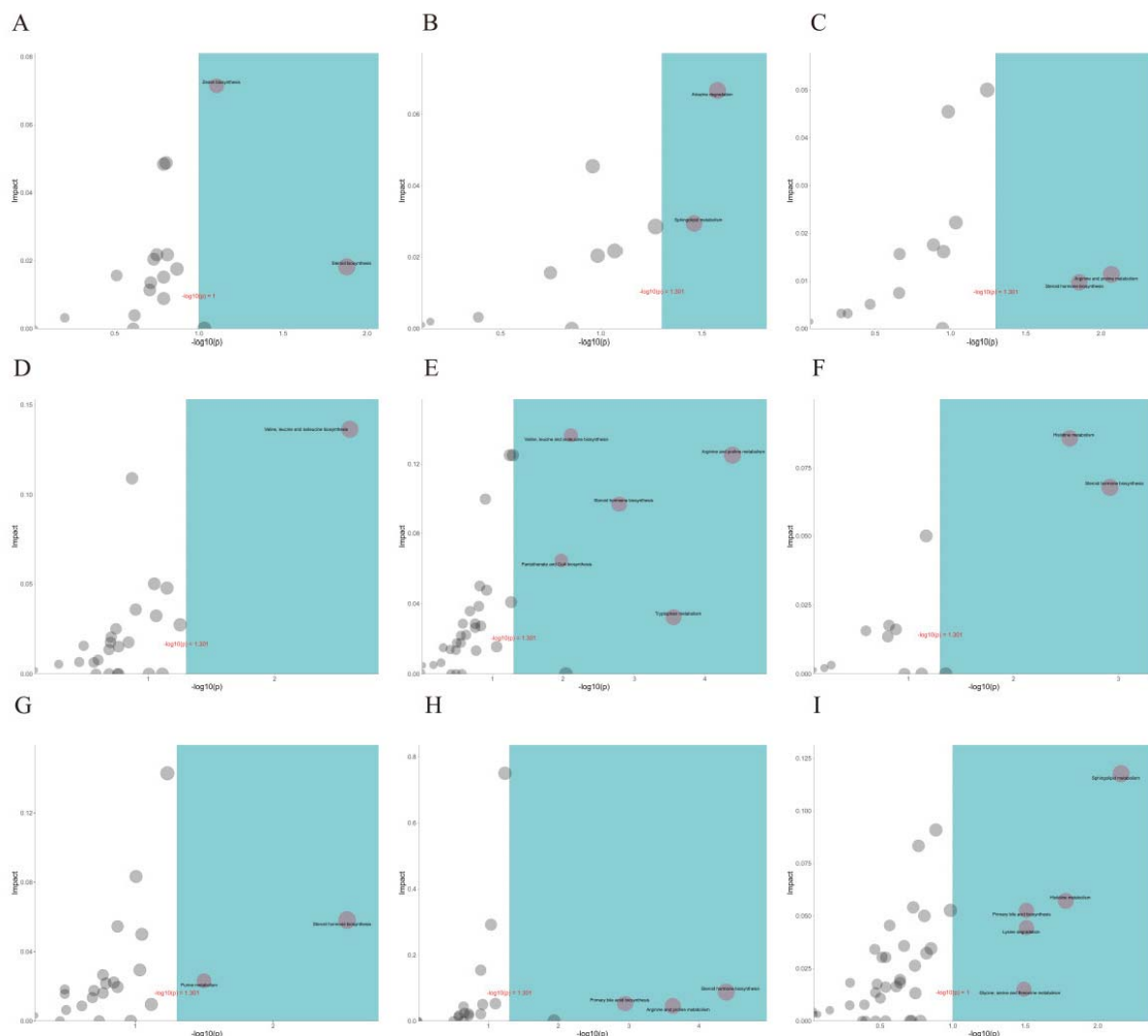


Figure 9. Effects of different edible oils in different dietary structures on visceral fat and body fat percentage in mice. Topological analysis in positive ion mode: X-axis: P-value; blue shaded area indicates significance ($P < 0.05$). Y-axis: Topological impact value. LID.VS.LOD (A), LLD.VS.LID (B), LID.VS.LRD (C), LID.VS.LSD (D), LID.VS.HID (E), HID.VS.HOD (F), HLD.VS.HID (G), HID.VS.HRD (H), HID.VS.HSD (I).

