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Fructooligosaccharides enhance the gut–liver effects of raspberry polyphenols in a rat model of diet-induced metabolic dysfunction-associated steatotic liver disease

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ABSTRACT

Background: MASLD is closely linked to gut–liver axis dysfunction, including altered microbial activity, disturbed bile acid metabolism, and hepatic lipid accumulation. Raspberry polyphenols may help counteract these disturbances, whereas fructooligosaccharides (FOS) may enhance their microbial transformation and biological activity.

Methods and results: Male Wistar rats were fed for 12 weeks with a control diet, a high-fat low-fiber diet (HF), or HF diets supplemented with raspberry polyphenols alone (HF + PP) or combined with FOS (HF + PP + FOS). Compared with HF feeding alone, the combined treatment produced the most favorable response, including lower hepatic triglyceride and cholesterol accumulation, improved plasma lipid indices, higher hepatic and plasma levels of polyphenol-derived metabolites, reduced hepatic Ahr/AHR expression, increased Cyp8b1/CYP8B1 expression, enhanced short-chain fatty acid production, altered cecal bile acid profile, lower activity of selected bacterial enzymes, and a distinct shift in cecal microbiota composition.

Conclusion: Combined supplementation with raspberry polyphenols and fructooligosaccharides partly alleviated selected metabolic and intestinal disturbances induced by a high-fat, low-fiber diet. The findings may support the involvement of the gut–liver axis and suggest that FOS may strengthen the functional effects of raspberry polyphenols by promoting microbial fermentation and increasing the formation and availability of smaller bioactive metabolites. Overall, the combination of a polyphenol-rich raspberry preparation with a microbiota-targeting prebiotic may represent a nutritionally relevant strategy for supporting metabolic homeostasis under MASLD-like conditions.

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD; formerly NAFLD) is currently one of the most prevalent chronic liver disorders in industrialized countries [1]. It is characterized by excessive lipid accumulation in the liver and is commonly accompanied by hypertriglyceridemia, elevated low-density lipoprotein (LDL) cholesterol, low-grade inflammation, and disturbances in redox homeostasis [2]. In a proportion of affected individuals, MASLD progresses to metabolic dysfunction-associated steatohepatitis (MASH; formerly NASH), a more severe stage marked by hepatic inflammation and fibrosis, which may eventually lead to cirrhosis and end-stage liver disease [3]. With the global burden of MASLD continuing to rise, MASH-related cirrhosis is expected to become one of the leading indications for liver transplantation in many developed countries [4,5]. Despite the scale of this problem, effective pharmacological options remain limited,

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highlighting the need for preventive and dietary strategies supported by clear biological rationale.

The development of MASLD should be viewed within a broader metabolic framework involving the gut–liver axis [6]. Dietary patterns rich in fat and poor in fermentable fiber, which are common in many modern societies, may impair gut microbial activity, disturb intestinal homeostasis, and promote the formation of metabolites that adversely affect host metabolism [7]. These alterations are increasingly associated with obesity-related disorders, including hepatic steatosis, insulin resistance, and chronic low-grade inflammation. Accordingly, the intestine should be considered not only as a site of digestion and absorption, but also as an important regulator of metabolic and inflammatory processes relevant to liver function.

Among the key mechanisms linking the intestine and liver is bile acid (BA) metabolism. In addition to facilitating intestinal lipid absorption, bile acids act as signaling molecules involved in the regulation of lipid and glucose metabolism and in the control of inflammatory responses [8]. These actions are mediated mainly through bile acid receptors, especially the farnesoid X receptor (FXR). Disturbances in BA–FXR signaling are increasingly recognized as contributors to hepatic lipid accumulation and the progression of metabolic liver disease [9]. In the liver, activation of FXR induces the small heterodimer partner (SHP), which regulates the transcription of enzymes responsible for the conversion of cholesterol into bile acids, thereby contributing to bile acid and cholesterol homeostasis [10]. At the same time, excessive accumulation of more hydrophobic and cytotoxic bile acids may promote epithelial injury, oxidative stress, and metabolic dysfunction, further strengthening the link between intestinal disturbances and hepatic damage.

Hepatic bile acid synthesis proceeds through two main pathways. The classical pathway involves cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1), whereas the alternative pathway depends primarily on sterol 27-hydroxylase (CYP27A1) and oxysterol 7α-hydroxylase (CYP7B1) [8]. CYP7A1 is the rate-limiting enzyme in bile acid biosynthesis and is tightly regulated by hepatic FXR signaling [11]. CYP8B1, in turn, determines the proportion of 12α-hydroxylated bile acids, thereby influencing bile acid hydrophobicity and intestinal cholesterol absorption [12,13]. Besides FXR- and CYP7B1-dependent regulation, bile acid homeostasis may also be modulated by the aryl hydrocarbon receptor (AHR) [14]. AHR is a ligand-activated transcription factor involved in lipid metabolism, oxidative stress responses, and inflammation, which makes it relevant to the pathogenesis of MASLD [15]. Altogether, these observations indicate that bile acid metabolism is not merely an endpoint of hepatic cholesterol turnover, but a regulatory hub integrating nuclear receptor signaling with inflammatory and redox pathways implicated in MASLD.

Polyphenols are dietary bioactives widely recognized for their antioxidant and anti-inflammatory properties, as well as for their interactions with gut microbiota and host metabolic signaling. Depending on their structure and biological context, they may also influence AHR activity, acting either as agonists or antagonists [16]. Berries are among the richest dietary sources of polyphenols, and raspberries are particularly abundant in ellagitannins and anthocyanins [17]. Previous in vitro and in vivo studies have shown that polyphenolic extracts obtained from raspberry juice can downregulate AHR expression in hepatocytes and hepatic tissue [18,19]. However, the physiological effectiveness of many berry polyphenols is limited by their low bioavailability. In foods, these compounds are often present as esters, glycosides, or polymers that are poorly absorbed in the small intestine and therefore reach the colon, where they undergo microbial hydrolysis and further transformation into smaller metabolites with distinct biological properties. After absorption, these metabolites are further conjugated in the intestine and liver through methylation, sulfation, and glucuronidation, which additionally affects their systemic availability and activity. As a result, the local effects of native polyphenols are likely to occur mainly in the gastrointestinal tract, whereas their systemic actions depend largely on microbiota-derived metabolites.

This limited bioavailability represents an important challenge for the development of effective and safe polyphenol-based dietary strategies. Simply increasing polyphenol intake is not an optimal solution, because high doses of ellagitannin-rich preparations may impair the bioavailability of essential nutrients and inhibit digestive or microbial enzymatic activity [20]. A more physiologically relevant approach may be to enhance the microbial conversion of complex polyphenols into smaller and potentially more bioavailable metabolites, including ellagic acid derivatives, hydroxybenzoic acids, urolithin-related compounds, and other low-molecular-weight products capable of reaching systemic organs in biologically meaningful amounts. Notably, polyphenols may also reciprocally shape gut microbiota composition by stimulating beneficial taxa and suppressing less favorable microbial groups, which further supports their relevance within the gut–liver axis [21].

Fructooligosaccharides (FOS) appear particularly promising in this context. As prebiotic substrates, they can stimulate microbial activity and may support the biotransformation of polyphenols into absorbable metabolites, thereby potentially enhancing their systemic and hepatic effects [19]. In addition, fermentable fibers such as FOS are utilized by colonic bacteria to produce short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, which improve intestinal physiology, modify luminal conditions, and influence host metabolism [22,23]. These microbial metabolites may contribute to the regulation of hepatic lipid metabolism, oxidative balance, inflammatory status, and possibly also AHR-related pathways. Therefore, the combination of raspberry polyphenols and FOS represents a mechanistically justified strategy to target the gut–liver axis by simultaneously enhancing microbial polyphenol transformation, increasing the availability of active metabolites, and providing SCFA-mediated support for intestinal and metabolic homeostasis.

Taken together, available evidence suggests that raspberry-derived polyphenols may influence not only oxidative and inflammatory status in the liver, but also bile acid-related and lipid-metabolic pathways relevant to MASLD. At the same time, prebiotic FOS may improve intestinal function, stimulate microbial activity, and enhance the formation of systemically active polyphenol metabolites. Based on this rationale, we hypothesized that combined supplementation with raspberry polyphenol extract and FOS would strengthen the beneficial effects of polyphenols along the gut–liver axis, including the modulation of intestinal environment, microbial metabolism, and hepatic lipid and bile acid homeostasis, thereby attenuating disturbances induced by a high-fat, low-fiber diet. Accordingly, the aim of this study was to determine whether the addition of fructooligosaccharides enhances the functional potential of raspberry polyphenols in an experimental model of diet-induced MASLD.

2. Materials and methods

2.1. Preparation of raspberry polyphenolic preparation

Clear raspberry juice, produced from frozen raspberries of the ‘Polana’ cultivar, was used to prepare a polyphenol-rich extract at the processing facility of the Institute of Horticulture in Skierniewice. The fruits were purchased from a commercial frozen food supplier, then thawed, enzyme-treated, and mechanically pressed. The resulting juice was centrifuged and subsequently pasteurized. Juice with a dry matter content of 11% (measured in degrees Brix) served as the starting material for the extraction of polyphenols. Polyphenols were extracted using a 10-l column packed with an 8-l Amberlite XAD sorption bed, preconditioned according to the manufacturer's instructions. A total of 48 l of juice—equivalent to six bed volumes (BVs)—was passed through the column at a flow rate of 1 BV per hour. The column was then washed with 2 BVs of water followed by 1 BV of 5% ethanol. Polyphenol desorption was achieved with 1.5 BVs of 25% ethanol applied in a counter-current flow. The collected eluate (total volume: 12 L) was

concentrated using a rotary evaporator in two stages: ethanol removal followed by volume reduction to 1 l. The resulting concentrated extract, with a dry matter content of 14.6% (Brix), was subsequently freeze-dried. A detailed description of the polyphenol preparation is provided in Supplemental File 1.

2.2. Polyphenolic profile of raspberry preparation

The polyphenol profile of the preparation was analyzed following the methodology previously described by Fotschki et al. (2018) [18]. Briefly, a Knauer Smartline HPLC system equipped with a diode array detector (DAD) was employed to quantify ellagitannins and anthocyanins in methanol-diluted samples (40 mg/10 mL for ellagitannins; 75 mg/10 mL for anthocyanins). Ellagitannin analysis was conducted using a Gemini C18 column (250 × 4.6 mm, 5 µm; Phenomenex, Torrance, CA, USA), while anthocyanins were separated with a Gemini C18 column (150 × 4.6 mm, 5 µm; Phenomenex). Detection of ellagitannins was performed at 250 nm, and quantification was based on calibration curves prepared using sanguiin H-6, lambertianin C, and ellagic acid standards (Extrasynthese, Genay, France), synthesized at the Łódź University of Technology according to Klewicka et al. (2016) [24]. Anthocyanins were detected at 520 nm and quantified using a calibration curve prepared with cyanidin-3-O-glucoside (Extrasynthese). Compound identification for both classes was supported by LC-MS analysis using a Q Exactive Orbitrap system (Thermo Fisher Scientific), as described in Fotschki et al. (2018) [18].