3.7.3 KEGG Pathway Analysis of Differential Metabolites Between Groups

Topological analysis of differential metabolites revealed diet-specific metabolic pathway perturbations^[46]. Under LFD condition, relaxing significance thresholds ($P < 0.1$) identified LID-linked modulation of atrazine degradation and valine/leucine/isoleucine biosynthesis, while LOD affected zeatin biosynthesis, and LLD altered sphingolipid metabolism. Steroid hormone biosynthesis was perturbed in both LID and LRD groups (Figure 9A-D). In HFD group, steroid hormone biosynthesis emerged as a common hub

across multiple oil comparisons. HID uniquely regulated lysine degradation and glycine/serine/threonine metabolism, contrasting with HSD-driven sphingolipid and bile acid pathway changes (Figure 9F-I).

Metabolic pathway analysis revealed that nucleotide metabolism (purine/pyrimidine) and pantothenate/CoA biosynthesis exhibited consistent dysregulation across most dietary comparisons (HOD vs. LOD, HLD vs. LLD, HRD vs. LRD, HSD vs. LSD), underscoring pervasive disruptions in energy metabolism and genetic material synthesis. Arginine/proline metabolism was frequently altered (e.g., HID vs. LID, HLD vs. LLD, HSD vs. LSD), indicating impaired nitrogen handling and osmotic regulation. Condition-specific variations emerged: branched-chain amino acid biosynthesis significantly changed in HID vs. LID and HRD vs. LRD; tryptophan metabolism was disrupted in HID vs. LID and HSD vs. LSD; steroid hormone biosynthesis was altered in HID vs. LID and HRD vs. LRD, while steroid degradation occurred exclusively in HOD vs. LOD. Highly specialized pathway disruptions included furfural degradation and α -linolenic acid metabolism (solely in HRD vs. LRD) and phenylpropanoid biosynthesis (only in HSD vs. LSD). Collectively, these results demonstrate that while core metabolic functions remain universally vulnerable, diet-dependent adaptive specialization manifests in lipid metabolism, xenobiotic processing, and secondary metabolite synthesis (Figure 9E, S5).

4. Discussion

Current research on *I. polycarpa* crude oil, both domestically and internationally, has predominantly focused on applied foundational studies. These encompass optimization of extraction techniques (e.g., pressing parameters affecting oil yield), comparative analyses of lipid composition across raw materials from diverse geographical origins, and characterization of physicochemical properties^[27, 45, 47-50]. As an emerging edible oil, the potential of *I. polycarpa* crude oil to replace common dietary oils (such as olive oil, lard, rapeseed oil, and soybean oil) and its drawbacks require further clarification through *in vivo* studies, with optimization targeting its shortcomings being crucial for its widespread adoption.

This study investigated the effects of different dietary fat sources on phenotype, gut microbiota, and related metabolites. Previous research has demonstrated that modifying lipid type and fatty acid composition, even under the same dietary regimen, can markedly influence lipid metabolism, oxidative stress balance, inflammatory signaling pathways, and gut microbiota function^[14, 51-53]. Our results showed that the physiological parameters of mice fed *I. polycarpa* crude oil did not differ significantly from those of mice fed olive oil, lard, rapeseed oil, or soybean oil, regardless of whether the diet contained 10% (low-fat) or 60% (high-fat) of energy from fat. Serum triglycerides, TC, and HDL-C levels were also comparable between the beechwood crude oil group and the lard, rapeseed, or soybean oil groups under both dietary conditions. However, LDL-C levels were significantly lower in the *I. polycarpa* crude oil group compared with the olive oil group under the low-fat diet, and in both the *I. polycarpa* crude oil and olive oil groups compared with others under the high-fat diet. This is consistent with the established LDL-C-lowering effects of both PUFA and MUFA, with PUFA being more effective^[54]. Nevertheless, under low-fat conditions, liver IL-1 β levels were significantly elevated in the *I. polycarpa* crude oil group

compared with the lard group, reaching levels similar to those in the soybean oil group, which is also rich in linoleic acid (LA). Previous studies have shown that excessive LA and palmitic acid (PA) can trigger inflammatory and metabolic disorders via distinct mechanisms: LA promotes NF- κ B activation, nuclear translocation, and pro-apoptotic gene expression, while PA enhances NLRP3 inflammasome activity through S-palmitoylation, leading to increased IL-1 β production^[55, 56]. Given the high PA content of *I. polycarpa* crude oil, it is plausible that PA-mediated NLRP3 activation explains the IL-1 β elevation relative to the lard group. Interestingly, soybean oil did not show a comparable significant increase in IL-1 β , likely because its high ω -6 PUFA content predisposes it to oxidation, and the resulting oxidation products themselves strongly induce IL-1 β ^[57] potentially masking differences. The relatively high LA content of beechwood crude oil suggests similar oxidative susceptibility, implying that oxidative stress-induced proinflammatory responses may further contribute to the observed IL-1 β increase.

Hepatic and intestinal pathological assessment demonstrated that under both dietary regimes, *I. polycarpa* crude oil - induced pathological damage was significantly more severe than that in the olive oil, lard, and soybean oil groups, but less severe than in the rapeseed oil group. Compared to the olive oil and lard groups, the more pronounced pathology in the *I. polycarpa* crude oil group likely stems from inflammation triggered by its high LA content and subsequent lipid metabolism dysregulation. Conversely, its milder pathology relative to the rapeseed oil group correlates with the detectable erucic acid content in rapeseed oil (which was undetectable in *I. polycarpa* crude oil in this study). Using the NAS score, we found that the rapeseed oil group consistently exhibited the most severe pathology, regardless of dietary fat level. Notably, under the high-fat diet, the NAS score was 0 due to extensive hepatic necrosis. Research indicates that erucic acid can induce hepatocyte pyroptosis via the autophagy-CTSB-NLRP3 inflammasome pathway^[58], consistent with the extensive hepatocellular necrosis, severe intestinal pathology, and characteristic pyroptotic morphological features (including nuclear pyknosis, DNA fragmentation, plasma membrane pore formation, cell swelling/rupture, and eosinophil infiltration) observed in the rapeseed oil group^[59, 60]. Notably, despite the significant reduction in erucic acid content in modern modified rapeseed oil (< 2 % in this study)^[61], erucic acid-associated pathological damage was still observed even under low-fat conditions and exacerbated under high-fat conditions, suggesting that intake dose requires careful consideration when using rapeseed oil as an edible oil. Collectively, these findings indicate that in the mouse model, *I. polycarpa* crude oil may possess relative advantages over rapeseed oil; however, the potential inflammatory risks associated with its high PA and LA content warrant attention. This conclusion necessitates further experimental validation, and the underlying molecular mechanisms, along with its potential applicability to human diets, require in-depth investigation.

Extensive research confirms that variations in dietary fat sources remodel gut microbiota structure and modulate metabolic phenotypes^[62, 63]. Our study found that under low-fat conditions, the *I. polycarpa* crude oil group exhibited significant enrichment of *Lactobacillus*, which has antibacterial, anti-inflammatory, antioxidant, and immunomodulatory effects^[64]. Control groups displayed protective microbiota signatures:

olive oil/lard groups enriched beneficial *Bacteroides*^[65]; the soybean oil group featured *Christensenellaceae-R-7-group*^[66]; the rapeseed oil group showed elevated *Verrucomicrobia* under low-fat conditions—aligning with non-alcoholic fatty liver disease (NAFLD) profiles^[67] and this group's most severe liver damage. Notably, high-fat *I. polycarpa* crude oil feeding, dominated by *Gastranaerophilales*, reduced fecal short-chain fatty acid (SCFA) levels^[68, 69], impairing SCFA-mediated suppression of LPS-induced inflammation. Conversely, all control groups enriched *Bacillus* (inversely correlated with $\text{TNF-}\alpha/\text{TNF-}\gamma/\text{IL-1}\beta$ ^[70]) and *Erysipelotrichaceae* (producing barrier-protective butyrate despite inflammatory links^[71, 72]).