Proanthocyanidin content in the preparation was determined via acid-catalyzed degradation of polymeric proanthocyanidins in the presence of phloroglucinol, following the method outlined by Sójka et al. (2013) [25]. Phloroglucinolysis was performed on 20 mg of freeze-dried preparation. The resulting degradation products were separated using identical chromatographic parameters (column type, gradient, mobile phases, and standards), and detected with a fluorescence detector (Shimadzu RF-10Axl; λ_ex = 278 nm, λ_em = 360 nm). A Shimadzu HPLC system comprising an LC-20 CE pump, DGU-20ASR degasser, CTO-10AS column oven, and SIL-20 AC autosampler was used. Concentrations of terminal units were calculated using standard curves for (−)-epicatechin and (+)-catechin, whereas extender units were quantified using (−)-epicatechin–phloroglucinol adduct. Free (−)-epicatechin and (+)-catechin were analyzed under the same HPLC conditions applied for polyphenol analysis. The average degree of polymerization (DP) was calculated from the molar ratio of total flavan-3-ol units (phloroglucinol adducts + terminal units) to terminal flavan-3-ols. Chemical characteristics of the analyzed preparation are presented in Table 1.

Table 1
Polyphenol composition of raspberry juice phenolic preparations.

Compound	Mean	SD
Ellagitannins and ellagic acid conjugates		
Lamb. C	1040.5	17.0
Lamb. C-EA	104.9	2.1
Sang. H-10 iso.1	595.8	3.2
Sang. H-10 iso.2	530.2	6.2
Sang. H-6	5043.2	10.1
EA	263.9	8.4
EA-PC	193.6	2.4
EA-APC	43.8	1.2
MEA-PC	71.6	3.3
Total ET	7314.6	22.9
Total EAC	572.9	15.2
Total ET + EAC	7887.5	10.9
Anthocyanins		
Cy-soph	4453.1	35.7
Cy-glu-rut	406.3	1.4
Cy-glu	1320.3	5.8
Cy-rut	153.4	0.5
Plg-glu	55.7	2.9
Total ACY	6388.7	39.4
Flavanols		
(+)-catechin	27.3	0.9
(−)-epicatechin	354.7	12.4
Proanthocyanidins	590.3	20.4
mDP	2.2	0.0
Terminal units [%]	44.8	0.6
(+)-catechin	4.5	0.1
(−)-epicatechin	40.4	0.5
Extension units [%]	55.2	0.6
(+)-catechin	45.8	0.9
(−)-epicatechin	9.3	0.3
Total FLAVA	972.4	7.9
Total phenolics content	15,248.6	50.5

The values are provided as a mean ± standard deviation (SD; mg/100 g). Analyses were performed in triplicate. Cy-soph, cyanidin-sophoroside; Cy-glu-rut, cyanidin-glucosyl-rutinoside; Cy-glu, cyanidin-glucoside; Cy-rut, cyanidin-rutinoside; Pel-gluc, pelargonidin-glucoside; EA, ellagic acid; EA-PC, ellagic acid pentose conjugate; EA-APC, ellagic acid acetyl pentose conjugate; MEA-PC, methyl ellagic acid pentose conjugate; Sang. H-10 and H-6, sanguiin H-10 and H-6; Lamb. C, Lambertianin C; TPH – total polyphenols. The content of ellagitannins and ellagic acid conjugates was calculated based on the ellagic acid, lambertianin C, sanguiin H-6 and lambertianin C standards. The content of anthocyanins was calculated based on the cyanidin-glucoside standard.

2.3. In vivo experiment and preparation of diet

All procedures complied with ARRIVE guidelines [26]. The in vivo experiment was approved by the regional Institutional Animal Care and Use Committee (Permission No. 51/2016; Olsztyn, Poland), and all procedures were carried out in accordance with the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes. A total of thirty-two male Wistar rats (56 days old) were enrolled in the study and randomly allocated to four groups ($n = 8$ per group). Animals were housed individually in metabolic cages (Tecniplast Spa, Buguggiate, Italy) under controlled environmental conditions: a 12-h light/dark cycle, ambient temperature of 21 ± 1 °C, relative humidity of 55% ± 10%, and a ventilation rate of 20 air changes per hour. The average initial body weight was 173.1 ± 2.1 g.

All rats received a modified version of the American Institute of Nutrition's semipurified casein diet for laboratory rodents, along with ad libitum access to tap water for 12 weeks. The control group (C) was fed a standard diet containing 5% cellulose as a fiber source. The high-fat (HF) diet group received a formulation enriched with 20% lard and 4% cellulose to induce MASLD -like hepatic dysfunction. Experimental groups included one receiving the HF diet supplemented with 0.3% raspberry polyphenols (HF + PP), and another receiving the same HF + PP diet enriched with 3% fructooligosaccharides (HF + PP + FOS). The experimental design was intentionally focused on evaluating the effect of combining raspberry polyphenols with FOS in the diet. This approach enabled assessment of the polyphenol-related response under microbiota-supporting conditions, while avoiding unnecessary duplication of previous studies that had already characterized the physiological effects of FOS administered alone. Accordingly, the study design was consistent with the 3R principle in animal experimentation, particularly Reduction, by restricting the number of groups to those required to address the specific objective of the study. Diets were administered ad libitum throughout the study period, and detailed diet compositions are provided in Supplemental Table 1.

2.4. Biological materials collection and animal protocol

Throughout the experimental period, rats were monitored daily for feed intake and changes in body weight. After 12 weeks of dietary intervention, animals were anesthetized with ketamine and xylazine (100 mg and 10 mg/kg body weight, respectively) diluted in physiological saline, in accordance with standard protocols for laboratory animal anesthesia. Euthanasia was performed by cervical dislocation. Following laparotomy, blood samples were collected from the vena cava

into heparinized tubes and centrifuged at 4 °C for 10 min at 350 ×g. Obtained plasma samples were stored at − 80 °C until analysis. After blood collection, the liver and caecum with digesta was excised, weighed, and immediately frozen in liquid nitrogen for subsequent further analyses.

2.5. Plasma biochemical parameters

Plasma biochemical parameters were analyzed using a Pentra C200 biochemical analyzer (Horiba, Tokyo, Japan). Quantification included plasma triglycerides, total cholesterol, HDL cholesterol fractions, and enzymatic activity of alanine transaminase (ALT) and aspartate transaminase (AST). The non-HDL cholesterol concentration for each sample was calculated by subtracting HDL cholesterol from total cholesterol, based on the obtained lipid profile.

2.6. Plasma antioxidant potential parameters

Plasma antioxidant-related parameters were assessed using a combination of commercial diagnostic kits and spectrophotometric assays. Catalase activity was measured using a commercial diagnostic kit (Oxis International, Inc., Portland, OR, USA). Ceruloplasmin activity in plasma was determined according to a modified *p*-phenylenediamine assay [19]. Briefly, 20 µL of plasma was diluted in 1.6 mL of 0.4 M acetate buffer (pH 5.5) and mixed with 0.2 mL of freshly prepared buffered *p*-phenylenediamine solution (27.6 mmol/L; Sigma, Poznań, Poland). The samples were incubated at 37 °C, and absorbance was measured spectrophotometrically at 530 nm after 5 min (blank) and 60 min (reaction). The enzymatic reaction was stopped by the addition of 0.2 mL of sodium azide solution (1.5 mol/L; Sigma, Poznań, Poland). Total antioxidant status and superoxide dismutase activity in blood were determined using commercial diagnostic kits from Randox Laboratories Ltd. (Crumlin, UK). Total glutathione content in blood was measured using ELISA kits from Cell Biolabs, Inc. (San Diego, CA, USA). Lipid hydroperoxides were quantified using competitive adduct ELISA kits from Blue Gene Biotech (Shanghai, China).

2.7. Liver lipid and antioxidant status

Liver lipids were extracted using the Folch method [27]. Quantification of hepatic fat, total cholesterol, and triglycerides within the isolated lipid fraction was performed using commercial assay kits (Cholesterol DST, Triglycerides DST; Alpha Diagnostics Ltd., San Antonio, TX, USA). Malondialdehyde (MDA) levels, an indicator of lipid peroxidation, were assessed according to the method described by Botsoglou et al. (1994) [28]. Spectrophotometric measurements were conducted at 532 nm, and MDA concentrations were expressed as nanograms per gram of liver tissue. Hepatic levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined using the enzymatic recycling procedure previously described [29].

2.8. Bile acids analysis formed through microbial metabolism in the cecum

Bile acids in the cecal digesta were quantified using a Shimadzu liquid chromatography system (Kyoto, Japan) coupled with a Shimadzu LCMS-2020 mass spectrometer. Chromatographic separation was carried out on an Avantor ACE C18-amide column (75 × 2.1 mm, 2.7 µm particle size) via gradient elution using solvent A (0.01% *v*/v formic acid in water) and solvent B (methanol:acetonitrile, 1:9 *v*/v). The column temperature was maintained at 45 °C, with a flow rate of 0.35 mL/min and an injection volume of 1 µL. The gradient program was as follows: 0–8 min, 40% B; 8–14 min, 50% B; 14–15.3 min, ramp from 50% to 100% B; and 15.3–17.4 min, return to 40% B. Mass data acquisition and analysis were performed using LabSolutions 5.109 software (Shimadzu). Quantification of bile acids was conducted using external standards (0.01–1 µM), and linear calibration curves were generated with correlation coefficients ranging from 0.994 to 0.997. All measurements were performed in triplicate for each sample to ensure analytical precision.