Interactions between the gut microbiota and the host are primarily mediated by microbial metabolites^[73]. To elucidate the differential effects of *I. polycarpa* crude oil and the four common dietary oils on the gut microbial metabolome in mice, we systematically compared dominant metabolites: Under low-fat conditions, intestinal abundance of Rumphellolide B (isolated from the gorgonian coral *Rumphella antipathies*^[74]) was significantly higher in the *I. polycarpa* crude oil group than in olive oil, lard, and rapeseed oil groups, though its biological function remains unknown. Compared to the soybean oil group, the *I. polycarpa* crude oil group showed significant enrichment of Tensyucic acid A and 7-Methoxydeoxymorellin, whose functions are also unelucidated. Under high-fat conditions, Vanillin abundance was generally higher in the *I. polycarpa* crude oil group than in the other groups, whereas Pectinolide B was commonly present in olive oil, rapeseed oil, and soybean oil groups (excluding lard); both metabolites possess anti-inflammatory effects^[75, 76]. This study clearly observed that differences in dietary lipids significantly altered gut microbiota structure and associated metabolite profiles; however, there is currently no evidence indicating that these changes directly caused the phenotypic differences between the *I. polycarpa* crude oil group and the others, nor that dominant gut microbiota drove phenotypic differences via specific metabolites. Therefore, these causal relationships require further validation through fecal microbiota transplantation (FMT) experiments.

It should be noted that, compared with high-fat/low-carbohydrate diet, low-fat/high-carbohydrate diet has no difference in fat sources, but there are differences in phenotype and gut microbiota structure^[35]. Our findings indicate that analyses of individual oils were constrained by limited sample sizes, resulting in insufficient statistical power to reliably detect small yet consistent effects. In contrast, pooled analyses significantly enhanced statistical power through effect integration, thereby enabling more accurate characterization of systemic impacts under distinct dietary conditions^[77]. To address analytical complexity, we implemented a stratified strategy: (1) phenotypic differences related to HFD group as a whole and LFD as a whole were assessed, consistent with our stratified analysis framework; (2) high-dimensional microbiota/metabolite features were evaluated using contextualized "same-oil diet" inter-group comparisons. This dual approach leverages fundamental principles of high-dimensional omics statistics, effectively mitigating false-negative risks induced by multiple testing correction^[78, 79].

An appropriate balance between dietary fat and carbohydrate intake is essential for maintaining glucolipid metabolic homeostasis, as both excessively high and excessively low proportions can disrupt metabolic regulation. This concept is corroborated by a 24-week study conducted by Zhang et al. [34], which reported no significant differences in serum levels of TG, TC, HDL-C, or LDL-C across groups with varying dietary fat energy contributions (15%, 25%, 35%, and 45%). The same study further demonstrated that a low-fat/high-carbohydrate diet did not induce weight gain in mice, whereas a high-fat diet increased the risk of hepatic steatosis and structural damage to the small intestine—findings consistent with the present research. Additionally, Zhang et al. revealed that the low-fat/high-carbohydrate diet primarily promotes endogenous cholesterol synthesis in the mouse liver through the ASGR1-mTORC1-USP20-HMGCR signaling pathway. Interestingly, however, diets containing rapeseed oil or olive oil within the HFD group actually led to a reduction in visceral fat compared to the LFD group. We speculate that this effect may be attributed to the high oleic acid content in these oils, as groups fed HFD enriched with oleic acid (e.g., olive oil, rapeseed oil) exhibited decreased visceral fat and lower body fat percentage. This observation aligns with previous reports suggesting that high-oleic acid foods may help reduce abdominal fat accumulation^[80, 81].

Under HFD the universal rise in serum TG can be attributed to excess energy intake driving hepatic TG synthesis and increased VLDL secretion together with impaired peripheral clearance of VLDL/chylomicrons linked to insulin resistance^[82]. Crucially, the fatty-acid profile of each oil (SFA vs MUFA vs PUFA), their antioxidant/polyphenol contents, and their differential regulation of hepatic lipid-metabolic genes (e.g. SREBP-1c, PPAR α) and lipoprotein components (ApoB, ApoA1, LDL receptor) determine whether TC, LDL-C and HDL-C rise or fall. For example, rapeseed oil — rich in oleic acid and ALA — can modulate apolipoprotein expression, lipoprotein remodeling and gut microbial diversity, producing phenotypes such as higher TC and HDL-C in some HFD contexts^[83]. Additionally, erucic acid accumulation is linked to oxidative stress and inflammatory signaling and can perturb cholesterol esterification, apolipoprotein and receptor expression, and bile-acid handling, which may explain the rapeseed-oil-specific increases in TC, LDL-C and even HDL-C under HFD by altering hepatic cholesterol processing and lipoprotein remodeling^[84]. In contrast, soybean oil (high in linoleic ω -6 PUFA) may promote higher LDL-C under HFD by altering hepatic cholesterol esterification, LDL-receptor mediated uptake, and inflammatory/oxidative pathways^[85]. Olive oil, although rich in oleic acid and polyphenols that protect against LDL oxidation and support HDL function, can still yield higher circulating TG when total fat/caloric load is excessive; its bioactives may, however, mitigate LDL oxidation and preserve HDL functionality^[86]. Finally, oil-specific effects on the gut microbiome, bile acid signaling and low-grade inflammation further modulate host lipid handling, explaining the oil-dependent differences in TC/LDL-C/HDL-C despite a common TG rise. In short, the interaction of fatty-acid composition $\rightarrow$ hepatic gene/protein regulation $\rightarrow$ lipoprotein production/clearance $\rightarrow$ oxidation/inflammation and microbiome effects offers a coherent mechanistic explanation for our observed results^[14].

After analyzing the phenotypic differences between the HFD group and the LFD group, we further focused on studying the differences in gut microbiota between low-fat/high-carbohydrate diets and high-fat/low-carbohydrate diets. Systematic comparison revealed that compared to their respective low-fat controls, the HFD groups of *I. polycarpa* crude oil (HID vs. LID), rapeseed oil (HRD vs. LRD), and olive oil (HOD vs. LOD) all exhibited significant enrichment of potentially beneficial bacterial taxa: the class *Clostridia* (enhancing anti-inflammatory responses by inducing regulatory T cells^[87]), the family *Lachnospiraceae* (alleviating inflammation, hepatic neutrophil infiltration, oxidative stress, while improving microbiota composition and intestinal permeability^[88]), and the family *Muribaculaceae* (whose abundance positively correlates with butyrate production and may influence barrier function and inflammation via butyrate modulation^[89], although some studies suggest a potential pro-inflammatory role via kynurenine metabolites^[90]; notably, neither study validated function via FMT, which may explain this contradiction). Furthermore, except for the lard group, the HFD groups of *I. polycarpa* crude oil (HID vs. LID), rapeseed oil (HRD vs. LRD), olive oil (HOD vs. LOD), and soybean oil (HSD vs. LSD) all showed a significant reduction in the relative abundance of *Bifidobacterium*, renowned for its multiple health benefits including pathogen resistance, hypertension reduction, inflammation alleviation, diabetes improvement, oxidative stress mitigation, and modulation of microbiome, immunity, and anti-cholesterol effects^[91]. Of particular note, within the *I. polycarpa* crude oil group during the low-fat to high-fat transition (HID vs. LID), the relative level of Homostachydrine—reported to possess anti-obesity, neuroprotective, renoprotective, and cardiovascular protective effects^[92]—significantly increased, while Tensyic acid A significantly decreased.