2.9. Cecal microbial enzymatic activity and short-chain fatty acids

SCFA profiles and concentrations in cecal digesta were determined using a modified protocol based on Yao et al. (2022) [30]. UHPLC-MS-grade water, methyl tert-butyl ether (MTBE), and hydrochloric acid were purchased from Sigma-Aldrich and Merck (Darmstadt, Germany). Liver samples stored at − 80 °C in Eppendorf tubes were thawed at 4 °C. Each 100 mg digesta sample was mixed with 24 µL of 2 N HCl and 976 µL of water, homogenized, and centrifuged at 3000 ×*g* for 10 min at 4 °C. From the resulting supernatant, 400 µL was transferred and combined with 24 µL of 2 N HCl and 376 µL of cold MTBE. The mixture was vortexed for 15 s and centrifuged again under the same conditions. Approximately 60 µL of the upper MTBE layer was recovered and stored at − 20 °C prior to analysis. For gas chromatography, 1 µL of MTBE extract was injected into a Shimadzu Nexis GC-2030 system coupled to a GCMS-QP2020NX mass spectrometer (Shimadzu, Kyoto, Japan), operating at a 5:1 split ratio. The injection port was maintained at 240 °C. SCFA were separated using a Sh-PolarWax-MS column (30 m × 0.25 mm × 0.25 µm; Shimadzu, USA). The oven program was set as follows: initial temperature 100 °C (held for 0.5 min), ramped to 175 °C at 10 °C/min, then to 240 °C at 40 °C/min with a final hold of 5 min, resulting in a total run time of 14.63 min. The helium carrier gas flow rate was 1.2 mL/min. Both the transfer line and ion source temperatures were maintained at 250 °C. Single Ion Monitoring (SIM) under electron impact ionization mode was used at a scan rate of 10 scans per second. Quantification was based on calibration curves prepared from a standard mixture of pure acetic, propionic, butyric, isobutyric, isovaleric, and valeric acids (Sigma Co., Poznań, Poland), with concentration ranges between 0.01 and 1 µM and correlation coefficients from 0.995 to 0.998.

In addition to SCFA analysis, cecal fermentation activity was assessed by measuring the activity of selected bacterial enzymes released into the cecal environment, namely α-galactosidase, α-glucosidase, β-galactosidase, β-glucosidase, and β-glucuronidase, according to a previously described method [31]. Enzyme activity was determined based on the rate of release of p- or o-nitrophenol from corresponding nitrophenyl substrates. The following substrates were used: *o*-nitrophenyl-β-D-galactopyranoside for β-galactosidase, *p*-nitrophenyl-α-D-glucopyranoside for α-glucosidase, *p*-nitrophenyl-β-D-glucopyranoside for β-glucosidase, *p*-nitrophenyl-α-D-galactopyranoside for α-galactosidase, and *p*-nitrophenyl-β-D-glucuronide for β-glucuronidase. For the determination of extracellular enzyme activity, the reaction mixture consisted of 0.3 mL of substrate solution (5 mM) and 0.2 mL of a 1:10 (*v*/v) dilution of cecal digesta in 100 mM phosphate buffer (pH 7.0), prepared after centrifugation at 7211 ×*g* for 15 min. Samples were incubated at 37 °C, and the released p-nitrophenol was quantified at 400 nm, whereas o-nitrophenol was measured at 420 nm following the addition of 2.5 mL of 0.25 M ice-cold sodium carbonate. Enzyme activity was expressed as µmol of product formed per hour per g of digesta.

2.10. Liver and plasma bioactive compounds derived from the raspberry polyphenolic preparation

The analysis was focused on plasma and liver metabolites in order to characterize polyphenol-derived compounds that reached the systemic circulation and hepatic tissue, and were therefore potentially relevant to liver function in the studied model. The analysis of bioactive compound profiles and concentrations in liver tissue and blood plasma was performed according to the methodology described previously by Fotschki et al. (2022) [19], with minor modifications for plasma samples. Briefly, liver samples (0.25 g) were lyophilized and cryogenically pulverized using a Freezer/Mill system (Spex SamplePrep 6870, Metuchen, NJ, USA). Plasma samples were analyzed directly. Both liver powder and

plasma aliquots were transferred into 5 mL test tubes and extracted with a solvent mixture consisting of methanol, water, and formic acid (80:15:5, *v*/v/v). Extraction was carried out by adding 4 mL of the solvent, followed by five alternating cycles of vortexing (30 s) and sonication (30 s; VC 750, Sonics & Materials, Newtown, CT, USA). Subsequently, samples were centrifuged at 13,200 ×*g* for 20 min at 4 °C using a 5415R centrifuge (Eppendorf, Wesseling, Germany). The resulting supernatants were evaporated to dryness under a nitrogen stream at 35 °C, and the residues were stored at −80 °C until further analysis. Dried sample residues were reconstituted in 100 μL of 80% (*v*/v) methanol containing 0.9% (v/v) formic acid, followed by centrifugation in Micro-Spin PES Filter Tubes (Ciro Manufacturing Corporation, Deerfield Beach, USA) for 20 min at 13,200 ×g at 4 °C. After additional dilution with the same solvent, if necessary, the samples were analyzed using a high-performance liquid chromatography (HPLC) system (LC-200, Eksigent, Canada) coupled to a QTRAP 5500 mass spectrometer (AB Sciex, Canada) equipped with a triple quadrupole, ion trap, and electrospray ionization (ESI) source.

Chromatographic separation was performed on a HALO C18 column (100 mm × 0.5 mm, 2.7 μm; Eksigent) maintained at 45 °C with a flow rate of 18 μL/min. Elution was carried out using a binary gradient consisting of solvent A (0.9% formic acid in water, *v*/v) and solvent B (0.9% formic acid in acetonitrile, v/v). The gradient program was as follows: 0–0.5 min, 0.4% B; 0.5–2.0 min, linear increase to 95% B; 2.0–3.0 min, isocratic at 95% B; 3.0–3.5 min, decrease to 0.5% B; 3.5–4.0 min, re-equilibration at 0.4% B.

Mass spectrometric analysis was conducted under positive ESI mode using the following parameters: curtain gas at 25 L/min, ion spray voltage at 5300 V, interface temperature at 350 °C, ion source gas 1 at 35 L/min, gas 2 at 30 L/min, declustering potential at 70–100 V, entrance potential at 10 V, collision energy at 30–40 eV, and collision cell exit potential at 15–20 V. Compound identification was based on retention time and matching multiple reaction monitoring (MRM) transitions to authenticated standards and published literature. Quantification was performed using external standards (0.01–1 μM), generating linear calibration curves with correlation coefficients ranging from 0.995 to 0.998. All analyses were conducted in triplicate for each sample to ensure reproducibility and accuracy.

2.11. Liver gene expression

Quantitative real-time PCR (qRT-PCR) was performed using a previously established protocol (Opyd et al.*, 2018). Total RNA was extracted from liver tissue using TRI Reagent (Thermo Fisher Scientific), following the manufacturer's instructions. The expression of target genes was normalized to β-actin*, which served as the reference gene. mRNA levels of aryl hydrocarbon receptor (Ahr), cholesterol 7α-hydroxylase (*Cyp7a1*), steroid 7α-hydroxylase (*Cyp7b1*), sterol 27-hydroxylase (*Cyp27a1*), sterol 12α-hydroxylase (*Cyp8b1*), and the nuclear receptors farnesoid X receptor (*Fxr*) and small heterodimer partner (*Shp*) were quantified using single-tube TaqMan® Gene Expression Assays (Life Technologies, CA). Amplification was carried out on a 7900HT Fast Real-Time PCR System (Applied Biosystems).

2.12. Protein isolation from the liver and Western blot analysis

Protein isolation was performed using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Rockford, USA), following the manufacturer's instructions. Frozen liver tissues from all experimental groups were homogenized and sonicated (3 × 5 s; Vibro-Cell VCX 130 PB, Sonics) in cytoplasmic extraction buffer CER1, supplemented with a protease inhibitor cocktail, phosphatase inhibitor cocktail, and phenylmethanesulfonyl fluoride (PMSF), all from Sigma-Aldrich. Protein concentrations were determined using the Bradford assay (Bradford Reagent, Sigma-Aldrich).

Aliquots containing 40 μg of protein were separated on 9% tricine SDS-PAGE gels and subsequently transferred onto polyvinylidene difluoride (PVDF) membranes (Merck Millipore). Membranes were incubated overnight at 4 °C with primary antibodies specific for: CYP8B1 (1:500, Cat# ab191910, Abcam), AHR (1:200, Cat# MA1–514, Invitrogen), and β-actin (1:1000, Cat# 8226 and Cat# 8227, Abcam). After washing, membranes were incubated for 1 h with fluorescent secondary antibodies: Alexa Fluor Plus 680 (1:10,000, Cat# A32729 and Cat# A32802, Invitrogen) and Alexa Fluor Plus 800 (Cat# A32735 and Cat# A32789, Invitrogen). Bands were visualized using the ChemiDoc Touch Imaging System (Bio-Rad Laboratories, Inc.) and quantified with Image Lab Software (Bio-Rad), based on molecular weight standards. β-actin was used as a loading control for normalization. To assess differences in protein expression, relative band intensities of target proteins were calculated as ratios to β-actin. One sample was designated as an internal quality control to normalize densitometric values across multiple blots, as described by Gawronska-Kozak (2011) [32].

2.13. Caecal bacterial sequencing

Caecal microbiota composition and diversity were analyzed by next-generation sequencing (NGS) of the bacterial 16S rRNA gene. Caecal digesta samples were collected into Eppendorf tubes and stored at −80 °C until further analysis. Total DNA was isolated using the QIAamp Fast DNA Stool Mini Kit (Qiagen) according to the manufacturer's instructions. The V3–V4 region of the 16S rRNA gene was amplified and used for library preparation following the recommended 16S metagenomic sequencing protocol. Sequencing was performed on an Illumina MiSeq instrument using a 2 × 250 bp paired-end approach. Raw sequencing data were deposited in the NCBI SRA database under BioProject accession number PRJNA853112. The sequencing results are presented in Fig. 9, and the full bioinformatic analysis pipeline is described in Supplementary File 2.

2.14. Statistics

Data are expressed as means ± standard error of the mean (SEM) or standard deviation (SD). Statistical analyses were performed using Statistica software (StatSoft Corp., Kraków, Poland). Differences among experimental groups were evaluated by one-way analysis of variance (ANOVA), followed by Duncan's multiple range post hoc test when assumptions of normality and homogeneity of variance were met. In cases of unequal variances, the Kruskal–Wallis test was applied, followed by Dunn's post hoc test with Bonferroni correction. For comparisons of liver and plasma bioactive compound concentrations between the HF + PP and HF + PP + FOS groups, an independent *t*-test was used. Differences were considered statistically significant at $P < 0.05$. For caecal bacterial sequencing data, beta diversity based on Bray–Curtis was analyzed using permutational multivariate analysis of variance (PERMANOVA), followed by pairwise PERMANOVA comparisons implemented in the vegan package and pairwiseAdonis in R. Differential abundance of amplicon sequence variants (ASVs) was assessed using DESeq2 based on a negative binomial distribution. To control for multiple testing, *P* values were adjusted using the Benjamini–Hochberg false discovery rate procedure.

3. Results

Dietary intake was significantly reduced (Table 2), whereas final body weight was increased in all groups fed obesogenic diets compared with control (C) rats ($P < 0.05$). The caecal tissue and digesta weights were significantly higher in the HF + PP + FOS than in the other groups ($P < 0.05$). In parallel, relative liver weight was significantly higher in the HF, HF + PP, and HF + PP + FOS groups than in the control group ($P < 0.01$), reflecting diet-induced hepatic alterations. The highest hepatic concentrations of total cholesterol and triglycerides were observed in the HF group ($P < 0.01$ vs. all other groups for cholesterol; $P < 0.05$ vs. C and HF + PP + FOS for triglycerides). In contrast, the lowest values were

Table 2
Growth parameters, liver lipid profile, and antioxidant potential of rats fed the experimental diets.

Parameters	Groups				ANOVA *p* Value
	C	HF	HF + PP	HF + PP + FOS	
Diet intake (g/day)	22.69 ± 0.6[a]	20.50 ± 0.69[b]	21.05 ± 0.58[b]	20.1 ± 0.47[b]	<0.05
Final body weight (g)	324.1 ± 3.3[b]	404.6 ± 5.2[a]	395.1 ± 7.3[a]	388.9 ± 12.7[a]	<0.01
Caecum					
Tissue mass (g)	0.512 ± 0.024[b]	0.526 ± 0.016[b]	0.557 ± 0.034[b]	0.691 ± 0.041[a]	<0.01
Digesta mass (g)	1.396 ± 0.114[b]	1.248 ± 0.113[b]	1.386 ± 0.164[b]	2.265 ± 0.156[a]	<0.01
Liver parameters					
Weight (g/100 g BW)	3.779 ± 0.097[b]	4.074 ± 0.091[a]	4.329 ± 0.189[a]	4.168 ± 0.185[a]	<0.01
Cholesterol (mg/g)	2.29 ± 0.15[c]	3.99 ± 0.52[a]	3.08 ± 0.27[b]	2.15 ± 0.13[c]	<0.01
Triglycerides (mg/g)	9.37 ± 1.1[b]	12.35 ± 1.42[a]	11.90 ± 1.71[ab]	9.83 ± 1.01[b]	<0.05
Fat content (%)	8.18 ± 0.35[c]	12.34 ± 0.75[a]	9.67 ± 0.49[b]	9.96 ± 0.36[b]	<0.01
GSH/GSSG (ratio)	25.47 ± 3.81[a]	11.13 ± 1.67[c]	9.58 ± 1.93[c]	15.17 ± 1.71[b]	<0.05
MDA (ng/g)	611.7 ± 32.3[b]	788.9 ± 37.5[a]	689.7 ± 20.4[b]	656.5 ± 23.1[b]	<0.05

Data with different superscripts in the same row are significantly different from each other at $P < 0.05$. Results are expressed as means ±SEM. C, control diet; HF, high-fat diet; HF + PP, high-fat diet supplemented with raspberry polyphenol preparation; HF + PP + FOS high-fat diet supplemented with raspberry polyphenol preparation and fructooligosaccharides. GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde.

recorded in the control and HF + PP + FOS groups (P < 0.01 vs. HF and HF + PP for cholesterol; $P < 0.05$ vs. HF for triglycerides). Consistently, hepatic fat content was highest in the HF group and lowest in the control group (P < 0.01 vs. all other treatments).

Relative to control animals, the hepatic reduced-to-oxidized glutathione (GSH/GSSG) ratio was significantly decreased in all high-fat-fed groups, with the most pronounced reduction observed in the HF and HF + PP groups (P < 0.05 vs. C and HF + PP + FOS). In line with this finding, hepatic malondialdehyde (MDA) levels were significantly elevated in the HF group compared with all other treatments ($P < 0.05$), whereas MDA concentrations in the HF + PP and HF + PP + FOS groups did not differ from those observed in control animals.

Consistent with the hepatic oxidative stress markers, plasma antioxidant indices were also significantly affected by the dietary treatments (Fig. 1). A significant increase in blood plasma total antioxidant status (TAS) followed the dietary addition of raspberry extract to a diet in comparison to the C and HF groups. Additionally, the HF + PP + FOS treatment had the highest value of plasma TAS ($P < 0.05$ vs. all other groups). The activity of superoxide dismutase in the plasma was the lowest in the HF rats ($P < 0.05$ vs. all other treatments). The activity of ceruloplasmin and catalase was significantly decreased in the plasma of rats fed the HF + PP + FOS diet as compared to other groups (P < 0.05 vs. C, HF, HF + PP). The highest and the lowest total glutathione contents in the plasma were noted in the C and the HF groups, respectively (in both cases P < 0.05 vs. remaining groups).

As shown in Fig. 2, plasma concentrations of total cholesterol, non-HDL cholesterol, and triglycerides were significantly increased in HF rats relative to all other experimental groups ($P < 0.05$ and $P < 0.01$). The HF + PP + FOS group exhibited the lowest total cholesterol levels, while the lowest non-HDL cholesterol concentrations were observed in both the control and HF + PP + FOS groups. Plasma triglycerides concentrations were lowest in control rats ($P < 0.01$ vs. HF and HF + PP). All high-fat-fed groups showed significantly reduced plasma HDL cholesterol concentrations compared with control animals ($P < 0.05$). Plasma aspartate aminotransferase activity was markedly increased in the HF and HF + PP groups (P < 0.05 vs. C and HF + PP + FOS), whereas alanine aminotransferase activity was elevated in all high-fat diet groups, irrespective of polyphenol or FOS supplementation (P < 0.01 vs. C).

Fig. 3 illustrates both cumulative and individual hepatic and cecal concentrations of bioactive compounds derived from dietary raspberry polyphenols in the HF + PP and HF + PP + FOS groups. Supplementation with FOS resulted in a significantly higher total hepatic concentration of polyphenol-derived metabolites compared with raspberry polyphenols alone ($P < 0.05$). Specifically, the HF + PP + FOS group exhibited significantly higher hepatic levels of ellagic acid (EA), methylcyanidin-sophoroside (Met-Cy-soph), and cyanidin-sophoroside (Cy-soph) than HF + PP rats ($P < 0.05$). Conversely, hepatic methylpelargonidin-glucoside (Met-Pel-gluc) concentrations were significantly lower in the HF + PP + FOS group than in the HF + PP group ($P < 0.05$). In parallel, the addition of FOS to the HF diet containing raspberry extract also increased the plasma concentration of polyphenol-derived metabolites, indicating greater systemic exposure to these compounds. Compared with the HF + PP group, rats receiving the HF + PP + FOS diet showed significantly higher plasma levels of DMEAG, nasutin glucuronide, ellagic acid, cyanidin, and the total sum of bioactive compounds ($P < 0.05$).

As presented in Fig. 4, hepatic expression of sterol 12α-hydroxylase (*Cyp8b1*) was significantly higher in the HF + PP + FOS group than in the HF and HF + PP groups ($P < 0.05$). Expression of farnesoid X receptor (*Fxr*) was increased in both HF + PP and HF + PP + FOS groups relative to HF rats (P < 0.05). In contrast, hepatic aryl hydrocarbon receptor (*Ahr*) expression was significantly lower in the HF + PP + FOS group than in the HF and HF + PP groups ($P < 0.05$). No significant differences were observed in the expression of *Cyp7a1*, *Cyp7b1*, *Cyp27a1*, or *Shp* among the high-fat-fed groups. Consistent with the gene expression results, protein analysis (Fig. 5) showed a significant reduction in hepatic AHR protein levels in the HF + PP + FOS group compared with the HF and HF + PP treatments. Conversely, the HF + PP + FOS group exhibited the highest hepatic CYP8B1 protein abundance among all experimental groups ($P < 0.05$ vs. C, HF, and HF + PP).

As shown in Fig. 6, the cecal concentration of cholic acid (CA) was significantly elevated in the HF + PP + FOS group ($P < 0.05$). The HF diet alone increased deoxycholic acid (DCA) and α-muricholic acid (α-MCA) concentrations compared with the control group (C). Notably, the highest levels of both DCA and α-MCA were observed in the HF + PP and HF + PP + FOS groups relative to the control animals (P < 0.05 vs. C and HF). In turn, β-muricholic acid (β-MCA) was significantly higher in all experimental groups than in the C group ($P < 0.05$). Hyodeoxycholic acid (HDCA) was significantly increased only in the HF + PP and HF + PP + FOS groups compared with controls (P < 0.05). Analysis of total bile acids further showed that both the HF and HF + PP diets increased the sum of measured bile acids formed through microbial metabolism, whereas the addition of FOS in the HF + PP + FOS group significantly enhanced this effect ($P < 0.05$ vs. C, HF, and HF + PP).

The dietary HF and HF + PP treatments caused a significant decrease in the cecal concentration of acetic, isobutyric, isovaleric, valeric acids, and total SCFA in comparison to the C and HF + PP + FOS groups (Fig. 7). The addition of raspberry extract and FOS to a high-fat diet resulted in the highest propionic acid concentration in the cecal digesta of rats ($P < 0.05$ vs. all remaining groups). The butyric acid cecal concentration was diminished by HF and HF + PP, but not by HF + PP + FOS dietary treatment as compared to the control C rats. A significant increase in the cecal digesta pH value followed the high-fat dietary regimen without and with the raspberry extract supplementation (P < 0.05 vs. C, HF + PP + FOS).