This study has several limitations. When comparing the efficacy of different dietary oils, fecal lipid excretion represents a major confounding factor, as it is affected by variations in food intake and energy balance. This, in turn, may obscure the accurate evaluation of phenotypes, gut microbiota, and metabolic profiles. In addition, the current experimental design included only high-fat and low-fat diet groups, without a normal-fat intake control group. As a result, the observed differences between *I. polycarpa* crude oil and other edible oils cannot be readily extrapolated to routine dietary practices in broader populations. Furthermore, the study duration was limited to eight weeks, which may have been too short to capture significant differences between low-fat/high-carbohydrate and high-fat/low-carbohydrate dietary patterns. It must be acknowledged that previous studies have demonstrated excessive LA can induce oxidative stress through direct pathways (e.g., mitochondrial damage and enzymatic aldehyde production)^[93, 94] or indirect pathways (dysbiosis-inflammation-gut leak cascade)^[95], or synergistically, ultimately causing Hepatointestinal injury. It is noteworthy that we observed the *I. polycarpa* crude oil group maintained a microbiota dominated by beneficial bacteria under low-fat dietary conditions compared to other oil groups. Based on this finding, we hypothesize that the more pronounced Hepatointestinal injury induced by *I. polycarpa* crude oil (relative to other oils) may stem from a combination of excess LA and microbiota-mediated indirect pathways, rather than from microbiota-mediated indirect pathways alone. Future research will focus on optimizing blending ratios of *I. polycarpa* crude oil to modulate its ω -6/ ω -3

PUFA ratio, thereby reducing excessive LA content. We will evaluate the efficacy of this intervention and employ FMT experiments to determine whether phenotypic improvements result not only from ω -6/ ω -3 ratio optimization—which reduces inflammation and ameliorates hepatic/intestinal pathologies—but also from beneficial gut microbiota modulation. Upon identifying the optimal ratio, the study will be extended in duration and comparative analyses will be performed against commonly consumed edible oils under low-, medium-, and high-fat dietary conditions to elucidate differential effects.

5. Conclusions

In summary, compared to the olive oil, lard, rapeseed oil, or soybean oil groups, the *I. polycarpa* crude oil group exhibited the following characteristics: (1) compared with the rapeseed oil group, it induced milder metabolic disorder-related damage, but more severe damage than the olive oil, lard, and soybean oil groups; (2) gut microbiota and metabolites showed diet-dependent differences: under low-fat conditions, the relative abundance of intestinal *Lactobacillus* was significantly higher in the *I. polycarpa* crude oil group than in the other four oil groups, its Rumphellolide B abundance was significantly higher than in the olive oil, lard, and rapeseed oil groups, while Tensyucic acid A and 7-Methoxydeoxymorellin abundances were significantly higher than in the soybean oil group; under high-fat conditions, both the relative abundance of intestinal *Gastranaerophilales* and Vanillin abundance were significantly higher in the *I. polycarpa* crude oil group than in the other four oil groups; (3) Compared with a low-fat diet (26% fat, 70% carbohydrate energy), a high-fat, low-carbohydrate diet (60% fat, 10% carbohydrate energy) induces more adverse metabolic outcomes, such as weight gain, visceral fat accumulation, dyslipidemia (elevated serum TG), hepatic steatosis, and impaired intestinal barrier integrity. Interestingly, no significant differences were observed in liver antioxidant enzymes (SOD, GSH-Px) or inflammatory cytokines (IL-4, IL-6, TNF- α). These findings suggest that a low-fat, high-carbohydrate diet does not necessarily confer the expected health benefits, and in some respects, its impact may not differ substantially from that of a high-fat, low-carbohydrate diet. This study systematically elucidates the differences in physiological effects between *I. polycarpa* crude oil and common edible oils, reveals its potential health risks, and provides science-based guidance for its health-oriented applications. By examining five different types of dietary oils, we found that when the type of oil intake remains constant, a low-fat/high-carbohydrate diet can also lead to metabolic syndrome, just as a high-fat/low-carbohydrate diet does. This finding suggests that regardless of the fat-to-carbohydrate ratio, an unbalanced dietary structure increases the risk of metabolic disorders.

Funding Sources

This work received funding from the National Natural Science Foundation of China (32472256), the Natural Science Foundation of Hubei Province (2023AFD080), The Open Fund of Hubei Engineering Research Centre of selenium food nutrition and health intelligent technology (Hubei Minzu University) (PT082401).

Acknowledgment

We would like to express our heartfelt gratitude to Prof. Kai Luo and Prof. Jianquan Kan for their meticulous guidance and invaluable insights. Additionally, we extend our sincerest appreciation to Wekemeng Bioincloud for its exceptional technical support.

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