All three high-fat treatments regardless of any dietary supplementation caused a significant decrease in the caecal activity of bacterial α-glucosidase (P < 0.05 vs. C; Fig. 8). The HF + PP + FOS group was characterized by the lowest cecal activity of bacterial β-glucosidase, α-

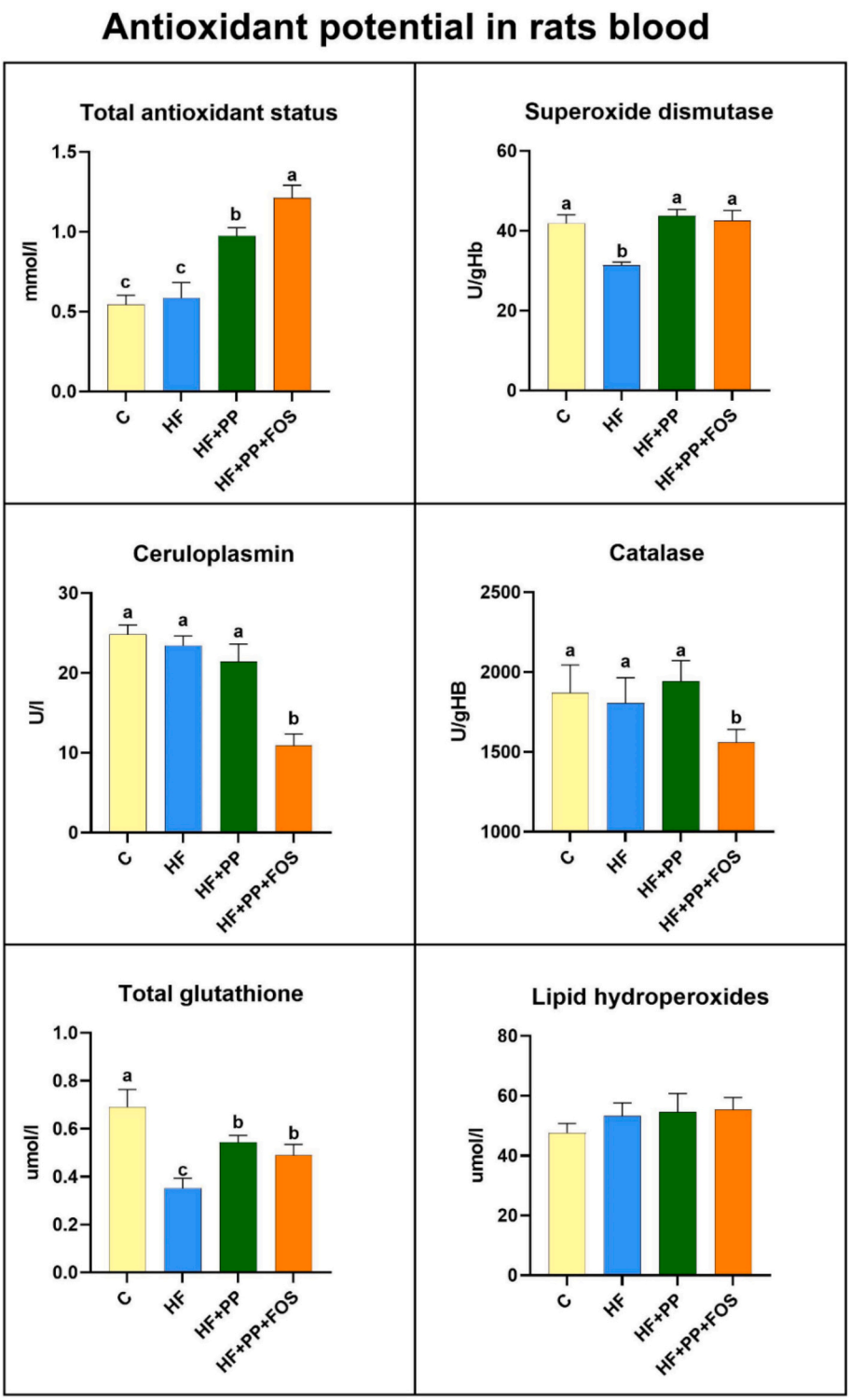

Fig. 1. Selected blood factors related to antioxidant potential in rats fed the experimental diets ($n = 8$). Within each parameter, bars marked with different superscript letters differ significantly at $P < 0.05$, whereas bars sharing at least one common letter are not significantly different. C, control diet; HF, high-fat diet; HF + PP, high-fat diet supplemented with raspberry polyphenol extract; HF + PP + FOS, a high-fat diet supplemented with raspberry polyphenol extract and fructooligosaccharides.

and β-galactosidase, and β-glucuronidase ($P < 0.05$ vs. all other groups). The highest cecal activity of α-galactosidase was observed in the control rats while that of β-galactosidase and β-glucuronidase in the HF group (in all cases $P < 0.05$ vs. remaining treatments).

The differences observed in cecal SCFA profile, digesta pH, and bacterial enzyme activity were paralleled by changes in cecal microbiota composition and diversity (Fig. 9). The cecal microbiota profile at the order level differed among the experimental groups, with visible shifts in the relative abundance of the dominant bacterial orders in rats receiving the HF + PP + FOS diet. Alpha diversity analysis, assessed using the Shannon index for all post hoc comparisons, showed no significant differences between the HF + PP + FOS group and either the control or HF

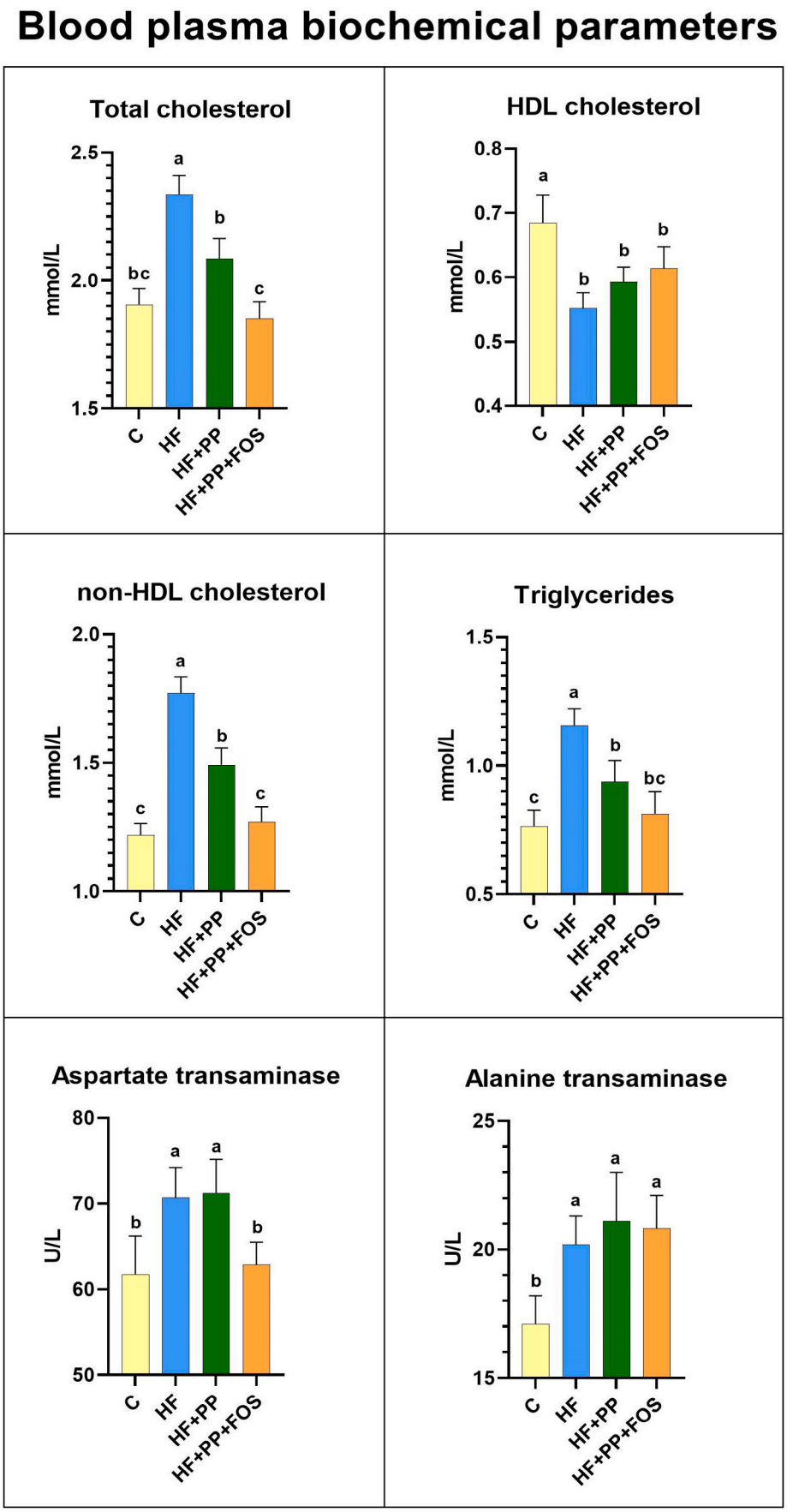

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Fig. 2. Blood plasma biochemical parameters of rats fed experimental diets (n = 8). Within each parameter, bars marked with different superscript letters differ significantly at P < 0.05, whereas bars sharing at least one common letter are not significantly different. Results are expressed as the means ± SEM. HF + PP, high-fat diet supplemented with raspberry polyphenol preparation; HF + PP + FOS high-fat diet supplemented with raspberry polyphenol preparation and fructooligosaccharides.

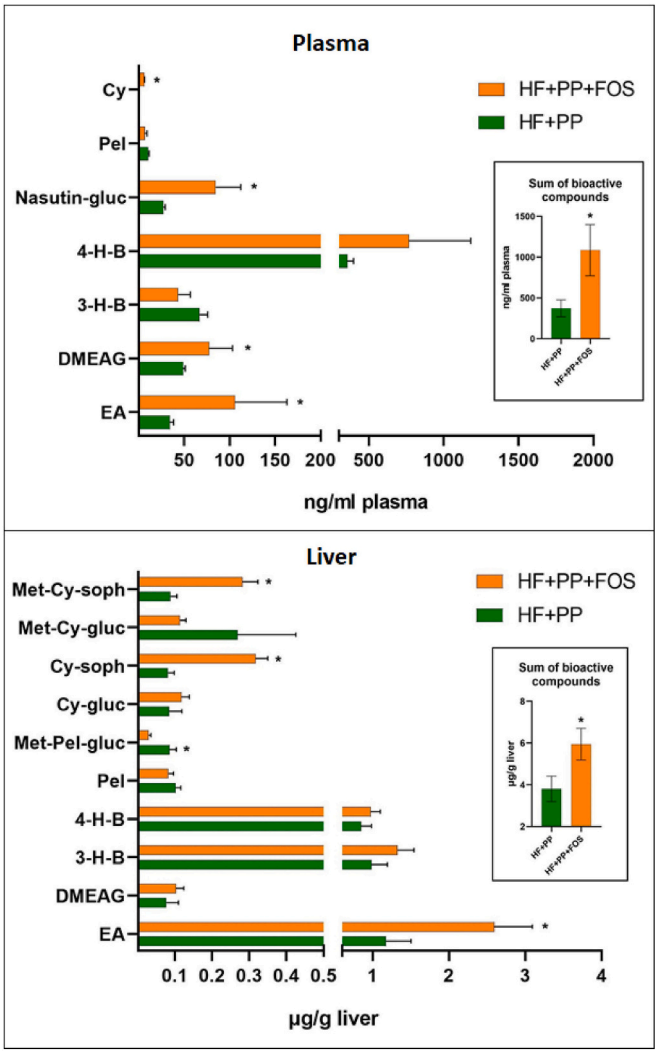

Fig. 3. Plasma and liver profiles of bioactive compounds derived from the raspberry polyphenol preparation in the rats fed the experimental diets (n = 8). Within each parameter, bars marked with different superscript letters differ significantly at P < 0.05, whereas bars sharing at least one common letter are not significantly different. Results are expressed as the means ± SEM. HF + PP, high-fat diet supplemented with raspberry polyphenol preparation; HF + PP + FOS high-fat diet supplemented with raspberry polyphenol preparation and fructooligosaccharides. DMEAG, ellagic acid dimethyl ether glucuronide; EA, ellagic acid; Pel, pelargonidin; Cy, cyaniding; Cy-soph, cyanidin-sophoroside; Cy-gluc, cyanidin-glucoside; Met-Cy-gluc, methyl-cyanidin-glucoside; Met-pel-gluc, methyl-pelargonidin-glucoside; Met-Cy-soph, methyl cyanidin-sophoroside; Nasutin-gluc, nasutin-glucuronide; 3-H-B, 3-hydroxybenzoic acid; 4-H-B, 4-hydroxybenzoic acid.

group, whereas Shannon diversity was significantly lower in the HF + PP + FOS group than in the HF + PP group ($P = 0.033$). In contrast, beta diversity analysis based on Bray–Curtis dissimilarity revealed significant separation of the HF + PP + FOS group from the C, HF, and HF + PP groups ($P = 0.002$, $P = 0.001$, and P = 0.001, respectively). At the genus level, the HF + PP + FOS treatment was associated with significant differences in several bacterial taxa relative to each comparison group.

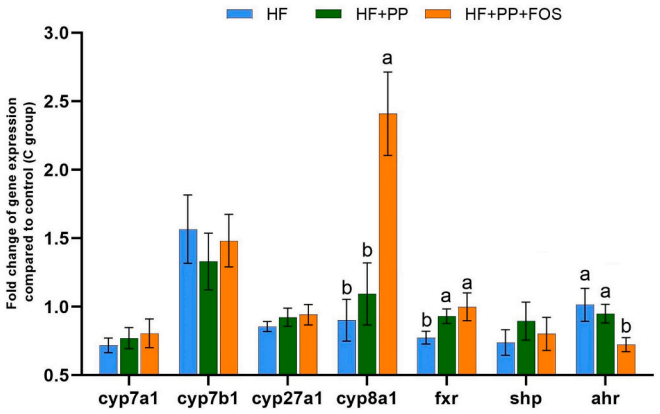

Fig. 4. Liver molecular factors of bile acid synthesis, and oxidative stress in the rats fed the experimental diets (n = 8). Within each parameter, bars marked with different superscript letters differ significantly at P < 0.05, whereas bars sharing at least one common letter are not significantly different. Results are expressed as the means ± SEM. HF + PP, high-fat diet supplemented with raspberry polyphenol preparation; HF + PP + FOS high-fat diet supplemented with raspberry polyphenol preparation and fructooligosaccharides. Ahr, aryl hydrocarbon receptor; cyp7a1, cholesterol 7 alpha-hydroxylase; cyp7b1, steroid 7-alpha-hydroxylase; cyp27a1, sterol 27-hydroxylase; cyp8b1, sterol 12alpha-hydroxylase; fxr, nuclear receptors farnesoid X receptor; shp, small heterodimer partner.

4. Discussion

Raspberry juice-derived preparations contain a complex mixture of polyphenols, mainly ellagitannins and anthocyanins, with smaller amounts of flavonols. In the present study, ellagitannins accounted for 52% of total polyphenols, anthocyanins for 42%, and flavonols for 6%, confirming enrichment in the phenolic classes commonly regarded as the main bioactive constituents of raspberries [33]. These compounds are increasingly recognized as modulators of processes linking intestinal function with hepatic metabolism, although their biological effects depend on their chemical structure, food matrix, and subsequent biotransformation. It should also be noted that the polyphenolic composition of raspberry-derived preparations is naturally variable and may be influenced by cultivar, ripeness, environmental conditions, and processing [33].

The present study suggests that combined supplementation with raspberry polyphenols and fructooligosaccharides (FOS) partly alleviated several adverse effects of a high-fat, low-fiber diet in Wistar rats. The obesogenic diet induced a phenotype consistent with MASLD-like dysfunction, including higher body and liver weight, hepatic lipid accumulation, dyslipidemia, and oxidative stress. Against this background, the combined intervention was associated with improvements in both intestinal and hepatic parameters, which is consistent with the involvement of the gut–liver axis rather than the liver alone. At the same time, the observed changes should be interpreted as coordinated associations within this axis rather than as evidence of a defined causal mechanistic sequence.

In the current study, liver concentrations of phenolic metabolites increased markedly, with ellagic acid (EA) as the predominant compound. Because EA has documented antioxidant and hepatoprotective properties and has been linked to improvements in steatosis and plasma lipid profile in rodent models [34,35], its higher hepatic level may have contributed to the lower triglyceride and cholesterol accumulation

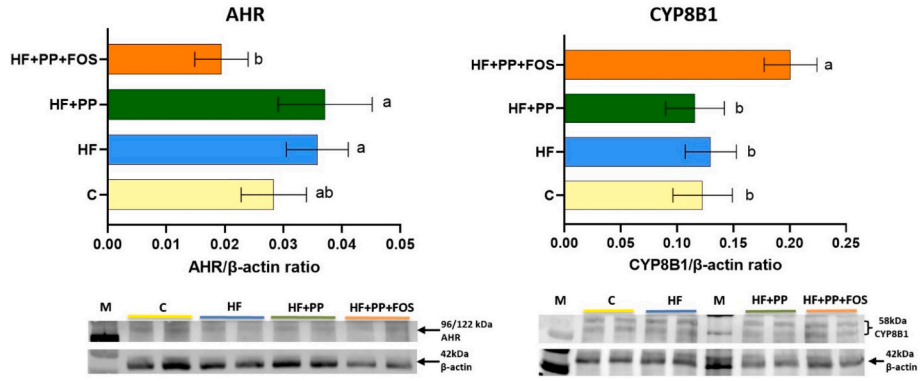

Fig. 5. The protein expression of AHR and CYP8B1 in the liver (n = 8). Within each parameter, bars marked with different superscript letters differ significantly at P < 0.05, whereas bars sharing at least one common letter are not significantly different. Results are expressed as the means ± SEM. HF + PP, high-fat diet supplemented with raspberry polyphenol preparation; HF + PP + FOS high-fat diet supplemented with raspberry polyphenol preparation and fructooligosaccharides. AHR aryl hydrocarbon receptor; CYP8B1, sterol 12alpha-hydroxylase.

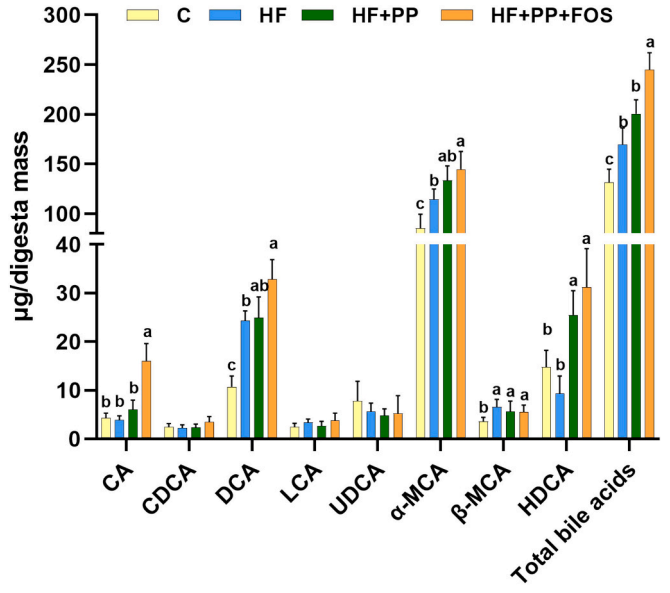

Fig. 6. Cecal concentrations of bile acids formed through microbial metabolism in the rats fed the experimental diets (n = 8). Within each parameter, bars marked with different superscript letters differ significantly at P < 0.05, whereas bars sharing at least one common letter are not significantly different. C, control diet; HF, high-fat diet; HF + PP, high-fat diet supplemented with raspberry polyphenol extract; HF + PP + FOS, high-fat diet supplemented with raspberry polyphenol extract and fructooligosaccharides. CA, cholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; LCA, litocholic acid; α-, β-MCA, α-, β-muricholic acid; UDCA, ursodeoxycholic acid; HDCA, hyodeoxycholic acid; Total bile acids, the sum of all measured bile acids formed through microbial metabolism.

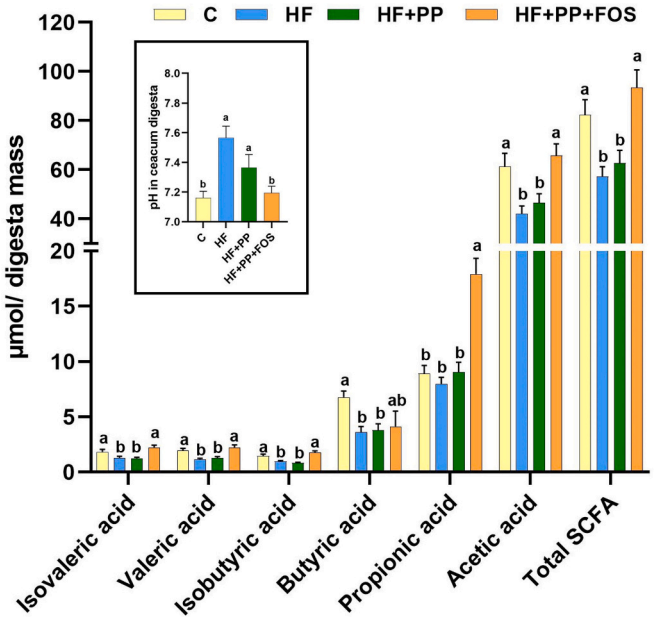

Fig. 7. Caecum concentration of short-chain fatty acids and pH of caecum digesta in the rats fed the experimental diets (n = 8). Within each parameter, bars marked with different superscript letters differ significantly at P < 0.05, whereas bars sharing at least one common letter are not significantly different. C, control diet; HF, high-fat diet; HF + PP, high-fat diet supplemented with raspberry polyphenol extract; HF + PP + FOS, high-fat diet supplemented with raspberry polyphenol extract and fructooligosaccharides. Total SCFA; the sum of all short-chain fatty acids.

observed in the HF + PP + FOS group. This interpretation is supported by the plasma results from the same experiment, where the combination of raspberry extract and FOS increased circulating levels of several phenolic metabolites, including ellagic acid, ellagic acid dimethyl ether glucuronide, nasutin glucuronide, cyanidin-derived compounds, and hydroxybenzoic acids. Taken together, the plasma and liver profiles suggest that FOS not only increased overall exposure to raspberry-derived metabolites, but may also have shifted their distribution toward greater hepatic availability, especially for ellagic acid and simpler phenolic acids such as 3- and 4-hydroxybenzoic acids [36]. Differences between plasma and liver metabolite profiles may reflect selective intestinal absorption, first-pass hepatic uptake, tissue-specific retention, and further biotransformation of individual compounds [37]. Together, these processes may determine whether a given metabolite remains detectable in circulation or preferentially accumulates in the liver. Overall, these findings suggest that FOS may enhance microbial transformation of complex raspberry polyphenols and facilitate systemic delivery of their smaller metabolites, thereby amplifying extra-intestinal effects.

The favorable hepatic changes were accompanied by an improved plasma lipid profile, reflected by lower cholesterol and triglyceride concentrations. One possible contributor to these effects is ellagic acid, which has been reported to exert antioxidant and anti-inflammatory activity and to modulate pathways involved in lipid metabolism [38,39]. This may be particularly relevant in MASLD and MASH, where disturbances in lipid handling, inflammatory signaling, and bile acid homeostasis are closely interconnected. In this context, recent evidence

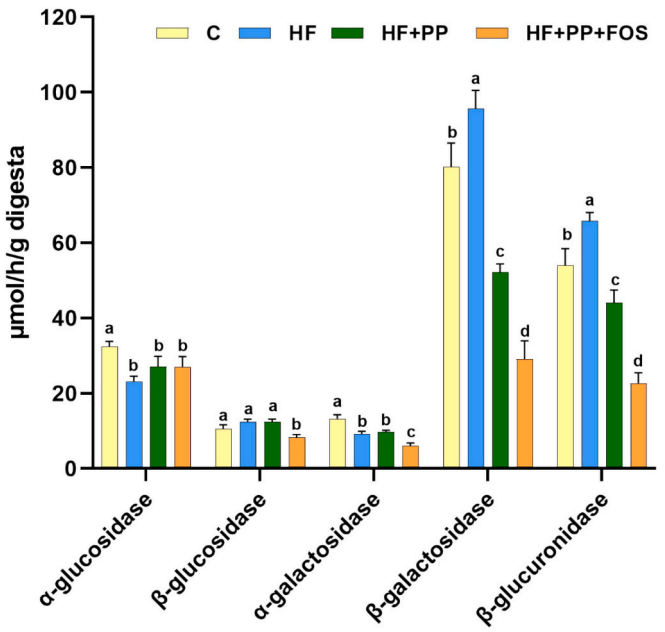

Fig. 8. The activity of selected bacterial enzymes in the caecum of rats fed the experimental diets (n = 8). Within each parameter, bars marked with different superscript letters differ significantly at $P < 0.05$, whereas bars sharing at least one common letter are not significantly different. C, control diet; HF, high-fat diet; HF + RE, high-fat diet supplemented with raspberry polyphenol extract; HF + RE + FOS, high-fat diet supplemented with raspberry polyphenol extract and fructooligosaccharides.

suggesting that EA may influence AHR-related responses in hepatocytes [40] is of interest, especially given the growing recognition of AHR as a factor involved in dysregulated lipid metabolism, inflammation, and bile acid signaling in these conditions [34,41]. Within this framework, the reduced hepatic *Ahr*/AHR expression together with elevated *Cyp8b1*/CYP8B1 expression in the HF + PP + FOS group may reflect coordinated changes in selected bile acid-related and cholesterol-related pathways; importantly, however, lower *Ahr*/AHR expression should not be interpreted as direct evidence of reduced AHR signaling activity, but rather as an expression change associated with the dietary intervention.

Changes in bile acid metabolism may be one of the processes associated with the observed effects. Bile acids play central roles in lipid digestion, cholesterol homeostasis, vitamin absorption, antimicrobial defense, and metabolic signaling, and their dysregulation is closely linked to obesity-related disorders and chronic liver disease [42–44]. In the present study, the HF + PP + FOS treatment increased cecal cholic acid (CA), which, together with elevated hepatic expression of *Cyp8b1* and *Fxr*, may be consistent with changes in selected bile acid-related markers. These findings should be interpreted together with the microbial data, because the intestinal microbiota contributes to the conversion of primary bile acids into secondary forms and may therefore be relevant to the intestinal bile acid profile observed in this study. Primary bile acids synthesized in the liver, including CA and CDCA, are transformed by intestinal microorganisms into secondary bile acids such as deoxycholic acid (DCA), hyodeoxycholic acid (HDCA), ursodeoxycholic acid (UDCA), and muricholic acid derivatives [42]. Within this framework, the high-fat diet itself increased DCA and α-MCA relative to controls, whereas the highest concentrations of both were observed in the HF + PP and HF + PP + FOS groups. β-MCA was elevated in all experimental groups, while HDCA increased significantly only in the polyphenol-supplemented groups. This pattern is noteworthy because DCA, formed from CA through bacterial 7α-dehydroxylation, is considered one of the more hydrophobic and potentially cytotoxic secondary bile acids, and its excessive intestinal accumulation has been associated with epithelial stress, oxidative imbalance, and metabolic disturbances [45–47]. Taken together, these findings suggest that the high-fat dietary background modified microbial bile acid turnover, while raspberry polyphenols, especially when combined with FOS, were associated with a further shift in these bile acid-related readouts. This interpretation is also consistent with the sum of the measured cecal bile acids, which increased in the HF and HF + PP groups and was elevated even more markedly in the HF + PP + FOS group. Such a scenario is biologically plausible because gut microbiota composition is closely related to intestinal bile acid transformation [48]. Since raspberry polyphenols may influence microbial activity and bile acid metabolism [49,50], and FOS provides a fermentable substrate that can modify the intestinal environment, the combined intervention may have been associated with differences in both bacterial composition and bile acid-transforming conditions in the cecum. In line with this broader concept, Zheng et al. (2017) [51] showed that combining FOS with polyphenols modified rat gut microbiota composition, supporting the view that such dietary combinations can reshape microbial ecology.

This interpretation is reinforced by the SCFA profile in the cecal digesta. The high-fat diet reduced total SCFA production compared with the control diet, whereas combined supplementation with raspberry polyphenols and FOS markedly increased total SCFA, restoring it to a level comparable to, or even exceeding, that of the control group. Acetate remained the predominant SCFA in all groups, but the HF + PP + FOS group also showed a clear increase in propionate and higher concentrations of isovaleric, valeric, and isobutyric acids compared with the HF + PP group. Notably, butyrate, which was reduced by the high-fat diet, was partially restored by the combined intervention. These changes coincided with the lowest cecal pH in the HF + PP + FOS group, supporting the view that FOS may have supported saccharolytic fermentation and modified metabolic conditions in the large intestine. This is consistent with the concept that carbohydrate fermentation promotes SCFA production and lowers luminal pH, which in turn may shape microbial composition and activity [52]. Propionate has been linked to protection against diet-induced obesity and insulin resistance through effects on gut hormones and food intake [53], whereas pH lowering associated with active fermentation may favor butyrate production and butyrate-producing bacteria while limiting the growth of *Bacteroides* spp. [54]. Altogether, these findings suggest that the combined polyphenol and FOS intervention improved the intestinal fermentation profile and bile acid-transforming environment in a way that may be relevant to both local gut homeostasis and metabolic responses observed in the liver.

This interpretation is further supported by the activity of microbial enzymes in the cecal digesta. Combined supplementation with raspberry polyphenols and FOS reduced the activity of several bacterial enzymes, particularly β-galactosidase and β-glucuronidase, and also lowered α-galactosidase activity. These changes may be beneficial because elevated β-glucuronidase activity can promote the deconjugation of glucuronidated compounds excreted with bile, thereby increasing intestinal exposure to metabolites that would otherwise be eliminated [55]. Likewise, high activities of β-glucosidase and related glycosidases are often associated with less favorable colonic metabolism. Their reduction, together with the improved SCFA profile and lower cecal pH, suggests that the combined intervention shifted microbial activity toward a more favorable functional state. Thus, the combined treatment with raspberry polyphenols and FOS appeared to involve not only a more favorable fermentation pattern, but also qualitative remodeling of microbial metabolism in a direction that may support intestinal homeostasis and, indirectly, hepatic metabolic responses.

The microbiome findings provide additional support for this interpretation. Compared with the other groups, HF + PP + FOS showed the clearest shift in cecal microbial composition, suggesting that the addition of FOS to raspberry polyphenols promoted broader microbial remodeling than polyphenols alone under high-fat dietary conditions. This group was characterized by a higher abundance of several genera that may be relevant to the metabolic changes observed elsewhere in the

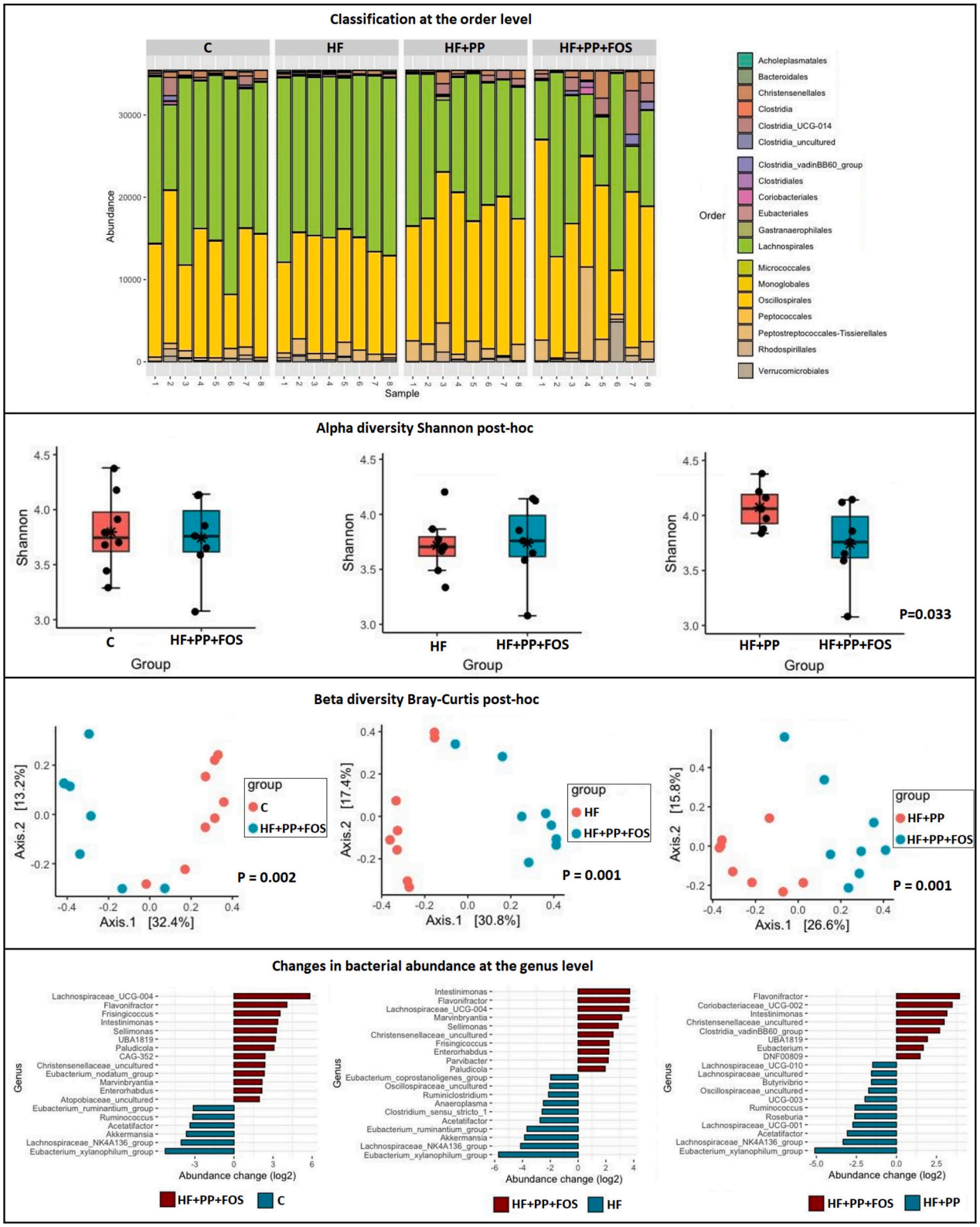

Fig. 9. Cecal microbiota composition and diversity in rats fed the control diet (C) or high-fat diet (HF) supplemented with raspberry polyphenols (PP) alone or combined with fructooligosaccharides (FOS), including the comparison highlighting the effect of the HF + PP + FOS dietary treatment (n = 8). The upper panel shows bacterial composition at the order level in cecal digesta. The middle panels present alpha diversity for all comparisons, assessed using the Shannon index; the horizontal line within each boxplot indicates the median, the asterisk indicates the mean, and individual points represent Shannon index values for individual samples. Beta diversity was assessed by Bray–Curtis dissimilarity, with *P* values indicating post hoc differences between the compared groups. The lower panel shows the bacterial genera in cecal digesta whose abundance differed significantly between groups in the post hoc analysis.

study, including *Flavonifractor*, *Intestinimonas*, Christensenellaceae-related taxa, *Lachnospiraceae_UCG-004*, *Sellimonas*, and, relative to HF + PP, *Coriobacteriaceae_UCG-002*. Although such compositional changes do not allow direct conclusions about function, they are still meaningful in the context of the other intestinal findings, since *Flavonifractor* and Coriobacteriaceae-related bacteria have been associated with the conversion of dietary polyphenols into smaller phenolic metabolites, whereas *Intestinimonas* and some Lachnospiraceae members are linked to intestinal fermentation and SCFA generation [49–52,54]. In this context, the distinct microbial profile of the HF + PP + FOS group was accompanied by a more favorable fermentation pattern, an altered bile acid profile in the cecal digesta, and a greater systemic availability of polyphenol-derived metabolites; however, the present study does not allow these relationships to be interpreted as causal. At the same time, the microbial response did not follow a simple pattern of uniformly increasing taxa regarded as beneficial and decreasing those considered less favorable. Instead, the intervention may have influenced the overall balance and metabolic activity of the microbial community rather than isolated bacterial groups alone. Overall, these observations are consistent with the broader results of the study and support the interpretation that combining raspberry polyphenols with FOS modified the intestinal milieu in a way that favored fermentation, bile acid transformation, and polyphenol bioconversion, thereby contributing to downstream improvements along the gut–liver axis [48,51,52,54].

Another important aspect of the observed response was oxidative balance, as oxidative stress is a central feature of obesity-related metabolic dysfunction and contributes to the progression of hepatic steatosis and associated liver injury [56]. In the present study, raspberry polyphenols improved systemic antioxidant status, and this effect appeared to be more pronounced when FOS was included in the diet. Total antioxidant status increased in the HF + PP group and reached its highest value in the HF + PP + FOS group, suggesting that the combined treatment was most effective in enhancing blood antioxidant capacity. This is consistent with the view that raspberry-derived polyphenol metabolites exert broad biological effects that are at least partly related to their antioxidant properties, and further supports the possibility that FOS may have supported these effects by promoting microbial transformation and systemic availability of phenolic metabolites. Additional support for this interpretation is provided by ceruloplasmin, a circulating multi-copper oxidase regarded as a biomarker of oxidative stress and, in some studies, also of obesity [57]. In the present study, ceruloplasmin was significantly reduced only in the HF + PP + FOS group, whereas no significant difference was observed between the HF and HF + PP groups. A similar pattern was noted for catalase. Although catalase is a key antioxidant enzyme involved in H_2O_2 detoxification [58], its lower activity in the HF + PP + FOS group, when considered together with the higher total antioxidant status, may reflect a lower need for compensatory enzymatic defense under conditions of reduced oxidative burden. This interpretation is further supported by the pattern observed for superoxide dismutase and total glutathione, both of which were impaired by the high-fat diet and improved in the raspberry-supplemented groups, whereas lipid hydroperoxides in blood remained unchanged. This suggests that the intervention more clearly affected antioxidant defense systems than circulating end-products of lipid oxidation.

These systemic effects were mirrored by changes in the liver. The hepatic reduced-to-oxidized glutathione ratio was decreased in all high-fat-fed groups relative to controls, confirming that the obesogenic diet imposed persistent oxidative pressure. However, this reduction was less pronounced in the HF + PP + FOS group than in the HF and HF + PP groups. At the same time, hepatic malondialdehyde levels were significantly elevated only in the HF group, whereas both raspberry-supplemented groups showed values comparable to controls. Taken together, these findings suggest that raspberry polyphenols, particularly when combined with FOS, may have alleviated hepatic lipid peroxidation and partly improved redox balance, even though not all oxidative markers were fully normalized under high-fat dietary conditions.

Taken together, the findings suggest that the addition of FOS to raspberry polyphenols was associated with a broader intestinal and hepatic response than supplementation with polyphenols alone. This response included greater systemic availability of polyphenol-derived metabolites, changes in cecal fermentation, bile acid profile, microbial enzyme activity, microbiota composition, hepatic molecular markers, and lipid-related outcomes. The overall pattern is biologically consistent with the concept that FOS may support the functional effects of raspberry polyphenols by improving intestinal conditions for fermentation and biotransformation. However, the present study does not establish direct causal links among these events, and the proposed relationships should therefore be regarded as hypothesis-supporting rather than mechanistically proven.

Several considerations should be kept in mind when interpreting these findings. The study was designed to provide an integrated view of intestinal and hepatic responses to the enrichment of a polyphenol-containing high-fat diet with FOS, rather than to establish direct causal relationships between individual endpoints. Accordingly, the liver-related response was characterized using biochemical, molecular, and oxidative stress-related markers, but without histological evaluation, and should be interpreted within that scope. In keeping with the microbiota-related aim of the work, bile acid analysis focused on selected cecal bile acids, particularly secondary bile acids linked to microbial metabolism, rather than on the entire bile acid pool, and therefore does not provide a direct assessment of bile acid flux or enterohepatic dynamics. Similarly, the microbiota data should be interpreted together with the SCFA, bile acid, and microbial enzyme results. In this context, the observed response is interpreted primarily in relation to the significantly higher levels of polyphenol-derived metabolites detected after the addition of FOS. However, since the experimental design did not include a high-fat + FOS-only group, the present data do not allow a formal distinction between combined and independent effects, and a more detailed assessment of the specific contribution of FOS would require a broader factorial design.

5. Conclusions

The present study suggests that combined supplementation with raspberry polyphenols and fructooligosaccharides may partly alleviate selected intestinal and metabolic disturbances induced by a high-fat, low-fiber diet in rats. The observed response included lower hepatic lipid accumulation, improvement in selected plasma lipid and oxidative stress-related parameters, and changes in cecal short-chain fatty acid production, bile acid profile, microbial enzyme activity, and microbiota composition. Taken together, these findings are consistent with the view that the combined intervention may have influenced both intestinal and hepatic processes, highlighting the relevance of the gut–liver axis in this model. The data further suggest that the addition of FOS to the polyphenol-containing diet may have contributed to the observed response by supporting microbial fermentation and by being associated with a greater systemic availability of smaller phenolic metabolites. In this context, ellagic acid and other low-molecular-weight polyphenol-derived compounds may have contributed to the observed hepatic and systemic responses, although the overall effect likely reflects the interaction of multiple mechanisms rather than a single dominant pathway. Overall, these results are consistent with the concept that combining polyphenol-rich raspberry preparations with a microbiota-targeting prebiotic may represent a nutritionally relevant strategy for supporting metabolic homeostasis under obesogenic dietary conditions. At the same time, given the animal nature of the study and the complexity of intestinal microbial and hepatic regulation, the findings should be interpreted with caution and viewed primarily as support for further mechanistic and translational research on dietary approaches relevant to MASLD-like disturbances.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used AI-assisted tools for language editing and clarity improvement. The authors reviewed and edited the content and take full responsibility for the final version.

CRediT authorship contribution statement

Bartosz Fotschki: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marta Kopcewicz:** Methodology, Investigation. **Joanna Fotschki:** Writing – review & editing, Writing – original draft, Methodology. **Sylwia Machcińska-Zielińska:** Writing – review & editing, Methodology, Investigation. **Mariola Słowińska:** Writing – review & editing, Investigation. **Tomasz Sawicki:** Methodology. **Wiesław Wiczkowski:** Formal analysis. **Michał Sójka:** Methodology. **Elżbieta Janda:** Investigation. **Katarzyna Ognik:** Investigation. **Adam Jurgoński:** Investigation. **Sara Silva:** Writing – review & editing. **Jerzy Juśkiewicz:** Writing – original draft, Supervision.

Ethics statement

We confirm that all the experiments were approved by the regional Institutional Animal Care and Use Committee (Permission No. 51/2016; Olsztyn, Poland), and all procedures were carried out in accordance with the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nfs.2026.100286.

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